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Invasive success of the fruit fly *Drosophila suzukii*: Role of polyphagy and thermal plasticity

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Abstract

Globally, the apparently inexorable increase of biological invasions and their resulting damage raise questions about the factors that influence invasion success. In this thesis, I considered the spotted wing drosophila, *Drosophila suzukii*, which provides an exemplar as a highly successful invasive species. It is an ideal model because of the rapidity of its invasion and the important economic losses resulting from its ability to oviposit in healthy fruit. I explored the mechanisms that could contribute to its rapid global invasion. In particular, I considered its polyphagy, the alternative resources it can use in seasons when cultivated fruit are not available, as well as the local, landscape-scale and climatic factors that influence its infestations. I also investigated its behavioural thermoregulation capacities and the plasticity of its thermal preference, traits that allow a better understanding of the ability of *D. suzukii* to exploit favourable microhabitats. Finally, I explored the effect of the interaction between food resource, temperature and the presence of the bacterial symbiont *Wolbachia* on the performance of *D. suzukii* and its oviposition choices. My thesis highlights the great plasticity of *D. suzukii* when confronted with environmental changes. This could represent a form of pre-adaptation facilitating the fly's rapid global spread. My work opens research avenues in applied research around crop protection by controlling the wild plants used by *D. suzukii*, and also in fundamental research around resource selection and the use of integrated approaches to understand invasion success.

Keywords: Behavioural thermoregulation, Biological invasions, Plant-insect interactions, Trophic ecology, *Wolbachia*

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General Introduction

1. CONTEXT AND CHALLENGES

Globally, biodiversity currently faces two main threats: global climatic change and biological invasions (Ward and Master 2007). Numbers of invasive species have escalated, especially those of plants and insects (Roques et al. 2009; Bonnamour et al. 2021). Primarily dispersed *via* human transport and trade (Levine and D'Antonio 2003; Fenn-Moltu et al. 2023b), their establishment can be facilitated by global change (Dukes and Mooney 1999; Ward and Master 2007). Invasive species have the potential to cause important economic losses when they disturb ecosystem services (Bradshaw et al. 2016; Henry et al. 2023), and also disrupt ecosystems and impact their biodiversity (Kenis et al. 2009; Tobin 2015; Yadav et al. 2023). The first part of this chapter introduces insect invasions and gives an overview of understanding of their economic and ecological impacts.

Many studies have addressed the invasion process and what makes a species a successful invader, particularly in the context of preventing or managing invasions (Tait et al. 2021; Zhao et al. 2023). To be successful, species have to pass through all stages of the introduction–naturalisation–invasion continuum (Chapple et al. 2012; Daly et al. 2023). Overall, successful invasive insects often have more pathways of introduction, occur in more habitats, have higher fecundity and voltinism, shorter lifespans and faster development from egg to adult, than is the case for non-successful species (Zhao et al. 2023). Adaptive abilities of the invaders are also determinants of invasion success (Alpert et al. 2000; Richardson and Pyšek 2006; Lee and Gelembiuk 2008). The second part of this chapter introduces concepts relating to the mechanisms facilitating and limiting the invasion of new environments, including the invasion continuum, the biotic and abiotic compartments, as well as the importance of symbionts.

Drosophila suzukii (Matsumura) is recognized as one of the world's 100 most serious invasive insect species (Zhao et al. 2023). Originating from Asia, it has successfully invaded all continents other than Antarctica in less than 20 years (Walsh et al. 2011; Fraimout and Monnet 2018). This fly ticks all boxes of successful invaders. It has taken advantage of multiple introduction pathways (Fraimout et al. 2017), occurs in cultivated, natural and urban habitats (Poyet et al. 2015; Urbaneja-Bernat et al. 2020; Ulmer et al. 2024), has high fecundity combined with a long adult lifespan (Kanzawa 1939) and is capable of producing several generations per year (Kanzawa 1939; Schlesener et al. 2020) thanks to its rapid development (Schlesener et al. 2020; Raynaud-Berton et al. 2024). *Drosophila suzukii* also possesses several relevant adaptive strategies including polyphagy, the use of a commonly vacant niche (i.e., ripening / healthy fruit; Atallah et al. 2014), and good acclimation capacities (Raynaud-Berton et al. 2024). The

final part of this chapter considers why *D. suzukii* provides an ideal model to study the mechanisms underlying invasion success.

While *D. suzukii* has been widely studied, key questions remained unaddressed or unresolved at the start of this thesis. Most studies on *D. suzukii* to date have focused on evaluating infestations in crop fruit or surrounding wild plants during the abundance peak of the population in summer / early autumn when most damage is caused (Burrack et al. 2013; Poyet et al. 2015; Kenis et al. 2016; Rombaut et al. 2017; Tait et al. 2021). However, *D. suzukii* is present in the environment year-round (Wang et al. 2016; Stockton et al. 2020; Tait et al. 2020b; Ulmer et al. 2024). Overwintering in a reversible reproductive dormancy, females resume oviposition when temperature rises sufficiently (Shearer et al. 2016; Grassi et al. 2018; Panel et al. 2018). In the first of the three main sections of this thesis, I address the potential resource continuum available for the fly from autumn, through winter, to spring. I first assessed whether fallen fruit could provide a reservoir for the fly at the end of the crop season as, even though *D. suzukii* oviposits preferentially in healthy fruit, it is also able to use decaying fruit, especially when other resources are scarce (Kienzle et al. 2021). Apple, *Malus domestica*, were chosen for this part of the study because this is one of the most important fruit crop species worldwide (FAOSTAT 2023), its fruit have a long decaying process and *D. suzukii* are known to utilise them (Weydert and Mandrin 2013; Asplen et al. 2015; Briem et al. 2015; Bal et al. 2017). I then studied the fly's use of mistletoe (*Viscum album*) at the national scale within France. The fruit of this parasitic plant are available in high quantities from the middle of winter to the end of spring (Thomas et al. 2023) and are, again, highly suitable for its development (Poyet et al. 2015) and known to be infested by *D. suzukii* in nature (Briem et al. 2016; Panel et al. 2018; Delbac et al. 2020a). Finally, I considered the role of ornamental plant species in late winter, as potential reservoirs which could catalyse fly population expansion in early spring before cultivated fruit crops become available. Throughout these studies, I assessed for the first time the role of climatic, local and landscape factors in shaping *D. suzukii* (and other drosophilids) infestation.

Drosophila suzukii is able to use many habitats (Santoiemma et al. 2018; Tait et al. 2020a). Adults are found in diverse landscapes including woodlands, hedges, crops and urban and domestic situations (Santoiemma et al. 2018; Ulmer et al. 2024). At a broader scale, it has invaded both temperate and tropical areas (Walsh et al. 2011). While the thermal biology of the fly has been thoroughly examined (Raynaud-Berton et al. 2024), its behavioural thermoregulation capacity and plasticity are yet to be investigated. As a polyphagous fly (Poyet et al. 2015; Kenis et al. 2016), its larvae are highly likely to experience variable

microclimates and I hypothesised that the behavioural thermoregulation abilities of female flies would be a determinant of overall offspring fitness. In the second part of this thesis, I studied these behavioural thermoregulation abilities and their fitness advantages. I first developed a conceptual framework to study the behavioural thermoregulation strategies of ectotherms using the related *D. melanogaster*. Then, I applied this framework to study the behavioural thermoregulation strategies of *D. suzukii*. Finally, I examined the adaptive value of an apparent 'hyperplasticity' in thermal preference (a metric used to assess behavioural thermoregulation).

The various host plants susceptible to *D. suzukii* infestation have been widely studied, including their influence on fly performance (Burrack et al. 2013; Poyet et al. 2014, 2015; Kenis et al. 2016; Olazcuaga et al. 2019). However, to date, performance in the winter / early spring period has not been assessed under realistic temperature regimes, and this forms the opening of the third part of this thesis. I then examined whether the strain of *Wolbachia* associated with *D. suzukii*, whose role is believed to be mutualistic (Mazzetto et al. 2015; Saeed et al. 2018), could help the fly to deal with some of the possible consequences of polyphagy. Finally, while *D. suzukii* performance is known to differ between different host fruit and attraction to fruit is also known to be influenced by multiple factors (Poyet et al. 2015; Olazcuaga et al. 2019), the influence of either temperature or *Wolbachia* on oviposition choice have also not previously been studied. Therefore, I assessed whether trade-offs existed between thermal and nutritional choice in female oviposition, in the presence or absence of infection by *Wolbachia*.

2. INSECT BIOLOGICAL INVASIONS

Dispersal is a natural process limited and influenced by multiple factors, particularly those relating to biogeography (Kühn and Klotz 2008). However, human-assisted (anthropogenic) dispersal has increasingly overcome all barriers, enabling access to all parts of the world. This has led to thousands of species introductions globally, with sometimes devastating effects on ecosystems (natural and human-associated), human health and economic impacts (Nentwig 2008a). In the last century, rapid globalisation of trade and human movement has further accelerated the rate of biological invasions (Nentwig 2008a; Bonnamour et al. 2021). The number of invasive species worldwide is unknown, but some estimates indicate that it includes up to 10,000 plant species, 300 vertebrates, more than 5,000 invertebrates and many thousands of microorganisms (Nentwig 2008a). Insects are the second most invasive organisms globally (Roques et al. 2009; Bonnamour et al. 2021). In this section I focus on insect invasions,

considering the stages in the invasion process, the economic costs of invasions and their impacts on ecosystems.

2.1. Invasive species

To be considered invasive, species must pass through all the stages of the introduction–naturalisation–invasion continuum (Figure 1) and, to do so, they can face multiple barriers (Chapple et al. 2012; Daly et al. 2023). First, the species must arrive in the new location, an event which often occurs along human transport and trade routes that help to overcome geographical barriers (Fenn-Moltu et al. 2023a). Second, it needs to survive and establish, which requires a sufficient population size, including reproductive individuals, and the survival of often novel environmental conditions (Wilson et al. 2009; Fauvergue et al. 2012; Bock et al. 2015). Third, if the species starts to expand its range in the novel location, at the same time displacing or impacting native species, it is considered invasive (Levine 2008). Amongst species that are transported to new areas, only a small fraction successfully establishes and, in turn, only a small fraction of these then successfully spread and impact native communities (Levine 2008).

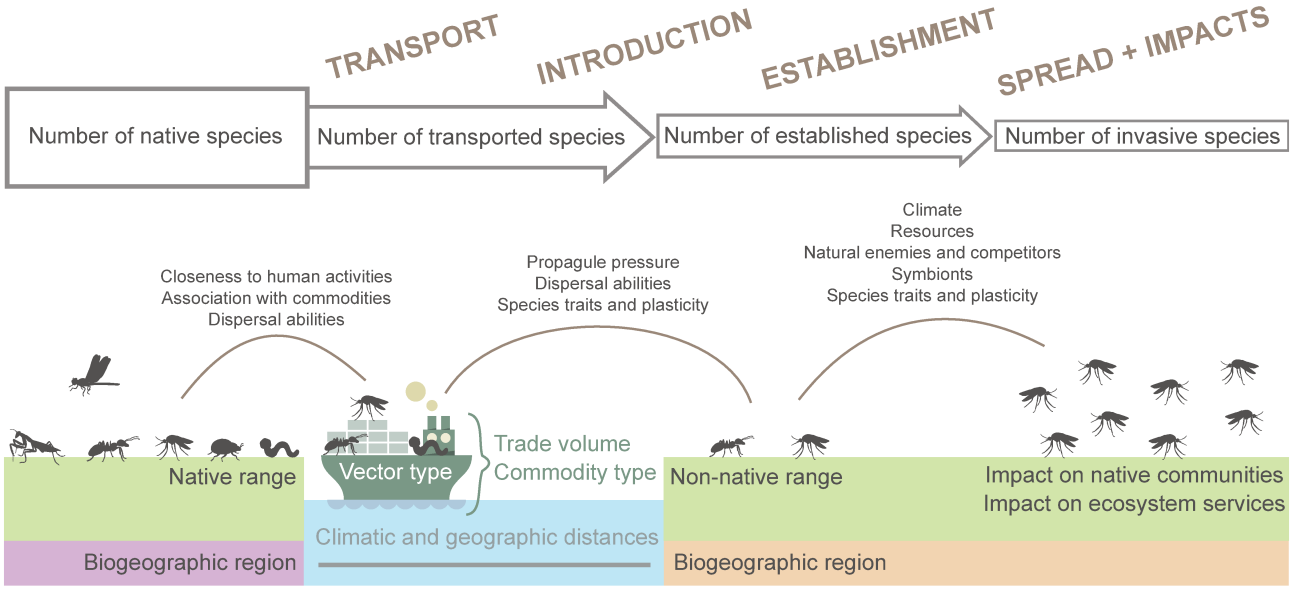

Figure 1 The invasion process for unintentional species introductions. The stages of transport, introduction, establishment and spread / impacts are shown, along with key mechanisms affecting success/failure at each stage. Transport includes both maritime, aerial and overland introduction pathways. Arrow size indicate the decrease in proportion of species that succeed to overcome all the steps of the invasion process. Adapted from Daly et al. (2023) and Fenn-Moltu et al. (2023a).

2.2. Introduction

The process of introduction includes both the transport of a non-native species to a new exotic range, and subsequent transport within that exotic range (Levine 2008). Most invasions start with an accidental introduction or as a result of an intentional introduction that then spreads beyond the intended use (Levine 2008; Nentwig 2008b). In most cases, insect introductions are accidental, with the exception of biological control agents or leisure/pet species (Levine 2008; Roques et al. 2009). In Europe, 90% of non-native invertebrates were introduced unintentionally through human activity, 9% were biological control agents and 1% were leisure escapes (Roques et al. 2009).

For deliberately released biological control agents, pathways of introduction are well known (Roques et al. 2009). Some were first released in glasshouses and escaped, others were directly released into the outdoor environment (Roques et al. 2009). Examples include several ladybird beetles, such as the Asian species *Harmonia axyridis*, several parasitoid wasps (e.g., the whitefly parasitoid *Encarsia formosa*) and predatory true bugs and mites (Roques et al. 2009). Other species were introduced for leisure or as pets and escaped, including various lepidopterans, such as saturniid moths (e.g., *Samia cynthia*), or cockroaches used as food for reptiles (Roques et al. 2009).

Accidental introduction pathways are often linked with human transport and trade (Levine and D'Antonio 2003; Fenn-Moltu et al. 2023a,b). Most non-native insects (about 38%) arrive as contaminants of a commodity, following horticultural and ornamental trade routes (Roques et al. 2009; Fenn-Moltu et al. 2023b). For instance, the Asian long-horned beetle, *Anoplophora chinensis*, was introduced associated with bonsai trees (Roques et al. 2009). Another 15% are stowaways (Roques et al. 2009). For instance, the Asian tiger mosquito, *Aedes albopictus*, transits as eggs and larvae in second-hand tyres containing water or inside bags used to water plants (Roques et al. 2009). Other introduction pathways include the trades of stored products, vegetables and fruit, wood materials and animal husbandry (Roques et al. 2009; Fenn-Moltu et al. 2023b). In Europe, since the 2000s, an average of 17.5 new introduced species of insects have been recorded per year (Roques et al. 2009).

2.3. Establishment

Establishment requires the development of viable reproducing populations in the invaded range (Blackburn et al. 2011; Chapple et al. 2012). Successful establishment is strongly dependent on the abiotic and biotic characteristics of the receiving environment (abiotic and biotic resistance, respectively; Levine

2008), referred to as the ecosystem invasibility (Daly et al. 2023), and on the possession of specific advantageous life history traits, referred to as the species invasiveness (Daly et al. 2023).

Abiotic features such as temperature, moisture and salinity are the major factors limiting invasive species establishment (Levine 2008). This is particularly true for insects. As ectotherms, their body temperature is directly linked to ambient temperature (Woods et al. 2015). Insects are also particularly vulnerable to desiccation because of their small size and limited capacity for storing water (Addo-Bediako et al. 2001). However, these abiotic filters during the invasion process have mostly been studied at large biogeographic scales and much less at local scales (Levine 2008).

The pre-existing communities into which non-native species are introduced include competitors, predators and pathogens that constitute biotic resistance (Levine 2008). There are conflicting views of whether or not a more diverse community is likely to be invaded (Daly et al. 2023). Spatial and temporal environmental heterogeneity is suggested to promote coexistence of both resident and invasive species (Melbourne et al. 2007), while abiotic resistance often overwhelms biotic resistance (Levine 2008). For instance, the distribution of invasive Argentine ants (*Linepithema humile*) in southern California is largely constrained by moisture availability while competition from the native harvester ants is of little importance (Heller et al. 2008).

Introduced populations initially comprise a small number of individuals, as a result of bottleneck events during transport and introduction (Sakai et al. 2001; Hufbauer and Torchin 2008). Insects, because of their small size, are often less easily detected than other, larger invasive species (Holway et al. 2002). Consequently, a larger number of individuals can be introduced in a single introduction event, i.e. higher propagule pressure, and more separate introduction events may occur, favouring admixture which, in turn, enhance the genetic diversity and the adaptive potential and chances of establishment of the introduced population (Wilson et al. 2009; Fauvergue et al. 2012; Bock et al. 2015). Increased propagule pressure has been used to improve success in establishing non-native pest regulatory species such as parasitoids (Roques et al. 2009).

Propagule pressure alone does not explain why some species establish successfully and others do not (Chapple et al. 2012). Species adaptive abilities, especially phenotypic plasticity which confers high performance across a broad range of environmental conditions (Richards et al. 2006), also influences establishment success (Alpert et al. 2000; Richardson and Pyšek 2006; Lee and Gelembiuk 2008). Plastic responses can allow invaders to thrive in environmental conditions that only partially or sub-optimally

match those of their native range (Richards et al. 2006; Renault et al. 2018). Behavioural abilities, while less studied, can also act at all stages of the invasion process, and especially during establishment (Chapple et al. 2012). For instance, the Argentine ant's (*Linepithema humile*) ability to modify its behaviour according to environmental conditions and resource availability was a better predictor of its establishment success than was propagule pressure (Sagata and Lester 2009).

2.4. Spread

After initial establishment, range expansion (spread) takes place from the point of introduction (Blackburn et al. 2011). This step is driven by demographic and ecological processes, including population growth and dispersal (Hastings et al. 2005). When a species spreads successfully, and also impacts native species, it is considered invasive (Blackburn et al. 2011).

Species dispersal abilities determine the speed of colonisation of new habitats (Renault et al. 2018). In Europe, many non-native insect species first reported in the late 1990s / early 2000s spread extremely rapidly, invading much of the continent within a few years (Roques et al. 2016). They include wood-associated species (e.g., the chestnut gall wasp, *Dryocosmus kuriphilus*, the honeylocust gall midge, *Obolodiplosis robiniae*, the elm zig-zag sawfly, *Aproceros leucopoda*, the box tree moth, *Cydalima perspectalis*), palm-tree-associated species (e.g., the palm weevil, *Rhynchophorus ferrugineus*, the palm moth, *Paysandisia archon*), crop pests (e.g., the tomato leafminer, *Tuta absoluta*, the fruit fly, *Drosophila suzukii*), predators (e.g., the Harlequin ladybeetle, *Harmonia axyridis*) and many others (Roques et al. 2016). In recent years, the rate of spread of introduced insects was three-fold greater than in the late 1900s and 5.7-fold higher than between 1950 and 1969 (Roques et al. 2016).

Temperature is an important predictor of range expansion for some insects (Evans and Gregoire 2007; Morin et al. 2009; Lantschner et al. 2014). Global warming, with increase of mean annual temperature and climate homogenisation, can reduce the biotic barriers faced by non-native insects and, thereby, facilitate their spread (Dukes and Mooney 1999; Thuiller et al. 2008). However, an even more important driver of non-native insect spread is now trade (Levine and D'Antonio 2003), and especially the horticultural trade (Roques et al. 2016). Plant trade facilitates long distance movement and increases the likelihood of associated inadvertent introduction of phytophagous insect species (Roques et al. 2016), consistent with herbivores being the major group of non-native insects in Europe (Roques et al. 2009).

3. ECOSYSTEM DISRUPTION AND ECONOMIC IMPACTS

Biological invasions in general are responsible for high economic losses including both damage costs (e.g., economic losses due to direct and/or indirect impacts, such as yield loss, damage and disease, land alteration, infrastructure damage or income reduction) and management expenditure (e.g., resource allocation to avoiding/mitigating invasion, prevention, control, research, long-term management or eradication) (Diagne et al. 2021). Below, examples of invasive insect species that have important economic costs linked to their disruption of ecosystem services are considered.

3.1. Overall cost of invasive insects

Invasive insect species have the potential to disrupt ecosystems at the community and ecosystem scales (Kenis et al. 2009). By entering a new community, invasive species modify species interaction networks, through complex mechanisms including competition for resources, disease transmission or pollination disruption, amongst others, with sometimes wide ranging impacts (Kenis et al. 2009). At the ecosystem scale, invasive insects, by affecting different ecosystem services, can disrupt nutrient fluxes and have wide-ranging impacts (Kenis et al. 2009). For instance, forest defoliation by the invasive gypsy moth *Lymantria dispar* affects the populations and reproductive success of forest-dwelling birds, as nests located in defoliated sites suffer a higher predation rate than those in non-defoliated areas (Thurber et al. 1994). Natural enemy species have been introduced to control *L. dispar* but several of these were generalists and attacked native species (Brown 1984; Elkinton and Boettner 2012). For example, the tachinid fly *Compsilura concinnata*, a polyphagous parasitoid species known to attack more than 200 hosts, was introduced to North America in 1906 in an attempt to control the gypsy moth population, but eventually decimated many of the larger moth species previously abundant in the region (Elkinton and Boettner 2012). This is an example of how many invasive pest insects can have cascading effects on ecosystem functioning (Kenis et al. 2009), leading to sometimes massive economic losses.

Between 1970 and 2017, the total reported cost of invasions has been estimated at US$1.288 trillion, with an annual mean cost of US$26.8 billion (Diagne et al. 2021). This figure is likely to be a considerable underestimation, as only a subset of invasive species has reported costs (Bradshaw et al. 2016; Henry et al. 2023). Amongst invasive species, invertebrates are the greatest contributors over this period, with a cumulative cost of US$416 billion (between 1970 and 2017) and a mean annual cost of US$8.7 billion, with insects accounting for 90% of these costs (Diagne et al. 2021). Seven of the ten most

economically damaging invasive taxa are insects (Figure 2). Invasive insects have consequences on all major classes of ecosystem services (i.e., provisioning, regulating, supporting and cultural; Millennium Ecosystem Assessment 2005) (Bradshaw et al. 2016; Henry et al. 2023). Taking all these consequences into account, Bradshaw et al. (2016) estimated the cost of invasive insects globally to be tenfold greater than Diagne et al. (2021), with a minimum cost of US$70.0 billion per year.

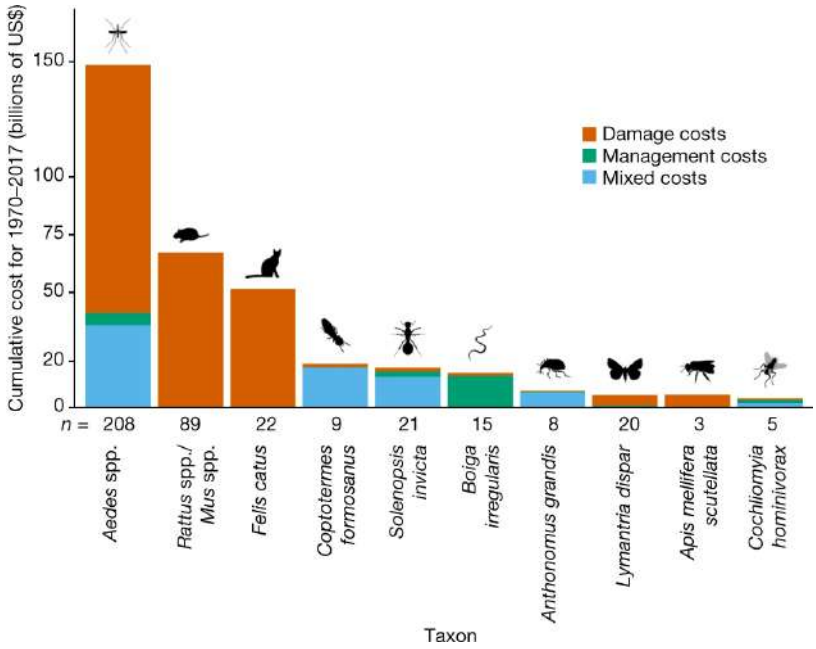

Figure 2 The 10 most economically damaging invasive taxa (including seven insect taxa) from the InvaCost database for both cumulative damage and management costs (in billions of US dollars, at 2017 value) between 1970 and 2017. Mixed costs are cost estimates for which the specific monetary contribution of damage and management costs could not be determined. Each bar represents a species or a complex of species (when different species were considered simultaneously to provide cost estimates). Numbers below the bars indicate the number of cost estimates (from Diagne et al. 2021).

3.2. Provisioning services

Provisioning services include food, fibre / materials, genetic resources, natural medicines, ornamental resources and fresh water (Millennium Ecosystem Assessment 2005). The impact of invasive insects on these services were estimated to cost US$25.2 to 70.0 billion per year globally (Bradshaw et al. 2016). The most expensive invasive insects are generally crop pests that devastate food production globally, but also include species that destroy materials, particularly wood (Bradshaw et al. 2016) (Figure 3).

Several invasive insect species are crop pests (Bradshaw et al. 2016; McLaughlin and Dearden 2021).

The diamondback moth, *Plutella xylostella*, is the most economically damaging invasive crop pest, with costs estimated at US$4.6 billion per year (Bradshaw et al. 2016). Its origin is hypothesised to be from the Mediterranean region but it now has a worldwide distribution (Shu-Jun et al. 2013). The larvae feed mainly on the leaves of cruciferous crops and often completely defoliate plants (Talekar and Shelton 1993). After the widespread use of DDT in the 1940s, all natural enemies of the moth were eliminated across large areas of cultivated regions (Talekar and Shelton 1993), while it also developed resistance to the pesticide in only four decades (Leibee and Savage 1992), further increasing the cost of both crop losses and management methods (Bradshaw et al. 2016). Invasive crop pests can also further impact human health by generating famines and malnutrition through crop failure (Mazza et al. 2014).

Wood is vulnerable to impacts from various invasive insect pests. For instance, the brown spruce longhorn beetle, *Tetropium fuscum*, infests dead and dying trees in its native range (Europe), while it also attacks healthy trees and timber in its invasive range (e.g., Canada, where it was introduced in the late 1990s; Smith and Hurley 2000) (Flaherty et al. 2011). Its costs are estimated at US$4.5 billion per year in Canada alone (Bradshaw et al. 2016). Another economically damaging species is the Formosan termite, *Coptotermes formosanus*, originating from China and invasive in the United States (Henderson 2014; Bradshaw et al. 2016). This species infests a wide variety of structures, including trees, boats and buildings (Hochmair and Scheffrahn 2010; Evans et al. 2019). A single colony can contain up to several million termites that can forage up to 100 m underground (Su and Scheffrahn 1988), making the use of termiticides inefficient (Henderson 2014). In the United States, along with another invasive species, *Coptotermes gestroi*, introduced from south-east Asia (Neoh and Lee 2023), these termites are responsible for considerable damage to property resulting in large treatment and repair costs (Suszkiw 1998; Neoh and Lee 2023).

3.3. Regulating services

Regulating services include air quality, climate (e.g., carbon storage), water, erosion, disease, pest control and pollination (Millennium Ecosystem Assessment 2005). Invasive insects primarily impact disease regulation, particularly through the spread of important viruses (Mazza et al. 2014), climate regulation via disruption of carbon storage, with many species weakening or killing trees via defoliation or damage to wood (see section 2.2.4 for an example), and pollination (Hanna et al. 2015; Vanbergen et al. 2018) (Figure 3).

Various invasive insect species threaten human health, by causing or facilitating diseases or

infections, exposure to wounds from bite/stings, biotoxins, allergens or toxicants, or by inflicting other negative effects on human livelihood (Mazza et al. 2014; Cuthbert et al. 2023). In Europe, biting invasive insects include seven mosquito species, such as the tiger mosquito *Aedes albopictus* that can transmit at least 22 different viruses (Kenis and Branco 2010), six flea species, such as the rat flea *Nosopsyllus fasciatus*, vector of plague and typhus (Zuritta et al. 2021), and 27 sucking louse species, such as *Gyropus ovalis* that can be a vector of phthiriasis (Stojcevic et al. 2004). In Central and South America, invasive hemipteran kissing bugs, vectors of Chagas disease, also cause important concerns (Loubinos 2002). Species that are vectors of viruses and pathogens to humans can have devastating consequences for personal health and at societal level, including leading to epidemics (Loubinos 2002). The global estimated costs associated with invasive *Aedes aegypti* and *A. albopictus* mosquitoes that transmit viruses such as dengue, chikungunya and Zika, were estimated to range between US$ 3.1 and 20.3 billion in 2013 (Roiz et al. 2024).

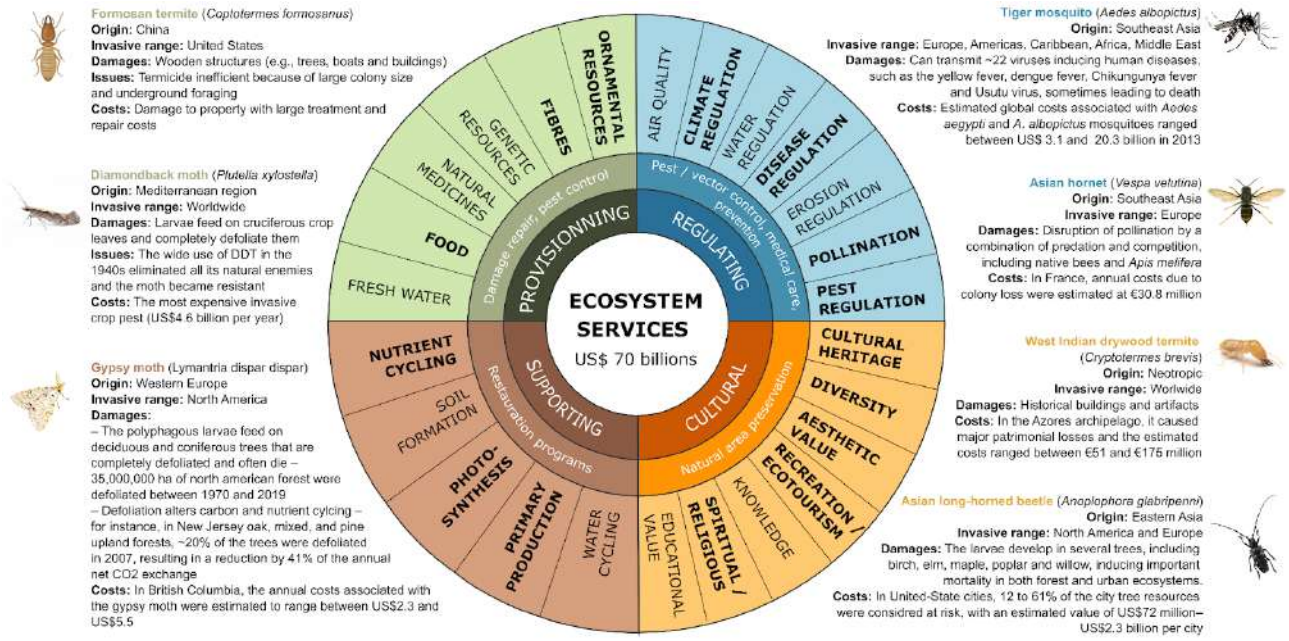

Figure 3 Invasive insects impact the four main categories of ecosystem services recognised in Millennium Ecosystem Assessment (2005), i.e. provisioning, regulating, supporting and cultural (in green, blue, brown and orange, respectively). The most impacted services are highlighted in bold in the outer circle. In each ecosystem service quadrant, one or two examples of invasive species associated with important economic and ecological costs are given, with the name font colour corresponding to the respective ecosystem service. Inspired by Bradshaw et al. (2016).

Pollination services can be impacted both by invasive pollinators and predators (Vanbergen et al. 2018). Invasive pollinators can compete directly with native pollinators, induce mating interference, or introduce alien pests and pathogens (Vanbergen et al. 2018). Humans have deliberately globally

translocated different bee species for apiculture and crop pollination (e.g., species of *Apis*, *Bombus*, *Osmia*, *Megachile*; Vanbergen et al. 2018), raising the risk of competition with native species (Dohzono et al. 2010; Morales et al. 2013; Hanna et al. 2014). Another outcome is the greater likelihood of pathogen and parasite transfer to new hosts, with the potential to cause population declines of native pollinators (Morales et al. 2013; Schmid-Hempel et al. 2014). Invasive predators, by directly consuming pollinators, alter pollination services (Vanbergen et al. 2018). The invasive hornet *Vespa velutina* is a well documented example (see section 2.2.5). In France, annual costs due to *Apis melifera* colony loss have been estimated at €30.8 million (Requier et al. 2023). Other examples are provided by invasive ant species which, by being more aggressive or competitive than native ants, deter pollinators and seed dispersers, thereby reducing plant fitness (Hansen and Müller 2009; Hanna et al. 2015). For instance, in Mauritius, the invasive white-footed ant *Technomyrmex albipes* monopolises flowers and fruit of the endangered plant, *Roussea simplex*, and prevents access by the endemic pollinator, the blue-tailed day-gecko, *Phelsuma cepediana*, by aggressive interference competition, leading to a reduction in pollination and seed-dispersal (Hansen and Müller 2009).

3.4. Supporting services

Supporting services include soil formation, photosynthesis, primary production, nutrient cycling and water cycling (Millennium Ecosystem Assessment 2005). Invasive insect species, especially defoliating species, impact photosynthesis and primary production (Roques et al. 2009), as well as nutrient cycling (Clark et al. 2010; Rubino et al. 2015) (Figure 3). The costs of such impacts are difficult to estimate (Bradshaw et al. 2016).

Forests and woodlands are the main ecosystems impacted in this way by invasive insects (Kenis et al. 2009). In these ecosystems, invasive herbivores can cause massive defoliation and mortality (Roques et al. 2009), altering both photosynthesis and the primary production achieved. Defoliation also causes cascading effects (Lovett et al. 2002, 2006; Kenis et al. 2009), including decreased transpiration, tree growth and seed production; increased mortality, light penetration to the forest floor and water drainage; alteration of tree community and, consequently, faunistic composition; and alteration of carbon allocation and nitrogen cycling, which may lead to further consequences such as acidification of stream waters.

As mentioned above, the gypsy moth, *Lymantria dispar*, is a typical example of a species altering supporting services. Native to temperate forest in western Europe, it was accidentally introduced to North

America in the late 1800s (Lovett et al. 2006). The larvae are polyphagous and feed on a variety of deciduous and coniferous trees, leading to complete defoliation and, often, tree death (Davidson et al. 1999). Since its introduction, it has caused significant ecological damage, defoliating about 35,000,000 ha of North American forest between 1970 and 2019 (USDA 2022). The defoliation also alters carbon and nutrient cycling (Lovett et al. 2002; Clark et al. 2010). For instance, in New Jersey oak, mixed and pine upland forests, the moth defoliated ~20% of trees in 2007, resulting in a reduction of 41% in annual net CO2 exchange (Clark et al. 2010). In British Columbia, the annual costs associated with gypsy moth damage have been estimated to range between US$2.3 and 5.5 million (Sun et al. 2019).

3.5. Cultural services

Cultural services include cultural diversity, spiritual and religious values, recreation and ecotourism, aesthetic values, knowledge systems and educational values (Millennium Ecosystem Assessment 2005). Invasive insects can influence cultural diversity by impacting native biodiversity (Hulme et al. 2010), recreation and ecotourism and aesthetic values, by modifying the structure of some ecosystems (Manachini 2017), and also by destroying cultural heritage artefacts (Manachini 2017) (Figure 3). The impacts of invasive species on these services are much less studied and known, as are the associated consequential costs (Bradshaw et al. 2016).

Landscape modification by invasive insect species can impact local aesthetic values and reduce their attractiveness to (eco)tourism (Hollenhorst et al. 1993). Defoliating species, such as the gypsy moth mentioned above, can decrease the aesthetics or recreation value of highly infested sites (Hollenhorst et al. 1993). The Asian long-horned beetle, *Anoplophora glabripennis*, also causes high tree mortality in both forest and urban ecosystems (Meng et al. 2015). Native to eastern Asia, it is invasive in North America and Europe (Meng et al. 2015), where the larvae can develop on several widespread trees, including birch, elm, maple, poplar and willow, inducing significant mortality (Haack et al. 2010). In cities in the United States, the potential tree resources at risk were estimated to range between 12 and 61% of the local tree populations, with an estimated value of US$72 million to 2.3 billion per city (Nowak et al. 2001).

Some invasive species could reduce the cultural diversity (biodiversity) of ecosystems. The invasive hornet *Vespa velutina* is native to south-east Asia and was accidentally introduced to Europe in 2004 (Monceau et al. 2014). Although formal studies are lacking about its impact on biodiversity (Lioy et al. 2022), it could disrupt native communities by a combination of predation, competition and alteration of the pollination ecosystem service (Monceau et al. 2014). This species is an aggressive predator and a single

colony was found to prey upon at least 159 different insect species, including important pollinators (Rome et al. 2021).

Finally, cultural heritage, including wood and paper, can also be at risk from invasive species (Manachini 2017). For instance, the invasive West Indian drywood termite *Cryptotermes brevis*, has been introduced to Italy and Sicily (Liotta and Matranga 1999) where it has caused great damage to historical buildings and artefacts (Manachini 2017). In the Azores archipelago, *C. brevis* has caused major economic and patrimonial losses, with the estimated costs of treating all infested buildings being US$56.5 million, while building reconstruction would increase these costs to US$193.8 million (Guerreiro et al. 2014).

Considering the acceleration of biological invasion, the major ecological impacts of some invasive species globally and their associated costs, it seems crucial to understand the mechanisms facilitating insect invasion both to prevent future invasive pest outbreaks and to deepen the knowledge onto physiology and ecology of invasive insects.

4. MECHANISMS FACILITATING INSECT ESTABLISHMENT IN NEW ENVIRONMENTS

During the invasion process, species face various barriers that need to be overcome to pass through the stages of the process and be successful (Figure 1). Any invading species encounters new combinations of abiotic and biotic environmental conditions which may be more or less compatible with their ecological requirements. Abiotic (e.g., temperature, humidity) as well as biotic (e.g., trophic niche, predators, competitors) stressors in the invaded area can limit the establishment of non-native species. In this section, I give an overview of the mechanisms that help insects to establish in novel environments, including the biotic and abiotic dimensions of the invaded ecosystem, as well as the potential role of symbionts.

4.1. Challenges provided by the new abiotic environment

Invasive species can face unfamiliar abiotic conditions when entering a new environment. Temperature and water availability are among the major climatic drivers of species distribution and performance globally (Kellerman et al. 2009; Hoffmann and Sgrò 2011; Ma et al. 2021). The ability to adapt to these conditions and/or to seek for favourable microhabitats and microclimates can be important determinants of invasive success (Loubinos et al. 2010; Colinet and Hoffmann 2012; Gippet et al. 2022).

4.1.1. Thermal stress

Temperature is one key factor affecting the geographical distribution of insects (Kellerman et al. 2009; Hoffmann and Sgrò 2011). Acting on biochemical rates (Hochachka and Somero 2002), it drives all physiological processes and influences insect growth, reproduction and survival (Kellerman et al. 2009; Hoffmann and Sgrò 2011; Ma et al. 2021). A species displaying resilience or tolerance to different thermal conditions (e.g., originating from temperate regions) is often considered better equipped, compared to a species originating from more thermally stable regions such as the tropics (i.e., thermal generalist vs thermal specialist), to become established in new environments (Lee and Gelembiuk 2008; Kelley 2014; Bates et al. 2020). Enhanced tolerance to temperature extremes can be acquired through plastic responses such as rapid cold or heat hardening (in the short term) and more gradual acclimation responses (Colinet and Hoffmann 2012; Kelley 2014). *Drosophila* species are well known to exhibit remarkable capacity for acclimation to thermal stress through plastic responses (Hoffmann et al. 2003). Acclimation responses are known to differ between cold and warm ends of the temperature gradient (Colinet et al. 2013).

Overwintering abilities are crucial for species invading temperate regions (Bale 2002). Cold stress, besides slowing development, can induce damage such as tissue freezing during prolonged exposure to sub-zero temperatures (Block 1990) or even death (Bale 1996) and cold tolerance or resistance abilities can, therefore, influence species distributions (Kellerman et al. 2009). Insects have evolved various physiological and biochemical mechanisms for coping with the detrimental effects of low temperature (Teets and Denlinger 2013). Amongst these is overwintering diapause, an environmentally-programmed period of dormancy characterised by developmental arrest and reduced oxygen consumption rates (Denlinger et al. 2005). In *Drosophila*, species with reproductive diapause (i.e., *D. bifasciata*, *D. obscura*) exhibit greater cold resistance and their distribution extends further into colder northern areas (Goto et al. 1999). The ability to use different overwintering strategies may contribute to invasion success, enabling species to exploit different ecological niches and survive diverse and changing environmental conditions (Doucet et al. 2009).

At the other end of the temperature spectrum, mean temperature increase, for instance due to climate change, is often considered to be likely to benefit invasive species (Ward and Master 2007; Skendžić et al. 2021), as it reduces or removes the environmental barriers to the initial colonisation and subsequent establishment of species (Dukes and Mooney 1999; Wards and Masters 2007; Skendžić et al. 2021). For instance, future scenarios of global warming predict a clear increase in climatic suitability and

corresponding invasive potential for the small hive beetle *Aethina tumida*, a parasite of honeybees whose pupation in soil depends on moisture and temperature (Cornelissen et al. 2019). However, heat stresses can also be challenging for insects (Ma et al. 2021). Heat stress can induce cellular damage and reduced metabolic efficiency (Krebs and Loeschcke 1994; Sørensen et al. 2003; King and Macrae 2015), as well as disrupt reproductive processes such as gamete production or embryo development, leading to lower performance (Araripe et al. 2004; van Heerwaarden and Sgró 2021; Grandela et al. 2024). Thermal performance curves illustrate how warm temperature can severely and rapidly impact fitness (Huey and Stevenson 1979; Dowd et al. 2015; Williams et al. 2016; Figure 4). Invasive species often show higher thermal tolerance than non-invasive species (Diez et al. 2012; Kelley 2014).

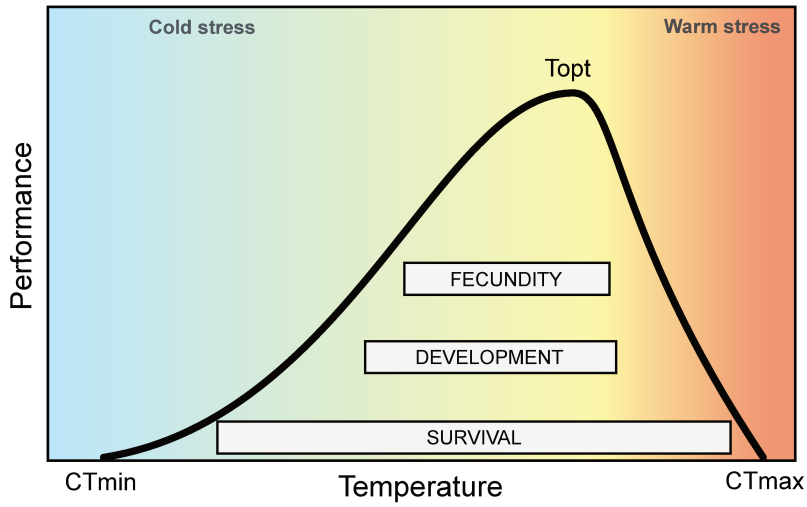

Figure 4 Thermal performance curve (TPC) for a given hypothetical trait (e.g., walking speed), with critical limits for other organismal functions indicated by bars. The TPC is asymmetric and bounded at the extremes by critical limits. Performance generally decreases on either side of a thermal optimum (Topt), with a shallow decrease towards the lower critical limit (CTmin) and a steep decline to the upper critical limit (CTmax). Outside these critical limits, survival is time and temperature dependent. Between CTmin and CTmax lie progressively narrower limits for higher level organismal functions such as development and fertility. Adapted from Williams et al. (2016).

4.1.2. Water stress and synergy with temperature

Insects are particularly at risk of water loss as they have high surface to volume ratios and limited water storage capacity because of their small size, along with varying resistance to water loss (Addo-Bediako et al. 2001; Chown et al. 2002, 2011). Thus, water availability plays a crucial role in defining the ecological niches of insects, influencing their physiology, stress responses, behaviour and life cycle (Harrison et al. 2012). This is particularly the case for aquatic insects or insects with aquatic life stages

(Filazzola et al. 2021). Exhibiting flexible responses to fluctuations in water availability and desiccation stress may help species to cope with new environmental conditions (e.g., alteration in precipitation patterns, seasonal variations) in invaded areas (Harrison et al. 2012) and give invasive species a significant advantage in colonising and establishing in new environments.

During the invasion process, invasive species also face synergistic interactions between thermal and water stress (Galic et al. 2018; Sara et al. 2018). High temperatures contribute to increased evaporation and reduced water availability, leading to desiccation stress (Addo-Bediako et al. 2001; Chown et al. 2011; Frank 2021), and inducing respiratory water loss in insects (Chown et al. 2002). These multi-stress conditions can synergistically affect insect physiology and behaviour (Forister et al. 2018; Hoffmann et al. 2003). Desiccation, along with cold and heat stress, but also starvation and anoxia, are among the principal stresses resulting in the production of heat shock proteins (HSPs), which are known to be linked with physiology (Krebs and Loeschcke 1994; Sørensen et al. 2003; King and Macrae 2015). Desiccating conditions in combination with thermal stress are often associated with reduced survival probability compared to thermal stress in the absence of desiccation, as noted in *D. melanogaster* (Bubliy et al. 2012) and *D. suzukii* (Enriquez and Colinet 2017). Species distribution is also impacted by the combination of desiccation and temperature extremes (Filazzola et al. 2021). For instance, inclusion of both temperature extremes and desiccation stress reduced the modelled suitable habitat range by 60% for two African butterfly species (*Busseola fusca* and *Bicyclus anynana*; Barton et al. 2019).

Physiological plasticity is expected to be a key aspect of tolerance to multiple stressors. The tropical drosophilid *Zaprionus indianus* has substantial capability to increase (> 2-fold) its tolerance to various stresses including desiccation, heat exposure and starvation after hardening/acclimation, which likely accounts for its invasion potential (Kalra et al. 2017). In general, plastic responses to warm and dry environments are a complex mixture of physiological responses to both variables (Sinclair et al. 2024).

4.1.3. Behavioural thermoregulation

When studying insect invasive potential, the new abiotic environment, including temperature and water availability, is often considered through the lens of macroclimate (Venette et al. 2010; Boher et al. 2016; Rebaudo et al. 2016). However, insects live and move across mosaics of micro-environments that can provide them with access to a wide range of abiotic conditions (Pincebourde et al. 2007; Woods et al. 2015; Pincebourde et al. 2016) and sometimes buffer the macroclimatic variations (Pincebourde et al. 2007; Suggitt et al. 2011; Woods et al. 2015; Ma and Ma 2022; Gril et al. 2023). Because of their small size,

insects have the ability to use these microclimatic conditions at local scales (Pincebourde and Woods 2020). Insect behavioural abilities to select the most appropriate micro-environment are determinant for both their own performance (e.g., survival, fecundity) and the success of their progeny (e.g., developmental rate, survival) (Ma et al. 2021; Figure 5).

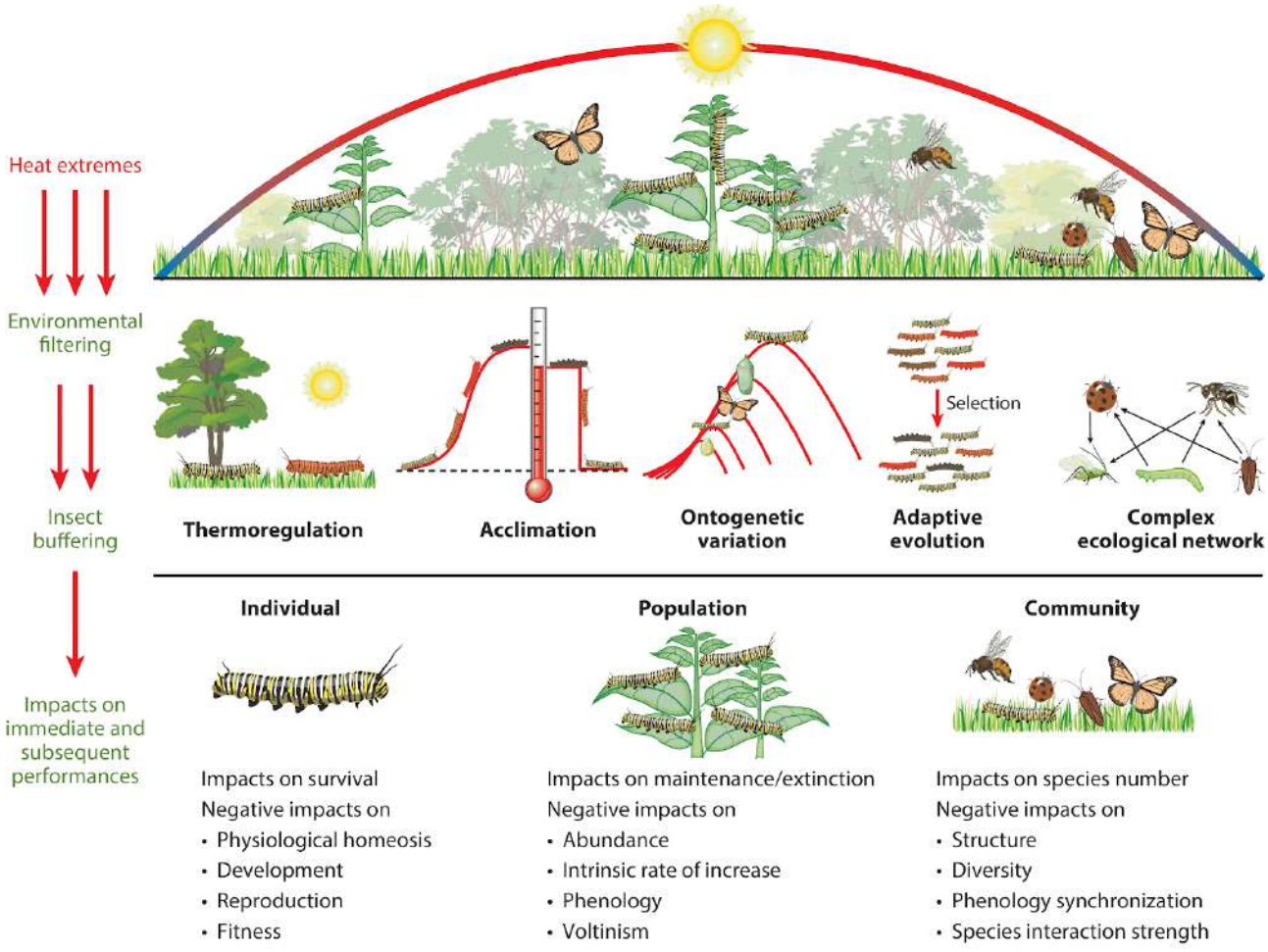

Figure 5 Natural thermal extremes are filtered (intensity is often lowered) by microhabitats and buffered by insects through behavioural thermoregulation, phenotypic plasticity, ontogenetic variations, and adaptive evolution to ultimately increase survival in response to heat. Even after filtering and buffering, thermal extremes still impact insect performance at the individual, population and community levels, within and across generations (from Ma et al. 2021).

Insects that are able to actively select microhabitats and associated microclimates, including temperature (and potentially relative humidity) are more likely to survive in a variable environment (Danks 1978; Ma and Ma 2022). Selection ability can be assessed using thermal preference studies (Angilletta 2009). Thermal preference is a measure of the temperature an organism chooses when provided with access to a wide range of temperatures (Angilletta 2009; Dillon et al. 2009) and is generally

assessed using a thermal gradient (Dillon et al. 2009). However, in many studies, the thermal preference range of a species is rarely correlated with the organism's ability to exploit its thermal environment, limiting understanding of its behavioural thermoregulation capacities (Gvozdik 2012).

Some invasive insect species display adaptations that limit direct exposure to stress. For instance, the invasive Asian ladybird, *Harmonia axyridis*, selects domestic dwellings as overwintering sites tin order to avoid harsh winter environmental conditions in North America (Labrie et al. 2008). In the driest field habitats, shaded microhabitats may provide sufficient refuges to enable insect's persistence in otherwise unsuitable areas (Tochen et al. 2016). Larval stages that develop within fruit or decaying organic materials can also buffer direct exposure to atmospheric conditions (Fekete et al. 2016), although in some situations the temperature variation can be amplified (Feder et al. 1997; Saudreau et al. 2011). Drosophilid species with low desiccation tolerance select moister sites for pupation (Kojima and Kimura 2003).

4.2. Benefits gained from the new biotic environment

Besides facing new combinations of abiotic conditions, invasive species need to access the living resources of the recipient ecosystems (trophic niche) and adapt to new biotic interactions (competition and facilitation) and local resistance (from natural enemies) in order to establish successfully.

4.2.1. Trophic niche

Invasion success of insect species is strongly influenced by their trophic niche width or adaptability (Zhao et al. 2023). The host range of an invasive species can differ considerably compared with that in its native range (Hu et al. 2017). Establishment can only take place if the species' trophic preferences and requirements match with the quality, quantity and availability of resources in the new environment (Alpert et al. 2000; Richardson and Pyšek 2006). The presence of invasive plant species can facilitate the establishment of invasive insects (Bella 2013; Engelkes and Mills 2013; Poyet et al. 2015). Experimental studies of the light brown apple moth, *Epiphyas postvittana*, a generalist herbivore, suggest that prior invasion of exotic plants can facilitate the moth's invasion, particularly if they have shared some history, thereby accelerating the invasion process and expansion into a new geographic range (Engelkes and Mills 2013). In this way, in Europe, the invasive American tree *Prunus serotina* provides a suitable reservoir for the invasive Asiatic fly *D. suzukii* (Poyet et al. 2014). Globally, the homogenisation of culture practices and trade, especially in areas sharing similar environments, is expected to lead new species assemblages dominated by few but widespread invasive generalist species (Capinha et al. 2015).

The trophic niche width of invasive species impacts their invasiveness potential (Crowder and Snyder 2010; Zhao et al. 2023). Monophagous species, except those that breed / feed on widely distributed species, are unlikely to become invasive. Amongst exceptions to this generalisation is a successful invasive monophagous olive fly, *Bactrocera oleae* (Malacrida et al. 2007). Tightly linked to cultivated olive trees and their wild relatives in the genus *Olea*, this pest has expanded its range to most places where olive trees have been introduced and are grown commercially (Nardi et al. 2005). Conversely, polyphagy, allowing a species to benefit from a diverse range of substrates/hosts, could be considered as a form of preadaptation leading towards the evolution of a cosmopolitan invader (Morse and Hoddle 2006). Polyphagy provides various advantages, such as reducing the time required to search for suitable resources, increase the success of locating them within and between years, release from predator pressure, or attenuate the potential toxic effects of some resources (Zhang et al. 2014; Poyet et al. 2015). However, polyphagous insects are also exposed to resources varying in quality which can impact offspring performance and fitness (e.g., development rate and survival; Janz 2003; Poyet et al. 2015) or even lead to erroneous oviposition on 'dead-end plants' which do not support larval development (Ulmer et al. 2020).

4.2.2. Reduced competition

Inter- and intraspecific competition are widespread among insects, particularly for trophic resources and reproduction. Interspecific competition is commonly regarded as one of the main drivers of distribution and abundance of phytophagous insects (Denno et al. 1995; Kaplan and Denno 2007; Bühlmann and Gossner 2022), although its importance relative to other ecological factors remains debated (Facon et al. 2021). In the context of biological invasions, limited attention has been paid to the role of competition compared to those of other biotic factors, such as the existence of ecological niches not exploited by local native species (Empty Niche Hypothesis; Herbold and Moyle 1986) or the lack of natural enemies in the invaded area (Enemy Release Hypothesis; Williamson and Fitter 1996; Keane and Crawley 2002). It is generally predicted that communities are more likely to be invaded when they contain significant unused resources (Davis et al. 2000), when the introduced species has greater acquisition efficiency of a given resource than do native species, or when, at equal acquisition efficiencies, the introduced species has lower requirements than the native species for a given resource (Shea and Chesson 2002).

The Empty Niche Hypothesis posits that species can exploit vacant niches to avoid interspecific competition (Herbold and Moyle 1986). Some invasive species have developed specific adaptations to do

so. For instance, both *Drosophila suzukii* and *Scaptomyza flava* have evolved a serrated ovipositor allowing them to insert eggs in a hard and firm substrate, a resource inaccessible to other species - unripe fruit for the former (Atallah et al. 2014) and fresh leaves for the latter (Peláez et al. 2022). Temporal partitioning of the resource, with the invasive species developing more rapidly than the native species, can also decrease the competitive pressure and facilitate establishment (Roughgarden 1976; Jarošík et al. 2015; Zhao et al. 2023). Similarly, spatial partitioning of the resource by exploiting the microclimatic heterogeneity can promote native and invasive species coexistence (Dangles et al. 2008; Lounibos et al. 2010; Gippet et al. 2022). For instance, the native ant *Lasius niger* shifted its foraging activity spatially to avoid the invasive *Tapinoma magnum* (Gippet et al. 2022). In general, habitat heterogeneity is expected to both increase invasion success and reduce the impact to native species in the community by promoting invasion and coexistence mechanisms that are not possible in homogeneous environments (Figure 6; Melbourne et al. 2007).

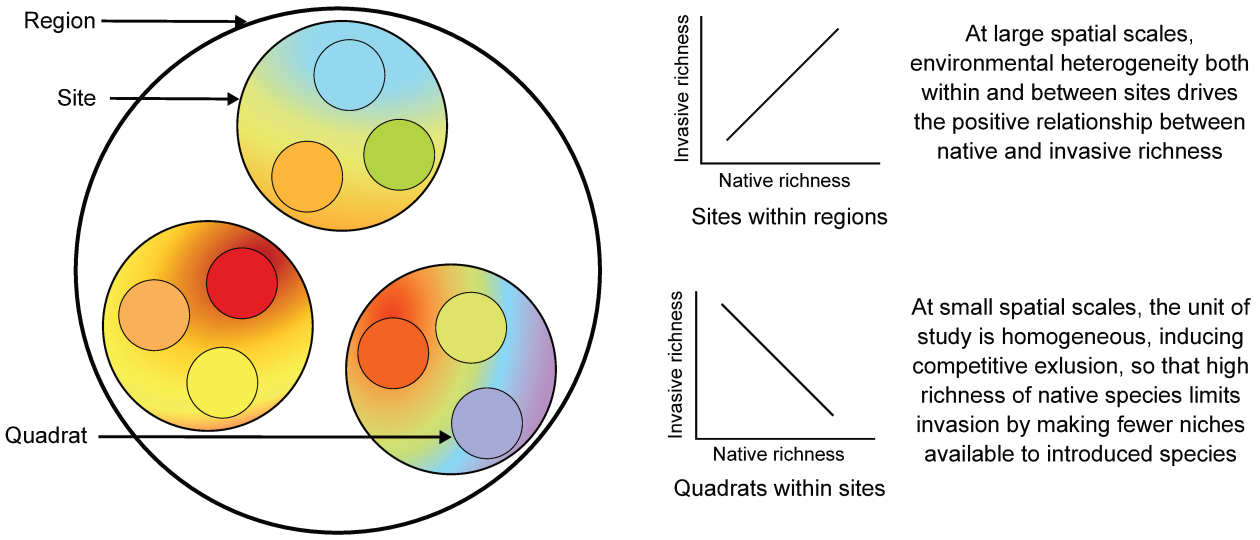

Figure 6 Heterogeneity (represented by colour gradients) at large spatial scales promotes coexistence mechanisms between native and invasive species while, at small spatial scales, competitive exclusion reduces the niches available for invasive species. Small spatial scales are those at which individuals interact (e.g. experience inter- and intraspecific competition). Large spatial scales are those greater than the scale of individual interaction. Shading represents variation in an environmental (exogenous) factor. Adapted from Melbourne et al. (2007).

4.2.3. Facilitation

There is growing research interest in understanding the impact of positive interactions between species and their role in shaping the community structure (Stachowicz 2001; Fridley et al. 2007; Cavieres 2021; Koffel et al. 2021). Interaction between native and invasive species can lead to facilitation, which is a

positive interaction between two species that results in an increase in the density or biomass of at least one of the species (Rodriguez 2006). Invasive species are known to facilitate infestations of native species, with sometimes wide-ranging impacts (Rodriguez 2006), but the question of whether or how native species can facilitate invasive species has received less attention (Fridley et al. 2007; Gribben et al. 2020). For instance, the invasive drosophilid *Chymomyza amoena* benefits from seed-boring insect parasites that damage and modify the fruit of several broad-leaved tree species, providing opportunities for the flies to oviposit (Band et al. 2005).

Microbes can also act as facilitators for invasive insect species (Amsellem et al. 2017). For example, the yeast *Hanseniaspora uvarum*, which proliferates on both unripe and ripe raspberries, creates favourable conditions for the invasive *D. suzukii* development, suppressing an antagonist grey mould (*Botrytis cinerea*) and producing less ethanol which would otherwise deter the fly from ovipositing (Chakraborty et al. 2022). More broadly, the expanding range of invasive species creates new opportunities for horizontal acquisition of symbionts from native species (Amsellem et al. 2017). This can be an immediate and powerful mechanism of rapid adaptation to acquire ecologically important traits, such as heat tolerance or parasite defence, that increase host fitness (Amsellem et al. 2017). This is illustrated by the whitefly *Bemisia tabaci,* where the presence of the symbiont, *Rickettsia,* increased survival to adulthood, shortened the time required for development and lead to a higher production of females (Himler et al. 2011).

4.2.4. Avoidance of natural enemies

The biotic resistance of the recipient ecosystem can influence the outcome of invasions (Daly et al. 2023). A species invading a new area encounters novel local communities and interacts with a diversity of species some of which may hinder its long term establishment. Among these are natural enemies, which include predators and parasitoids, but also entomopathogenic nematodes, fungi, viruses or bacteria (Keane and Crawley 2002; Woltz et al. 2015; Wang et al. 2021). These can affect invasive species through a consumptive effect (i.e., directly affecting the prey population by killing the individuals) and/or by a non-consumptive effect (i.e., influencing the performance of the invasive species) (Ulmer et al. 2021).

The Enemy Release Hypothesis (ERH) suggests that the establishment of a new invading species can be facilitated by lack of regulation from natural competitors and enemies (Williamson and Fitter 1996; Keane and Crawley 2002) as, even if potentially appropriate native species are present, they are generally naïve and unable to recognise/detect and then predate/parasitise the new species. The ERH is

one of many hypotheses that set out to explain the abundance and/or impact of a given invading species (see Colautti et al. 2004 for review). Polyphagy and the use of fruit with a large diversity of colours can also be incorporated in enemy avoidance strategy by disorienting those attracted to a specific range of colours (Vargas et al. 1991).

4.3. Symbiont influence

Microbial symbionts are widespread, including in insects (Douglas 2015). They are 'plasticity generators', inducing a genetic variability in the hologenome which creates phenotypic variability of the holobiont (composed of the insect and its symbionts) (Rosenberg 2021; Zilber-Rosenberg and Rosenberg 2021). There is a tremendous diversity of symbionts with variable effects (Feldhaar 2011) which can enhance the invasive success of insect hosts (Lu et al. 2016; Liu et al. 2022). Some of them are able to help insects overcome abiotic and/or biotic stress (Douglas 2009; Lemoine et al. 2020; Cornwallis et al. 2023).

4.3.1. Symbiont diversity among insects

Microbial symbionts encompass a diverse array of bacteria, fungi and viruses (Douglas 2015). In insects, they colonise the cuticle, gut and cells (Douglas 2015). Symbionts can influence various aspects of the insect phenotype, ranging from physiology to behaviour (reviewed by Feldhaar 2011). Recent studies have emphasised the role of these microbes in enhancing the invasion success of their insect hosts (Lu et al. 2016; Liu et al. 2022). Throughout the introduction-establishment-invasion process, microbial symbionts can play a crucial role by influencing propagule pressure, resource acquisition, resistance to pathogens, tolerance to abiotic factors and the competitive capacity of insects (Lu et al. 2016; Liu et al. 2022). These effects may stem from symbionts already present in the native population or arise from others acquired during the invasion process by horizontal transfer from native insects or directly from the environment (Wooding et al. 2013).

4.3.2. The role of symbionts in species establishment

Some symbionts have evolved mechanisms to manipulate the reproduction of their host to favour their own transmission. The best-known example is the bacterial symbiont *Wolbachia*, which has been described in various invasive *Drosophila* species including *D. suzukii*, *D. simulans* and *D. immigrans*. *Wolbachia* can also spread by providing a fitness advantage to infected host individuals compared to uninfected (Kriesner and Hoffmann 2018). For instance, in *D. simulans* and *D. suzukii*, *Wolbachia*

infection is associated with increased fecundity and/or offspring survival (Mazzetto et al. 2015; Cao et al. 2019). Such symbiont-induced effects could increase the propagule pressure of their host and, consequently, increase the likelihood of invasive events.

Some facultative symbionts can also turn a non-native insect into an invasive one (Lu et al. 2016). This is the case for the whitefly (*Bemisia tabaci*), where only a few biotypes with specific associations with microbial symbionts are responsible for global invasions, specifically biotypes Q and B (Gueguen et al. 2010). Similarly, a microsporidium closely related to *Nosema thompsoni* facilitated the invasion of the harlequin ladybird *Harmonia axyridis* through Europe, as this symbiont is lethal for native ladybird species (Vilcinskas et al. 2013).

Bacterial symbionts can also enhance host resistance to natural enemies like viruses, fungi and parasitoids (Douglas 2015). In aphids, the secondary symbiont *Hamiltonella defensa* provides resistance against parasitoids (Oliver et al. 2003) and *Regiella insecticola* gives protection against a fungal pathogen (Scarborough et al. 2005). *Wolbachia* provides, again, a well-known example as it has been associated with resistance to viruses in *D. melanogaster* and *D. suzukii* (Hedges et al. 2008; Teixeira et al. 2008; Martinez et al. 2014; Cattel et al. 2016b; Cogni et al. 2021). *Wolbachia* has also been shown to increase resistance towards entomopathogenic fungi in *D. melanogaster* (Panteleev et al. 2007). Another bacteria, *Spiroplasma*, is associated with parasitoid resistance in *D. hydei* (Xie et al. 2010).

4.3.3. The role of symbionts in buffering thermal and nutrient stress

Symbionts can increase insect tolerance to abiotic stresses (Corbin et al. 2017; Lemoine et al. 2020) which may, in turn, enhance the invasive potential of species. This increased tolerance can include tolerance of temperature extremes. For instance, the gut microbiota of *D. subobscura* induces greater heat tolerance (Jaramillo and Castañeda 2021) and that of *D. melanogaster* greater cold tolerance (Henry and Colinet 2018). In the invasive whitefly *Bemisia tabaci*, the maternally inherited bacterial endosymbiont *Cardinium* increased the fly's thermal tolerance, but is also influenced by the host's genetic background (Yang et al. 2021). Similarly, in the saw-toothed grain beetle, *Oryzaephilus surinamensis*, an ancient clade of intracellular, bacteriome-located Bacteroidetes symbionts affects cuticle thickness, melanisation and hydrocarbon profile, enhancing desiccation resistance and thereby strongly improving fitness under dry conditions (Engl et al. 2018).

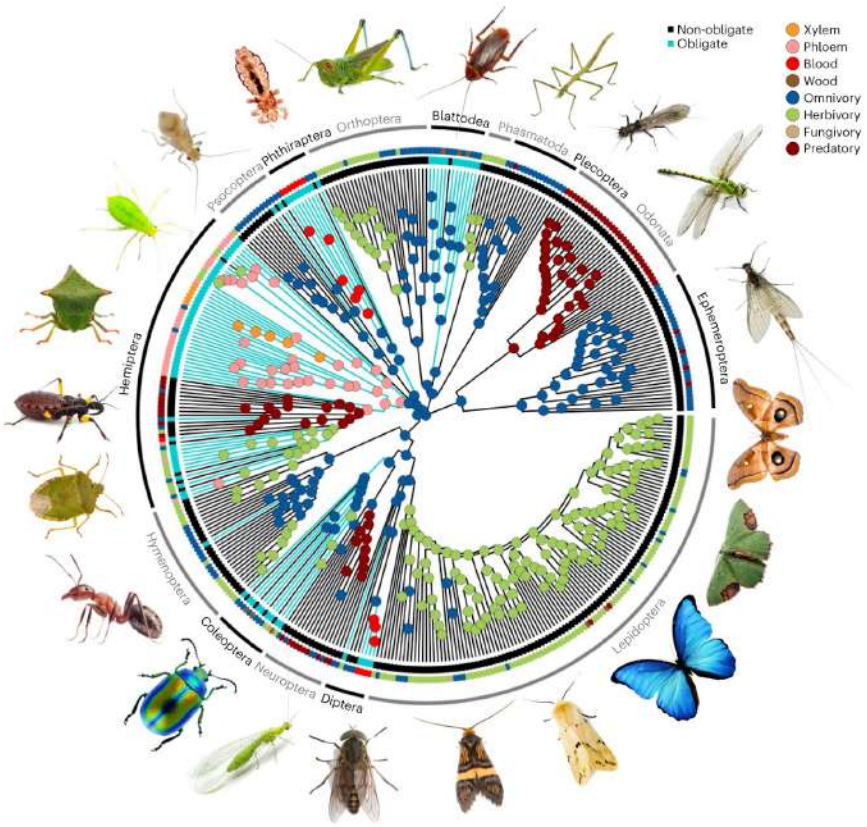

Figure 7 Phylogenetic distribution of obligate symbionts across insect families investigated for symbiosis and their feeding niches. Turquoise tips and branches represent obligate symbiosis and different coloured dots represent different feeding niches (from Cornwallis et al. 2023).

The acquisition of symbionts can enable insects to exploit new hosts (Hansen and Moran 2014; Sudakaran et al. 2017; Liu et al. 2022; Cornwallis et al. 2023), further contributing to invasion success (Liu et al. 2022; Hafsi and Delatte 2023). For instance, ambrosia beetles are associated with fungi and several introduced beetle-fungus combinations can become invasive by shifting from colonising dead and dying trees to attacking living trees (Hulcr and Dunn 2011). This new niche has allowed them to rapidly expand their geographical ranges (Hulcr and Dunn 2011). However, invasive species host shifts are not always directly linked with changes in symbiotic microbes (Hu et al. 2017). For instance, the invasive Argentine ants, *Linepithema humile,* have shifted their diet towards greater consumption of sugar-rich, nitrogen-poor foods, but with no apparent contribution of symbiotic microbes help (Hu et al. 2017).

Symbionts can also be beneficial to their host under nutrient-deficient conditions (Cornwallis et al. 2023; Figure 7). For example, under iron stress, *D. melanogaster* females infected with *Wolbachia* have a higher fecundity than do uninfected females (Brownlie et al. 2009). In the invasive ant, *Cardiocondyla obscurior*, a novel intracellular bacterium, *Westeberhardia*, seems to play a role in host cuticle formation, possibly facilitating an invasive lifestyle in nutrient-poor arboreal microenvironments (Klein et al. 2016).

Recent studies have shown that the gut microbiota can also affect food choice and foraging decisions under laboratory conditions in *D. melanogaster*, and allow tolerance of unfavourable nutritional situations (Yamada et al. 2015; Leitão-Gonçalves et al. 2017; Henriques et al. 2020). Thus, symbiotic microbiota could impact the adaptation capacity of their host to available food resources and, more generally, to abiotic and biotic conditions.

5. A CASE STUDY OF A SUCCESSFUL INVASION PROCESS

Among recently-established invasive species, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) has been one of the most impressive and best-documented (Walsh et al. 2011; Cini et al. 2012). Originating from Asia, its range expansion was swift, from first reports in Europe and America in 2008 to an almost global distribution today (Fraimout and Monnet 2018). The female oviposits in ripening and healthy fruit, causing important economic impacts for fruit growers (Yeh et al. 2020) and making the fly an important recognised pest of soft fruit (Walsh et al. 2011). Its remarkable invasive success is due to many factors, including the use of a vacant niche (i.e., healthy fruit; Atallah et al. 2014), extensive polyphagy (Poyet et al. 2015), high thermal plasticity and acclimation abilities (Tait et al. 2021; Raynaud-Berton et al. 2024) and the absence of effective parasitoid species in its invading range (Poyet et al. 2013). Research is ongoing to develop effective integrated pest management practices appropriate for this species (Haye et al. 2016; Tait et al. 2021). *Drosophila suzukii* provides an excellent model to improve understanding of invasion success (see Appendix X - Deconninck et al. under review Cracking the code of Drosophilidae invasive success).

5.1. Description

5.1.1. Taxonomy

Drosophila suzukii is a member of the Drosophilidae family, sub-family Drosophilinae. The genus contains over 2000 described species and is divided in two subgenera: *Drosophila* and *Sophophora* (Bock 1980; van der Linde and Houle 2008). *Drosophila suzukii* is part of the second, and is classified in the *melanogaster* species-group and *suzukii* subgroup (Ometto et al. 2013; Rota-Stabelli et al. 2013). *Drosophila suzukii* is phylogenetically close to *Drosophila melanogaster*, a well described laboratory model species extensively used in genetic studies (Ashburner et al. 2005), with which it shares many biological features (Yang et al. 2012; Rota-Stabelli et al. 2013; Iacovone et al. 2015).

5.1.2. Morphology

Adults measure between 2 and 3.5 mm, have a pale brown or yellowish-brown thorax and abdomen with red eyes, and shows a sexual dimorphism with females larger than males (Figure 8; Kanzawa 1939; Walsh et al. 2011). Morphologically, *D. suzukii* is separable from other *Drosophila* species by two main characteristics. First, females have a well-developed sclerotised serrated ovipositor (Hauser et al. 2011; Atallah et al. 2014). This morphological adaptation evolved twice in the *suzukii* subgroup (Rota-Stabelli et al. 2013) and allows *D. suzukii* females to puncture the intact skin of fruit (Figure 8; Kanzawa 1939; Atallah et al. 2014). Second, males have one melanized dot on each wing that appears after the teneral stage (Figure 8; Hauser et al. 2011), giving the species its vernacular name of spotted-wing drosophila. Males also have two sets of black tarsal combs (Hauser et al. 2011). Over different seasons and their temperature variations, *D. suzukii* presents a continuum of morphological changes: in summer, individuals are light brown, relatively large with shorter wings while, in winter, individuals are dark brown, almost black, with smaller body size and relatively larger wings (Figure 8; Shearer et al. 2016; Leach et al. 2019).

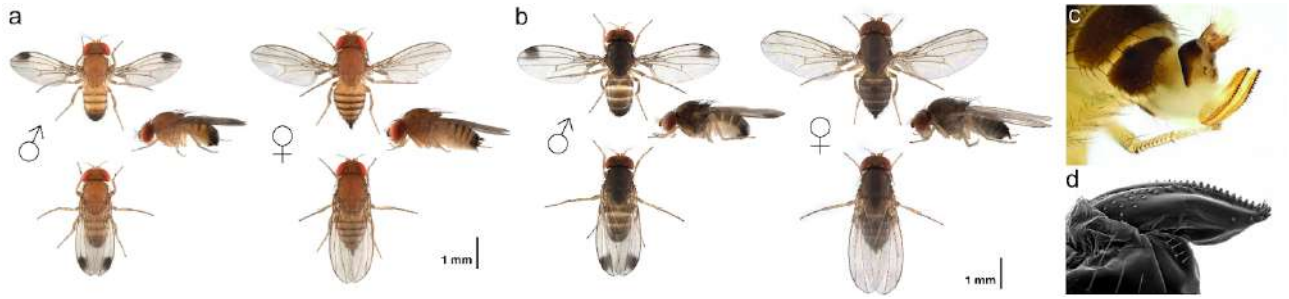

Figure 8 Morphology of *D. suzukii* (a) in summer and (b) in winter (pictures by Nicolas Gompel's lab). (c) Female serrated ovipositor picture (by Chris Thomas) and (d) scanning electron microscope image (from Hauser et al. 2011).

5.1.3. Life cycle

Drosophila suzukii occurs from temperate to subtropical climates (Mitsui et al. 2010). The life cycle has four main stages: egg, larva (three instars), pupa and adult (Figure 9; Kanzawa 1939; Walsh et al. 2011). A female lays ~1–3 eggs per oviposition site, with an average of 380 eggs laid in her lifetime (Kanzawa 1939). Most eggs are laid directly into fruit (Kanzawa 1939; Walsh et al. 2011). Eggs hatch inside the fruit where larval development occurs (Kanzawa 1939; Walsh et al. 2011). The larvae feed on the fruit flesh and develop through three instars until pupation (Kanzawa 1939; Walsh et al. 2011).

Pupation occurs both within and outside the fruit (Kanzawa 1939; Walsh et al. 2011), but pupae are mostly found on the ground (Ballman et al. 2017; Woltz and Lee 2017). Adults, and especially females, can live up to several months, with an average of 200 days and records of up to 300 days for females (Kanzawa 1939).

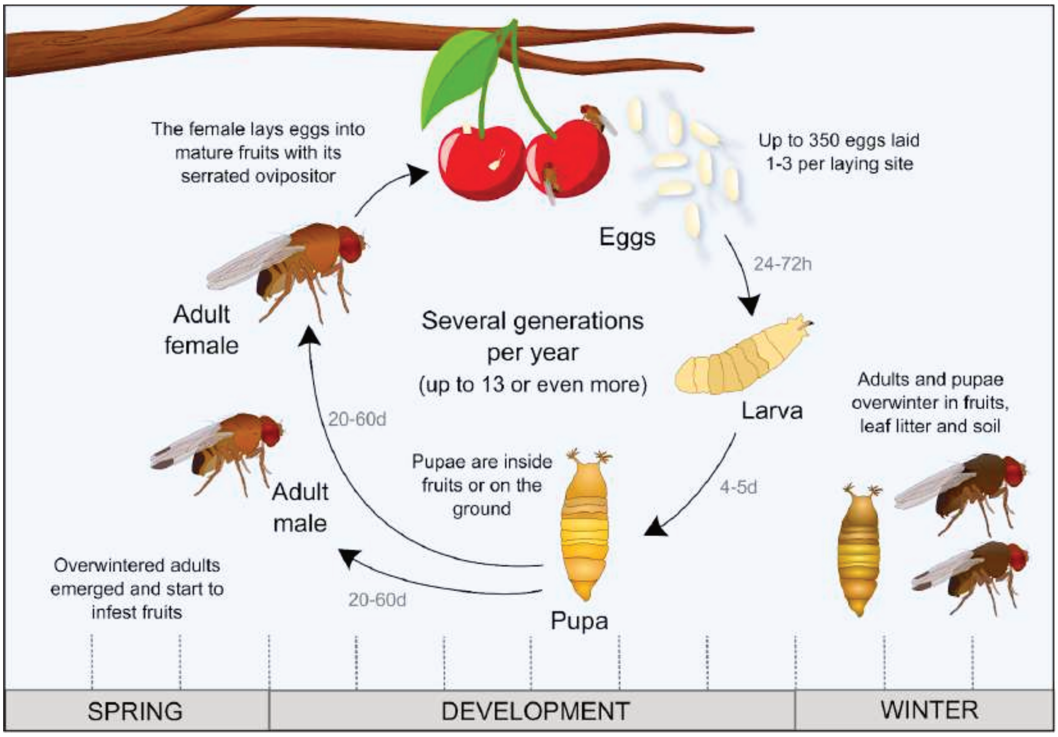

Figure 9 Life cycle including overwintering stages. Adapted from Spears et al. (2017).

The development time from egg-to-adult depends on temperature and can be as short as nine days at 30°C (constant) or as long as 74.5 days at 9°C (constant) (Raynaud-Berton et al. 2024). Because of its overall rapid development, *D. suzukii* can complete several generations a year, with up to 13 generations recorded in the field in Japan (Kanzawa 1939) and estimates of up to 17 and 27 generations in cold and warm regions, respectively (Schlesener et al. 2020).

Most parts of the life cycle occur in spring, summer and autumn in temperate regions (Arnó et al. 2016; Wang et al. 2016; Panel et al. 2018). During winter, it is generally thought that the adult stage overwinters (Kanzawa 1939; Mitsui et al. 2010), but recent data suggest that a small proportion of larvae and pupae could survive, especially if insulated (Kaçar et al. 2016). Cold-acclimation generates cold-tolerant females which show reproductive dormancy that ends spontaneously when temperatures rise (Shearer et al. 2016; Grassi et al. 2018; Panel et al. 2018). At the end of winter, overwintering individuals that have successfully mated in autumn are the first to search for food and egg-laying sites and establish spring populations (Panel et al. 2018).

5.2. Invasion history

5.2.1. From continental to global distribution

Drosophila suzukii was first described in Japan in 1916 (Kanzawa 1939). It is a native species of south-east Asia, including a large part of China, bordering countries (e.g., India, Myanmar, Pakistan, Thailand) and Japan (Walsh et al. 2011; Calabria et al. 2012; Ometto et al. 2013; Asplen et al. 2015; Ørsted and Ørsted 2019). It was first reported outside of its native zone in Hawaii in the 1980s (Kaneshiro 1983). About 20 years later, European and North American invasions apparently commenced simultaneously (Walsh et al. 2011; Cini et al. 2012; Asplen et al. 2015), with first reports in 2008 in California (USA) (Hauser et al. 2009) and Barcelona (Spain) (Calabria et al. 2012). Its subsequent expansion in these continents has been remarkably rapid and, today, the fly is reported in most European and North American countries and states (IPCC 2024). In 2013, the fly reached South America, where it was first reported from Brazil (Deprá et al. 2014). Invasion of the African continent then commenced in 2017, first with northern Africa, the neighbouring Indian Ocean islands of the Comores and La Réunion (Fraimout et al. 2017; Hassani et al. 2020; Boughdad et al. 2021), Kenya in 2019 (Kwadha et al. 2021), and, most recently, South Africa in 2023 (IPCC 2024). It appears to be only a matter of time before *D. suzukii* reaches other parts of Africa, and even Australia, that have remained free of the species to date (Dos Santos et al. 2017; Ørsted and Ørsted 2019). Global warming also is likely to contribute to further expansion to higher northern latitudes (Fraimout and Monnet 2018). The known current distribution in 2024 is presented in Figure 10.

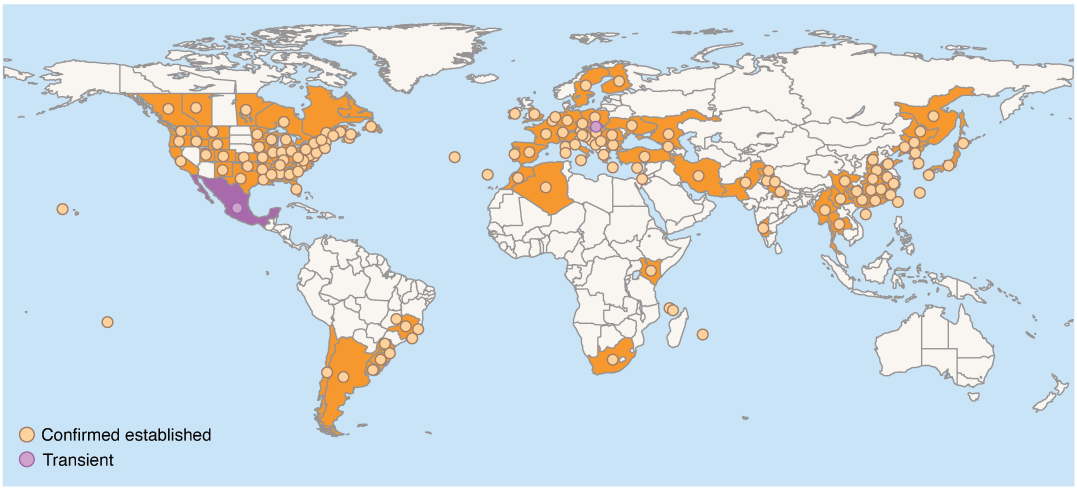

Figure 10 States and countries were establishment is confirmed are in orange while purple means that the pest status is actionable and under eradication (transient). Adapted from the European and Mediterranean Plant Protection Organization (EPPO) distribution map (https://gd.eppo.int/taxon/DROSSU/distribution).

5.2.2. Invasion pathways

During the species' invasion process, there have been multiple introductions from Asia to Europe and the Americas (Fraimout et al. 2017). *Drosophila suzukii* expansion has benefited from globalisation, which has seen the inexorable growth of international trade and human movement, as well as from some of the consequences of global environmental changes (Cini et al. 2014; Haye et al. 2016; Tait et al. 2021). The fly can survive the transport process in all developmental stages (i.e., eggs, larvae, pupae, or adults) within fruit or in containers (Tait et al. 2021). Using microsatellite data combined with first observation dates, Fraimout et al. (2017) reconstructed the probable invasion routes of *D. suzukii* (Figure 11). The first introductions in Hawaï, Europe and North America originated from different initial Asian populations. Then, commercial trade routes between, for instance, France and La Réunion, North America and South America, and within individual nations and region countries, further contributed to fly dispersal (Cini et al. 2014; Fraimout et al. 2017). Even if these introduced populations faced several bottleneck events and lost some genetic variability, the genetic diversity present in established populations remains high, not least as there were at least five admixture events during the invasion history which likely contributed to maintaining genetic variability (Fraimout et al. 2017).

5.3. Ecology

5.3.1. Habitat diversity

Drosophila suzukii is found in both natural and anthropogenic habitats (Thistlewood et al. 2018; Kanda et al. 2019; Santoiemma et al. 2019; Tait et al. 2020a; Ulmer et al. 2024). Across seasons, its distribution and population fluctuations are driven by combinations of biotic and abiotic factors including temperature, water and nutrient availability (Tochen et al. 2016; Evans et al. 2017; Eben et al. 2018; Plantamp et al. 2019; Rendon et al. 2019; Delbac et al. 2020a; Little et al. 2020; Stockton et al. 2020; Winkler et al. 2020; Ørsted et al. 2021). During the commercial fruit producing season, flies are found both in cultivated areas and surrounding semi-natural habitats (Klick et al. 2016; Tonina et al. 2018; Santoiemma et al. 2018, 2019; Delbac et al. 2020b; Urbaneja-Bernat et al. 2020), while, in winter, they find shelters in semi-natural habitats and wooded areas (Rossi-Stacconi et al. 2016; Tonina et al. 2018; Santoiemma et al. 2019; Delbac et al. 2020b), or synanthropic locations (Rossi-Stacconi et al. 2016; Ulmer et al. 2024).

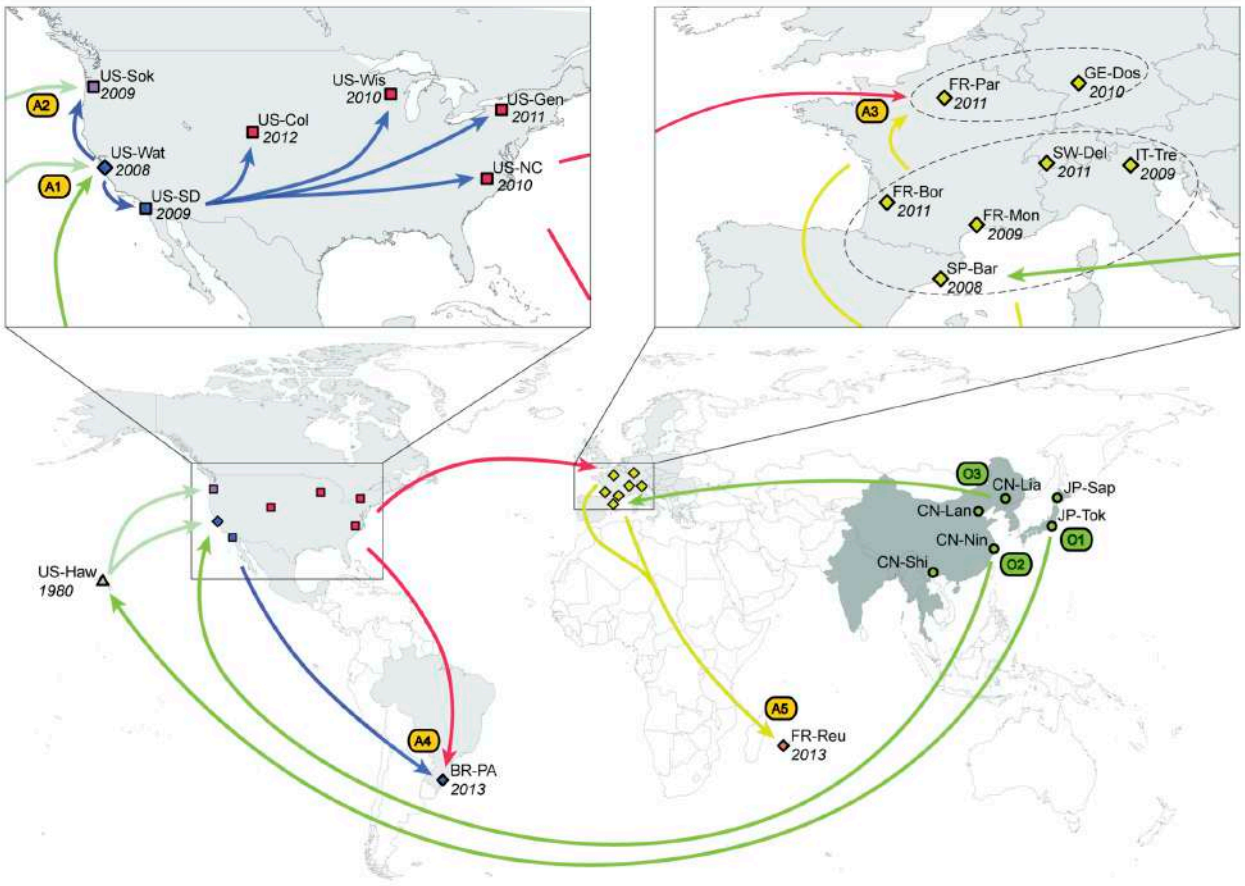

Figure 11 Invasion routes of *D. suzukii*. Global invasion routes of *D. suzukii* inferred from microsatellite data and dates of first observation. The native and invasive ranges are shown in dark- and light-grey, respectively. The year in which *D. suzukii* was first observed in each new region is indicated in italics. Squares and diamonds indicate populations that experienced bottlenecks. The colours of symbols and arrows correspond to different genetic groups. The arrows indicate the most likely invasion pathways. A1 to A5 indicate five separate admixture events. O1 to O3 indicate the most likely geographic locations from which primary introductions from the native range occurred. Figure from Fraimout et al. (2017).

5.3.2. Trophic niche

As a synovigenic species, *D. suzukii* females need to feed in order to mature their eggs (Plantamp et al. 2017). Adults feed mainly on decaying fruit but can also use alternative nutrient sources such as flower nectar, bird guano or fungi when other resources are scarce (Plantamp et al. 2017; Wallingford et al. 2018; Cai et al. 2019; Stockton et al. 2019).

In contrast with most *Drosophila* species, *D. suzukii* can oviposit in ripening and healthy fruit thanks to its serrated ovipositor (Atallah et al. 2014). The fly can also lay a non-negligible part of its eggs in unripe green fruit (Ulmer et al. 2022). With this feature, the fly has access to a wider trophic niche in which ripening fruit are almost free of competitors (Lee et al. 2011; Atallah et al. 2014; Keesey et al. 2015). The fly is considered highly polyphagous as it can develop in numerous fruit species, both cultivated and wild, healthy and damaged, as well as occasionally in fungi or flowers (Mitsui et al. 2010; Lee et al. 2011; Walsh et al. 2011; Lee et al. 2015; Poyet et al. 2015; Briem et al. 2016; Kenis et al. 2016; Cai et al. 2019; Stockton et al. 2019; Kienzle et al. 2020). In Europe, field surveys of *D. suzukii* host range have reported more than 80 suitable host plant species belonging to 19 plant families (Poyet et al. 2015; Kenis et al. 2016). Similarly, in North America, host plants belonging to 12 plant families were susceptible to *D. suzukii* (Lee et al. 2015). These even include some plants that are generally (highly) toxic, such as *Atropa belladona* and *Viscum album* (Poyet et al. 2015; Poyet et al. 2017; Panel et al. 2018).

5.3.3. Natural enemies

Drosophila suzukii is predated by a range of macroarthropods (Ulmer et al. 2021; Siffert et al. 2021). As the larval development occurs mostly within fruit, the pupal and adult stages are more exposed to predation than larvae, even if eggs can be detected by some species (Englert and Hertz 2019). Commonly reported predatory species include earwings, spiders, ants, bugs and lacewings (Gabarra et al. 2015; Englert and Hertz 2019; Ulmer et al. 2021; Siffert et al. 2021).

Parasitoids are also amongst the natural enemies that can control *D. suzukii* populations (Fleury et al. 2009). In its native range, *D. suzukii* is susceptible to 19 larval parasitoids, such as *Ganaspis xanthopoda*, *Asobara tabida* and *A. japonica* (Mitsui et al. 2007; Wang et al. 2021). However, in its invaded range, there are no local larval drosophila parasitoids known to be able to develop in *D. suzukii* (Wang et al. 2021). The fly seems particularly resistant to resident parasitoids due to a strong cellular immune response leading to encapsulation (Chabert et al. 2012; Poyet et al. 2013). Studies are ongoing to test the susceptibility of *D. suzukii* to several *Drosophila* parasitoids in invaded regions (e.g., Gabarra et al. 2015; Miller et al. 2015; Shaw et al. 2023; reviewed by Lee et al. 2019; Wang et al. 2021). However, to date, only six species have been confirmed to attack *D. suzukii,* including three widely studied generalist pupal parasitoids, *Pachycrepoideus vindemiae*, *Trichopria drosophilae* and *T. anastrephae* (Wang et al. 2021).

5.3.4. Symbionts

Research attention has been given to describing microbial communities associated with *D. suzukii* (reviewed by Hamby and Becher 2016). In *Drosophila* species, the gut bacterial community depends on fly genotype, diet, and host bacterial community (Hamby and Becher 2016). The bacterial microbiome is dominated by a limited number of groups such as Enterobacteriales, Rhodospirillales and Lactobacillales (Chandler et al. 2011; Wong et al. 2011; Broderick and Lemaitre 2012; Chandler et al. 2012). In *D. suzukii*, bacterial communities have been reported to be dominated by *Tatumella*, a genus of Enterobacteriales previously not considered a common associate of drosophilid species (Chandler et al. 2014).

Amongst important symbionts in insects are members of the bacterial genus *Wolbachia* (Zug and Hammerstein 2015). In *D. suzukii*, the *Wolbachia* strain *w*Suz has been reported (Cattel et al. 2016b). It has imperfect maternal transmission in nature (Hamm et al. 2014), although perfect in laboratory (Cattel et al. 2016a). It does not induce reproductive manipulation in American populations (Hamm et al. 2014; Cattel et al. 2016b), although induces moderate cytoplasmic incompatibility in European populations (Cattel et al. 2016b, 2018). The prevalence of *Wolbachia* in natural populations is greater than expected by chance, indicating that *w*Suz is likely to provide benefits to its host (Cattel et al. 2016b), even if costs and drift (Oliver et al. 2014) can act against its maintenance. Reported effects are inconclusive, with reports of both increase (Mazzetto et al. 2015) and decrease (Hamm et al. 2014) in fecundity, and inconsistent changes in thermal tolerance (Saeed et al. 2018) or protection against viruses (Cattel et al. 2016b).

5.4. Impacts and management strategies

5.4.1. Damage to cultivated fruit

Drosophila suzukii is a pest of cultivated fruit, especially soft fruit (Walsh et al. 2011). Using its serrated ovipositor, the female causes physical injuries to ripening and healthy fruit (Atallah et al. 2014). The holes created by egg-laying provide entry points for bacteria, fungi and yeast that contribute to fruit decay (Cini et al. 2012). They can also facilitate the oviposition of other *Drosophila* species without this ability, such as *D. melanogaster* in grapes (Rombaut et al. 2017). Direct damage to fruit is caused by larvae feeding on the fruit flesh and mining galleries (Cini et al. 2012). Such damaged fruit unsuitable for sale (Bolda et al. 2010). Commonly impacted soft fruit crops include cherries, raspberries, strawberries,

blackberries and blueberries (Walsh et al. 2011; De Ros et al. 2013; Olazcuaga et al. 2019), but a wide range of other fruit can also be impacted (e.g., apricots, apples, figs, grapes, kiwi, peaches, pears, persimmons, plums; Walsh et al. 2011; Olazcuaga et al. 2019)

Since the first reports of the species in invaded areas, there have been a number of assessments of its potential economic impacts on the cultivated soft fruit industry (Bolda et al. 2010; De Ros et al. 2013). In the single canton of Trentino (Italy), the total damage caused to the five most impacted crops was estimated at ~3.3 million euros in 2011 (De Ros et al. 2013). In Minnesota, raspberry growers lost between 2 and 100% of their production, with a median yield loss of 20% corresponding to an estimated loss of US$ 2.36 millions in 2017 (Digiacomo et al. 2019). More recently, the costs and benefits of different management strategies have also started to be assessed (Farnsworth et al. 2017; Yeh et al. 2020).

5.4.2. Management strategies

Several management strategies have been developed to limit *D. suzukii* infestations of crops, although this is an ongoing field of research (Haye et al. 2016; Tait et al. 2021; Figure 12). Selection of particular strategies can also depend on the economic means and production scale of specific farmers (Farnsworth et al. 2017). Cultural practices are often the first methods applied that can reduce or help control infestation (Tait et al. 2021). These include sanitation measures (e.g., removal of damaged or decaying fruit; Leach et al. 2018), pruning of the crop plant, mulching and change in irrigation practice to modify the surrounding microclimate (Rendon and Walton 2019; Schöneberg et al. 2020), appropriate timing of harvest (Leach et al. 2018), and exclusion netting (Leach et al. 2016; Ebbenga et al. 2019). Biological control is the next most important method widely applied to reduce crop infestation (Tait et al. 2021; Wang et al. 2021). This includes the use of predators (Gabarra et al. 2015; Englert and Hertz 2019; Ulmer et al. 2021), parasitoids (Chabert et al. 2012; Gabarra et al. 2015; Fellin et al. 2023), entomopathogens (Woltz et al. 2015; Bing et al. 2021) or dead-end trap plants (Ulmer et al. 2020).

Cultural practices and biological control are often not sufficient alone (Diepenbrock et al. 2016) and many fruit producers still rely on insecticides to protect their crops (Tait et al. 2021). Common and effective conventional chemical insecticides include organophosphates, pyrethroids, carbamates, and diamides (Shawer et al. 2018), while reduced-risk chemical groups include spinosyns, and the certified organic insecticide spinosad (Gress and Zalom 2019). These are sprayed following calendar scheduling, with application based on the duration of protection provided (Van Timmeren and Isaacs 2013). Other methods based on behavioural manipulation (e.g., mass trapping, attract and kill, arrestants, spatial

repellents and oviposition deterrents, push-pull techniques, etc) or developmental disruption (e.g., sterile insect technique, gene drive systems) are being developed and show promise in the context of integrated pest management (Tait et al. 2021).

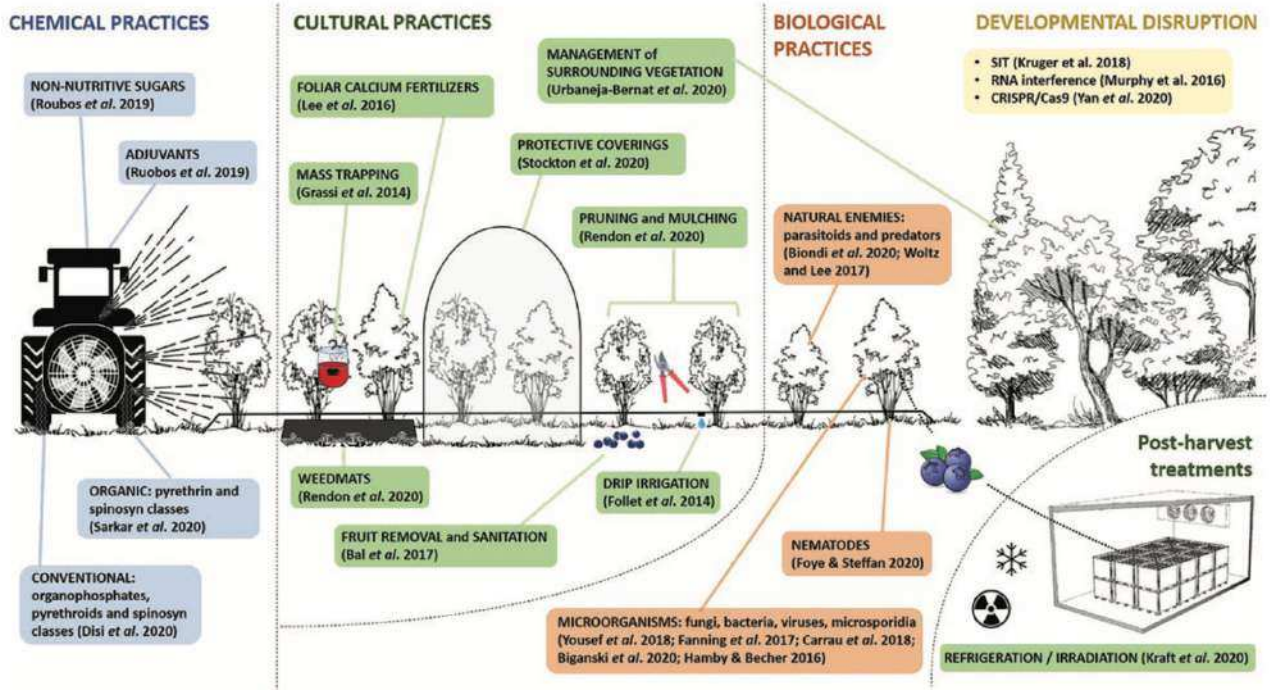

Figure 12 Integrated pest management strategies for *D. suzukii*. Methods applied to reduce *D. suzukii* infestation in fruit crops. None of these methods are entirely effective alone and there is a need to use integrated management practices to optimise pest control. From Tait et al. (2021).

5.4.3. Ecological disruption

Most research to date on *D. suzukii* impacts has focused on agronomic systems, while the ecological consequences of the fly on the habitats in its invaded range are still poorly documented (Kenis et al. 2009; Poyet et al. 2014). However, with the species' widespread distribution, extensive polyphagy and resistance to parasitoids, it has the ability to disrupt both agronomic and natural ecosystem functioning. Furthermore, by using ripening and healthy fruit, again in crop systems and natural habitats, it can impact the use of these fruit by other frugivorous insect species and by seed dispersers (e.g., birds, mammals; Bühlmann and Gossner 2022). In forest ecosystems, *D. suzukii* can be by far the most abundant Drosophilidae species, indicating strong competitive ability with other frugivorous species (Bülmann and Gossner 2022). The puncture damage created by ovipositing females facilitates other drosophilid species, such as *D. subobscura* in wild fruit (Poyet et al. 2014) or *D. melanogaster* in cultivated grapes (Rombaut et al. 2017). The species' activity can, thereby, increase the availability of resources for other fruit fly

decomposers (Poyet et al. 2014; Bernardi et al. 2017), although the premature decay caused by larval feeding and microbial development can repel seed dispersers, with knock-on negative consequences for plant populations (Poyet et al. 2014; Bülmann and Gossner 2022).

6. THESIS OBJECTIVES

In the context of biological invasions, fundamental research is essential. Improved knowledge of biology, ecophysiology, community ecology, and trophic ecology will contribute to better prediction of species range expansion, disruptive potential to ecosystems and its economic impacts, and often to developing prevention or mitigation strategies that can be included in integrated pest management programmes. *Drosophila suzukii* was clearly preadapted to be the successful invasive pest it has now become, facilitated by its extreme polyphagy, wide thermal niche and proximity to human activities, all features known to favour invasions (Zhao et al. 2023). In my thesis, I set out to research more deeply into the remarkable abilities of *D. suzukii*, with a focus on its trophic and thermal ecology (Figure 13). Most studies of *D. suzukii* available to date have focused on its impact on cultivated crops, while the use of other fruit outside the crop season and immediate commercial fruit farmers has been much less studied. The questions on which fruit may be used and how these may support *D. suzukii* populations through the winter period was remaining unaddressed. Observational study of this subject forms **the first part of my thesis**. In terms of thermal biology, *D. suzukii* has the capacity to disperse and select microhabitats offer protection of buffering from harmful conditions, in particular warm or cold temperatures. I hypothesised that this capacity would be linked with important behavioural thermoregulation abilities that have not been assessed, and **the second part of my thesis** focuses on experimental studies of this trait. I also hypothesised that the influence of temperature and trophic resources would overlap, because the life cycle of *D. suzukii* is completed mostly in fruit in which the temperature microclimate varies according to the larger-scale environmental conditions. **The last part of my thesis**, therefore, questioned the relationship between temperature and trophic resources, investigating the existence of trade-offs between trophic and thermal choices. Furthermore, the influence of the *D. suzukii Wolbachia* strain, *w*Suz, on the fly's biology is still debates. As other *Wolbachia* strains are known to supply nutrients to their host under conditions of nutritional deprivation or to influence the host's thermal preference, I predicted that it would influence both behavioural thermoregulation and trophic choices of *D. suzukii.* Experimental studies and analyses of the role of *Wolbachia* are, therefore, included in both the second and third parts of my thesis.

6.1. Polyphagy

The first part of my thesis consists of three distinct chapters in which I investigate the use of non-crop plants by *D. suzukii* in the challenging periods for the fly in autumn, winter and early spring. I considered the influence of local, climatic and landscape variables on *D. suzukii* infestation of several fruit-bearing plant species, and into the existence of linkages between *D. suzukii* and other drosophilid species associated with these fruit.

The first chapter documents the use of fallen fruit, especially apples (*Malus domestica*). After harvest, most damaged fruit remain on the ground and can constitute a reservoir for drosophilid species (Bal et al. 2017; Leach et al. 2018). Indeed, while *D. suzukii* preferentially oviposits on ripening and healthy fruit (Atallah et al. 2014), it can also use decaying fruit (Lee et al. 2011; Cai et al. 2019). Apples are a major fruit crop worldwide, but a considerable proportion of the crop can be damaged and commercially lost (Jeannequin et al. 2015). *Drosophila suzukii* has been reported to infest apples (Walsh et al. 2011; Weydert and Mandrin 2013; Asplen et al. 2015; Briem et al. 2015; Bal et al. 2017) as well as to be attracted by apple by-products (e.g., apple cider vinegar; Lasa et al. 2020) or to develop in substrates containing apples (Wallingford et al. 2018; Stockton et al. 2019; Jiménez-Padilla et al. 2020). I predicted that fallen apples would provide a back-up resource for the fly as autumn advanced. I also predicted that other Drosophilidae would be facilitated by the initial damage caused by *D. suzukii*. This chapter aimed at disentangling the relative roles of microclimatic, landscape and local factors in driving the diversity of the drosophilid community in decaying apples during the autumn-to-spring non-cropping seasons, and was published in *Agricultural and Forest Entomology* in January 2024 (Deconninck et al. 2024b).

The second chapter documents the use of mistletoe (*Viscum album* L.) fruit by *D. suzukii* between the end of winter and the end of spring. Mistletoe provides an important reservoir of fruit that mature slowly over this period. The ability of *D. suzukii* infest *V. album* has previously been assessed in field monitoring studies (Briem et al. 2016; Panel et al. 2018; Delbac et al. 2020a) and in laboratory experiments (Poyet et al. 2015; Briem et al. 2016; Wolf et al. 2020). However, the influence of environmental factors on infestation rate has not been explored. I hypothesised that not only would temperature be an important predictor of infestation in *V. album*, but also the proximity to natural habitat such as woodlands which provides shelter for the fly (Briem et al. 2018; Rossi-Stacconi et al. 2016; Ulmer et al. 2022). The study took place at national scale within France, with seven French laboratories involved in the field sampling. This chapter was published in *Arthropod-Plant Interactions* in May 2024

(Deconninck et al. 2024a).

The third chapter addresses the use of exotic ornamental plants and mistletoe by *D. suzukii*, and took place at regional scale in northern France. Relatively few plant species with fruit are available to support *D. suzukii* during winter and early spring. This chapter focuses on the known susceptible species, *Aucuba japonica*, *E.* ×*submacrophylla*, *Mahonia* spp. and *V. album* (Poyet et al. 2015). These plants are commonly found in parks and gardens and could act as catalysts for the initial expansion of the spring population of *D. suzukii* at the end of the winter period, before the first commercial fruit crops such as cherries, strawberries or raspberries become available. In this study I assessed the relative roles of climatic, landscape and local factors on infestation rates of the fruit of these host plants by *D. suzukii*. This chapter was submitted to *Ecological Entomology* in August 2024 (Deconninck et al. under review a).

6.2. Thermal plasticity

In the second part of my thesis, I investigated the behavioural thermoregulation capacities of *D. suzukii* and their potential adaptive value. This part is again divided into three distinct chapters.

The first chapter set out to develop a new conceptual framework to study the thermal preference plasticity in ectotherms and, more generally, behavioural thermoregulation abilities. To do so, I analysed a dataset produced in 2017 by Nicolas Meyer, an intern that was co-supervised by Sylvain Pincebourde and Hervé Colinet. This dataset was obtained in order to quantify the plasticity of *D. melanogaster* thermal preference. Thermal preference was studied across life stages and generations under the influence of different acclimation temperatures. Instead of developing previous analysis of this dataset, I investigated the relationship between the thermal preference mean and its variance. This allowed the development of several hypotheses relating to behavioural thermoregulation strategies that could (co)exist in ectotherms. This chapter was submitted to *The American Naturalist* in September 2024 (Deconninck et al. under review b).

The second chapter addresses thermal preference (hyper)plasticity in *D. suzukii*. *Drosophila suzukii* is able to actively select protected microhabitats according to season, circadian rhythm and environmental conditions (Tait et al. 2020a,b). Consequently, I predicted its thermal preference would be highly plastic. I investigated the influence of acclimation temperature on the thermal preference of *D. suzukii* across life stages and generations, and considered other variables such as sex, age and reproductive status. I used the framework above to describe the behavioural thermoregulation abilities of *D. suzukii*. This chapter will be submitted to *Proceedings of the Royal Society series B* in late 2024 (Deconninck et al. in prep a).

The third chapter in this part of the thesis set out to address the question of whether thermal preference plasticity in *D. suzukii* is adaptive. I did this by planning experiments through the lens of the bioenergetics of the fly. First, I examined the influence of acclimation temperature on *D. suzukii* metabolism and thermal sensitivity over three distinct generations, and also investigated the influence of *Wolbachia*. I measured the energy content (total carbohydrates, proteins, lipids) of flies from the different acclimation treatments. Then, I investigated if there was a relationship between their thermal preference and metabolism. I predicted that, if a fly showed a thermal preference, this would be linked to its metabolism. For instance, a fly may select a low temperature and thereby reduce its standard metabolic rate. This chapter will be submitted in late 2024.

6.3. Integrative approach

The final part of my thesis addresses the interaction between thermal and trophic choices, again considering the influence of *Wolbachia* in this, and is divided into three chapters.

The first chapter of this part addresses the influence of temperature on *D. suzukii* performance in different winter-available fruit. This differs from Part 1 of my thesis, in which I studied the infestation of these winter-available fruit under field conditions from January to June. While obtaining information on the phenological use of these fruit, as well as on the influence of several climatic, landscape and local factors on their infestation, the performance of *D. suzukii* in winter-available fruit under realistic winter/early spring temperatures was not addressed. Panel et al. (2018) compared fly performance in these same fruit under laboratory conditions (~20°C) and found differences in terms of development time, fecundity and survival. Under realistic winter/early spring temperatures, we predicted differences in fly performance would be apparent because of different nutritional requirements when under cold conditions (Rendon et al. 2019). This chapter was submitted to *Insect Science* in July 2024 (Larges and Deconninck et al. under review).

The second chapter addresses the potential role of *Wolbachia* in polyphagy. Polyphagy can be costly because of the varying nutritional quality of different fruit can impact overall fitness (Janz 2003). This cost can be offset by a female laying eggs in different fruit (bet-hedging strategy), but *Wolbachia* could also play a role in this respect. As other strains of *Wolbachia* were already are known to be nutritional mutualists (e.g. Hosokawa et al. 2010), I hypothesized, stimulated by a discussion with Aurélien Vigneron, that *w*Suz may enhance nutrient supply to *D. suzukii* in nutrient-depleted conditions. This chapter was published in *Journal of Pest Science* in January 2024 (Deconninck et al. 2024c).

In the final chapter, I investigated the existence of any trade-off between trophic choice and thermal choice. In the field, *D. suzukii* faces two practical constraints when choosing a resource in which to oviposit. First, the nutritional quality of the resource should maximize the performance of the offspring, according to the preference-performance hypothesis (Gripenberg et al. 2010). Following this same hypothesis, I also predicted that the temperature of the resource would be of importance when selecting oviposition fruit, as this also determines the performance of the offspring (Raynaud-Berton et al. 2024). This chapter will be submitted in late 2024 (Destierdt and Deconninck et al. in prep).

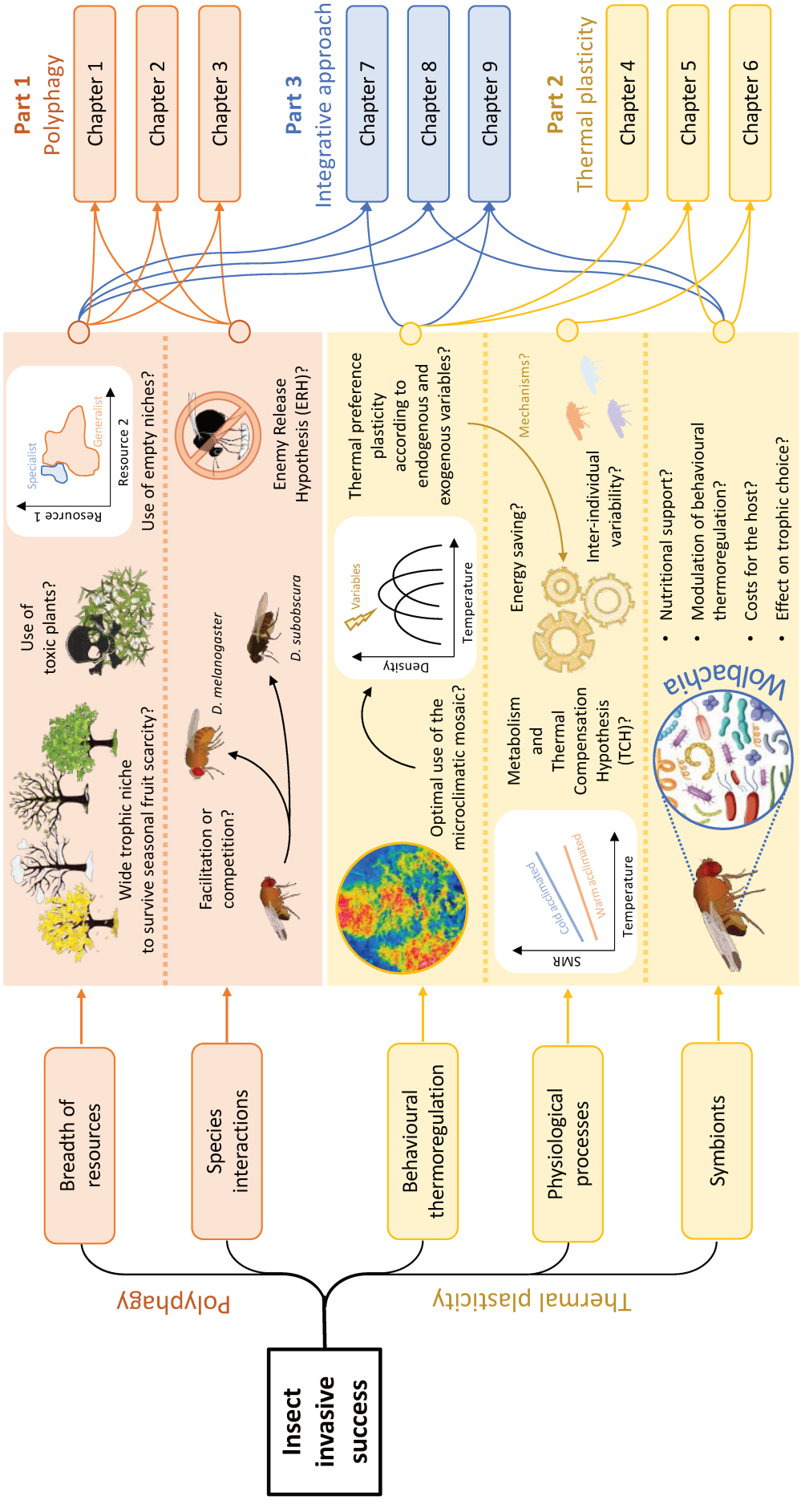

Figure 13 Conceptual figure of the thesis.

Part 1

Trophic resources through seasons

INTRODUCTION

Part 1
Trophic resources through seasons

The challenging conditions of the winter season can generate bottlenecks for populations. In this first part, I was interested in the resources used by *D. suzukii* outside of the commercial cropping season, from autumn to spring. Previous studies have already documented the wide trophic niche of *D. suzukii* (Poyet et al. 2015; Kenis et al. 2016), which includes both wild and ornamental fruit. However, the factors driving infestation of these non-commercial species were unknown, especially from autumn to spring.

In the first chapter of Part 1 (Chapter 1), I explored the use of fallen fruit by *D. suzukii* from autumn to winter. Fallen fruit can play a significant role as feeding and breeding substrates for arthropod communities (Alamiri 2000; Rohlfs and Hoffmeister 2004; Mészárosné Póss et al. 2022) and can also provide a potential reservoir for insect pests when cultivated fruit are scarce, providing a seasonal refuge and potentially initiating future pest outbreaks (Liquido 1991). This chapter explored the use of fallen apples by *D. suzukii*, a food source that is still present in significant amounts after the commercial harvest is completed (Jeannequin et al. 2015), and that can remain on the ground for several months until decomposition has completed (Myczko et al. 2013; Stockton et al. 2019; Folwarczny et al. 2022).

In Chapter 2, I focused on the use of mistletoe, a natural crop that is known to be susceptible to *D. suzukii* (Briem et al. 2016; Panel et al. 2018) and constitutes an important fruit reservoir in winter and spring (Thomas et al. 2023). Mistletoe is one of the few fruit available during this period but its infestation dynamics has not previously been explored before. The study was conducted at national scale in France in order to increase understanding of the biogeographic dynamics of *D. suzukii* infestation and the macroecology of the fly.

In Chapter 3, I further explored the resource continuum available to *D. suzukii* from winter to spring when, again, fruit resources are limited (Grassi et al. 2018). Examples include various ornamental species in gardens and parks as well as wild plant species (Poyet et al. 2015; Kenis et al. 2016). While we hypothesized that these could provide the starting point for the build-up of early spring populations, their role as potential egg-laying sites for *D. suzukii* has been poorly documented. In this chapter, infestations in the ornamental plants *Aucuba japonica*, *Elaeagnus* ×*submacrophylla*, *Mahonia aquifolium* and *M. japonica* agg. and in the wild parasitic shrub *V. album* were studied. This study was made at a smaller, regional, scale than that described in Chapter 2, using a multi-resources landscape approach (and a space-for-time substitution, SFTS; Pickett 1989) which allow the reconstitution of temporal dynamics.

Chapter 1

Fallen fruit as autumn resources

Chapter 1 • Objectives and main outcomes

In this chapter, I examined the use of fallen fruit as a potential back-up resource for *D. suzukii* at the end of the commercial cropping season. Decaying fruit on the ground provides an important feeding and breeding substrate for small mammals and insects, including several Drosophilidae species. Invasive species such as *D. suzukii* and *Chymomyza amoena* were predicted to use these resources and to modify the decomposer community.

The study was carried out in northern France between September 2021 and April 2022. Decaying apples were sampled monthly from 19 sites randomly selected within a landscape window of 30 × 45 km. Several microclimatic, landscape-scale and local factors were recorded in order to assess their role in driving the drosophilid community associated with decaying fruit across seasons. Drosophilid flies emerging from the fallen apples were identified to species level.

Fallen apples were used by 14 drosophilid species, including the invasive *D. suzukii* and *C. amoena*. Even though *D. suzukii* was only found in late summer, it was able to use fallen fruit, confirming that they can be part of the resource continuum allowing the fly to overwinter. The abundance of *D. suzukii* decreased with the presence of *Penicillium* in the apples and with increasing apple tree canopy size, while it increased with higher local air temperature during the week before sampling and with mowing of the local vegetation. These findings highlight the importance of considering multiple scales and factors when studying the interactions between invasive species, native species and their shared trophic resources.

A novel approach used in this chapter was the hierarchical continuum model, which is primarily used in plant community studies, to study Drosophilidae species coexistence in decaying apples. Four strategies were identified ('core', 'satellite', 'urban' and 'rural' species), based on the patterns of their abundance (i.e., dominance) and distribution (i.e., frequency changes between months) with seasonal progression. I concluded that *Drosophila suzukii* was a 'satellite' species, as it was found at low density and restricted to a short period of time, probably applying an opportunistic strategy in fallen apples, using the freshest and sweetest damaged fruit.

Contributions

Olivier Chabrerie conceptualised the study. Méghan Boulembert did the field work.

I analysed the data, produced the graphics and tables. I wrote the original draft, revised the manuscript according to reviewers' comments and wrote the response letter to the editor and reviewers.

Other co-authors contributed to the conceptualisation and revision of the manuscript.

Fallen fruit: A backup resource during winter shaping fruit fly communities

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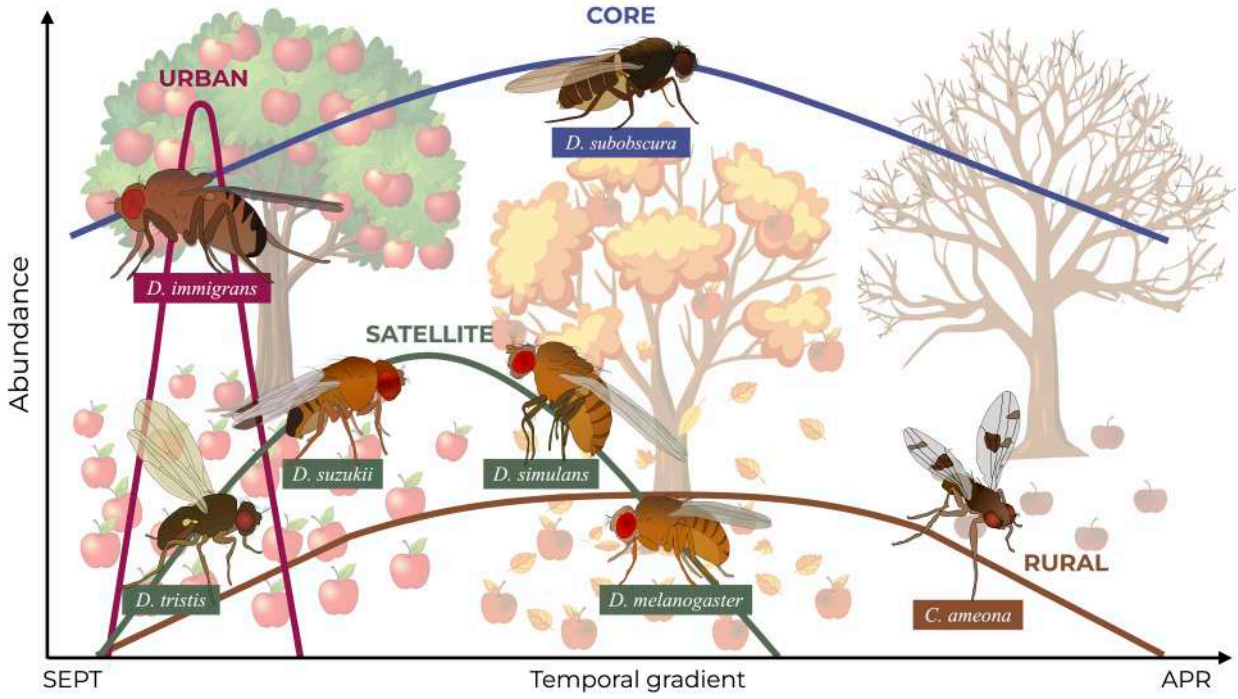
§: equal contribution

Status: Published in *Agricultural and Forest Entomology* (2024).

GRAPHICAL ABSTRACT



ABSTRACT

1. Fallen fruits provide important feeding and breeding substrates for insects such as Drosophilidae and can be a potential trophic reservoir when usual host fruits become scarce.
2. Recently, two invasive fruit fly species, *Drosophila suzukii* and *Chymomyza amoena*, have become established in Europe and are expected to alter existing Drosophilidae communities.
3. In this study, carried out between September 2021 and April 2022 in northern France, we aimed to disentangle the relative roles of microclimatic, landscape and local factors driving the diversity of the Drosophilidae community in decaying fruit across seasons.
4. Minimum site temperature during the week preceding sampling and the proportion of rotten fruit tissue had the strongest positive influence on Drosophilidae abundance and species richness. Drosophilidae abundance also increased with urbanisation (portion of building cover) around the sampled trees.
5. Decaying apples were important breeding sites for *C. amoena* across seasons, but provided a suboptimal substrate for *D. suzukii*, which was only present in late summer.
6. This study sheds light on the important role of unharvested fallen crop fruit in maintaining the diversity of an insect family that is generally overlooked in field studies. It also emphasises the importance of considering multiple scales and factors when studying the interactions between invasive species, native species and their shared trophic resources. Finally, our data highlight the importance of the Drosophilidae community in recycling agricultural waste.

Keywords: insect invasion, *Chymomyza amoena*, *Drosophila suzukii*, apple fruit, orchard, landscape composition, temperature, crop fruits, plant traits

INTRODUCTION

In agricultural systems, herbivorous insects attract attention because of their pest status and their damaging impacts on crop production and storage (Manosathiyadevan *et al*., 2017). Most research studying fruit pests focuses on the effects of insect attacks before or during fruit ripening, when the economic consequences are greatest (Herrera, 1982; Alford, 2017). However, what happens after the harvest period is often neglected including, for instance, the role of insects in organic matter recycling in crop fields. Fallen fruit can play a significant role as feeding and breeding substrates for arthropod communities (Alamiri, 2000; Rohlfs & Hoffmeister, 2004; Mészárosné Póss *et al.*, 2022). They could also represent a potential reservoir for insect pests when cultivated fruits become scarce, providing a seasonal refuge and potentially initiating future pest outbreaks (Liquido, 1991). Nevertheless, the temporal dynamics of arthropod communities across the 'lifespan' of a decaying fruit have largely been overlooked.

Drosophilidae species are of particular interest due to their significant role in the recycling process of decaying fruit. The success of fruit infestation by Drosophilidae species is influenced by numerous environmental factors. At the regional scale, climatic and microclimatic conditions influence their presence. In particular, temperature and precipitation patterns affect thermal limits and desiccation tolerance (Hoffman, 2009; Kellerman *et al*., 2009; Ulmer *et al*., 2022). At finer scale, landscape composition, i.e., the cover of natural and anthropic habitats surrounding a site, is a key driver of their diversity and abundance (Poppe *et al*., 2016; Furtado & Martins, 2018; Delbac *et al.*, 2020; Wen *et al*., 2023). At local microclimate scale, during cold seasons or cold parts of the day, decomposing fruit can provide niches that are warmer than the surrounding environment, as microbial communities produce heat during organic decomposition (Ryckeboer *et al*., 2003). All these factors can independently influence the infestation rates of different Drosophilidae species. Finally, the structure of Drosophilidae communities is likely to be highly dynamic given that fruit can display profound gradual changes over the period of decomposition, affecting the quality and quantity of substrate they can provide for insects.

Drosophilidae community structure is also strongly influenced by biotic interactions. Individual species may limit or facilitate infestation by other species, depending on their ability or preference to oviposit and develop in a fruit at a given stage of decomposition and on their competitive ability (Davis *et al*., 1998; Hoffman, 2009). The number and identity of competitors can influence the range and abundance of species (Davis *et al*., 1998). Furthermore, invasive species can disrupt the native community assemblage and cause cascading effects across trophic networks, sometimes also facilitating native species

infestations (Rodriguez, 2006; Poyet *et al*., 2014). Worldwide, communities of frugivorous species have been impacted by invasive exotic species. For instance, in Europe, the invasive members of the Drosophilidae family, *Drosophila suzukii* Matsumara and *Chymomyza amoena* Loew, are highly polyphagous and could profoundly alter the structure of Drosophilidae communities developing in fresh and decaying fruit by using the same resources as native species (Band *et al*., 2005; Poyet *et al*., 2014, 2015; Pajač Živković *et al*., 2017).

On a short timescale, changes in the nutritional composition of fruit during the decomposition process can affect fly foraging decisions and adult oviposition choices (Silva-Soares *et al*., 2017). Fruit with characteristically slower and longer decomposition allow study of the temporal dynamics and environmental drivers of the Drosophilidae community depending on them. Prolonged decomposition could include successive decomposition stages characterised by different chemical composition and texture, sometimes spanning different seasons and potentially offering successional niches to different Drosophilidae species. Apples (*Malus domestica* Borkh) provide an excellent candidate fruit for such studies. Apples are a major crop fruit in Europe and worldwide (FranceAgriMer, 2021; FAOSTAT, 2023). However, a considerable proportion of many apple crops are damaged and commercially lost (Jeannequin *et al*., 2015). The large quantity of apples remained on the ground through damage or as windfalls at the end of summer/beginning of autumn can provide an important potential trophic resource for both vertebrates (Myczko *et al*., 2013; Bouvier *et al*., 2020; Castañeda *et al.*, 2022) and a range of invertebrates (Papadopoulos *et al*., 2001; Morimoto & Pietras, 2020), including exotic species (Bal *et al*., 2017). The apple decomposition process can extend throughout the winter, providing both food for many organisms when other sources are scarce (Myczko *et al*., 2013; Stockton *et al.*, 2019; Folwarczny *et al*., 2022) and a microclimatic environment protecting from environmental extremes (Jiménez-Padilla *et al*., 2020). Rotting apples contribute to maintaining the diversity of decomposer insects in the ecosystem, amongst which frugivorous Drosophilidae species (MacMillan *et al*., 2016; Morimoto & Pietras, 2020) are of particular interest because of (i) their significant role in the decomposition of decaying fruit and (ii) the recent establishment in Europe of two major invasive Drosophilidae that can utilise these decaying fruit.

Originating from Asia, the invasive *D. suzukii* fly spread to Europe in the late 2000s (Asplen *et al*., 2015). Because of their serrated ovipositor, females can attack healthy, ripening, fruit making this species a major threat to fruit crops including berries and stone fruit (Walsh *et al.*, 2011; Tait *et al.*, 2021). However, interactions between *D. suzukii* and apples have been poorly documented, due to the low (if

not imperceptible) level of damage it causes, leading to very little commercial impact. However, decaying apples are likely to be attractive to adult flies, as *D. suzukii* is attracted to apple juice (Feng *et al*., 2018), products of apple degradation (Feng *et al*., 2018) and apple cider vinegar (Lasa *et al*., 2020). Few studies have reported infestations of apples by *D. suzukii* in orchards (Walsh *et al*., 2011; Weydert & Mandrin, 2013; Asplen *et al*., 2015; Briem *et al*., 2015; Bal *et al*., 2017) and these infestations probably primarily occurred on already damaged fruit with easily accessible flesh for oviposition (Lee *et al*., 2011; Cai *et al*., 2019). Under laboratory conditions, although poor larval performance was generally recorded (Cai *et al*., 2019; Stockton *et al*., 2019; Jiménez-Padilla *et al*., 2020), *D. suzukii* has been successfully reared on various apple substrates, including cut apples (Jiménez-Padilla *et al*., 2020), fallen apples (Wallingford *et al*., 2018), apple puree (Stockton *et al*., 2019) and artificial medium made from apple pulp (Englert & Herz, 2019) or apple juice (Sato *et al*., 2021). Apples may therefore provide a back-up resource when other fruit are scarce.

The Nearctic invasive species *C. amoena* is known to oviposit on already damaged or parasitised fleshy fruit and nuts (Band *et al*., 2005), but its contribution to fruit decomposition has not been studied. It is not considered as a true pest (Band & Band, 1980; Pajač Živković *et al*., 2017) at present and has received much less research attention than *D. suzukii*. Native to North America, it has become widely established in Europe since its first detection in 1975 in Czechia (Jong & van Zuijlen, 2003; Band *et al*., 2005; Pajač Živković *et al*., 2017). The species' spread across Europe is likely to have been facilitated by association with regional transport of fruits, probably apples, across the region (Burla & Bächli, 1992; Withers & Allemand, 1998; Band *et al*., 2006). Questions about its potential impact on the native Drosophilidae community have been raised previously (Withers & Allemand, 1998), but no further investigations have been made. The trophic resources used by *C. amoena* are varied, spanning multiple wild and domestic fruit including apples (Band, 1991; Burla & Bächli, 1992; Band *et al*., 1998, 2005, 2006). Oviposition mostly takes place in fruit scars, codling moth (*Cydia pomonella* Linnaeus) tunnels or frass (Band, 1988; Band *et al*., 2005) and is usually aggregated (Band, 1989). *Chymomyza amoena* takes advantage of previous physical damage to fruit, including from previous pest infestations (Band *et al*., 1998, 2005). Both *C. amoena* and *D. suzukii* have been reported co-occurring in apple orchards in Italy (Amiresmaeili *et al*., 2019).

This study aimed to disentangle the relative roles of landscape and local factors in controlling the diversity and composition of Drosophilidae communities using decaying apples as breeding sites,

including these two invasive species. We hypothesised that (i) the highly polyphagous *D. suzukii* would preferentially utilise apples in the early stages of decomposition at the end of summer, as it is a pioneer species preferentially infesting ripening fruit (Poyet *et al*., 2014), and (ii) *C. amoena* would use this fruit resource in autumn, as this fly is commonly found infesting apples that has been first attacked by codling moth *Cydia pomonella* (Band *et al*., 2005). We also hypothesised that, (iii) both native and exotic species would be positively associated with the proximity of natural habitats (particularly woodland) which generally provide a refuge (and thus reservoir) for *Drosophila* species (Basden, 1954), (iv) they would be affected by low temperature and particularly frost days, and (v) they would be influenced by apple tree traits, such as the presence of large canopy trees providing shelter or high apple production for feeding and oviposition. It is worth mentioning that we focused on apples for the abovementioned reasons but other fruits could play this back-up resource role for Drosophilidae (e.g., pears, grapes, figs, persimmons, etc).

METHODS

Study area

The study was conducted between September 2021 and April 2022 in the region of Amiens (49°53′40″ N, 2°18′07″ E) in northern France. The region's climate is oceanic with a mean annual temperature of 10.7°C and average rainfall of 691.9 mm (data from meteorological station Dury-les-Amiens, StatIC network). The landscape of this region is largely characterised by agricultural production and is a mosaic of open fields cultivated for cereals, rapeseed and sugar beet, interspersed by apple orchards, grasslands and woodland patches. The apple orchards are managed using various strategies including conventional and organic fruit production. However, apple trees are also commonly present in domestic gardens, green spaces in towns and villages, along roadsides and in pastures and woodlands. In the study area, only one study assessed the Drosophilidae diversity, recording 25 species (Ulmer, 2022).

Apple collection and fruit and tree trait measurements

Decaying apples were obtained from 19 sites randomly selected within a landscape window of 30 × 45 km (see Table S1 for site characteristics and apple varieties). To capture the temporal dynamics of communities (apples mainly fall in September and October, and some apples still remain on the ground in early Spring), eight sampling sessions took place between September 2021 and April 2022 (21-22 September 2021, 19-20 October 2021, 16-17 November 2021, 14-15 December 2021, 18-19 January

2022, 16 February 2022, 15 March 2022 and 12 April 2022). Each month, at each site, six decaying apples were randomly collected from the ground below the canopy of a single apple tree. After the last session in April, no decaying apples remained on the ground at any of the sites. A total of 633 apples were collected over the study period.

To evaluate the fruit resources available for the Drosophilidae, the following quantitative and qualitative traits were measured for each apple collected: height (from the flower scar to the fruit peduncle), diameter, mass, volume (mean radius^3 × π × 4/3), proportion of healthy skin of the fruit (based on colouration), proportion of rotting surface (based on visual aspect), proportion of fruit surface with no skin (removed by physical wounds or animal foraging), presence of mould (i.e., *Penicillium* and *Monilinia* fungal sporophores following the identification keys of FREDON, 2002), and the number of holes associated with galleries drilled by pests (including those from the codling moth *Cydia pomonella*). Since fruit sugar levels are known to be associated with egg laying choices and/or larval development of some *Drosophila* species (Lee *et al*., 2008), Brix values (% sugar content) were measured both in the healthy and rotting parts of each collected fruit using a refractometer (Bellingham+Stanley sugar 0%-50%, serial number 019568 for low volume). The pH was also measured in both parts of the apples using pH indicator paper (Special indicator paper, REF 902 05, pH 0.5-5.5 (MACHEREY-NAGEL) and pH indicator paper, pH 5.5-9.0 (Supelco). In all cases, pH and sugar contents were used as proxies for the general decaying stage of the apple, including both internal and external decays.

The following vegetative and reproductive traits were recorded for each apple tree under which fruit was collected in order to estimate their influence on community dynamics: tree height, minimum and maximum canopy diameter, canopy area (π × max canopy radius × min canopy radius) and trunk circumference (at 1.30 m height). The numbers of fruit hanging on the tree and on the ground surface under the tree canopy in a 5 m radius around the trunk were recorded. The relative proportions of healthy vs. damaged fruit on the ground were measured. For each apple tree, the mean volume of an individual fruit (mean radius^3 × π × 4/3) and the total volume of fruit on the tree were calculated. The apple variety was also noted (Table 1).

Emergence and identification of Drosophilidae flies

Immediately after collection, apples were individually placed on wet cotton wool in cylindrical plastic transparent containers (diameter = 118 mm, height = 135 mm, volume = 1,476 cm^3), covered with

Table 1 Site, apple tree and apple characteristics. Site: site code, Coordinate: geographic coordinates of the apple tree (coordinate system: WGS 84), Altitude: mean altitude of the apple tree, Variety: variety of the apple tree, Sum collected apples: total of apples collected, Mean sugar content: mean sugar content in healthy part of apples sampled ± standard error, Mean pH: mean pH in healthy part of apples sampled ± standard error.

Site	Coordinate	Altitude (m)	Variety	Total collected apples	Mean sugar content (Brix)	Mean pH
PrV	49.823692 N ; 2.385196 E	59.836	Marie Mesnard	14	17.283 ± 0.393	3.783 ± 0.101
PrC	49.822715 N ; 2.392622 E	38.947	Golden	20	15.9 ± 0.1	2.9 ± 0.4
JL	49.897864 N ; 2.319218 E	24.346	Falstaff	42	12.925 ± 1.649	2.875 ± 0.125
BsPR	49.765967 N ; 2.002825 E	92.223	Colapuy	28	13.533 ± 0.769	3.433 ± 0.067
BsPM	49.771729 N ; 1.994607 E	96.904	Cardinal rouge	42	16.4 ± 0	3.5 ± 0
Bg	49.742929 N ; 2.021757 E	120.06	Colapuy	42	13.45 ± 0.15	2.6 ± 0.1
NaV	49.791208 N ; 2.094462 E	139.983	Belle Fille	44	17.3 ± 1.8	3.7 ± 0
T	49.802371 N ; 2.136424 E	110.422	Racine	48	17.7 ± 0.866	4.25 ± 0.144
N	49.839968 N ; 2.165911 E	78.762	Reine des reinettes	17	13.8 ± 1.795	4.333 ± 0.167
CF	49.823927 N ; 2.149882 E	123.489	Médaille d'Or	48	14.8 ± 1.401	3.633 ± 0.067
CS	49.859573 N ; 2.170174 E	115.905	Reinette étoilée	18	15.45 ± 0.55	3.4 ± 0.1
A	49.879944 N ; 2.318306 E	59.741	Reine des reinettes	36	11.25 ± 1.25	3.1 ± 0.4
Pc	49.637533 N ; 2.427752 E	136.781	Rateau	34	11 ± 0	3.3 ± 0
H	49.987074 N ; 2.055347 E	28.451	Kermerrien	24	21.325 ± 0.99	3.4 ± 0.117
Bl	49.993649 N ; 2.121355 E	64.168	Noël des Champs	38	16.15 ± 0.85	4 ± 0
W	49.92154 N ; 1.947491 E	55.99	Groseille	12	16.8 ± 0.4	2.5 ± 0
C	49.922992 N ; 2.08597 E	109.09	Boskoop	42	14.7 ± 0	2.5 ± 0
LlA	49.922801 N ; 2.26735 E	35.414	Natural seedling (close to Golden)	48	17 ± 1.966	2.333 ± 0.167
F	49.883266 N ; 2.170291 E	105.483	Doux Blanc	36	17.15 ± 0.55	4.4 ± 0.1
Total				633		

a nylon mesh, and maintained in a temperature-controlled room at 20°C under a 16:8 L:D regime until all insects had emerged. Adult insects emerging were collected daily and stored in 70% ethanol until identification. All collected fruit were maintained in the emergence room for 100 days to check for any late emergence of imagos. Emerged insects were subsequently sorted to select taxa representing Drosophilidae, which were identified to species level using a Leica M205C stereomicroscope (equipped with a Leica MC170 HD camera and Leica Application Suite software) following the keys provided by Bächli *et al*. (2005) and a collection of standard specimens available in the laboratory.

Environmental variables

Local, landscape and meteorological variables were measured at each sampling site or obtained from online databases to examine their relationships with the Drosophilidae community emerging from the apples. The full list of variables, their units and their codes are given in Suppl. Mat. (Table S1).

For each sampled tree, the altitude and the slope of the site were recorded. Within a 5 m-radius plot centred on the tree trunk, the cover and height of the tree layer, shrub layer and herbaceous layer were estimated, as well as soil litter thickness. Leaf litter, dead wood debris and the presence of a composting agent, such as apple pomace waste from cider factories, were also noted. The proportions of local habitats surrounding the apple tree (wood, hedgerow, orchard, grassland, crop, garden, building, road, river and pond) within 10 m and 50 m radii were also noted. The management of the site herbaceous vegetation (mown vs. grazed grasslands) and apple trees (yearly pruned vs. not or rarely pruned, pesticide treated vs. non-treated) were noted.

The landscape composition around each sampled apple tree was characterised. A geographic database was created using a Geographic Information System (GIS; ArcGIS Pro v.2.5, ESRI). The sampled apple trees were positioned in the GIS and buffers of 10 m, 20 m, 50 m, 100 m, 250 m, 500 m, 750 m and 1000 m radii around each tree were created for subsequent analyses of landscape composition. Landscape elements (woodland, grassland, crop, building, road, water) were extracted from the OSO database (Centre d'Expertise Scientifique OSO, 2021) and updated using aerial photographs and field observations (in buffers < 100 m). The BD-Carto® database from French National Geography Institute was used to refine mapping and georeferencing. For each sampled tree, the cumulative area of the different habitats composing the landscape was then computed in each concentric buffer.

Macroclimatic conditions were characterised for each sampling site using regional measurements. The daily meteorological data were retrieved from the three nearest meteorological stations to each site (https://www.historique-meteo.net/france/), representing large-scale climatic conditions. Daily minimum and maximum temperatures, rainfall and snowfall were calculated for all sites using inverse-distance weighting (IDW) interpolation (Willmott *et al*., 1985) from the data from the three nearest weather stations (detailed in Table S3 and S4). Daily temperatures were extracted for each day from January 1 2021 to the date of sampling, allowing us to calculate the mean daily temperature range (daily maximum - daily minimum) as well as the number of frost days since the beginning of the year. Accumulated degree-days (Growth Degree Days, GDD) were calculated using a lower threshold of 0°C between January 1 2021 and the date of sampling (Baskerville & Emin, 1969). The same calculation was also performed from September 1 2021 to the date of sampling in order to better match the ripening and collection period of the apples. The threshold value of 0°C is a standard threshold commonly used in insect and plant studies (White *et al*., 2012; McNeil *et al*., 2020), although it may not represent the precise

threshold for all Drosophilidae. This threshold is particularly appropriate for study of temporal synchrony between flies and plant resources (Iler *et al*., 2013). It was also selected as several *Drosophila* species are known to be active at very low temperatures (<5°C) during winter days (January-February in Amiens and Bordeaux, Ulmer *et al*., 2022). Cumulative precipitation between January 1 2021 and the date of sampling were also calculated from daily precipitation values.

Microclimatic temperatures were recorded at each sampling site, noting the temperature of the ground surface on which the apples were laying and the air temperature within the apple tree canopies. Hobo loggers (TIDBIT data logger V2 TEMP TBI-001, ONSET Company, Bourne MA, USA) were used, recording every 60 min. Each sampling site was equipped with two loggers (soil surface and within tree canopy). The first was placed on the ground surface among decomposing apples ("bottom hobo"). The second was suspended 1.5 m above the ground in the tree canopy, under the shade of a branch to avoid direct exposure to solar radiation ("top hobo") and oriented northward. The minimum, mean and maximum temperatures for ground and air were extracted every day to compute the mean daily minimum, mean and maximum temperatures, and used to assess the buffering ability of the soil relative to the local air temperature. Accumulated degree-hours (Growth Degree Hours, GDH) were calculated using a lower threshold of 0°C (Baskerville & Emin, 1969) during the week preceding the day of each sampling.

Statistical analyses

General Linear Mixed Models (GLMMs) were used to test the influence of environmental factors (apple traits, local conditions, landscape composition and meteorological variables; see list in Table S1) on the temporal dynamics of species richness and abundance of the Drosophilidae community emerging from the decomposing apples (n = 633 fruit). Part of the analysis specifically focused on the number of *D. suzukii* and *C. amoena* individuals emerging per fruit. "Site" was included as a random effect in the GLMMs to account for the non-independence of apples sampled from the same site. Response variables were checked for normality and transformed ($\log_{10}$+1) when necessary prior to analyses (Quinn & Keough, 2002). In the models, only the environmental variables that were significantly correlated with the response variables, and with $R > 0.2$ (Pearson correlation), were considered in order to avoid overfitting and excessive complexity. After backward selection of explanatory variables, the Akaike information criterion (AIC) was used to select the most parsimonious model, i.e., the most significant model with the

Table 2 Distribution of the Drosophilidae species in the study. N ind: total number of individuals emerged from apples, Min: minimum number of individuals per apple, Max: maximum number of individuals per apple, Mean: mean number of individuals per apple, SE: standard error, %: percentage of individuals of the species among all individuals that emerged from apples, AFreq: frequency of apples infested by the species among all apples sampled.

		Total study (633 apples)						
Species	**Code**	**N ind**	**Min**	**Max**	**Mean**	**SE**	**%**	**AFreq**
Chymomyza amoena	CHYAMO	498	0	34	0.787	0.121	7.926	0.197
Drosophila ambigua	DROAMB	6	0	2	0.009	0.046	0.095	0.008
Drosophila bifasciata	DROBIF	2	0	1	0.003	0.04	0.032	0.003
Drosophila hydei	DROHYD	24	0	21	0.038	0.172	0.382	0.003
Drosophila immigrans	DROIMM	2578	0	375	4.073	0.561	41.031	0.1
Drosophila kuntzei	DROKUN	1	0	1	0.002	0.04	0.016	0.002
Drosophila melanogaster	DROMEL	318	0	136	0.502	0.329	5.061	0.047
Drosophila obscura	DROOBS	2	0	1	0.003	0.04	0.032	0.003
Drosophila simulans	DROSIM	171	0	61	0.27	0.242	2.722	0.033
Drosophila subobscura	DROSUB	2499	0	115	3.948	0.206	39.774	0.384
Drosophila suzukii	DROSUZ	87	0	16	0.137	0.112	1.385	0.043
Drosophila tristis	DROTRI	87	0	16	0.137	0.122	1.385	0.032
Hirtodrosophila cameraria	HIRCAM	2	0	2	0.003	0.056	0.032	0.002
Scaptomyza pallida	SCAPAL	8	0	3	0.013	0.056	0.127	0.008
Total		6283						

Note: N ind: total number of individuals emerged from apples, Min: minimum number of individuals per apple, Max: maximum number of individuals per apple, Mean: mean number of individuals per apple, SE: standard error, %: percentage of individuals of the species among all individuals that emerged from apples, AFreq: frequency of apples infested by the species among all apples sampled.

lowest AIC (Harrison *et al*., 2018). Model residuals were checked for homoscedasticity using biplots of model predictions. Kruskal-Wallis tests were used to test whether the numbers of species and individuals of Drosophilidae emerging from apples varied between months. *Post-hoc* pairwise comparisons between months were performed using Mann-Whitney U tests ($\alpha = 0.05$).

The influence of environmental conditions on Drosophilidae community composition was examined using a redundancy analysis (RDA; Jongman *et al*., 1995; McCune & Grace, 2002) of the Drosophilidae species matrix constrained by environmental variables. Before performing the RDA, a detrended correspondence analysis (DCA) (Hill & Gauch, 1980) was run on the species matrix to assess

(on the basis of the gradient lengths depicted by DCA axes) whether a linear or unimodal model was appropriate in the subsequent multivariate analyses. DCA results on the species matrix showed short gradients (< 3 S.D.), supporting the use of an ordination technique based on a linear model, such as RDA (Jongman *et al.*, 1995). The Drosophilidae species matrix was built using the mean abundance of Drosophilidae species calculated from the six apples collected per site for each month (91 samples x 7 Drosophilidae species). The Drosophilidae species matrix was standardised with a general relativisation by sites (McCune & Grace, 2002) and $\log_{10}$+1-transformed prior to analyses to decrease the influence of extreme variability in the numbers of individuals (Baar & ter Braak, 1996). Drosophilidae species present in < 0.8% of the sampled apples (i.e., in < 6 apples amongst the total of 633 apples collected) were removed from the matrix prior to analysis to avoid outlier effects. To avoid multicollinearity among the numerous environmental variables measured at the different spatial scales of the study (see list in Table S1), only the variables that correlated with DCA axes and were ecologically non-redundant or statistically not inter-correlated were retained, generating a final subset of 91 samples x 18 environmental variables.

Univariate analyses were performed using IBM-SPSS v.24.0 (IBM, Armonk, NY, USA) and multivariate analyses using PC-ORD v.7.03 (McCune & Mefford, 2016).

RESULTS

Variations in quantity and quality of the apple resource

Across all the sampling sites, apple resources declined progressively over the study period (Fig. 1). Initially, 7,969 apples were present in September 2021, of which 7,040 were hanging on the trees and 929 were laying on the ground. The numbers of apples progressively declined until April, when no apples remained on the trees and only 65 on the ground.

The quality of the apples also changed across the study period, with a gradual decrease in sugar content and increase in pH, especially in the rotten parts of the fruit (Fig. 2). Overall mean sugar content decreased from 12.28% ± 0.28 to 4.5% ± 1.03 in the rotten parts of the fruit between September and April, with the lowest concentration measured in February (2.84% ± 0.5). In the healthy parts of the fruit, sugar content decreased from 16.41% ± 0.85 to 10.9% ± 2.12 between September and March (apples collected in April were entirely rotten). Mean pH of the rotten parts increased from 2.86 ± 0.07 to 3.36 ± 0.09 between September and April, with the maximum value observed in February (4.66 ± 0.1). In the

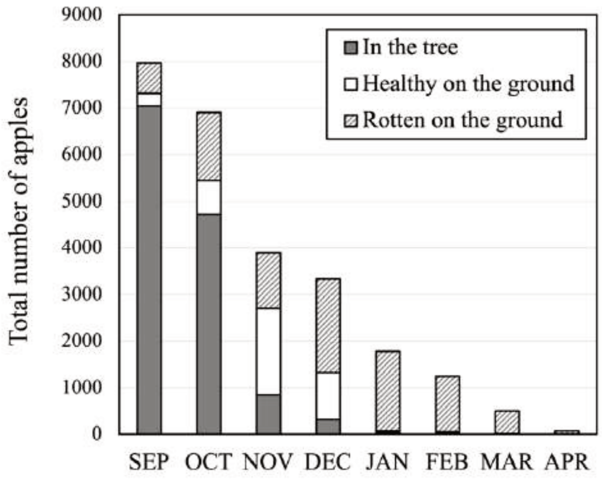


Figure 1 Variation in apple abundance between September 2021 and April 2022, in relation to their position (in the trees vs. on the ground) and the status of the latter (healthy vs. rotten).

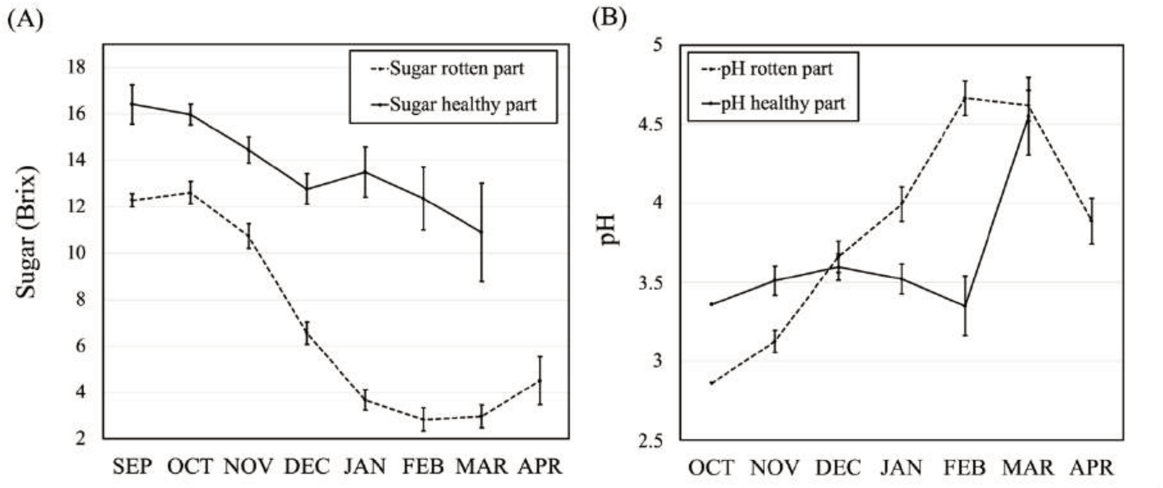


Figure 2 Variation of mean (± SE) sugar content (A) and apple pH (B) between September 2021 and April 2022, with healthy (plain line) and rotten parts (dotted line) of the apples presented separately.

healthy parts, pH remained relatively stable from September to February (between 3.35 and 3.60), but then increased to reach 4.55 ± 0.51 in March.

Microclimatic differences between soil and air over the study period

Ground surface and canopy air temperature measurements confirmed that the soil acted as a thermal buffer, especially during the coldest months. From February to April, differences in mean monthly temperature between air and ground were observed (Fig. S1A). Notably, even in months where the mean temperatures were similar, temperature variability was higher in the air than on the ground (Fig. S1B), reaching both higher and lower temperatures daily than the soil with differences of up to ~10°C. At

the site scale, temperature variation was even more apparent when comparing the weekly variations between autumn, winter and spring (Figs. S1C, S1D, S1E, respectively). In winter and spring, negative canopy air temperatures were experienced, while the ground surface was protected from frost by the soil's buffering effect.

Drosophilidae species abundance and richness

From the 633 apples collected on the ground, a total of 6,283 individual flies representing 14 Drosophilidae species emerged (Table 2, detailed by month in Table S2). The Drosophilidae community was dominated by two species, *D. immigrans* (41.03% of individuals) and *D. subobscura* (39.77%). Five other species contributed > 1% of total individuals: *C. amoena* (7.93%), *D. melanogaster* (5.06%), *D. simulans* (2.72%), *D. suzukii* (1.39%) and *D. tristis* (1.39%). The remaining species (< 1% of total) were *Scaptomyza palida*, *D. ambigua*, *D. bifaciata*, *D. cameraria*, *D. obscura*, *D. kunzei*, and *Hirtodrosophila cameraria*. Excepting *D. suzukii* and *C. amoena*, all species were native.

The abundance patterns of Drosophilidae species were compared between months over the study period (Fig. 3). The total number of individuals recorded per month rapidly and consistently declined, from 3,635 emerging from apples collected in September to 84 from apples collected in April. *Drosophila subobscura* was the only species that emerged from apples collected throughout the sampling campaign. *Chymomyza amoena* emerged from apples collected in all months except April. *Drosophila immigrans* emerged from apples collected from September to December and *D. suzukii* only emerged from apples collected between September and November.

Drosophilidae species richness per apple was 0.86 ± 1.24 species (n = 633 apples), but differed significantly between months (X^2 = 92.08, p < 0.001; Fig. 4A) with a maximum of 2.02 ± 0.17 in September and a minimum of 0.38 ± 0.09 in March. Up to eight different species were present in one apple in September. Drosophilidae abundance also decreased markedly over the study period and also showed high variability, averaging 9.93 ± 36.66 emerging individuals per apple overall, with a maximum in September of 33.66 ± 76.81 (Fig. 4B) and a minimum in March of 2.43 ± 9.28. Drosophilidae species richness and abundance were significantly influenced by numerous variables (GLMMs, Table 3), increasing with apple size and the percentage of rotten surface (MASSAV2, BROWNROTTEN2) in sites where the minimum ground temperature was mild (i.e., highest local daily minimum temperature on the ground during the week before sampling, MINTH7hb). The total abundance of Drosophilidae also increased with variables related to direct human influence, such as the area represented by buildings

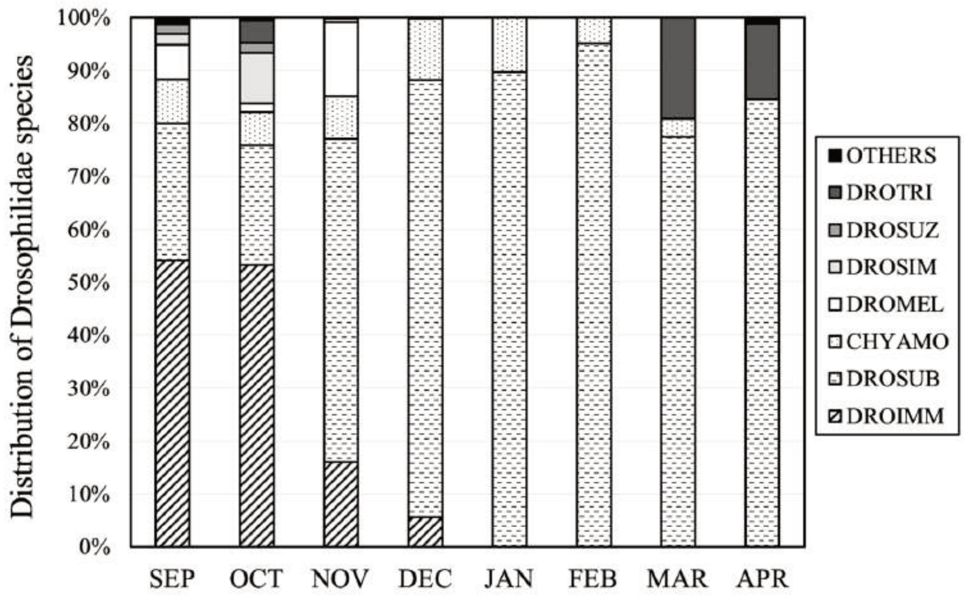


Figure 3 Distribution of the most abundant Drosophilidae species emerging from apples collected during the study, from September 2021 to April 2022. DROTRI: *Drosophila tristis*, DROSUZ: *Drosophila suzukii*, DROSIM: *Drosophila simulans*, DROMEL: *Drosophila melanogaster*, CHYAMO: *Chymomyza amoena*, DROSUB: *Drosophila subobscura*, DROIMM: *Drosophila immigrans*, OTHERS: species representing less than 1% of the emerged flies.

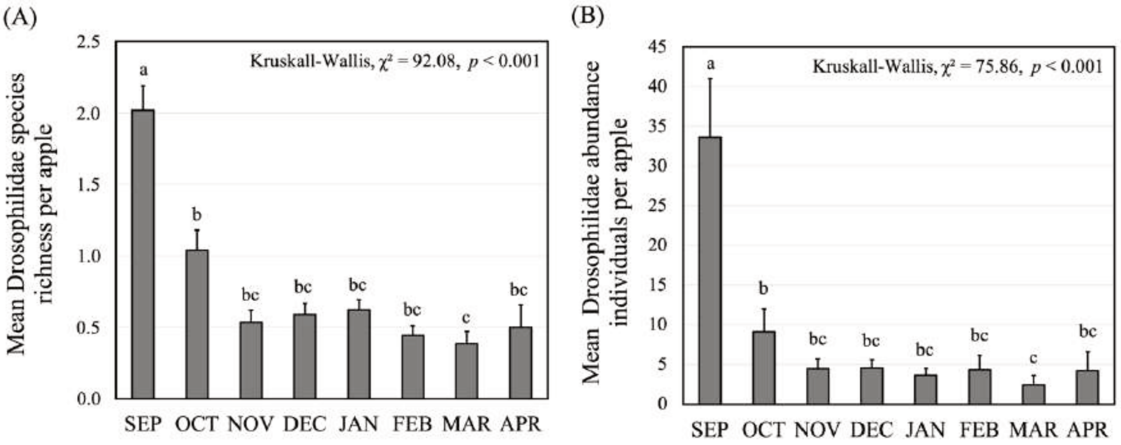


Figure 4 Mean Drosophilidae species richness (A) and abundance (B) per apple (± SE) in the 19 sites sampled from September 2021 to April 2022.

within a 50 m radius around the apple tree (BUILD50). The abundance of *C. amoena* was positively correlated with apple size and percentage of rotten surface, as well as the presence of codling moth holes (CARPOCAPSE2). The abundance of *D. suzukii* decreased with the presence of *Penicillium* (PENICILI2) and with increasing apple tree canopy size (CANOPY). Both *C. amonena* and *D. suzukii* abundances increased with higher local air temperature during the week before sampling (MINTH7hh, MAXTH7hh) and with mowing of the local vegetation (MOW).

Influence of environment on Drosophilidae community composition

Individual Drosophilidae species presence could be differentiated according to their ecological niche in terms of substrate acidity and sugar content (Fig. 5). Two groups of species were apparent: one, including *D. suzukii*, *D. melanogaster*, *D. simulans* and *D. immigrans*, was associated with the sweetest and more acidic apples that were freshly fallen in early autumn; the second, comprising *C. amoena*, *D. subobscura* and *D. tristis*, was mostly present in rotten apples that had lower sugar content and increased pH.

In the redundancy analysis (RDA) coupling environmental factors and Drosophilidae species matrices (Fig. 6), the eigenvalues of the first two ordination axes explained 54.8% of the data variance. In the ordination diagrams, the plots formed groups associated with the successive seasons (Fig. 6A). Along

Table 3 Effect of environmental variables on the species abundance and richness of the full Drosophilidae community, *Chymomyza amoena* abundance and *Drosophila suzukii* abundance, analysed by GLMMs.

		Model parameters						
Dependent variables	**Explanatory variables**	**F**	**Estimates**	**SE**	**d.f.**	**t**	**p**	**AIC**
Drosophilidae abundance (log10, n = 633 apples)	Model constant	16.665	-0.2332	0.0571	47.00	-4.082	0.000	892.16
	BROWNROTTEN2	66.609	0.0041	0.0005	593.02	8.161	0.000	
	MASSAV2	44.404	0.0044	0.0007	323.61	6.664	0.000	
	BUILD50	14.610	0.0180	0.0047	17.01	3.822	0.001	
	MINTH7Jhb	49.331	0.0333	0.0047	505.88	7.024	0.000	
Drosophilidae species richness (log10, n = 633 apples)	Model constant	0.128	-0.0091	0.0256	53.80	-0.358	0.722	-273.05
	BROWNROTTEN2	65.421	0.0016	0.0002	627.49	8.088	0.000	
	MASSAV2	20.649	0.0012	0.0003	517.87	4.544	0.000	
	MINTH7Jhb	73.374	0.0165	0.0019	607.50	8.566	0.000	
Chymomyza amoena abundance (log10, n = 633 apples)	Model constant	0.285	-0.1329	0.0452	194.53	-2.940	0.004	87.55
	BROWNROTTEN2	9.838	0.0008	0.0003	620.92	3.137	0.002	
	VOL2	8.476	0.0009	0.0003	375.85	2.911	0.004	
	MAXTH7Jhh	13.832	0.0078	0.0021	610.42	3.719	0.000	
	MOW	4.293	0.1210	0.0584	585.35	2.072	0.039	
	CARPOCAPSE2	5.209	0.0769	0.0337	626.98	2.282	0.023	
Drosophila suzukii abundance (log10, n = 633 apples)	Model constant	22.827	0.0637	0.0183	18.61	3.479	0.003	-998.57
	CANOPY	10.782	-0.0002	0.0001	19.18	-3.284	0.004	
	MINTH7Jhh	38.693	0.0069	0.0011	617.17	6.220	0.000	
	MOW	24.130	0.1217	0.0248	609.07	4.912	0.000	
	PENICILI2	3.871	-0.0406	0.0206	625.35	-1.968	0.050	

Note: BROWNROTTEN2: % of rotten part on apple surface, MASSAV2: apple mass, BUILD50: % of buildings in a radius of 50 m, MINTH7Jhb: soil minimum temperature in the preceding 7 days, VOL2: apple volume, MAXTH7Jhh: air maximum temperature in the preceding 7 days, MOW: mowing of herbaceous vegetation, CARPOPAPSE2: presence of codling moth borehole, CANOPY: radius of the apple tree canopy, MINTH7Jhh: air minimum temperature in the preceding 7 days, and PENICILI2: presence of *Penicillium* sporophore on apple

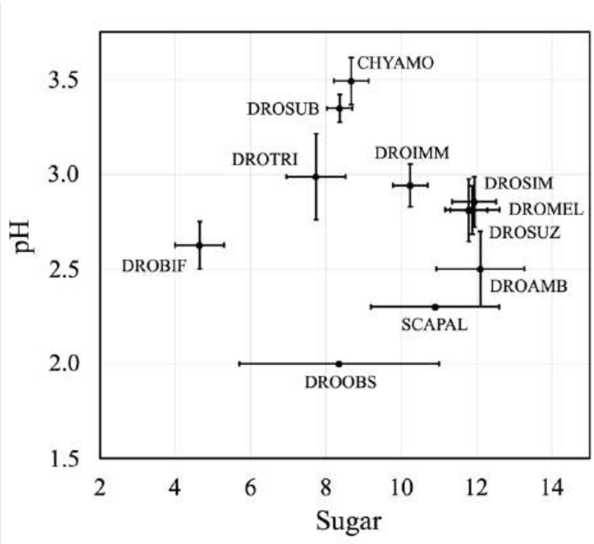


Figure 5 Trophic preferences of Drosophilidae species based on mean (± SE) sugar content and apple pH. For each fly species, only apples in which the considered species was present were used to calculate the sugar and pH means presented in the figure. CHYAMO: *Chymomyza amoena,* DROAMB: *Drosophila ambigua,* DROBIF: *Drosophila bifasciata,* DROIMM: *Drosophila immigrans,* DROMEL: *Drosophila melanogaster,* DROOBS: *Drosophila obscura,* DROSIM: *Drosophila simulans,* DROSUB: *Drosophila subobscura,* DROSUZ: *Drosophila suzukii,* DROTRI: *Drosophila tristis,* SCAPAL: *Scaptomyza pallida.*

axis 1 (27.8%) groupings discriminated samples from autumn (empty circles, positive part), associated with the warmest temperatures (MAXMAX7Js; Fig. 6B), and winter and early spring samples (grey and black squares, respectively, negative part). The negative part of axis 1 was associated with *D. subobscura*, damaged apples (BIOORIGIN2), apple trees close to roads (ROAD10_%) and apple trees surrounded by grasslands and shrubs (GRASS50_%, SHRUB50_%). Axis 2 (26.9%) discriminated apple samples with *C. amoena*, collected under tall apple trees (HEIGHT), growing in orchards (ORC10_%), and surrounded by woodland (WOOD50_%). The positive part of axis 2 was associated with autumnal species (*D. immigrans*, *D. suzukii*, *D. melanogaster*) present in the warmest periods (MAXMAX7Js), apples with the highest sugar content (SUGAR2) and mass (MASSAV2), degree of rot (BROWNROTTEN2), and collected from a human-influenced environment (BUILD50_%). In summary, the RDA showed that local and seasonal thermal variation, habitat composition and fruit quality and quantity were the major drivers of Drosophilidae species assemblages.

DISCUSSION

Our study showed that four main categories of environmental drivers systematically influenced the diversity and composition of the Drosophilidae community present in fallen apples (i) local and seasonal thermal variation, (ii) habitat composition, and (iii) fruit quality and quantity.

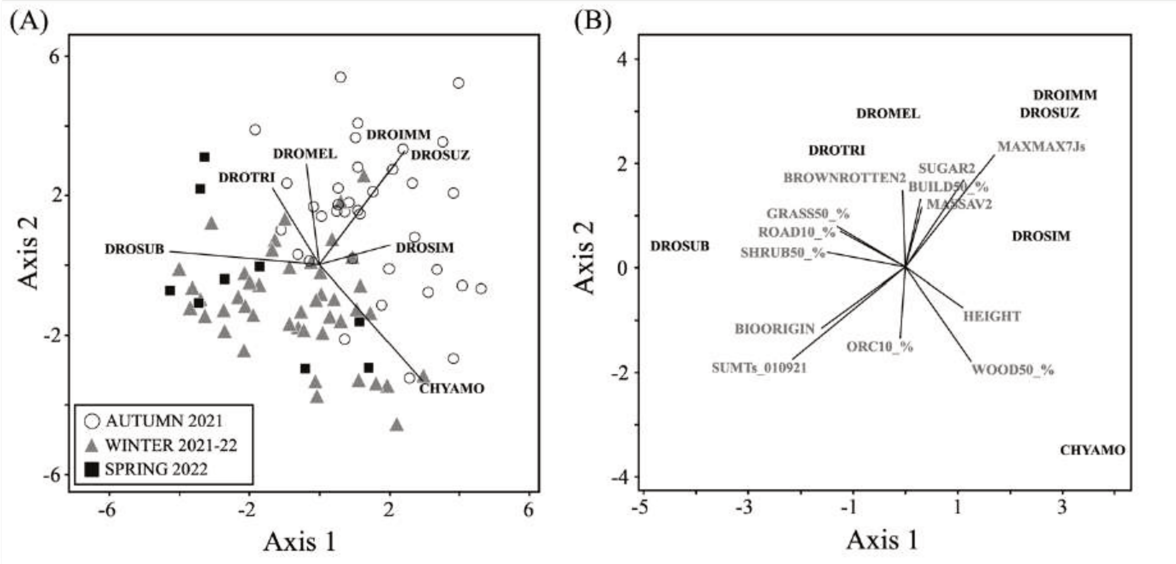


Figure 6 Redundancy analysis (RDA) on (A) months and (B) environmental variables influencing the Drosophilidae community present in fallen apples. Only the most significant environmental variables are shown (cut-off r^2 value = 0.15). Months: AUTUMN: September and October; WINTER: November, December, January, and February; SPRING: March and April. Drosophilidae: CHYAMO: *Chymomyza amoena,* DROIMM: *Drosophila immigrans,* DROMEL: *Drosophila melanogaster,* DROSIM: *Drosophila simulans,* DROSUB: *Drosophila subobscura,* DROSUZ: *Drosophila suzukii,* DROTRI: *Drosophila tristis*. Environmental variables: BIOORIGIN2: Presence of damage from biotic origin (birds, mammals, molluscs, codling moth); BUILD50_%: Cover of building within 50 m; BROWNROTTEN2: Percentage of apple surface being brown because of rotting; HEIGHT: Height of the apple tree; GRASS50_%: Cover of grassland within 50 m; MASSAV2: Apple weight; MAXMAX7Js: Warmest temperature of the maximum temperatures during the 7 days before the apple sampling from meteorological stations); ORC10_%: Cover of apple orchard within 10 m; ROAD10_%: Cover of road within 10 m; SHRUB50_%: Cover of shrub within 50 m; SUGAR2: Mean sugar content in apple; SUMTs_01092021: Sum of positive temperatures from meteorological stations between 01/09/2021 and the sampling day; WOOD50_%: Cover of woodland within 50 m.

Climatic variation and the seasonal dynamics of the Drosophilidae community

Local-scale variations in microclimate temperature appeared to influence Drosophilidae diversity and species abundance more than regional-scale variations derived from meteorological stations. Local and hourly microclimatic data are likely to provide a better indicator of the impact of temperature extremes on the diversity and abundance of Drosophilidae species, especially that of negative temperatures (freezing events). In temperate regions, temperature is known to be a major driver of insect population dynamics and geographic range (Sinclair *et al*., 2003) because most insects are chill-susceptible and die due to injuries caused by ice formation (Lee, 1991; Stephens *et al*., 2015). Soil appeared to buffer the ground surface temperatures experienced across seasons, especially during the coldest months. In autumn, canopy shading may explain the smaller difference between air and ground surface temperature. During winter and early spring, when the trees have lost their leaves, the soil becomes the last compartment of the ecosystem capable of buffering the colder open air temperatures and protecting on or near the ground surface from freezing temperatures.

The Drosophilidae community was structured by season, likely to be strongly influenced by the different species' cold-tolerance strategies. Some species were present in apples collected throughout the sampling, in particular *D. subobscura* and *C. amoena*, which are known to be cold-tolerant (Band & Band, 1984; David *et al*., 2003). Species known to be chill-susceptible or freeze-intolerant were found only in apples collected in early autumn. These included *D. suzukii* (Jakobs *et al*., 2015; Winkler *et al*., 2020), *D. immigrans* (Strachan *et al*., 2011; MacMillan *et al*., 2015; Tamang *et al*., 2017), *D. melanogaster* (Strachan *et al*., 2011; Koštál *et al*., 2012; MacMillan *et al*., 2015) and *D. simulans* (Strachan *et al*., 2011; Lubawy *et al*., 2022). Finally, *D. tristis* was found only in apples collected during the early months of autumn and in March, probably indicating freeze-intolerance, although studies of this species' cold-tolerance are lacking (Basden, 1954).

The cold tolerance strategies of different species are likely to structure seasonal community composition, while partitioning may also take place when two species sharing the same fruit have different fitness values (e.g., developmental time, number of eggs laid, percentage of adult emergence, etc) when developing at the same temperature (Grimaldi, 1985). For example, *D. melanogaster* lays more eggs than *D. simulans* at 15°C (McKenzie, 1978), and their development times vary according to the season, with *D. melanogaster* developing more rapidly in September than *D. simulans* (Behrman *et al*., 2015). Laying more eggs and developing more rapidly would favour *D. melanogaster* over *D. simulans*, as it results in

increased larval competitive ability (Grimaldi, 1985; Sevenster & Van Alphen, 1993). This could explain why, in our study, the abundance of *D. melanogaster* was greater than that of *D. simulans* in September but that the reverse was apparent in October after the temperature dropped. Exploiting the resource first and having a short development time is another strategy that would benefit a species (Nunney, 1990). This is the strategy adopted by *D. suzukii*, which can utilise the fruit as it ripens, before any decomposition takes place (Walsh *et al*., 2011; Clemente *et al*., 2018).

Over the thermally-governed seasonal gradient extending from September to April, the decaying apples were exploited by a succession of Drosophilidae species, which supports the hypothesis of temporal partitioning of the use of this food resource. This strategy adopted by organisms using a common resource has been reported in studies of *Drosophila* species (Hodge *et al*., 1996; Matavelli *et al*., 2015) and in insects in general (Wettlaufer *et al*., 2021; Vindstad *et al*., 2020). The sequential use of resources allows coexistence through minimising competitive interactions, potentially avoiding exclusion. This temporal separation of the use of decaying apples is not only determined by temperature changes differentially affecting Drosophilidae species over time across seasons, but is also associated with the gradual modification of fruit quality, which varies with its stage of decay, as shown in other guilds of insects (Koskinen *et al*., 2022). The hierarchical continuum model, primarily used in plant communities (Collins *et al*., 1993; Hanski 1982), may be applicable to Drosophilidae species coexistence in decaying apples. Based on this concept, four strategies can be identified and applied to species, termed 'core', 'satellite', 'urban' and 'rural' species, based on the responses of their abundance (i.e., dominance) and distribution (i.e., frequency changes between months) to the seasonal progression (Fig. 7). Only one species, *D. subobscura*, was typically dominant and was present across the full seasonal sampling period (see Fig. 3 and Table S2) and, thus, can be considered a 'core' species (*sensu* Collins *et al*., 1993). This species plays a major role in apple decomposition and its role in structuring the community by transforming the substrate is fundamental. 'Satellite' species are found at low density and restricted to a smaller element of the seasonal progression. This was the case for late-summer / early autumn species (*D. suzukii*, *D. melanogaster*, *D. simulans, D. tristis*), that are probably opportunistic species ovipositing and developing in fallen apples, using the freshest and sweetest wounded fruits (Lee *et al*., 2011; Cai *et al*., 2019). *Drosophila immigrans* conformed to the description of an 'urban' species in the hierarchical continuum model, with a very high abundance peak (particularly in September and October) restricted to the beginning of the seasonal progression. This strategy indicates that *D. immigrans* is a strong performer in

early autumn but is also very dependent on the substrate quality (high sugar levels; Fig. 5). The invasive *C. amoena* was the only 'rural' species in the current, present throughout most of the seasonal progression from September to March, but at low to moderate densities. Even though not dominant, its strategy of persisting through the seasons could contribute to its invasion success. Its persistence in decaying apples could indicate higher tolerance to acidic substrates (Fig. 5).

Landscape and local factors driving community diversity and invasive populations

Insect communities are affected by both local conditions and landscape composition (Mitchell *et al*., 2014). At the landscape scale, variables associated with direct human activities and semi-natural habitats were significant drivers of the Drosophilidae community in our study. Abundance and species richness increased with the cover of buildings in a 50 m radius (even though this variable was not retained in the final GLMM of species richness). Anthropogenic stress factors, such as urbanisation, reduce the overall abundance and richness of insect communities (Vaz *et al*., 2023) and may lead to ecological homogenisation (McKinney, 2006; Knop, 2016; Eggleton, 2020) with specialist species being negatively impacted (Shuisong *et al*., 2013; Knop, 2016). However, some species benefit from urbanisation, including non-native or invasive species (McKinney, 2006; Bertelsmeier, 2021), generalist species (Shuisong *et al*., 2013), or human commensal species such as mosquitoes (Perrin *et al*., 2022). Some members of Drosophilidae could also benefit from urbanisation. *Drosophila immigrans*, *D. melanogaster*, and *D. suzukii* were strongly correlated with the cover of buildings. The former two species are known to be associated with human activities (David, 1979; Kojima & Kimura, 2003), especially *D. melanogaster*, which is considered as the most commensal *Drosophila* species associated with humans (Lachaise & Silvain, 2004). In winter, all three of these species may find overwintering refuges around human structures (Ulmer, 2022). Other species in this study were affected by the presence of semi-natural habitats. *Drosophila subobscura* was found in apples collected under trees close to roads. Road edges impact community structure, yet can have positive effects on biodiversity (Rotholz & Mandelik, 2013). *Chymomyza amoena* abundance was positively correlated to the presence of orchards with woodland within a 50 m radius, consistent with previous studies (Band *et al*., 2005).

At the local scale, Drosophilidae richness and abundance were mostly influenced by the quantity and quality of the food resource, the management of the site's herbaceous vegetation and the tree canopy. The larger the apples, the greater the richness and abundance present. This could be explained by higher detectability of larger fruit, either visually or by olfaction, a selection process similar to that of other

frugivorous insects (Sallabanks, 1993), or by a decreased larval competition in larger fruits. In association with a higher detectability, grass mowing was associated with increased Drosophilidae abundance. Mowing could also negatively impact the presence of some predators (Horton *et al*., 2003; Dobbs & Potter, 2014), contributing further to increased Drosophilidae abundance. The quality of the resource, that is, the percentage of rotten surface, the presence of carpocapse or *Penicillium* and the presence of damage of biotic origin, also influenced the Drosophilidae community. Apple skin is waxy and robust and most Drosophilidae ovipositors may not be able to penetrate it (Atallah *et al*., 2014). Damage of biotic origin provides a point of entry for oviposition. This is especially the case for *C. amoena*, which uses codling moth carpocapse tunnels to oviposit in walnuts and apples (Band, 1988). Similarly, *D. melanogaster* needs naturally damaged grapes to oviposit but can also benefit from *D. suzukii*'s oviposition sites (Rombaut *et al*., 2017). In contrast, the presence of *Penicillium* negatively affected *D. suzukii* abundance. *Penicillium commune* is found in *D. suzukii* artificial diet (Gao *et al*., 2017). It has been isolated from *D. suzukii* and had no effect on host survival (Bing *et al*., 2020). The odour profile of *Penicillium* spp. contains geosmin and octenol, molecules associated with other fruit-associated plant pathogens, which could act as an indicator of unsuitable substrate for oviposition for *D. suzukii* (Wallingford *et al*., 2016). Large trees were also negatively associated with *D. suzukii* presence, possibly as their canopy shading is unsuitable for this species which may be more attracted by thermophilic and sites exposed to full sun for oviposition as autumn approaches.

Trophic niche partitioning and the role of Drosophilidae in fruit decomposition

Trophic niche partitioning occurs when different species share the same nutritional resource but use it differently, limiting competition (Roughgarden, 1976; Pocheville, 2015). In our study, up to eight species were able to coexist in one fallen apple. This suggests temporal niche partitioning. Temporal partitioning in relation to seasonal temperature variation was addressed above. Another driver of temporal partitioning may relate to the fruit decay process and physical and chemical changes over shorter timescales. When fruit is damaged, either by the fall from the tree or by insect oviposition, microbial communities, mostly bacteria and yeasts, immediately start to proliferate (Rombaut *et al*., 2017), inducing changes in biochemical composition (Nunney, 1990; Awmack & Leather, 2002; Matavelli *et al*., 2015). In particular, the protein:carbohydrate (P:C) ratio increases, allowing flies with different dietary requirements for protein to use the resource sequentially (Matavelli *et al*., 2015). For instance, the successive stages of decay in figs are exploited sequentially by different Drosophilidae species as the sugar

and protein contents change (Lachaise *et al*., 1982; Matavelli *et al*., 2015). In early autumn, apples are sweeter and more acidic than in winter and spring. Drosophilidae species appear to be sensitive to sugar concentration and pH variation. Sugar and pH are known to impact fecundity, survival and microbiota composition (Deshpande *et al*., 2015; Fellous & Xuéreb, 2017). For example, *D. suzukii* prefers low sugar content substrate in which to oviposit and shows lower survival and fecundity at higher sugar concentrations (Fellous & Xuéreb, 2017). In adult *D. melanogaster*, acidic food increases palatability, food intake and survival (Deshpande *et al*., 2015). As decay continues, the fruit texture changes, and firmness of the substrate can be of importance for oviposition (Kienzle & Rohlfs, 2021; Sato *et al*., 2021). For example, *D. suzukii* oviposits in firm substrates (Kienzle & Rohlfs, 2021; Sato *et al*., 2021) while *D. melanogaster* selects softer substrates (Sato *et al*., 2021). Decaying apple can be considered as ephemeral resource patches, as their chemical and structural compositions vary in space and time, and therefore the diversity of niches they provide can be large, thereby enriching local community composition and interspecific interactions at the individual scale (Butterworth *et al*., 2023). A study on the Drosophilidae succession across the decaying stages of the apple would nicely complement our results in the future. We discuss in the Supplementary Material how spatial partitioning may also have occurred between and within apples.

Conclusions and perspectives

Ecological processes during the post-harvest period in agricultural systems is of particular interest but has received limited research attention. Unharvested fruits can remain on the ground for long periods of time, providing potential breeding sites, food resources and/or microclimatic refuges across seasons. In our study, decomposing apples hosted up to eight species of Drosophilidae, including two important invasive species, *D. suzukii* and *C. amoena*. The fly community structure was strongly influenced by local and seasonal thermal variation, habitat composition, and fruit quality and quantity. Decomposing fruits are a dynamic substrate allowing resource niche partitioning, both temporal and spatial, with competition and facilitation processes occurring. Our study raises questions relating to post-harvest pest management strategies. Known serious pest species such as *D. suzukii* can use these post-harvest resources to increase overwinter survival and accelerate subsequent proliferation after the cold season. As it is already recommended to reduce other apple fruit pathogens and parasites (brown rot fungi scab), actively removing these 'waste' fruits could, therefore reduce the pest's abundance in the subsequent spring. However, the native Drosophilidae community, which plays a key role in the recycling process of organic

matter, would clearly also be impacted if this management strategy was applied. Similarly, while *C. amoena* is generally considered as a pest species, by being present throughout the winter, it also contributes to reducing available resources in spring for *D. suzukii* (see Wilson *et al*., 2012). Finally, although this study focused on Drosophilidae, other species will certainly benefit from these fallen fruits, including parasitoids and other arthropods. Future studies should focus on the dynamics of these multitrophic relationships across winter time in relation with these ephemeral resources.

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Supplementary material

Table S1 List of the variables assessed in the study. Abbreviations: hh: local air temperature measure from high HOBO, hb: local soil temperature measure from low HOBO, h: mean temperature measures from high and low HOBO, s: measure from weather station, 7J and 14J: measures collected from the 7 and 14 days before apple collection, P/A: Presence/Absence

CATEGORY	CODE	DESCRIPTION	UNIT
DROSOPHILIDAE COMMUNITY			
DROSOPHILIDAE DIVERSITY	SRdroso	Species richness of Drosophilidae	Species
	ABUNdroso	Abundance of Drosophilidae (number of individuals)	Individuals
MEASURES ON SAMPLED APPLES			
SKIN COLOR	RED2	Percentage of apple surface being red	% area
	YELLOW	Percentage of apple surface being yellow	% area
	GREEN2	Percentage of apple surface being green	% area
	BROWNROTTEN 2	Percentage of apple surface being brown because of rot	% area
	BLACK2	Percentage of apple surface being black	% area
INFECTION	SPOROPH2	Presence of fungal sporophore on apple surface	P/A
	MONILIOS2	Presence of *Moniliasis*	P/A
	PENICILI2	Presence of *Penicillium*	P/A
	OTHERMOLD2	Presence of other mould species	P/A
DAMAGE	SURFOPEN	Percentage of openings (i.e., removed skin) in the apple surface	% area
	NBAPERTURE	Number of openings in the apple surface	Number
	NBTUNNEL	Number of insect galleries	Number
	ABIOORIGNE	Presence of damage from abiotic origin (trampling, apple fall)	P/A
	BIOORIGIN	Presence of damage of biotic origin (birds, mammals, molluscs, codling moth)	P/A
	CARPOCAPSE	Presence of openings from codling moth (*Cydia pomonella*)	P/A
APPLE SIZE	HEIGHT2	Apple height after collection (pedicel-calyx axis)	cm
	LENGHT2	Apple width (diameter) after collection	cm
	RMEAN2	Mean apple radius: ((HEIGHT / 2) + (LENGTH2 / 2)) / 2	cm
	VOL2	Apple volume = RMEAN2^3 × π × 4/3	cm^3

CATEGORY	CODE	DESCRIPTION	UNIT
	MASSAV2	Apple mass	g
CHEMICAL PARAMETERS	SUGARROTTEN2	Sugar concentration measured in a rotten part of the apple after collection using a refractometer	Brix degree
	SUGARHEALTHY 2	Sugar concentration measured in a healthy part of the apple after collection using a refractometer	Brix degree
	SUGARMIXED2	Sugar concentration measured between rotten and healthy part of the apple after collection using a refractometer	Brix degree
	SUGAR2	Mean sugar concentration in apple	Brix degree
	pHROTTEN2	pH measured in a rotten part of the apple after collection using pH paper	/
	pHHEALTHY2	pH measured in a healthy part of the apple after collection using pH paper	/
	pHMIXED2	pH measured between rotten and healthy part of the apple after collection using pH paper	/
	pH2	Mean pH in apple	/
SITE AND LANDSCAPE CHARACTERISTICS			
LOCAL RESOURCES AND APPLE TREE TRAITS	TRONC	Circumference of the apple tree trunk	cm
	CANOPY	Radius of the apple tree canopy	m
	HEIGHT	Height of the apple tree	m
	TAPPLE	Number of apples on the tree	Number
	TAPPLE5	Number of apples on all the apple trees in 5 m radius	Number
	SAPPLE	Number of apples on the ground	Number
	HSAPPLE	Number of healthy apples on the ground	Number
	RSAPPLE	Number of rotten apples on the ground	Number
	APPLET10	Number of apple trees in 10 m radius	Number
	APPLET50	Number of apple trees in 50 m radius	Number
	APPLET100	Number of apple trees in 100 m radius	Number
	DES_CID	"DA" = "Dessert Apple" or "CA" = "Cider Apple"	Category
	MTN_AB	"M" = "Managed" or "A" = "Abandoned"	Category
	ALTM	Site altitude	m
	SLOM	Site slope	degree
	CTREE	Cover of tree layer (height > 8 m)	%
	CSHRUB	Cover of shrub layer (1-8 m)	%
	CHERB	Cover of herb layer (< 1 m)	%

CATEGORY	CODE	DESCRIPTION	UNIT
LOCAL ENVIRONMENT (5 m radius around apple tree)	HHERBMEAN	Height of herbaceous layer	cm
	HLIGN	Height of ligneous species (shrub and tree)	m
	COMP	Cover of compost material	%
	DWOOD	Cover of dead wood debris on the ground	%
	TLITTER	Depth of leaf litter	cm
	LITTER	Cover of leaf litter	%
	GRAZE	Presence of grazing by cows, horses or donkeys during the sampling month	P/A
	MOW	Presence of grass mowing	P/A
LOCAL HABITATS (from field measures within 10 and 50 m radii around apple tree)	BUILD	Cover of buildings	%
	WOOD	Cover of woodland	%
	GROVE	Cover of grove (small woodland patch with diameter < 50 m)	%
	HEDGE	Cover of hedgerow	%
	ORC	Cover of apple trees in orchard	%
	ORCTOT	Cover of orchard area (apple trees and associated grassland)	%
	MGARD	Cover of market garden	%
	CROP	Cover of crop	%
	RIVER	Cover of river	%
	POND	Cover of pond	%
	HEDGE	Cover of hedgerow	%
	GARDEN	Cover of private garden	%
	GRASS	Cover of grassland	%
	SHRUB	Cover of shrub	%
	SOIL	Cover of bare soil	%
	RUBB	Cover of rubble	%
	ROAD	Cover of road and paved surface	%
	PATH	Cover of (unpaved) pathway	%
	URB	Sum cover of urban area = BUILD + ROAD	%
LANDSCAPE COMPOSITION (from OSO data basis; 10, 20, 50,	WATE%	Cover of water	%
	GRASS%	Cover of grassland	%
	ROAD%	Cover of road	%

CATEGORY	CODE	DESCRIPTION	UNIT
100, 250, 500, 750 and 1000 m radii around the apple tree)	CROP%	Cover of crop	%
	FORE%	Cover of woodland	%
	URB%	Cover of urban area	%
		METEOROLOGY	
HOURLY METEOROLOGY (hour scale, from local HOBO dataloggers)	GDH7J	Sum of positive temperature hours (Growth Degree Hours) in the 7 days before apple sampling (from hh, hb and h)	°C
	SMTH7J	Sum of negative temperature hours in the 7 days before apple sampling (from hh, hb and h)	°C
	NFH7J	Number of hours with negative temperatures (≤0 °C) (Number of freezing hours) in the 7 days before apple sampling (from hh, hb and h)	Number
	MINTH7J	Minimum temperature in the 7 days before apple sampling (from hh, hb and h)	°C
	MAXTH7J	Maximum temperature in the 7 days before apple sampling (from hh, hb and h)	°C
	MEANTH7J	Mean temperature over the 7 days before apple sampling (from hh, hb and h)	°C
DAILY METEOROLOGY (day scale, from regional weather stations)	GDDM7J	Sum of daily positive temperature (Growth Degree Days) calculated from the mean temperatures of the 7 days before apple sampling (from s, hh, hb and h)	°C
	GDDMC7Js	Sum of daily positive temperature (Growth Degree Days) from the calculated mean temperatures of the 7 days before apple sampling (from s)	°C
	NFD7J	Number of days with negative temperatures (≤ 0 °C) during the 7 days before apple sampling (from s, hh, hb and h)	Number
	RAIN	Sum of daily precipitation from meteorological stations in the 7 days before the apple sampling (7J)	cm
	SNOW	Sum of daily snowfall from meteorological stations in the 7 days before the apple sampling (7J)	cm
	MMIN7J, MMIN14J	Mean minimum temperature during the 7 and 14 days before apple sampling (from s, hh, hb and h)	°C
	MMAX7J	Mean maximum temperature during the 7 days before apple sampling (from s, hh, hb and h)	°C
	MMEAN7Jhh	Mean of mean temperatures of the 7 days before apple sampling (from s, hh, hb and h)	°C
	MMEANCALC7Js	Calculated mean of mean temperatures of the 7 days before the apple sampling (from s)	°C

CATEGORY	CODE	DESCRIPTION	UNIT
	MINMIN7J	Lowest minimum temperature during the 7 days before apple sampling (from s, hh, hb and h)	°C
	MAXMAX7J	Warmest maximum temperature during the 7 days before apple sampling (from s, hh, hb and h)	°C
	SUMTs_01012021	Sum of positive temperatures from meteorological stations between 01/01/2021 and the sampling date	°C
	SUMTs_01092021	Sum of positive temperatures from meteorological stations between 01/09/2021 and the sampling date	°C

Table S2 Distribution of the Drosophilidae species in the study, detailed by month. N ind: total number of individuals emerging from apples, Occ: number of collected apples hosting a given Drosophilidae species, Mean: mean number of Drosophilidae individuals per apple, SE: standard error, Min: minimum number of Drosophilidae individuals per apple, Max: maximum number of Drosophilidae individuals per apple, %: percentage of individuals of a given species amongst all individuals that emerged from apples, AFreq: frequency of apples infested by a given species amongst all apples sampled.

Species	**Code**	**SEP (108 apples)**								**OCT (106 apples)**								**NOV (103 apples)**							
		N ind	Occ	Mean	SE	Min	Max	%	AFreq	N ind	Occ	Mean	SE	Min	Max	%	AFreq	N ind	Occ	Mean	SE	Min	Max	%	AFreq
Chymomyza amoena	CHYAMO	305	50	2.824	0.519	0	34	8.391	0.463	60	19	0.566	0.182	0	16	6.211	0.179	37	18	0.359	0.09	0	5	8.9	0.175
Drosophila ambigua	DROAMB	3	3	0.028	0.016	0	1	0.083	0.028	3	2	0.028	0.021	0	2	0.311	0.019	0	0	0	0	0	0	0	0
Drosophila bifasciata	DROBIF	0	0	0	0	0	0	0	0	1	1	0.009	0.009	0	1	0.104	0.009	0	0	0	0	0	0	0	0
Drosophila hydei	DROHYD	24	2	0.222	0.196	0	21	0.66	0.019	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Drosophila immigrans	DROIMM	1966	41	18.204	6.023	0	375	54.085	0.38	515	15	4.858	2.372	0	213	53.313	0.142	74	3	0.718	0.557	0	55	16.017	0.029
Drosophila kuntzei	DROKUN	1	1	0.009	0.009	0	1	0.028	0.009	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Drosophila melanogaster	DROMEL	237	17	2.194	1.287	0	136	6.52	0.157	16	8	0.151	0.065	0	5	1.656	0.075	65	5	0.631	0.455	0	43	14.069	0.049
Drosophila obscura	DROOBS	1	1	0.009	0.009	0	1	0.028	0.009	1	1	0.009	0.009	0	1	0.104	0.009	0	0	0	0	0	0	0	0
Drosophila simulans	DROSIM	75	10	0.694	0.454	0	48	2.063	0.093	93	10	0.877	0.587	0	61	9.627	0.094	3	1	0.029	0.029	0	3	0.649	0.01
Drosophila subobscura	DROSUB	940	66	8.704	1.373	0	69	25.86	0.611	218	35	2.057	0.526	0	40	22.567	0.33	282	27	2.738	0.775	0	63	61.039	0.262
Drosophila suzukii	DROSUZ	68	17	0.63	0.228	0	16	1.871	0.157	18	9	0.17	0.072	0	6	1.863	0.085	1	1	0.01	0.01	0	1	0.216	0.01
Drosophila tristis	DROTRI	6	5	0.056	0.026	0	2	0.165	0.046	40	9	0.377	0.193	0	16	4.141	0.085	0	0	0	0	0	0	0	0
Hirtodrosophila cameraria	HIRCON	2	1	0.019	0.019	0	2	0.055	0.009	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scaptomyza pallida	SCAPAL	7	4	0.065	0.035	0	3	0.193	0.037	1	1	0.009	0.009	0	1	0.104	0.009	0	0	0	0	0	0	0	0
Total		3635								966								462							

Table S2 (continued) Distribution of the Drosophilidae species in the study, detailed by month. N ind: total number of individuals emerging from apples, Occ: number of collected apples hosting a given Drosophilidae species, Mean: mean number of Drosophilidae individuals per apple, SE: standard error, Min: minimum number of Drosophilidae individuals per apple, Max: maximum number of Drosophilidae individuals per apple, %: percentage of individuals of a given species amongst all individuals that emerged from apples, AFreq: frequency of apples infested by a given species amongst all apples sampled.

Species	**Code**	**DEC (90 apples)**								**JAN (76 apples)**								**FEB (70 apples)**							
		N ind	Occ	Mean	SE	Min	Max	%	AFreq	N ind	Occ	Mean	SE	Min	Max	%	AFreq	N ind	Occ	Mean	SE	Min	Max	%	AFreq
Chymomyza amoena	CHYAMO	47	12	0.522	0.203	0	14	11.52	0.133	29	14	0.382	0.127	0	6	10.357	0.184	15	7	0.214	0.109	0	7	4.967	0.1
Drosophila ambigua	DROAMB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Drosophila bifasciata	DROBIF	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Drosophila hydei	DROHYD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Drosophila immigrans	DROIMM	23	4	0.256	0.158	0	11	5.637	0.044	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Drosophila kuntzei	DROKUN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Drosophila melanogaster	DROMEL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Drosophila obscura	DROOBS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Drosophila simulans	DROSIM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Drosophila subobscura	DROSUB	337	36	3.744	0.948	0	49	82.598	0.4	251	33	3.303	0.83	0	42	89.643	0.434	287	24	4.1	1.856	0	115	95.033	0.343
Drosophila suzukii	DROSUZ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Drosophila tristis	DROTRI	1	1	0.011	0.011	0	1	0.245	0.011	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hirtodrosophila cameraria	HIRCON	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scaptomyza pallida	SCAPAL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total		408								280								302							

Table S2 (continued) Distribution of the Drosophilidae species in the study, detailed by month. N ind: total number of individuals emerging from apples, Occ: number of collected apples hosting a given Drosophilidae species, Mean: mean number of Drosophilidae individuals per apple, SE: standard error, Min: minimum number of Drosophilidae individuals per apple, Max: maximum number of Drosophilidae individuals per apple, %: percentage of individuals of a given species amongst all individuals that emerged from apples, AFreq: frequency of apples infested by a given species amongst all apples sampled.

Species	**Code**	**MAR (60 apples)**								**APR (20 apples)**								**TOTAL (633 apples)**							
		N ind	Occ	Mean	SE	Min	Max	%	AFreq	N ind	Occ	Mean	SE	Min	Max	%	AFreq	N ind	Occ	Mean	SE	Min	Max	%	AFreq
Chymomyza amoena	CHYAMO	5	5	0.083	0.036	0	1	3.425	0.083	0	0	0	0	0	0	0	0	498	125	0.787	0.107	0	34	7.926	0.197
Drosophila ambigua	DROAMB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	5	0.009	0.004	0	2	0.095	0.008
Drosophila bifasciata	DROBIF	0	0	0	0	0	0	0	0	1	1	0.05	0.05	0	1	1.19	0.05	2	2	0.003	0.002	0	1	0.032	0.003
Drosophila hydei	DROHYD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	2	0.038	0.034	0	21	0.382	0.003
Drosophila immigrans	DROIMM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2578	63	4.073	1.133	0	375	41.031	0.1
Drosophila kuntzei	DROKUN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0.002	0.002	0	1	0.016	0.002
Drosophila melanogaster	DROMEL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	318	30	0.502	0.233	0	136	5.061	0.047
Drosophila obscura	DROOBS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0.003	0.002	0	1	0.032	0.003
Drosophila simulans	DROSIM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	171	21	0.27	0.126	0	61	2.722	0.033
Drosophila subobscura	DROSUB	113	14	1.883	1.026	0	56	77.397	0.233	71	8	3.55	1.829	0	35	84.524	0.4	2499	243	3.948	0.41	0	115	39.774	0.384
Drosophila suzukii	DROSUZ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	87	27	0.137	0.042	0	16	1.385	0.043
Drosophila tristis	DROTRI	28	4	0.467	0.257	0	10	19.178	0.067	12	1	0.6	0.6	0	12	14.286	0.05	87	20	0.137	0.045	0	16	1.385	0.032
Hirtodrosophila cameraria	HIRCON	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0.003	0.003	0	2	0.032	0.002
Scaptomyza pallida	SCAPAL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	5	0.013	0.006	0	3	0.127	0.008
Total		146								84								6283							

Table S3 Weather station information. Department: Insee code and department name, station city: postcode and the municipality name of the location of the weather station, station coordinates: geographic location of the city where the weather station is located, label city; postcode and municipality name used by Historique-Météo.net. Coordinate system: WGS 84

Department	Station city	Station city coordinates	Label city	Label city coordinates
60_Oise	60 130_Angivillers	49.5 N, 2.5 E	80 500_Montdidier	49.65 N, 2.56667 E
60_Oise	60 490_Antheuil-Portes	49.5 N, 2.75 E	80 700_Roye	49.697797 N, 2.788831 E
80_Somme	80 160_Essertaux	49.75 N, 2.25 E	80 000_Amiens	49.894067 N, 2.295753 E
80_Somme	80 260_Flesselles	50.0 N, 2.25 E	80 600_Doullens	50.15724 N, 2.34019 E
80_Somme	80 890_Condé-Folie	50.017 N, 2.017 E	80 420_Flixecourt	50.01465 N, 2.08095 E

Table S4 The three nearest weather station cities and their distance from the sampling sites

	Weather station city			Distances (in meters)		
Site code	First station	Second station	Third station	First station	Second station	Third station
PrC	Essertaux	Flesselles	Condé-Folie	13,069	22,231	34,596
PrV	Essertaux	Flesselles	Condé-Folie	12,723	21,892	34,112
JL	Flesselles	Essertaux	Condé-Folie	12,408	17,170	25,447
BsPR	Essertaux	Condé-Folie	Flesselles	17,888	27,958	31,514
BsPM	Essertaux	Condé-Folie	Flesselles	18,548	27,345	31,328
Bg	Essertaux	Condé-Folie	Flesselles	16,461	30,505	32,967
T	Essertaux	Flesselles	Condé-Folie	10,026	23,450	25,393
NaV	Essertaux	Condé-Folie	Flesselles	12,091	25,747	25,774
CF	Essertaux	Flesselles	Condé-Folie	10,917	20,865	23,527
N	Essertaux	Flesselles	Condé-Folie	11,677	18,799	22,435
CS	Essertaux	Flesselles	Condé-Folie	13,455	16,640	20,706
F	Flesselles	Essertaux	Condé-Folie	14,19	15,875	18,532
H	Condé-Folie	Flesselles	Essertaux	4,348	14,023	29,833
C	Condé-Folie	Flesselles	Essertaux	11,596	14,552	22,553
W	Condé-Folie	Flesselles	Essertaux	11,736	23,388	28,924
Bl	Condé-Folie	Flesselles	Essertaux	7,950	9,242	28,616
LlA	Flesselles	Essertaux	Condé-Folie	8,684	19,244	20,825
A	Flesselles	Essertaux	Condé-Folie	14,234	15,253	26,491
Pc	Angivillers	Essertaux	Antheuil-Portes	16,159	17,933	27,882

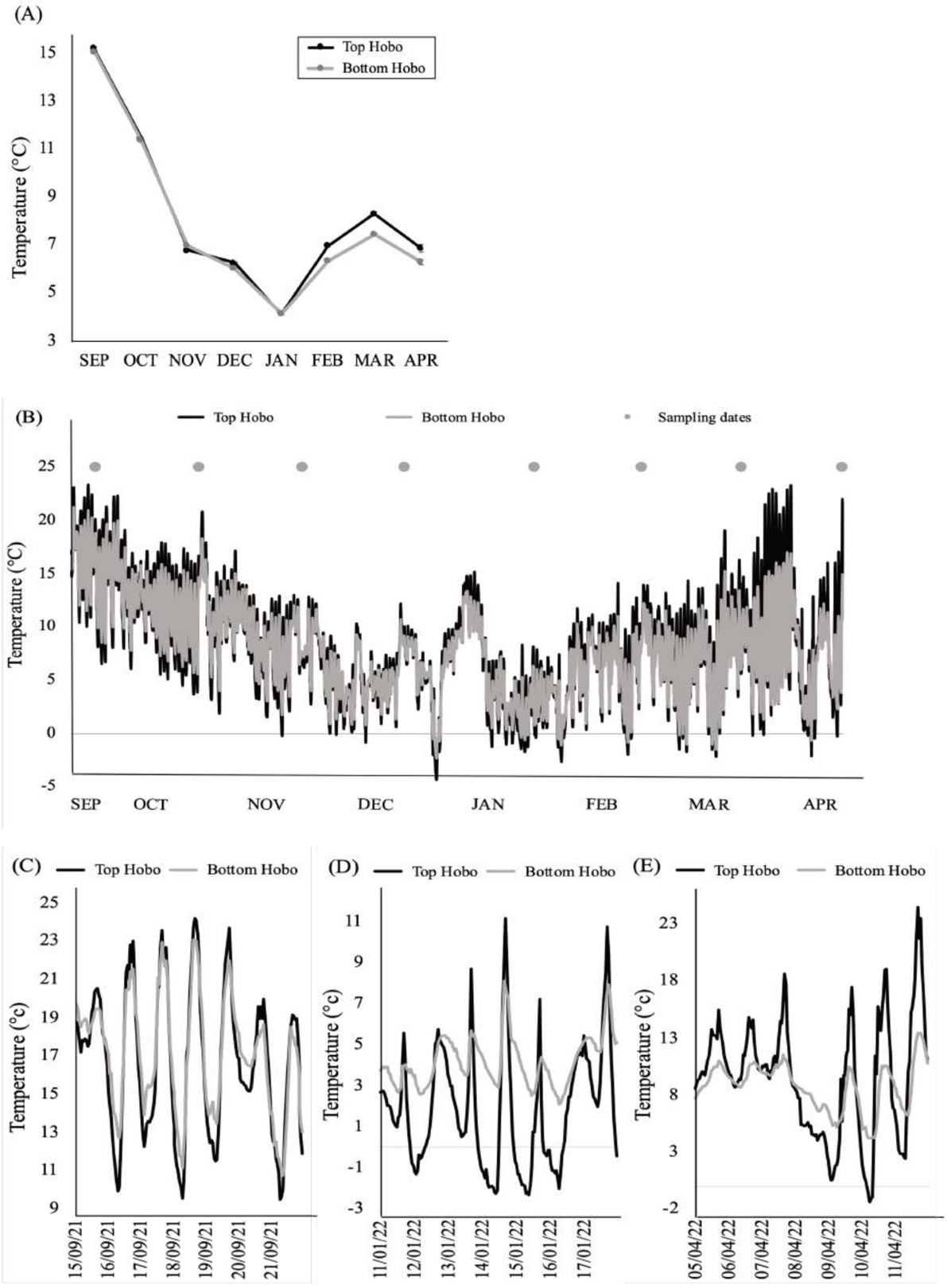

Figure S1 Comparison between top and bottom Hobo temperature across the study months, (A) monthly mean temperature for all sites; (B) hourly mean temperatures for all sites; (C) hourly temperatures 7 days before September apple sampling in LlA site; (D) hourly temperatures 7 days before January apple sampling in LlA site; (E) hourly temperatures 7 days before April apple sampling in LlA site

Spatial trophic niche partitioning and the role of Drosophilidae in fruit decomposition

Spatial partitioning occurs between different fruit and within individual fruit. According to the ephemeral resource patch concept, each apple (considered as a patch) will have unique characteristics including size, shape, ephemerality, and compositional heterogeneity that will shape both local community composition within the patch and the interspecific interactions occurring in the patch (Butterworth *et al.*, 2023). Apple fruit has a large volume relative to the size of Drosophilidae larvae. The larger this volume, the greater the emerged fly abundance and richness. This means that each species could use a different zone of the fruit to oviposit. This choice is made (a) at the adult stage influenced by the fruit color, firmness, and presence or absence of mould or (b) at the larval stage, by choosing to move deeper in the fruit to avoid competition with other larvae (Grimaldi, 1985). As with most herbivores, Drosophilidae use olfactory and visual stimuli for host selection from a distance (Little et al, 2019; Silva & Clarke, 2020), then tactile and gustatory cues at close range (Gregg et al, 2018). In our study, apple fruit skins had different proportions of green, red, yellow and brown areas. These colors may indicate the level of fruit maturity/decay and help Drosophilidae species making oviposition choices according to their ecology, explaining the segregation of the freshest fruit Drosophilidae from the rotting fruit Drosophilidae in our results. The composition of volatile organic compounds may also play an important role in attracting gravid females in the field. In particular, microbial organic compounds produced by yeasts, bacteria and other fungi, are known to act as aggregation pheromones, oviposition stimulants and a means to differentiate food resources from unfavorable environmental conditions (Becher et al, 2012; Gregg et al, 2018). During the early months of sampling, Drosophilidae had a choice between healthy and rotting apples. Later, most apples had a rotten part, either due to the initial fall from the tree or to insect oviposition (e.g., from codling moth). Biochemical compositions of healthy and rotting parts differed, as did firmness. Rotting parts were softer, more or less acidic in different months and had lower sugar content. *Drosophila* species genotypes are adapted to natural resources differing in their micro- and macronutrient content that optimize their metabolic pool sizes (Matzkin et al, 2009; Nazario-Yepiz, 2017). Consequently, each part could be used by different species with specific requirements. For example, if *D. suzukii* and *D. melanogaster* were coexisting, one would expect the former to oviposit in the healthy part of the apple, as *D. suzukii* oviposits in firm healthy substrate (Kienzle & Rohlfs, 2021; Sato et al, 2021), and the latter in the rotting part, as *D. melanogaster* is found in soft rotting material (Sato et al, 2021). The presence of specific yeast species is also known to repel or attract specific

Drosophilidae species (Becher et al, 2012; Sato et al, 2021). For example, *Acetobacter* and *Gluconobacter* inoculated on artificial diet enhanced oviposition of *D. melanogaster* and *D. biarmipes*, but discouraged that of *D. suzukii* (Sato et al, 2021). Becher et al. (2012) showed that *Saccharomyces cerevisiae* alone is sufficient to attract *D. melanogaster*. Yeast succession during apple decay could also provide a mechanism of resource partitioning, as has been reported in the Amazonian fruit *Parahancornia amapa* (Morais et al, 1995). Responses to the presence of yeast can also be mediated by competition (Matavelli et al, 2015; Rombaut et al, 2021).

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Chapter 2

Mistletoe as winter resource

Chapter 2 • Objectives and main outcomes

In this chapter, I studied the use of mistletoe (*Viscum album*) by overwintering *D. suzukii* at the national scale, across a wide climatic gradient. Mistletoe is a keystone species in winter, as this often abundant fruit constitute one of very few available resources. Previous studies have reported that *D. suzukii* is capable of infesting mistletoe, suggesting that its fruit could support overwintering *D. suzukii* populations after the end of the commercial cropping season.

The study was carried out in seven regions of France between January and July 2022. Mistletoe fruit were sampled and emerging drosophilids identified to species level. Microclimatic, landscape-scale and local factors were recorded in order to assess their role in driving infestation in mistletoe fruit during winter and early spring.

Mistletoe was infested by both *D. suzukii* and *D. suboboscura* between February and June. Infestations by both flies were positively associated with the presence of fallen mistletoe fruit on the ground and semi-natural (forest, hedgerow) and anthropogenic (garden, park) habitats. The mistletoe's host tree species also influenced the fruit infestation rate, with those growing on poplar being more infested.

This study shed light on the potential agricultural and ecological consequences in of *D. suzukii* infestation in mistletoe, especially in the context of climate change. Host trees can bear numerous mistletoe individuals (e.g., a maximum of 110 mistletoe individuals was counted in a single poplar tree in this study) that can generate a high number of flies. Consequently, commercial fruit fields surrounded by trees bearing mistletoe could be exposed to a wave of *D. suzukii* once the first crops mature sufficiently to attract these flies. Furthermore, *D. suzukii* infestation was positively correlated with the accumulated thermal energy ('degree days') in the weeks preceding assessment. Therefore, with climate change, *D. suzukii* may be able to use mistletoe fruit earlier in the year, in turn impacting fruit availability for other frugivores and causing cascading consequences on the trophic network equilibrium in the ecosystem.

Contributions

Olivier Chabrerie conceptualised the study. Field work was performed by most co-authors.

I analysed the data, produced the graphics and tables. I wrote the original draft, revised the manuscript according to reviewers' comments and wrote the response letter to the editor and reviewers.

All co-authors contributed to the revision of the manuscript.

Environmental factors driving infestations of a keystone winter fruit by an invasive and a native fruit fly

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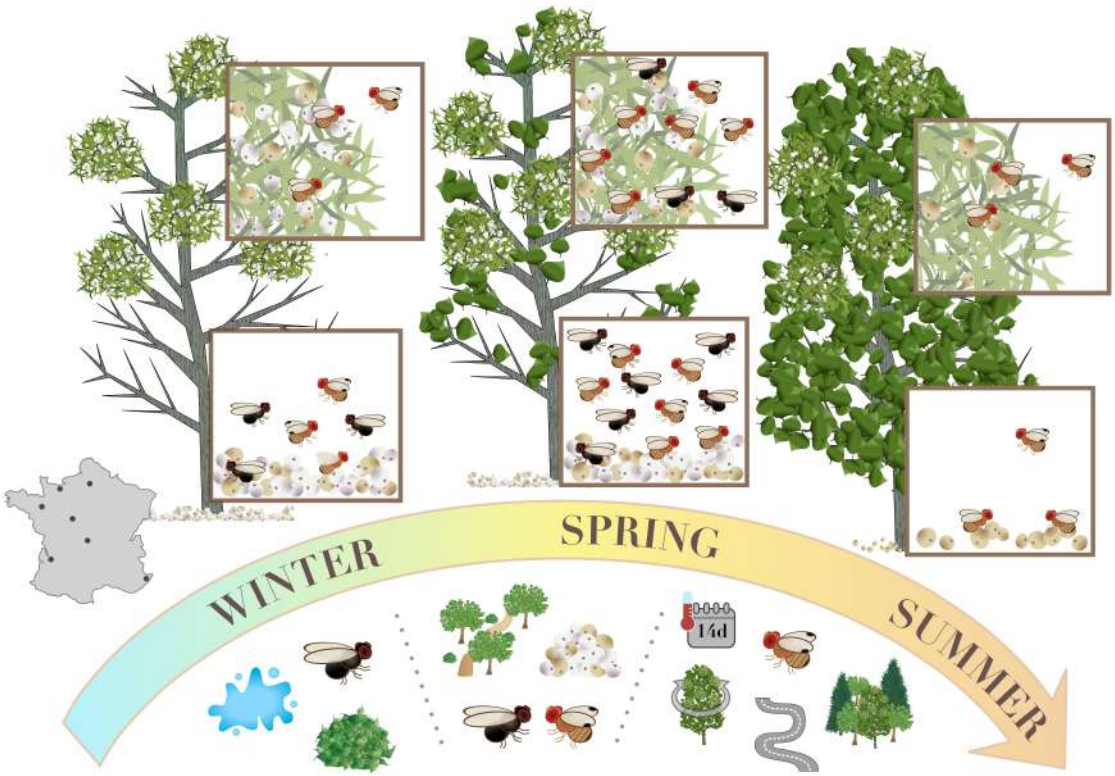
Status: Published in *Arthropod-Plant Interaction* (2024).

GRAPHICAL ABSTRACT



ABSTRACT

In temperate regions, most insect species overwinter in diapause while others continue to be active, feed, and possibly reproduce despite adverse climatic conditions. For fruit flies which remain active winter long, the presence of winter-available fruit is crucial for population persistence. This study aimed to disentangle the relative effects of climatic, landscape, and local factors on infestation rates of an important winter trophic resource, mistletoe (*Viscum album*) fruit, by drosophilid flies. Mistletoe fruits were sampled between January and July 2022 in seven regions of France, across a wide range of climatic conditions from Mediterranean to temperate oceanic. The fruits were used both by the invasive *Drosophila suzukii* and by the native *D. subobscura* in the latter part of winter and throughout spring, suggesting that this resource may assist these species to overcome the winter bottleneck. Infestations by both flies were positively associated with the presence of fallen mistletoe fruit on the ground and semi-natural (forest, hedgerow) and anthropogenic (garden, park) habitats. The mistletoe's host tree species also influenced the fruit infestation rate. *Drosophila suzukii* infestation rate was positively impacted by the accumulated thermal energy ('degree days') in the previous 14 days. Mistletoe could act as a catalyst for the development of spring *D. suzukii* generations and should be considered in the context of integrative pest management strategies to prevent early infestation of crop fruit.

Keywords: insect pest; biological invasion; mistletoe; *Viscum album*; non-crop host plants; *Drosophila suzukii*; *Drosophila subobscura*; landscape

INTRODUCTION

In temperate regions, many insect species successfully overwinter through adopting specific strategies for synchronizing their cycle with food availability, such as diapause (Bale and Hayward 2010; Gill et al. 2017; Lehmann et al. 2017). During diapause, development is suspended, which means no or little feeding during larval and adult stages (Sinclair 2015; Gill et al. 2017) and reliance on food reserves acquired in the pre-diapause phase for survival (Storey and Storey 2012; Gill et al. 2017). Other species, however, continue to feed and sometimes even reproduce throughout the winter (Danks 1978; Moore and Lee 1991; Wharton 2011), despite facing unfavorable climatic conditions. Resource selection in these species is therefore a crucial factor determining the extent to which a population is maintained (Danks 1978). The ability of insect herbivores to infest fruit resources during winter remains largely underexplored. These resources may also benefit invasive pest species, for which low temperature and resource scarcity make winter a critical bottleneck (Storey and Storey 2012).

Drosophila suzukii (Matsumura 1931) is a globally important invasive fly and a fruit pest (Asplen et al. 2015). Contrasting with other *Drosophila* species, *D. suzukii* is able to oviposit and develop in unripe and ripening fruit, causing important economic losses (Walsh et al. 2011; Tait et al. 2021). This fly is highly polyphagous and uses wild as well as cultivated fruit (Lee et al. 2015; Poyet et al. 2015; Kenis et al. 2016; Ulmer et al. 2022), including fruit of invasive plants (Poyet et al. 2014). Winter is a demographic bottleneck period for *D. suzukii*, mainly because the low temperatures and the lack of trophic resources reduce the population pool (Asplen et al. 2015; Hamby et al. 2016; Stockton et al. 2019). Even if remaining *D. suzukii* individuals are active during winter (e.g., foraging, displacement; Ulmer et al. 2024), so that they do not have a diapause sensu stricto, cold temperatures cause an ephemeral reproductive diapause (quiescence), which is reversible when climatic conditions become more favorable (Toxopeus et al. 2016; Wallingford et al. 2018). A major challenge for the fly is thus to find suitable hosts (usually wild fruit in natural habitats) both to provide food to allow populations that developed in the previous year to persist between winter and late springand to initiate the establishment of new generations (Poyet et al. 2015; Panel et al. 2018) before cultivated fruit become available as hosts. Among the few plant species bearing fruit in winter and early spring, *Viscum album* (mistletoe) is expected to be an important resource for *D. suzukii* since the fly can develop in the berries it produces (Panel et al. 2018; Poyet et al. 2015; Kenis et al. 2016) and because *V. album* and closely related species are widely distributed and often abundant across temperate regions worldwide (Watson 2001).

Viscum album L., 1753 is recognized as a keystone resource for various animal species in most temperate forests and woodlands (Watson 2001). It is an obligate hemiparasitic shrub that grows on a wide range of angiosperm and gymnosperm trees (Barney et al. 1998; Zuber 2004; Thomas et al. 2023). Native to Europe, it has a patchy distribution which depends on host availability, bird dispersal of seeds, and human management (Zuber 2004; Thomas et al. 2023). Its fruits develop slowly, ripening from November to December in western Europe, with some remaining attached until spring (Thomas et al. 2023). Depending on the host tree, the number of berries per kg of mistletoe ranges between 96 and 237 (Preston 1977), providing resources for birds and small mammals (Thomas et al. 2023). It could also represent a potentially important breeding reservoir for insects in winter. Several arthropod species take advantage of *V. album* stems and leaves (Zuber 2004; Briggs 2011; Thomas et al. 2023). However, only a few utilize the fruit, among which are the larvae of *D. suzukii* (Thomas et al. 2023).

Viscum album is widely distributed across the area invaded by *D. suzukii* in Europe (Hultén and Fries 1986) and occurs in woodlands and hedges (Briggs 2021; Thomas et al. 2023) where *D. suzukii* overwinters (Rossi-Stacconi et al. 2016; Briem et al. 2018; Santoiemma et al. 2019). The relationship between *V. album* and *D. suzukii* has been explored using field monitoring of infestation at the end of winter (Briem et al. 2016; Panel et al. 2018; Delbac et al. 2020a) and in laboratory experiments assessing the ability of *D. suzukii* to infest *V. album* berries (Poyet et al. 2015; Briem et al. 2016; Wolf et al. 2020). Its ability to utilize the fruit suggests that *D. suzukii* is able to overcome the toxicity of *V. album* berries (Gaidamashvili et al. 2012), as has been shown with other toxic fruit (Poyet et al. 2015, 2017). In winter and early spring, mistletoe fruit also constitutes a food resource for adult *D. suzukii* females that are active but with underdeveloped oocytes in ovaries (Briem et al. 2016). As the fly's reproductive diapause ends and the female's oocytes mature, the mistletoe fruit may provide suitable breeding sites at a time when other commercial fruit are not yet available (Poyet et al. 2015; Briem et al. 2016; Panel et al. 2018; Delbac et al. 2020a). Seed populations of *D. suzukii* in these fruit could therefore catalyze the development of new generations in spring.

Drosophila suzukii is the only Drosophilidae species known to oviposit and develop in *V. album* berries (Briem et al. 2016; Thomas et al. 2023) which may, thus, represent a low or free competition niche for the fly. This contrasts with other fruits where *D. suzukii* is known to facilitate other fly species: the fruit skin perforation by ovipositing *D. suzukii* females accelerates fruit decomposition and provides a point of entry for other species ovipositing preferentially in decaying organic matter (Poyet et al. 2014;

Rombaut et al. 2017). This is the case for *D. melanogaster* in grapes (Rombaut et al. 2017) or *D. subobscura* in *Prunus serotina* (Poyet et al. 2014). The same process may occur in *V. album*, with *D. suzukii* facilitating other overwintering Drosophilidae species. Among them, the native European species *D. subobscura* could be expected to share *D. suzukii* winter niche. This species is highly cold tolerant (David et al. 2003; Delbac et al. 2020b), specialized on decomposing fruit (Begon 1975; Shorrocks 1975), often dominant in Drosophilidae communities (Delbac et al. 2020b; Deconninck et al. 2024 - Chapter 1), and has already been found associated with *D. suzukii* in fallen *Prunus serotina* fruit (Poyet et al. 2014) and apple fruit (Deconninck et al. 2024 - Chapter 1).

To date, the influence of environmental factors on *V. album* infestation rate has not been explored, although they are expected to act at multiple scales. Global warming, resulting in mild winter temperatures, may promote early winter fruit infestation, including those of *V. album*, by shortening the reproductive diapause period of *D. suzukii*. Thus, among climatic variables, those related to temperature may be important predictors of *V. album* infestation by *D. suzukii*. Landscape variables are also expected to influence fruit infestation. As wintering *D. suzukii* find shelter in hedges and woodland (Briem et al. 2018; Rossi-Stacconi et al. 2016; Ulmer et al. 2022), *V. album* individuals close to forest habitats are therefore likely to have a higher risk of infestation. Local variables such as the quantity and quality of berries are also likely to influence infestation levels (Ulmer et al. 2022). Conversely, bird foraging can reduce the availability of *V. album* fruit, especially when temperatures are low and alternative resources are scarce (Briggs 2021). There is, therefore, a risk that *D. suzukii* may face a lack of this key resource in late winter, before the fly's eggs mature in early spring. In general, *D. suzukii* prefers to oviposit in ripening and ripe fruits (Walsh et al. 2011; Atallah et al. 2014). In laboratory assays, flies laid more eggs in artificially damaged than in undamaged berries (Briem et al. 2016). Damaged fruits are commonly observed in the field after the birds have foraged or after a storm event, but the distribution of *D. suzukii* between damaged or undamaged mistletoe berries in the field remains unknown.

This study aimed to disentangle the relative roles of climatic, landscape, and local factors that could affect infestation rates of a wild host plant by *D. suzukii*. We focused on the mistletoe *V. album* because this major host plant is one of the few species bearing fruit in winter and early spring. This non-crop host plant could also support overwinter seed populations of *D. suzukii*, catalyzing the development of new generations in spring in advance of the availability of the first commercial fruit crops such as cherries, strawberries, or raspberries. We hypothesized that (i) *D. suzukii* presence in *V. album* would be positively

associated with the abundance of natural habitats such as woodland, which provide winter refuge for *Drosophila* species (Basden 1954) and particularly for *D. suzukii* (Ulmer et al. 2024); and, (ii) *D. suzukii* infestation of *V. album* fruit would facilitate infestation by other *Drosophila* species such as *D. subobscura* (Poyet et al. 2014; Deconninck et al. 2024 - Chapter 1) or *D. melanogaster* (Rombaut et al. 2017; Deconninck et al. 2024 - Chapter 1). We determined the infestation rates across a large geographical area spanning 1000 km in France and including a large range of climatic conditions. At each sampling location, we also measured several local (e.g., mistletoe host tree and mistletoe traits) and landscape variables (e.g., vegetation cover, proportion of habitats) to identify the environmental drivers of mistletoe infestations.

METHODS

Sampling design

Mistletoe fruits were sampled in seven regions of France across a wide range of climatic conditions from Mediterranean in the south to temperate oceanic in the north (Fig. 1, Table 1). In Amiens, Bordeaux, Rennes, and Tours, sampling took place every month between January and July 2022, while in Caen, Clermont-Ferrand, and Nice, sampling took place only once at the optimum timing of fruit maturity in February/March. Indeed, no fruit remained at these sampling locations after March, likely as a result of bird foraging. An average of 10 mistletoe individuals were randomly sampled at each location and on each sampling day, with some variation depending on fruit availability. Where possible, a minimum of 100 berries were sampled from each individual mistletoe, but this number was not always reached on some sites at the end of the fruiting period.

Traits of mistletoe and host trees

Collected fruits were separated into three subsets to monitor *Drosophila* emergence: undamaged ('healthy') and damaged fruit collected on the plant and fruit collected on the ground (if present). Several traits of the sampled mistletoe individuals and their host trees were measured to characterize the local resources available for the flies and the microhabitat. The following reproductive and vegetative traits were measured: the maximum and minimum diameter of the mistletoe individual sampled, and an estimate of the number of fruits present. For each sampled mistletoe individual, five berries were randomly selected to measure their length and width and calculate their volume ($4/3 \times \pi \times \text{mean radius}^3$) and fruit skin area (4

× π × mean radius^2). Five leaves were also taken; their length and width were measured and the leaf surface index (length × width; Ulmer et al. 2022) was calculated. The height of the mistletoe individual on the host tree (from the ground) was also recorded. The tree species hosting the mistletoe was identified (Table S1) and its height, crown diameter, and trunk circumference were measured. The number of mistletoe individuals on the host tree was recorded. Variables, units, and codes are listed in Table S2.

Table 1 Main features of the studied regions from north to south of France. Latitude and longitude are the mean values of geographic coordinates (WGS84 projection system) of the sampling locations in each region. Mean daily T°C and rainfall (mm): mean daily temperatures and cumulative rainfall calculated with daily data from meteorological stations in 2022.

Region (main city)	Latitude (north)	Longitude (east)	Mean daily T°C	Rainfall (mm)	Sampling period (year 2022)
Amiens	49.843391	2.161312	12.8	154.8	26/01–19/07
Caen	49.098724	-0.167531	12.92	208.8	28/03–24/05
Rennes	48.168850	-1.743138	13.8	135.7	17/01–05/04
Tours	47.373393	0.818669	14.6	164.6	21/01–18/05
Clermont-Ferrand	45.814265	3.205791	13.2	225.8	14/03–19/03
Bordeaux	44.787455	-0.595867	16.8	113.1	21/01–14/06
Nice	43.786175	6.802034	17.53	128.2	23/02–23/03

Environmental variables

Local environmental, landscape, and climatic variables were measured at each sampling location or extracted from online databases to examine the effects of regional and local environmental conditions on infestation rates (Table S2). Local environmental conditions were described as follows. First, within a 5 m-radius plot centered on the mistletoe host tree, the cover, and height of the tree, shrub, and herbaceous layers were estimated, as well as soil litter thickness (Table S2). Second, within a 20 m radius, the percentage of local habitat elements surrounding the host was recorded (e.g., orchard, woodland, grassland, swamp, crop, garden, shrub, building, hedgerow, river, pond, poplar plantation, park, road; see Table S2), as well as the number of *V. album* individuals around the host tree (i.e., on other trees than the host tree itself) and any presence of other plant species with maturing fleshy fruit.

The landscape composition around each sampled mistletoe was then characterized. A geographic database was created using a Geographic Information System (GIS; ArcGIS Pro v.2.5, ESRI). The sampled host trees were positioned in the GIS and buffers of 50, 100, 250, 500, 750, 1000, 1250, 1500,

1750, 2000, 2500, and 3000 m radii around each host tree were created for subsequent analyses of landscape composition. Landscape elements (crop, water, woodland, heartland, grassland, road, urban area, orchard, industrial zone) were extracted from the OSO 2022 database (THEIA, 2023) and updated using aerial photographs and field observations (in buffers < 100 m).

Macroclimatic conditions were characterized for each sampling site using regional measurements. The daily meteorological data were retrieved from the three nearest meteorological stations to each site, from January 1st 2022 to each day of sampling (https://www.historique-meteo.net/ france/, details in Table S3). Daily minimum, mean, and maximum temperatures, rainfall, and snowfall were calculated for all sites using inverse-distance weighting (IDW) interpolation (Willmott et al. 1985) from the data from the three nearest weather stations (Table S3). Accumulated degree days ("Growing Degree Days", GDD) were calculated using a lower threshold of 0 °C between January 1st, 2022, and the day of sampling (Baskerville and Emin 1969). The baseline value of 0 °C is a standard threshold commonly used to calculate GDD in insect and plant studies (White et al. 2012; McNeil et al. 2020). It is particularly suitable to study the temporal synchrony between insects and plant resources (Iler et al. 2013). It was also chosen because active *D. suzukii* can be observed even at very low positive temperature (< 5 °C) during winter, including during periods of snowfall (Ulmer et al. 2022, 2024) and because mistletoe fruit can undergo freeze–thaw cycles before ripening ends. From daily precipitation values, we also calculated mean daily and cumulative precipitation between January 1st, 2022, and the day of sampling and within the 7- or 14-day periods before each fruit sampling day.

Emergence of *Drosophila* species

After collection, the mistletoe fruits were individually placed on wet cotton wool in cylindrical plastic transparent containers (diameter = 118 mm, height = 135 mm, volume = 1476 cm^3), covered with a nylon mesh, and maintained in a temperature-controlled room at 20 °C under a 16:8 L:D regime. Adult flies emerging from the fruits were placed in 70% ethanol. They were identified to species level using Bächli et al. (2005) and Withers and Allemand (2012). Individuals of the two major species found (*D. suzukii* and *D. subobscura*) were sexed and counted using a Leica M205C stereomicroscope equipped with a Leica MC170 HD camera and the Leica Application Suite software.

Infestation variables

We examined the relationships between environmental variables and two common infestation variables (Benavídez et al. 2021) that where either centered on the fruit (Fruit Infestation Rate: FIR = 100 × number of emerged *Drosophila* individuals from fruit collected from a given *V. album* individual / total number of fruit collected from the same *V. album* individual) or on the plant (Plant Infestation Rate: PIR = 100 × number of infested *V. album* individuals in a region or month / total number of *V. album* individuals sampled in the same region or month). A high FIR indicates that the majority of fruit on the plant are infested by the fly and a high PIR indicates that the majority of the plants in a region are infested by the flies. These variables can be interpreted as follows: a high FIR reflects the plant auto-contamination by the flies while a high PIR reflects fly dispersal (e.g., when the FIR and PIR are both high, there is both auto-contamination of the plant and dispersal of the flies; when the FIR is high and the PIR is low, there is mostly plant auto-contamination; when the FIR is low and the PIR high, there is mostly fly dispersal; when both FIR and PIR are low, there is an absence of both contamination and dispersal). These infestation variables were also calculated for each fruit category (healthy or damaged fruit on the plant and fruit on the ground, following Deconninck et al. (2024) - Chapter 1).

Statistical analyses

For each of the two *Drosophila* species, we tested the influence of environmental variables (fruit and plant morphology, host tree characteristics, local abiotic conditions, habitat composition in the surrounding landscape, and climatic variables) on FIR using mixed models (GLMMs). Region was also introduced as a random effect in GLMMs to account for the non-independence among sampling locations within the same region. For both *D. suzukii* and *D. subobscura* models, a preselection of explanatory variables was made by using the non-redundant variables that correlated (Pearson correlation) most strongly with fruit infestation rates at the assessed spatial scales (buffers from 5 to 3000 m radius around each sampling point), as recommended by Ulmer et al. (2022). Backward selection of explanatory variables and the secondorder Akaike information criterion (AICc; Hurvich and Tsai 1989) were used to select the most parsimonious model, i.e., the model with the lowest AICc. Homoscedasticity was checked using biplots of residuals and model predictions. As sample numbers were not balanced between mistletoe host trees (Table S1; due to high host tree diversity and random sampling, many host tree species included only one or a few samples in the dataset), separate GLMMs were performed to test the effect of host tree

identity on FIR by *Drosophila* species using the four dominant host tree species, i.e., those for which the occurrence was greater than 5% of the total number of samples. As with previous GLMMs, region was introduced as a random effect in the models. To test whether the FIR varied across months, we used Kruskal–Wallis tests. When significant, post hoc pairwise comparisons between months were then performed using Mann–Whitney *U*-tests. All statistical analyses were performed using SPSS version 24.0 (IBM Corporation).

Table 2 Number of mistletoe individuals sampled in all studied regions between January and July 2022 with number of fruit sampled, total number of emerged *Drosophila* species, mean ± SE and maximum fruit infestation rate. Regions are listed from north to south of France. Fruit Infestation Rate: FIR = 100 × number of emerged *Drosophila* sp. from all fruit collected from a *Viscum album* individual / number of fruit collected from the same *V. album* individual. Plant Infestation Rate: PIR = 100 × number of infested *V. album* individuals in a month / number of sampled *V. album* individuals in the same month.

			D. suzukii				***D. subobscura***				***D. repleta***
Region (main city)	**No mistle-toes**	**No fruit**	**No emerged**	**Mean FIR ± SE (%)**	**Max FIR (%)**	**PIR (%)**	**No emerged**	**Mean FIR ± SE (%)**	**Max FIR (%)**	**PIR (%)**	**No emerged**
Amiens	126	16723	341	2.15 ± 0.77	72.86	20.63	33	0.31 ± 0.15	16.67	7.94	1
Caen	19	1870	16	2.66 ± 2.63	50.00	10.53	0	0.00	0.00	0.00	0
Rennes	30	3211	0	0.00	0.00	0.00	0	0.00	0.00	0.00	0
Tours	22	3526	43	1.11 ± 0.61	11.63	31.82	8	0.26 ± 0.23	4.96	9.09	0
Clermont-Ferrand	15	1550	0	0.00	0.00	0.00	0	0.00	0.00	0.00	0
Bordeaux	46	5673	335	6.11 ± 1.83	53.00	39.13	12	0.19 ± 0.07	1.96	15.22	0
Nice	6	983	0	0.00	0.00	0.00	0	0.00	0.00	0.00	0
Total	**264**	**33536**	**735**	**2.37 ± 0.53**	**72.86**	**20.08**	**53**	**0.20 ± 0.08**	**16.67**	**7.20**	**1**

RESULTS

Dynamics of *Viscum album* fruit resource

A total of 33,536 fruit from 264 mistletoe individuals were sampled across all regions between January and July 2022 (Table 2). The number of fruit decreased progressively over the study period, from an average of 167 collected per mistletoe in January to an average of only 26 in July (Fig. 1). From January to June, the majority of fruit were healthy on the plant (47–63%) and the proportion of damaged fruit on the plant progressively declined from 33 to 16% over the same period. The proportion of fruit on the ground initially decreased from 18 to 9% between February and March, then increased by June to reach

37%. In July, no healthy fruit remained on the plants and most fruits were either damaged (88%) or on the ground (12%).

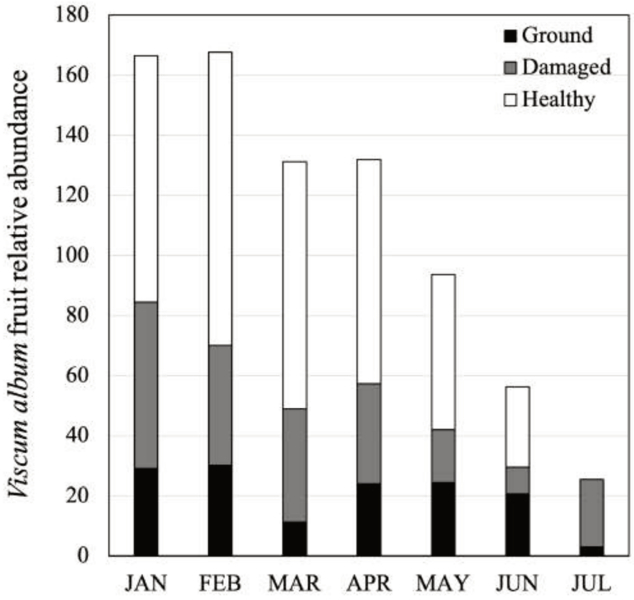


Figure 1 Variation in *Viscum album* fruit relative abundance (number of collected fruit divided by the number of plants sampled) between January and July 2022, in relation to their position and status: on the ground (Ground), on the tree damaged (Damaged) or healthy (Healthy).

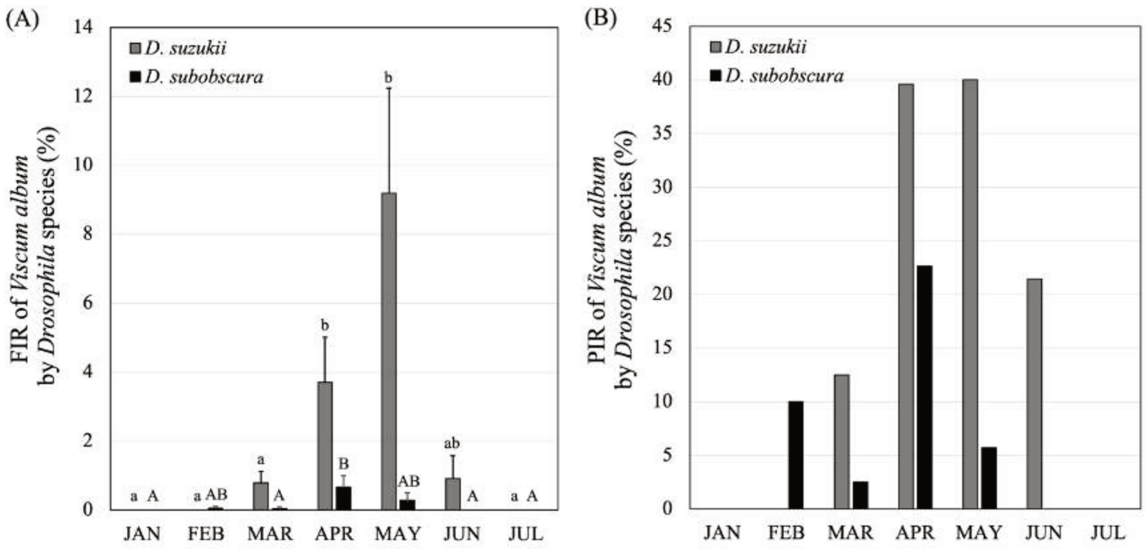


Figure 2 Variation in FIR (A) and PIR (B) of *Viscum album* by *Drosophila suzukii* and *D. subobscura* between January and July 2022. Fruit Infestation Rate: FIR = 100 × number of emerged *Drosophila* sp. from all fruit collected from a *Viscum album* individual / number of fruit collected from the same *V. album* individual. Plant Infestation Rate: PIR = 100 × number of infested *V. album* individuals in a month / number of sampled *V. album* individuals in the same month. Significance of differences between months are represented by different lowercase and capital letters for *D. suzukii* and *D. subobscura* respectively.

Regional infestation of *Viscum album*

A total of 735 *D. suzukii*, 53 *D. subobscura* and 1 *D. repleta* individuals emerged from the fruits sampled in all regions between January and July 2022 (Table 2). No flies emerged from fruits sampled in Clermont-Ferrand, Nice, and Rennes. *Drosophila suzukii* mean infestation rate (FIR) was the highest in Bordeaux (6.11 ± 1.83%), with a mean of 2.37 ± 0.53% across all regions. Among regions showing fruit infestation, the maximum fruit infestation rate (max FIR) by *D. suzukii* ranged between 11.63% (Tours) and 72.86% (Amiens). Up to 39.13% of the *V. album* individuals sampled were infested by *D. suzukii* (Bordeaux), with a mean plant infestation rate (PIR) of 20.08% across all regions.

Drosophila subobscura was only found in Amiens, Bordeaux and Tours, with mean FIR ranging between 0.19 ± 0.07% in Bordeaux and 0.31 ± 0.15% in Amiens. The maximum FIR by *D. subobscura* ranged between 1.96% in Bordeaux and 16.67% in Amiens. Up to 15.22% of the *V. album* individuals sampled were infested by *D. subobscura*, with a mean PIR of 7.20% across all regions. The single individual of *D. repleta* was obtained in Amiens.

Seasonality of *Viscum album* infestation

Drosophila subobscura was the earliest fly species to emerge from *V. album* fruits, emerging from fruits sampled in February in Amiens and Tours (Fig. 2A, B). In February, its FIR was low (0.06 ± 0.04%) then increased significantly in April and May to reach 0.67 ± 0.33% and 0.28 ± 0.23%, respectively (Kruskal–Wallis test, $\chi^2 = 26.145$, $p < 0.001$; Fig. 2A). This species did not emerge from fruits collected after May and was mostly found in fruits collected on the ground (95% of individuals), with only two individuals emerging from healthy fruits sampled on the plant (Fig. 3B). The sex ratio of emerging *D. subobscura* flies was unbiased (chi-squared test: $\chi^2 = 0.647$, $p = 0.42$), with 30 females against 23 males.

Drosophila suzukii first emerged from fruits sampled in March, showing a FIR of 0.79 ± 3.71%, with infestation increasing significantly to reach 9.20 ± 3.05% in May, followed by a decrease in June to 0.92 ± 0.66%, and no further emergence in July (Kruskal–Wallis test, $\chi^2 = 37.951$, $p < 0.001$; Fig. 2A). A majority (58.3%) of *D. suzukii* emerged from fruits collected on the ground, then 36.8% from healthy fruits on the plant, and 4.9% from damaged fruits on the plant. The highest fruit infestation rate was observed in May, with 15% for fruits on the ground, 8% for healthy fruits on the plant, and 0.1% for damaged fruits on the plant (Fig. 3B). Emerging *D. suzukii* flies had an unbiased sex ratio overall (chi-squared test: $\chi^2 = 0.647$, $p = 0.42$), with 374 females and 361 males.

In February, 10% of *V. album* individuals were infested by *D. subobscura*, which decreased to 3% in March, then increased to 23% in April, and finally decreased to 6% in May (Fig. 2B). In March, 13% of *V.*

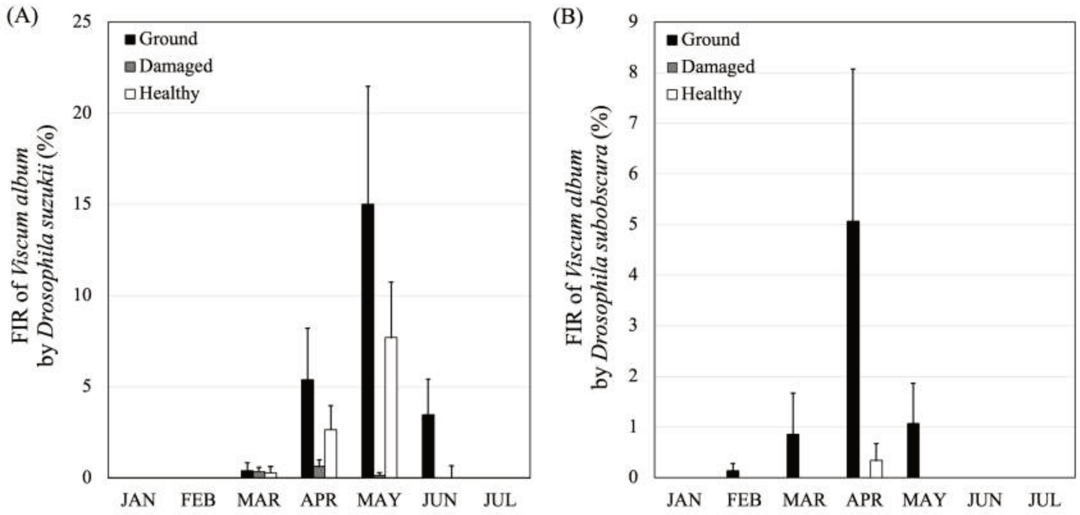


Figure 3 Variation in FIR of *Viscum album* by *Drosophila suzukii* (A) and *D. subobscura* (B) between January and July 2022, in relation to their position and status: on the ground (Ground), on the tree damaged (Damaged) or healthy (Healthy). Fruit Infestation Rate: FIR = 100 × number of emerged *Drosophila* sp. from all fruit collected from a *Viscum album* individual / number of fruit collected from the same *Viscum album* individual, as described in Figure 2.

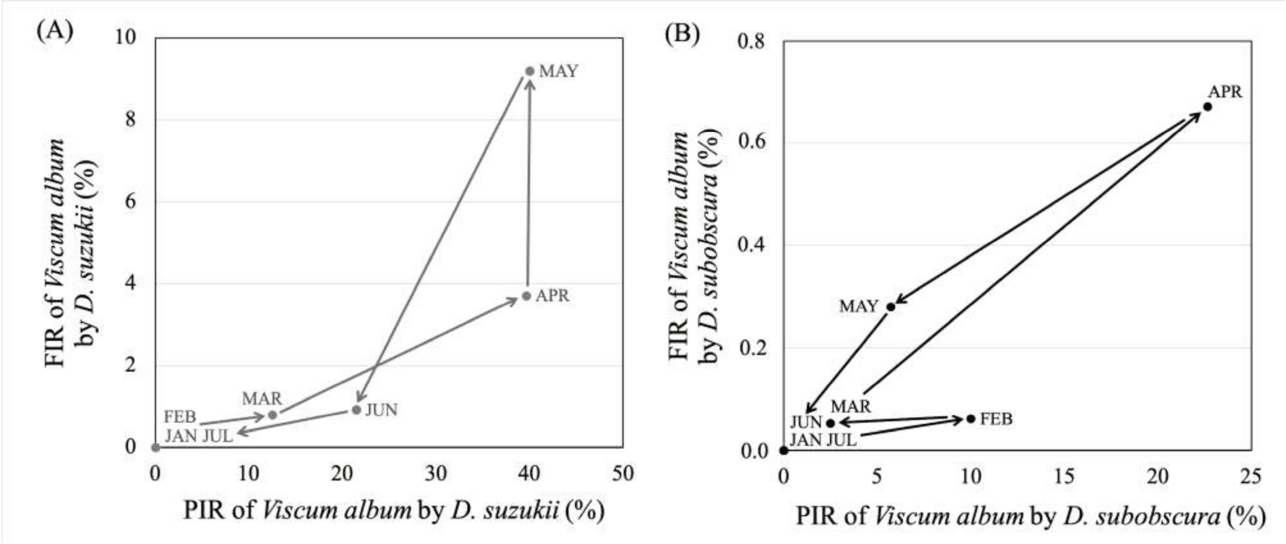


Figure 4 Mean fruit infestation rate (FIR) in relation to plant infestation rate (PIR) of *Viscum album* according to months between January and July 2022 for (A) *Drosophila suzukii* and (B) *D. subobscura*. Interpretation: high FIR + high PIR = dispersion + auto-contamination; high FIR + low PIR = auto-contamination; low FIR + high PIR = dispersion; low FIR + low PIR = low auto-contamination / dispersion. Dispersal and auto-contamination are maximized in May for *D. suzukii* and in April for *D. subobscura*.

album individuals were infested by *D. suzukii*, increasing to 39% and 40% in April and May, respectively, and then decreasing in June to reach 22%.

Throughout the seasons, the dynamics of infestation by *D. suzukii* and *D. subobscura* changed (Fig. 4A, B). In February and March, both species had low FIR and PIR, suggesting that both auto-contamination (local infestation) of *V. album* individuals and dispersal were low. Then, in April, both species started to disperse. In May, *D. suzukii* was both autocontaminating *V. album* individuals and dispersing while *D. subobscura* infestation decreased. In June, local infestation of *D. suzukii* increased again. Finally, starting from June for *D. subobscura* and July for *D. suzukii*, no further infestation was found in *V. album* fruit.

Effect of environmental variables on *Viscum album* fruit infestation rate

Drosophila suzukii fruit infestation rate was positively associated with the percentage of fruit collected on the ground, the canopy width of the host tree, the path cover in a 5 m radius, the garden and forest cover in a 20 m radius around sampling points, and the GDD in the preceding 14 days (GLMM, Table 3). *Drosophila subobscura* fruit infestation was positively associated with the percentage of fruit collected on the ground, the cover of water, hedgerow, and urban park in a 20 m radius around sampling points (GLMM, Table 3). The identity of the dominant host trees (*Crataegus monogyna*, *Malus domestica*, *Populus nigra*, *Robinia pseudoacacia*) significantly influenced FIR by *Drosophila* species (Table S1 and Figure S1). Mistletoe growing on *P. nigra* had the most heavily infested berries, with a FIR of 4.11 ± 1.57% and 0.65 ± 0.32 by *D. suzukii* and *D. subobscura*, respectively. *Drosophila suzukii* FIR was significantly reduced in *M. domestica* and *R. pseudoacacia* compared to *P. nigra*, and the lowest FIR was for mistletoe growing on *C. monogyna* (GLMM: $F = 3.00$, $p = 0.04$). *Drosophila subobscura* FIR was also significantly decreased in mistletoe growing on *C. monogyna* and *R. pseudoacacia* and was zero on *M. domestica* (GLMM: $F = 2.63$, $p = 0.05$).

DISCUSSION

Both *D. suzukii* and *D. subobscura* emerged from *Viscum album* fruit in the latter part of winter and throughout spring, confirming that this food resource likely helps them to overcome the winter bottleneck induced by low temperatures and limited availability of fruit of other species. Below, we address (i) how local and landscape factors affect *Drosophila* infestation, (ii) the potential role of *V. album* as a catalyst for the development of spring *D. suzukii* generations, (iii) the potential for *D. suzukii* to

impact trophic fluxes in the context of invasion and climate change, and (iv) the relevance of our findings in the context of pest management strategies.

Table 3 Effect of environmental variables on *Viscum album* fruit infestation rate (FIR) by *Drosophila suzukii* and *D. subobscura* analyzed using GLMMs. GROUTOT: % fruit collected on the ground, HLENGH: Canopy width of the host tree (m), PATH: Path cover (in a 5 m radius), GARDEN20: Garden cover (in a 20 m radius), FOREST20: Forest cover (in a 20 m radius), GDDMC14J: Growing Degree Days (of the last 14 days), WATE20: Water cover (in a 20 m radius), HEDGE20: Hedgerow cover (in a 20 m radius), PARK20: Urban park cover (in a 20 m radius)

Dependent variables	**Explanatory variables**	**Model parameters**						
		F	**Estimates**	**SE**	**d.f.**	**t**	***p***	**AICC**
Viscum album fruit infestation rate by *Drosophila suzukii* (n=264 samples)	Model constant	18.895	-7.6485	1.7596	257.00	-4.347	<0.001	1848.96
	GROUTOT	14.684	0.0797	0.0208	257.00	3.832	<0.001	
	HLENGH	3.933	0.2230	0.1124	257.00	1.983	0.048	
	PATH	8.023	0.1402	0.0495	257.00	2.833	0.005	
	GARDEN20	15.847	0.1637	0.0411	257.00	3.981	<0.001	
	FOREST20	4.680	0.0887	0.0410	257.00	2.163	0.031	
	GDDMC14J	13.476	0.0402	0.0110	257.00	3.671	0.000	
Viscum album fruit infestation rate by *Drosophila subobs*cura (n=264 samples)	Model constant	3.075	-0.1829	0.1043	259.00	-1.753	0.081	856.94
	GROUTOT	12.400	0.0101	0.0029	259.00	3.521	0.001	
	WATE20	17.729	0.1980	0.0470	259.00	4.211	<0.001	
	HEDGE20	6.514	0.0125	0.0049	259.00	2.552	0.011	
	PARK20	9.348	0.0092	0.0030	259.00	3.057	0.002	

Local and landscape drivers of fruit infestation

Levels of infestation by both *D. suzukii* and *D. subobscura* were locally positively associated with the percentage of fruit on the ground, most likely a result of wind, bird foraging, or abscission due to fruit maturity. *Drosophila subobscura* has not previously been reported to complete its life cycle in *V. album*, although it is known to utilize fallen fruit for breeding (Shorrocks 1975; Poyet et al. 2014; Deconninck et al. 2024 - Chapter 1). Begon (1975) showed that the density of berries in an area increased the chances of breeding by *D. subobscura*. He studied *D. subobscura*'s peak of abundance in November and sampled several fleshy-fruited plants (e.g., hawthorn, rowan, woody nightshade), but not mistletoe which matures later. These plants are also present in our sampling regions (Poyet et al. 2015) and *D. subobscura* may have

switched from them to *V. album* in February. *Drosophila subobscura* is also known to favor soft fruit for oviposition (Begon 1975), which is consistent with its preference for softer decaying fruit on the ground. In contrast, *D. suzukii* is known for its ability to oviposit in ripening and ripe fruit (Walsh et al. 2011) using its serrated ovipositor (Atallah et al. 2014), although it was still more attracted by damaged than intact mistletoe berries in a laboratory experiment (Briem et al. 2016). The field patterns revealed by our study are consistent with this experimental result as the largest number of *D. suzukii* was found in fallen fruit (damaged), even if, on the plant itself, healthy fruits were preferred over damaged. Several hypotheses may support these observations: (i) the dietary requirements of *D. suzukii* may vary seasonally (Rendon et al. 2019; Jiménez-Padilla et al. 2020); ripe and rotting berries may be more suitable during winter as winter morphs seek resources richer in sugar (Rendon et al. 2019); (ii) the absence or low levels of interspecific competition even in decaying *V. album* berries may allow the larvae to complete their development; it has been suggested that the presence of other drosophilids in other decaying fruit may result in levels of competition that *D. suzukii* cannot tolerate (Kidera and Takahashi 2020); and (iii) more extreme winter environmental conditions may restrict the ability of flies to reach mistletoe individuals higher in the shrub or tree vegetation (Dillon and Frazier 2006; Frazier et al. 2008); this will result in the flies staying close to the ground where the temperature is buffered (Deconninck et al. 2024 - Chapter 1) and, therefore, in fallen fruit infestation.

Landscape features affected the two *Drosophila* species differently. Garden and forest cover in the area of the sampling locations positively influenced the levels of infestation by *D. suzukii*, while the cover of water, hedgerow, and urban park positively influenced those of *D. subobscura*. Seminatural habitats including woodland, grassland, hedgerow, and shrubs are known to positively affect the abundance of Drosophilidae species by providing food resources, breeding sites, and shelters with beneficial microclimatic conditions (Santoiemma et al. 2018; Delbac et al. 2020b). The differences between the semi-natural habitats affecting each species may be due to their diet and breeding preferences, as *D. suzukii* is mostly frugivorous (Walsh et al. 2011; Poyet et al. 2015; but see Stockton et al. 2019 for alternative resources), while *D. subobscura* is both frugivorous and fungivorous (Begon 1975; Delbac et al. 2020b). The food and breeding resources the flies are seeking may not be found in the same semi-natural habitats. Similarly, the canopy width of the host tree only affected *D. suzukii*, which has a strong preference for the canopy layer (Tanabe 2002).

The importance of semi-natural habitats varied between the two species, with *D. subobscura* less affected by their presence than *D. suzukii* (Delbac et al. 2020b). This is consistent with the 'generalist' ecology of *D. subobscura*, as it is found across multiple habitats (Shorrocks 1975; Gross and Christian 1994). This species is also considered a 'core' species invariably dominating fruit fly species assemblages from autumn to early spring, at least in apples (Deconninck et al. 2024 - Chapter 1). The impact of semi-natural habitats on Drosophilidae abundance also varied between seasons.

Mistletoe as a host for seed populations catalyzing new generations of *D. suzukii* in spring?

Viscum album fruiting commences in November and berries can remain on the plant until May (Thomas et al. 2023; Delbac et al. 2020a). It is one of the few plant species providing resources to birds, mammals, and some insect species in winter (Watson 2001; Thomas et al. 2023). *Drosophila suzukii* adults have previously been reported to feed on *V. album* berries in winter and to start using them as a breeding substrate in April (Briem et al. 2016; Panel et al. 2018; Delbac et al. 2020a). In our study, *V. album* started to be infested by *D. suzukii* in March, slightly earlier than previously reported (Delbac et al. 2020a). Early infested *V. album* berries could therefore generate the first generations of *D. suzukii* that will later attack early maturing commercial fruit such as cherries. In this part of Europe, cherries begin to mature mid-April (Santoiemma et al. 2018) and are one of the most heavily infested commercial plants by *D. suzukii* (Beers et al. 2011).

The fruit infestation rate (FIR) was quite low in our data, but the plant infestation rate (PIR) was very high (up to 40%). This discrepancy between the two metrics may be explained by a combination of the low survival rate of *D. suzukii* at the end of winter and by the high quantity of fruit on each plant, diluting the overall infestation rate. Nonetheless, a host tree bearing numerous mistletoe individuals (e.g., a maximum of 110 mistletoe individuals was counted in a single poplar tree in this study) will generate a high number of flies even if its mean FIR is low. Therefore, commercial fruit fields surrounded by trees bearing mistletoe individuals may be exposed to a wave of *D. suzukii* around mid-April once the first crop fruit mature sufficiently to attract these flies, although this effect will depend on the location.

There were clear differences between the regions studied in the persistence of mistletoe fruit across months and in infestation rates. Regional contrasts may primarily result from overall climatic conditions impacting mistletoe phenology (Fontúrbel et al. 2018), as it is the case for flowering and fruiting periods of many plant species (reviewed by Menzel et al. (2006, 2020)). More specifically, earlier and more rapid fruit maturation in some regions may not match the fly's phenology, leading to a phenological mismatch

(Renner and Zohner 2018). Second, even if berries are at an appropriate stage of maturity for the flies to oviposit, the latter may not be able to do so due to reproductive diapause (Abarca and Spahn 2021). Finally, in some regions, mistletoe berries may be more rapidly consumed by birds due to a lack of availability of other resources (Visser et al. 2012). In our study locations, wood pigeons (*Columba palumbus*) and blackbirds (*Turdus merula*) were observed to eat mistletoe berries. Thrushes are known dispersers (Thomas et al. 2023), while it is unclear whether wood pigeons generally feed on berries or graze *V. album* leaves (Briggs 2021). Such mismatches between fruit and *Drosophila* sp. physiology and phenology have been reported between elderberries and *D. suzukii* (Ulmer et al. 2022). In southern France, *Sambucus nigra* produces fruits in July and experiences a low infestation rate because the typically hot and dry weather is unfavorable for *D. suzukii* (mismatch); on the contrary, in northern France, *S. nigra* produces fruits in September and experiences considerable infestation, as the mild and humid weather is favorable for the fly (match). In the context of contemporary climate change, which is already impacting *V. album* distribution (Bertin 2008), any advance in timing leading to an earlier match between fruit maturity and fly ability to reproduce may amplify the possibility of infestation and damage to spring-cultivated commercial fruit, but this requires further investigation.

Potential impact of *D. suzukii* on ecosystem functioning

The timing and pattern of mistletoe utilization differed between *D. suzukii* and *D. subobscura*. Both species initially adopted a similar strategy of local infestation at the end of winter, probably reducing energetic costs associated with dispersal. In April, when temperatures are milder, dispersal reaches a peak. As spring progressed, mistletoe utilization started to diverge: *D. suzukii* showed both a high level of local infestation and dispersal in May, coinciding with abundance at that time when various cultivated fruits are maturing (Estrella et al. 2007). *Drosophila suzukii* thus seemed to favor dispersal strategies while *D. subobscura* continued to infest at a local scale. Our data contrast with those of Poyet et al. (2014), who suggested facilitation of *D. subobscura* by *D. suzukii* in *Prunus serotina* fruits, thanks to the ability of *D. suzukii* to pierce fruit skin with its serrated ovipositor, a feature not possessed by *D. subobscura*. However, *D. subobscura* started to emerge from mistletoe fruit in February, one month earlier than *D. suzukii*, and was mainly found in fruit on the ground. This is likely because of *D. subobscura* oviposition requirements (Begon 1975; Shorrocks 1975) and because this fly species is unusual among the *Drosophila* community in being able to infest decaying fruit throughout the coldest period of the year (Deconninck et al. 2024 - Chapter 1). As *D. subobscura* emerged before *D. suzukii*, our results did not provide evidence of a

facilitating effect of *D. suzukii* benefiting *D. subobscura*, at least up to March. Other environmental factors (e.g., winds, birds, fruit maturity) caused mistletoe fruit damage and/or fall, facilitating *D. subobscura* infestation.

The number of GDD in the previous 14 days (GDDMC14j, Table 3) strongly influenced *D. suzukii* infestation rate. As temperature increases as a result of climate change, the increased thermal energy available offers *D. suzukii* the opportunity to start utilizing mistletoe fruit earlier in the year. This resource diversion benefiting *D. suzukii* is likely to impact fruit availability for other frugivores and could have cascading consequences on the trophic network equilibrium in the ecosystem (Roche et al. 2023). Decaying fruit could be unattractive to some bird species (Manzur and Courtney 1984; Poyet et al. 2014) and/or become attractive to others (Greenleaf et al. 2023). Premature fruit abscission because of *D. suzukii*-induced fruit decay may also reduce ornithochoric seed dispersal (Bühlmann and Gossner 2022) and, thus, the colonization capacity of mistletoe. Further behavioral studies are required to experimentally test the attractiveness of infested versus uninfested fruit and their visual attractiveness and palatability for the birds that disperse their seeds.

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Considerations about pest management strategies

The data obtained in this study suggest that the use of mistletoe fruit by *D. suzukii* could catalyze an increase in fly populations available to infest early maturing cultivated fruit in spring. Developing integrated pest management strategies is essential (Tait et al. 2021) and the consideration of winter processes in these will become increasingly important. Better understanding of *D. suzukii* overwintering

strategies is required in order to identify the potential avenues for the development of pest management strategies applicable in winter (Panel et al. 2018). *Viscum album* fruit being an important trophic resource not only for *D. suzukii* but also for birds and mammals; it is not viable or acceptable to eradicate mistletoe generally. However, removal could be considered from trees adjacent to certain fruit crop production areas (e.g., cherry, strawberry, raspberry, etc.), which could help reduce or delay the initial crop infestation. Notably, *V. album* host tree species influenced the fruit infestation rate: mistletoe on poplars were most heavily infested compared to those on hawthorn, apple, or black locust which were eight, two and 0.33 times less infested, respectively. Pest management strategies should initially focus on the most heavily infested host plant species.

Further research is now required to better understand the differences in *D. suzukii* infestation between the various mistletoe host tree species. They could be a result of the variability in biologically active phenolic compounds in mistletoe berries depending on the host tree species (Pietrzak et al. 2017). For example, mistletoe growing on *Populus nigra* trees are particularly rich in phenolic acids (Pietrzak et al. 2017). Such compounds may act as repellents or attractants for insects (Pratyusha 2022), including *D. suzukii* (Hussain et al. 2023). Further research could improve monitoring and behavior-based management tools. More generally, commercial fruit cultivation should be revisited from the lens of landscape structure, for instance, by integrating the configurations that are least favorable for winter population growth of this invasive pest insect.

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Supplementary material

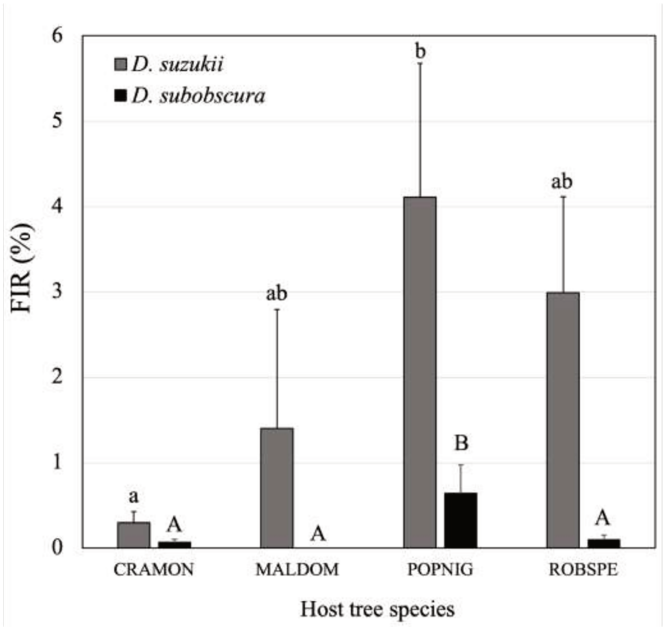

Figure S1 Mean fruit infestation rate (FIR) ± SE (%) by *D. suzukii* and *D. subobscura* in *Viscum album* individuals according to the major host tree species of *Viscum album*. CRAMON: *Crataegus monogyna* (n = 66 samples), MALDOM: *Malus domestica* (n = 36), POPNIG: Populus nigra (n = 60), ROBSPE: *Robinia pseudoacacia* (n = 43). Significance of differences between months are represented by different lowercase and capital letters for *D. suzukii* (GLMM: F = 3.00, p = 0.04) and *D. subobscura* (GLMM: F = 2.63, p = 0.05) respectively.

Table S1 Host trees of *Viscum album* individuals sampled in the study and mean FIR ± SE (%) by *D. suzukii* (*Dsuz*) and *D. subobscura* (*Dsub*).

Species	Code	Number	%	***Dsuz*** **mean FIR ± SE (%)**	***Dsub*** **mean FIR ± SE (%)**
Acer campestre	ACECAM	13	4.9%	0.00 ± 0.00	0.00 ± 0.00
Acer opalus	ACEOPA	3	1.1%	0.00 ± 0.00	0.00 ± 0.00
Acer saccharinum	ACESAC	4	1.5%	9.64 ± 4.14	0.00 ± 0.00
Acer sp.	ACESP	7	2.7%	4.41 ± 4.10	0.28 ± 0.28
Carpinus betulus	CARBET	5	1.9%	12.28 ± 10.31	0.20 ± 0.20
Corylus avellana	CORAVE	1	0.4%	0.00 ± 0.00	0.00 ± 0.00
Crataegus monogyna	CRAMON	66	25.0%	0.30 ± 0.13	0.07 ± 0.03
Fraxinus sp.	FRASP	4	1.5%	0.97 ± 0.97	0.33 ± 0.33
Malus domestica	MALDOM	36	13.6%	1.40 ± 1.39	0.00 ± 0.00
Populus alba	POPALB	3	1.1%	0.00 ± 0.00	0.29 ± 0.29
Populus nigra	POPNIG	60	22.7%	4.11 ± 1.57	0.65 ± 0.32
Populus × *canadensis*	POPCAN	1	0.4%	0.00 ± 0.00	0.00 ± 0.00
Prunus avium	PRUAVI	1	0.4%	0.00 ± 0.00	0.00 ± 0.00
Prunus sp.	PRUNUS	3	1.1%	0.00 ± 0.00	0.00 ± 0.00
Pyrus communis	PYRCOM	1	0.4%	0.00 ± 0.00	0.00 ± 0.00
Quercus ilex	QUEILE	3	1.1%	14.67 ± 14.67	0.00 ± 0.00
Quercus pubescens	QUEPUB	2	0.8%	0.00 ± 0.00	0.00 ± 0.00
Quercus rubra	QUERUB	1	0.4%	0.00 ± 0.00	0.00 ± 0.00
Quercus sp.	QUERCU	1	0.4%	0.00 ± 0.00	0.00 ± 0.00
Robinia pseudoacacia	ROBSPE	43	16.3%	2.99 ± 1.12	0.10 ± 0.05
Tilia platyphyllos	TILPLA	2	0.8%	0.64 ± 0.23	0.00 ± 0.00
Tilia sp.	TILSP	4	1.5%	0.34 ± 0.34	0.00 ± 0.00
TOTAL		264	100%	2.37 ± 0.53	0.20 ± 0.08

Table S2 List of the variables assessed in the study. Abbreviations: s: measure from weather station, 7J and 14J: measures collected from the 7 and 14 days prior to sample collection.

CATEGORY	CODE	DESCRIPTION	UNIT
		DROSOPHILIDAE SPECIES	
INFESTATION INDICES	FIR	Fruit Infestation Rate = 100 × number of emerged *Drosophila* individuals from fruit collected from a *Viscum album* individual / total number of fruit collected from the same *Viscum album* individual	%
	PIR	Plant Infestation Rate = 100 × number of infested *Viscum album* individuals in a region or month / total number of *Viscum album* individuals sampled in the same region or month	%
		MISTLETOE FRUIT CATEGORIES	
NUMBER	HEAFRUIT	Number of healthy fruit collected on a *Viscum album* individual	Number
	DAMFRUIT	Number of damaged fruit (rotten or with open skin) collected on a *Viscum album* individual	Number
	PLAFRUIT	Sum of healthy and damaged fruit collected on a *Viscum album* individual	Number
	GROFRUIT	Number of fruit collected on the ground below a *Viscum album* individual	Number
	TOTFRUIT	Sum of *Viscum album* fruit collected on the plant and on the ground	Number
PERCENTAGE	%HEALPLA	100 × HEAFRUIT / PLAFRUIT	%
	%DAMAPLA	100 × DAMFRUIT / PLAFRUIT	%
	%HEALTOT	100 × HEAFRUIT / TOTFRUIT	%
	%DAMATOT	100 × DAMFRUIT / TOTFRUIT	%
	%FPLANT	100 × PLAFRUIT / TOTFRUIT	%
	%GROUTOT	100 × GROFRUIT / TOTFRUIT	%
		LIFE TRAITS OF MISTLETOE INDIVIDUALS	
FRUIT TRAITS	MFWIDTH	Mean fruit width (n = 5 fruit)	cm
	MFVOL	Mean fruit volume (n = 5 fruit)	cm^3
	MFSURF	Mean fruit skin area (n = 5 fruit)	cm^2
LEAVE TRAITS	MLLENGTH	Mean leaf length (from the base of the petiole to the top/apex of the blade; n = 5 leaves)	cm
	MLWIDTH	Mean leaf width (n = 5 leaves)	cm
	MLSURF	Mean leaf area (area = length × width; n = 5 leaves)	cm^2
	LENGTH	Maximum diameter of V*iscum album* individual	m

INDIVIDUAL MORPHOLGY	WIDTH	Minimum diameter of V*iscum album* individual	m
	INDFRUITS	Total number of fruit present on a sampled *Viscum album* individual	Number
	HEIGHT	Height of *Viscum album* individual from the ground	m
MISTLETOE HOST TREE			
MISTLETOE HOST TREE	HOSTSPE	*Viscum album* host species	Species
	HHEIGHT	Height of the host tree	m
	HLENGH	Maximum diameter of the host tree canopy	m
	HWIDTH	Minimum diameter of the host tree canopy	m
	HTRUNK	Circumference of the host tree trunk	cm
	HMISTLETOE	Number of *Viscum album* individuals on the host tree	Number
	HMISTLETOE20	Number of *Viscum album* individuals in a 20 m radius around the host tree	Number
	SUMHMIST20	Sum of *Viscum album* individuals on the host tree and in a 20 m radius around the host tree	Number
SITE AND LANDSCAPE CHARACTERISTICS			
LOCAL ENVIRONMENT (in a 5 m radius around the mistletoe host tree)	ALTM	Site altitude	m
	SLOM	Site slope	Degree
	CTREE	Cover of tree layer (height > 8 m)	%
	CSHRUB	Cover of shrub layer (1-8 m)	%
	CHERB	Cover of herb layer (< 1 m)	%
	HHERBMEAN	Height of herbaceous layer	cm
	HLIGN	Height of woody species (shrubs, trees and lianas)	m
	COMP	Cover of biowaste composter	%
	SOIL	Cover of bare soil	%
	DWOOD	Cover of dead wood debris on the ground	%
	TLITTER	Depth of leaf litter	cm
	LITTER	Cover of leaf litter	%
	RIVER	Cover of stream	%
	POND	Cover of pond	%
	PATH	Cover of (unpaved) pathway	%
	ROAD	Cover of road and paved surface	%
	BUILD	Cover of building	%
	ORC20	Cover of orchard area (fruit trees and their associated grassland)	%

LOCAL HABITATS (in a 20 m radius around the mistletoe host tree)	FOREST20	Cover of woodland	%
	PASTURE20	Cover of grazed grassland	%
	MOW20	Cover of mown grassland	%
	GRASS20	Cover of grassland (PASTURE20+MOW20)	%
	SWAMP20	Cover of swamp	%
	CROP20	Cover of conventional agriculture crop	%
	VEGGAR20	Cover of market garden	%
	GARDEN20	Cover of private garden	%
	SHRUB	Cover of shrub	%
	BUILD20	Cover of building	%
	HEDGE20	Cover of hedgerow	%
	RIVER20	Cover of river	%
	POND20	Cover of pond	%
	POPLARP20	Cover of poplar plantation	%
	GREESP20	Cover of urban green space (small urban planted herbaceous or shrub strips)	%
	PARK20	Cover of park	%
	GGSP20	Total urban vegetated area (sum of garden, green space and park cover)	%
	RP_FC	Fleshy-fruited plant species richness	Number
LANDSCAPE COMPOSITION (from OSO 2022 data basis; cover percentages calculated within the following radii around the mistletoe host tree: 50 m, 100 m, 250 m, 500 m, 750 m, 1000 m, 1250 m, 1500 m, 1750 m, 2000 m, 2500 m and 3000 m)	CROP%	Cover of conventional agriculture crop	%
	WATE%	Cover of river and other water surface	%
	FORE%	Cover of woodland	%
	HETH%	Cover of heathland	%
	MOW%	Cover of grassland	%
	ROAD%	Cover of road and paved surface	%
	URB%	Cover of urban area	%
	ORC%	Cover of orchard area (fruit trees and their associated grassland)	%
	INDU%	Cover of industrial zone	%
METEOROLOGY			
	GDDMC	Sum of daily positive temperature (Growing Degree Days) calculated from the mean temperatures of the 7 or 14 days before fruit sampling	°C
	NFD	Number of days with negative temperatures (≤ 0 °C) during the 7 or 14 days before fruit sampling	Number

TEMPERATURE AND PRECIPITATION VARIABLES (from weather stations data; meanings of suffixes added to variable names: 7J = data from the 7 days preceding fruit sampling, 14J = data from the 14 days preceding fruit sampling)	RAIN	Sum of daily precipitation in the 7 or 14 days before the fruit sampling	cm
	SNOW	Sum of daily snowfall in the 7 or 14 days before the fruit sampling	cm
	MMIN	Mean minimum temperature during the 7 and 14 days before fruit sampling	°C
	MMAX	Mean maximum temperature during the 7 days before fruit sampling	°C
	MMEANCALC	Calculated mean of daily mean temperatures of the 7 or 14 days before the fruit sampling	°C
	MINMIN	Lowest minimum temperature during the 7 or 14 days before fruit sampling	°C
	MAXMAX	Highest maximum temperature during the 7 or 14 days before fruit sampling	°C
	SUMT_010122	Sum of daily positive temperature (Growing Degree Days) between 01/01/2022 and the sampling date	°C
	RAIN_010122	Sum of rain precipitation between 01/01/2022 and the sampling date	cm

Table S3 Weather station information. Geographic area: city of the research laboratory, station city: postcode and the municipality name of the weather station location, Station coordinates: geographic location of the city where the weather station is located, Label city: postcode and municipality name according to Historique-Météo.net, Label city coordinates: geographic location of the label city, Mean distance: mean distance between the weather station and sampled locations (in kilometers). Geographic coordinate system: WGS 84.

Geographic area	Station city	Coordinates	Label city	Coordinates	Mean distance (m)
Amiens	80260_Flesselles	50.0 N, 2.25 E	80600_Doullens	50.15724 N, 2.34019 E	16,288
	80160_Essertaux	49.75 N, 2.25 E	80000_Amiens	49.894067 N, 2.295753 E	17,516
	80890_Condé-Folie	50.017 N, 2.017 E	80420_Flixecourt	50.01465 N, 2.08095 E	22,404
	80300_Albert	50.0 N, 2.65 E	80300_Albert	50.00091 N, 2.65096 E	28,868
	80140_Doudelainville	50.0 N, 1.767 E	80100_Abbeville	50.105467 N, 1.836833 E	30,264
	60130_Angivillers	49.5 N, 2.5 E	80500_Montdidier	49.65 N, 2.56667 E	29,046
Bordeaux	33400_Talence	44.817 N, -0.6 O	33400_Talence	44.802614 N, -0.588054 O	3,520
	33360_Quinsac	44.75 N, -0.5 O	33000_Bordeaux	44.837789 N, -0.57918 O	8,853
	33440_Ambarès-et-Lagrave	44.983 N, -0.517 O	33390_Blaye	45.13333 N, -0.66667 O	22,444
Caen	14000_Caen	49.183 N, -0.35 O	14000_Caen	49.182863 N, -0.370679 O	11,027
	14170_Saint-Pierre-en-Auge	49.0 N, 0.0 E	14170_Saint-Pierre-en-Auge	49.02022 N, -0.0316086 O	4,852
	14860_Amfreville	49.25 N, -0.248 O	14150_Ouistreham	49.276656 N, -0.258658 O	15,936
	14430_Danestal	49.25 N, 0.017 E	14950_Beaumont-en-Auge	49.278363 N, 0.111144 E	27,512
	14400_Bayeux	49.277 N, -0.704 O	14400_Bayeux	49.276437 N, -0.70314 O	22,532
	27270_Saint-Aubin-du-Thenney	49.017 N, 0.5E	27300_Bernay	49.08888 N, 0.59858 E	30,614
Clermont-Ferrand	03000_Moulins	46.567 N, 3.333 E	03000_Moulins	46.568059 N, 3.334417 E	12,255
	63410_Charbonnières-les-Vieilles	46.0 N, 3.0 E	03800_Gannat	46.10015 N, 3.19886 E	31,664
	03500_Louchy-Montfand	46.317 N, 3.25 E	03500_ Saint-Pourçain-sur-Sioule	46.30927 N, 3.28787 E	26,725
	03320_Le Veurdre	46.75 N, 3.0 E	58000_Nevers	46.990896 N, 3.162845 E	25,782
	63122_Saint-Gènes-Champanelle	45.75 N, 3.017 E	63000_Clermont-Ferrand	45.777222 N, 3.087025 E	18,178

	63120_Courpière	45.75 N, 3.55 E	63120_Courpière	45.75689 N, 3.54216 E	18,261
	63740_Gelles	45.767 N, 2.75 E	63230_Saint-Ours	45.817793 N, 2.947995 E	18,059
	63320_Courgoul	45.517 N, 3.033 E	63450_Saint-Saturnin	45.660653 N, 3.090363 E	22,473
	63500_Le Broc	45.5 N, 3.25 E	63500_Issoire	45.54151 N, 3.248128 E	25,697
Nice	04120_Castellane	43.85 N, 6.517 E	04120_Castellane	43.85 N, 6.51667 E	5,755
	04120_Gorges-du-Verdon	43.7613593 N, 6.3788372 E	04120_La-Palud-sur-Verdon	43.783000 N, 6.333000 E	20,377
	04000_Digne-les-Bains	44.1 N, 6.233 E	04000_Digne-les-Bains	44.09252 N, 6.23199 E	35,130
	06130_Grasse	43.667 N, 6.917 E	06130_Grasse	43.66667 N, 6.91667 E	34,072
	06140_Vence	43.717 N, 7.117 E	06140_Vence	43.72254 N, 7.11183 E	16,624
	06250_Mougins	43.6 N, 7.0 E	06250_Mougins	43.6023319 N, 7.006491 E	18,610
	06700_Saint-Laurent-du-Var	43.667 N, 7.183 E	06700_Saint-Laurent-du-Var	43.67097 N, 7.17606 E	13,915
Rennes	35360_Landujan	48.25 N, -2.0 O	22100_Lanvallay	48.450000 N, -2.033330 O	20,409
	35520_Montreuil-le-Gast	48.25 N, -1.717 O	35630_Hédé-Bazouges	48.3 N, -1.8 O	11,064
	35340_Ercé-prés-Liffré	48.25 N, -1.5 O	35270_Combourg	48.41267 N, -1.74424 O	20,780
	35150_Amanlis	48.0N, -1.483 O	35000_Rennes	48.117266 N, -1.6777926 O	18,869
Tours	37190_Azay-le-Rideau	47.267 N, 0.467 E	37190_Azay-le-Rideau	47.26177 N, 0.46574 E	20,161
	37400_Amboise	47.417 N, 0.983 E	37400_Amboise	47.413326 N, 0.984407 E	13,491
	37190_Cheillé	47.25 N, 0.483 E	37000_Tours	47.394144 N, 0.68484 E	24,074
	37310_Sublaines	47.25 N, 1.0 E	37150_Château de Chenonceau	47.3248696 N, 1.0703005 E	17,622
	37600_Loches	47.133 N, 1.0 E	37600_Loches	47.128158 N, 0.997664 E	27,232
	41120_Candé-sur-Beuvron	47.5 N, 1.25 E	41000_Blois	47.5860921 N, 1.3359475 E	25,144

Chapter 3

Fruit of ornamental plants as early-spring resources

Chapter 3 • Objectives and main outcomes

In this chapter, I examined the use of fruit available in winter and early spring as a potential resource and catalyst for D. suzukii at the end of winter before commercial fruit become available. Females, ending their reproductive dormancy as temperature rises, seek for food in order to mature their eggs and fruit in which to oviposit. Native mistletoe, along with some ornamental plants, are among the only fruit-bearing plants at this time of the year. We predicted them to be the first to be infested by D. suzukii and, often grow in parks and areas close to crop production, to act as a reservoir for the first spring crop fruit infestations.

This experimental study was carried out in northern France between January and July 2022. Fruit of *Aucuba japonica*, *Elaeagnus* ×*submacrophylla* (syn. *Elaeagnus* ×*ebbingei*), *Mahonia aquifolium, M. japonica* and *Viscum album* were sampled monthly within a landscape window of 30 × 45 km. Microclimatic, landscape and local factors were recorded in order to assess their role in driving *D. suzukii* infestation in these fruit.

Drosophila suzukii was able to use all fruit trialled except *M. aquifolium*. It was the only drosophilid species to infest ornamental plants, but shared *V. album* fruit with *D. subobscura*. These host plant species effectively provide a resource continuum for the flies between the end of winter and start of summer. Climatic factors, landscape composition and local variables influenced *D. suzukii* infestation, with effects varying between host plants.

Based on this study, we developed a global overview of the successive challenges that *D. suzukii* must face over the seasonal cycle, shaping the dynamics of its populations subjected to winter conditions. The scheme is centred on the winter season bottleneck but also integrates the upstream processes of population initiation in autumn and the downstream consequences of winter processes on the first cultivated fruit that mature in spring. This integrated and multiscale approach to the study of *D. suzukii* winter ecology highlights the importance of understanding the winter season for the development and early application of effective pest management strategies.

Contributions

Olivier Chabrerie conceptualised the study. Field work was performed by Méghan Boulembert.

I analysed the data, produced the graphics and tables. I wrote the original draft, revised the manuscript according to reviewers' comments and wrote the response letter to the editor and reviewers.

All co-authors contributed to the revision of the manuscript.

Winter fleshy-fruited plants are the catalysts for spring populations of an invasive fruit fly

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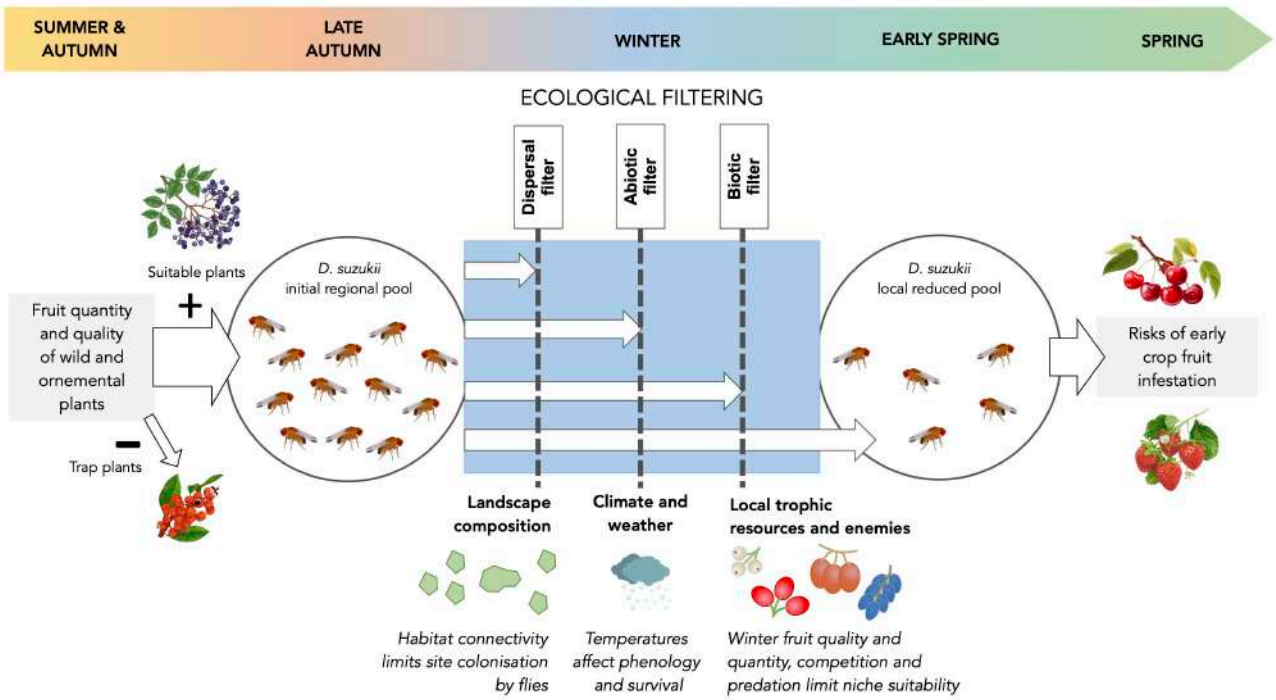
GRAPHICAL ABSTRACT



ABSTRACT

1. Winter is a critical bottleneck period for many insects and is particularly pivotal for pest species, being a major regulator of their populations. In winter, insect fruit pests such as the invasive fly, *Drosophila suzukii*, not only have to cope with harsh environmental conditions but also need to find alternative resources when cultivated fruit are scarce.
2. This study aimed to disentangle the relative roles of climatic, landscape and local factors affecting fruit infestation rates of winter and spring host plants by *D. suzukii*. We assessed infestation in *Aucuba japonica*, *Elaeagnus* ×*submacrophylla* (syn. *Elaeagnus* ×*ebbingei*), *Mahonia aquifolium, M. japonica* and *Viscum album* fruit in the north of France, between January and July 2022.
3. *Drosophila suzukii* emerged from fruit of all plant species except *M. aquifolium*. The fly was the only species that emerged from fruit of *A. japonica*, *E.* ×*submacrophylla* and *M. japonica*, but co-occurred in *V. album* fruit with *D. subobscura*.
4. Climatic factors (e.g., cumulative precipitation, the number of frost hours), landscape composition (e.g., grassland, shrubland and water cover) and local variables (e.g., resource abundance, vegetation architecture) influenced *D. suzukii* infestation, with effects varying between host plants.
5. The fruiting succession of these five plants effectively provides a resource continuum from March to June, in which *E.* ×*submacrophylla*, in particular, plays a pivotal role before commercial fruit become available (e.g., strawberries, cherries). Non-crop plants should be considered in integrative pest management strategies as they could catalyse *D. suzukii* populations early in the season.

Keywords: insect pest; biological invasion; mistletoe; *Viscum album*; non-crop host plants; *Drosophila suzukii*; *Drosophila subobscura*; landscape

INTRODUCTION

Winter is a critical bottleneck period for most temperate insects (Danks 1978; Moore and Lee 1991). Driven by the harsh environmental conditions, many species overwinter by entering diapause (Bale and Hayward 2010; Gill et al. 2017; Lehmann et al. 2017), during which development is slow or suspended, do not feed or reproduce and rely on reserves accumulated in the pre-diapause phase to survive (Storey and Storey 2012; Sinclair 2015; Gill et al. 2017). Other species, however, continue to be active throughout winter (Danks 1978; Moore and Lee 1991; Wharton 2011). These species must find resources in order to survive and sometimes to complete their development. For species relying on seasonal fruit, this can be a challenge, as they will likely need to be polyphagous, switching between different resources over time (Kennedy and Storer 2000; Butterworth et al. 2023). Winter is also critical for insect pest species, as it plays a role in population regulation (Kennedy and Storer 2000; Gajski et al. 2023; Cuff et al. 2024). Insect fruit pests that are able to survive the harsh environmental conditions of winter, avoid predation and find alternative resources when cultivated fruit are scarce, are likely to gain an advantage at the beginning of the growth season. In this study we examine some elements of this hypothesis in the widespread pest species, *Drosophila suzukii* (Matsumura, 1931).

Drosophila suzukii is an invasive fruit fly and a pest worldwide (Asplen et al. 2015). Originating in Asia, it was first reported in Europe in 2008, followed by very rapid range expansion (Walsh et al. 2011; Cini et al. 2012; Asplen et al. 2015). While winter is undeniably challenging for its survival (Hamby et al. 2016; Stockton et al. 2019; Ulmer et al. 2024), *D. suzukii* shows remarkable behavioural and physiological adaptations (Tait et al. 2021). With the onset of winter, it is able to switch from cultivated fruit to the late maturing fruit of wild and/or non-native plants (Poyet et al. 2014, 2015; Lee et al. 2015; Kenis et al. 2016; Ulmer et al. 2022), at the same time changing its distribution in the landscape, finding sheltered microhabitats in woodland and hedgerows (Rossi-Stacconi et al. 2016; Tait et al. 2021; Ulmer et al. 2024). The adult fly's circadian rhythm changes, with maximum activity at mid-day, when temperatures are generally warmest, thereby reducing the risk of exposure to extreme cold (Shaw et al. 2019). Winter temperatures and short photoperiod also induce a winter morphotype which shows increased cold tolerance (Panel et al. 2020). Nevertheless, finding suitable host plants on which to feed and reproduce during winter remains a major challenge for *D. suzukii* (Stockton et al. 2019).

In natural habitats, wild fruit provide food and allow the persistence of seed populations of *D. suzukii* between winter and late spring, enabling the establishment of new generations (Poyet et al. 2015;

Panel et al. 2018), before cultivated fruit become available as hosts. Nutrition is a critical driver of cold tolerance in *Drosophila* species (Henry et al. 2020; Jiménez-Padilla et al. 2020). Protein-rich diets can enhance the melanization response (Lee et al. 2008) which would help the reparation process and protect tissues after cold exposure (Sinclair et al. 2013). Specific amino-acids such as proline and arginine are also known to have a cryoprotective effect in *D. melanogaster* (Koštál et al. 2012, 2016). Finally, sugar-rich diets can improve resistance to starvation, by increasing the lipid content of the fly (Ballard et al. 2008), thus enabling flies to survive longer when resources are scarce (Henry et al. 2020). Consequently, availability of winter fruit resources may be an important determinant of *D. suzukii* persistence in winter. However, not only do few plant species bear fruit in winter and early spring, but only part of them are known to be susceptible to *D. suzukii* infestation. In Europe, these include *Aucuba japonica, Elaeagnus* ×*submacrophylla, Hedera helix, Lonicera nitida, Mahonia* spp., *Rosa canina, Skimmia japonica, Symphoricarpos* spp. and *Viscum album* (Grassi et al. 2018; Panel et al. 2018; Poyet et al. 2015; Kenis et al. 2016). The phenology of these plants, however, varies tremendously.

Among these winter plants susceptible to *D. suzukii*, *Aucuba japonica*, *E.* ×*submacrophylla*, *Mahonia* spp. and *V. album* fruit are among the most heavily infested in laboratory experiments and allow both larval development and a higher rate of adult emergence than from any other plant (Poyet et al. 2015). *Aucuba japonica* (Thunb.), *Mahonia japonica* (Thunb.) DC. and *E.* ×*submacrophylla* (Servett) (syn. *E.* ×*ebbingei* (J.Door)) are native to Asia while *M. aquifolium* (Thunb.) is native to North America (Prior 1881; Bayton 2017; Edwards 2017). All are introduced ornamental shrubs that have long been planted in urban parks, partly because of their resistance to harsh environmental conditions (e.g., pollution, wind exposure; Prior 1881; Coklar and Akbulut 2017; Edwards 2017; Abdalla 2019; Blanuša et al. 2020; Quadir et al. 2021). *Viscum album* L., 1753 (mistletoe) is an obligate hemiparasitic shrub native to Europe that grows on a wide range of angiosperm and gymnosperm trees (Barney et al. 1998; Zuber 2004; Thomas et al. 2023). All these plants can be found from winter to spring, with overlapping periods of fruiting (Terabayashi 1978; Abe 2001; Francis 2004; Edwards 2017; Thomas et al. 2023).

Most of these fruit contain compounds that are toxic to insects (e.g., aucubin; Rombouts and Links 1956), but *D. suzukii* seems to be able to overcome at least some of these plant species' defences. Poyet et al. (2015) confirmed that *D. suzukii* can develop on several toxic plants and that it even uses toxic compounds to perform medication when in the presence of parasitoids (Poyet et al. 2017). Knowledge of the detoxification processes involved is lacking and research on which compounds *D. suzukii* is able to

overcome could help improve pest management strategies. Although chemical (Asplen et al. 2015; Dam et al. 2019; Tait et al. 2021) and biological (Iacovone et al. 2015; Ulmer et al. 2020, 2021) control strategies have been and are still widely explored, knowledge relating to the phenology and ecology of *D. suzukii* species in natural systems is limited and is required to better predict and possibly control the risks of early infestation in cultivated crops. In particular, the species' population dynamics at the end of winter, several months before the fruiting period of most commercially cultivated plants, are largely unknown.

This study aimed to disentangle the relative roles of climatic, landscape and local factors that could affect infestation rates of winter host plants by *D. suzukii*. We focused on *A. japonica*, *E.* ×*submacrophylla*, *M. aquifolium*, *M. japonica* agg. and *V. album* because they are known to be susceptible to *D. suzukii* and are representatives of the very limited group of plant species bearing fruit in winter and early spring. We hypothesised that (i) *D. suzukii* infestation rates would differ between plant species as they produce different types of fruit varying in quantity, quality and phenology; (ii) *D. suzukii* presence in wild fruit would be positively associated with the abundance of natural habitats such as woodland which are known to provide a winter refuge for *Drosophila* species (Basden 1954), and specifically for *D. suzukii* (Ulmer et al. 2024); (iii) environmental factors controlling fruit infestation may vary between plant species differing in their ecology and fruiting phenology; and (iv) these non-crop host plants could support overwintering seed populations of *D. suzukii*, catalysing the development of new generations in early spring in advance of the availability of the first commercial fruit crops such as cherries, strawberries or raspberries. To test these hypotheses, we measured fruit infestation rates in these four plant species in northern France from winter to summer. Each sample was accompanied by measurements of climatic and local and landscape variables in order to identify the environmental drivers of host fruit infestations.

MATERIALS AND METHODS

Study area

The study was conducted between January and July 2022 in the region of Amiens (49°53′40″ N, 2°18′07″ E) in northern France. The region's climate is oceanic with a mean annual temperature of 10.7°C and an average annual rainfall of 691.9 mm (data from meteorological station Dury-les-Amiens, StatIC network). The landscape of this region is generally characterised by agricultural production and consists

of a mosaic of open fields cultivated for cereals, rapeseed and sugar beet, interspersed with orchards, grasslands, woodland patches and rivers.

Sampling design

Aucuba japonica var. *variegata*, *Elaeagnus* ×*submacrophylla*, *Mahonia aquifolium*, *Mahonia japonica* agg. (including a complex of related cultivars and hybrids) and *V. album* fruit were sampled every month, with individual plants of each species randomly sampled within a landscape area of 35 × 45 km (Fig S1). The number of individuals sampled per species and per month varied depending on fruit availability (from 8 to 119 individuals, see Table 1 and results below). When possible, a minimum of 100 fruit were randomly sampled from each plant individual, with some variation depending on seasonal availability of fruit.

Traits of host plant species and fruit

Collected fruit were separated into three subsets to monitor *Drosophila* emergence: undamaged ('healthy') and damaged fruit collected on the plant ('damaged') and on the ground ('ground'; if present). The fruits were categorized as 'damaged' when they were opened / injured (incised skin) and/or rotten (brown spots). Several traits of the sampled plants were measured to characterise the local resources available for the flies and the local microhabitat. For each sampled plant, five berries were randomly selected to measure length and width and calculate volume ($4/3 \times \pi \times \text{mean radius}^3$) and fruit skin area ($4 \times \pi \times \text{mean radius}^2$). Five leaves were also taken, their length and width measured and the leaf surface index (length × width; Ulmer et al. 2022) calculated. Individual morphology of each plant species was characterised by measuring the minimum and maximum plant canopy diameter, the circumference of the largest trunk (except for *V. album*) and the total number of fruit present on the plant. Mistletoe being a parasitic shrub, we also recorded the host tree species and measured the height of the mistletoe individual on the tree (from the ground), the tree height, the crown diameter, the trunk circumference, and the number of mistletoe individuals present on the host tree and in a 20 m radius around it. Variables, units and codes are listed in Table S1.

Environmental variables

Local, landscape and climatic variables were measured at each sampling site or extracted from online databases to examine the influence of regional and local environmental conditions on infestation rates

(Table S1).

Local environmental conditions were described as follows. First, within a 5 m radius plot centred on the sampled plant, the cover and height of the tree, shrub and herbaceous layers were estimated, as well as soil litter thickness (Table S2). Second, within a 20 m radius, the percentage of local habitats surrounding the host was recorded (e.g., orchard, woodland, grassland, swamp, crop, garden, shrub, building, hedgerow, river, pond, poplar plantation, park, road; see Table S1), as well as the percentage of other plant species with maturing fruit.

The landscape composition around each sampled plant was then characterised. A geographic database was created using a Geographic Information System (GIS; ArcGIS Pro v.2.5, ESRI). The sampled plants were positioned in the GIS and buffers of 50, 100, 250, 500, 750, 1000, 1250, 1500, 1750, 2000, 2500 and 3000 m radii around each host tree were created for subsequent analyses of landscape composition. Landscape elements (crop, water, woodland, shrubland, grassland, road, urban area, orchard, industrial zone) were extracted from the OSO 2022 database (THEIA 2023) and updated using aerial photographs and, in buffers <100 m, field observations.

Macroclimatic conditions were characterised for each sampling site using regional measurements. Daily meteorological data were retrieved from the three meteorological stations closest to each site, from 1 January 2022 to each sampling date (https://www.historique-meteo.net/france/; details in Table S2). Daily minimum, mean and maximum temperatures, rainfall and snowfall were calculated for all sites using inverse-distance weighting (IDW) interpolation (Willmott et al. 1985) from the data from the three nearest weather stations (Table S2). Accumulated degree-days ("Growing Degree Days", GDD) were calculated using a lower threshold of 0°C between 1 January 2022 and the sampling date (Baskerville and Emin 1969). The baseline value of 0°C is a standard threshold commonly used to calculate GDD in insect and plant studies (White et al. 2012; McNeil et al. 2020). It is particularly suitable to study the temporal synchrony between insects and plant resources (Iler et al. 2013; Ulmer et al. 2022). It was also chosen because (i) active *D. suzukii* can be observed even at very low positive temperature (< 5°C) during winter, including during periods of snowfall (Ulmer et al. 2024), (ii) flies are able to recover from chill coma after exposure to −1°C (Wallingford et al. 2016) and (iii) mistletoe fruit can undergo freeze-thaw cycles before ripening ends (Thomas et al. 2023). From daily precipitation values, we also calculated mean daily and cumulative precipitation between 1 January 2022 and each sampling date, and within the 7- or 14-days periods preceding each sampling date.

Microclimate temperatures were recorded at each sampling site using Hobo loggers (TIDBIT data logger V2 TEMP TBI-001, ONSET Company, Bourne MA, USA), recording every 60 min. In each sampling site a logger was suspended 1.5 m above the ground in the plant canopy, under the shade of a branch to avoid direct exposure to solar radiation and oriented northward. The minimum, mean and maximum air temperatures were extracted every day to compute the mean daily minimum, mean and maximum temperatures. As described for macroclimate data, GDD were calculated using Hobo logger data to characterise the local microclimate under the canopy of the sampled plants.

Emergence of *Drosophila* species

After collection, the fruit sampled from each plant were placed on wet cotton wool in cylindrical plastic transparent containers (diameter = 118 mm, height = 135 mm, volume = 1,476 cm^3), covered with a nylon mesh, and maintained in a temperature-controlled room at 20°C under a 16:8 L:D regime. Adult flies emerging from the fruit were placed in 70% ethanol. They were identified to species level using Bächli et al. (2004) and specific criteria published for *D. suzukii* (Withers and Allemand 2012). Individuals of each species were sexed and counted using a Leica M205C stereomicroscope equipped with a Leica MC170 HD camera and the Leica Application Suite software.

Infestation variables

We examined the relationships between environmental variables and two common infestation variables (Benavídez et al. 2021) that were either centred on the fruit (Fruit Infestation Rate: FIR = 100 × number of emerged *Drosophila* individuals from fruit collected from a given plant individual / total number of fruit collected from the same plant individual) or on the plant species (Plant Infestation Rate: PIR = 100 × number of infested plant individuals of a species in a month / total number of plant individuals of this same species sampled in a month). These variables can be interpreted as follows: FIR reflects the plant auto-contamination by the flies while PIR reflects fly dispersal between host plant individuals (e.g., when the FIR and PIR are both high, both auto-contamination within the plant individual and dispersal of the flies between plant individuals take place; when the FIR is high and the PIR is low, there is mostly plant auto-contamination; when the FIR is low and the PIR high, there is mostly fly dispersal; when both FIR and PIR are low, there is an absence of both auto-contamination and dispersal). These infestation variables were calculated for each fruit category (healthy or damaged fruit on the plant and fallen fruit on the ground) following Deconninck et al. (2024b - Chapter 1).

Statistical analyses

For each of the two *Drosophila* species (*D. suzukii* and *D. subobscura*) that emerged from the samples, we tested for the influence of environmental variables (fruit and plant morphology, local abiotic conditions, habitat composition in the surrounding landscape and climatic variables) on FIR using mixed models (GLMMs) with host plant species included as a random effect in models to identify environmental variables responsible for fly infestations whatever the host plant species considered. Then, to identify environmental variables that specifically influenced infestation of each plant species, generalized linear models (GLMs) were built for each of the host plant species that were infested by *D. suzukii*. For infestation of *V. album*, we used mixed models (GLMMs) with host tree species parasitised by *V. album* included as a random effect to identify environmental variables responsible for fly infestations whatever host tree of *V. album* considered. For both *D. suzukii* and *D. subobscura* models, preselection of explanatory variables was made by using the variable that most strongly correlated (Pearson correlation) with fruit infestation rates across the spatial scales assessed (buffers from 5 m to 3,000 m radius around each sampling point), as recommended by Ulmer et al. (2022). A backward selection of explanatory variables and the second-order Akaike information criterion (AICc; Hurvich and Tsai 1989) were used to select the most parsimonious model, i.e. the model with lowest AICc. Homoscedasticity was checked using biplots of residuals and model predictions. We used Kruskal-Wallis tests to test whether the FIR varied across months or between host plant species. *Post-hoc* pairwise comparisons between months were then performed using Mann-Whitney U tests. All statistical analyses were performed using SPSS version 24.0 (IBM Corporation).

RESULTS

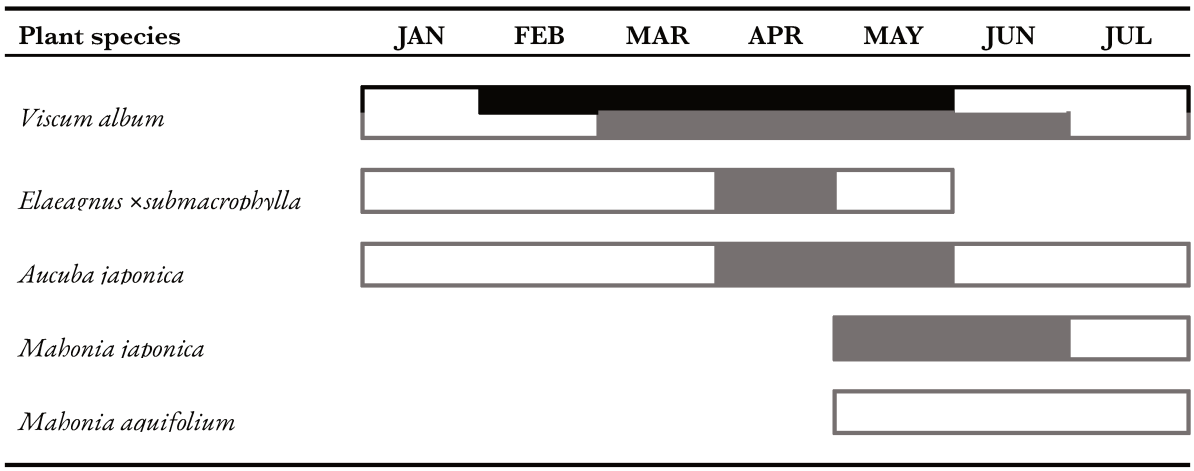
Availability of fruit resources across winter and spring

Between January and July 2022, totals of 1385, 1357, 690, 2280 and 16,632 fruit were sampled from 43, 25, 8, 17 and 119 plants of *A. japonica*, *E.×submacrophylla*, *M. aquifolium*, *M. japonica* and *V. album*, respectively (Table 1). Plant phenology differed between species (Table 2). *Aucuba japonica* and *V. album* bore ripe fruit throughout the entire sampling period, *E. ×submacrophylla* bore fruit from January to May, and *Mahonia* spp. only from May to July.

Table 1 Number of plant individuals sampled in the study area between January and July 2022 with number of fruit sampled, total number of emerged *Drosophila* individuals, mean ± SE and max FIR and PIR. Fruit Infestation Rate: FIR = 100 × number of emerged *Drosophila* individuals from all fruit collected from a given plant individual / number of fruit collected from the same plant individual. Plant Infestation Rate: PIR = 100 × number of infested plant individuals in the study area / number of sampled plant individuals in the study area.

			D. suzukii				*D. subobscura*				*D. repleta*
Plant species	**No plant**	**No fruit**	**No emerged**	**Mean FIR ± SE (%)**	**Max FIR (%)**	**PIR (%)**	**No emerged**	**Mean FIR ± SE (%)**	**Max FIR (%)**	**PIR (%)**	**No emerged**
Aucuba japonica	43	1385	24	0.81 ± 2.32	10.00	13.95	0	0.00	0.00	0.00	0
Elaeagnus ×submacrophylla	25	1357	392	17.68 ± 38.74	146.97	32.00	0	0.00	0.00	0.00	0
Mahonia aquifolium	8	690	0	0.00	0.00	0.00	0	0.00	0.00	0.00	0
Mahonia japonica	17	2280	4	0.30 ± 1.07	4.41	11.76	0	0.00	0.00	0.00	0
Viscum album	119	16232	216	1.46 ± 5.87	42.55	18.49	27	0.23 ± 1.58	16.67	6.72	1
Total	**212**	**21944**	**636**	**3.09 ± 14.83**			**27**	**0.13 ± 1.19**			**1**

Table 2 Fruit seasonality of winter and spring host plant species of *D. suzukii* (grey) and *D. subobscura* (black) in the study area in 2022. Filled and empty bars correspond to months when adult flies emerged from fruit and to months when ripe fruit were present and sampled but no flies emerged, respectively.



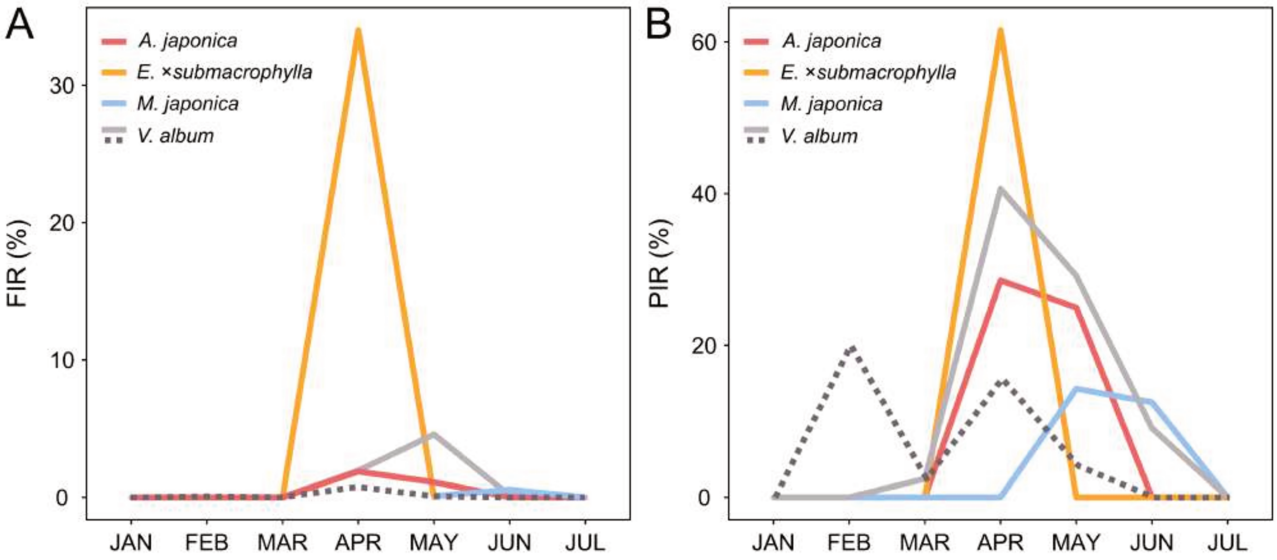


Figure 1 Variation of fruit infestation rate (FIR) (A) and plant infestation rate (PIR) (B) of *Aucuba japonica*, *Elaeagnus ×submacrophylla*, *Mahonia japonica* and *Viscum album* by *Drosophila suzukii* (solid line) and *D. subobscura* (dotted line) between January and July 2022. Fruit Infestation Rate: FIR = 100 × number of emerged *Drosophila* individuals from all fruit collected from a plant individual / number of fruit collected from the same plant individual. Plant Infestation Rate: PIR = 100 × number of infested plant individuals in a month / number of sampled plant individuals in the same month.

Host plant fruit infestation

Between January and July 2022, a total of 636 *D. suzukii*, 27 *D. subobscura*, and 1 *D. repleta* emerged from all the sampled fruit (Table 1). No flies emerged from *M. aquifolium* fruit. *Drosophila subobscura* was only found in *V. album* fruit, with a mean FIR of 0.23 ± 1.58%. Fruit of *E.* ×*submacrophylla* were the most heavily infested by *D. suzukii* (FIR = 17.68 ± 38.74%), followed by *V. album*, *A. japonica* and *M. japonica*, in decreasing order. Plant infestation rate (PIR) ranged between 11.76% and 32.00%, consistent with the FIR pattern, with *E.*×*submacrophylla* plants having the highest PIR.

Seasonality in host plant fruit infestation

Although some fruit were available from January, fruit fly infestation began only in February (Table 2, Figure 1). *Viscum album* fruit were the earliest to be infested, first by *D. subobscura* from February to May, then by *D. suzukii* from March to June. The FIR of *V. album* fruit remained low (mean < 2%), with highest values of 16.67% for *D. subobscura* and 42.55% for *D. suzukii* (Table 1). PIR was relatively high, 18.49% for the entire study, and increasing to 40.63% for *D. suzukii* in April (Figure 1B). Infestations of *A. japonica* and *E.*×*submacrophylla* by *D. suzukii* started in April (Table 2, Figure 1A, B). *Aucuba japonica* fruit were used until May, with a low FIR below 10.00%, but a relatively high global PIR of 13.95% (Table 1), peaking at 28.57% in April (Figure 1B). *Drosophila suzukii* that emerged from *E.*×*submacrophylla* fruit were only found in fruit collected in April (Table 2; Figure 1) but showed the highest FIR (146.97%; i.e., the number of emerging flies was ~1.5× greater than the number of collected fruit in a sample; Table 1) and PIR (32.00%; Table 1, Figure 1B) compared to the other species (Kruskal-Wallis test on FIR, χ^2 = 7.00, p = 0.030). Finally, *M. japonica* was used in May and June by *D. suzukii*, with low FIR values of 0.61% and 4.41%, respectively, and the lowest PIR of 11.76% (Table 1).

Environmental variables correlating with fruit infestation

The quality of the fruit from which *D. suzukii* emerged ('healthy', 'damaged', or 'ground') differed significantly according to the host plant species (Kruskal-Wallis test, χ^2 = 323.42, p < 0.0001; Figure 2). In *M. japonica*, adult flies only emerged from healthy fruit collected on the plant. In *A. japonica*, flies emerged only from fruit collected on the plant, both healthy (54%) and damaged (46%). In *V. album*, adult flies emerged from healthy fruit on the plant (43%) and from fruit on the ground (57%). Finally, in

Table 3 Effect of environmental variables on fruit infestation rate (FIR) by *Drosophila suzukii* analysed by GLMM. Host plant species were used as a random factor. Fruit Infestation Rate: FIR = 100 × number of emerged *Drosophila suzukii* individuals from all fruit collected from a host plant individual / number of fruit collected from the same host plant individual.

		Model parameters						
Dependent variables	**Explanatory variable**	***F***	**Estimates**	**SE**	**d.f.**	***t***	***p***	**AICC**
Fruit Infestation Rate (FIR) by *D. suzukii* (log10, *n* = 204 plants individual sampled, random factor = plant species)	Model constant	54.061	0.4729	0.0643	198.00	7.353	<0.001	206.97
	RAIN7J	11.574	-0.0524	0.0154	198.00	-3.402	0.001	
	NFH7Jh	12.890	-0.0109	0.0030	198.00	-3.590	<0.001	
	GRASS20	9.282	-0.0033	0.0011	198.00	-3.047	0.003	
	WATE100	17.461	0.0265	0.0063	198.00	4.179	<0.001	
	HETH3000	13.896	-5.8668	1.5738	198.00	-3.728	<0.001	

Note: RAIN7J: cumulative precipitation in the last 7 days, NFH7Jh: number of frost hours in the last 7 days, GRASS20: cover of grassland in a radius of 20 m, WATE100: cover of water in a radius of 100 m, HETH3000: cover of shrub land in a 3 km radius.

Table 4 Effect of environmental variables on fruit infestation rate (FIR) of each host plant species (*Aucuba japonica*, *Elaeagnus ×submacrophylla*, *Mahonia japonica* and *Viscum album*) by *Drosophila suzukii* analysed by GLMs.

		Model parameters					
Dependent variables	**Explanatory variable**	**Wald χ^2**	**Estimates**	**SE**	**d.f.**	***p***	**AICC**
Fruit Infestation Rate (FIR) of *Aucuba japonica* by *D. suzukii* (log10, n = 43 plant individuals sampled)	Model constant	0.385	0.058	0.0929	38	0.535	-11.539
	SOIL	17.247	0.011	0.0027	38	<0.001	
	GRASS20	23.771	-0.009	0.0019	38	<0.001	
	RP_FC	26.718	0.320	0.0619	38	<0.001	
	WATE250	14.152	-0.151	0.0401	38	<0.001	
Fruit Infestation Rate (FIR) of *Elaeagnus ×submacrophylla* by *D. suzukii* (log10, n = 25 plant individuals sampled)	Model constant	50.310	1.141	0.1608	21	<0.001	35.000
	NFD14Js	19.659	-0.190	0.0428	21	<0.001	
	RAIN7J	45.122	-0.327	0.0487	21	<0.001	
	ROAD250	7.019	0.290	0.1095	21	0.008	
Fruit Infestation Rate (FIR) of *Mahonia japonica* by *D. suzukii* (log10, n = 17 plant individuals sampled)	Model constant	5.802	0.752	0.3122	15	0.016	-7.337
	STRUNK	5.053	-0.051	0.0228	15	0.025	

Note: SOIL: cover of bare soil in a radius of 10 m, GRASS20: cover of grassland in a radius of 20 m, RP_FC: richness of freshly-fruited plant species in a radius of 20 m, WATE250: cover of water in a radius of 250 m, NFD14Js: number of frost days in the last 14 days, RAIN7J: cumulative precipitation in the last 7 days, ROAD250: cover of road in a radius of 250 m, STRUNK: trunk circumference.

E. ×*submacrophylla*, flies emerged from fruit sampled either on the plant (88%, comprising healthy (17%) or damaged (71%)), or on the ground (12%; Figure 2).

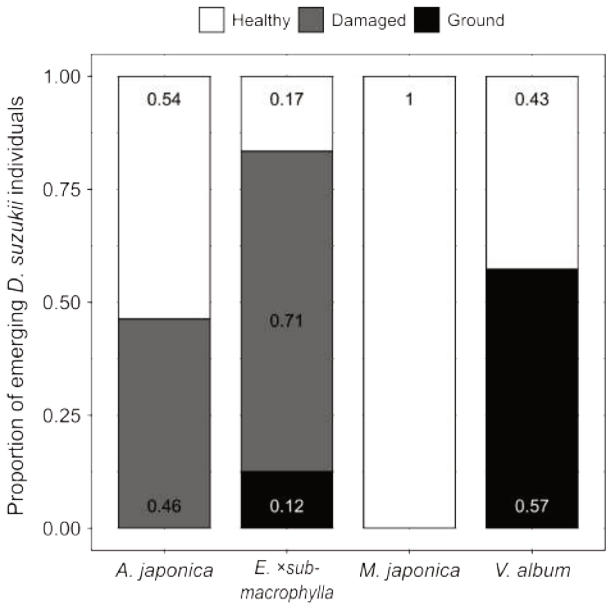


Figure 2 Proportion of *Drosophila suzukii* individuals emerging from the different fruit categories of *Aucuba japonica* (n = 1385), *Elaeagnus* ×*submacrophylla* (n = 1357), *Mahonia japonica* (n = 2280) and *Viscum album* (n = 16,232) between January and July 2022, in relation to their position and health status: healthy on the plant (Healthy), damaged on the plant (Damaged) or fallen on the ground (Ground).

Independent of the host plant species, *D. suzukii* fruit infestation was negatively associated with the cumulative precipitation and the number of frost hours recorded by Hobo microclimatic sensors in the 7 d preceding fruit sampling, with the cover of grassland in a radius of 20 m, and with the cover of shrubland in a 3 km radius; infestation was positively associated with the area of water in a radius of 100 m (GLMM, Table 3). Environmental variables affecting *D. suzukii* FIR differed between plant species (GLM, Table 4; GLMM, Table 5). In *A. japonica*, the cover of bare soil in a radius of 10 m and the richness of freshly-fruited plant species in a radius of 20 m had a positive association, while the cover of grassland in a radius of 20 m and the area of water in a radius of 250 m had a negative association (GLM, Table 4). In *E.* ×*submacrophylla*, the number of frost days recorded by weather stations in the preceding 14 d and the cumulative precipitation in the preceding 7 d were negatively associated with *D. suzukii* FIR, while the cover of road surface in a radius of 250 m had a positive association (GLM, Table 4). In *M. japonica*, only plant trunk circumference was associated with *D. suzukii* FIR, with a negative correlation (GLM, Table 4). In *V. album*, *D. suzukii* FIR was positively associated with the percentage of fruit collected on the ground, the mean leaf area index of *V. album* and the cumulative precipitation between 1 January 2022 and the sampling date (GLMM, Table 5). The cover of poplars (POPLARP20; R = 0.292; p = 0.001) and hedges (HEDGE20; R = 0.252; p = 0.006) in a radius of 20 m were positively associated with *D. suzukii* infestation in *V. album,* although the variables were not retained in the final model. Finally, *D.*

Table 5 Effect of environmental variables on fruit infestation rate (FIR) by *Drosophila suzukii* and *D. subobscura* in *Viscum album* analysed by GLMM. Host tree species parasitised by *Viscum album* was used as a random factor (see Table S3 and Fig S2 for details). Fruit Infestation Rate: FIR = 100 × number of emerged *Drosophila suzukii* individuals from all fruit collected from a *Viscum album* individual / number of fruit collected from the same *Viscum album* individual.

		Model parameters						
Dependent variables	**Explanatory variable**	***F***	**Estimates**	**SE**	**d.f.**	***t***	***p***	**AICC**
Fruit Infestation Rate (FIR) of *Viscum album* by *D. suzukii* (log10, n = 119 host plants)	Model constant	28.678	-0.601	0.1123	20.676	-5.355	<0.001	58.013
	GROUTOT	18.794	0.006	0.0015	113.961	4.335	<0.001	
	MLSURF	20.111	0.050	0.0112	13.968	4.485	0.001	
	RAIN_010122	20.844	0.007	0.0016	111.239	4.566	<0.001	
Fruit Infestation Rate (FIR) of *Viscum album* by *D. subobscura* (log10, n = 119 host plants)	Model constant	4.422	0.059	0.028	115	2.103	0.038	-107.704
	HSHRUB	4.866	-0.015	0.007	115	-2.206	0.029	
	POPLARP20	4.986	0.003	0.002	115	2.233	0.027	
	SNOW14J	11.748	0.088	0.026	115	3.428	0.001	

Note: GROUTOT: % of fruit collected on the ground, MLSURF: mean leaf area index of *Viscum album*, RAIN_010122: cumulative precipitation between 01/01/22 and the sampling date, HSHRUB: mean shrub height in a radius of 5 m, POPLARP20: % of poplar grove in a radius of 20 m, SNOW14J: cumulative snow precipitation in the last 14 days.

subobscura infestation in *V. album* fruit was positively associated with the cover of poplar in a radius of 20 m and the cumulative snow precipitation in the preceding 14 d, and negatively associated with the mean shrub height in a 5 m radius (GLMM, Table 5).

Microclimatic variables also correlated with *D. suzukii* FIR, but were not retained in the final models after the backward selection of competing explanatory factors. For example, FIR of *V. album* was positively correlated with the highest hourly maximum temperature (MAXMAX7Jh; R = 0.293; $p < 0.001$) and with Growing Degree Days (GDDM7Jh; R = 0.276; $p = 0.002$) recorded by Hobo loggers during the 7 d preceding fruit sampling. FIR of *A. japonica* was negatively correlated with the number of frost days recorded by weather stations during the 14 d preceding fruit sampling (NFD14Js; R = −0.350; $p = 0.022$) but it was not retained in the final model. Finally, recent weather metrics, i.e. temperatures during the preceding two weeks, were more correlated with infestation than the overall climate of the entire study period, i.e. the cumulative temperature (GDD) calculated from 1 January 2022, which showed no significant correlation with FIR.

DISCUSSION

Our study demonstrates that *D. suzukii* is able to use the vernal fruit of the plant species *A. japonica*, *E. ×submacrophylla*, *M. japonica* and *V. album* in the latter part of winter and throughout spring, confirming that these plants are likely to help the fly to overcome the winter bottleneck induced by low temperatures and fruit resource scarcity. Here, we (i) address how multi-scale environmental factors influence *Drosophila* infestation, (ii) discuss the role of ornamental and non-crop plant species as catalysts for the development of spring *D. suzukii* populations, and (iii) summarise the hierarchy of ecological filters influencing the dynamics of *D. suzukii* populations in winter, highlighting its value in the context of early pest management strategies.

Climate and weather effects on fruit infestation

Several climatic variables correlated with *D. suzukii* infestation. These variables acted at different temporal scales (cumulative climate of the year *versus* weather in the weeks immediately preceding sampling) and spatial scales (regional macroclimate *versus* sub-canopy microclimate). Cumulative precipitation and the number of frost hours locally measured under the canopy of the host plants in the week preceding sampling had an overall negative correlation with infestation. Rain is known to limit the flight ability of flies (Dickerson et al. 2014) and frost may have induced mortality (Ryan et al. 2016). The association between climatic variables and *D. suzukii* infestation differed between plant species. *Aucuba japonica* and *M. japonica* infestations were less associated with climatic variables than with the other environment features (i.e., by landscape composition or local conditions). These ornamental species are often planted in gardens close to houses and parks in an urbanised environment, which may have protected them from extreme low temperatures through the "heat island effect" (Oke 1973; Meineke et al. 2017), making climatic variations a secondary factor in the explanation of the FIR. *Elaeagnus ×submacrophylla* infestation was negatively correlated with frost in the 14 days preceding sampling and with rain in the previous week. Fruit of *E.×submacrophylla* are soft and very sensitive to rot (pers. obs. reported in an experimental study: Larges et al. 2023); the alternation of frost followed by rain may have caused damage to fruit (Ladaniya 2015), thereby accelerating the rotting process (Rodrigo 2000) and reducing fruit suitability for *D. suzukii*, which prefers ripening and ripe fruit (Walsh et al. 2011; Atallah et al. 2014). *Drosophila subobscura* infestation in *V. album* was positively associated with cumulative snowfall in the 14 days preceding sampling. This is consistent with *D. subobscura* ecology and phenology, given that

it is a cold tolerant species and often found in winter (David et al. 2003; Delbac et al. 2020), when it can even be dominant (Deconninck et al. 2024b - Chapter 1). Snow may also provide an ephemeral shelter against freezing for the flies, as reported for some other arthropods (Aitchison 2001; Pauli et al. 2013). Surprisingly, precipitation had contrasting effects on *D. suzukii* infestation between *E.×submacrophylla* and *V. album* fruit. Precipitation in the previous week (i.e., on a short-term scale) had a negative correlation with *D. suzukii* infestation in *E. ×submacrophylla* fruit, while cumulative precipitation from the beginning of the year to the sampling date (i.e., on a long-term scale for an insect) positively correlated with *D. suzukii* infestation in *V. album* fruit. The first precipitation variable (RAIN7J; see Table 3), reflecting the most recent weather conditions, besides limiting the fly's flight ability, may also favour the rot of *E. ×submacrophylla* fruit, as noted above. Conversely, the second precipitation variable (RAIN_01012022; see Table 5) reflects the regional climate and/or the progression of seasons; at this longer temporal scale, rain would have had a positive impact on plant phenology, growth and fruit production (Vergotti et al. 2019), increasing the number of ripe fruit available for *D. suzukii* on *V. album* plants. These contrasting correlations of climatic variables with infestation over different timescales also means that single-scale climatic models are insufficient to predict infestation rates, and that other variables related to the ecology of the focal insect and of the host plant identity need to be considered. Finally, it is notable that recent weather conditions were more associated with infestation than the cumulative climate experienced from the start of the year, as also shown by Wiman et al. (2016). This suggests that the end of the winter reproductive diapause in flies could be triggered more strongly by short-term spring heat peaks than by the accumulation of heat since the beginning of the year, in contrast to that of plants (Zavalloni et al. 2006). This also highlights an ongoing need to identify the true cues, and their timing, controlling *Drosophila* entry into and exit from diapause (Numata and Shintani 2023). The use of macroclimate alone in predictive models would tend to mask the reality of microclimate and weather variations, potentially explaining the discrepancies between predictions and actual population densities. Thus, annual measures of temperature variation may be more appropriate to model the infestation of summer fruit (Dalton et al. 2011; Kamiyama et al. 2020), but we suggest that winter fruit infestation may be better predicted by shorter timescale warming events occurring between winter and spring.

Association between landscape and local factors and infestation differ according to host plant species

Habitats and landscape elements that correlated with overall *D. suzukii* infestations were the

grassland, water and shrubland covers. Local grass cover (in a 20 m radius) negatively correlated with the infestations. This result is consistent with those of Santoiemma et al. (2019) showing that during winter, at lower temperatures, *D. suzukii* had a greater density in forest than in grassland. The presence of mown grassland under or near the fruiting plants, frequently observed in our sites, was also identified as an efficient management tool reducing infestation (Santoiemma et al. 2020). Grasslands also host predatory species, including macroarthropods such as spiders, centipedes or ants (Ulmer et al. 2021), which may control *D. suzukii* overwintering populations (Ulmer et al. 2021; Perrot et al. 2023). Surprisingly, the cover of shrubland at a large spatial scale (in a 3 km radius) also negatively correlated with infestations. Shrubland is an excellent overwintering area for *D. suzukii* as it includes several fruiting shrubs which provide both food resources and shelter (Panel et al. 2018; Tait et al. 2021; Ulmer et al. 2024). Thus, the presence of large shrub patches in the landscape mosaic may cause a dilution effect on the fly's density. Shrubland and other wooded habitats are used as a winter refuge by the flies and represent sink-habitats for their population during the cold season (Santoiemma et al. 2019; Ulmer et al. 2024). They may thus compete in terms of attractiveness to flies with our sampling sites, reducing the numbers present in the latter (see Ulmer et al. 2024 showing changes in the fly distribution between preferred habitats during the cold seasons). Finally, the area of water within a 100 m radius was positively correlated with infestation. This may be an indirect consequence of water provisioning for the preferred host plants of *D. suzukii* (Predieri et al. 2003), further illustrating the influence of landscape structure on infestation.

The association between landscape elements and infestation was also modulated by the plant species. *Aucuba japonica* infestations were more strongly correlated with local variables than with landscape variables: the cover of bare soil in a 10 m radius and the richness of freshly-fruited plant species in a radius of 20 m positively correlated with *D. suzukii* infestation of this species, while the cover of grass in a 20 m radius and water area in a 250 m radius were negatively correlated with infestation. Bare soil, primarily a result of local trampling or even ploughing, is an indicator of anthropogenic impacts (Previati et al. 2007). *Aucuba japonica* is mainly found in gardens where soil is frequently and intensively disturbed by owners for the management of their ornamental plants. Our findings are consistent with those of Rossi-Stacconi et al. (2016), confirming the affinity of *D. suzukii* for anthropogenic habitats. Here, freshly-fruited species around the sampled plant may have provided a food resource and shelter for *D. suzukii*, increasing its population in the sampling area and inducing greater infestation of *A. japonica* fruit. Begon (1975) similarly showed that the density of berries of several wild plant species in an area

increased the success of breeding in *D. subobscura*. Ulmer et al. (2024) also demonstrated that the local diversity in fruit-bearing plant species promoted *D. suzukii* populations. The negative association between infestation and grass cover in this instance may be analogous to that described above for overall *D. suzukii* infestation, suggesting that flies avoid open areas and short vegetation in winter. However, the negative association between water area and *A. japonica* infestation contrasts with its overall positive association with infestation across all plant species, demonstrating that the same environmental factor may have divergent effects on different plant species. *Aucuba japonica* is commonly planted taking advantage of its very high resistance to drought (Percival and Sheriffs 2002) and it is negatively impacted by fluvial flooding in its natural range (Kamisako et al. 2007). Thus, excessive water in its environment (from the Somme valley in our study) contrasts with its natural ecological niche and performance, including fruit production. Water is also commonly associated with fog in our study area, particularly in the Somme valley. This may, in turn, have been associated with higher humidity and colder temperatures which could negatively impact *D. suzukii*, as fog alters flight abilities in insects with thin wings (Dickerson et al. 2015). Furthermore, increased water area within the landscape area inevitably results in decreased vegetation cover, which may reduce the spatial opportunities for *D. suzukii* to find resources as shown by Ulmer et al. (2024).

In *E.* ×*submacrophylla*, *D. suzukii* infestation was positively associated with the road area in a 250 m radius. Roads are an indicator of urbanisation and *D. suzukii* is known to be an anthropogenically-associated species whose occurrence has been correlated with the local area of buildings (Deconninck et al. 2024b - Chapter 1) and presence of artificial habitats (Rossi-Staconi et al. 2016). In *V. album*, the presence of poplar plantations and hedges in a 20 m radius positively correlated with infestation. As an important mistletoe host tree species (Briggs 2021), and the most heavily infested by *D. suzukii* (Deconninck et al. 2024a - Chapter 2), the presence of surrounding poplars increases the density of available fruit for *D. suzukii*, increasing the chances of breeding by the flies as shown by Begon (1975). Finally, *M. japonica* infestation by *D. suzukii* were not associated with landscape elements, but exclusively and negatively correlated with the sampled plant trunk circumference. When young, *M. japonica* plants are bushy, compact and close to the ground but, as they age and if they are not pruned, the stems become larger, erect and their foliage becomes much sparser basally (Silver 2023). This architecture of older *Mahonia* plants would be unfavourable for *D. suzukii*, which relies on shelter within the shrub canopy close to the ground (Evans et al. 2017).

Drosophila subobscura infestations in *V. album* negatively correlated with the mean height of shrubs in a 5 m radius and positively correlated with the cover of poplar plantation in a 20 m radius, as noted above for *D. suzukii*. Argañaraz et al. (2018) showed that a vertical structure of vegetation at least up to 1 m in height contributes to higher richness and abundance of insect predators in urban green areas, potentially explaining the negative association between vegetation height and *D. subobscura* infestation. As *D. subobscura* mainly infests fruit on the ground, this same feature of local vegetation architecture may have also masked the presence of small fallen fruit of *V. album* to the fly. Conversely, as *V. album* is frequently found on poplars (Briggs 2021), the cover of poplar plantation might indicate the size of the mistletoe reservoir available for *D. subobscura*, as this species is known to overwinter in mistletoe (Deconninck et al. 2024a - Chapter 2).

In *V. album*, the percentage of fruit collected on the ground and the mean leaf area were positively associated with *D. suzukii* infestation. The former measure has already been reported to positively correlated both with *D. suzukii* and *D. subobscura* infestation in *V. album* at a larger national scale (Deconninck et al. 2024a - Chapter 2). In addition, the combination of low FIR and high PIR for *V. album* highlights the importance of dispersal over auto-contamination, suggesting that this resource attracts flies from a distance. The leaf surface area of a *V. album* individual is a proxy for the compactness of the mistletoe individual, which forms a more or less dense ball of evergreen leaves and branches. This compactness varies considerably over climatic gradients and with mistletoe age, and inter-individual differences can be important within a region. The denser an individual (i.e., greater leaf surface area), the more it is protected from wind influence, even if the host tree itself is bare. This may create a favourable microhabitat for *D. suzukii*, explaining the positive correlation of this plant trait with infestations. *Viscum album* provides both a food resource and shelter for *D. suzukii*, as do the dense evergreen leaves of *Hedera helix* (ivy) in winter (Ulmer et al. 2024). Future research should explore the role of plant architecture in the maintenance of winter *D. suzukii* populations, as has previously been surveyed in blackberry field for summer *D. suzukii* infestations (Diepenbrock and Burrack 2016), or even in more details for other species (e.g., see Pincebourde et al. 2007). Overall, the role of plant architecture on plant-insect interactions during winter remains understudied.

Trade-off between auto-contamination and dispersal depending on the host plant

The FIR (autocontamination) / PIR (dispersal) ratio differed between host plant species. This ratio represents the trade-off between intra- and inter-individual host plant infestation within a plant species.

When FIR > PIR (i.e., mean fruit infestation rate of a host plant species is higher than the infestation rate of the individuals within a population of this same host plant species), the fly could be opting for a strategy of auto-contaminating the fruit within a host plant individual, rather than dispersing to new sites to infest other host plant individuals, as observed in *D. suzukii* infesting *E. ×submacrophylla* and *V. album* (Max FIR > PIR; Table 1). In contrast, a FIR < PIR (i.e., infestation rate of individuals within a population of a host plant species is higher than the mean fruit infestation rate of this same host plant) can suggest that flies disperse between individuals of the same plant species, rather than auto-contaminating a single plant individual, as observed in *D. suzukii* infesting *A. japonica* and *M. japonica*. These contrasts between host plants could result from varying fruit quality and indicate a modulation of the fly's choices to adapt to the variable developmental success of its offspring in these fruits. *Elaeagnus ×submacrophylla* and *V. album* exhibited a high FIR / PIR ratio and *D. suzukii* is known to perform well in these species in terms of larval development (Poyet et al. 2015; Panel et al. 2018; Deconninck et al. 2024a - Chapter 2). However, when developing on *A. japonica*, *D. suzukii* shows higher mortality and a longer developmental time compared to the previous two species (Panel et al. 2018; Poyet et al. 2018). No data are available on *M. japonica* utilisation by *D. suzukii* but a similar pattern to that of *A. japonica* could be expected because of similar fruit characteristics.

Our findings suggest that dispersal is favoured by the flies when the host plant is of low quality. Dispersal is well-known in insects (Clobert et al. 2009), allowing avoidance of environmental stressors and access to seasonally-available habitats (Asplen 2018) or ephemeral resources (Butterworth et al. 2023). In the case of *D. suzukii*, dispersal allows the exploitation of spatially and temporally variable fruit resources. However, increased levels of mobility also impose costs in terms of survival and/or reproductive success (Chapman et al. 2015). Therefore, we suggest that *D. suzukii* individuals will tend to leave their fruit/host plants (i.e., Max FIR < PIR) only when their quality is low (illustrated by the case of *A. japonica* in our study). The fruit quality could also be assessed by measuring the fruit penetration force, which may influence the fruit susceptibility to *D. suzukii* (Burrack et al. 2013). At a daily scale, flies are able to move between neighbouring habitats, driven by temperature, humidity and the availability of alternative hosts (Tait et al. 2020), limited to distances below 100 m (experimental measures from Vacas et al. (2019)). However, long-distance dispersal at a regional scale of up to 9,000 m has also been recorded (Tait et al. 2018), while passive dispersal over multiple generations of the species over more than 1,400 km in one year has been documented (Vacas et al. 2019). In the current study, landscape influenced *D. suzukii*

infestation within a radius of up to 250 m, consistent with previous research. *Drosophila suzukii* dispersal ability is hard to evaluate, mainly because of the difficulty to follow individuals in nature (Klick et al. 2016), even if marking-recapture techniques were successfully applied (Klick et al. 2015, 2016; Clymans et al. 2020). However, studying the role of climate and landscape features (e.g., barriers) on active flight performance, as well as passive dispersal, could improve our understanding of the fly's use of resources and its potential for range expansion.

Ornamental and wild plants as catalysts of rapid population expansion of *D. suzukii* in spring?

In recent years, the ecological impacts of the ornamental plant trade, its important economic value (Oxford Economics 2018), have received much less attention than the impacts of the commodity exchange (Hinsley et al. 2023). Ornamental horticulture is a major pathway for plant invasions, but also co-transports associated exotic microbes and invertebrate species, including pests (Hu et al. 2023). These already serious biosecurity risks are further exacerbated by contemporary climate change (Tubby and Webber 2010; Hinsley et al. 2023). For instance, in Portugal, 13 alien species were discovered associated with allochthonous ornamental plants, including several pest species (Bella 2013). Most of the species reported by Bella (2013) were specialists, limiting the risk of their further successful dispersal, but this limitation may not apply or be so stringent in the case of generalist species such as *D. suzukii*. Notably, most species in our study, with the exception of *V. album*, are ornamental plants. The only plant species not infested by *D. suzukii* in our study was *M. aquifolium* (but see Poyet et al. (2015), who reported low levels of infestation in this species) and we provide the first record of infestation in *M. japonica*. The highest infestation rate was observed in *E.* ×*submacrophylla*, whose fruit availability takes over from *V. album* and *A. japonica* fruit in April and, in turn, are succeeded by commercial strawberries and cherries. We suggest that the presence of *E.* ×*submacrophylla* in the vicinity of commercial crop sites gives this plant species a pivotal role in the early initiation of *D. suzukii* infestation in cultivated fruit. To limit the risk of *D. suzukii* infestation in spring, pruning of *E.* ×*submacrophylla* could be carried out after flowering in the autumn (Edwards 2017), which would reduce the amount of fruit formed.

Ecological filtering of *D. suzukii* populations throughout winter

Figure 3 illustrates a global summary of the successive challenges that *D. suzukii* must face over the seasonal cycle and which, in particular, shape the dynamics of its populations subjected to winter conditions. Based on existing conceptual studies of species assembly rules in plant communities linked to

environmental filtering (Grime 1998; Rahel 2002; Lortie et al. 2004; Lake et al. 2007; Kraft et al. 2015; Germain et al. 2018), this theoretical framework is adapted here to insects at the population scale. It summarises the hierarchy of ecological filters identified as influencing fruit infestation by *D. suzukii* in the present study and in other relevant studies (Poyet et al. 2014, 2015; Ulmer et al. 2020, 2021, 2022, 2024; Deconninck et al. 2024b - Chapter 1). Here, environmental filtering refers to ecological factors that prevent the establishment and/or persistence of *D. suzukii* individuals in a particular location. The large set of influencing factors are grouped into three types of ecological filter (i.e., dispersal, abiotic and biotic filters; Rahel 2002; Lortie et al. 2004) that act at different spatial and temporal scales and may affect the fly's migration, activity, fitness and survival. The scheme is centred on the winter season bottleneck but also integrates the upstream processes of population formation in autumn and the downstream consequences of winter processes on the first cultivated fruit that mature in spring.

Regional *D. suzukii* populations increase throughout the summer months, reaching a maximum in late autumn (Ulmer et al. 2024) coincident with the peak in diversity and abundance of fruit that are compatible with the fly's physiological requirements (peak in October in temperate regions; Poyet et al. 2014, 2015; Ulmer et al. 2022). In nature, about half of fruit bearing plants produce fruit that can be utilised by *D. suzukii* (Poyet et al. 2015). However, some usually rare fruit in which the fly's development is impaired, named dead-end trap fruit (Ulmer et al. 2020), may reduce its population in autumn (Ulmer et al. 2020; Fragnière et al. 2024). *Drosophila suzukii* may also suffer from competition with other Drosophilidae in late cultivated fruit, such as apples (Deconninck et al. 2024b - Chapter 1). Nevertheless, globally, autumn-maturing fruit generate a large population of *D. suzukii* (Poyet et al. 2014), primarily winter morphs (Panel et al. 2018; Ulmer et al. 2024) that are ready to face the winter season. This initial large pool of overwintering adult flies is then subjected to three ecological filters during winter before the first commercial fruit ripen in late spring or early summer. First, only a portion reaches locations harbouring suitable winter-available fruit. This first "dispersal filter" immediately reduces the number of arriving *D. suzukii* individuals, depending on landscape variables favouring or limiting access to fruit sites and affecting connectivity between semi-natural habitats –that provide winter shelters (e.g., forests, hedges, anthropogenic habitats; Rossi-Stacconi et al. 2016)– and sites that provide spring fruit. The risk of fruit infestation increases with the extent of wooded habitats available in the surrounding landscape (Pelton et al. 2016; Urbaneja-Bernat et al. 2020; Ulmer et al. 2022). For individuals that successfully pass this first filter, they then face two other ecological filters, abiotic and biotic.

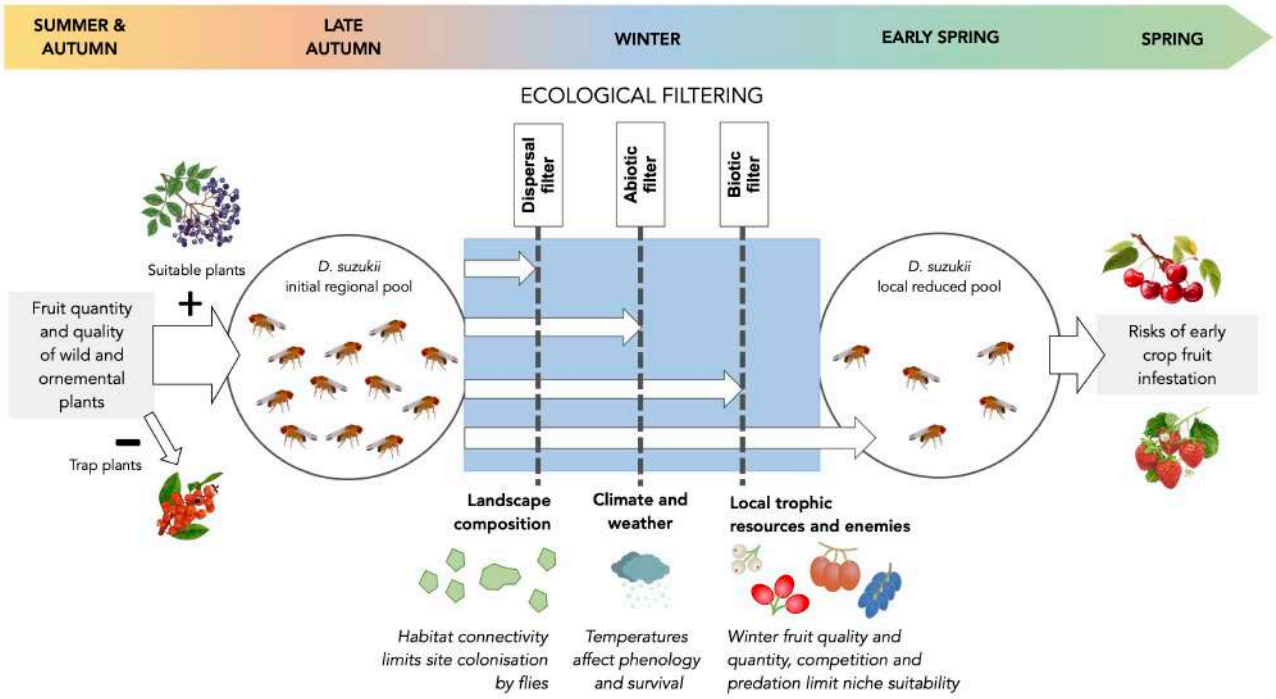


Figure 3 Conceptual summary of the successive ecological challenges that *Drosophila suzukii* must face over the seasonal cycle and which, in particular, shape the dynamics of populations subjected to winter conditions. It provides an organised view of the hierarchy of ecological filters ("environmental filtering", adapted from theories in plant ecology: Lake et al. 2007; Lortie et al. 2004; Rahel 2002) influencing vernal fruit infestations by *D. suzukii* and preceding the arrival of the earliest commercial fruit (e.g., strawberries, cherries). First, in autumn, an initial large pool of *D. suzukii* individuals is produced as the result of emergences from a high diversity of summer and autumnal fruit (Poyet et al. 2015) present in various habitats of the region (even if some of the fruit are not favourable to the fly, Ulmer et al. 2020). Second, a dispersal filter determines the sub-pool of *D. suzukii* individuals (among the autumnal regional pool) which arrives at a particular site, depending principally on the abundance of semi-natural (forests and hedgerows) or anthropic habitats in the surrounding landscape (Deconninck et al. 2024a - Chapter 2). Third, abiotic factors, mainly climatic conditions (e.g., frost, cumulative temperature, microclimatic refuges, recent rain events...), affect flies's activity (e.g., end of winter diapause, flight and oviposition) and survival in each local site. Fourth, biotic filtering can occur when a fly arrives at a focal site but fails to lay eggs, produce offspring and persist, depending on biotic interactions with local plant (i.e., types of fruit, their availability and suitability) and animal diversity (e.g., potential presence of enemies such as predators (Ulmer et al. 2021) or competitors (Deconninck et al. 2024b - Chapter 1)). Finally, the legacy of these multiscale ecological filters results in a reduced pool of *D. suzukii* at the end of winter (Ulmer et al. 2024). This spring fly population emerging from vernal fruit (*Elaeagnus* ×*submacrophylla*, *Viscum album*, *Aucuba japonica*, *Mahonia japonica*) is the main source for infestations of the first fruit crops (Panel et al. 2018).

During winter and the immediate post-winter period, the abiotic filter preventing fruit infestation by *D. suzukii*, is primarily composed of macroclimatic and microclimatic variables. Macroclimatic variables, as measured by free-air meteorological stations at a regional scale over several months from the beginning of the year, determine the female's ability to start producing eggs and oviposit. Female *D. suzukii* experience a reproductive diapause in winter (Rossi-Stacconi et al. 2016) when their ovaries become atrophied (Zerulla et al. 2015; Langille et al. 2016; Toxopeus et al. 2016; Wiman et al. 2016). The timing or cues for the end of this diapause period in nature are unclear. However, post-diapause, the ovaries become mature as early as March in our study area (Ulmer 2022), so that fruit attacks by flies can start at the very end of winter. Local microclimatic conditions differ from those of the regional macroclimate (Suggitt et al. 2011) because of the shading and transpiration provided by trees and shrubs (Geiger et al. 2003). Beneath the tree and shrub canopy, temperature extremes are moderated, with higher minimum and lower maximum air temperatures (Gril et al. 2023b), as shown in Fig S3. Conversely, air temperature extremes can be amplified near the ground in open or sparse vegetation (with lower minima and higher maxima; Gril et al. 2023a), as shown in Fig S3. Soil/litter surface temperatures can be amplified to an even greater extent (Pincebourde and Salle 2020). Thus, during winter, the phenology of flies and fruit in open areas can be delayed by the microclimatic amplification of cold, while it can be advanced in warmer sites protected by a forest canopy (Pelton et al. 2016).

Finally, local biotic filters must be surpassed. In particular, predation must be avoided (Siffert et al. 2021; Ulmer et al. 2021), most likely through habitat choice, as suggested by the influence of the grass cover in a radius of 20 m for *D. suzukii* and the mean shrub height in a radius of 5 m for *D. subobscura*. They may also face competition for available fruit resources with birds (Briggs 2011). Having overcome these filters, infestation success will then depend on the quantity and quality of suitable fruit (Figure 2), which varies depending on the plant species.

This integrated and multiscale approach to the study of *D. suzukii* winter ecology highlights the importance of understanding the winter season for the development and early application of effective pest management strategies. The identification and quantification in time and space of winter host plants in regions of commercial production of early maturing fruit will provide an important first step in the control of this pest. Avoiding the inclusion of specific non-native ornamental plants in local hedgerows and garden/shrub areas in the vicinity of fruit farms will also provide a benefit to producers. We further recommend pruning these exotic fruit sources in autumn after flowering. Beyond the direct economic

benefits this would achieve for fruit producers, such actions could also limit bird dispersal competition between native and non-native plants (Aslan and Rejmanek 2012).

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Table S1 List of the variables assessed in the study. Abbreviations: h: local air temperature measurement from HOBO, s: measurement from weather station, 7J and 14J: measurements taken 7 and 14 days before fruit collection, P/A: Presence/Absence. The columns 'Ecological relevance' and 'Expected correlation' give information about what was the expected link between the potential explanatory variables and the fruit infestation by *Drosophila suzukii*.

CATEGORY	CODE	DESCRIPTION	UNIT	ECOLOGICAL RELEVANCE	EXPECTED CORRELATION
		DROSOPHILIDAE SPECIES			
INFESTATION INDICES	FIR	Fruit Infestation Rate = 100 × number of emerged *Drosophila* individuals from fruit collected from target species individual / total number of fruit collected from the same target species individual	%		
	PIR	Plant Infestation Rate = 100 × number of infested target species individuals in a region or month / total number of target species individuals sampled in the same region or month	%		
		FRUIT CATEGORIES			
NUMBER	HEAFRUIT	Number of healthy fruit collected on a plant individual	Number	Developmental substrate	+
	DAMFRUIT	Number of damaged fruit (rotten or with open skin) collected on a plant individual	Number	Developmental substrate	+
	PLAFRUIT	Sum of healthy and damaged fruit collected on a plant individual	Number	Developmental substrate	+
	GROFRUIT	Number of fruit collected on the ground under the canopy of a plant individual	Number	Developmental substrate	-
	TOTFRUIT	Sum of fruit collected on the canopy and on the ground of a plant individual	Number	Developmental substrate	+
PERCENTAGE	%HEALPLA	100 × HEAFRUIT / PLAFRUIT	%	Resource quality	+
	%DAMAPLA	100 × DAMFRUIT / PLAFRUIT	%	Resource quality	+
	%HEALTOT	100 × HEAFRUIT / TOTFRUIT	%	Resource quality	+
	%DAMATOT	100 × DAMFRUIT / TOTFRUIT	%	Resource quality	+
	%FPLANT	100 × PLAFRUIT / TOTFRUIT	%	Resource quality	+
	%GROUTOT	100 × GROFRUIT / TOTFRUIT	%	Resource quality	-

Table S1 (Continued)

CATEGORY	CODE	DESCRIPTION	UNIT	ECOLOGICAL RELEVANCE	EXPECTED CORRELATION
LIFE TRAITS OF THE D. SUZUKII'S HOST PLANT INDIVIDUALS					
FRUIT TRAITS	MFWIDTH	Mean fruit diameter (n = 5 fruit measured)	cm	Resource quantity	+
	MFVOL	Mean fruit volume ($4/3 \times \pi \times$ mean radius^3) (n = 5 fruit measured)	cm^3	Resource quantity	+
	MFSURF	Mean fruit skin area ($4 \times \pi \times$ mean radius^2) (n = 5 fruit measured)	cm^2	Resource quantity	+
LEAVE TRAITS	MLLENGTH	Mean leaf length (from the basis of the petiole to the top/apex of the blade; n = 5 leaves measured)	cm	Protection against weather disturbances	+
	MLWIDTH	Mean leaf width (n = 5 leaves measured)	cm	Protection against weather disturbances	+
	MLSURF	Mean leaf area index (area index = length × width; n = 5 leaves measured)	cm^2	Protection against weather disturbances	+
INDIVIDUAL MORPHOLGY	LENGTH	Maximum diameter of the target species individual	m	Attractiveness / shelter	+
	WIDTH	Minimum diameter of the target species individual	m	Attractiveness / shelter	+
	STRUNK	Circumference of the largest trunk (not measured for *Viscum album* individuals)	cm	Attractiveness / resource permanence	+
	INDFRUITS	Total number of fruit present on a sampled target species individual	Number	Resource quantity	+
	HEIGHT	Height of *Viscum album* individual from the ground	m	Resource accessibility	+/-
MISTLETOE HOST TREE					
MISTLETOE HOST TREE	HOSTSPE	*Viscum album* host species	Species		
	HHEIGHT	Height of the host tree	m	Attractiveness / resource accessibility	+/-
	HLENGH	Maximum diameter of the host tree canopy	m	Attractiveness / shelter	+
	HWIDTH	Minimum diameter of the host tree canopy	m	Attractiveness / shelter	+

Table S1 (Continued)

CATEGORY	CODE	DESCRIPTION	UNIT	ECOLOGICAL RELEVANCE	EXPECTED CORRELATION
		MISTLETOE HOST TREE			
MISTLETOE HOST TREE	HTRUNK	Circumference of the host tree trunk	cm	Attractiveness / resource permanence	+
	HFRUITS	Number of fleshy fruit of the host tree	Number	Resource quantity	+
	HMISTLETOE	Number of *Viscum album* individuals on the host tree	Number	Resource quantity	+
	HMISTLETOE20	Number of *Viscum album* individuals in a 20 m radius around the host tree	Number	Resource quantity	+
	SUMHMIST20	Sum of *Viscum album* individuals on the host tree and in a 20 m radius around the host tree	Number	Resource quantity	+
		SITE AND LANDSCAPE CHARACTERISTICS			
LOCAL ENVIRONMENT (in a 5 m radius around the mistletoe host tree)	ALTM	Site altitude	m		+/-
	SLOM	Site slope	Degree		+/-
	CTREE	Cover of tree layer (height > 8 m)	%	Shelter	+
	CSHRUB	Cover of shrub layer (1-8 m)	%	Shelter or resource / predator reservoir	+/-
	CHERB	Cover of herb layer (< 1 m)	%	Shelter / predator reservoir	+/-
	HHERBMEAN	Height of herbaceous layer	cm	Shelter / predator reservoir	+/-
	HLIGN	Height of ligneous species (shrubs, trees and lianas)	m	Shelter / predator reservoir	+/-
	COMP	Cover of biowaste composter	%	Resource	+
	SOIL	Cover of bare soil	%	Reduced presence of potential resource / anthropogenic disturbance indicator	-
	DWOOD	Cover of dead wood debris on the ground	%	Shelter	+

Table S1 (Continued)

CATEGORY	CODE	DESCRIPTION	UNIT	ECOLOGICAL RELEVANCE	EXPECTED CORRELATION
		SITE AND LANDSCAPE CHARACTERISTICS			
LOCAL ENVIRONMENT (in a 5 m radius around the mistletoe host tree)	TLITTER	Depth of leaf litter	cm	Shelter	+
	LITTER	Cover of leaf litter	%	Shelter	+
	RIVER	Cover of stream	%	Reduced presence of potential resource / water availability	+/-
	POND	Cover of pond	%	Reduced presence of potential resource / water availability	+/-
	PATH	Cover of (unpaved) pathway	%	Reduced presence of potential resource	-
	ROAD	Cover of road and paved surface	%	Reduced presence of potential resource	-
	BUILD	Cover of building	%	Shelter / anthropization	+/-
LOCAL HABITATS (in a 20 m radius around the mistletoe host tree)	ORC20	Cover of orchard area (fruit trees and their associated grassland)	%	Resource reservoir	+
	FOREST20	Cover of woodland	%	Shelter / predator reservoir	+/-
	PASTURE20	Cover of grazed grassland	%	Shelter / predator reservoir	+/-
	MOW20	Cover of mown grassland	%	Shelter / predator reservoir	+/-
	GRASS20	Cover of grassland (PASTURE20+MOW20)	%	Shelter / predator reservoir	+/-
	SWAMP20	Cover of swamp	%	Reduced presence of potential resource	-
	CROP20	Cover of conventional agriculture crop	%	Resource reservoir / pesticide risk	+/-

Table S1 (Continued)

CATEGORY	CODE	DESCRIPTION	UNIT	ECOLOGICAL RELEVANCE	EXPECTED CORRELATION
		SITE AND LANDSCAPE CHARACTERISTICS			
LOCAL HABITATS (in a 20 m radius around the mistletoe host tree)	VEGGAR20	Cover of market garden	%	Shelter or resource reservoir	+
	GARDEN20	Cover of private garden	%	Shelter or resource reservoir	+
	SHRUB	Cover of shrub	%	Shelter or resource / predator reservoir	+/-
	BUILD20	Cover of building	%	Shelter / anthropization	+/-
	HEDGE20	Cover of hedgerow	%	Shelter or resource / predator reservoir	+/-
	RIVER20	Cover of river	%	Reduced presence of potential resource / water availability	+/-
	POND20	Cover of pond	%	Reduced presence of potential resource / water availability	+/-
	POPLARP20	Cover of poplar plantation	%	Resource reservoir	+
	GREESP20	Cover of urban green space (small urban planted herbaceous or shrub strips)	%	Shelter or resource / predator reservoir	+/-
	PARK20	Cover of park	%	Shelter or resource / predator reservoir	+/-
	GGSP20	Total urban vegetated area (sum of garden, green space and park cover)	%	Shelter or resource / predator reservoir	+/-
	RP_FC	Fleshy-fruited plant species richness	Number	Resource reservoir	+

Table S1 (Continued)

CATEGORY	CODE	DESCRIPTION	UNIT	ECOLOGICAL RELEVANCE	EXPECTED CORRELATION
SITE AND LANDSCAPE CHARACTERISTICS					
LANDSCAPE COMPOSITION (from OSO 2022 data basis; cover percentages calculated within the following radii around the mistletoe host tree: 10 m, 20 m, 50 m, 100 m, 250 m, 500 m, 750 m, 1000 m, 1250 m, 1500 m, 1750 m, 2000 m, 2500 m and 3000 m)	CROP%	Cover of conventional agriculture crop	%	Resourced reservoir / pesticide risk	+/-
	WATE%	Cover of river and other water surfaces	%	Reduced presence of potential resource / water availability	+/-
	FORE%	Cover of woodland	%	Shelter or resource / predator reservoir	+/-
	HETH%	Cover of heathland	%	Shelter or resource / predator reservoir	+/-
	MOW%	Cover of grassland	%	Shelter or resource / predator reservoir	+/-
	ROAD%	Cover of road and paved surface	%	Anthropization	-
	URB%	Cover of urban area	%	Shelter / anthropization	+/-
	ORC%	Cover of orchard area (fruit trees and their associated grassland)	%	Resource	+
	INDU%	Cover of industrial area	%	Anthropization	-
METEOROLOGY					
HOURLY METEOROLOGY (hour scale, from h: HOBO data)	GDH7J	Sum of positive temperature hours (Growth Degree Hours) in the 7 days before sampling	°C	Larval development and adult activity	+
	SMTH7J	Sum of negative temperature hours in the 7 days before sampling	°C	Larval development and adult activity	+
	NFH7J	Number of hours with negative temperatures (≤0 °C) (Number of freezing hours) in the 7 days before sampling	Number	Larval development and adult activity	-
	MINTH7J	Minimum temperature in the 7 days before sampling	°C	Larval development and adult activity	+

Table S1 (Continued)

CATEGORY	CODE	DESCRIPTION	UNIT	ECOLOGICAL RELEVANCE	EXPECTED CORRELATION
		METEOROLOGY			
HOURLY METEOROLOGY (hour scale, from h: HOBO data)	MAXTH7J	Maximum temperature in the 7 days before sampling	°C	Larval development and adult activity	+
	MEANTH7J	Mean temperature over the 7 days before sampling	°C	Larval development and adult activity	+
DAILY METEOROLOGY (day scale, from h: HOBO data, s: weather stations data; meanings of suffixes added to variable names: 7J = data from the 7 days preceding fruit sampling, 14J = data from the 14 days preceding fruit sampling)	GDDMC	Sum of daily positive temperature (Growth Degree Days) calculated from the mean temperatures of the 7 or 14 days before fruit sampling	°C	Larval development and adult activity	+
	NFD	Number of days with negative temperature (≤ 0°C) during the 7 or 14 days before fruit sampling	Number	Larval development and adult activity	-
	RAIN	Sum of daily precipitation in the 7 or 14 days before the fruit sampling	cm	Plant growth / adult activity	+/-
	SNOW	Sum of daily snowfall in the 7 or 14 days before the fruit sampling	cm	Larval development and adult activity	-
	MMIN	Mean minimum temperature during the 7 and 14 days before fruit sampling	°C	Larval development and adult activity	+
	MMAX	Mean maximum temperature during the 7 days before fruit sampling	°C	Larval development and adult activity	+
	MMEANCALC	Calculated mean of daily mean temperatures of the 7 or 14 days before the fruit sampling	°C	Larval development and adult activity	+
	MINMIN	Lowest minimum temperature during the 7 or 14 days before fruit sampling	°C	Larval development and adult activity	+
	MAXMAX	Highest maximum temperature during the 7 or 14 days before fruit sampling	°C	Larval development and adult activity	+
	SUMT_010122	Sum of daily positive temperature (Growth Degree Days) between 01/01/2022 and the sampling date	°C	Plant growth, seasonal progression / adult activity	+
	RAIN_010122	Sum of rain precipitation between 01/01/2022 and the sampling date	cm	Plant growth, seasonal progression / adult activity	+/-

Table S2 Weather station information. Geographic area: city of the research laboratories, station city: postcode and the municipality name of the weather station location, Station coordinates: geographic location of the city where the weather station is located, Label city: postcode and municipality name according to Historique-Météo.net, Label city coordinates: geographic location of the label city, Mean distance: mean distance between the weather station and sampled sites (in kilometers). Geographic coordinate system: WGS 84.

Geographic area	Station city	Station city coordinates	Label city	Label city coordinates	Mean distance (m)
Amiens	80260_Flesselles	50.0 N, 2.25 E	80600_Doullens	50.15724 N, 2.34019 E	16,288
	80160_Essertaux	49.75 N, 2.25 E	80000_Amiens	49.894067 N, 2.295753 E	17,516
	80890_Condé-Folie	50.017 N, 2.017 E	80420_Flixecourt	50.01465 N, 2.08095 E	22,404
	80300_Albert	50.0 N, 2.65 E	80300_Albert	50.00091 N, 2.65096 E	28,868
	80140_Doudelainville	50.0 N, 1.767 E	80100_Abbeville	50.105467 N, 1.836833 E	30,264
	60130_Angivillers	49.5 N, 2.5 E	80500_Montdidier	49.65 N, 2.56667 E	29,046

Table S3 Host trees of *Viscum album* individuals sampled in the study and mean FIR ± SE (%) by *D. suzukii* (*Dsuz*) and *D. subobscura* (*Dsub*).

Species	Code	Number	%	Dsuz mean FIR ± SE (%)	Dsub mean FIR ± SE (%)
Acer campestre	ACECAM	6	5.0%	0.00 ± 0.00	0.00 ± 0.00
Acer sp.	ACER	3	2.5%	0.00 ± 0.00	0.00 ± 0.00
Crataegus monogyna	CRAMON	59	49.6%	0.33 ± 0.15	0.08 ± 0.04
Malus domestica	MALDOM	7	5.9%	0.00 ± 0.00	0.00 ± 0.00
Populus nigra	POPNIG	33	27.7%	4.41 ± 1.83	0.69 ± 0.52
Robinia pseudoacacia	ROBSPE	11	9.2%	0.81 ± 0.74	0.00 ± 0.00
TOTAL		119	100.0%	1.46 ± 0.54	0.23 ± 0.15

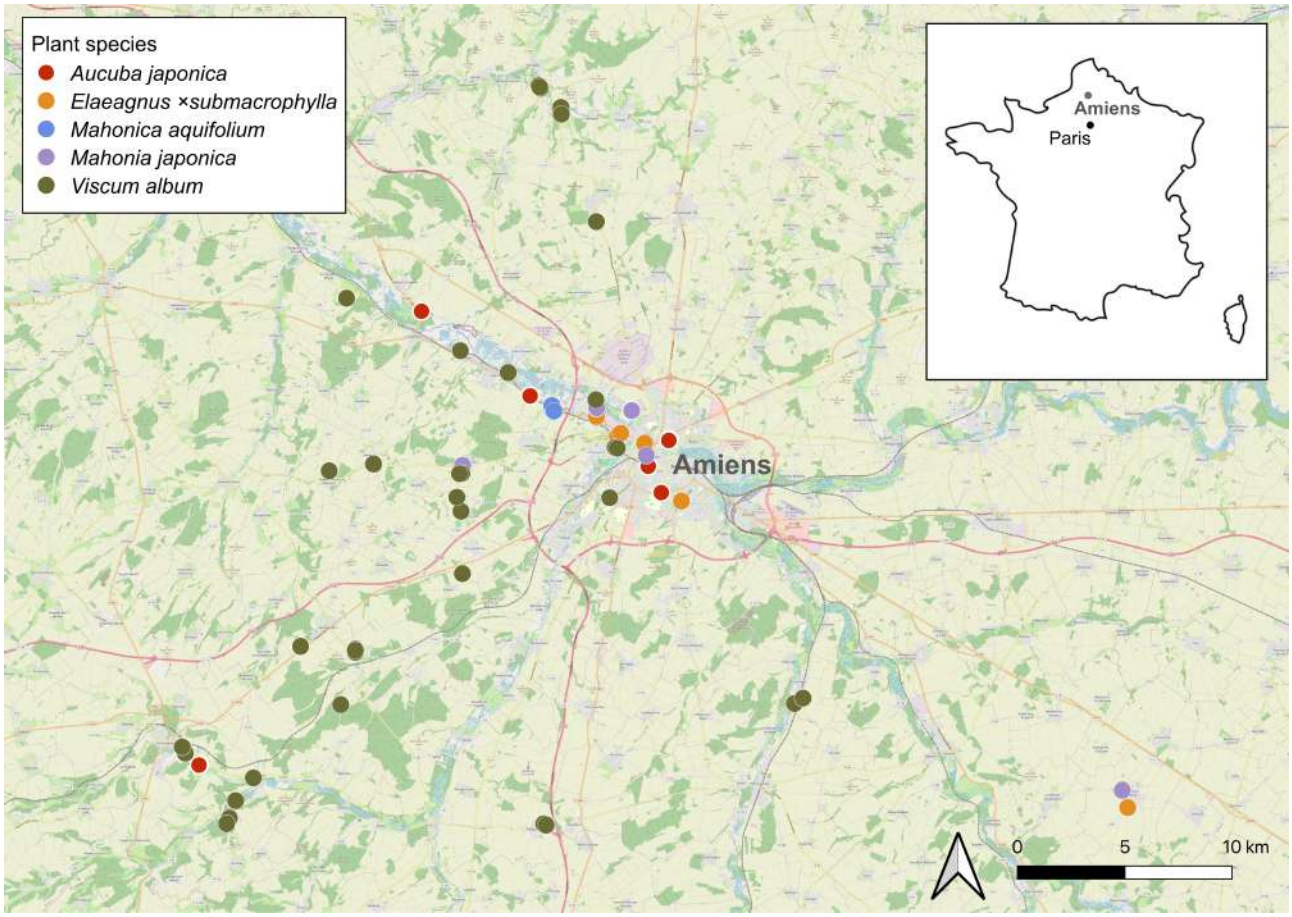

Figure S1 Map of the sampling locations of host plants of *Drosophila suzukii* in Amiens region in the north of France. Made with QGIS using OpenStreetMap base map.

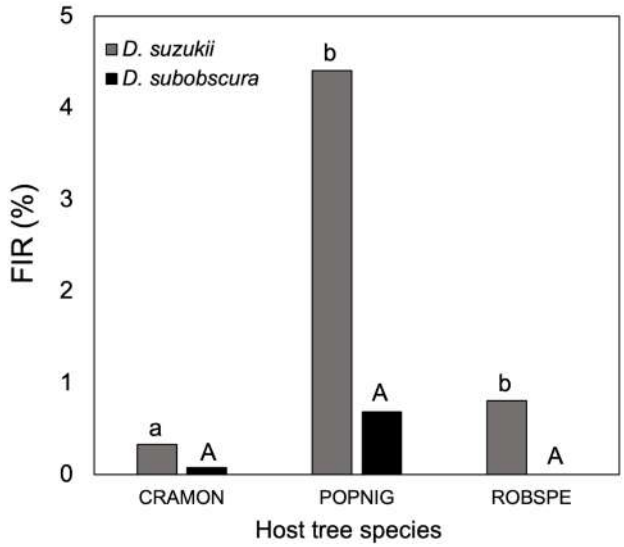

Figure S2 Mean FIR ± SE (%) by *D. suzukii* and *D. subobscura* in *Viscum album* individuals according to its major host tree species. CRAMON: *Crataegus monogyna*, POPNIG: *Populus nigra*, ROBSPE: *Robinia pseudoacacia*. Significant differences ($p < 0.05$) between months are represented by different lowercase and capital letters for *D. suzukii* and *D. subobscura* respectively (Kruskal-Wallis tests).

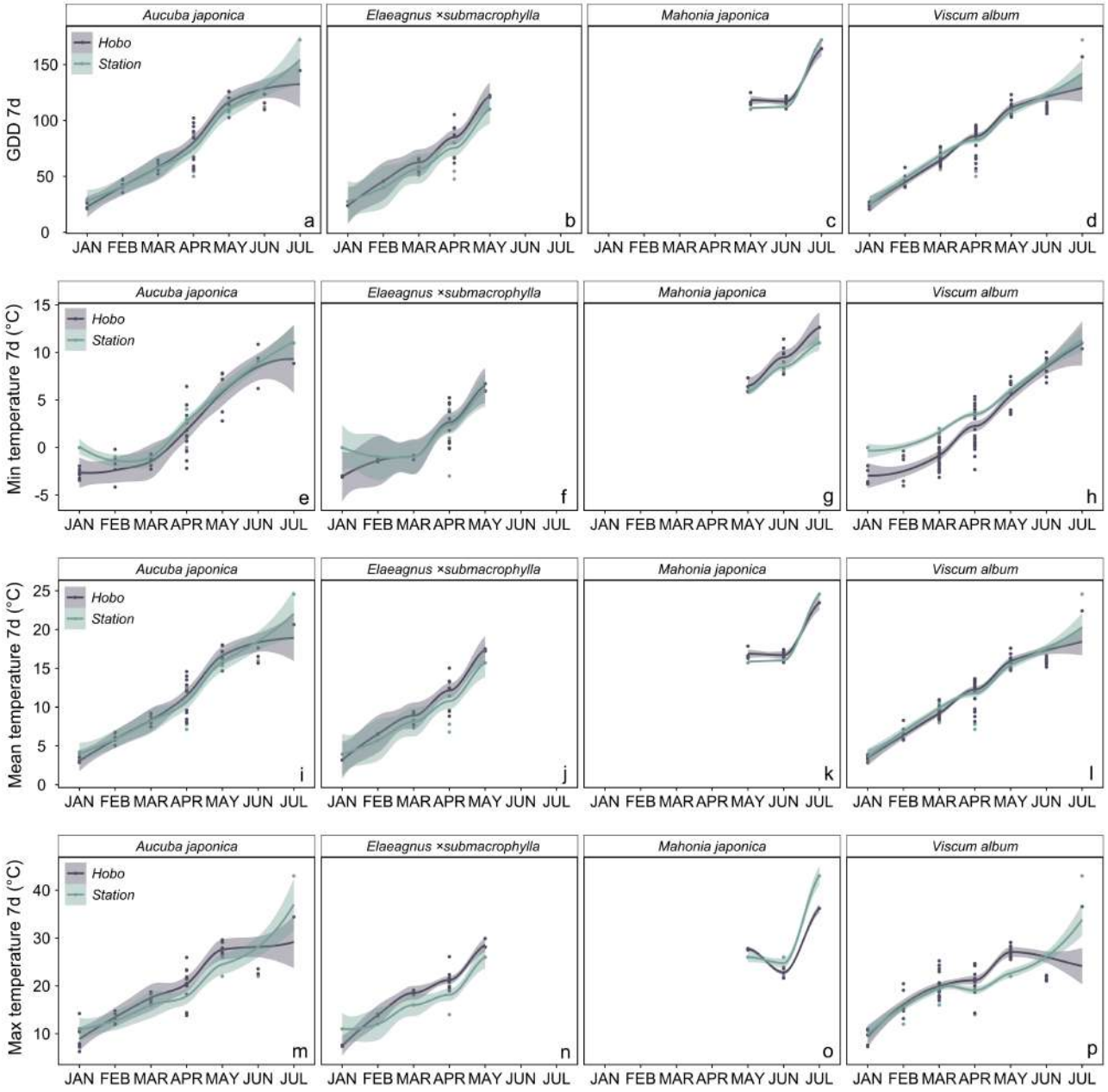

Figure S3 Difference in Growth Degree Days (a-d), minimum (e-h), mean (i-l) and maximum (m-p) temperatures (°C) of the last 7 days preceding fruit sampling between regional weather stations ('Station'; macroclimate) and local dataloggers under plant canopy ('Hobo'; microclimate) throughout the months and according to plant species (*Aucuba japonica*: a, e, i. m; *Elaeagnus ×submacrophylla*: b, f, j, n; *Mahonia japonica*: c, g, k, o; *Viscum album*: d, h, l, p).

DISCUSSION

Part 1
Trophic resources through seasons

The three chapters in Part 1 highlighted the seasonal use of resources by *D. suzukii* and the local, landscape and climatic drivers of its infestations.

In autumn, *D. suzukii* is able to use the ephemeral resource patches (ERP; Butterworth et al. 2022) provided by fallen apples. Infestations occurred only when the fruit were early in the decay process, consistent with *D. suzukii* ecology (Kienzle et al. 2021). The use of other similar ERP during autumn remains to be explored but it could be predicted that other fallen fruit such as plums, pears or, later in the season, persimmons, would be infested and support the fly population in a period of fresh fruit scarcity.

In winter and spring, recent microclimatic measurements were better predictors of *D. suzukii* infestations than larger-scale regional measurements, likely reflecting the direct effect of temperature on female reproductive dormancy (Colinet and Kustre 2024). Further research is required to identify the drivers of the termination of this dormancy (Numata and Shintani 2023).

In all three chapters, the main factors influencing infestation differed between host plants. Two, however, were consistently identified: local temperature in the preceding days and plant architecture. Temperature clearly impacts fly development and emergence success (Raynaud-Berton et al. 2024), in turn influencing the development of the population available to infest surrounding host plants. The role of climate (and landscape) on *D. suzukii* active flight performance and passive dispersal is worth exploring to disentangle effects on population size and the ability to reach the resource. Plant architecture can also provide protection from harmful environmental conditions. Again, further studies on the role of plant architecture in the persistence of *D. suzukii* in winter are required.

Finally, Part 1 raised questions about pest management strategies and their ecological consequences. To reduce *D. suzukii* infestation in crops, possible solutions include removal of fallen fruit, extermination of mistletoe or pruning of ornamental plants. However, these plants and their fruit are part of the ecosystem and not only support *D. suzukii* infestation but also provide important seasonal resources for other arthropods, birds, small mammals, etc (Watson 2001; Myczko et al. 2013; Thomas et al. 2023). While the presence of *D. suzukii* may also compete with or disrupt native species using these fruit, complete removal of these resources would clearly be deleterious to them. To limit impacts on native species, management strategies should, therefore, focus on areas in the vicinity of crop production, with monitoring of surrounding potential host plants and limiting the inclusion of ornamental plants.

Part 2

Thermal preference plasticity and underlying physiological mechanisms

Part 2
Thermal preference plasticity and underlying physiological mechanisms

Species that are able to select favourable microhabitats during the invasion process can potentially be more successful than those that are not. However, the behavioural thermoregulation abilities of invasive species have only rarely been explored. In Part 2, I aimed to characterise the thermal preference (Tp) plasticity of *D. suzukii* and its potential adaptive value.

The first chapter of Part 2 (Chapter 4) describes the development of a new method to study Tp in ectotherms. Most studies of Tp focus on the mean (e.g., Labra et al. 2009; Takeuchi et al. 2009) because it represents the temperature targeted by a population in the context of behavioural thermoregulation (Camacho and Rusch 2017), but the mean alone reveals little about the thermoregulation abilities of a species. Drawing on examples from the literature, I led the development of a new conceptual framework based on hypotheses corresponding to behavioural thermoregulation strategies that integrate the level of plasticity in Tp, and tested this framework using the model organism *D. melanogaster*.

In the second chapter of Part 2 (Chapter 5), I used this new framework to study Tp plasticity in *D. suzukii.* As the fly shows several seasonal adaptations (Tait et al. 2021a,b) and abilities to exploit different habitats (e.g., crops, woodland, urban areas; Ulmer et al. 2024), has considerable acclimation flexibility (Raynaud-Berton et al. 2024), and can invade temperate as well as tropical areas (Walsh et al. 2011), we predicted that its behavioural thermoregulation abilities (and, hence, Tp) would be highly plastic.

In the third chapter of Part 2 (Chapter 6), I investigated the inter-individual variability in Tp integrating four distinct but complementary steps. First, I questioned the individual repeatability of *D. suzukii* Tp at a short time scale. Second, I quantified the acclimation abilities of *D. suzukii* by measuring the metabolism (i.e., oxygen consumption) of flies that developed in different temperature regimes at different temperatures. Third, I measured the energetic content of *D. suzukii* developing under the same temperature regimes. Fourth, I explored the existence of any relationship between Tp and metabolism. Finally, I integrate these steps by calculating the inter-individual variability of each trait to compare them.

In Chapters 5 and 6, I integrated the presence / absence of the bacteria *Wolbachia* as a variable to provide insights about the role of *D. suzukii*'s strain, *w*Suz, in the fly biology, a subject that remains puzzling. In the closely related *D. melanogaster*, several *Wolbachia* strains have been reported to lower Tp (Truitt et al. 2019), although not in all studies (Strunov et al. 2023). Hosting symbionts can also be costly and result in impacts on the host's energetic content or metabolism (Clavé et al. 2022).

Chapter 4

A new conceptual framework to study thermal preference plasticity

Chapter 4 • Objectives and main outcomes

In this chapter, I used an existing dataset obtained several years ago on the thermal preference of *Drosophila melanogaster* to conceptualise the relationship between thermal preference and behavioural thermoregulation. Thermal preference (Tp) is a metric that is often used to discuss the behavioural thermoregulation abilities of ectotherms. However, thermal preference can be influenced by various parameters, notably the acclimation temperature but also related to the physiological state of individuals. This limits the possibility of generalising findings at the species level, for instance. Therefore, we designed a novel conceptual framework that includes competing hypotheses about Tp plasticity relating two important components of temperature preference: its mean, which indicates temperature affinity, and its variance, which informs on the precision of behavioural thermoregulation.

To test this framework, the Tp of *D. melanogaster* was measured across different life stages (L3, L3W, 2-day-old virgin adults and 12-day-old adults) and generations under various developmental temperatures (fluctuating and constant). The data obtained highlight that life stages differ in their behavioural response to temperature regimes, potentially exposing less plastic stages to challenging thermal conditions. Generally, we found a bell-shaped relationship between temperature affinity and precision of thermoregulation, reducing the requirement to thermoregulate precisely within the range of suboptimal temperatures, but not in all life stages.

In herbivorous insects, previous studies stated that behavioural thermoregulation is a strategy primarily to escape overheating and improve survival, rather than a means of approaching the optimal temperature for reproduction. This is consistent with our "Precision at extreme hypothesis", in which thermoregulation is less precise within the permissive temperature range than near the thermal limits. However, this strategy might not be used by all ectotherms. The application of our conceptual framework in the context of a comparative approach across species should provide deeper knowledge of how Tp plasticity translates into thermoregulation strategies. Finally, our framework could be extended by integrating more variables of interest to explore the maximum level of Tp plasticity for any given species.

Contributions

Sylvain Pincebourde and Hervé Colinet conceptualised the study. Nicolas Meyer did the lab work.

I analysed the data, produced the graphics and tables. I wrote the original draft, revised the manuscript according to reviewers' comments and wrote the response letter to the editor and reviewers.

All co-authors contributed to the revision of the manuscript.

Thermal preference plasticity in ectotherms: integrating temperature affinity and thermoregulation precision

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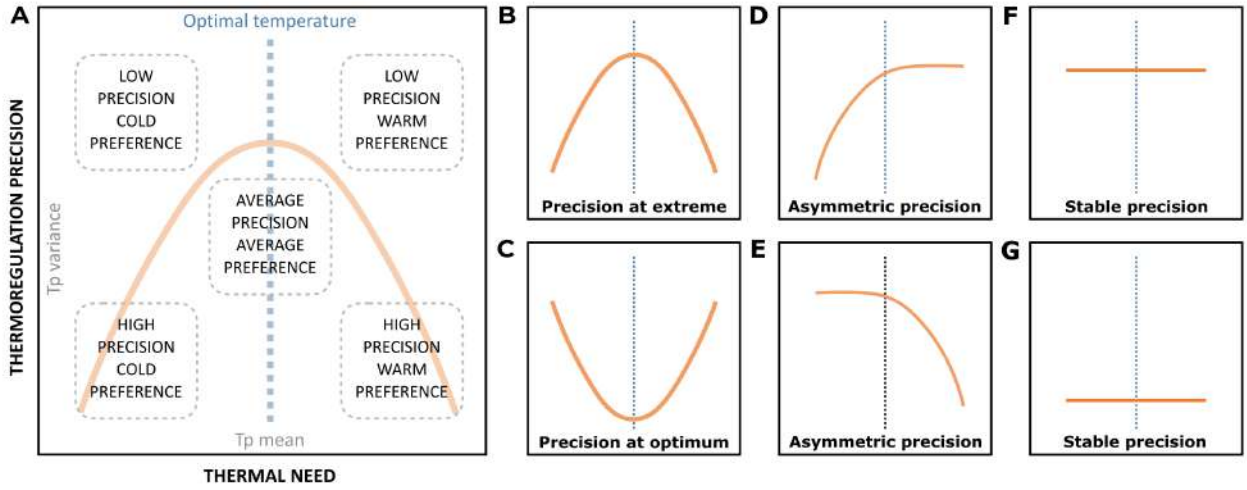
GRAPHICAL ABSTRACT



ABSTRACT

Thermal preference (Tp) prevents ectotherms from encountering sublethal temperatures. Its plasticity likely modulates the importance of behavioral thermoregulation under changing conditions. Ectotherms' thermal sensitivity typically varies across life stages but the dynamics of Tp, and its plasticity level, across life stages remains overlooked. We propose a novel conceptual framework relating two plastic components of Tp: its mean, which indicates temperature affinity, and its variance, which informs on the precision of behavioral thermoregulation. We tested this framework by measuring Tp variations across life stages of an insect model after several generations under contrasting developmental temperatures. Tp plastic responses differed among life stages. Generally, we obtained a bell-shape relationship between temperature affinity and precision of thermoregulation, reducing the requirement to thermoregulate precisely around optimal temperature, but not in all life stages. We highlight the need to change the paradigm underlying the study of Tp beyond the simple use of mean or median.

Keywords: *Drosophila melanogaster*, behavioral thermoregulation, phenotypic plasticity, selected temperature

INTRODUCTION

The thermal biology of organisms provides crucial information to assess the potential impacts of climate change on the physiology, ecology and distribution of ectotherms. Thermal limits are probably the most documented thermal biology metrics for ectotherms (Bennett et al. 2021; Sunday et al. 2011) because they can be compared directly to environmental temperatures to quantify the vulnerability of ectotherms to climate warming (Sunday et al. 2011, 2012; García-Robledo et al. 2016; Pincebourde and Casas 2019). The typically weak plasticity of thermal limits was argued to produce limited opportunities for ectotherms to escape either gradual warming or extreme events such as heat waves (Gunderson et al. 2017). However, species can use behavioral thermoregulation to actively search for and exploit particular microclimates in their natural habitat that buffer against thermal extremes (Caillon et al. 2014; Huey et al. 2012; Sunday et al. 2014; Leith et al. 2024). Indeed, species that exhibit precise thermoregulation are not expected to have evolved particularly high or plastic tolerance limits (Logan et al. 2019; Munoz and Bodensteiner 2019). Therefore, behavioral thermoregulation abilities could be more relevant than thermal limits when assessing responses to environmental changes (Sears et al. 2016; Leith et al. 2024; Pincebourde 2024).

Thermal preference (Tp) is a comprehensive trait that directly reflects an organism's ability to thermoregulate behaviorally (Angilletta 2009). In its simplest definition, Tp is the body temperature an ectotherm selects when provided with a large range of temperatures. This trait, usually measured under controlled conditions in the laboratory (Dillon et al. 2009), is often used together with field body temperature patterns and the distribution of operative temperatures to assess the efficiency of behavioral thermoregulation (Angilletta 2009). Thus, a large body of existing literature has focused on the Tp of ectotherms to determine its adaptive value, for instance with warm-adapted species displaying greater Tp than cold-adapted. In general, the Tp matches relatively well the optimal temperature of various performance metrics, although this is not always the case (Angilletta 2009). Tp also varies along environmental gradients, supporting a local optimal adaptation (Trochet et al. 2018), a counter-gradient pattern of Tp (Freidenburg and Skelly 2004) or no variation indicative of a Bogert effect (Logan et al. 2019). In addition, numerous factors influence Tp, including infection by *Wolbachia* (Truitt et al. 2019; Hague et al. 2021), circadian rhythm (Goda et al. 2014), social behavior (Stapley 2006) and water constraints (Rozen-Rechels et al. 2019). Globally, however, the level of plasticity of the Tp remains understudied and poorly conceptualized (Gvozdik 2012).

Tp is potentially very plastic at various levels. Seasonal plasticity was demonstrated in the Tp of a lizard, increasing the effectiveness of thermoregulation across seasons (Hadamova and Gvozdik 2011; Ortega et al. 2016). Tp plasticity resulting from acclimation to developmental temperatures was also demonstrated in several *Drosophila* species (Rajpurohit and Schmidt 2016; MacLean et al. 2019). In addition, the level of plasticity may vary according to age and life stage, the latter reflecting the ontogenetic variation that is often observed in thermal adaptation (Bowler and Terblanche 2008; Kingsolver et al. 2011; Colinet et al. 2013; Pincebourde and Casas 2015; Lockwood et al. 2018). Life stage also influences the degree of plasticity in thermoregulation. For instance, adults might rely more on behavioral thermoregulation to manage thermal extremes, reducing their need for physiological plasticity, while less mobile larvae might depend more on physiological acclimation due to their reduced behavioral options (Marais and Chown 2007). In *Drosophila* species, both the Tp and its level of plasticity generally differs between larvae and adults (MacLean et al. 2019). Nonetheless, studies reporting information on the variation and level of plasticity of Tp across life stages remain rare.

Tp can be divided into two components: its mean (or median) and its variance (or range), either at the within-group level or across time at the individual level. Most studies focus on the mean Tp (e.g., Labra et al. 2009; Takeuchi et al. 2009) because it represents the temperature targeted by a population in the context of behavioral thermoregulation (Camacho and Rusch 2017). Within populations, inter-individual variability in the Tp is quantified by the variance component. Inter-individual Tp variation can result from a bet-hedging strategy that impedes heritability of the Tp, allowing adaptation to seasonal environments (Kain et al. 2015). The relationship between mean and variance of the Tp is rarely investigated. The level of covariation between the two metrics can inform on the ability to perform behavioral thermoregulation with high precision in challenging thermal environments. In newts, seasonal acclimation resulted in an inverse relationship between the mean and range of Tp: higher mean Tp was associated with a decreased range of Tp (Hadamova and Gvozdik 2011), although this result was not directly interpreted. Both metrics can also respond differently, and independently, to environmental change, as reported in adult *versus* juvenile *Pardosa* spiders (Cabon et al. 2023). This complexity may result from differential levels of plasticity of the two metrics across life stages.

In this study, we propose a conceptual framework that considers both the mean and the variance of Tp. This framework examines plastic changes in thermoregulatory capacities by addressing both the precision of thermoregulation and the thermal needs (Fig 1). The variance in Tp inversely reflects the

precision of thermoregulation: higher variance indicates lower precision (Fig. 1A). The mean Tp informs on the thermal need (or affinity): a high or low thermal need corresponds to a warm or cold preference, respectively (Fig. 1A). The Tp is expected to change in both variance and mean in response to many factors. Several hypotheses are plausible: (i) ***Precision at extreme hypothesis***; The need for precise thermoregulation is high at extreme temperatures and relatively lower near the optimal temperature. When the mean Tp is close to the optimum, the variance in Tp is high (i.e. the organism does not need to be very precise in temperature selection). However, at more extreme temperatures, precise temperature selection (lower variance) is required to avoid lethal temperatures (Fig. 1B); (ii) ***Precision at optimum hypothesis***; Conversely, the need for precise thermoregulation is low at extreme temperatures and relatively high near the optimum temperature (Fig. 1C); (iii) ***Asymmetric precision hypothesis***; The constraints for precise thermoregulation only apply at one end of the temperature range, either at low (Fig. 1D) or high (Fig. 1E) temperature; (iv) ***Stable precision hypothesis***; The thermal need does not impact the thermoregulation precision, which can be either low (Fig. 1F) or high (Fig. 1G) according to physiological requirements.

Various examples exist in the literature that can be interpreted as providing indirect support for one or more of these hypotheses. For example, because of the Jensen inequality (Martin and Huey 2008), it is riskier for organisms to be close to their critical thermal maximum, leading to a prediction of low variance when mean Tp is high ('Asymmetric precision hypothesis'). A spider had the same mean Tp across different acclimation temperatures, but the Tp variance was lower when reared at high temperature (Alfaro et al. 2013). Another indirect line of evidence for a negative covariation between mean and variance of Tp was reported for several drosophilid species at the egg-laying stage (MacLean et al. 2019). Contrasting results, with either positive or negative covariation between the mean and variance of Tp, were reported in two marine snails (Lah et al. 2017). Additionally, acclimation to various temperatures can induce shifts in mean and variance of Tp independently (Reiser et al. 2014), producing a favorable context for a mix of hypotheses (i) and (iv). Nevertheless, none of these studies set out to correlate changes in mean and variance of Tp.

We tested this new framework with the fly *Drosophila melanogaster*. We measured the mean and variance of Tp throughout the life stages of *D. melanogaster*. We applied several treatments and factors (developmental temperatures, mating status: mature vs young virgin adults), and across different generations (G1 to G3) to stimulate plastic responses and obtain the largest range of Tp mean and

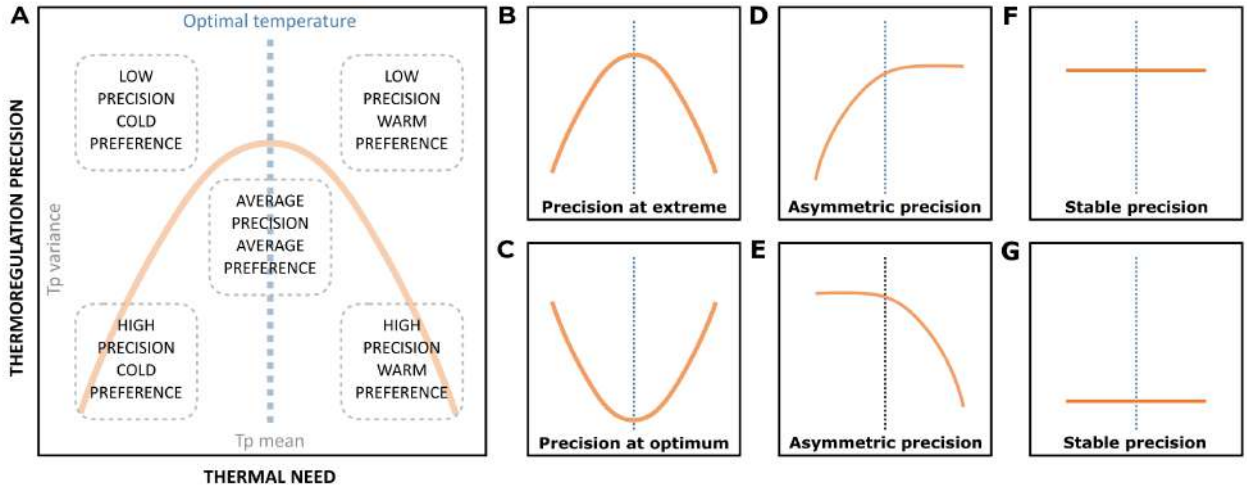


Figure 1. A conceptual view of plastic changes in thermal preference (Tp) metrics. (A) Variation of the thermoregulation precision (Tp variance), according to the thermal need (mean Tp) of a population. The orange line represents a hypothetical relationship between the two variables. The dashed line represents a hypothetic optimal temperature. The five dashed cases indicate the thermoregulation precision and the thermal preference in each area of the plot. Plastic responses can modulate the thermoregulation precision and not change the thermal need, modulate the thermal need and not change the thermoregulation precision, or they can modulate both the thermal need and the thermoregulation precision. (B, C, D, E, F, G) Hypothetical responses of plasticity-induced changes in Tp metrics: (B) *Precision at extreme hypothesis,* the requirements for thermoregulation precision are high at both temperature extremes and relatively lower at the optimal temperature; (C) *Precision at optimum hypothesis*, the requirements for thermoregulation precision are low at both temperature extremes and relatively higher at the optimal temperature; (D, E): *Asymmetric precision hypothesis*, the requirements for precise thermoregulation only apply at one end of the temperature range, either at low (D) or high (E) temperature ; (F, G): *Stable precision hypothesis*, the thermal need does not impact the thermoregulation precision, which is either low (F) or high (G).

variance as possible. We had various levels of comparison: (1) across life stages for a given developmental temperature (ontogenic or age effect), (2) among thermal treatments for a given life stage (level of plasticity), and (3) between generations to assess any intragenerational plasticity influence. We predicted that adults display more plasticity than larvae, with Tp more responsive to the temperature treatments in terms of both mean and variance. Adult flies are more likely to have more opportunity for behavioral thermoregulation than larvae which are confined to the fruit where eggs were deposited. We found that the strength of covariation between mean and variance of Tp is lower in larvae than adults, and that life stages followed different strategies for behavioral thermoregulation (see Fig. 1). The level of plasticity varied widely and independently across the two components of Tp.

MATERIALS AND METHODS

Origin and maintenance of experimental flies

We used a laboratory population of *D. melanogaster* from the ECOBIO laboratory in Rennes, France. It was initially established from a large number of flies captured in Rennes and Plancoët (Brittany, France) in 2010. Every year, new wild individuals collected in September were added to the mass population to limit inbreeding (Colinet et al. 2012). Therefore, this line has a wild phenotype and contained a natural level of genetic variability at the time of experiments in 2017. Several hundred individuals were reared at 25 ± 1°C, LD 12:12 h and 75% RH, in 200 mL bottles containing 25 mL of standard fly medium consisting of sugar (saccharose, 50 $g.L^{-1}$), brewer's yeast (80 $g.L^{-1}$), agar (15 $g.L^{-1}$), propionic acid (3 $mL.L^{-1}$) and a fungicide solution (methyl-4-hydroxybenzoate-ethanol 95% solution, 8 $mL.L^{-1}$). To generate flies for the experiments, groups of 15 mated females were allowed to lay eggs in 200 mL bottles during 6 h, to avoid overcrowding (Colinet et al. 2013).

Population generations and developmental temperature treatments

From the population reared at 25 ± 1°C, about 500 adults were collected to constitute five sub-populations distributed among five climatic chambers (STRADER, Angers, France). Groups of 4-to-5-day-old adults were placed in 200 mL bottles containing medium for females to oviposit during 6 h. Adults were then removed and bottles with eggs were randomly assigned to one of five thermal treatments: three constant treatments (**C1**: 22°C, **C2**: 25°C, and **C3**: 28°C) and two fluctuating regimes differing in their thermal variance (**F1**: 23.5–26.5 °C and **F2**: 22–28°C, both with a mean of 25°C), all under LD 12:12 h and 75% RH. In fluctuating regimes, the coldest and warmest temperatures were reached at midnight and midday, respectively, and maintained for almost 11.5 hours (the transition lasted about 30 min). We measured the Tp of virgin young flies (**V**), which were isolated at emergence and left to age for 2 days, and of mature adults (**A**) of 12 days of age, as well as those of third instar larvae (**L3**) and wandering third instar larvae (**L3W**). To assess whether the responses to thermal treatments shift over generations, these measurements were repeated in generations one (**G1**) and three (**G3**). For adults, both sexes were tested.

Thermal gradient apparatus

Tp was measured by placing the insects in a linear thermal gradient encompassing temperatures

from 10 to 35°C. Our system was similar to the apparatus used in previous studies on Tp of *Drosophila* (Goda et al. 2014; Takeuchi et al. 2009). Tp assays were performed using a custom made linear thermal gradient involving an aluminum slab (400 × 200 × 2 mm) heated on one hand by an auto-adhesive resistance (40W), and cooled on the other hand by a Peltier device (45W) (Supplemental Material S1). The system was placed in a climatically controlled laboratory at a constant air temperature of 22°C. A system of closed ramps was used to allow flies – both adults and larvae – to move along this temperature gradient without escaping, and to easily determine the temperature experienced at each point from the insect's position along the gradient. For larvae, the ramps were filled with a homogeneous nutritional gel (composed of saccharose 50 $g.L^{-1}$ and agar $7g.L^{-1}$) which guaranteed the displacements of larvae. Finally, the displacements of the insects inside the ramps of the thermal gradient were recorded with a camera (HDC-SD200, Panasonic) positioned above the system. The thermal gradient was calibrated before the experiments to determine the temperature at any position (Supplemental Material S1).

Tp measurement

The setup above was used to measure the Tp of L3, L3W, V and A. For each trial, 20 individuals were introduced inside each ramp to assess a populational Tp. The six ramps were considered as independent replicates because no communication or contact was possible among individuals from different ramps. The individuals were filmed over 60 min. In all instances the movements were much less frequent after about 20 min. Therefore, we considered the Tp of every individual to be the temperature selected after 40 min. The experimental assay started slightly differently between adults and larvae. For adults, the thermal gradient was off (i.e., 22°C across ramps) when introducing the flies into each ramp such that flies were randomly distributed inside each ramp. The system was switched on after 10 min and the gradient took another 10 min to stabilize. We measured males and females separately to detect any influence of sex. For larvae, we used the same approach as described by Takeuchi et al. (2009): larvae were deposited in the agar gel at the position corresponding to 28°C on each ramp. The ramping system containing larvae was then applied onto the aluminum plate with the thermal gradient already active. Larvae were allowed to move for 60 min in the gradient before their position (Tp) was recorded. All Tp assays were run within the same daily time window (between 10:00 and 18:00) to minimize any influence of circadian rhythm of Tp. The light environment was homogeneous to avoid any directional attraction (Dillon et al. 2009). Flies were handled with a mouth aspirator.

Statistical analyses

All data were analyzed in R (R Core Team, 2022). Tp below 13°C were removed from the analyses (7.9% of all data) as they corresponded to flies entering a quasi-comatose state (i.e., cold stupor; Vannier 1994) when encountering the cold end of the gradient. Tp were compared among treatments using ANOVA. The explanatory variables were the developmental temperature, the life stage, the sex and the generation. When ANOVA was significant, *post-hoc* comparisons were performed using Tukey's test. Patterns of the relationship between Tp mean and variance were inferred applying a LOESS procedure (with tension 10) which allowed us to retrieve the pattern without *a priori* assumption of shape.

RESULTS

Tp variations: general patterns

Drosophila melanogaster's Tp was influenced by all explanatory variables and most of their interactions (Table 1). In larvae, Tp increased with constant developmental temperatures, except for L3W in G3 (Fig. 2). For instance, L3 larvae in G1 had a Tp of 23.6 ± 3.0 °C (mean ± sd) when developing at C22 and a Tp of 26.5 ± 4.5 °C at C28. Conversely, in V adults, Tp decreased with increasing developmental temperature from 23.9 ± 5.7 °C to 21.7 ± 6.3 °C when reared at C22 and C28 (in G1), respectively (Fig. 2). In A adults, Tp increased with developmental temperature in G1 but remained constant in G3 (Fig. 2). Sex had inconsistent influence, with no differences, or females having higher Tp than males, or vice versa.

Life stage plasticity in Tp components

In G1, mean Tp decreased while Tp variance increased across life stages in all temperature treatments (Fig. 3). Mean Tp increased with the constant temperature treatment in all life stages, with the exception of V adults in the C28 treatment having the lowest mean Tp. Meanwhile, the Tp variance increased with constant temperature treatments in all life stages, with the exception ofA adults at C28 being intermediate. The mean Tp of individuals that experienced fluctuating temperature regimes followed a similar relationship as at C25. Their Tp variance however differed. The Tp variance of L3 and L3W was similar to that at C22 and C25, respectively. For V and A adults, their Tp variances were lower compared to constant treatments, with A adults having the lowest Tp variance.

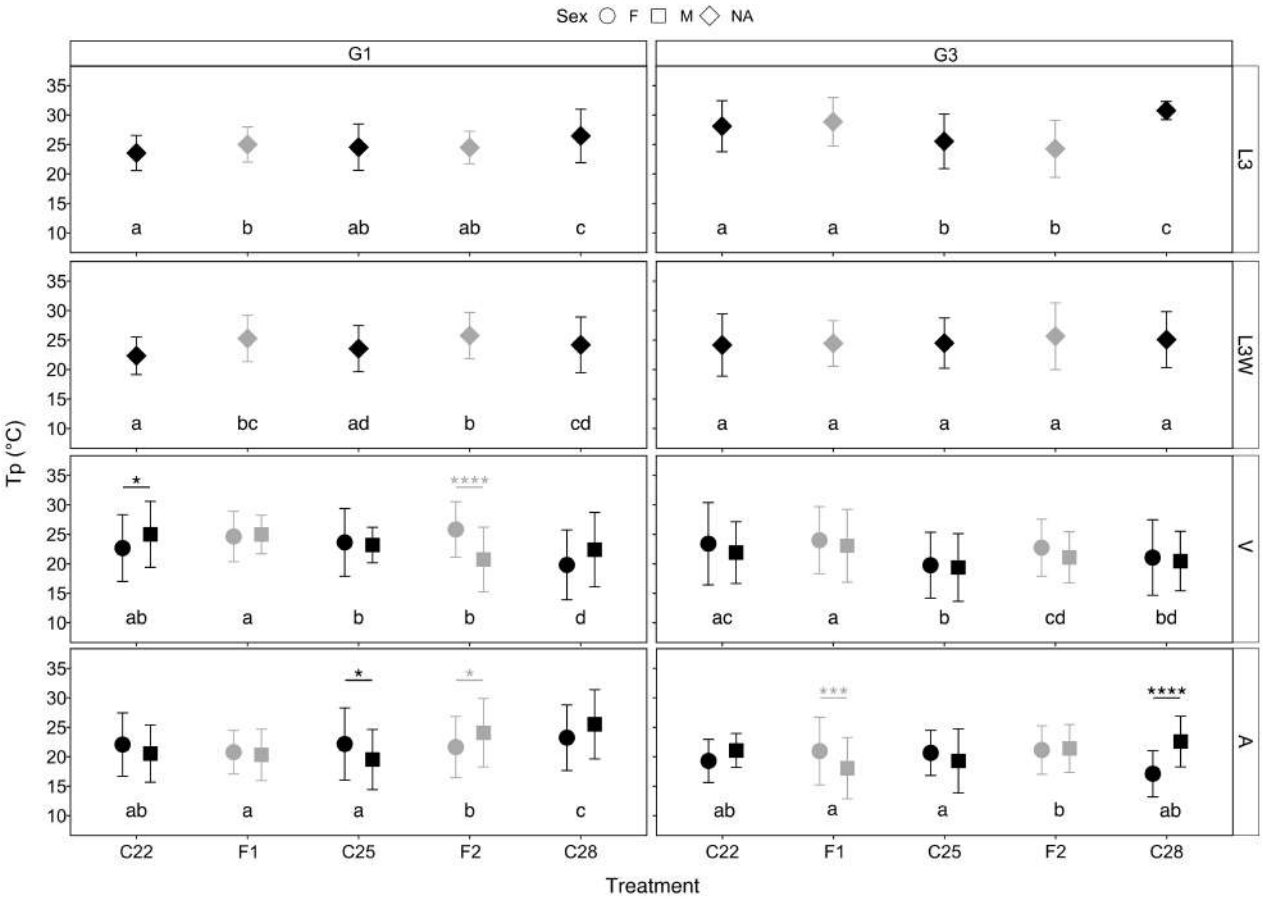


Figure 2. Plastic responses of thermal preference. Thermal preference (Tp) of *Drosophila melanogaster* (n = 6,174) according to developmental temperature treatment (constant regimes in black: 22, 25 and 28°C (C22, C25, C28); fluctuating regimes in grey: 23.5–26.5 and 22–28°C, F1 and F2). Panels show mean Tp with respect to generation (G1 or G3) vertically and life stages (L3: third larval stage; L3W: wandering larval stages; V: 2-day-old virgin adults; or A: 12-day-old adults) horizontally. Each symbol represents the mean Tp for a replicate of a given sex (circles: females; squares: males; diamonds: undetermined sex, larvae). The error bars represent standard errors for mean Tp at a given temperature across all replicate experiments carried out for each life stage, generation and sex. Significance of differences between temperature treatments and sex are represented by different letters (a, b, c, d; bottom of each panel) or by asterisks, respectively (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$; top of each panel).

In G3, the decrease in mean Tp across life stages remained but the relationship was much steeper than in G1 (Fig. 3), with an average decrease of 7.7°C between L3 and A adults (compared to 2.8°C in G1). Individuals at C28 had the steepest drop in mean Tp, from 30.8°C in L3 to 17.1°C in A adults. The relationship between constant temperature treatments also changed relative to G1: in L3, the mean Tp was the lowest at C25 and the highest at C28. In A adults, conversely, it was the lowest at C28 and the highest at C25. In fluctuating regimes, the mean Tp patterns were similar to those of C22, except for L3 in

Table 1. Summary of the ANOVA results on the influence of developmental temperature, life stage, sex, generation and their interactions on thermal preference of *D. melanogaster*.

Explanatory variables	Df	Sum Sq	Mean Sq	F value	*P-value*
Developmental temperature (DevT)	4	4695	1174	50.585	< **0.0001**
Life stage (LS)	3	24435	8145	351.057	< **0.0001**
Sex	1	298	298	12.857	**0.0003**
Generation (Gen)	2	722	361	15.566	< **0.0001**
DevT × LS	12	6693	558	24.041	< **0.0001**
DevT × Sex	4	1027	257	11.064	< **0.0001**
LS × Sex	1	290	290	12.505	**0.0004**
DevT × Gen	8	3518	440	18.953	< **0.0001**
LS × Gen	5	4446	889	38.330	< **0.0001**
Sex × Gen	2	34	17	0.738	0.4781
DevT × LS:Sex	4	1569	392	16.909	< **0.0001**
DevT × LS:Gen	20	10864	543	23.413	< **0.0001**
DevT × Sex:Gen	8	500	62	2.692	**0.0059**
LS × Sex × Gen	1	44	44	1.910	0.1670
DevT × LS × Sex × Gen	4	1304	326	14.051	< 0.**0001**
Residuals	8173	189624	23		

F2 that had the lowest mean Tp. The Tp variance differences among life stages were also much more pronounced than in G1. In constant temperature regimes, the Tp variance increased from L3 to V adults, and then dropped steeply in A adults. The Tp variance of individuals in the F1 regime followed the same pattern as that of C25 until V stage but did not drop in A adults; while individuals from the F2 regime followed the same pattern as those from C22, with the exception of L3W.

The relationship between Tp variance and mean

Overall, the relationship between Tp mean and variance was modulated by the plastic responses to all temperature treatments, both at intra- and inter-generational levels. The relationships between Tp variance and mean differed between life stages and changed after two generations (Fig. 4). The Tp variance of L3 from G1 increased with the mean Tp, corresponding to the 'asymmetric precision hypothesis' (Fig. 1D), indicating a requirement for precise thermoregulation behavior at low temperature. This relationship was reversed in L3 from G3, with a requirement for precise thermoregulation at high temperature (Fig. 1E). L3W from G1 had a higher precision of thermoregulation behavior around the

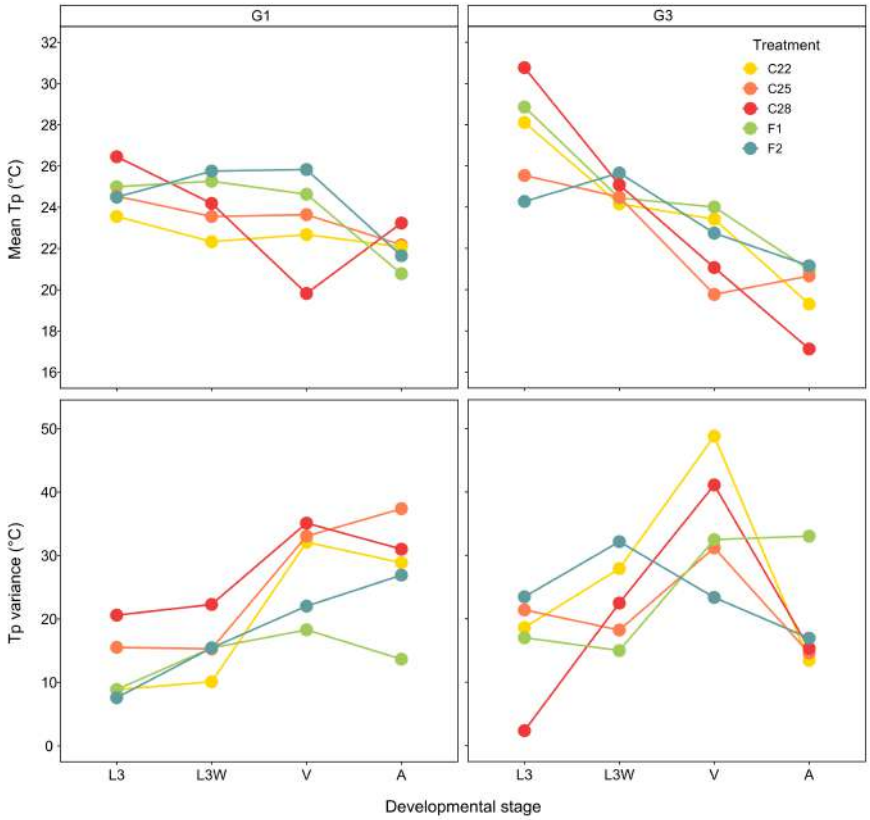


Figure 3. Life stage specificity of plasticity in Tp components. Mean (top panels) thermal preference (Tp) and variance (bottom panels) of female *Drosophila melanogaster* (n = 4,216) according to life stages (L3: third larval stage; L3W: wandering larval stage; V: 2-day-old virgin adults; or A: 12-day-old adults). Panels show averaged Tp mean and variance with respect to generation (G1 or G3) vertically. Each symbol represents the averaged Tp for a replicate at a given factor level of temperature treatment (constant C22, C25 and C8, in yellow, orange and red, respectively; fluctuating regimes: F1: 23.5–26.5 and F2: 22–28°C, in green and blue, respectively).

thermal optimum, corresponding to the 'precision at optimum hypothesis' (Fig. 1C). For G3 L3W, the thermoregulation precision was relatively high overall, corresponding to the 'stable precision hypothesis' (Fig. 1G). Nevertheless, the mean Tp of L3W displayed the lowest plasticity, constraining the assignment to any hypothesis. G1 and G3 V adults had the same plastic response: the need for thermoregulation precision was high at temperature extremes and low around the thermal optimum, corresponding to the 'precision at extreme hypothesis' (Fig. 1B). Older A adults from G1 also showed this response, but shifted in G3 to the 'asymmetric precision hypothesis' (Fig. 1D).

DISCUSSION

The Tp provides crucial information on ectotherms' ability to thermoregulate behaviorally and therefore to survive and/or reproduce in heterogeneous environments (Leith et al. 2024). The level of

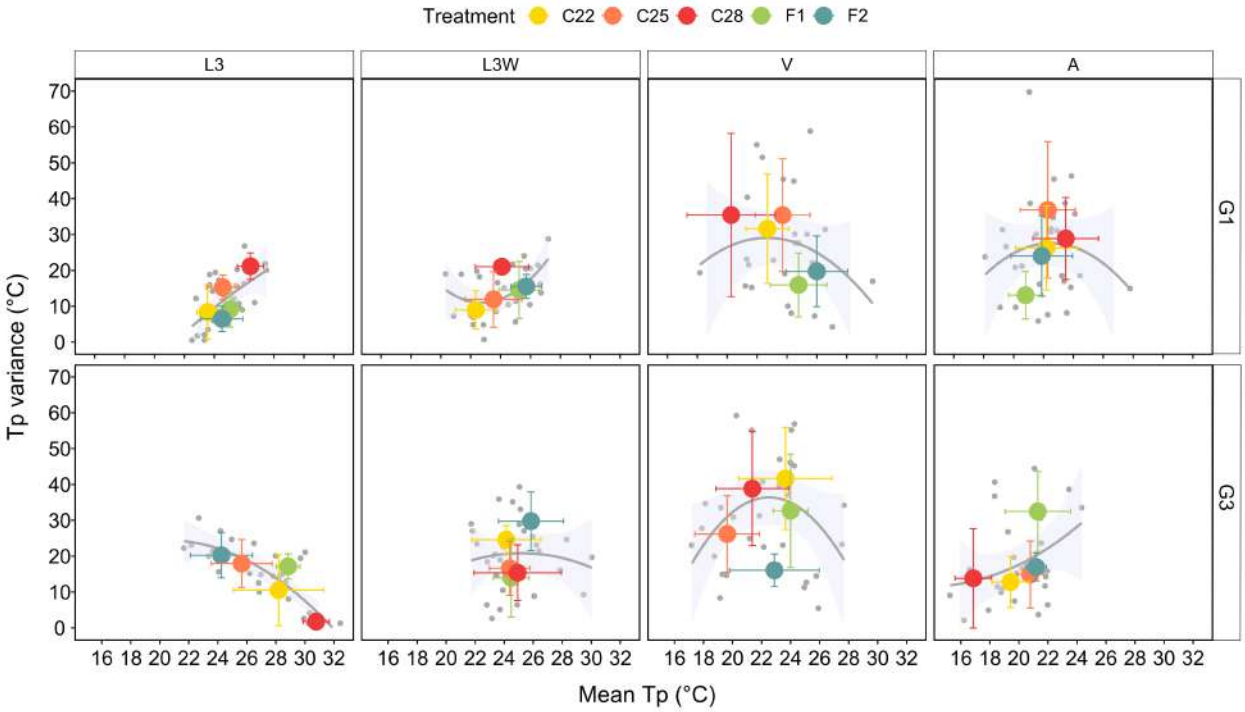


Figure 4. The relationship between Tp variance and mean. Variance of *Drosophila melanogaster* female thermal preference (Tp) according to mean Tp. Panels show averaged Tp mean and variance (n = 6) with respect to life stages (L3: third larval stage; L3W: wandering larval stage; V: 2-day-old virgin adults; or A: 12-day-old adults) vertically and generation (G1 or G3) horizontally. Each symbol represents the averaged Tp for a replicate at a given level of temperature treatment (constant C22: 22°C, C25: 25°C and C28: 28°C, in yellow, orange and red, respectively; fluctuating regimes: F1: 23.5–26.5 and F2: 22–28°C, in green and blue, respectively). The error bars represent standard errors for both mean and variance of Tp. The grey dots are the raw data for each replicate and the grey curves are drawn using LOESS procedure (with 95% confidence intervals).

plasticity of Tp, if adaptive, should reflect an ectotherm's propensity to buffer against the negative effects of challenging thermal conditions. However, in contrast with widely used traits such as thermal limits, the level of plasticity of Tp is poorly documented (Gvozdik 2012). Our data illustrate that Tp can display high levels of plasticity, both at intra- and inter-generational levels and across different life stages with variation of up to 15°C in the mean Tp. Both mean Tp (a metric of thermal affinity or need) and Tp variance (a proxy of thermoregulation precision) change depending on the current and previous thermal environment, suggesting that the ability to adjust thermoregulation precision is as plastic as the mean Tp itself. This highlights the need for a change in the paradigm underlying the study of Tp beyond the simple use of mean or median Tp (see Fig. 1). Below we consider (i) how the plastic Tp shifts can be interpreted based on the ecology of the species both across life stages and generations, (ii) the possible adaptive value of hyper-plasticity in Tp, and (iii) avenues for the further development of our conceptual framework.

Interpreting Tp across life stages

The high plasticity of Tp complicates the interpretation of any change driven by multiple factors. The conceptual framework we propose simplifies this process by integrating all Tp responses along only two axes, the thermal need (Tp mean) and the thermoregulatory precision (Tp variance). For instance, virgin V adults (at G1) follow the 'precision at extreme hypothesis' according to which any plastic response should induce an increase in thermoregulatory precision when the thermal need approaches sublethal temperatures. Now, the priority is to investigate the mechanisms determining the Tp strategy (Fig. 1). Our data highlight that the different life stages have different thermoregulatory strategies (see Fig. S1) and, also, that the strategy can vary due to transgenerational acclimation.

In contrast to flying adults that may rely more on behavioral thermoregulation than physiological plasticity to manage thermal extremes, the less mobile larvae may rely more on physiological acclimation due to reduced behavioral options within their decomposing fruit (Feder and Krebs 1998; Marais and Chown 2007). Larval stages are therefore expected to have evolved less precise thermoregulation and broader thermal needs than adults, and probably also less plastic Tp (Marais and Chown 2007). In agreement with this hypothesis, we found that the Tp plasticity (Tp differences between treatments) was lower in larvae than in adults overall (see also Fig. S1). However, overall, larvae (L3) showed higher precision (lower Tp variance) than adults, especially when the mean Tp was high (e.g., larvae at C28 in G3). This counter-intuitive finding is consistent with previous observations in several *Drosophila* species (MacLean et al. 2019). A plausible explanation is that fly larvae evolved the ability to exploit fine-scale temperature gradients that can occur across small structures such as fruit. In apples, for instance, thermal gradients of up to 15°C can occur across a single fruit (Saudreau et al. 2007) and codling moth larvae use this gradient for behavioral thermoregulation (Kührt et al. 2005). The wandering stage L3W was intermediate between L3 and adults. This is the stage at which larvae leave their food source to pupate within the soil or on the plant surface (Sokolowski et al. 1984), and this stage can be expected to have evolved thermoregulatory abilities to exploit microhabitats that are optimal for the maturation of pupae. The role of fine-scale microclimatic variation in the evolution of invertebrate Tp is likely to be underestimated and underappreciated (Pincebourde et al. 2016; Convey et al. 2018).

In adult flies, the thermal need and the range of permissive temperatures differ according to their nutritional or reproductive status and these shifts help explain our results. Virgin adults had the highest Tp variance (i.e., low thermal precision), especially in G3. Virgin adults can loosely thermoregulate around

the optimal temperature with little or no cost to their future reproduction because they are viable across a broad temperature range (10–33°C), and only temperatures >30°C are deleterious for reproduction (Hoffman 2010). In contrast, fertile females are more restricted in their options because they have to find suitable oviposition sites for both the maturation of their eggs (Huey et al. 1995) and the development of their offspring (Fogleman 1979; MacLean et al. 2019). Indeed, oviposition site preference has a narrow temperature range (Fogleman 1979) and fertility is more constrained than viability, with a temperature range of 14–29°C (Hoffman 2010). This thermal constraint would explain the high level of thermoregulation precision in G3 fertile females. The narrower temperature range for fertility would also explain the drop in mean Tp of fertile females in G3, especially in the constant 28°C temperature regime. After three generations being exposed to temperatures (28°C) close to that at which fertility starts to be compromised (around 29°C), choosing lower temperatures would not only protect the females from harmful temperatures but also favor physiological repair processes (Ørsted et al. 2022). The production of offspring is known to be more sensitive to elevated temperatures than other fitness-related traits in *Drosophila* and other insects (van Heerwaarden and Sgrò 2021; Parratt et al. 2021; Ørsted et al. 2024). Therefore, adjustments of Tp similar to those we describe here for *D. melanogaster* may be widespread across insects.

Tp plasticity as a means to counteract the Bogert effect?

Our data demonstrate significant transgenerational plasticity in Tp with, for instance, an increase of more than 4°C in Tp mean under C28 and F2 regimes from G1 to G3. Three generations under C28 were sufficient to produce larvae with more pronounced preference for elevated temperature and with high thermoregulation precision. This rapid change due to transgenerational plasticity is supported by the very low heritability of Tp in general (Castañeda et al. 2019), but contrasts with the relatively slow rates of evolution of Tp across 20,000 years in lizards (Ibargüengoytía et al. 2021), although amongst classical thermal traits selection pressure may be greatest on Tp (Gilbert and Miles 2017). It remains unclear from our study to what extent this transgenerational plasticity is adaptive, but we speculate that high plasticity across very few generations may obstruct the Bogert effect. The Bogert effect explains how behavior (e.g., selecting the same narrow temperature range whenever it is available) can generate physiological inertia limiting the evolution of higher tolerance or warmer Tp under changing environmental conditions (Logan et al. 2019; Munoz and Bodensteiner 2019). However, the transgenerational plasticity of Tp opens the potential for the thermal physiology of the organism to respond rapidly when novel thermal

environments are experienced. Future studies are required combining experimental approaches and modeling of selection pressures to help address this question.

Tp plasticity as a means to escape sublethal effects under fluctuating conditions?

The partitioning of thermal need (Tp mean) and thermoregulatory precision (Tp variance) allows us to unravel responses that are otherwise unclear. The fluctuating temperature treatments that we applied with the same temperature average of 25°C were informative. In general, the fluctuating thermal regimes induced larger changes in Tp variance than in Tp mean compared to C25. Therefore, daily temperature fluctuations can modulate the thermoregulation precision of the organism, but not necessarily its thermal need. This is consistent with other studies showing how thermal traits (mainly CTmax or metrics of the thermal performance curve) can vary with the amplitude of daily fluctuations while mean temperature remains identical (Paaijmans et al. 2013; Foray et al. 2014; Sinclair et al. 2016). By contrast, a shift in the thermal need in response to thermal fluctuations was reported in species living in highly fluctuating environments (Podrabsky et al. 2008). The influence of fluctuating temperature on the mean Tp should depend on the abiotic context (Denny 2017). Surprisingly, our data indicate that increased amplitude of daily fluctuation improved the precision of thermoregulation after several generations, but very little after the first generation. Higher precision should increase the probability of avoiding high temperatures that can induce sublethal effects, including physiological stress and decreased survival (Koussoroplis et al. 2017). Therefore, this response is likely adaptive but is based mostly on transgenerational plasticity instead of developmental plasticity.

Our inferences are necessarily bounded by the experimental treatments that we applied. The temperatures in our treatments were all within the permissive temperature range for the development of *D. melanogaster*. The thermal limits of *D. melanogaster* are well beyond these temperature treatments (Hoffman 2010), and fluctuating treatments could include short exposures to sublethal / near lethal temperatures to possibly increase the amplitude of plastic responses in Tp (Colinet et al. 2015; Koussoroplis et al. 2017). A broader range of plasticity should help to better delineate the response curves of the relationships between mean Tp and Tp variance. Similarly, we assumed that the optima, taken as a global trait, remains at or near 25°C, but recognize that optimal temperature for life-history traits such as growth rate or egg laying can also change slightly in response to environmental temperature changes (Fischer et al. 2011; Buckley et al. 2022). Future analysis of Tp plasticity should be integrated with

assessment of thermal performance curves, or combined with estimates of optimal temperature and limits for specific traits (Huey et al. 2012), to refine the extent to which the plasticity we describe is adaptive.

Future perspectives for this novel conceptual framework of Tp

Recently, behavioral thermoregulation was revealed to be a strategy primarily to escape overheating and improve survival, rather than a means of approaching the optimal temperature for reproduction in herbivorous insects (Leith et al. 2024). This is consistent with our "***Precision at extreme hypothesis***" in which thermoregulation is less precise within the permissive temperature range in adults than near the thermal limits. Nevertheless, this may not be the case for all ectotherms. For instance, reptiles rely on behavioral thermoregulation to approach their optimum for reproduction-related traits as much as to escape extreme temperatures (Ladyman et al. 2003; Taylor et al. 2021). The application of our conceptual framework in the context of a comparative approach across ectotherms should provide deeper knowledge of how interactions between reproduction and thermoregulation strategies translate into Tp plasticity.

Finally, we manipulated the level of plasticity by focusing on a few key variables (developmental/adult temperature, life stage, age, sex). Our framework can now be extended by integrating more variables of interest with the aim of exploring the maximum level of Tp plasticity for a given species. In ectotherms, Tp variation depends on numerous factors including body mass (Shepherd et al. 2008; Cabon et al. 2023), reproductive status (MacLean et al. 2019), short-term acclimation (Mascaro et al. 2019), water status (Rozen-Rechels et al. 2019), diet quality or quantity (Hardison and Eliason 2024), altitude/latitude (Hodgson and Schwanz 2019), pathogens/disease (Beukema et al. 2021), the presence of symbionts such as *Wolbachia* (Arnold et al. 2019), and the presence of prey/predator cues (Smolinsky and Gvozdik 2012). The precise sensory and neuronal pathways controlling Tp are known in *Drosophila* (Hamada et al. 2008), paving the way to comparisons in Tp plasticity across genotypes differing at this level. We anticipate that the amplitude of Tp responses to these factors modulate to a large extent the ecological responses to climate warming.

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Supplementary Materials

Thermal gradient

An aluminum slab (400 × 200 × 2 mm) was heated at one end and cooled on the other, which resulted in a linear temperature gradient. The high thermal conductivity of the aluminum material guarantees a stable gradient as long as the heating and cooling devices work at a constant rate. A Peltier device (45W, Quick-cool®, Germany) coupled with a rheostat (Quick Cool Pelletier Controller, Conrad), a temperature probe and a display device were used to control the cold end. A hand-made dissipater device was used to avoid overheating of the Peltier element. An aluminum block was placed against the warm side of the Peltier, and cool water circulated continuously through holes across this block. This cooling liquid system was connected to a large water tank (about 50 L) using a water pump. The warm end of the gradient was heated by a 200 × 80 mm auto-adhesive resistance (40W) coupled with a temperature probe and a temperature controller (ET2011, ENDA, Industrial Electronics, Conrad). The entire system was placed in a climatically controlled laboratory at a constant air temperature of 22°C.

A system of closed ramps was used to allow flies – both adults and larvae – to move along this temperature gradient without escaping, and to easily determine the temperature experienced at each point from the insect's position along the gradient. This system was composed of a Plexiglas plate (340 × 130 × 4 mm) with seven aluminum rods (7 mm in width) glued parallel to each other such that they formed six ramps of 7 mm width joining the two sides of the thermal gradient. This system was positioned upside down onto the aluminum plate of the thermal gradient. Small holes were created through the Plexiglas plate to allow the introduction of adults inside the ramps.

For the calibration procedure, we used a weather station (CR10X, Campbell Scientific) with a multiplexer (AMT25, Campbell Scientific) to connect 25 thermocouples (T-type copper Constantan, diameter 0.2mm, TC Direct, Dardilly, France). The ramp system detailed above was modified to have multiple holes each separated by 1 cm along each of the six ramps. Thermocouples were introduced into these holes to record the temperatures along the ramps every minute for one hour. For each ramp, a linear regression model was applied to relate the spatial coordinate and the mean temperature during this experimental hour. The thermal gradient was strongly linear in all ramps (all $R^2 > 0.99$), allowing us to predict the temperature from the position along the ramp using these linear models.

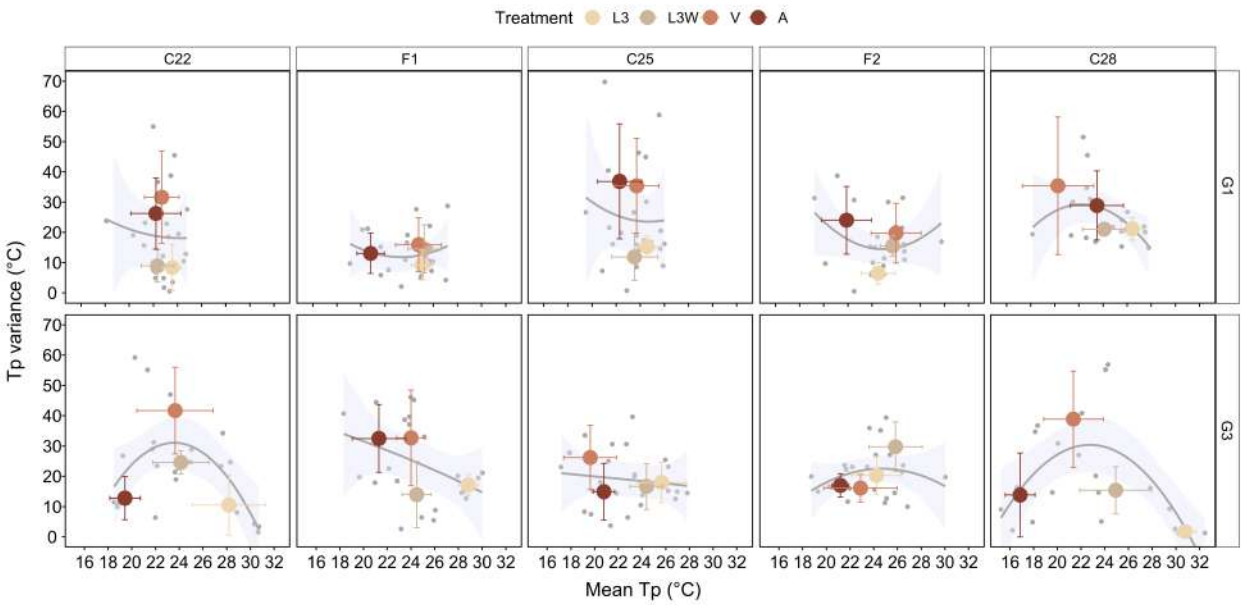

Figure S1. The relationship between Tp variance and mean. Variance of *Drosophila melanogaster* female thermal preference (Tp) according to mean Tp. Panels show averaged Tp mean and variance (n = 6) with respect to temperature (constant C22: 22°C, C25: 25°C and C28: 28°C; fluctuating regimes: F1: 23.5–26.5 and F2: 22–28°C) vertically and generation (G1 or G3) horizontally. Each symbol represents the averaged Tp for a replicate at a given life stage (L3: third larval stage; L3W: wandering larval stage; V: 2-day-old virgin adults; or A: 12-day-old adults in yellow, light-brown, orange and red, respectively). The error bars represent standard errors for both mean and variance of Tp. The grey dots are the raw data for each replicate and the grey curves are drawn using LOESS procedure (with 95% confidence intervals).

Chapter 5

Thermal preference hyperplasticity

Chapter 5 • Objectives and main outcomes

The aim of this chapter was to study the behavioural thermoregulation abilities of *Drosophila suzukii*, in the context of the framework developed in Chapter 4. As a fly that has successfully invaded all continents except Antarctica in less than 15 years, we predicted that its thermal preference (Tp) would be highly plastic and would respond to various internal and external parameters.

Tp variations of *D. suzukii* were measured using a thermal gradient considering the effects of several variables including developmental temperature, life stage, sex, age, reproductive status and infection by the endosymbiont *Wolbachia* across multiple generations. We also used the framework developed in Chapter 4 to decipher the behavioural thermoregulation strategies of *D. suzukii* according to these same variables.

As expected, the Tp of *D. suzukii* was highly plastic and all variables tested showed significant effects. The variables with the strongest effects were the developmental temperature, the life stage and sex of adults, as well as *Wolbachia* infection. The behavioural thermoregulation strategies of the fly changed according to its physiological state and to the environmental conditions. Most life stages showed a strategy of 'precision at extreme' that would protect them from exposure to harmful high temperatures.

The high plasticity of *D. suzukii* could provide an important contribution to its invasion success, acting as a pre-adaptation to the new environmental conditions experienced in its invasive range. Integrating studies of behavioural thermoregulation strategies with those of critical thermal limits could also help identify the organisms most vulnerable to climate change.

Contributions

Sylvain Pincebourde and I conceptualised the study. I contributed to the construction of the thermal gradient with the help of the Gustave Eiffel secondary school.

I performed the experimental work and collected the data. I was assisted by students (Jules Lachery, Nina Lemattre, Katrina Madr, Julien de Bièvre, Alexandre Brogi, Florian Simao) and an engineer assistant (Marina Millet) that I supervised for both data collection and data curation.

I analysed the data, produced the graphics and tables and wrote the original draft.

Thermal preference hyperplasticity allows adjustment of behavioural thermoregulation strategies in an invasive fly

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ABSTRACT

Climate change threatens ectotherms worldwide. Conversely to adaptations that act over large timescales, the ability to express plastic responses could potentially overcome negative impacts of warming at shorter timescales. However, while has addressed the level of plasticity of thermal limits, that of plasticity of behavioural thermoregulation has received much less attention. Thermoregulation strategies are expected to improve survival before thermal limits are approached under changing environments. In this study, we investigated the behavioural thermoregulation plasticity of *Drosophila suzukii* by measuring its thermal preference (Tp). As an invasive fly that has successfully invaded most continents in less than 15 years, we expected its Tp to be highly plastic through adjustments to various endogeneous and exogeneous factors.

We measured Tp using a linear thermal gradient and considered its response to several variables including developmental temperature, life stage, sex, age, reproductive status and infection by the endosymbiont *Wolbachia* across multiple generations. Then, using the precision-thermal need framework, we determined the behavioural thermoregulation strategies of *D. suzukii* life stages in order to identify the most vulnerable stages to warming.

As predicted, the Tp of *D. suzukii* was highly plastic and all variables tested showed significant effects. The variables with the strongest influence were developmental temperature, life stage and sex of adults, as well as *Wolbachia* infection. The behavioural thermoregulation strategies of the fly were influenced by both endogeneous and exogeneous factors. Most life stages showed a strategy of 'precision at extreme' that would protect them from exposure to harmful high temperatures. However, *Wolbachia* seemed to shift this strategy into 'precision et optimum' in the larval stage.

The high plasticity of *D. suzukii* likely contributed to its invasion success, acting as a pre-adaptation to the new environmental conditions experienced as it progressed in its invasive range. More generally, integrating studies of behavioural thermoregulation strategies with those of critical thermal limits could also help identify the organisms most vulnerable to climate change.

Keywords: Climate change, *Drosophila suzukii*, phenotypic plasticity, selected temperature

INTRODUCTION

Ectotherms are threatened by climate change (Pecl et al. 2017; Renner and Zohner 2018; Harvey et al. 2020). Adaptation can help buffer the effects of climate change on organisms over longer timescales (Williams et al. 2008; Hoffmann and Sgró 2011; Ma et al. 2021) while plastic responses could potentially overcome negative impacts of warming at shorter timescales, within and across only few generations (Kingsolver and Buckley 2018; Buckley and Kingsolver 2021). Many studies have focused on the level of plasticity of thermal limits (e.g., Gunderson et al. 2017; Fey et al. 2019) while some have addressed the plasticity of various variables influencing thermal performance curves such as optimal temperature (Sinclair et al. 2016; Kingsolver and Buckley 2017; Johnson et al. 2023). All these studies suggest that the level of plasticity may not be sufficient to buffer against warming and that some life stages may be particularly at risk because of lower plasticity in thermal limits than others (Marochi et al. 2021; Sun et al. 2021). Nevertheless, before approaching its thermal limit, an ectotherm can rely on behavioural thermoregulation to escape overheating (Leith et al. 2024) and/or to find its optimal temperature within heterogeneous natural habitats (Martin and Huey 2008). However, the level of plasticity in behavioural thermoregulation has received much less attention than that of thermal limits (but see Gvozdík 2012; Sinclair et al. 2016), despite the fact that it can be more important than the thermal limits as a factor influencing survival under changing environmental conditions (Pincebourde and Woods 2020; Ma et al. 2021).

Organisms that are able to actively select microhabitats and associated microclimates, including temperature, are more likely to survive in a variable environment, including under climate change (Danks 1978; Ma and Ma 2022). Selection ability can be assessed using thermal preference (Tp) approaches that measure the temperature an organism chooses when provided with access to a wide range of temperatures (Angilletta 2009; Dillon et al. 2009). Tp can respond to many physiological and environmental factors including seasonality (Hadamova and Gvozdik 2011; Ortega et al. 2016), acclimation (Rajpurohit and Schmidt 2016; MacLean et al. 2019), age and life stage (Bowler and Terblanche 2008; Kingsolver et al. 2011; Colinet et al. 2013; Pincebourde and Casas 2015; Lockwood et al. 2018), sex and reproductive status (Deconninck et al. under review - Chapter 4), or symbiosis (Truitt et al. 2019). Recently, a novel framework has been proposed to partition the plasticity in thermal need (Tp mean) versus thermoregulation precision (Tp variance), allowing the definition of thermoregulation strategies such as 'precision at optimum' versus 'precision at extremes' where the selection pressure acts to thermoregulate

precisely around optimal and sublethal temperatures, respectively (Deconninck et al. under review - Chapter 4). This framework could help define the vulnerability of organisms to climate change by describing the plasticity of thermoregulation and the strategies used according to endogeneous and environmental conditions.

Amongst species impacted by climate change, invasive species are particularly striking. Climate change can facilitate biological invasions (Ward and Masters 2007; Diez et al. 2012; Robinson et al. 2020), including those of pest species (Cornelissen et al. 2019; Skendžić et al. 2021). The plastic capacities of invasive species, especially behavioural, developmental, physiological and morphological plasticity, allow them to cope with some of the environmental modifications induced by climate change (Chown et al. 2007; Manfredini et al. 2019; Ashra and Nair 2022). *Drosophila suzukii* is one example of a successful invader benefiting from climate change thanks to its exceptional plasticity (Little et al. 2020). This pest species of soft fruit shows several seasonal adaptations (Tait et al. 2020, 2021) and abilities to exploit different habitats (e.g., crops, woodland, urban areas; Ulmer et al. 2024), has considerable acclimation flexibility (Raynaud-Berton et al. 2024), is highly polyphagous (Poyet et al. 2015; Kenis et al. 2016) and can invade temperate as well as tropical areas (Walsh et al. 2011). Therefore, its behavioural thermoregulation abilities (and, hence, Tp) are expected to be highly plastic as a means to adjust its behavioural thermoregulation strategies according to the multifactorial context it faces when arriving in a novel environment.

Here we quantified the plasticity of *D. suzukii* Tp following a multifactorial approach to rank various environmental and endogenous factors from the most to the least impactful on Tp responses. Then, focusing on the most impacting variables, we determined the thermoregulation strategies of different life stages to identify which stage(s) is more likely to buffer against climate warming.

MATERIALS AND METHODS

Origin and maintenance of experimental flies

Drosophila suzukii flies originated from a field collection made from infested raspberries in Rennes, France (48°7′2.158″N, 1°40′40.053″W), in October 2020, by the ECOBIO Laboratory (University of Rennes). Separate lines differing by the presence (W+) or absence (W–) of *Wolbachia*, were obtained as described in Deconninck et al. (2024 - Chapter 8). The microbiota of W– flies was restored as described by Strunov et al. (2022). The two lines were reared in 100 mL plastic bottles containing ~30 mL of

standard cornmeal diet and incubated at 20°C, LD 12:12 h and 75% RH (STRADER, EV1300, Angers, France). At least 30 bottles of 50–200 flies per line were used for continuous maintenance. Before and after experiments, *Wolbachia* presence in the two lines was assessed using specific PCR assays with *Wolbachia*-specific primers targeting the *wsp* gene (81F/691R), following Braig et al. (1998) (see also Deconninck et al. 2024 - Chapter 8).

Developmental temperature regimes

From the source lines reared at 20°C, an equal number of individuals were collected to constitute five sub-populations (about N=100 adult flies each) each allocated to five climatic chambers (STRADER, Angers, France). Groups of around a hundred of 4-to-5-day-old adults were placed in 200 mL bottles containing oviposition medium for 5 days. Adults were then removed and (at least 12) bottles with eggs were randomly distributed between the five thermal regimes. The regimes included three constant temperatures (16, 20 and 24°C) and two fluctuating temperatures differing in their thermal variance (18–22 and 16–24°C, both with mean temperature of 20°C) under LD 12:12 h and 75% RH. In the fluctuating regimes, the coldest and warmest temperatures were reached at midnight and midday, respectively, and maintained for almost 11.5 hours (the transition between the two temperatures occurred within 30 minutes). Populations were maintained for 15 generations, except for 18-22°C (four generations) because of a maintenance issue.

Thermal gradient apparatus

Thermal preference was measured by placing the insects in a linear thermal gradient encompassing temperatures from 13°C to 28°C. Our system was similar to the apparatus used in previous studies on thermal preference of *Drosophila* (Takeuchi et al. 2009; Goda et al. 2014). Thermal preference assays were performed using a custom-made linear thermal gradient. An aluminium slab (500 × 250 × 20 mm) was heated at one end and cooled at the other, resulting in a linear temperature gradient. The high thermal conduction of the aluminium material guarantees a stable gradient as long as the heating and cooling devices are set at a constant level. Peltier devices (45W, Quick-cool®, Germany) coupled with two Arduino controllers (UNO R3), a temperature probe and a display device were used to control the temperatures at both ends of the gradient. A hand-made dissipater device was used to avoid overheating of the Peltier element: an aluminium block was placed against the warm side of the Peltier, and cool water circulated continuously inside it. This cooling system was connected to a large water tank (about 50 L)

using a water pump. The entire system was placed in a climatically controlled laboratory at a constant air temperature of 22°C.

In the aluminium slab, 10 ramps (400 × 10 × 5 mm) were dug and covered with a plastic strip to allow the flies – both adults and larvae – to move along the temperature gradient without escaping. The plastic strips were transparent to enable determination of the insects' position along the gradient. Small holes were created through the plastic strip for the introduction of adult flies into the ramps. For larvae, the ramps were filled with a homogeneous nutritional gel (solution composed of distilled water, saccharose 50 $g.L^{-1}$ and agar $7g.L^{-1}$) which permitted the movement of larvae. Finally, the movement of insects within the ramps was recorded using a camera (Kodak PixPro AZ422) positioned above the system, which took an image every 5 min.

The thermal gradient was calibrated before the experiments to determine the temperature at any position along the thermal gradient. The thermal gradient was very stable over time and between assays. For calibration, we used several HOBO devices (UX120-014M, ONSET) connected to thermocouples (T-type copper Constantan, diameter 0.2 mm, TC Direct, Dardilly, France). Plastic strips were designed with multiple holes each separated by 4 cm. Thermocouples were introduced into these holes to record the temperatures along the ramps every 30 sec for 60 min. For each ramp, a linear regression model was applied to relate the spatial coordinate and the mean temperature record over this experimental hour. The thermal gradient was strongly linear in all ramps (all $R^2 > 0.99$), allowing the accurate prediction of temperature from the position along the ramp.

Thermal preference measurement

The apparatus described above, with all electrical parameters kept constant, was used throughout the study to measure the thermal preference of larvae (L3), young adults (3 day-old) and old adults (13 day-old), including virgin and fertile females and individuals infected or uninfected by *Wolbachia*. The life stage, sex and reproductive status of adults were grouped under 'Life stage' for analyses. Measurements were made on individuals from generations 1, 2, 3, 4, 10 and 15. All variables are in Table 1. For each trial (adults or larvae), 10 individuals (with a unique combination of variables) were introduced inside each ramp to assess the range of thermal preference of that source population. A total of 50 individuals with a unique combination of variables were measured. The 10 ramps were considered as independent replicates because no communication or contact was possible among individuals from different ramps. As a control,

Table 1 Variables considered to induce plasticity in *D. suzukii* thermal preference

Environmental	
Developmental temperature	Constant (16, 20, 24°C)
	Fluctuant (18-22, 16-24°C)
Life stage	
Age	Larvae (L3)
	3-day-old adult (D3)
	13-day-old adult (D13)
Sex	Larvae (None)
	Male (M)
	Female (F)
Reproductive status	Larvae (L3)
	Virgin female (Female_V)
	Fertile female (Female_F)
	Male (Male)
LifeS (integrative variable of Sex, Age and Reproductive status)	Larvae (L3)
	3-day-old virgin females (Fem_V_3d)
	3-day-old fertile females (Fem_F_3d)
	13-day-old virgin females (Fem_V_13d)
	13-day-old fertile females (Fem_F_13d)
	3-day-old males (Male_3d)
	13-day-old males (Male_13d)
Symbionts	
Wolbachia infection	Uninfected (Wm)
	Infected (Wp)
Transgenerational	
Generation	G1, G2, G3, G4, G10, G15

we placed flies inside each ramp in the absence of thermal gradient, which resulted in a random distribution (data not shown). Before starting the assays, the gradient was switched on for 15 min in order to stabilise. Adult flies or larvae were placed in the centre of the ramps at a temperature around 20°C. The ramps were photographed every 5 min during 60 min to document individual positions. Initial trial runs indicated that the movements of individuals were much less frequent after about 20 min. Therefore, we considered the preferred temperature of every individual to be the temperature along the thermal gradient at their position after 45 min. All thermal preference assays were run within the same daily time window (between 0900 and 1200) to minimise any influence of circadian rhythm in preferred temperature. The

light environment was homogeneous to avoid any attraction toward a given direction along the thermal gradient (Dillon et al. 2009). All flies were handled with a mouth aspirator to limit stress due to manipulation.

Statistical analysis

Data were analysed in R program ver. 4.1.1 (R Core Team, 2021). Tp data were analysed using a maximum likelihood approach for fitting a mixed effects model using the lmer function in the lme4 package (Bates et al. 2015). Models included developmental temperature, generation, *Wolbachia* infection, life stage and their interactions as fixed factors, and ramp in the gradient as a random factor. Effect sizes (beta) of fixed and random variables on Tp were obtained and plotted using the ggcoef_model function in the ggstats package (Larmarange 2024). Patterns of the relationship between Tp mean and variance (calculated on five ramps) were inferred applying a LOESS procedure (with tension 10) which allowed us to retrieve the pattern without a priori assumption of shape, following the procedure described by Deconninck et al. (under review - Chapter 4). These patterns were used to assess the behavioural thermoregulation strategies according to Deconninck et al. (under review - Chapter 4).

RESULTS

Thermal preference plasticity

Drosophila suzukii Tp was highly plastic and all variables, including most of their interactions, showed significant effects (Table 2). Tp increased with constant developmental temperature from 19.2 ± 3.3 to 20.5 ± 2.3 °C when *D. suzukii* was reared at 16 and 24°C, respectively. The life stage, sex and reproductive status of adults significantly impacted the Tp, effects which are grouped under 'Life stage' in Table 1. Males had the lowest Tp (18.8 ± 2.9 °C), followed by virgin females (19.7 ± 3.0 °C), while larvae and fertile females had the highest Tp (20.6 ± 3.5 and 20.7 ± 2.9 °C, respectively). *Wolbachia* infection reduced Tp from 20.1 ± 3.1°C to 19.6 ± 3.1 °C. Finally, Tp first decreased with generation, from 20.0 ± 3.1 °C in G1 to 19.6 ± 3.2 °C in G3, and then, in G15, returned to values equivalent to those in G1 (20.0 ± 2.9 °C). Effect sizes of all variables allowed the ranking of the most influencing variables, namely the developmental temperature, the life stage and *Wolbachia* infection (Figure 1).

Table 2 Effect of rearing temperature, generation, *Wolbachia* infection, life stage and their interactions on *D. suzukii* thermal preference.

	Chisq	Df	Pr(>Chisq)	
Rearing temperature (E_temp)	512.0302	4	< 2.2e-16	***
Generation (Gen)	41.7171	5	6.720e-08	***
Wolbachia (Wol)	143.2163	1	< 2.2e-16	***
Life stage (LifeS)	1238.6328	6	< 2.2e-16	***
E_temp × Gen	75.0995	17	2.806e-09	***
E_temp × Wol	6.9830	4	0.1368	
Gen × Wol	5.6123	5	0.3458	
E_temp × LifeS	211.1774	24	< 2.2e-16	***
Gen × LifeS	128.2454	30	4.114e-14	***
Wol × LifeS	53.2608	6	1.040e-09	***
E_temp × Gen × Wol	30.5951	17	0.0223	*
E_temp × Gen × LifeS	231.0820	99	1.439e-12	***
E_temp × Wol × LifeS	20.7842	24	0.6514	
Gen × Wol × LifeS	51.5960	30	0.008417	**
E_temp × Gen × Wol × LifeS	134.7048	98	0.008248	**

Behavioural thermoregulation strategy

The behavioural thermoregulation strategies of *D. suzukii* were assessed in relation to the most impactful variables, including life stage, developmental temperature and *Wolbachia* infection (Figure 2). Four main strategies were apparent: (i) 'precision at extreme' (inverted U-shape), (ii) 'precision at optimum' (U-shape), (iii) 'precision at high temperature' and (iv) 'stable high precision' (Figure 2). In W– individuals, 'precision at extreme', including 'precision at high temperature', was the most represented strategy, shared by larvae and adults, with the exception of males and virgin females in G3 that showed 'precision at optimum strategy' and 'stable high precision', respectively. In W+ individuals, strategies were less consistent between life stages. Larvae showed a 'precision at optimum strategy', shared with fertile females in G3, while virgin females in G3, fertile females in G4 and males in G3 showed a 'precision at extreme' strategy. Virgin females and males in G4 did not show clear association with any particular strategy.

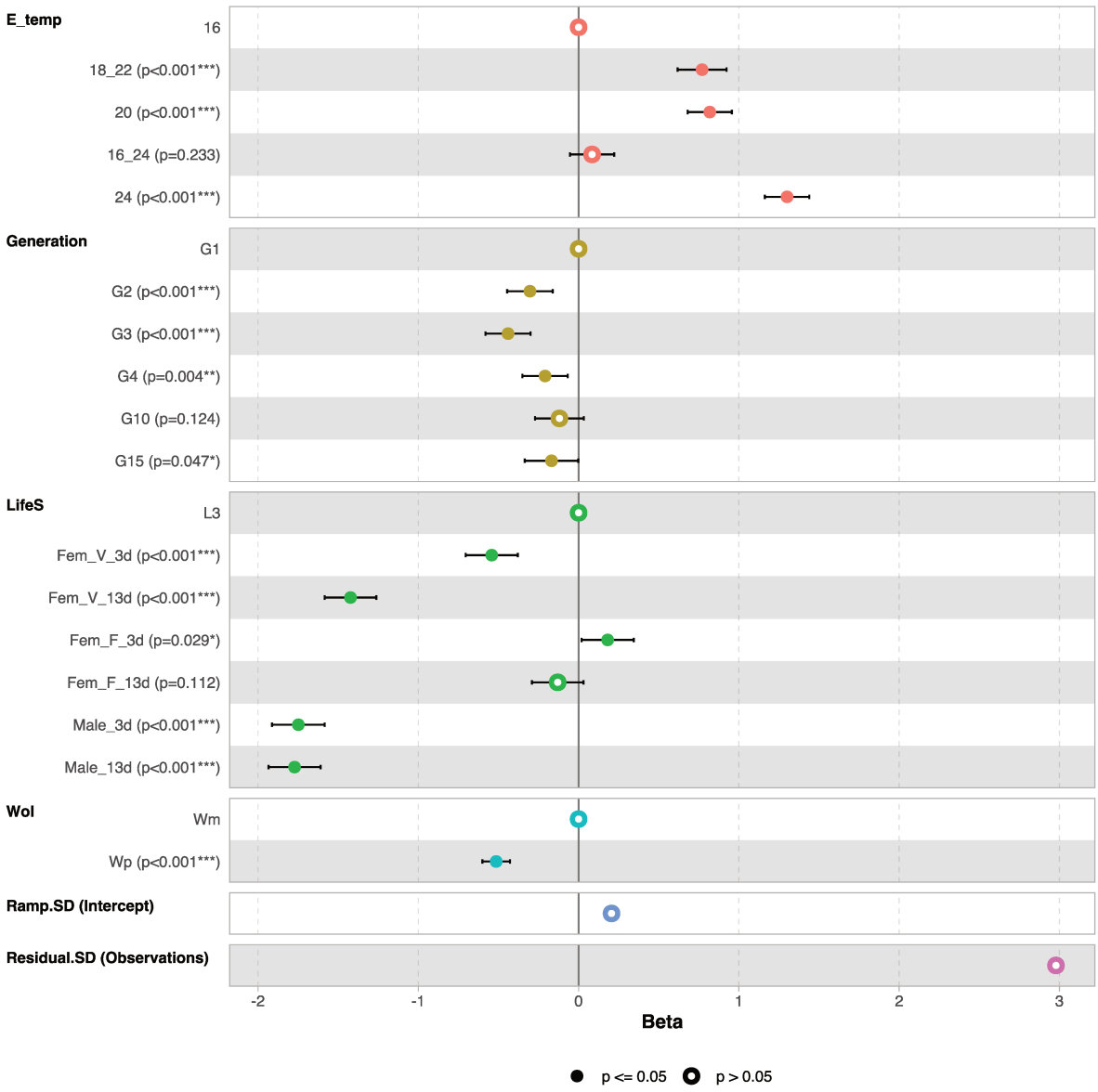

Figure 1 Effect sizes (beta) of fixed and random variables on thermal preference (Tp) of *D. suzukii* with 95% confidence intervals. Positive beta values indicate an increase in Tp and negative values a decrease.

DISCUSSION

Behavioural thermoregulation can allow ectotherms to buffer against environmental changes, before they reach their own thermal limits, and the Tp informs on thermoregulatory abilities. Our data suggest that the Tp of *D. suzukii* is 'hyperplastic'. The ranking of effect sizes allowed us to identify developmental temperature, sex and life stage as being the factors most strongly influencing Tp. The various levels of plasticity, depending on the changing variable, were linked to different thermoregulatory strategies across life stages. 'Precision at extreme' was the most common behavioural thermoregulation strategy. Notably, *Wolbachia* infection completely reversed the behavioural thermoregulation strategy of larvae.

Thermal preference plasticity

Developmental temperature was one of the most important factors influencing Tp, consistent with previous studies (MacLean et al. 2019). Higher constant temperature regimes resulted in increased Tp. Analogous positive correlations have been reported in several ectotherms, including seahorses (Mascaró et al. 2019), shrimps (Reiser et al. 2014), killifishes (Podrabsky et al. 2008) and spiders (Alfaro et al. 2013). In studies of *Drosophila*, effects are more inconsistent with either no effect on adult Tp (MacLean et al. 2019), decrease in Tp when acclimation temperatures are above thermal optima (Krstevska and Hoffmann 1994), or increase in Tp in some species (Yamamoto and Ohba 1984; McDaniel et al. 1995). It is likely that the effect of developmental temperature depends on several factors including how stressful temperatures are to organisms (McDaniel et al. 1995; Dillon et al. 2009). Yamamoto and Ohba (1984) reported that *D. suzukii* Tp increased with acclimation temperature (from 15 to 25°C), consistent with our data.

Fluctuating temperature regimes showed contrasting effects on *D. suzukii* Tp. A low amplitude of fluctuation (18-22°C) gave the same outcomes as an absence of fluctuation with similar mean (constant 20°C) while a large amplitude of fluctuation (16-24°C) led to Tp reduction. Relatively low thermal fluctuations around an optimal temperature might not need an adjustment of the selected temperature as shown in *D. melanogaster* (Deconninck et al. under review - Chapter 4). However, above a certain magnitude of temperature fluctuation organisms might be regularly exposed to sublethal temperatures, which could make a reduction of Tp a viable strategy to protect from potentially harmful conditions, as reported in killifishes (Podrabsky et al. 2008). This outcome is also expected from Jensen's inequality, as being exposed to temperature above the thermal optimum is riskier than being exposed to lower temperatures due to the asymmetry of most thermal performance curves (Martin and Huey 2008).

Life stage, sex and reproductive status also influenced Tp. In *Drosophila* species, both the Tp and its level of plasticity generally differ between larvae and adults (MacLean et al. 2019). Larvae had an overall high Tp, although equivalent to that of fertile females. Being able to remain at a warmer temperature would allow faster development which could give a competitive advantage to larvae (Nunney 1990; Grainger and Levine 2022) and reduce their risk of predation (Grainger and Levine 2022). Females had higher Tp than males, even more so when they were fertile. Fertile females might seek higher temperatures to accelerate oogenesis (Cohet and David 1978; Gandara and Drummond-Barbosa 2022) while virgin females may select relatively lower temperatures to maximise their longevity (Marshall and Sinclair 2010).

Males had the lowest Tp, consistent with reports of elevated temperatures being deleterious to male fertility (Jørgensen et al. 2006; van Heerwaarden and Sgrò. 2021; Ørsted et al. 2024).

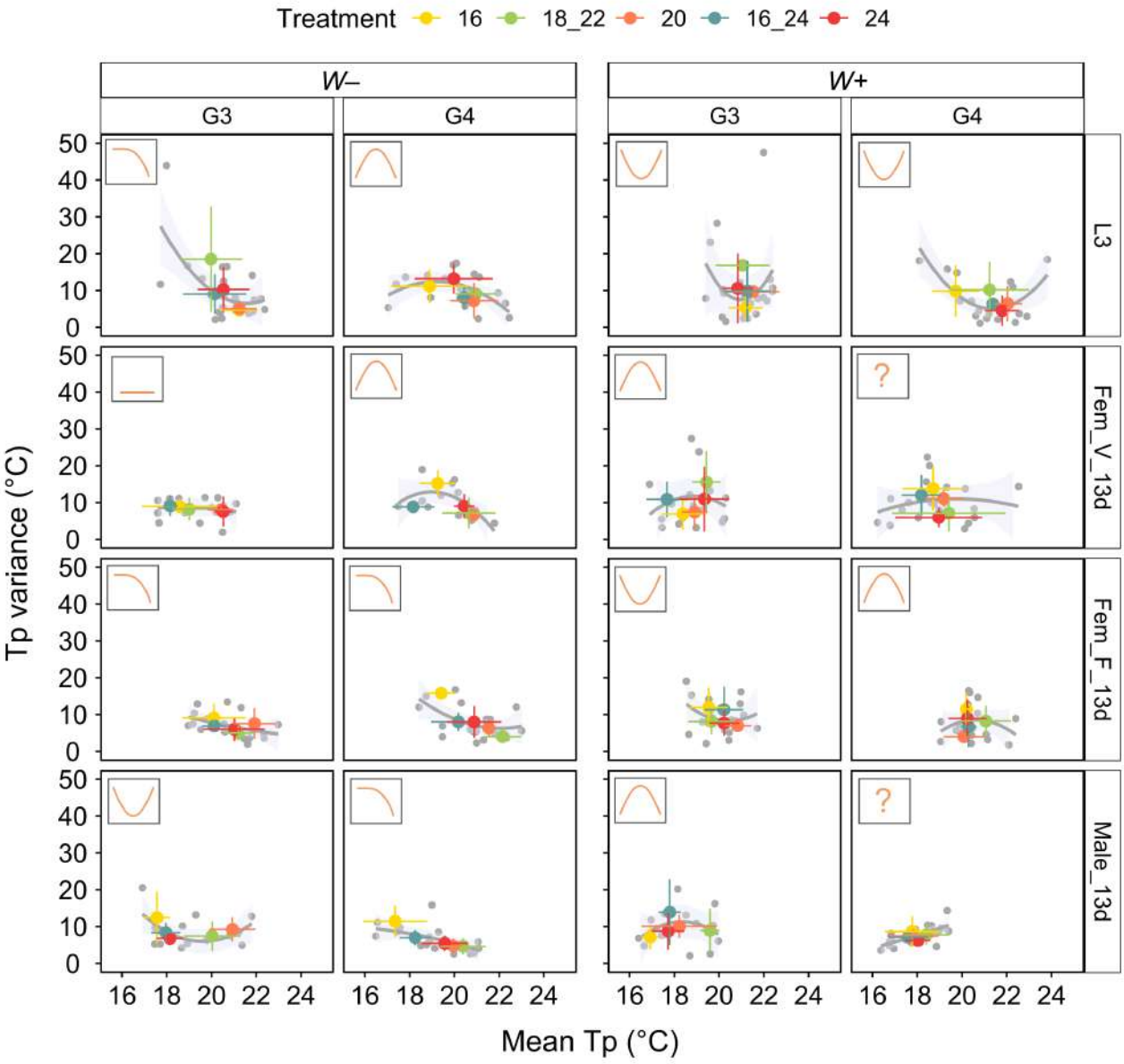

Figure 2 Variance of *D. suzukii* thermal preference (Tp) according to mean Tp. Panels show averaged Tp mean and variance (n = 5) with respect to life stages (L3: third larval stage; L3W: wandering larval stage; V: 2-day-old virgin adults; or A: 12-day-old adults) horizontally and across generations (G3 or G4). Each symbol represents the averaged Tp for a replicate at a given temperature regime (constant 16, 20 or 24°C, in yellow, orange and red, respectively; fluctuating regimes: 18-22 and 16-24°C, in green and blue, respectively). Error bars represent standard errors for both mean and variance of Tp. The grey dots are the raw data for each replicate and the grey curves are drawn using a LOESS procedure (with 95% confidence intervals).

Our data indicated that *Wolbachia* infection lowered the Tp. To date, reports available in the literature on the effect of *Wolbachia* on Tp have been inconclusive. Studies of several *Wolbachia* strains in *D. melanogaster* have reported either a significant decrease in Tp, with the magnitude of the reduction ranging from 2 to 8°C according to the strain (Truitt et al. 2019), or concluded that differences in Tp with or without *Wolbachia* were rather a result of confounding effects (Strunov et al. 2023). Nevertheless, our findings are consistent with Truitt et al. (2019). A reduced Tp when infected with *Wolbachia* (behavioural chill; Fedorka et al. 2016) could be a self-medicating behaviour to reduce the proliferation of the bacteria and attenuate any deleterious effects and fitness costs associated with over-proliferation (Strunov et al. 2013a,b).

Generation was the least impacting factor on Tp. During the first generations, Tp decreased but then stabilised after G4 and went back to the initial measurements. Such transgenerational dynamics of Tp has been rarely demonstrated but was found to exhibit a low heritability and to be mostly influenced by maternal effects (Castañeda et al. 2019; Paranjpe et al. 2019).

Thermoregulation strategies

Behavioural thermoregulation strategies of larvae and adults were similar in the *Wolbachia*-free line, consistent with a 'precision at extreme' strategy. This probably reflects, first, the need of adults to avoid sublethal temperatures, particularly due to impacts on their reproduction system, and, second, the constraint of larvae that are embedded into the substrate where they develop but could seek the less harmful temperature for their development.

The 'precision at extreme' strategy is consistent with recent studies demonstrating that behavioural thermoregulation primarily exists to escape overheating and improve survival, rather than to approach optimal temperatures for reproduction in herbivorous insects (Leith et al. 2024). Moreover, adults must avoid sublethal thermal stress, especially at higher temperatures, as they negatively impact fertility (Parratt et al. 2021; van Heerwaarden and Sgrò 2021; Ørsted et al. 2024), at relatively lower temperatures than their survival (Parratt et al. 2021). *Drosophila suzukii* larvae, even if physically constrained within the fruit they infest, might still be able to benefit from within-fruit thermal heterogeneity to escape overheating (Kührt et al. 2005).

Wolbachia infection reversed the behavioural thermoregulation strategy of both *D. suzukii* larvae and adults. Infected larvae exhibited a 'precision at optimum' strategy. This would allow them to select temperatures that optimise their foraging and accelerate their development, which is a strategy also

reported in reptiles (Ladyman et al. 2003; Taylor et al. 2021). In turn, faster development may reduce the risk of competition or predation (Nunney 1990; Grainger and Levine 2022). *Wolbachia* may also benefit from the fly adopting this strategy, through maximising its transmission and prevalence. Although the effects in adults were less clear, they mostly reflected 'precision at extreme' strategy, including in cold temperature. This could suggest that *Wolbachia* may effectively manipulate the thermoregulation strategy of the fly for its own benefit, seeking warm temperature to maximise its proliferation inside cells (Fedorka et al. 2016; Strunov et al. 2013a,b). This would require further investigation.

Under climate change

In a warming world, we predict that *D. suzukii* larvae may be more at risk of heat-induced mortality than adults as they will have less ability to select different microhabitat conditions. With low to moderate temperature increase, larvae might be able to achieve behavioural thermoregulation within some fruit, where within-fruit thermal heterogeneity could buffer warming (Saudreau et al. 2011). In apples, for instance, codling moth larvae can use behavioural thermoregulation within individual fruit, where thermal gradients can span up to 15°C (Kührt et al. 2005; Saudreau et al. 2007). However, in small and dark fruit, larvae would likely be at higher risk of reaching their thermal limit (Pincebourde et al. 2007; Saudreau et al. 2009), reducing their survival probability. With temperature increase, oviposition choice of *D. suzukii* females may become a more important determinant of larval survival (see Chapter 9).

Previous studies have suggested that males, in *Drosophila* and other insect species, could be more at risk from climate change, as exposure to high temperature is deleterious for sperm production (Jørgensen et al. 2006; van Heerwaarden and Sgrò. 2021; Ørsted et al. 2024). Our data demonstrate that *D. suzukii* males have a potentially important ability to select low temperatures, thereby reducing risk of damage. This suggests that, in the natural environment, *D. suzukii* males may be able to select microclimatic shelters with lower temperatures that would protect them from the impact of warm conditions. Indeed, overall, we found that the level of thermoregulatory precision in *D. suzukii* is high compared to *D. melanogaster* for instance (Deconninck et al. under review - Chapter 4). The local thermal heterogeneity available to them is, therefore, probably more important in defining their susceptibility to warming than larger scale mean or maximum temperatures.

While critical thermal limits are often used to predict vulnerability to climate change, our findings suggest that behavioural thermoregulation strategies deserve serious consideration as they could buffer the effect of warming on life stages or species previously considered as being most at risk. More generally,

integrated studies coupling assessment of critical thermal limits, behavioural thermoregulation strategies and better description of experienced microclimates would allow a better identification of species and life stages vulnerability to climate change.

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Chapter 6

Inter-individual variability of thermal preference

Chapter 6 • Objectives and main outcomes

In this chapter, I carried out further study of the thermal preference (Tp) of *D. suzukii* to decipher the inter-individual variability in Tp in *D. suzukii* through applying and integrating four distinct experimental steps.

I first addressed the Tp repeatability at a short time scale (over three consecutive days). The data obtained indicated that, although showing some variation over time in some individuals, overall, the Tp measurement was consistent with no significant differences between days even though the level of intra-individual variability between days was similar to the inter-individual variability.

Second, I quantified the acclimation ability of *D. suzukii* by measuring the metabolism (i.e., oxygen consumption) of flies reared under different temperature regimes (constant 16, 20 and 24°C and fluctuating16-24°C) at three temperatures (16, 20 and 24°C). These experiments used *Wolbachia*-infected and uninfected flies to address the potential cost of hosting the bacteria. The data obtained demonstrated that *D. suzukii* metabolism responded to temperature and that *Wolbachia* infection lowered the metabolism under some thermal regimes.

Third, I addressed the impact of developmental temperature and infection by *Wolbachia* on the total energy content of *D. suzukii*. Fluctuating temperatures increased the total energy content while *Wolbachia* infection had no detectable effect on the total energy content, contrary to expectation.

Fourth, I examined whether Tp showed any correlation with metabolism addressing contrasting hypotheses linked to costs of maintaining a high metabolism. In parallel, I assessed the correlation between Tp and fitness (i.e., fecundity). Overall, the data obtained suggested that Tp is decoupled from the metabolic status of the flies.

In a last part, I integrated all these results by calculating the overall inter-individual variability of Tp, metabolism rate and energy content and found that Tp variations cannot be explained by inter-individual variability in metabolic rate nor energetic levels, suggesting stochastic behavioural selection of temperature with implication in invasive success.

Contributions

Sylvain Pincebourde, Vincent Foray and I conceptualised the study.

I performed the experiment and collected the data. I was assisted by students (Katrina Madr, Matéo Millet) that I supervised for both data collection and data curation.

I analysed the data, produced the graphics and tables. I wrote the original draft.

Underlying the mechanisms behind thermal preference plasticity in *Drosophila suzukii*

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ABSTRACT

Thermal preference (Tp) is the body temperature an ectotherm selects when provided with access to a large range of temperatures. Tp can be highly variable and depends on numerous factors but despite numerous studies of Tp in various taxa, the physiological mechanisms underlying Tp plasticity are poorly resolved. The ability to select a temperature is linked with the capacity to be active with evidence that acclimation temperature is a major factor influencing Tp, further supporting that metabolism and bioenergetic processes may drive inter-individual variations in Tp.

In this study, we set out to decipher the mechanisms underlying the inter-individual variability in Tp in *D. suzukii.* This invasive fly has a highly plastic Tp and shows significant inter-individual variability, allowing comparison with variation in metabolic rate. We applied and integrated four distinct experimental steps: (i) assessment of the level of repeatability of the Tp measurement in individual flies; (ii) assessment of the level of inter-individual variability in metabolic rate and (iii) in overall energy content using thermal acclimation to induce plastic responses; and (iv) assessment of relationship between metabolism and Tp and between fitness and Tp. In (ii) and (iii), we also considered the effect of *Wolbachia* infection on metabolic rate and energy content, as hosting a symbiont is expected to result in metabolic costs and greater energy content. Finally, we integrated the plasticity of Tp, metabolic rate and energetic content of flies by comparing their levels of inter-individual variability. We hypothesised that, if Tp plasticity resulted from variation in metabolic rate and energetic content, their standardized variations would not differ.

Contrary to our expectations, we show that Tp variations cannot be explained by inter-individual variability in metabolic rate nor energetic levels. Although the Tp is repeatable at the group or population scale, we detect apparent stochasticity in individual choice generating intra-individual variability of the same amplitude than the inter-individual differences. This stochastic behavioural selection, combined with very precise thermoregulatory abilities in *D. suzukii*, may be a crucial component of the invasion success of this fruit fly.

Keywords: Behavioural thermoregulation, energy content, metabolic rate, phenotypic plasticity, *Wolbachia*

INTRODUCTION

Thermal preference (Tp) is the body temperature an ectotherm selects when provided with access to a large range of temperatures. This trait also allows the assessment of the behavioural thermoregulation efficiency (Angilletta 2009). In ectotherms, Tp can be highly variable and depends on numerous factors including body mass (Shepherd et al. 2008; Cabon et al. 2023), reproductive status (MacLean et al. 2019), acclimation (Mascaro et al. 2019), water status (Rozen-Rechels et al. 2019), circadian rhythm (Kaneko et al. 2012), diet quality or quantity (Hardison and Eliason 2024), altitude/latitude (Hodgson and Schwanz 2019), pathogens/disease (Beukema et al. 2021), the presence of symbionts such as *Wolbachia* (Arnold et al. 2019), or the presence of prey/predator cues (Smolinsky and Gvozdik 2012). Despite numerous studies of Tp in various taxa, the physiological mechanisms underlying Tp plasticity are poorly resolved (Killen 2014). The ability to select a temperature is linked with the capacity to be active (Dillon et al. 2012), with evidence that acclimation temperature is a major factor influencing Tp in laboratory studies (MacLean et al. 2019; Deconninck et al. under review, in prep; Chapters 4 and 5), further supporting that metabolism and bioenergetic processes may drive inter-individual variations in Tp.

Metabolic rate represents the costs of living in a specific environment (Chown and Gaston 1999). Metabolic rate and its variation have received considerable attention for many years (reviewed in Chown and Nicolson 2004; Clarke 2006; Denlinger and Lee 2010; Clarke et al. 2017), especially in the context of the 'metabolic theory of ecology' (Brown et al. 2004). In insects, metabolic rate can vary with several factors, including size (Lighton and Fielden 1995), activity (Bartholomew et al. 1985; Reinhold 1999), ambient temperature (Gillooly et al. 2001), as well as age and ontogeny, diapause, gender, feeding status, reproductive status, time of day and season (Chown and Nicolson 2004). Metabolic rate and its temperature-dependence (or rate-temperature (R–T) relationships) are also responsive to environmental variation, and the metabolic response to temperature can differ between populations within a species and between species (Addo-Bediako et al. 2002). inter-individual variation in metabolic rate has been widely documented, but the underlying physiological mechanisms remain unclear (Norin et al. 2015; Norin and Metcalfe 2019; Réveillon et al. 2019). Nonetheless, this inter-individual variability can be linked to behavioural differences between individuals, including when selecting temperature (Norin et al. 2024). Organisms with elevated basal metabolism could actively select low temperatures (i.e., low Tp) to decrease the demands on energetic reserves induced by higher respiration rates (Miquel et al. 1976; Chaabane et al. 1999; Colinet et al. 2015), according to the thermal compensation hypothesis (TCH; Clarke 1993; Le

Lann et al. 2011). Therefore, the inter-individual differences in metabolic rate could be related to inter-individual variability in Tp.

Metabolic rate variations can also depend on the energetic status of organisms (Le Lann et al. 2011; Foray et al. 2014). Temperature, by acting on metabolic rate (Miquel et al. 1976; Terblanche et al. 2005; Mołoń et al. 2020), is expected to impact the rate of energy usage and, hence, energy content of insects (Miquel et al. 1976; Chaabane et al. 1999; Colinet et al. 2015). Elevated temperatures accelerate the respiration rate, which accounts for 50% of insect energy expenditure (Chaabane et al. 1999), and consequently lead to energy depletion., unless compensated by increased foraging Temperature influences energy acquisition during development (Foray et al. 2014) and can modify the allocation of nutrient reserves, in order to maximise relevant life history traits (e.g., fecundity, survival, dispersal; Le Lann et al. 2011; Foray et al. 2014; Tamang et al. 2017). Temperature fluctuations can also generate greater energy demands than constant thermal regimes (Colinet et al. 2015), often resulting in higher lipid storage (Hahn and Denlinger 2007) or variation in fatty acid composition (Roy et al. 1991; Rozsypal et al. 2014; Colinet et al. 2016). inter-individual variation in Tp could result from both the variability in metabolic rate and in energetic content.

Drosophila suzukii provides a good model to study the relationship between Tp, metabolic rate and energetic status. This invasive fly is an important crop pest of soft fruit that causes significant economic losses (Walsh et al. 2011; Tait et al. 2021). The Tp of *D. suzukii* is highly plastic (Deconninck et al. in prep; Chapter 5) and shows significant inter-individual variability, allowing comparison with variation in metabolic rate. The metabolic rate of *D. suzukii* has rarely been a subject of study, but was assessed by Putero et al. (2023) under different types of anaesthesia. Furthermore, some populations of *D. suzukii* host the bacteria *Wolbachia*, which is known to induce a reduction in Tp (Deconninck et al. in prep; Chapter 5). *Wolbachia* presence is expected to induce energetic costs that could result in reduced metabolism (Truitt et al. 2019; Clavé et al. 2022) and lead to increased host energetic content, as reported in a study of *D. melanogaster* (Karpova et al. 2023).

In this study, we set out to decipher the inter-individual variability in Tp in *D. suzukii* through applying and integrating four distinct experimental steps (Figure 1). The first step was to assess the level of repeatability of the Tp measurement in individual flies. The second step was to assess the level of inter-individual variability in metabolic rate. We manipulated acclimation temperature to produce plastic responses on the metabolic rate of *D. suzukii*, allowing us to quantify the inter-individual variability in

metabolic rate. We further examined whether the TCH applied to *D. suzukii* and if fluctuating temperature differentially impacted metabolism. In this step, we also considered the effect of *Wolbachia* on metabolic rate, as hosting a symbiont is expected to result in metabolic costs (Truitt et al. 2019; Clavé et al. 2022). The third step was to estimate the inter-individual variability in overall energy content, again using thermal acclimation to induce plastic responses, and also testing whether *Wolbachia*-infected flies would have greater energy content than uninfected flies (cf. results of Karpova et al. 2023). In the fourth step, we tested correlations between Tp and metabolism, and the impact of Tp on *D. suzukii* fitness, using female fecundity as a proxy measure. Finally, we integrated the plasticity of Tp, metabolic rate and energetic content of flies by comparing their levels of inter-individual variability. We hypothesised that, if Tp plasticity resulted from variation in metabolic rate and energetic content, their variations would not to be significantly different.

MATERIALS AND METHODS

Fly stock

Drosophila suzukii flies originated from a field collection made from infested raspberries in Rennes, France (48°7′2.158″N, 1°40′40.053″W), in October 2020, by the ECOBIO Laboratory (Rennes). Two fly lines differing in the presence (W+) or absence (W−) of *Wolbachia* were obtained by using tetracycline treatment following Hague et al. (2020). The W− fly microbiota was restored to reduce confounding effects from the antibiotic treatment as described by Strunov et al. (2022). Before and after experiments, *Wolbachia* presence in the two lines was assessed by diagnostic PCR using *Wolbachia*-specific primers targeting the wsp gene (81F/691R) (Braig et al. 1998). Flies were reared in 100 mL plastic bottles containing ~30 mL of standard cornmeal diet and incubated at 16°C (MIR-254-PE, PHC Europe, Netherlands), 20°C (STRADER, EV1300, Angers, France), 24°C (MIR-254-PE, PHC Europe, Netherlands) or 16-24°C (MIR-254-PE, PHC Europe, Netherlands), LD 12:12 h and 75% RH. At least 30 bottles of 50–200 flies per line were used for continuous maintenance.

Step 1 • Thermal preference repeatability

Fly stock - Only W− flies reared at 20°C were used in this experiment.

Thermal preference measurement - Three-day-old adult flies (N = 20 males and 20 females) were used to assess the repeatability of Tp measurement over three successive days. The same thermal gradient as described in Deconninck et al. (in prep - Chapter 5) was used. After gradient stabilisation, an individual

fly was introduced individually in the centre of each ramp (10 ramps in total) to obtain individual measures. Pictures were taken every 5 min over 45 min. The position of the fly in the last picture was used to assess the Tp. Then, each fly was placed individually in an annotated tube that was placed back at 20°C

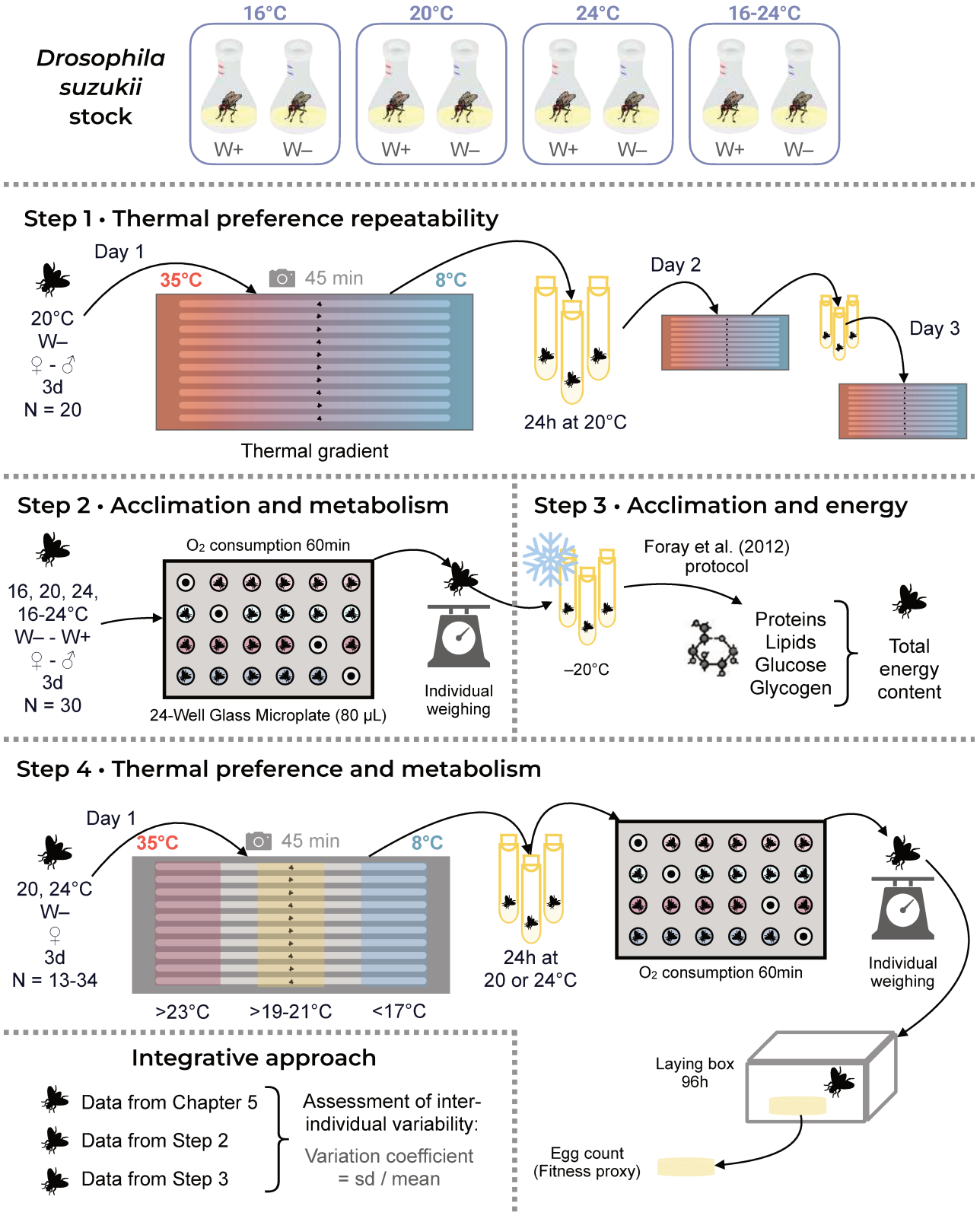

Figure 1 Schematic representation of the protocol steps including the experimental treatments and *D. suzukii* flies used in each step.

until the next measurement. The Tp measure was repeated a further two times on each fly at daily intervals. On each day, flies were randomised in the gradient ramps to avoid confounding effects. A total of 20 males and 20 females were tested.

Statistical analyses - Individuals that escaped or died during the experiment were removed from the analyses (four females and two males). Differences in Tp means between days, with males and females separated, were assessed using RM-ANOVA after verification of data normality and homoscedasticity. Statistical analyses were performed using R version 4.1.3 (R Core Team 2021).

Step 2 • Acclimation and metabolic rate

Fly stock - In this experiment, W– and W+ flies reared at 16, 20, 24 and 16-24°C were used.

Metabolism measurement - Oxygen consumption of individual flies was assessed using 24-well glass microplates (80 µL) (Loligo Systems, Denmark) connected to an oxygen sensor (SDR SensorDish Reader, PreSens, Germany). Three-day-old males and females from the four temperature regimes (N = 30 each) were placed individually in cells and their oxygen consumption was measured over one hour in dark conditions. Flies were randomly allocated to a climatic chamber set at either 16, 20 or 24°C (MIR-254-PE model, PHCbi, The Netherlands). Then, each individual was weighted using a laboratory precision balance (accuracy 0.01 mg; QUINTIX125D-1S; Sartorius, Göttingen, Germany) and stored individually at –20°C for subsequent measurement of total energy content (see Step 3).

Statistical analyses - To obtain the standard metabolic rate (SMR, $pmol.min^{-1}.mg^{-1}$), we divided the measurement of individual oxygen consumption with their mass raised to the 0.856 power (equation 1), which is the interspecific mass scaling exponent for tracheate arthropods (Lighton et al. 2001) and has already been applied in studies of *D. suzukii* (Putero et al. 2023):

$$SMR = MO_2/Mass^{0.856} \quad (1)$$

With MO_2: oxygen consumption in $pmol.min^{-1}$ and mass in mg.

For each rearing temperature regime (16, 20, 24 and 16-24°C), we used a generalised linear model (GLM) to assess the relationship between log-transformed SMR and measurement temperature according to sex and *Wolbachia* infection status. Statistical analyses were performed using R version 4.1.3 (R Core Team 2021).

Step 3 • Acclimation and energy content

Fly stock - In this experiment, W– and W+ flies reared at 16, 20, 24 and 16-24°C and originating from the experiment described in Step 2 were used.

Energy content measurement - The whole-body energy content was calculated for individual W+ and W– males and females from the four rearing thermal regimes. The amounts of proteins, free sugars, lipids and glycogen were quantified individually using an adjusted version of Foray et al.'s (2012) protocol. Individuals from each treatment combination were first solubilised in a phosphate-lysis buffer. Then, total proteins were assayed using Bradford's reagent (B6916, Sigma-Aldrich) with BSA as a standard. In a second step, a methanol:chloroform mixture and a sodium sulphate solution were added to the homogenate to reach the proportions 2:5:10 v/v/v of water, chloroform and methanol, respectively. After vortexing and centrifugation, glycogen was quantified from the pellet while free-carbohydrates and total lipids were assessed from the supernatant. Carbohydrates (free sugars and glycogen) were quantified using Anthrone reagent (319899, Sigma-Aldrich) with D-glucose as a standard, and total lipid quantification was performed using Vanillin (V1104, Sigma-Aldrich), with triolein as a standard. Optical density measurements were performed using a spectrophotometer (NanoQuant, TECAN). Total energy content of the flies was evaluated by converting the mean nutrient content into Joules (conversion factors: proteins 16.0 J/mg; carbohydrates 16.0 J/mg; lipids 37.65 J/g; see Giron and Casas 2003). Thirty flies were analysed per combination of acclimation temperature × *Wolbachia* × sex.

Statistical analyses - To assess the effect of acclimation temperature, sex and *Wolbachia* infection on the flies' energy content, ANOVAs were performed on log-transformed glucose, lipid, glycogen and total energy content, while Kruskall-Wallis tests were performed on protein content which data did not follow a normal distribution. Statistical analyses were performed using R version 4.1.3 (R Core Team 2021).

Step 4 • Thermal preference and metabolism

Fly stock - In this experiment, only W– female flies reared at 20 and 24°C were used.

Thermal preference measurement - Two-to-four-day-old fertile females were used to assess the correlation between thermal preference and metabolism, and the same thermal gradient was used. The gradient was divided into three zones: high (>23°C), moderate (19-21°C) and low (<17°C) Tp. After gradient stabilisation, a fly was introduced in the centre of each ramp to obtain an individual Tp measure. Pictures were taken every 5 min over 45 min. The last picture was taken to assess the Tp and only flies

positioned in the three zones were kept for further measurements. Each fly was then placed individually in an annotated tube and returned back to 20 or 24°C (according to initial rearing temperature) for 24 h before measurement of metabolism.

Metabolism measurement - Individual fly oxygen consumption was assessed as described above. Flies were randomly allocated to a climatic chamber set at either 16, 20 or 24°C (MIR-254-PE model, PHCbi, The Netherlands). Then, females were weighted using a laboratory precision balance (0.01 mg accuracy; QUINTIX125D-1S; Sartorius, Göttingen, Germany) and placed in individual laying boxes in which a 3D-print cylinder containing ~5 mL of standard cornmeal diet for fecundity measurement.

Fitness measurement - Females were kept for 96 h in laying boxes at the temperature corresponding to their metabolism measurement. The total number of eggs laid was counted and used as a proxy for fitness.

Statistical analyses - Oxygen consumption was standardised to the individual mass using equation 1 from Step 2 to obtain the SMR. To determine if there was a relationship between SMR and Tp, we used GLM with Tp and rearing temperature as explicative variables. We performed separated models on females reared at 20 and 24°C. To assess the effect of Tp on fitness, we used GLM with quasi-Poisson distribution. A minimum of 13 females per Tp category × measurement temperature was used. Statistical analyses were performed using R version 4.1.3 (R Core Team 2021).

Integrative approach

To integrate the inter-individual variation in Tp, SMR and energetic content, we used the SMR and the total energy content obtained in this study as well as the variation in Tp data obtained in a previous study (Deconninck et al. in prep; Chapter 5). Only individuals sharing the same treatments were considered for Tp data, i.e. 3-day-old adults, males and fertile females, reared at 16, 20, 24 or 16-24°C, from a single generation.

A variation coefficient was calculated for Tp, SMR and energetic content and for each combination of variables, including rearing temperature, sex, *Wolbachia* infection, by dividing the standard deviation of each by its mean (e.g., variation coefficient for females reared at 20°C and uninfected by *Wolbachia*). We took advantage of this variation coefficient to compare the level of dispersion (inter-individual variability) between the different variables that have contrasting values and ranges. Differences between variation coefficients were assessed using Kruskal-Wallis test followed by Wilcoxon pairwise comparisons.

RESULTS

Step 1 • Thermal preference repeatability

Flies that died during the experiments were discarded (final sample sizes are given in Table S1). Individual Tp did not differ significantly across the 3 d measurement period for either sex ($p = 0.349$ and $p = 0.605$ for males and females, respectively; pairwise comparisons in Table S1). Nevertheless, Tp varied by up to 6°C from day to day in several individuals (Figure 2). The level of intra-individual variability over time was similar to that of inter-individual variability throughout the study.

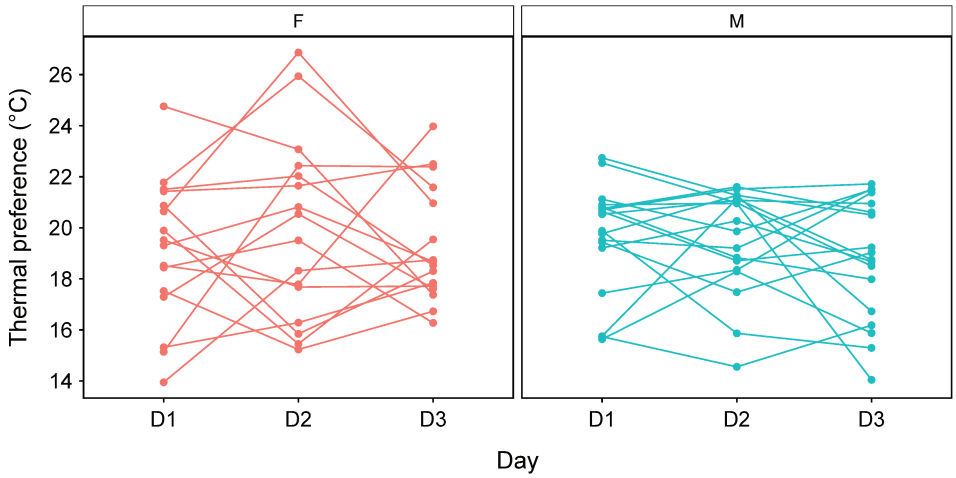

Figure 2 Thermal preference of *D. suzukii* females (F) and males (M) measured during three consecutive days. Females are in red, males in blue.

Step 2 • Acclimation and metabolic rate

Oxygen consumption and mass - MO_2 increased linearly with fly mass (Figure S1; $p < 0.0001$).

SMR temperature sensitivity - Results for SMR are standardised by fly mass as described in Methods. Temperature sensitivity of SMR increased with the acclimation temperature (Table S2) and females were less sensitive than males at 16°C.

Sex - Males had a higher SMR than females ($p < 0.0001$; Table S2), especially when reared at 24°C (Figure 3).

Wolbachia effect - W+ females and males had significantly lower SMRs than W− flies when acclimated at 16-24°C ($p = 0.0003$ and $p = 0.0012$, respectively). W+ males also had a lower SMR when acclimated at 16 and 20°C compared to W− males ($p = 0.0232$ and $p = 0.0012$, respectively). Generally, however, the level of inter-individual variability in SMR was always larger than the effect size of these factors, and this variation was most pronounced when the flies were reared at 24°C (Figure 3).

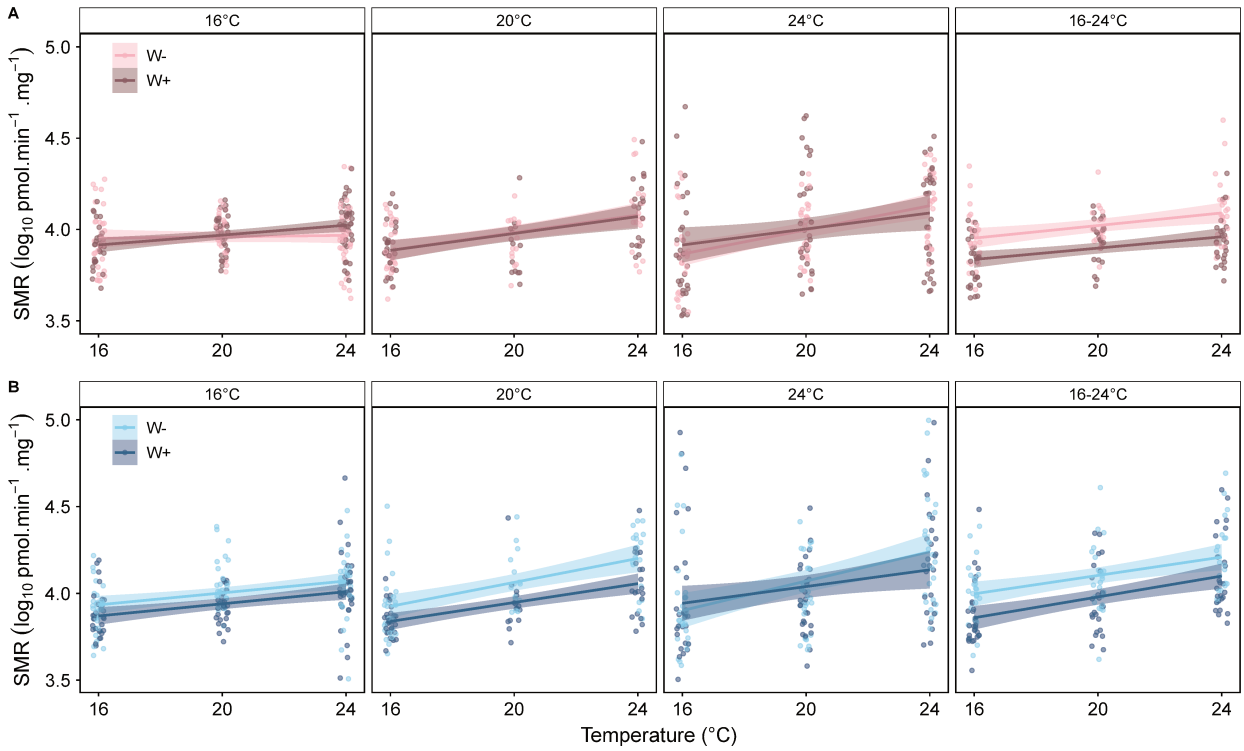

Figure 3 *Drosophila suzukii*'s log-transformed standard metabolic rate (SMR) according to measurement temperature (16, 20 or 24°C). Panels correspond to flies acclimated at different temperatures (constant: 16, 20, 24°C; fluctuant 12h/12h: 16-24°C). Lines represent linear models. Females are in pink, males in blue; *Wolbachia*-infected flies (W+) are darker than uninfected ones (W−).

Step 3 • Acclimation and energy content

Males vs females - Females contained significantly more glucose and lipids compared to males, resulting in overall higher total energy content (mean ± sd: 18.5 ± 9.2 J/mg against 15.3 ± 9.2 J/mg; $p < 0.0001$; Table S3).

Wolbachia - *Wolbachia* infection had no detectable influence on individual macronutrient or total energetic content in *D. suzukii* (Table S3).

Developmental temperature - In males, individual macronutrient content and total energy content did not differ significantly between constant temperature developmental regimes (Figure 4). In females, protein and lipid contents increased with temperature in these regimes, resulting in a slightly greater, but not significant, total energetic content at 24°C compared to 16 and 20°C (Figure 4A). Under the fluctuating temperature regime (16-24°C), lipid contents were significantly greater in both males and females, resulting in significantly greater total energy content in the fluctuating regimes compared to the constant regimes (Figure 4). Glycogen content in males was also slightly higher, but not significant, under the fluctuating regime (Figure 4B).

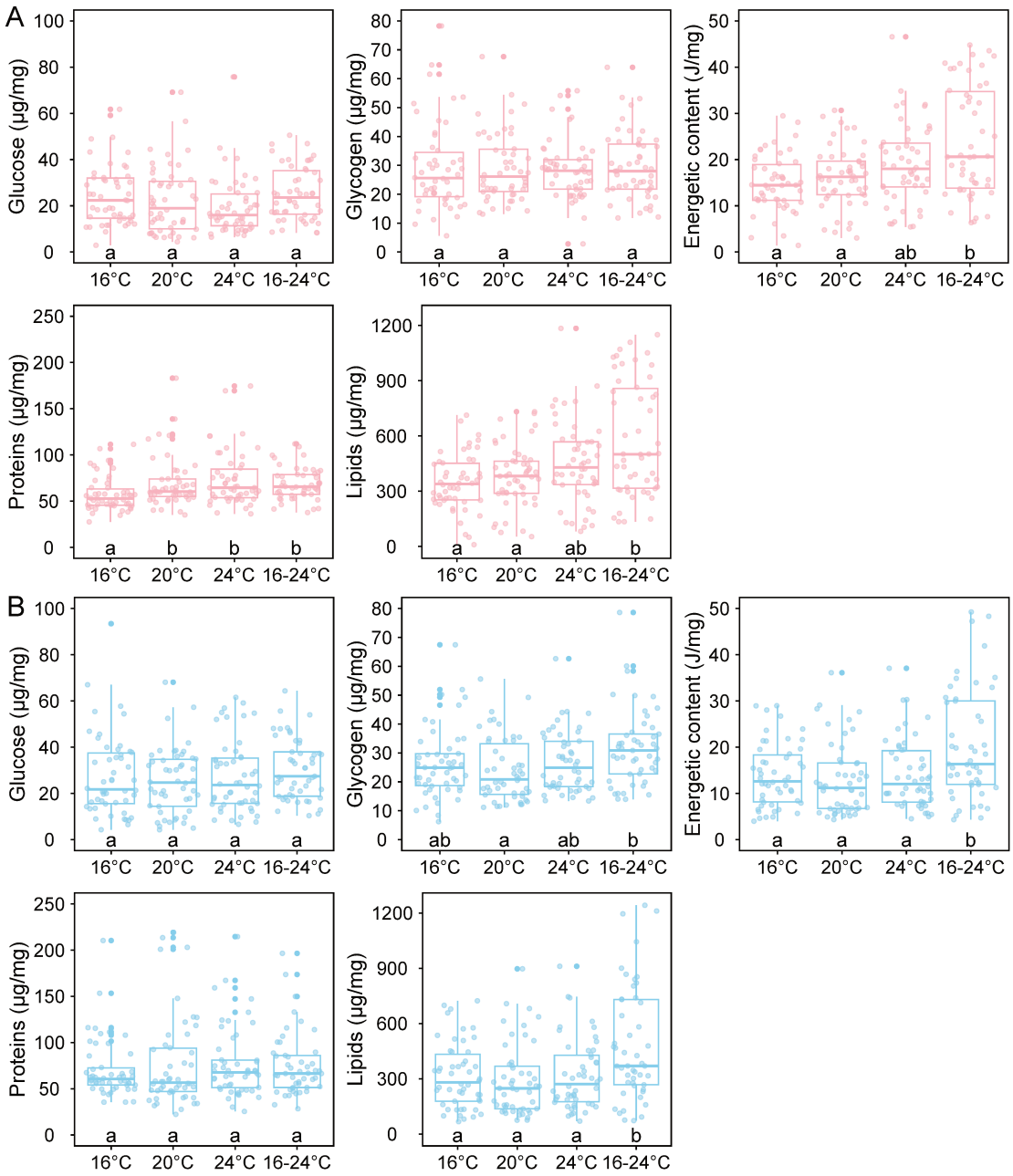

Figure 4 *Drosophila suzukii* (A) female and (B) male macronutrient content (glucose, glycogen, proteins and lipids in µg/mg) and total energetic content (J/mg) according to development temperature (constant: 16, 20, 24°C; fluctuant 12h/12h: 16-24°C). Significant differences between means are indicated by different letters at $p < 0.05$.

Step 4 • Thermal preference and metabolism

Relationship between Tp and metabolism - Final sample sizes are given in Table S4. There was no effect of thermal preference on SMR (Figure 5). The SMR significantly decreased with measurement temperature (Figure 5; Table S5).

Relationship between Tp and fitness - Drosophila suzukii females had comparable fecundity whatever their thermal preference (20°C: $p = 0.540$; 24°C: $p = 0.976$; Figure 6, Table S6).

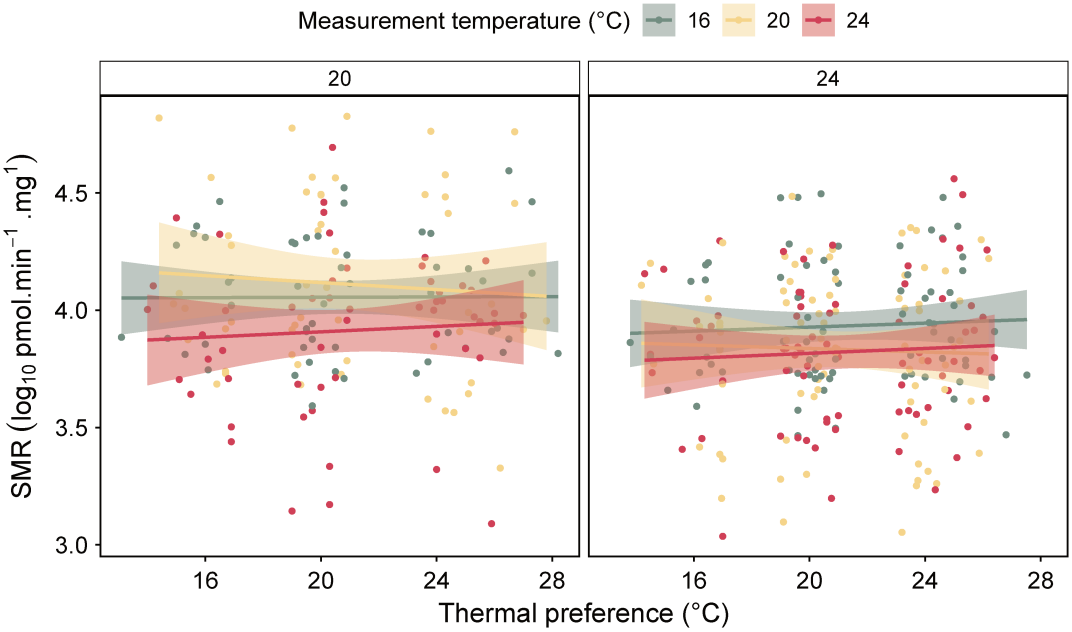

Figure 5 *Drosophila suzukii*'s log-transformed standard metabolic rate (SMR) according to thermal preference and measurement temperature (16, 20 or 24°C in green, yellow and red, respectively). Panels correspond to flies acclimated at different temperatures (constant: 20 or 24°C).

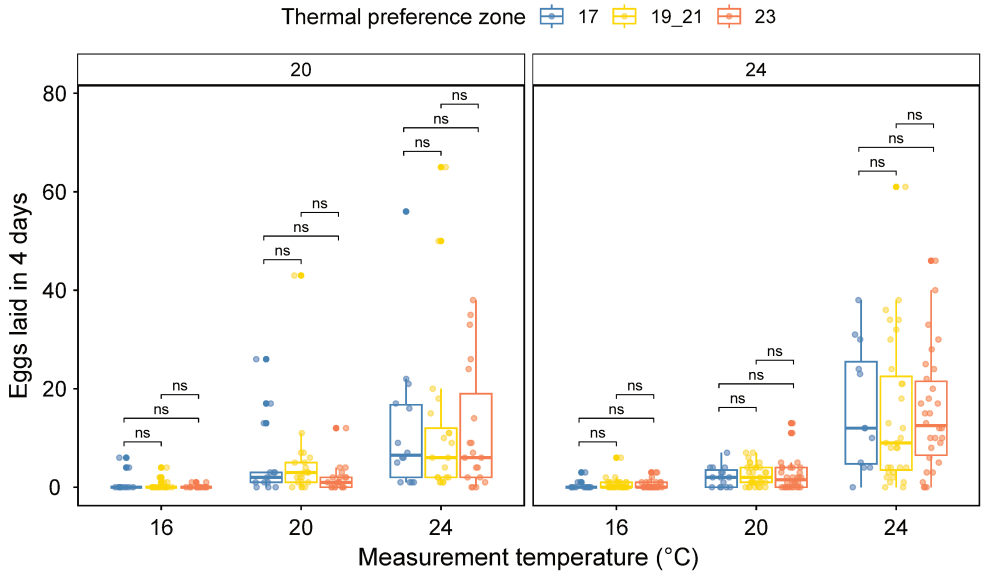

Figure 6 *Drosophila suzukii*'s fecundity after 4 d according to measurement temperature (16, 20 or 24°C) and thermal preference zone (17, 19-21 or 24°C, in blue, yellow and red, respectively). Panels correspond to flies acclimated at different temperatures (constant: 20 or 24°C). ns: non-significant difference at $p < 0.05$.

Integrative approach

All variation coefficients were significantly different ($p < 0.0001$), with Tp having the lowest (0.14 ± 0.02), SMR intermediate (0.23 ± 0.09) and energy content the highest (0.50 ± 0.08) (Figure 7).

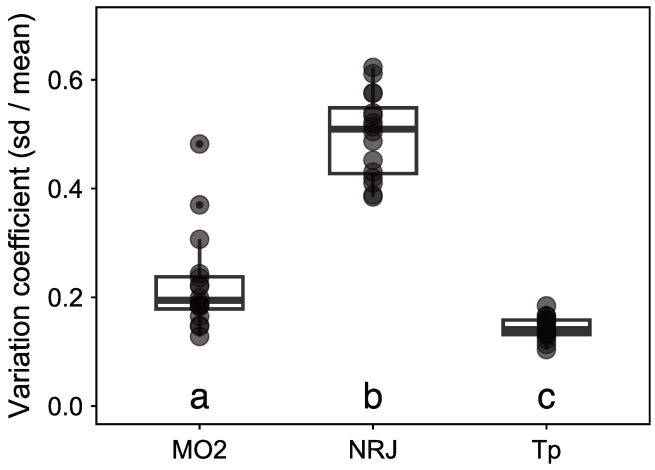

Figure 7 Variation coefficients of metabolic rate (MO2), total energy content (NRJ) and thermal preference (Tp) in *D. suzukii*. Different letters indicate significant differences at $p < 0.05$ (Kruskal-Wallis test followed by Wilcoxon pairwise comparisons).

DISCUSSION

The Tp of ectotherms often displays significant amounts of inter-individual variability, but the underlying physiological processes remain largely understudied. Inter-individual variations in basal or standard metabolic rate, and more generally the bioenergetic, were proposed as explanatory factors of these Tp variations (Killen et al. 2014). Our results do not support this assumption. We show that the measurement of Tp is repeatable statistically, but the inter-individual variability seems to include some level of stochasticity. We further show that in *D. suzukii*, the SMR responds to temperature as expected but the rate of change in MO2 as a function of temperature remains relatively small. This low response is reflected by the lack of differences in energy content across acclimation temperatures. Finally, the metabolic rate could not be correlated to the Tp of flies. An indirect cue of the absence of link between Tp, energy content and SMR is provided by the differences in the amplitude of inter-individual variability between these metrics. We conclude that the inter-individual variation in Tp does not seem to be related to the bioenergetic and metabolism of flies.

Thermal preference repeatability

As described in Deconninck et al. (in prep; Chapter 5), several factors including sex, age, reproductive status, and infectious status showed significant influences on Tp and significant inter-individual variability was observed in all instances. In the current study, individual Tp was statistically repeatable on average over a short time scale of three days, even though the level of intra-individual variability between days was similar to the inter-individual variability. This suggests that Tp variability within a population may be linked primarily to the temporal variation in the physiological status of individuals rather than to particular phenotypes. A similar experiment performed using *D. subobscura* reported that Tp was repeatable for females but not for males (Rego et al. 2010). In much larger ectotherms, such as lizards, the repeatability of Tp seems to be species-dependent (Clusella-Trullas et al. 2007; Truter et al. 2014; Goulet et al. 2017). A major drawback of these repeatability assessments, however, is that it remains challenging (if even possible) to partition the effect of age, physiological status and stochasticity or precision of the behaviour of flies. The experimental procedure used may also induce some level of stress through manipulating the same fly several times per day may not be pertinent. Such challenges may explain the discrepancies observed between species studied to date.

Acclimation and metabolic rate

The measured SMR increased with temperature as would be expected (Gillooly et al. 2001). Our data indicated that males had a higher SMR than females in *D. suzukii*, contrasting with the results reported by Putero et al. (2023). This might be a consequence of the measurement method and materials used. Although MO_2 measurements were performed in the dark, individuals could still have been able to move inside the wells, and especially males that are more active than females in the dark (G. Deconninck, unpublished data). The two studies also used different respiratory quotients, as Putero et al. (2023) measured SMR via CO_2 release while O_2 consumption was measured here. In *D. melanogaster*, the respiratory quotient does not differ between males and females (Brown et al. 2022), but in *D. suzukii* respiratory quotients were never assessed.

Female SMR was less sensitive to temperature than that of males, as also reported by Putero et al. (2023), at least at 16°C. The TCH was not verified by our data, with the SMR of individuals reared at low temperature not being higher than that of individuals reared at warmer temperatures. However, the range

of temperatures tested might have not been sufficient to induce thermal compensation and modifications in energy allocation (Le Lann et al. 2011).

The SMR of *Wolbachia*-infected flies was either comparable with or lower than that of uninfected flies. In most studies on the effect of *Wolbachia* on metabolic rate, infection increases the metabolism of the host organism because of the energetic costs of hosting this symbiont. For instance, *Wolbachia* increased the metabolism of the mosquito *Aedes aegypti* (Evans et al. 2009). In pharaoh ant colonies, *Wolbachia* effect depended on the queen's age, either having no effect or increasing metabolism (Singh et al. 2024). Our data suggest that *Wolbachia* infection does not have detectable energetic costs in *D. suzukii*, although we cannot discount costs being offset through nutritional symbiosis with *Wolbachia*, as found in the bed bug, *Cimex lectularius* (Hosokawa et al. 2010; Nikoh et al. 2014).

Acclimation and energy content

As predicted, the total energy content of flies increased when reared in fluctuating regimes (Colinet et al. 2015). This was particularly a result of higher lipid contents, consistent with previous studies (Roy et al. 1991; Hahn and Denlinger 2007; Rozsypal et al. 2014; Colinet et al. 2016). Total energy content also increased with temperature but only in females. In *Aedes albopictus*, the energy content of male and female mosquitoes was impacted by temperature, especially for proteins and lipids, but without clear directional patterns (Carlassara et al. 2024).

Wolbachia infection did not impact the total energy content of flies, contrary to our expectations. However, the effects of *Wolbachia* on energy content are often inconsistent in the literature. In *D. melanogaster*, *Wolbachia* increased overall lipid and glucose contents, although the effect was strain dependent (Karpova et al. 2023). In *Trichogramma brassicae* asexual females, lipid and protein contents increased in the presence of *Wolbachia* while carbohydrates decreased and glycogen was not impacted, but the authors did not discuss these results in the context of *Wolbachia* infection (Rahimi-Kaldeh et al. 2019). In *Aedes aegypti*, *Wolbachia*-infected mosquitoes had higher metabolic rates and activity compared to uninfected mosquitoes, which was expected to induce increased energy demand (Evans et al. 2009). In another study, *Wolbachia*-infected mosquitoes had a shorter lifespan than uninfected flies, which could be a result of depletion of energy content but this hypothesis has not been tested directly (Ross et al. 2016). The effect of *Wolbachia* infection on insect energy content clearly require further investigation.

Thermal preference and metabolism

The SMR does not seem to be related to the Tp of flies, suggesting that the inter-individual variability in metabolic rate is not related to that of the Tp. Nevertheless, this result is based on metabolic measurements demonstrating a weak temperature-dependence which contrasts with previous studies (Gillooly et al. 2001) and also to the results in step 2 to some extent. Taken together these results seem to indicate that the metabolism of *D. suzukii* is less sensitive to temperature than expected for an ectotherm. The absence of any relationship can be explained by several hypotheses. The first one is linked to opposite strategies of flies that cancelled each other when plotted: if *D. suzukii* has a high metabolism, the fly could select a low temperature to reduce its metabolism or, on the contrary, select a high temperature to maintain this high metabolism and therefore their activity level, and conversely for flies with low metabolism that could choose high temperature to raise their metabolism or low temperature to maintain this basal level. The other hypothesis would be that the potential adaptive value of Tp is not linked directly to metabolism. Indeed, we may be challenged by the problem of temporal scale of the physiological and behavioural integration. The Tp corresponds to a behaviour that is expressed at a relatively short time scale of minutes. By contrast, the metabolism physiology integrates various processes over a longer time scale. At one extreme, the absence of correlation between Tp variations and fitness proxies can certainly be explained by this scaling mismatch. Finally, the level of stochasticity that was detected in the repeatability analysis (Step 1) seems to indicate that a significant part of the Tp variations cannot be explained by physiological processes at the individual scale. This is reinforced by the variation coefficient analysis since the Tp displayed much less dispersion than the SMR and the energetic level. More research is required here, especially to uncover the adaptive value of Tp variations linked to plastic responses.

CONCLUSION

In this study, we searched for a link between the inter-individual variability in Tp, its plasticity and the physiology of metabolism. Contrary to our expectations, we show that Tp variations cannot be explained by inter-individual variability in metabolic rate nor energetic levels. Although the Tp is repeatable at the group or population scale, we detect apparent stochasticity in individual choice generating intra-individual variability of the same amplitude than the inter-individual differences. This stochastic behavioural selection, combined with very precise thermoregulatory abilities in *D. suzukii*

(Deconninck et al. in prep; Chapter 5), may be a crucial component of the invasion success of this fruit fly. *Drosophila suzukii* is extremely polyphagous and can develop in a large spectrum of host plants (Poyet et al. 2015; Kenis et al. 2016), implying that the probability to encounter a host plant is high. Therefore, the stochastic behaviour may facilitate dispersion, thereby increasing the speed of the invasion. Albeit complex, these interactions between behaviour, metabolism physiology, fitness and invasion potential should be integrated to better understand what makes a species a successful invasive species.

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Supplementary Materials

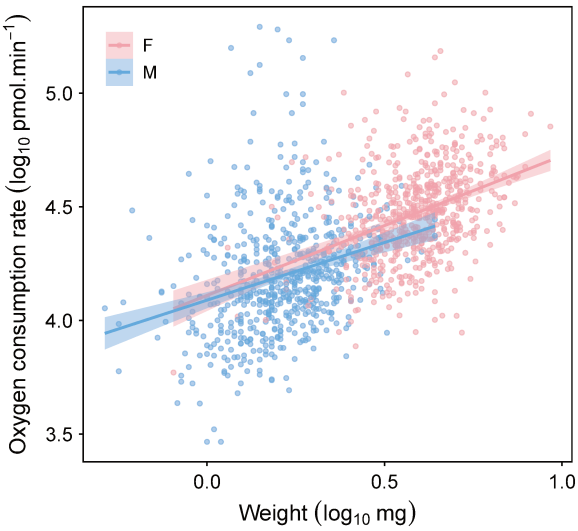

Figure S1 *Drosophila suzukii*'s log-transformed oxygen consumption rate according to log-transformed fly's mass. Lines represent linear models. Females are in pink, males in blue.

Table S1 Summary of RM-ANOVA comparing the thermal preference of *D. suzukii* males or females measured over three consecutive days (D1, D2, D3).

Sex	.y.	group1	group2	n1	n2	statistic	df	*p*	*p*.adj	signif
F	Tpref	D1	D2	16	16	-0.9550228	15	0.355	1.000	ns
F	Tpref	D1	D3	16	16	-0.2007508	15	0.844	1.000	ns
F	Tpref	D2	D3	16	16	0.7680357	15	0.454	1.000	ns
M	Tpref	D1	D2	18	18	0.2096378	17	0.836	0.836	ns
M	Tpref	D1	D3	18	18	1.1633327	17	0.261	0.729	ns
M	Tpref	D2	D3	18	18	1.2106689	17	0.243	0.729	ns

Table S2 Result summary of the GLM on the SMR of *D. suzukii* flies.

	LR Chisq	df	*p*-value	signif
Acclimation temperature (Acclim)	25.102	3	1.470E-05	***
Measurement temperature	187.813	1	< 2.2e-16	***
Sex	17.248	1	3.280E-05	***
Wolbachia (Wol)	15.401	1	8.696E-05	***
Acclim × M_temp	25.285	3	1.346E-05	***
Acclim × Sex	9.141	3	0.0275	*
M_temp × Sex	9.937	1	0.0016	**
Acclim × Wol	16.988	3	0.0007	***
M_temp × Wol	0.343	1	0.5581	
Sex × Wol	2.891	1	0.0891	.
Acclim × M_temp × Sex	0.343	3	0.9517	
Acclim × M_temp × Wol	2.263	3	0.5196	
Acclim × Sex × Wol	4.120	3	0.2488	
M_temp × Sex × Wol	0.000	1	0.9826	
Acclim × M_temp × Sex × Wol	1.995	3	0.5734	

Table S3 Result summary of ANOVA on glucose, glycogen, lipid and and energetic content and Kruskal-Wallis test on protein content of *D. suzukii* flies.

	df	Sum Sq	Mean Sq	F value	*p*-value	signif
Glucose						
Developmental temperature	3	4.04	1.348	3.890	0.00924	**
Wolbachia	1	0.05	0.054	0.155	0.69401	
Sex	1	3.52	3.517	10.148	0.00156	**
Residuals	393	136.21	0.347			
Glycogen						
Developmental temperature	3	1.49	0.4962	2.631	0.0497	*
Wolbachia	1	0.04	0.0423	0.224	0.6360	
Sex	1	0.48	0.4841	2.567	0.1099	
Residuals	393	74.11	0.1886			
Lipids						
Developmental temperature	3	13.02	4.339	10.705	8.89E-07	***
Wolbachia	1	0.04	0.039	0.095	0.758	
Sex	1	7.06	7.055	17.405	3.72E-05	***
Residuals	393	159.31	0.405			
Total energy content						
Developmental temperature	3	10.39	3.464	11.522	2.96E-07	***
Wolbachia	1	0.02	0.020	0.067	0.797	
Sex	1	4.74	4.745	15.782	8.46E-05	***
Residuals	393	118.15	0.301			
	df		**Chi-squared**		*p*-value	**signif**
Proteins						
Developmental temperature	3		12.807		0.0051	**
Wolbachia	1		0.59171		0.4418	
Sex	1		0.83976		0.3595	

Table S4 Sample size for Step 4 - Thermal preference and metabolism.

T_MO2	Tp zone	N - 20°C	N - 24°C
16°C	< 17°C	13	14
	19-21°C	22	30
	> 23°C	18	31
20°C	< 17°C	13	15
	19-21°C	21	34
	> 23°C	20	33
24°C	< 17°C	15	13
	19-21°C	20	31
	> 23°C	21	31

Table S5 Summary of GLM on SMR of *D. suzukii* females according to developmental temperature.

Developmental temperature	**Explicative variable**	**Df**	**Sum Sq**	***F* value**	***p*-value**
20°C	Thermal preference (Tp)	1	0.0003	0.0030	0.9566
	Measurement temperature (T_MO2)	2	1.1428	5.2647	0.0061
	Tp × T_MO2	2	0.0632	0.2912	0.7478
	Residuals	155	16.8222		
24°C	Thermal preference (Tp)	1	0.0112	0.1204	0.7289
	Measurement temperature (T_MO2)	2	0.5574	3.0029	0.0517
	Tp × T_MO2	2	0.0367	0.1979	0.8206
	Residuals	223	20.6970		

Table S6 Summary of GLM with quasi-Poisson distribution on total number of eggs laid by D. suzukii females according to developmental temperature.

Developmental temperature	**Explicative variable**	**Df**	**Chi-squared**	***p*-value**
20°C	Thermal preference (Tp)	2	1.232	0.5401
	Measurement temperature (T_MO2)	2	65.859	5E-15
	Tp × T_MO2	4	3.084	0.5439
24°C	Thermal preference (Tp)	2	0.048	0.9764
	Measurement temperature (T_MO2)	2	285.471	< 2E-16
	Tp × T_MO2	4	0.588	0.9644

DISCUSSION

Part 2

Thermal preference plasticity and underlying physiological mechanisms

This Part 2 of my thesis explores the plasticity of the thermal preference (Tp), its consequences for thermoregulation behaviour and its link to metabolism and bioenergetic.

The Tp provides crucial information on ectotherms' ability to thermoregulate behaviourally and therefore to survive and/or reproduce in heterogeneous environments (Leith et al. 2024). We developed a conceptual framework allowing the determination of behavioural thermoregulation strategies by integrating the Tp mean and the Tp variance, which correspond to the thermal need and the thermoregulatory precision, respectively. By testing this framework on *D. melanogaster*, we were able to determine that a strategy of 'precision at extreme' was dominant in all life stages, which was consistent with recent studies stating that behavioural thermoregulation was primarily used to escape overheating and improve survival, rather than a means of approaching the optimal temperature for reproduction in herbivorous insects (Leith et al. 2024). It is also likely that the amplitude of Tp responses to various variables, both endogenous and exogenous, modulate the ecological responses to climate warming.

We applied our framework on *D. suzukii* by inducing plastic responses of Tp with many more variables than previously with *D. melanogaster*. 'Precision at extreme' was again the predominant behavioural thermoregulation strategy. However, *Wolbachia* infection shifted the strategy, which could be a result of either *D. suzukii* selecting temperatures to limit *Wolbachia* proliferation (as suggested in *D. melanogaster* by Truitt et al. 2019) or of *Wolbachia* effectively manipulating the thermoregulation strategy of the fly for its own benefit, hypotheses that require further investigation. Our data also showed that males were highly precise in selecting low temperature that would protect them against heat-induced sterility. This suggested that they could be less at risk with warming than other life stages, contrary to what was supported by other studies (van Heerwaarden and Sgrò 2021; Ørsted et al. 2024). Critical thermal limits are not sufficient to assess organisms' vulnerability to climate change and need integrative approach with determination of the local thermal heterogeneity available and of their thermoregulation ability.

Finally, we investigated the physiological mechanisms underlying the inter-individual variability of Tp and we detected apparent stochasticity in individual choice that generated intra-individual variability of the same amplitude than the inter-individual differences. This stochastic behavioural selection, combined with very precise thermoregulatory abilities in *D. suzukii* and its exceptional polyphagy, may be a crucial component of its invasion success, potentially facilitating dispersion.

Part 3

Polyphagy, temperature and resource selection

Part 3
Polyphagy, temperature and resource selection

This Part 3 integrates the first (trophic relationships) and the second (thermal properties) Parts by tackling the three-way interaction between trophic resources, temperature and *Wolbachia* infection.

In the first chapter of Part 3 (Chapter 7), I investigated the influence of low temperature on the performance of *D. suzukii* in winter-available fruit. Most studies of *D. suzukii* performance using wild fruit substrates have used a constant experimental temperature of ~20-25°C (Poyet et al. 2015; Panel et al. 2018) which may not be ecologically relevant when considering winter and spring periods. Furthermore, temperature influence both *D. suzukii* life history traits (Raynaud-Berton et al. 2024) and nutritional requirements (Rendon et al. 2019). Therefore, I studied the effect of fluctuating winter/early spring-like experimental temperatures on several life history traits of *D. suzukii* reared using a sub-set of the winter-available fruit studied in Chapter 3 (*A. japonica, E* ×*submacrophylla* and *V. album*).

In the second chapter of Part 3 (Chapter 8), I focused on the potential mutualistic role played by *Wolbachia* infection. As most available studies on *D. suzukii*'s *Wolbachia* strain seem to indicate that it plays a mutualistic rather than a parasitic role (Saeed et al. 2018; Mazzetto et al. 2018; also supported by the findings of Part 2), this chapter examines the existence of a nutritional role. As a polyphagous species, *D. suzukii* faces appreciable variations in the nutritional quality of its breeding and nutritional substrates which *Wolbachia* could help overcome. I used infected and uninfected flies and two diets differing in their nutritional quality and measured several life history traits to assess the effect of *Wolbachia*.

In the third chapter of Part 3 (Chapter 9), I offset out to integrate the influences of temperature, winter-available fruit and *Wolbachia*, specifically, in relation to oviposition substrate choice of *D. suzukii*. Several previous studies have examined the influence of nutritional quality on oviposition preference and performance, considering different fruit species (Cai et al. 2019), protein:carbohydrate ratio (Silva-Soares et al. 2017), presence of damage (Kienzle and Rohlfs 2021) or microbes (Sato et al. 2021). However, no studies have yet examined the combined influence of temperature and nutritional quality on *D. suzukii* oviposition choice. Similarly, although *Wolbachia* influence host choice in some but not all species (Wiwatanaratanabutr et al. 2010; Abrun et al. 2021), its role in oviposition has not been studied in *D. suzukii*. This chapter examined the influence of temperature and nutritional quality on *D. suzukii* oviposition choice, both of *Wolbachia*-infected and uninfected females. I also assessed if the preference-performance hypothesis (Gripenberg et al. 2010) could be verified in *D. suzukii* under these conditions.

Chapter 7

Temperature, nutritional resources and performance

Chapter 7 • Objectives and main outcomes

In this chapter, I quantified the performance of *D. suzuki* in winter- / early spring-available fruit in the laboratory under realistic environmental temperature conditions. Building on Part 1 - Chapter 3, which confirmed that *D. suzukii* was able to develop in *A. japonica*, *E.* ×*submacrophylla* and *V. album* fruit (and others) and how external factors drove infestation in those fruit, we examined how those fruit influence the performance of *D. suzukii* under winter-early spring temperature regimes.

We experimentally infested the three winter- / early spring-available fruit mentioned above with *D. suzukii* larvae under three rearing temperature regimes: constant 20°C, fluctuating controlled 8–15°C 12h:12h mimicking early-spring conditions, and uncontrolled outdoor regime during spring. Development time, emergence success and sex ratio were assessed. Further life history traits were measured on emerging individuals, including starvation resistance, survival with food, ovarian load and total energy content. Behavioural assays were also performed to address the preference-performance hypothesis.

Fly development was impaired by early spring-like environmental conditions. Furthermore, the suitability of the different fruit tested differed compared to that of the artificial control diet and between temperature regimes, supporting the presence of physiological trade-offs. Under constant 20°C, *D. suzukii* females preferentially oviposited in *A. japonica* fruit where their performance was the lowest, refuting the preference-performance hypothesis. Surprisingly, under early spring-like environmental conditions, all three fruit were comparable in terms of performance.

Drosophila suzukii's ability to overwinter and reactivate reproduction as temperatures increase in early spring is remarkable. The use of any of the three fruit trialled here, after reactivating reproduction, would lead to the production of adults that are viable and ready to initiate the next generation and infest early season commercial crop fruit. From the data obtained here, fruit choice by *D. suzukii* females clearly varies not only according to fruit availability and characteristics but also to the field temperature regimes experienced. Field and laboratory experiments provide complementary approaches to understanding the drivers of fruit infestation in the natural environment and the existence of trade-offs between life history traits linked to environmental conditions such as temperature or trophic resources.

Contributions

Aude Couty conceptualised the study. Lab work was performed by Jordy Larges.

I analysed the data, produced the graphics and tables. I wrote the original draft.

All co-authors contributed to the revision of the manuscript.

Winter fruit contribution to the performance of the invasive fruit fly *Drosophila suzukii* under different thermal regimes

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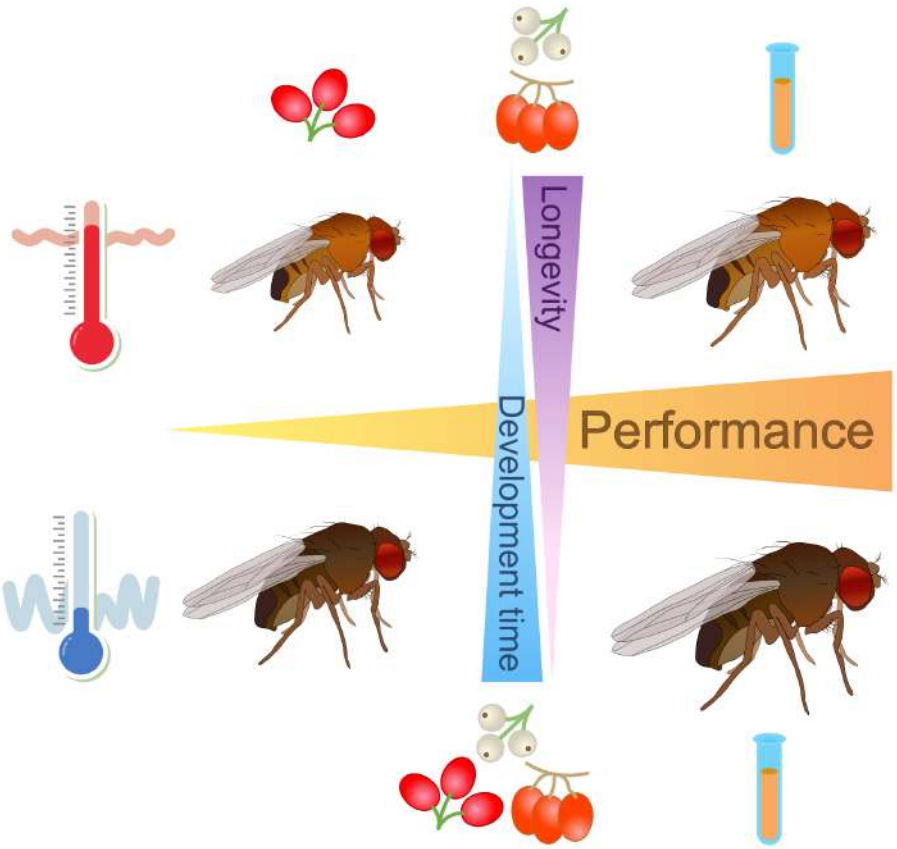
GRAPHICAL ABSTRACT



ABSTRACT

Polyphagous insect species develop using multiple host plants. Often considered beneficial, polyphagy can also be costly as host nutritional quality may vary. *Drosophila suzukii* (Matsumura) is an invasive species that can develop on numerous fruit species over the annual cycle. Here, we assessed the contribution of winter-available fruits to the development of seasonal populations of *D. suzukii*, under fluctuating end of winter/early spring temperature regimes. We infested an artificial diet and three suitable fruit species available in winter/early spring (*Aucuba japonica*, *Elaeagnus* ×*submacrophylla*, *Viscum album*) with *D. suzukii* larvae under three temperature regimes: constant 20°C, fluctuating controlled regime of 8–15°C 12h:12h, and uncontrolled outdoor regime during spring. Fly development was impaired by early spring-like environmental conditions. Furthermore, the suitability of the fruit species tested differed compared to that of the artificial diet and between temperature regimes, highlighting the presence of physiological trade-offs. Our study highlights the importance of combining field and experimental studies to better understand the ecological consequences of an invasive species.

Keywords: Life history traits, Overwintering, Polyphagy, Preference-performance hypothesis, Thermal fluctuations, Trade-off

INTRODUCTION

In temperate regions, winter is a critical period of food scarcity for most organisms (Danks, 1978; Moore & Lee, 1991; Folwarczny *et al.*, 2022). The number and diversity of native fruit-bearing plants at this time is limited (Thompson & Willson, 1979; Herrera, 1982), even if some invasive species provide additional resources (Greenberg & Walter, 2010). Most frugivorous insects enter diapause to overwinter (Bale & Hayward, 2010; Gill *et al*., 2017; Lehmann *et al*., 2017), with survival depending on reserves accumulated pre-diapause (Storey & Storey, 2012; Sinclair, 2015; Gill *et al.*, 2017). However, insects that remain active rely on seasonal fruit and most are polyphagous and able to switch between different resources over time (Kennedy & Storer, 2000; Butterworth *et al*., 2023). For instance, fruit flies switch their feeding preference from yeast to plants at low winter temperature (Brankatschk *et al*., 2018). The flexibility of polyphagy is often considered beneficial, however, it can also have costs, as offspring performance (e.g., developmental rate, survival) can vary between host plants (Janz, 2003; Poyet *et al*., 2015). The 'bet-hedging' strategy (Simons, 2011) suggests this cost can be offset by females ovipositing on numerous host plants, ensuring that at least some offspring survive (Jaenike, 1990; Singer, 2008). This could lead to a trade-off between host quality and availability if the most abundant host does not allow the best performance (Singer, 2008). Many successful pest insects are polyphagous (Singer 2008; Wilson *et al*., 2012) and some, such as the invasive fruit fly *Drosophila suzukii,* can be active during winter if temperatures are favorable (Ulmer *et al*., 2024).

Drosophila suzukii Matsumura (Diptera: Drosophilidae) is an agricultural pest originating from Asia which, since 2008, has gradually invaded Europe, North America and, more recently, Africa (Ørsted & Ørsted, 2019; Aouari *et al.,* 2022). This fly infests numerous crop and wild fruit species (Poyet *et al.*, 2015; Kenis *et al.*, 2016). In addition to its polyphagy, phenotypic plasticity induced by temperature variations (Fraimout *et al.*, 2018), high fertility (Rota-Stabelli *et al*, 2013) and low biotic pressures (competition, predation, parasitism) in its invaded area (Poyet *et al.*, 2013; Miller *et al.*, 2015) are key elements underlying its invasion success. Possessing a serrated ovipositor, *D. suzukii* is able to oviposit in unripe or ripening fruits, unlike most drosophilids (Clemente *et al.*, 2018). In Europe it is active throughout the year at varying population densities according to temperature, humidity and the availability of food resources (Lee *et al.*, 2015; Arnó *et al*., 2016; Shearer *et al.*, 2016; Tochen *et al*., 2016; Wang *et al*., 2016; Ulmer *et al.*, 2022).

The population present under winter suboptimal temperatures mainly comprises winter-acclimatized flies (Colinet & Kustre, 2024; Ulmer *et al*., 2024) and flies enter a reproductive dormancy. As temperatures begin to warm in spring (around March; Ulmer *et al*., 2024), the ovaries mature, egg production restarts, and females start searching for suitable winter/early spring host plants to oviposit. However, the density of suitable naturally occurring fruit varies considerably (Poyet *et al.,* 2015; Deconninck *et al.*, 2024a,b - Chapters 1,2) as does their nutritional composition (Shu *et al.*, 2022), with limited choice at the end of winter (Grassi *et al.*, 2018; Deconninck *et al.*, under review - Chapter 3). Examples of suitable species include various ornamental species from gardens and parks as well as wild species bearing fruit during winter. While we hypothesize that these could provide the starting point for the build-up of spring populations, their role as potential egg-laying sites for *D. suzukii* is poorly documented.

In Europe, potentially suitable winter-available species include *Aucuba japonica, Elaeagnus* ×*submacrophylla, Hedera helix, Lonicera nitida, Mahonia* spp., *Rosa canina, Skimmia japonica, Symphoricarpos* spp. and *Viscum album* (Grassi *et al*., 2018; Panel *et al*., 2018; Poyet *et al*., 2015; Kenis *et al*., 2016). Amongst these, *A. japonica*, *E.* ×*submacrophylla*, *Mahonia* spp.and *V. album* fruits are the most heavily infested in laboratory experiments and allow complete development from egg to adult emergence (Poyet *et al*., 2015; Panel *et al*., 2018). However, these studies were generally performed at a constant 20°C which is not representative of natural environmental conditions. Furthermore, depending on the protein:carbohydrate (P:C) ratio, their fruit are likely to vary in their contribution to *D. suzukii* larval development and adult performance (Jaramillo *et al.*, 2015; Silva-Soares *et al.*, 2017; Young *et al*., 2018). The 'preference-performance' (also known as 'mother knows best') hypothesis posits that insect females will have evolved preference for oviposition in hosts that confer the highest fitness to their offspring (Jaenike, 1990; Gripenberg *et al*., 2010; García-Robledo & Horvitz, 2012). However, contradictory findings have been reported and there is no consensus about the application of this hypothesis to *D. suzukii* (Burrack *et al.*, 2013; Poyet *et al*., 2015; Olazcuaga *et al.*, 2019). Other biotic and abiotic parameters in the natural environment might also influence the host preference of females (e.g., the female microbiota; Henry *et al*., 2020).

Our study investigated the performance of *D. suzukii* in winter fruits under realistic fluctuating temperature regimes reflecting the end of winter/beginning of spring. We used three temperature regimes (constant 20°C, fluctuating controlled regime of 8–15°C and an outdoor uncontrolled regime during the

early spring) and four developmental diets (the winter fruit of *A. japonica*, *E.* ×*submacrophylla* and *V. album*, and an 'optimal' artificial diet) and assessed fly performance. We addressed five hypotheses: (1) Under cold fluctuating temperature regimes, *D. suzukii* development would be impaired and slower than at constant 20°C; (2) Under constant 20°C, *D. suzukii* development would be better in artificial diet compared to winter fruit, with *E.* ×*submacrophylla* and *V. album* being intermediate quality and *A. japonica* the least favorable as reported by Poyet *et al.* (2015) and Panel *et al.* (2018); (3) Winter fruit suitability would differ under cold fluctuating temperatures compared to constant 20°C; (4) Trade-offs between development and reproductive traits may take place depending on the fruit in which the larvae developed (Chakraborty *et al*., 2023); and (5) if the preference-performance hypothesis applies, *D. suzukii* females would oviposit on the most suitable fruit, with preference changing depending on the temperature regime, because the nutritional requirements of winter and summer individuals may differ (Rendon *et al*., 2019).

MATERIALS AND METHODS

D. suzukii rearing

The *D. suzukii* strain used was provided by ECOBIO laboratory (Rennes, France) and was initiated in 2019 from adults collected on raspberries in Rennes, France (48°7′2.158″N, 1°40′40.053″W). This population is a common standard *Wolbachia*-free strain shared and used for experiments by all laboratories involved in the DroThermal national project (https://www.drothermal.cnrs.fr). The population was maintained on a banana-based substrate (water 50 mL, fresh banana 200 g, yeast 15 g, ethanol 15 mL, sugar 10 g, agar 10 g, Nipagin™ 2 g) at 20°C, L:D 12:12h.

Fruit collection

Branches of *V. album*, *A. japonica* and *E.* ×*submacrophylla* bearing fruit were collected weekly between 20 January and 20 April 2023 in the region of Amiens, northern France (49°54′0.01″N, 2°18′0″W) (Table S1). They were kept hydrated with moistened cotton wool around their base and stored outside in insect-proof nylon mesh cages (30×30×30 cm) placed in plastic greenhouses (150×90×60 cm) for experimental use in the week of collection. Only ripe and intact fruit were selected for the experiments. Maturity was visually assessed based on fruit color and firmness. For each artificial infestation session, a randomly selected batch of 10 fruit was isolated and stored at −80°C for nutritional analyses (proteins and carbohydrates; method in Supplementary Materials).

Developmental diets and temperature regimes

Four diets were used to examine the combined effects of fruit and temperature on *D. suzukii* development and adult performance: *A. japonica*, *E.* ×*submacrophylla* and *V. album* fruit (henceforth referred to as *Aucuba*, *Elaeagnus* and *Viscum* in Materials and Methods and Results for convenience) and a control artificial carrot-based diet (water 250 mL, sucrose 12.5 g, agar 3.75 g, brewer's yeast 7.5 g, cornmeal 5 g, carrot powder 12.5 g, Nipagin™ 0.4 g) commonly used as a rearing medium and considered optimal for *D. suzukii* development (Enriquez *et al*., 2018). We chose a diet that differed from the initial rearing diet, so that flies were naïve to all four experimental diets.

Each diet was manually infested with five first instar larvae (L1) of *D. suzukii* that were obtained as follows: one- to six-day-old females were left to oviposit for 8 h on cherry medium (agar 1.5 g, water 50 mL, cherry juice 50 mL) thinly spread on a 75×25 mm glass slide inserted into a 90×30 mm plastic vial and sealed with a foam lid. Females were then removed, and eggs were kept 48 h at 20°C until L1 emerged. To mimic *D. suzukii* infestation, each fruit was incised with a scalpel (incision length ~4 mm) under a stereomicroscope (Leica M205C) and larvae were directly deposited in the wound. Before each infestation, the fruits were individually weighed (d = 0.01 g; Traveler TA152, Ohaus Corporation, USA). Infested fruits were then placed in 30×55 mm plastic vials on top of 0.6 g distilled water-saturated cotton. For controls, the larvae were placed directly onto 1 mL of the carrot diet in a 2 mL Eppendorf tube closed with a piece of cotton wool.

Vials containing fruit or artificial diet were then randomly allocated to one of three temperature regimes: constant 20°C (CR), L:D 12:12h, 75 % RH (in a Snijders ECP01E phytotron); fluctuating 8–15°C (FCR), L:D 12:12h, 75% RH (in a Memmert HPP200 phytotron); and an uncontrolled outdoor regime (UNR) corresponding to field temperatures between 24 March and 30 June 2023 (vials were placed in insect-proof nylon mesh cages within open plastic greenhouses). Temperature inside the greenhouses was recorded using HOBO dataloggers every 15 min.

Life history trait measurements

In order to assess the combined effect of thermal regime and larval diet on *D. suzukii* fitness, several life history variables were measured. Adult fly emergence was checked daily and newly emerged individuals were sexed and randomly allocated to one of the following assays: (1) starvation resistance, (2) survival with food (males and females), (3) ovarian load (females only) and (4) energy content (females

only). Development time (i.e., time from L1 deposition to emergence), emergence success and sex ratio were recorded for all individuals.

Starvation resistance and survival with food for 15 days. Upon emergence, each adult was introduced into a 5 mL plastic tube (12×75 mm), either with a piece of distilled water-saturated cotton wool (starvation) or a piece of 7.5×15 mm filter paper soaked in a nutrient solution (water + 2% sugar + 5% yeast; as described by Plantamp *et al.* (2017)) replaced every two days, and kept at 20°C, L:D 12:12h, 75% RH (in a Snijders ECP01E phytotron). Mortality was checked daily and the cotton wool of the surviving individuals rewetted. Dead individuals were stored individually at room temperature in 70% ethanol for further morphometric measurements.

Ovarian load. Because *D. suzukii* is a synovigenic species, where females must feed to mature their eggs, each female was maintained as described in the section 'Survival with food for 15 days'. After three days, females were individually introduced into 2 mL Eppendorf microtubes and stored at −20°C (Reyes-Ramirez *et al.*, 2023). Ovarian load was subsequently assessed by dissecting their abdomen in 1% PBS solution. Using a stereomicroscope and following Plantamp *et al.* (2017), the number of mature eggs (i.e., at stage 14, with visible and well-formed respiratory filaments; Figure S1) was counted in both ovaries. Remains of dissected individuals were kept at room temperature in 70 % ethanol for further morphometric measurements.

Energy content. Upon emergence, females were frozen at −20°C and weighed using a laboratory precision balance (0.01 mg; MODELE; Sartorius, Göttingen, Germany). The whole-body energy content was calculated for individual females from each of the 12 combinations of diet and temperature. The amounts of proteins, free sugars, lipids and glycogen were quantified individually using an adjusted version of Foray *et al.*'s (2012) protocol (Supplementary Materials). Total energy content of the flies was evaluated by converting the mean nutrient content into Joules (conversion factors: proteins 16.0 J/mg; carbohydrates 16.0 J/mg; lipids 37.65 J/g; see Giron and Casas, 2003; Clavé *et al*., 2022). Twenty flies were analyzed per treatment, except for flies that emerged from *Elaeagnus* in the UNR (n = 16).

Morphometric analyses. Wing length, wing width and tibia length of flies from 'Starvation resistance', 'Survival with food for 15 days' and 'Ovarian load' assays were measured as described by Gilchrist *et al.* (2001), using a Leica M205C stereomicroscope equipped with a Leica MC170 HD camera and the software Leica Application Suite (Figure S2a,b). The wing area index ((wing length 1 + wing length 2) × wing width) was calculated as described by Ulmer *et al.* (2024). The number of fully

melanized abdominal tergites was recorded for both males and females from 'Starvation resistance' and 'Survival with food for 15 days' assays (Figure S2b).

Behavioral assays

To mimic *D. suzukii* choice at the end of winter when facing early-spring-like conditions and fruit, we produced winter-acclimated individuals by rearing flies on a banana-based substrate at 14°C, 12:12h, 75% RH (in a Panasonic MLR-352H phytotron). An oviposition choice experiment was conducted to determine the fruit preference according to the temperature regime (CR, FCR, UNR). One fruit of each of the three plant species was used per test (i.e., a total of three fruit from different plant species per test) and randomly placed in one of the corners of a 10×10×7.5 cm plastic box. The stem of each fruit was pierced with a needle and fixed in the box corner to avoid displacement when checking the boxes. A piece of water-soaked cotton was placed in the fourth corner to provide a water source for the flies. A 5×5 cm square hole was drilled in the lid of the box and covered with a piece of nylon mesh. Three females and two males (aged 3–8 d) were introduced to each box and left for 24 h. Once the flies were removed, the number of eggs laid on each fruit was counted. Twenty replicates were performed for each experimental condition. The experiment under UNR took place between 24 and 25 April 2023, while those under CR and FCR took place between 13 and 17 May 2023 (Figure S3).

Data analyses

All data were analyzed in R version 4.2.2 (R Core Team, 2021). Differences in nutritional composition between fruit species were analyzed using ANOVA on square-root-transformed protein content and P:C ratio and log-transformed glucose content. When significant, pairwise differences between means were identified using Tukey's HSD tests. To examine the impact of temperature regime, diet and their interaction on the emergence rate and sex ratio of emerged individuals, generalized linear models using a binomial distribution with logit link function were used. ANOVA was used to analyze the effects of diet, temperature regime and their interaction on morphometric measurements (wing area index, tibia size), mass of emerging individuals, development time and energy content (both log-transformed), followed by Tukey's HSD tests when significant. The effect of temperature regime, diet and their interaction on female ovarian load was analyzed using general linear models with quasi-Poisson error distributions. The differences in numbers of melanized tergites between temperature regimes were also analyzed using general linear models with Poisson error distributions. Survival rates with and without

Table 1 Number of *D. suzukii* females used in statistical analyses (see Table S2 for total number of individuals that emerged and were available for experiment). CR: 20°C; FCR: fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor regime between 24 March and 31 June 2023.

Temperature regime	Diet	Longevity	Starvation resistance	Ovarian load	Energy content	Morphometric measurement	Development time
CR	**Artificial**	40	58	30	20	94	191
	Aucuba	42	35	30	20	90	151
	Elaeagnus	38	28	33	20	80	139
	Viscum	60	106	32	20	92	258
FCR	**Artificial**	48	35	30	18	88	160
	Aucuba	26	23	20	17	66	95
	Elaeagnus	15	16	14	20	39	71
	Viscum	61	46	30	20	90	242
UNR	**Artificial**	35	34	33	19	89	147
	Aucuba	60	31	31	19	91	208
	Elaeagnus	30	0	0	16	23	47
	Viscum	56	36	32	19	80	266

food were analyzed using Kaplan-Meier curves and log-rank tests. To decipher the influence of temperature regime and diet on the performance of *D. suzukii*, a Principal Component Analysis (PCA) was performed using the mean of the studied life history traits of female *D. suzukii*, except starvation resistance (data were missing for *Elaeagnus* under UNR), in the different combinations of experimental conditions (12 experimental combinations × 11 life traits). Oviposition preference analysis was performed by computing the proportion of eggs laid in each fruit in each box and the means of these proportions were used to perform Chi-square tests. The number of flies that were retained for statistical analyses is given in Table 1 and the total number of flies that were used in experiments in Table S2.

RESULTS

Fruit composition

The fruit differed in their nutritional composition ($p < 0.0001$; Table S3). *Aucuba* fruit had the highest protein content (2.30 ± 1.03 mg/g fresh fruit) and an intermediate glucose content (3.43 ± 1.51 mg/g), as well as an intermediate P:C ratio (0.74 ± 0.35) (Table 2). *Viscum* fruit had an intermediate protein content (1.82 ± 0.79 mg/g) and the highest glucose content (4.16 ± 1.35 mg/g), leading to the lowest P:C ratio (0.44 ± 0.19). With only 0.63 ± 0.34 mg/g protein and 0.48 ± 0.19 mg/g glucose,

Elaeagnus was the nutritionally poorest fruit although it had the highest P:C ratio (1.40 ± 0.68).

Table 2 Nutritional composition of the three fruit types used in the study. Differences between the three fruit types were analyzed using ANOVA. Different letters indicate significant differences at $p < 0.05$ (Tukey's HSD).

	Fruit	N	Min	Mean ± sd	Max	Differences ($p < 0.01$)
Protein (mg/g fresh fruit)	*Aucuba*	70	0.80	2.30 ± 1.03	5.52	**a**
	Elaeagnus	50	0.04	0.63 ± 0.34	1.90	**b**
	Viscum	70	0.25	1.82 ± 0.79	3.30	**c**
Glucose (mg/g fresh fruit)	*Aucuba*	70	1.6	3.43 ± 1.51	8.4	**a**
	Elaeagnus	50	0.2	0.48 ± 0.19	1.3	**b**
	Viscum	70	1.7	4.16 ± 1.35	7.3	**c**
P:C ratio	*Aucuba*	70	0.20	0.74 ± 0.35	1.73	**a**
	Elaeagnus	50	0.08	1.40 ± 0.68	3.60	**b**
	Viscum	70	0.07	0.44 ± 0.19	1.10	**c**

Emergence rate and sex ratio

The emergence rate of *D. suzukii* was impacted by temperature, diet and their interaction ($p < 0.0001$; Table S4). Under CR, the emergence rate was maximized when developing in artificial diet and *Viscum* fruit (mean ± sd: 0.83 ± 0.30 and 0.88 ± 0.18, respectively), while it was significantly lower in *Aucuba* and *Elaeagnus* fruit (0.39 ± 0.29 and 0.36 ± 0.38, respectively; Figure 1a). Under fluctuating regimes, emergence rates were significantly reduced in all fruit diets compared to the artificial control diet. The artificial diet produced the highest emergence rate both in FCR and UNR (0.82 ± 0.23 and 0.76 ± 0.27, respectively). *Viscum* fruit had the second highest emergence rate (0.70 ± 0.30 under FCR and 0.64 ± 0.33 under UNR). *Aucuba* and *Elaeagnus* fruit emergence rates were similarly low under FCR (0.15 ± 0.21 and 0.16 ± 0.29, respectively). The emergence rate from *Aucuba* fruit was significantly higher under UNR (0.21 ± 0.27) than under FCR (0.15 ± 0.21), but the one of *Elaeagnus* fruit was significantly lower (0.07 ± 0.21).

The mean sex ratio of emerged individuals was 0.48 ± 0.33 (M:F). Diet had no effect on the sex ratio ($p = 0.1679$; Table S5). Temperature regime had a significant influence ($p = 0.0008$) with a lower proportion of males emerging under FCR than under CR (mean sex ratio ± sd: 0.45 ± 0.34 and 0.50 ± 0.30, respectively; $p = 0.0125$). Details are provided in Table S6.

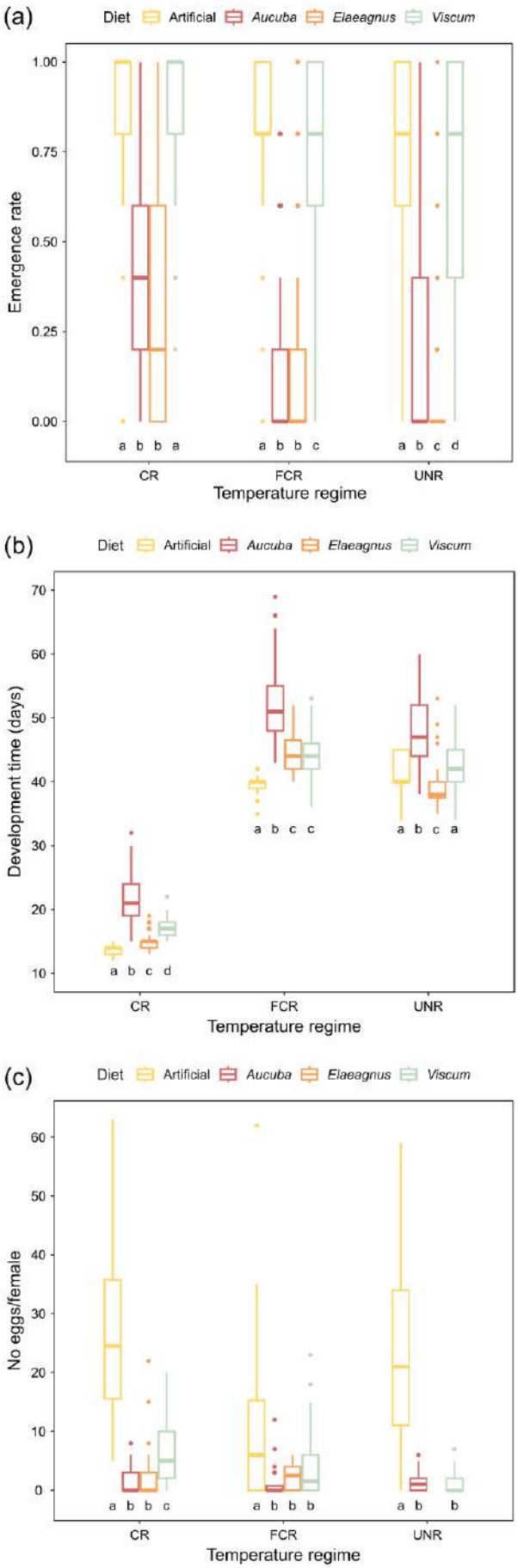


Figure 1 Emergence rate (a), female development time (b) and ovarian load (c) of *D. suzukii* that developed in artificial diet (control), *Aucuba japonica*, *Elaeagnus ×submacrophylla* or *Viscum album* fruit under different temperature regimes (CR: 20°C; FCR: fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor regime between 24 March and 31 June 2023). Different letters indicate significant differences at $p < 0.05$ (a: GLM using a binomial distribution with logit link function; b: ANOVA followed by Tukey's HSD; c: GLM with quasi-Poisson error distributions).

Development time

Development time was influenced by temperature, diet and their interaction ($p < 0.0001$; Table S7). It was shorter under CR (mean 16.4 ± 3.1 d), than under FCR and UNR (44.3 ± 5.0 and 43.3 ± 5.3 d, respectively; Figure 1b). Under CR, the artificial diet allowed the shortest development time (13.6 ± 0.8 d), followed by *Elaeagnus* (14.7 ± 1.2 d), *Viscum* (16.7 ± 1.4 d) and *Aucuba* (21.0 ± 3.3 d). Under FCR, although considerably longer, the development time remained the shortest on the artificial diet (39.6 ± 1.2 d) and the longest on *Aucuba* (51.8 ± 5.2 d), while it was intermediate in *Elaeagnus* and *Viscum* (44.4 ± 3.0 and 44.1 ± 2.8 d, respectively). The relationship changed under UNR with *Elaeagnus* fruit allowing the shortest development time (39.7 ± 3.9 d), while the artificial diet and *Viscum* fruit induced an intermediate development time (41.5 ± 3.8 and 41.3 ± 3.6 d, respectively), and development in *Aucuba* remained the longest (48.0 ± 5.3 d).

Morphometric measurements

The wing area index and the tibia size of female *D. suzukii* were impacted by temperature, diet and their interaction ($p < 0.0001$; Table S7). The wing area index was the smallest under CR (2.75 ± 0.51 mm^2), intermediate under UNR (3.14 ± 0.48 mm^2) and largest under FCR (3.35 ± 0.44 mm^2). The tibia size was also the smallest under CR (0.77 ± 0.09 mm), but was the longest under UNR (0.82 ± 0.08 mm) and intermediate under FCR (0.80 ± 0.08). Under all temperature regimes, *D. suzukii* females had the largest wings and the longest tibias when developing on the artificial diet (3.44 ± 0.33 mm^2 and 0.86 ± 0.05 mm, respectively), the smallest when developing on *Aucuba* fruit (2.61 ± 0.56 mm^2 and 0.72 ± 0.08 mm, respectively), and intermediate when developing on *Elaeagnus* (2.97 ± 0.49 mm^2 and 0.79 ± 0.08 mm, respectively) or *Viscum* (3.12 ± 0.39 mm^2 and 0.81 ± 0.06 mm, respectively) fruit (wing area index results in Figure 2).

Cold temperatures significantly increased the number of melanized tergites ($p < 0.0001$; Table S8). Under FCR and UNR, emerging flies were darker than under CR (Figure S4).

Mass and energy content

The mass of emerged females was influenced by temperature ($p = 0.0332$), diet ($p < 0.0001$) and their interaction ($p = 0.0003$; Table S7). Under all temperature regimes, females were the heaviest when developing on the artificial diet (1.92 ± 0.22 mg; Figure 2). Under CR, females that developed on *Aucuba* fruit were the lightest (0.73 ± 0.21 mg), and those developing on from *Elaeagnus* and *Viscum* fruit were

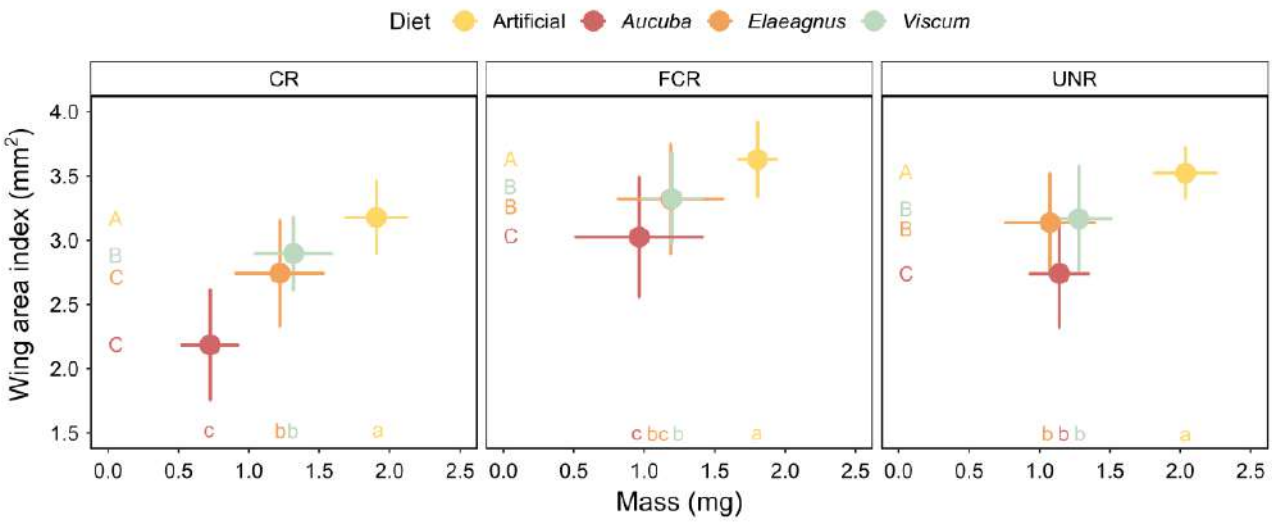


Figure 2 Wing area index (mean ± sd; mm^2) and mass (mean ± sd; mg) of *D. suzukii* females that developed and emerged from artificial diet (control), *Aucuba japonica*, *Elaeagnus* ×*submacrophylla* or *Viscum album* fruit under different temperature regimes (CR: 20°C; FCR: fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor regime between 24 March and 31 June 2023). Different letters indicate significant differences at $p < 0.05$ (ANOVA followed by Tukey's HSD tests).

of intermediate mass (1.22 ± 0.32 and 1.32 ± 0.28 mg, respectively). Under FCR and UNR, there was no significant difference in the mass of females that developed on the three fruit types, but all were lighter than those developing on the artificial diet (Figure 2).

The energy content of emerged females was influenced by temperature ($p < 0.0001$), diet ($p = 0.0001$) and their interaction ($p = 0.0014$; Table S7). Under CR, energy content did not differ significantly between diets, with an overall mean of 4.31 ± 1.28 J/mg (Figure 3; details in Table S9). However, under FCR and UNR, energy content varied between diets, being maximized in flies developing on the artificial diet and *Aucuba* fruit (UNR: 5.82 ± 0.94 and 6.03 ± 1.96 J/mg, respectively), intermediate in those on *Elaeagnus* (UNR: 4.85 ± 1.34 mg/J) and least in those on *Viscum* (4.39 ± 1.57 J/mg).

Ovarian load

Female ovarian load was influenced by temperature ($p < 0.0001$), diet ($p < 0.0001$) and their interaction ($p = 0.0008$; Table S10). Overall, the number of mature eggs was reduced under UNR and FCR compared to CR (Figure 1c). Under all temperature regimes, the ovarian load was greatest in females developing on the artificial diet (20.4 ± 16.7), even though the number of eggs was significantly lower under FCR (10.8 ± 13.7). Under CR, females developing on *Viscum* fruit had more eggs (6.9 ± 6.0) than

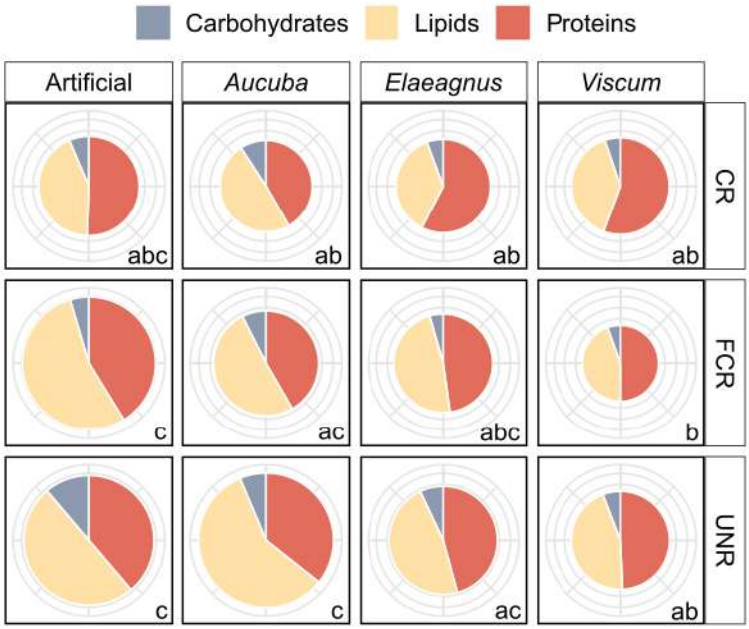


Figure 3 Energy content of *D. suzukii* females emerging from artificial diet, *Aucuba japonica*, *Elaeagnus ×submacrophylla* or *Viscum album* fruits under different temperature regimes (CR: 20°C; FCR: fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor regime between 24 March and 31 June 2023). N = 20, except for Artificial and *Aucuba* in FCR (n = 18 and n = 17, respectively), Artificial, *Aucuba* and *Viscum* in UNR (n = 19), and *Elaeagnus* in UNR (n = 16). The lower sample size is due to removal of aberrant values. Differences between fruit types and temperature treatment were analyzed by ANOVA followed by Tukey's HSD tests (energy content) or by Kruskal-Wallis tests followed by Wilcoxon pairwise comparisons. When significant ($p < 0.05$), differences are indicated with different letters.

those on *Aucuba* (1.7 ± 2.4) or *Elaeagnus* (2.4 ± 4.8). Under FCR and UNR, there were no significant differences between the ovarian loads of females developing on the different fruit. Insufficient females emerged to measure the ovarian load of those developing on *Elaeagnus* fruit under UNR (Table S2).

Starvation resistance and 15 days survival

Starvation resistance was influenced by temperature, diet, sex and their interaction ($p < 0.0001$). Females had higher starvation resistance than males ($p < 0.0001$) and we only considered females for further analyses. Under all temperature regimes, starvation resistance was greatest in females developing on the artificial diet (median: 4 d with 95%CI = [4,4]) compared to all fruit (median: 3 d [3,3]; $p < 0.0001$). Starvation resistance was also slightly greater under UNR (median: 3 d [3,4]) compared to CR (median: 3 d [3,3]; $p = 0.0062$) and FCR (median: 3 d [3,3]; $p = 0.0083$). Starvation resistance was differentially influenced by the diet depending on the temperature regime (Figure 4a; Table S11): under

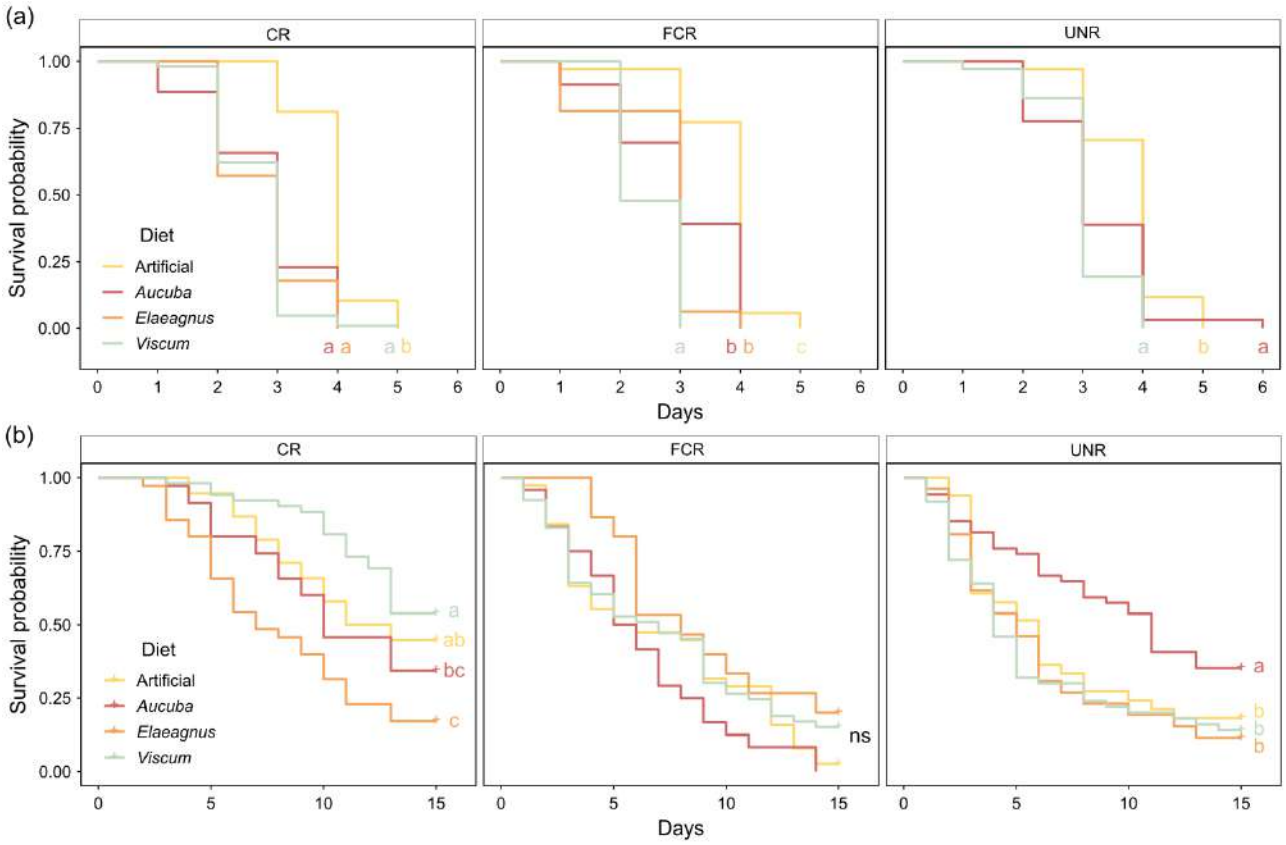


Figure 4 Survival probability of *D. suzukii* females that developed and emerged from artificial diet (control), *Aucuba japonica*, *Elaeagnus* ×*submacrophylla* or *Viscum album* fruit under different temperature regimes (CR: 20°C; FCR: fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor regime between 24 March and 31 June 2023): (a) Survival of individuals that only had access to water ('Starvation resistance'); (b) 15 days survival of individuals that were hydrated and fed *ad libitum* with a nutrient solution. Different letters indicate significant differences at $p < 0.05$ (log-rank test).

CR, it was greatest in females that emerged from the artificial diet (median: 4 d [4,4]) and less in all other fruit (median: 3 d [3,3] for *Aucuba* and *Viscum*, 3 d [2,3] for *Elaeagnus*; $p < 0.0001$); under FCR, it was greatest under the artificial diet (median: 4 d [4,4]), intermediate in *Aucuba* and *Elaeagnus* (both median: 3 d [3,3]), and least under *Viscum* (median: 2 d [2,2]; $p < 0.0001$); finally under UNR, it was again greatest for females that developing on the artificial diet (median: 4 d [4,4]) and less under *Aucuba* and *Viscum* (median: 3 d [3,4] and 3 d [3,3], respectively; $p = 0.0002$), with no data for *Elaeagnus*.

Survival after 15 days was influenced by temperature, diet and their interaction ($p < 0.0001$). Sex also had an effect, with males surviving longer ($p = 0.039$). We only considered females for further analyses. Female survival was influenced by temperature ($p < 0.0001$) and the interaction between temperature and diet, whilst diet alone had no significant effect ($p = 0.14$). Females survived longer under CR (median: 12 d) compared to FCR and UNR (median: 6 d both; $p < 0.0001$). The influence of diet on

female survival differed between temperature regimes (Figure 4b; Table S11): under CR, survival was greatest in females that developed in *Viscum* fruit (median: 15 d), intermediate under the artificial diet (median: 12 d) and *Aucuba* fruit (median: 10 d), and lowest in *Elaeagnus* fruit (median: 7 d); however, under FCR, diet had no influence on female survival (p = 0.17) and, under UNR, females developing in *Aucuba* had highest survival (median: 11 d) compared to all other diets (median: 4–6 d; p = 0.025).

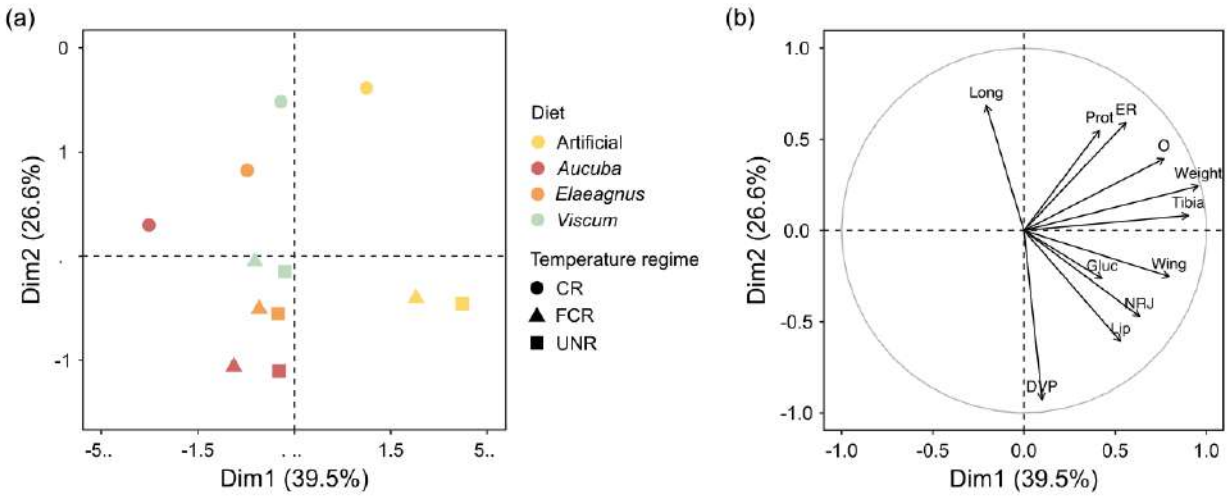


Figure 5 (a) Diagram of the experimental modalities from principal component analysis (PCA) performed on the mean values of life history traits in *D. suzukii* flies that developed and emerged in each combination of experimental treatment, i.e. from artificial diet (control), *Aucuba japonica*, *Elaeagnus* ×*submacrophylla* or *Viscum album* fruit under different temperature regimes (CR: 20°C; FCR: fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor regime between 24 March and 31 June 2023). The percentages of explained variance for each axis are in parentheses. (b) Correlation circle of the 11 life history traits of *D. suzukii* flies (DVP: development time; ER: emergence rate; Gluc: glucose concentration; Lipid: lipid concentration; Long: longevity; NRJ: energy content; O: ovarian load; Prot: Protein concentration; Tibia: tibia size; Weight: mass; Wing: wing area index).

Effect of temperature regime and diet on *D. suzukii* performance

In the PCA, the first two principal components (PC) explained 66.6% of variation and grouped the diet and temperature regimes according to the life history traits of *D. suzukii* (Figure 5). PC1 (39.5% of variation) represented a trophic resource quality gradient, with the artificial diet on its positive part and the three fruit species on its negative part. Along PC1, flies developed on the artificial diet were positively correlated with the energy content (glucose, protein and lipid content), morphometric measurements (mass, tibia size, wing length), emergence rate and ovarian load. Conversely, flies developing on *Aucuba*

fruit under CR were negatively correlated with those factors. Flies developing on *Aucuba* (under FCR and UNR), *Elaeagnus* and *Viscum* fruit overlapped considerably, with intermediate values for most of these factors and along PC1. PC2 (26.6% of variation) represented a gradient of thermal regime suitability from fluctuating and colder to constant and warmer regimes. On PC2, flies that developed under CR showed rapid development but low longevity while those that developed under the fluctuating regimes (FCR and UNR) had long development and extended longevity. For each type of fruit substrate, plots of FCR and UNR were closely overlaid in the PCA diagram.

Behavioral assays

Behavioral assays under UNR took place between 24 and 25 April 2023, during which time the temperature was 9.8 ± 2.2 °C (min: 6.0°C; max: 15.9°C; Figure S3), conditions broadly similar to those of FCR (mean: 11.5°C; min: 8°C; max: 15°C). Oviposition preference of winter-acclimated *D. suzukii* females differed between the experimental temperature regimes (Figure 6). Under CR and UNR, *Aucuba* fruit were preferred over *Elaeagnus* and *Viscum* fruit ($p < 0.0001$). On average, 73 and 65% of eggs were laid in *Aucuba* under CR and UNR, respectively. However, under FCR, *Elaeagnus* was the preferred fruit

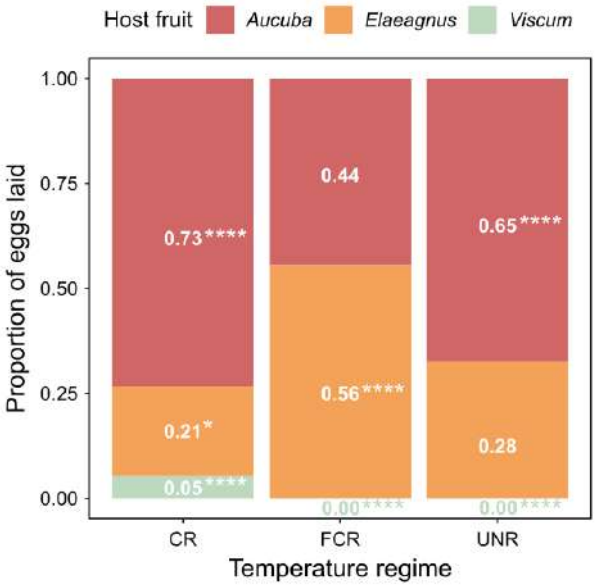


Figure 6 Oviposition preference of winter-acclimated *D. suzukii* females in the presence of fruit of *Aucuba japonica*, *Elaeagnus* ×*submacrophylla* and *Viscum album* (one of each) for 24 hours under different temperature regimes (CR: 20°C; FCR: fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor regime between 24 March and 31 June 2023). Each bar plot corresponds to the mean proportion of eggs laid per box (n = 20) in each fruit. Asterisks represent significant differences between expected (⅓ without preference) and observed proportions identified using Chi-square test (*: $p < 0.05$, ****: $p < 0.0001$).

($p < 0.0001$), with 56% of eggs laid. Under all temperature regimes, *Viscum* fruit were least preferred, with zero or < 5% of eggs laid ($p < 0.0001$).

DISCUSSION

Our aim was to assess the contribution of winter-available fruit to the development of spring populations of *D. suzukii*, under fluctuating end of winter/early spring temperature regimes. Here, we consider (1) how *D. suzukii* development was influenced by early spring-like conditions; (2) the suitability of the three fruit species trialed compared to an optimal artificial diet at 20°C; (3) how this suitability changed under early spring-like conditions and physiological trade-offs which may contribute to this; (4) the importance of combining field and laboratory studies to better understand the ecological consequences of an invasive species.

Impaired and extended development under colder and fluctuating temperature regimes

Low temperatures can impact the development and physiology of ectotherms. Generally, growth rates are slower, larger final body size is reached and survival is longer (Angilletta *et al.*, 2004; Denlinger & Lee, 2010). Thermal performance curves, that depict the performance of a species across temperatures, predict that, below an optimal temperature, the overall performance decreases (Huey & Kingsolver 1989; Colinet *et al.*, 2015; MacLean *et al.*, 2019). The effects of low temperatures on *D. suzukii* performance have been explored both under laboratory conditions (Tochen *et al.*, 2014; Shearer *et al*., 2016; Wallingford *et al*., 2016; Rendon *et al.*, 2019; Panel *et al.*, 2020; Schlesener *et al.*, 2020; Raynaud-Berton *et al.*, 2024) and in the field (Shearer *et al*., 2016; Wallingford *et al.*, 2016; Grassi *et al.*, 2018). Based on these studies, we predicted that *D. suzukii* fitness would be impaired under end of winter/early spring temperatures, independent of developmental diet.

Both fluctuating temperature regimes applied here reduced fly emergence rate and lengthened development time compared to CR, in accordance with previous studies. For instance, a decrease in temperature from 18 to 13°C reduced the egg-to-adult survival of *D. suzukii* by 40% and increased the development time by ~20 days (Schlesener *et al*., 2020). Sánchez-Ramos *et al.* (2019) compared the effect of constant and fluctuating temperatures on *D. suzukii* development time, finding that both constant and fluctuating cold regimes slowed the development and that development time was shorter in a 4-16°C fluctuating regime (55.19 ± 0.25 days) than in a constant 10°C regime (65.6 ± 0.5 days). Raynaud-Berton et al. (2024), in a study of several life history traits in *D. suzukii*, reported the same trend.

The sex ratio of emerged individuals was slightly influenced by temperature here, with less males under FCR compared to CR. Other studies have not shown an effect of temperature (Schlesener *et al.*, 2020; Raynaud-Berton *et al.*, 2024). Under FCR and UNR, the flies that emerged were darker than in CR, indicating a shift towards the production of winter-acclimated individuals as reported in the literature. Development of *D. suzukii* at low temperatures (<10°C) is known to induce the production of winter-acclimated individuals that exhibit higher melanization of the abdominal and thoracic tergites (Toxopeus *et al.*, 2016; Fraimout *et al.*, 2018) and are commonly observed in the field (Ulmer *et al*., 2024). There is substantial support for the hypothesis that increased melanism at low temperatures could confer some fitness advantages such as thermoregulation in line with the thermal melanism theory, camouflage against predators, or improved pathogen resistance due to melanin's involvement in immune response (Clusella Trullas *et al.*, 2007; Colinet & Kustre, 2024). Higher melanization of insects developing at low temperature is thus considered a cold adaptation (Toxopeus *et al.*, 2016; Fraimout *et al.*, 2018).

Under all diets trialed, the cold temperature regimes did not affect the final mass of flies, although they grew larger wings. The wing area index values of *D. suzukii* obtained under FCR and UNR were comparable to those of field-captured flies at the end of winter/beginning of spring reported by Ulmer *et al.* (2024). In *Drosophila*, wing morphology is a trait involved in flight (David *et al.*, 1997; Ray *et al.*, 2016) and mating behavior (Ewing, 1964; Yeh & True, 2014). *Drosophila suzukii* individuals that developed in colder temperatures exhibited increased flight speed and acceleration (Fraimout *et al*., 2018). Furthermore, cold-acclimated *D. melanogaster* (during development) have improved flying ability at lower temperatures due to increased wing area and length (Frazier e*t al.*, 2008; Huisamen *et al*., 2022). Hence, better flight performance may increase the foraging range of winter-acclimated individuals, helping find food resources that are scarce in early spring (Poyet *et al.*, 2015; Panel *et al.*, 2018). *Drosophila suzukii* males with larger wings also have higher mating success and, hence, potentially fitness (Menezes *et al.*, 2013).

Winter-acclimated individuals generally exhibit greater longevity at lower temperatures (Tochen *et al*., 2014; Schlesener *et al.*, 2020). In our study, however, we investigated the longevity (survival probability for 15 days) of *D. suzukii* individuals by placing them at 20°C after their emergence from the different experimental conditions. Those developing under winter/early spring temperatures showed reduced longevity at 20°C compared to flies from the control constant 20°C. This may result from acclimation, with flies developing under cold regimes not being adapted to constant 20°C temperature

(Kristensen *et al*., 2008). There could also be additional costs associated with developing larger wings and/or the production of melanin (Talloen *et al*., 2004; Roff & Fairban, 2013). Conversely to the longevity data, flies developing under UNR had greater starvation resistance than those under CR or FCR. This results from the longer development time increasing the flies' energy content, allowing them to survive longer (Ballard *et al*., 2008). A trade-off between development time and adult survival has been established in several frugivorous *Drosophila* species (Sevenster & van Alphen, 1993; Tu & Tatar 2003).

The negative impact of temperature on ovarian load was most noticeable in females developing on the artificial diet. Low temperatures during development reduced the number of mature eggs in 3-day-old females, consistent with Wallingford *et al*. (2016), who reported a reduction from eight to less than two mature eggs in the ovaries of seven-day-old *D. suzukii* females reared at 20°C and 15°C, respectively. In our study, the reduction in mature eggs was greater in females developing under FCR than UNR, which may be related to the differences in female energy content, which was higher in FCR and could have positively impacted egg maturation (cf. Berger *et al*., 2008).

Ranking of *D. suzukii* performance in different winter-available fruit

Performance of *D. suzukii* was maximized when developing on the artificial diet compared to the three winter-available fruit, supporting our second hypothesis. The ranking of the three fruit in terms of performance was consistent with previous studies (Poyet *et al*., 2015; Panel *et al*., 2018). Under CR, when thermal conditions are not a limiting/constraining developmental factor, performance was intermediate in *V. album* and *E.* ×*submacrophylla* and was lowest in *A. japonica*. There was no difference in emergence rate between flies developing in *V. album* and those in the artificial diet (0.83 and 0.88, respectively), while the rate was reduced by around half in *A. japonica* and *E.* ×*submacrophylla* (0.39 and 0.38, respectively). Previous studies have reported low emergence rates from *A. japonica* (Lee *et al*., 2015; Poyet *et al*., 2015; Panel *et al*., 2018), but the result for *E.* ×*submacrophylla* is surprising as it is usually considered as a suitable host for *D. suzukii* (Poyet *et al*., 2015; Panel *et al*., 2018). However, the low performance is consistent with the high P:C ratio in the collected *E.* ×*submacrophylla* fruit (1.40), which was 2-3 times greater than in the other fruit used. Compared to other *Drosophila* species, *D. suzukii* is known to perform better in diets with relatively low concentrations of protein (Silva-Soares *et al*., 2017; Rendon *et al*., 2018). As noted, the emergence rate was maximized in *V. album*, which had the lowest P:C ratio (0.44), but was also low in *A. japonica*, which had an intermediate P:C ratio (0.74).

Based on development time and body mass data alone, *E.* ×*submacrophylla* fruit could be considered a higher quality resource than those of *V. album*. Development time was shorter in the former, without impacting the body mass achieved or the wing area index. More rapid development without reduction in body size is considered an indicator of a higher larval resource quality (Edgar, 2006; Jaramillo *et al*., 2015). Panel *et al.* (2018) reported similar results, with even larger wing size in larvae that developed in *E.* ×*submacrophylla*, while Poyet *et al*. (2015) reported more rapid development in *V. album*. However, to rank the fruit resource quality under the constant 20°C temperature regime, other parameters were also taken into account, including ovarian load, starvation resistance and survival. There was no difference in starvation resistance between the three fruit types, which is consistent with an overall similar energy content for flies under CR under all diets. Ovarian load was greatest when developing in *V. album* fruit, again likely to be linked with P:C ratio as the number of ovarioles is maximized at intermediate P:C ratio (Silva-Soares *et al*., 2017). Finally, survival was maximized when developing on *V. album*, intermediate on *A. japonica* and least on *E.* ×*submacrophylla*. Survival in *D. suzukii* is favored by a higher concentration of sugar in its diet, which is expressed as a lower P:C ratio (Shu *et al*., 2022), consistent with the low survival probability of flies emerging from *E.* ×*submacrophylla*. In our PCA analysis most life history traits, except development time and longevity, ranked the three fruit in line with our initial hypothesis 2 (*E.* ×*submacrophylla* and *V. album* of intermediate quality and *A. japonica* the least favorable).

Other fruit morphological and biophysical properties could contribute to the variability in *D. suzukii* performance. Despite having a suitable P:C ratio, *A. japonica* fruit appeared to be the least suitable in terms of most of the life history traits examined. Fruit of this species are fibrous, making crawling and access to nutrients difficult for feeding larvae (House, 1969). They also contain secondary metabolites such as aucubin, an iridoid glycoside which has been shown to exhibit deterrent (Biere *et al*., 2004) and toxic (Vivekanandhan *et al*., 2022) properties against insects, in addition to its antimicrobial activity (Rombouts & Links, 1954), which could also be detrimental to *D. suzukii* larval development.

Fruit suitability differs under colder and fluctuating temperature regimes

Under colder fluctuating temperature regimes, the ranking of fruit supporting the best performance changed as illustrated by the PCA, with *A. japonica* fruit becoming comparable in most life history traits to those of *E.* ×*submacrophylla* and *V. album*, and even supporting greater longevity than these other fruit. This may be linked with the high energy content of flies developing in *A. japonica* under FCR and UNR, which was equal to that in the artificial diet. However, the ovarian load was the lowest,

highlighting a possible trade-off between survival and reproduction which has been recognized in the literature (e.g., Zera & Harshman, 2001; Hunt *et al*., 2004; Lee *et al*., 2008; De Loof, 2011; Attisano *et al*., 2012; Blacher *et al*., 2017). Under a low-quality diet, survival is favored at the expense of reproduction (Holliday, 1989; Boggs & Ross, 1993; Zera & Harshman, 2001; Attisano *et al.*, 2012) and can be influenced by specific nutrients. In *D. melanogaster*, adding methionine to a restricted diet maximized fecundity without reducing lifespan (Grandison *et al*., 2009). Temperature can also influence the trade-off between fecundity and longevity (Marshall & Sinclair, 2009). For *D. suzukii*, fruit selection maximizing longevity over reproduction could increase fitness of the first generation of females emerging at the end of winter/beginning of spring when resources are still scarce. Indeed, for a synovigenic species, being able to survive longer and having larger wings could clearly contribute to foraging success (Poyet *et al*., 2015; Panel *et al.*, 2018). Following this logic, although *A. japonica* provides a sub-optimal diet under a constant mild temperature of 20°C, it could represent a potentially valuable diet for pioneer *D. suzukii* females early in the season.

Maladaptive fruit choice?

In our study, we investigated the egg-laying behavior of winter-acclimated individuals when faced with a choice between three common and suitable available fruit. We hypothesized that food preference would change depending on the temperature regime, because the food requirements of winter and summer individuals could differ (Rendon *et al*., 2019). Various parameters can influence insect fruit choice, and *D. suzukii* is known to be sensitive to color (Bühlmann & Gossner, 2022), shape and size (Bühlmann & Gossner, 2022), firmness (Sato *et al*., 2021), skin texture (Bühlmann & Gossner, 2022) and odor (Cha *et al*., 2012), amongst other traits. We did not test for odor differences but the fruit used here differed in all of the other traits: *A. japonica* fruit are oblong (~1 cm), red, with smooth skin (Abe, 2001); *E.* ×*submacrophylla* fruit are oblong (~1.5 cm), soft, orange-deep-red silvery color, with rough skin (Edwards, 2017; Abdalla, 2019); *V. album* fruit are ovoid (~ 0.9 cm), white and tend to become translucent with ripening, with smooth skin (Thomas *et al*., 2023). *Viscum album* fruits combine small size and a low contrast color, while *D. suzukii* favors contrasting colors (Little *et al.*, 2019), as well as red, orange or black, and larger fruit (Bühlmann and Gossner, 2022). Future studies should include such traits in behavioral assays.

Our results did not generally provide support for the preference-performance hypothesis, agreeing with Poyet *et al.* (2015) and Olazcuaga *et al.* (2019). Under all thermal regimes, most eggs were laid on *A.*

japonica and *E.* ×*submacrophylla* fruit. Females only oviposited in *V. album* fruit in the constant 20°C regime, even then being least preferred. This appears maladaptive, as *V. album* optimized fly performance while *A. japonica* gave the worst results in terms of emergence rate, development time, morphometric characteristics, ovarian load and survival. However, under FCR, *A. japonica* fruit maximized fly energy content, resulting in longer survival than under any other diet. Under the same regime, *V. album* fruit would lead to the lowest energy content, but an increased emergence rate. Thus, there might be a trade-off whereby adult survival capacity could be favored at the expense of the risk of non-emergence, following a K strategy (Taylor & Condra, 1980), where *D. suzukii* selecting *A. japonica* fruit would have fewer offspring but they would survive longer. Alternatively, the preference for *A. japonica* could also be a result of coevolutionary history between *D. suzukii* and the plant in their native ranges (Mitsui *et al*., 2010). The preference-performance hypothesis might also not apply to *D. suzukii*, as has been demonstrated in other polyphagous species (Gripenberg *et al.*, 2010).

Ecological consequences

Drosophila suzukii's ability to overwinter and re-initiate reproduction as temperatures increase in early spring is remarkable. Overwintering individuals contribute to the initial build-up of the new year's population. In this study we have demonstrated that appropriate low temperature regimes impaired the fly's overall performance relative to a standard commonly-used rearing regime, while the three fruit trialed gave equivalent performance under the natural field fluctuating temperature regime. Use of any of three fruit after re-initiating reproduction would lead to the production of adults that are viable and ready to generate the next fly generation and infest early season commercial crop fruit.

In natural field conditions, *V. album* fruit can be heavily infested (Deconninck *et al*., 2024a - Chapter 2), sometimes even more so than those of *A. japonica* or *E.* ×*submacrophylla* (Deconninck *et al.*, under review - Chapter 3). Under natural conditions fruit choice is also dependent on resource abundance and availability. The phenology of fruit ripeness can limit the fly to the readily available suitable fruit, even if performance is not then maximized. *Viscum album* fruit are available for several months over winter/spring (Thomas *et al.*, 2023; Deconninck *et al.*, 2024a - Chapter 2) and can therefore become heavily infested when this is one of the only available fruit-bearing plants (Deconninck *et al.*, under review). The abundance of a given fruit in an area will also influence its infestation rate (Begon, 1975). This point is also linked with the phenology, but it is also notable that trees infested by *V. album* typically host about 20 individuals, and can approach almost 300 individuals per tree in *Pinus nigra*

plantations, providing a tremendous reservoir of fruit (Thomas *et al*., 2023) compared with *A. japonica* or *E.* ×*submacrophylla* bushes that are often planted in urban areas with a patchy distribution and are regularly pruned (Prior, 1881; Abe, 2001; Edwards, 2017; Abdalla, 2019).

The fruit selection made by *D. suzukii* under FCR is consistent with infestation rates reported in the field in the Netherlands in April and May 2017, with *A. japonica* fruit being more infested than those of *E.* ×*submacrophylla* and no eggs found in those of *V. album* (Panel *et al*., 2018). However, it differed from field data obtained in France in April and May 2022, where the most heavily infested fruit were those of *E.* ×*submacrophylla*, followed by *V. album* and *A. japonica* (Deconninck *et al.*, under review - Chapter 3). Field infestation rates are more representative of a population choice, and could also be modulated by the overall abundance of fruit in an area, so that it might not be straightforward to compare the results of these studies with our experimental data. However, combining the findings of these studies, it appears that fruit choice by *D. suzukii* females can vary not only according to fruit availability and characteristics but also to experienced temperature regimes. Field and laboratory experiments are complementary approaches to understanding the drivers of fruit infestation in the natural environment and the existence of trade-offs between life history traits linked to environmental conditions (e.g., temperature or trophic resources). Based on the results of our study, further research on the performance of flies under fluctuating natural and semi-natural conditions seems particularly relevant.

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Supplementary Material

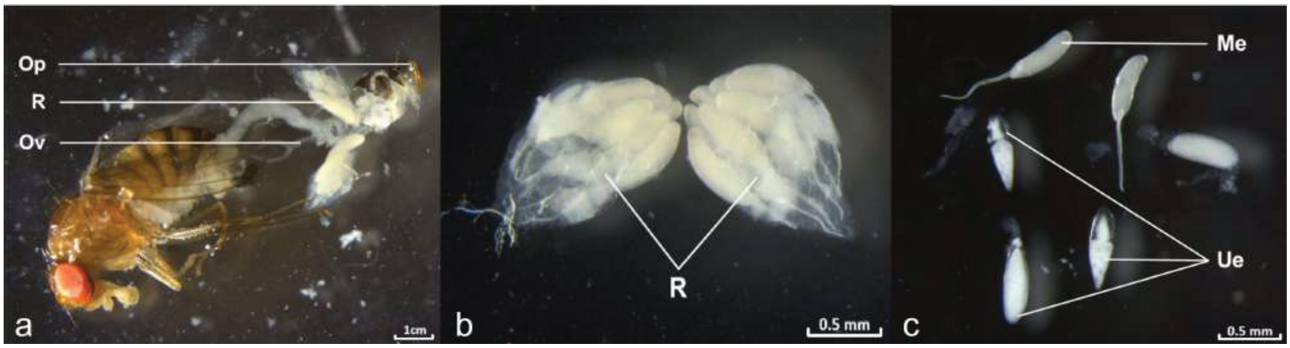

Figure S1 Ovarian load of female *D. suzukii*: (a) Dissected female with visible ripe ovaries; (b) ripe ovaries; (c) eggs. Legend: ripe ovaries (R), unripe (Ue) and mature eggs (Me), oviduct (Ov) and ovipositor (Op).

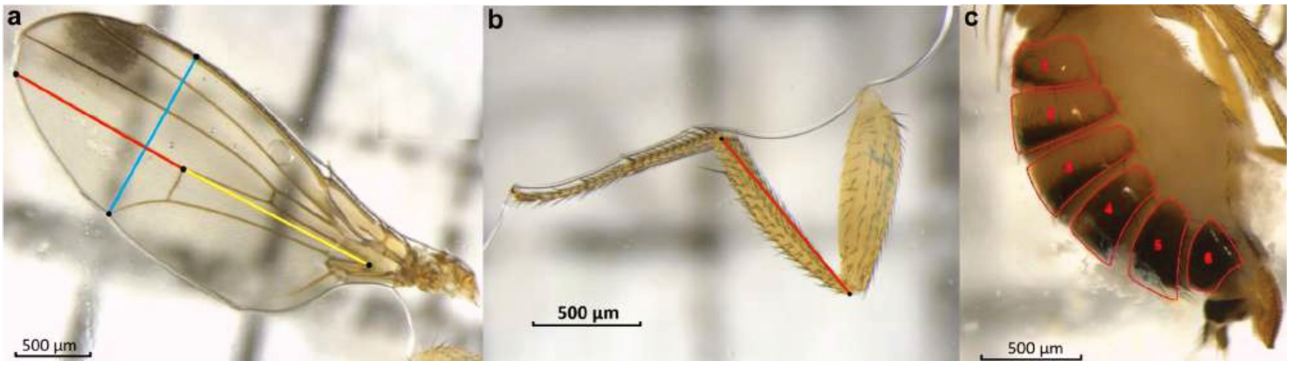

Figure S2 (a) Wing size measurement (red: wing length 1, yellow: wing length 2, blue: wing width); wing area index = (wing length 1 + wing length 2) × wing width (after Ulmer *et al*., 2024), (b) tibia size measurement and (c) count of the melanized abdominal tergites in *D. suzukii*.

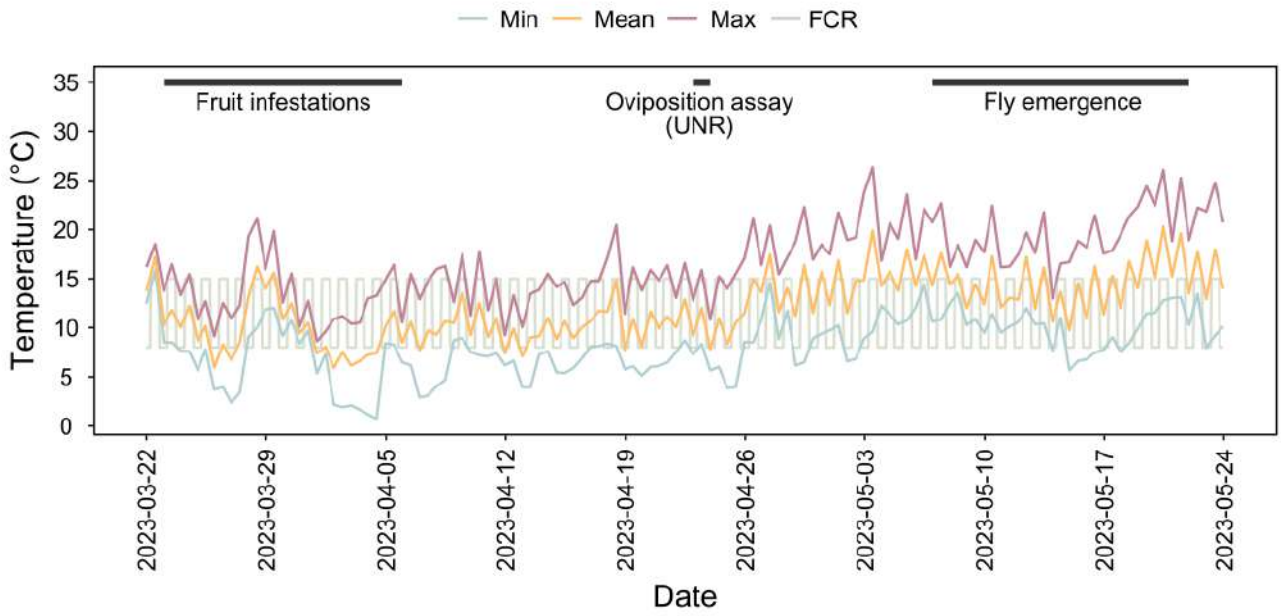

Figure S3 Field temperature records during the experiment, between March 22 and May 24, 2023. Colored lines represent minimum, mean and maximum temperatures every 12 h in the uncontrolled outdoor regime (UNR). The gray line corresponds to the temperature under the fluctuating controlled regime (8–15°C, FCR). Fruit infestation under UNR took place between March 24 and April 7, 2023. The oviposition assay under UNR took place between April 24 and 25, 2023. Fly emergence started 8 and ended 23 May, 2023.

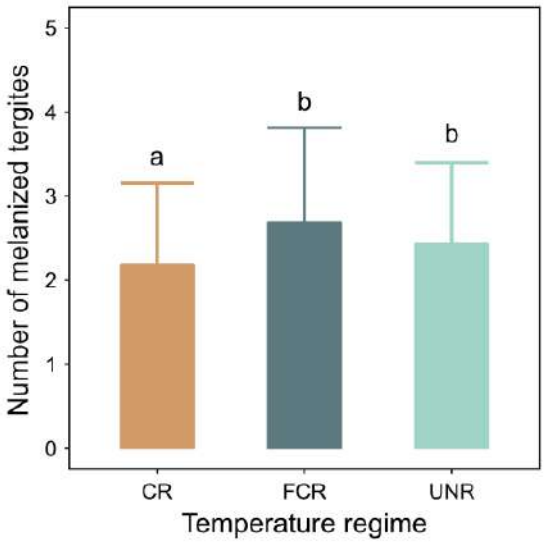

Figure S4 Number of melanized tergites (mean ± sd) of *D. suzukii* flies that emerged under the different temperature regimes (CR: 20°C; FCR: fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor regime between 24 March and 31 June 2023). Different letters indicate significant differences at $p < 0.05$ (GLM with Poisson error distributions).

Table S1 Period of fruit collection.

Collection date	Fruits
25.01.2023- 27.01.2023	*Aucuba* + *Viscum*
01.02.2023- 03.02.2023	*Aucuba* + *Viscum*
08.02.2023- 10.02.2023	*Aucuba* + *Viscum*
15.02.2023- 17.02.2023	*Aucuba* + *Viscum*
24.03.2023	*Aucuba* + *Viscum* + *Elaeagnus*
29.03.2023- 31.03.2023	*Aucuba* + *Viscum* + *Elaeagnus*
05.04.2023- 07.04.2023	*Aucuba* + *Viscum* + *Elaeagnus*
12.04.2023	*Elaeagnus*
19.04.2023	*Elaeagnus*

Table S2 Total number of individuals available for each experiment (CR: 20°C; FCR: fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor regime between 24 March and 31 June 2023).

Temperature regime	Diet	Longevity	Starvation resistance	Ovarian load	Energy content	Morphometric measurement
CR	**Artificial**	49♂/40♀	64♂/58♀	30♀	20♀	65♂/94♀
	Aucuba	65♂/42♀	59♂/35♀	30♀	20♀	69♂/90♀
	Elaeagnus	65♂/38♀	44♂/28♀	33♀	20♀	57♂/80♀
	Viscum	126♂/60♀	149♂/106♀	32♀	20♀	62♂/92♀
FCR	**Artificial**	40♂/48♀	46♂/35♀	30♀	20♀	60♂/88♀
	Aucuba	37♂/26♀	33♂/23♀	20♀	20♀	58♂/66♀
	Elaeagnus	28♂/15♀	31♂/16♀	14♀	20♀	50♂/39♀
	Viscum	73♂/61♀	61♂/46♀	30♀	20♀	60♂/90♀
UNR	**Artificial**	35♂/35♀	33♂/34♀	33♀	20♀	60♂/90♀
	Aucuba	66♂/60♀	32♂/31♀	31♀	20♀	61♂/91♀
	Elaeagnus	27♂/30♀	0♂/0♀	0♀	16♀	22♂/23♀
	Viscum	41♂/56♀	40♂/36♀	32♀	20♀	57♂/80♀

Table S3 Differences in nutritional composition (proteins, glucose, P:C ratio) between fruits.

Proteins (square-root -transformed)					
Effect	Df	Sum of Squares	Mean Square	F	$P > \|\chi^2\|$
Fruit	2	15.64	7.822	80.95	< 2e-16
Residuals	187	18.07	0.097		
Glucose (log-transformed)					
Effect	Df	Sum of Squares	Mean Square	F	$P > \|\chi^2\|$
Fruit	2	156.22	78.11	600.6	< 2e-16
Residuals	186	24.19	0.13		
P:C (square-root-transformed)					
Effect	Df	Sum of Squares	Mean Square	F	$P > \|\chi^2\|$
Fruit	2	6.944	3.472	65.74	< 2e-16
Residuals	186	9.823	0.053		

Data were analyzed using ANOVA on square-root-transformed proteins and P:C ratio, and log-transformed glucose. Df: degrees of freedom; $P > |\chi^2|$: *p*-value.

Table S4 Influence of diet, temperature and their interaction on *D. suzukii* emergence rate.

Emergence rate			
Effect	Df	LR Chisq	$P > \|\chi^2\|$
Diet	3	2974.64	< 2.2e-16
Temperature	2	457.25	< 2.2e-16
Diet × Temperature	6	85.29	2.9e-16

Data were analyzed using generalized linear models (GLM) on binomial data with logit link function. Df: degrees of freedom; LR Chisq: likelihood ratio Chi-square; $P > |\chi^2|$: *p*-value.

Table S5 Influence of diet, temperature and their interaction on *D. suzukii* sex ratio (M:F).

Sex ratio			
Temperature regime	Df	LR Chisq	$P > \|\chi^2\|$
Diet	3	5.0542	0.1679
Temperature	2	14.3665	0.0008
Diet × Temperature	6	6.0839	0.4139

Data were analyzed using generalized linear models (GLM) on binomial data with logit link function. Df: degrees of freedom; LR Chisq: likelihood ratio Chi-square; $P > |\chi^2|$: *p*-value.

Table S6 Sex ratio (M:F) of emerging *D. suzukii* under different combinations of temperature regime (CR: 20°C; FCR: fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor regime between 24 March and 31 June 2023) and diet. The sex ratio was calculated in each experimental tube to obtain the mean sex ratio ± sd.

Temperature regime	Diet	Ntot	Females	Males	Mean sex ratio ± sd (M:F)
CR	**Artificial**	393	200	193	0.49± 0.25
	Aucuba	292	151	141	0.50 ± 0.37
	Elaeagnus	321	164	157	0.48 ± 0.32
	Viscum	556	258	298	0.53 ± 0.23
FCR	**Artificial**	303	182	121	0.39 ± 0.28
	Aucuba	168	95	73	0.44 ± 0.42
	Elaeagnus	147	76	71	0.52 ± 0.35
	Viscum	543	303	240	0.45 ± 0.29
UNR	**Artificial**	334	176	158	0.49 ± 0.29
	Aucuba	410	220	190	0.48 ± 0.38
	Elaeagnus	77	48	29	0.38 ± 0.39
	Viscum	643	322	321	0.50 ± 0.30

Table S7 Influence of diet, temperature and their interaction on *D. suzukii* morphometric measurements (wing area index and tibia size), mass, energy content (J/mg of fly biomass) and development time.

Wing area index					
Effect	Df	Sum of Squares	Mean Square	F	*P* > F
Diet	3	87.87	29.291	201.858	< 2.2e-16
Temperature	2	52.31	26.153	180.231	< 2.2e-16
Diet × Temperature	6	4.61	0.768	5.292	2.24e-05
Residuals	911	132.19	0.145		
Tibia size					
Effect	Df	Sum of Squares	Mean Square	F	*P* > F
Diet	3	2.534	0.8446	205.037	< 2.2e-16
Temperature	2	0.406	0.2032	49.336	< 2.2e-16
Diet × Temperature	6	0.129	0.0215	5.228	2.64e-05
Residuals	910	3.749	0.0041		
Mass					
Effect	Df	Sum of Squares	Mean Square	F	*P* > F
Diet	3	31.279	10.426	131.987	< 2.2e-16
Temperature	2	0.523	0.262	3.312	0.0382
Diet × Temperature	6	2.111	0.352	4.455	0.0003
Residuals	224	17.695	0.079		
Energy content (log-transformed)					
Effect	Df	Sum of Squares	Mean Square	F	*P* > F
Diet	3	2.853	0.9511	11.357	6.02e-07
Temperature	2	1.575	0.7877	9.406	0.0001
Diet × Temperature	6	1.884	0.3140	3.749	0.0014
Residuals	216	18.089	0.0837		
Development time (log-transformed)					
Effect	Df	Sum of Squares	Mean Square	F	*P* > F
Diet	3	83.9	28.0	3634.5	< 2.2e-16
Temperature	2	854.9	427.5	55585.4	< 2.2e-16
Diet × Temperature	6	8.7	1.4	187.7	< 2.2e-16
Residuals	3822	29.4	0.0		

Data were analyzed using ANOVA; if a transformation was required to achieve normality, the transformation is indicated in parentheses. Df: degrees of freedom; *P* > F: *p*-value.

Table S8 Influence of temperature regime on the number of melanized tergites of *D. suzukii*.

Number of melanized tergites				
Effect	Df	Sum of Squares	F	*P* > F
Temperature	2	22.86	26.452	5.47e-12
Residuals	1310	566.07		

Data were analyzed using general linear models with Poisson error distributions. Df: degrees of freedom; *P* > F: *p*-value.

Table S9 Energy content (mean ± sd) of flies emerging from artificial diet, *Aucuba japonica*, *Elaeagnus ×submacrophylla* or *Viscum album* fruits under different temperature regimes (CR: 20°C; FCR: fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor regime between 24 March and 31 June 2023). N = 20, except for Artificial and *Aucuba* in FCR (n = 18 and n = 17, respectively), Artificial, *Aucuba* and *Viscum* in UNR (n = 19), and *Elaeagnus* in UNR (n = 16). The reduced sample size is due to removal of aberrant values. Differences between fruit types in each temperature treatment were analyzed using ANOVA followed by Tukey HSD's tests (energy content) or by Kruskal-Wallis tests followed by Wilcoxon pairwise comparisons. Different letters indicate significant differences at $p < 0.05$.

	Temperature	Artificial		*Aucuba*		*Elaeagnus*		*Viscum*		Test	*p-value*
Energy content (J/mg fly biomass)	CR	4.50 ± 1.21	a	4.13 ± 1.24	a	4.22 ± 1.52	a	4.37 ± 1.19	a		
	FCR	5.96 ± 1.55	a	4.70 ± 1.60	ab	4.43 ± 0.99	b	3.41 ± 1.03	c	Anova	**0.0014**
	UNR	5.82 ± 0.94	a	6.03 ± 1.96	a	4.85 ± 1.34	ab	4.39 ± 1.57	b		
Glucose (mg/mg fly biomass)	CR	10.23 ± 4.43	a	12.45 ± 5.21	a	9.05 ± 3.27	a	8.47 ± 3.95	a		0.163
	FCR	9.12 ± 1.42	a	12.52 ± 9.57	a	7.01 ± 4.01	a	7.13 ± 3.57	a	Kruskal-Wallis	**0.0163**
	UNR	20.73 ± 11.32	a	11.26 ± 5.17	b	11.12 ± 8.14	ab	9.04 ± 5.32	b		**0.0052**
Fructose (mg/mg fly biomass)	CR	1.10 ± 2.12	a	1.78 ± 2.24	a	0.91 ± 0.92	a	0.76 ± 1.12	a		0.488
	FCR	0.69 ± 1.05	a	0.39 ± 0.39	a	0.44 ± 0.85	a	0.19 ± 0.25	a	Kruskal-Wallis	**0.0498**
	UNR	3.55 ± 3.52	a	2.11 ± 2.45	ab	1.83 ± 2.14	ab	0.92 ± 0.91	b		**0.0137**
Total carbohydrates (mg/mg fly biomass)	CR	11.33 ± 6.41	a	14.23 ± 6.60	a	9.96 ± 3.63	a	9.23 ± 4.21	a		0.134
	FCR	9.81 ± 1.87	a	12.92 ± 9.85	ab	7.46 ± 4.11	ab	7.33 ± 3.47	b	Kruskal-Wallis	**0.0105**
	UNR	24.28 ± 13.70	a	13.37 ± 6.53	ab	12.95 ± 9.32	ab	9.96 ± 5.38	b		**0.0037**
Glycogene (mg/mg fly biomass)	CR	8.09 ± 4.52	a	14.85 ± 6.20	b	10.00 ± 4.35	a	8.15 ± 2.45	a		**< 0.0001**
	FCR	6.07 ± 2.36	a	11.79 ± 9.57	a	9.16 ± 9.59	a	5.97 ± 1.24	a	Kruskal-Wallis	0.0857
	UNR	15.73 ± 8.27	a	17.15 ± 16.08	ab	17.99 ± 13.37	ab	8.64 ± 2.67	b		**0.0301**
Proteins (mg/mg fly biomass)	CR	89.84 ± 26.35	a	64.85 ± 27.91	b	101.37 ± 73.83	a	99.29 ± 51.43	ab		**0.0071**
	FCR	88.58 ± 20.09	a	72.67 ± 45.41	bc	80.35 ± 14.78	ab	66.62 ± 24.97	c	Kruskal-Wallis	**0.0003**
	UNR	84.15 ± 15.87	a	75.38 ± 17.13	a	84.63 ± 56.07	a	84.32 ± 41.83	a		0.383
Lipids (mg/mg fly biomass)	CR	76.43 ± 27.28	a	76.01 ± 27.97	a	64.91 ± 22.45	a	70.04 ± 22.96	a		0.625
	FCR	116.37 ± 37.90	a	88.54 ± 32.29	bc	80.23 ± 23.04	ab	59.26 ± 23.14	c	Kruskal-Wallis	**< 0.0001**
	UNR	108.42 ± 25.33	a	122.47 ± 47.84	a	87.26 ± 38.55	a	76.66 ± 31.61	a		**0.0008**

Table S10 Influence of diet, temperature and their interaction on *D. suzukii* female ovarian load.

Female ovarian load				
Effect	Df	Sum of Square	F	*P* > F
Diet	3	2449.15	110.16	< 2.2e-16
Temperature	2	220.11	14.85	7.03e-07
Diet × Temperature	5	159.77	4.31	0.0008
Residuals	304	2252.98		

Data were analyzed using general linear models with quasi-Poisson error distributions. Df: degrees of freedom; *P* > F: *p*-value.

Table S11 Influence of different diets under each temperature regime (CR: 20°C; FCR: fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor regime between 24 March and 31 June 2023) on *D. suzukii* starvation resistance and longevity ('Survival with food for 15 days').

Starvation resistance	
Temperature regime	*p*-value
CR	2.37e-19
FCR	6.24e-12
UNR	2.13e-04
Longevity	
Temperature regime	*p*
CR	2.40e-05
FCR	0.17
UNR	2.49e-03

Data were analyzed using log-rank tests.

Table S12 Differences in oviposition choice between fruit types under each temperature regime (CR: 20°C; FCR: fluctuating controlled regime of 8–15°C 12h:12h; UNR: uncontrolled outdoor regime between 24 March and 31 June 2023).

Oviposition choice				
Temperature regime	n	Df	Chisq	$P > \|\chi^2\|$
CR	3	2	76.0	3.08e-17
FCR	3	2	51.9	5.49e-12
UNR	3	2	59.7	1.07e-13

Data were analyzed using Chi-squared tests with an expected proportion of 1/3 without any choice. Df: degrees of freedom; Chisq: chi-square; $P > |\chi^2|$: *p*-value.

Nutritional composition of collected fruit

For each artificial infestation experiment, a randomly selected batch of 10 fruit was isolated and stored at −80°C for further nutritional analysis of proteins and carbohydrates. The frozen fruit were sliced on an ice-cold crystallizer. The material was weighed and then homogenized in tubes with ice-cold PBS (Sigma P4417) and four tungsten beads. The tubes were placed in a bead-beater for 2 min at 25 Hz. Samples were then centrifuged for 5 min at 5000 ×g at 4°C. The clear supernatant was removed and stored at −80°C before glucose and protein quantification. For glucose quantification, samples were first deactivated by heating the tubes for 10 min at 70°C. Then, we used glucose oxidase/peroxidase reagent (Sigma G3660) and o-dianisidine reagent (Sigma D2679). The procedure involved the oxidation of glucose to gluconic acid and hydrogen peroxide by glucose oxidase. The hydrogen peroxide then reacted with o-dianisidine in the presence of peroxidase to form a colored by-product. The oxidized o-dianisidine then reacted with sulfuric acid to form a stable-colored product whose intensity (pink coloration) was measured at 540 nm using a microplate reader (SAFAS Flx-Xenius). A standard curve was generated using a glucose standard solution (Sigma G3285). For protein quantification, we used the Bradford assay, a colorimetric protein assay based on an absorbance shift of the dye Coomassie Brilliant Blue G-250 proportional to protein concentration. The blue coloration was measured at 595 nm using a microplate reader (SAFAS Flx-Xenius). A standard curve was prepared using a stock solution of BSA (bovine serum albumin).

Analysis of D. suzukii energy content

Individual females from each treatment combination were first solubilized in a phosphate-lysis buffer. Then, total proteins were assayed using Bradford's reagent (B6916, Sigma-Aldrich) with BSA as a standard. In a second step, a methanol:chloroform mixture and a sodium sulfate solution were added to the homogenate to reach the proportions 2:5:10 v/v/v of water, chloroform and methanol, respectively. After vortexing and centrifugation, glycogen was quantified from the pellet while free-carbohydrates and total lipids were assessed from the supernatant. Carbohydrates (free sugars and glycogen) were quantified using Anthrone reagent (319899, Sigma-Aldrich) with D-glucose as a standard, and total lipid quantification was performed using Vanillin (V1104, Sigma-Aldrich), with triolein as a standard. Optical density measurements were performed using a spectrophotometer (NanoQuant, TECAN).

Chapter 8

Polyphagy and symbionts

Chapter 8 • Objectives and main outcomes

In this chapter, I explore the role that the *Wolbachia* strain *w*Suz could play in helping *D. suzukii* to respond to the variation in its fruit resources resulting from polyphagy. A female can encounter several different fruit resources in the field and, therefore, oviposition can occur on fruit that differ from those in which the fly developed (i.e., a diet shift). In the natural environment, this *w*Suz shows imperfect maternal transmission while, in the laboratory, maternal transmission is perfect. While the bacteria does not manipulate the fly's reproduction, its prevalence in natural populations is higher than expected by chance, indicating that *w*Suz must provide benefits to its host. Studies have attempted to identify these beneficial effects, but to date the information available remains inconclusive. In other insect species, *Wolbachia* can offer nutritional benefits, but this role has not previously been explored in *D. suzukii*.

I used *Wolbachia*-infected and uninfected flies, maintained on a corn-based (low-starch) or a grape-based (high-starch) diet for at least 10 generations. Individuals from each line were then placed on one of the two diets for 30 days to simulate a diet shift. After the diet shift, *Wolbachia*-infected females had a higher fecundity than uninfected ones and their offspring were heavier. *Wolbachia* presence also increased survival during larval development regardless of the diet, supporting its mutualistic role. The data obtained support *w*Suz having a positive influence on *D. suzukii* diet tolerance and performance.

These findings suggest that *Wolbachia* could have a positive influence on supplementing the host's diet when developing on a low-quality resource, although further investigation is required to identify how this takes place. Such a role could also explain the maintenance of the *w*Suz strain in *D. suzukii* globally. However, caution is needed when interpreting these findings, as they are currently specific to a single *Wolbachia* genotype and *D. suzukii* population. To confirm broader applicability, it would be necessary to carry out assessments with various *D. suzukii* and *Wolbachia* genotypes, given that numerous studies have revealed that the influence of *Wolbachia* is contingent on both its own genotype and that of its host. An integrated approach is required to understand in detail the role *Wolbachia* plays in polyphagous insects.

Contributions

Sylvain Pincebourde, Vincent Foray and I conceptualised the study.

I performed the experiment and collected the data. I was assisted by a student (Jordy Larges) that I supervised for both data collection and data curation. Hélène Henri performed the microbiota analysis

I analysed the data, produced the graphics and tables. I wrote the original draft, revised the manuscript according to reviewers' comments and wrote the response letter to the editor and reviewers.

All co-authors contributed to the revision of the manuscript.

Wolbachia improves the performance of an invasive fly after a diet shift

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ABSTRACT

Polyphagy is an adaptative strategy allowing species to develop and survive on multiple diets. Phytophagous insects can be exposed to sub-optimal resources, as host plants vary in nutritional quality. Some insects may rely on symbiotic bacteria to better utilize less favorable substrates. The invasive fruit fly *Drosophila suzukii* is a highly successful pest worldwide. The use of ripening and ripe fruits, a niche neglected by other drosophilids, and its polyphagy may have contributed to its global spread. The role of *D. suzukii*'s *Wolbachia* strain (*w*Suz) remains unconfirmed, although a mutualistic role has been hypothesized *via* virus protection or dealing with abiotic stress. In some insect species, *Wolbachia* acts as a nutritional mutualist. This study explored the role of *w*Suz in *D. suzukii* adaptation to fluctuating diet regimes. To simulate a diet shift, we used *Wolbachia*-infected and uninfected flies, maintained on a corn-based (low-starch) or a grape-based (high-starch) diet for at least 10 generations. Then, we placed individuals from each line on one or the other diet for 30 days. The effects of *Wolbachia* and the diet treatments were assessed by quantifying female fecundity, egg-to-adult development time and survival, and offspring mass. The presence of *Wolbachia* positively affected female fecundity and offspring mass after a diet shift. *Wolbachia* also increased survival during larval development regardless of the diet, supporting its mutualistic role. Our results underline the role of *w*Suz on *D. suzukii* diet tolerance and performance. A better understanding of the role of symbionts in invasive species could help to improve management strategies.

Keywords: *Drosophila suzukii*, endosymbiont, fecundity, mutualism, nutrition, polyphagy

INTRODUCTION

The ability to develop and reproduce on several diets – known as polyphagy – is an adaptive strategy that is often successful in heterogeneous environments (Singer 2008). However, polyphagy among phytophagous insects is the exception rather than the rule as most hypotheses on niche breadth relate on host specialization (reviewed by Jousselin and Elias 2019). While specialists can only develop and survive on a very limited number of plant species, polyphagous insects can complete their cycle on a large spectrum of plants with varying quality. Encountering both low- and high-quality resources, the generalist strategy can be costly for individuals as offspring performance (e.g., developmental rate, survival) may vary between host plants (Janz 2003; Poyet et al. 2015). This cost could be offset by a female laying eggs on numerous host plants, ensuring that at least some offspring survive or achieve optimal development (Jaenike 1990; Singer 2008) and leading to a trade-off between host quality and availability (Singer 2008). Many successful pest insects are polyphagous (Singer 2008). This is the case for the fruit flies (Diptera: Tephritidae), which include some species with extreme polyphagy, for which selection pressure acts towards the ability of individuals to use any fruit that are readily available (Wilson et al. 2012).

Extreme polyphagy may act in synergy with other strategies to help improve performance when encountering a poor-quality host plant. A particularly important factor affecting insect host range is their associated microbiome (Frago et al. 2012; Sugio et al. 2015; Giron et al. 2018). For example, facultative secondary endosymbionts broaden the range of suitable host plants for the whitefly *Bemisia tabaci* (Benhamou et al. 2021). Associations between microbes and insect hosts can be beneficial or deleterious, facultative or obligatory (Sugio et al. 2015; Gurung et al. 2019). Amongst those with beneficial interactions, bacteria are the most studied organisms. They have a role in insect immunity, protection against parasitoids and parasites, helping insects coping with abiotic stress, as well as with nutritional stress (Dillon and Dillon 2004; Oliver et al. 2010; Eleftherianos et al. 2013; Sannino et al. 2018; Gurung et al. 2019). Bacteria can play multiple nutritional roles in insects, including improving their ability to live on suboptimal diets, increasing the efficiency of digestion, providing digestive enzymes, detoxifying defense compounds and provisioning vitamins (Dillon and Dillon 2004; Ashra and Nair 2022). For example, *Candidatus Erwinia dacicola*, an obligate symbiont of the tephritid olive fruit fly, *Bactrocera oleo* (Rossi), provides adult flies with essential amino acids that are absent in unripe olive fruits (Ben-Yosef et

al. 2014). In *Drosophila melanogaster* larvae, *Acetobacter pomorum* and *Lactobacillus plantarum* allow them to better cope with changes in nutrient availability, optimizing host fitness (Consuegra et al. 2020).

Among bacterial endosymbionts, *Wolbachia* is widely acknowledged to be the most abundant and prevalent in insect species (Zug and Hammerstein 2015). Initially known for their reproductive parasitism that allows them to spread, research has increasingly shed light on *Wolbachia*'s complex effects on hosts, ranging from parasitism to mutualism (Brownlie et al. 2009; Zug and Hammerstein 2015; Newton and Rice 2020; Pimentel et al. 2021). As mutualists, *Wolbachia* can protect the host against viruses (Chrostek et al. 2013; Cattel et al. 2016b; Pimentel et al. 2021), increase thermal tolerance (Saeed et al. 2018) and provide nutritional benefits (Brownlie et al. 2009; Hosokawa et al. 2010; Zug and Hammerstein 2015; Newton and Rice 2020; Lindsey et al. 2023). Nucleotides and vitamins have been suggested to be supplemented or modulated by *Wolbachia* (Newton and Rice 2020; Lindsey et al. 2023). In the bed bug *Cimex lectularius*, the *Wolbachia* strain *w*Cle provides B vitamins to its host that are essential for growth and reproduction (Hosokawa et al. 2010; Nikoh et al. 2014). In *D. melanogaster*, *Wolbachia* plays a role in iron metabolism (Brownlie et al. 2009) and was recently found to be highly beneficial under nutrient limited conditions (Lindsey et al. 2023). The nutritional role of *Wolbachia* could explain its prevalence in some populations even when no reproductive manipulation is found (Newton et al. 2020). Overall, however, the role of *Wolbachia* as a potential promotor of invasion and/or pest success has received little attention (but see Lu et al. 2016). In this study, we explored its role in an important invasive fruit fly.

Originating from Asia, *Drosophila suzukii* Matsumara spread in the late 2000s into Europe, America and, more recently, Africa (Asplen et al. 2015; Kwadha et al. 2021). Unlike other *Drosophila* species that oviposit on rotting fruit, *D. suzukii* lays eggs on still ripening and ripe fruits using a serrated ovipositor (Atallah et al. 2014; Clemente et al. 2018). This ability, in addition with multiple generations per year, makes *D. suzukii* an important pest of soft and thin-skinned crops, resulting in significant economic losses for fruit growers (Walsh et al. 2011). Being highly polyphagous, the fly exploits a wide variety of cultivated (e.g., red fruits, cherry, plum, peach, grape; Walsh et al. 2011) and wild fruits (e.g., elderberry, mistletoe, wild black cherry; Poyet et al. 2015; Kenis et al. 2016; Ulmer et al. 2022), ensuring its persistence year-round (Poyet et al. 2015). However, this polyphagy exposes the fly to diets of differing quality, which results in various effects on its development (Poyet et al. 2015). The *Wolbachia* strain *w*Suz has been reported in *D. suzukii* (Cattel et al. 2016b). In nature, this strain has an imperfect maternal transmission (Hamm et al. 2014), while in laboratory the maternal transmission is perfect (Cattel et al.

2016a). It does not induce reproductive manipulation in American populations (Hamm et al. 2014; Cattel et al. 2016b), yet it induces moderate cytoplasmic incompatibility in European populations (Cattel et al. 2016b, 2018). Its prevalence in natural populations is higher than expected by chance, indicating that *w*Suz must provide benefits to its host (Cattel et al. 2016b), buts costs (see below) and drift (Oliver et al. 2014) certainly can play against the maintenance of *Wolbachia* within populations. Studies have attempted to identify these beneficial effects, but the available information remains inconclusive. Some studies have reported an increase in fecundity (Mazzetto et al. 2015) and others a decrease (Hamm et al. 2014) associated with *Wolbachia* presence. Hamm et al. (2014) found no effect of *w*Suz on starvation tolerance or desiccation resistance while Saeed et al. (2018) reported an increase in thermal tolerance. These results must be taken with caution as different host genetic backgrounds or the presence/absence of antimicrobial agents in the diet may have led to confounding effects (Cattel et al. 2016b). Cattel et al. (2016b) revealed protection against two viruses, Drosophila C virus and Flock House virus. However, no studies have yet addressed the potential effects of *w*Suz on nutritional provisioning or diet tolerance. Taking into account the high level of polyphagy displayed by *D. suzukii*, the variable resource availability across time and space, and the difference in *w*Suz prevalence across geographical ranges, we hypothesized that *w*Suz benefits *D. suzukii* by improving the fly's ability to deal with the diet shifts it experiences.

This study therefore investigated the role of the *D. suzukii Wolbachia* strain *w*Suz in dealing with nutritional shifts. We used two fly lines sharing the same genetic background but differing in the presence or absence of *Wolbachia*. Two diets were tested: a corn diet and a grape diet, which differed mainly in their starch content. The numbers of eggs produced, developmental time, offspring survival to adulthood and offspring mass were assessed following different combinations of nutritional diet shifts. We predicted differences in fecundity and developmental parameters between the two diets, regardless of the *Wolbachia* infection status of the flies. Also, we hypothesized that the presence of *Wolbachia* would increase fly survival after a diet shift, especially if *Wolbachia* plays a nutritional mutualistic role in the context of polyphagy, as described above.

MATERIAL AND METHODS

Rearing of *D. suzukii* stocks

Drosophila suzukii flies originated from a field collection made on infested raspberries in Rennes, France (48°7'2.158"N, 1°40'40.053"W), in October 2020, by the ECOBIO Laboratory (Rennes). Five

hundred fruits from multiple plants were individually placed in vials for *D. suzukii* emergence. One hundred and ninety fly pairs were obtained and maintained separately on artificial diet (banana-based). In November 2020, nine lines infected by *Wolbachia* (*w*Suz) were detected using specific PCR assays with *Wolbachia*-specific primers targeting the *wsp* gene (81F/691R), following Braig et al. (1998). These infected lines were mixed in cages, and the population amplified. To obtain separate lines differing by the presence (W+) or absence (W–) of *Wolbachia*, half of the population was reared on a diet supplemented with tetracycline for four generations, as previously described by Hague et al. (2020). These two lines were used in the experiment. They were reared in 100 ml plastic bottles containing ~30 ml of standard cornmeal diet and incubated at 20°C, LD 12:12 h and 75% RH (STRADER, EV1300, Angers, France). At least 30 bottles of 50–200 flies per line were used for continuous maintenance. Before and after experiments, *Wolbachia* presence in the two lines was assessed by diagnostic PCR following the abovementioned method. To evaluate the impact of the tetracycline treatment on the other microbiota of the flies, we restored afterwards the microbiota of W– flies that have been raised for about 20 generation in the laboratory using W+ flies' faeces for three generations, as described by Strunov et al. (2022). Then we compared W– flies with or without microbiota restoration by performing a metabarcoding analysis with primers targeting the variable region V3-V4 of the 16S rRNA gene to characterize Bacteria diversity and ITS2 to assess the diversity of fungi (see Supplementary Material).

Experimental diets

We used two diets to analyze the influence of different nutritional substrates on *D. suzukii* development, a standard cornmeal diet (Corn) and an industrial diet made from grape (Grape). These two media were contrasted in their composition, both were artificial but the Grape diet included grape powder, a fruit that *D. suzukii* can use for its development (e.g., Shu et al. 2022). The flies were divided into two populations reared on each diet and maintained for several generations (~10, six months) before starting the experiment. A detailed analysis of macronutrients (carbohydrates, lipids and proteins) in each diet was performed as described by Foray et al. (2012). For analyses of amino acid, B vitamins and iron content, samples were sent to Eurofins©.

Effect of diet on *D. suzukii* development

Egg laying boxes were designed for the experiment: a hole was drilled in 60×45×50 mm crystal polystyrene boxes to receive a 6×30 mm 3D print egg laying cylinder containing 5 ml of diet. One female

and three males aged 24 h and collected randomly from the culture vials were placed in each laying box. There were four diet combinations for *Wolbachia*-infected and uninfected flies, resulting in eight treatments (Table 1). Each treatment had 25 replicates (i.e., 25 females). C/C and G/G treatments were used as control as they correspond to the routine rearing. A pinch of active yeast was dusted on each laying cylinder to stimulate egg laying (Plantamp et al. 2017). The laying boxes were maintained under standard culture conditions (see above). Every day over 30 days, eggs were counted on each laying cylinder under a stereomicroscope. The laying cylinder was replaced every 48 h and the one removed was placed on a Petri dish containing ~30 ml of diet to continue larval development. Developmental conditions were the same as for the rearing (see above). For each Petri dish, the numbers of developed pupae and the numbers of emerged flies were recorded daily. Egg-to-pupa, pupa-to-adult and egg-to-adult survival and development time were assessed. The emerging adults were counted and frozen at −20°C for later weighing. From each female and treatment, 120 emerging individuals were weighed on a laboratory precision balance (0.01 mg; QUINTIX125D-1S; Sartorius, Göttingen, Germany).

Table 1 Treatment codes corresponding to diet combinations for *Wolbachia*-infected and uninfected flies. N_i = initial sample size; N_f = final sample size after removing the data from females that died before the end of the experiment.

Code	Treatment	N_i	N_f
W+ C/C	*Wolbachia*-infected flies from corn culture placed on Corn	25	21
W+ G/C	*Wolbachia*-infected flies from grape culture placed on Corn	25	21
W+ G/G	*Wolbachia*-infected flies from grape culture placed on Grape	25	22
W+ C/G	*Wolbachia*-infected flies from corn culture placed on Grape	25	23
W− C/C	*Wolbachia*-uninfected flies from corn culture placed on Corn	25	19
W− G/C	*Wolbachia*-uninfected flies from grape culture placed on Corn	25	14
W− G/G	*Wolbachia*-uninfected flies from grape culture placed on Grape	25	22
W− C/G	*Wolbachia*-uninfected flies from corn culture placed on Grape	25	22

Statistical analyses

All data were analyzed in R version 4.1.2 (R Core Team 2022). Differences in macronutrient composition between diets (i.e., glucose, starch, proteins and lipids) were assessed using the Wilcoxon-Mann-Whitney test. Data from females that died before the end of the experiment were removed from the analyses (Table 1). The total numbers of eggs laid and numbers of offspring were compared among

treatments with generalized linear models using a Poisson distribution. The chosen explanatory variables were: the *Wolbachia* infectious status, the laying diet and the presence of a diet shift between the diet from which the female originated and the one it was made to oviposit on. Egg-to-pupa, pupa-to-adult and egg-to-adult survival, mean development time and offspring mass were compared using analysis of variance (ANOVA) after square root transformation to achieve normality and homoscedasticity of error variance. Offspring sex-ratio was also analysed with ANOVA. Where ANOVA was significant, significant differences between means were then identified using Tukey's HSD tests or the emmeans() function from the emmeans package.

RESULTS

Diet analyses

Macronutrient analysis indicated that the Grape diet was enriched in carbohydrates (more starch) compared to the Corn diet, but that both diets provided a similar amount of proteins and lipids (Table S1). Therefore, the Grape diet provided more energy than the Corn. Analyses of amino acid, B vitamins and iron contents revealed that both diets were comparable in these three compound groups (Table S2). Therefore we concluded that any differences found in fly performance between the diet treatments must be interpreted as a consequence of varying starch levels, as the protein:total carbohydrate ratios were similar between the two diets (P:C Corn = 0.011 ± 0.006; P:C Grape = 0.010 ± 0.005; $p = 0.84$).

Microbiota analysis

The microbiota consisted almost exclusively of *Acetobacter* for bacteria and *Saccharomyces* and *Wallemia* for fungi (Fig. 1, see Supplementary Material for more details). No significant difference was found between W– flies with or without microbiota restoration (Wilcoxon tests, see Supplementary Material for more details). Because this assessment is indirect, we still cannot totally exclude the confounding effect of microbiota. However, the absence of difference in the microbiota of W– flies (many generations after exposure to antibiotic) before and after exposure to W+ faeces strongly suggests that our W+ and W– lines shared the same microbiota. Moreover, the protocol to restore the microbiota of W– flies was used in previous studies and we are therefore confident that it provides adequate outputs. Therefore, we considered that the two lines W+ and W– share similar microbiota and that the results reported hereafter reflect the presence/absence of *Wolbachia*.

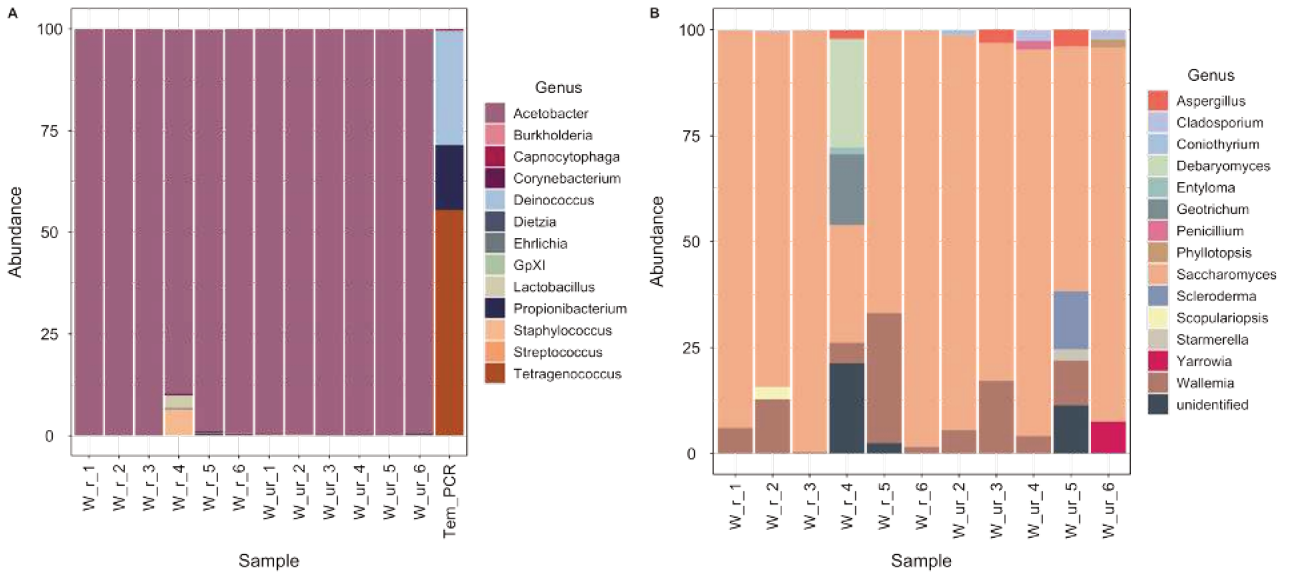

Fig.1 Metabarcoding analysis to characterize Bacteria (A) and fungi (B) diversity in *Drosophila suzukii*. W_r samples correspond to W– flies with microbiota restoration using W+ flies' faeces for three generations as described by Strunov et al. (2022) and W_ur to W– flies without microbiota restoration (see Supplementary Material for more details). Each column corresponds to one individual.

Effect of diet treatment and *Wolbachia* status on female fecundity

Overall, there was a large variation between females in the total numbers of eggs laid, from 18 to 568 eggs during the 30 days of the experiment. The total number of eggs laid by a female was significantly influenced by the laying diet, the diet shift, *Wolbachia* presence and the interaction terms (Table 2, Fig. 2A). Females laid significantly more eggs on Grape (mean ± sd: 214 ± 134 eggs) than on Corn (157 ± 96 eggs). When confronted to a diet shift, females from the Corn line laid more eggs on Grape than they did on Corn (263 ± 143 vs. 201 ± 111 eggs; $z = -32.4$, $p < 0.0001$) and females from the Grape line laid fewer eggs on Corn than they did on Grape (107 ± 38 vs. 163 ± 105 eggs; $z = 31.3$, $p < 0.0001$). In addition, W+ females laid fewer eggs than W–, although the difference was not significant for females from C/C treatment.

Effect of diet treatment and *Wolbachia* status on offspring survival

Wolbachia presence was the only variable to impact significantly the egg-to-adult survival (Table 2, Fig. 2B). Mean egg-to-adult survival was about 66 ± 14% and did not differ significantly between diet treatments. *Wolbachia* presence increased survival across diet conditions ($F = 39.2$, $p < 0.0001$), although this was not significant for the C/C treatment, with a mean increase of 17%. *Wolbachia* presence

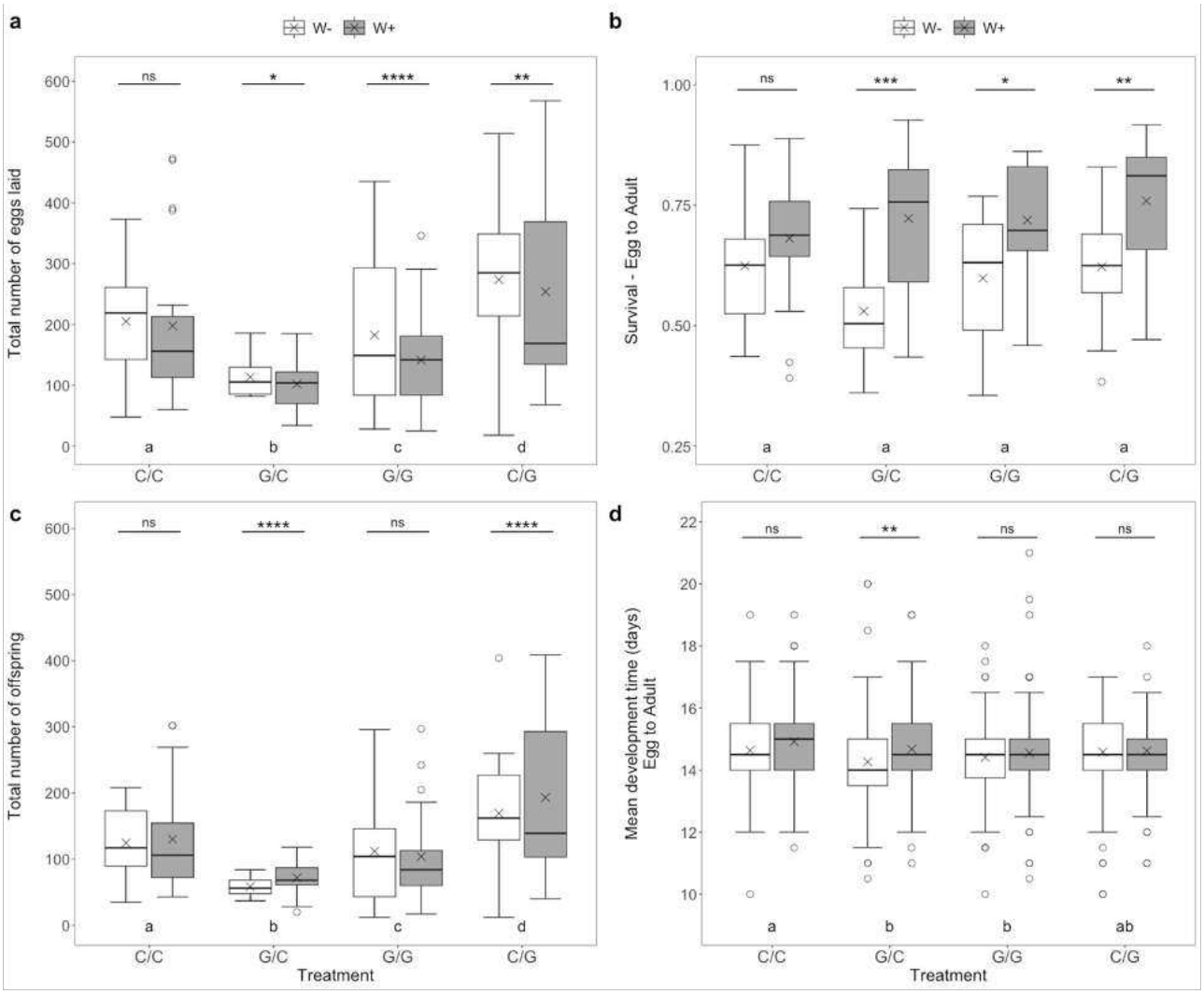

Fig. 2 Influence of diet treatment and *Wolbachia* infectious status (W+ or W-) on *Drosophila suzukii* reproductive parameters: (A) Total number of eggs laid in 30 d, (B) Egg to adult survival, (C) Total number of offspring per female (emerged adult flies), and (D) Mean development time from egg to adult emergence in days. The diet treatments were: flies from corn culture placed on corn diet (C/C), flies from grape culture placed on corn diet (G/C), flies from grape culture placed on grape diet (G/G), and flies from corn culture placed on grape diet (C/G). Significance of differences between diet treatments and *Wolbachia* infection status are represented by different letters (a, b, c, d; bottom of each panel) or by asterisks, respectively (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$, ns: non-significant; top of each panel)

primarily acted on larval survival (egg-to-pupa) and did not have a significant effect on pupal survival (Table S3, Fig. S1).

Overall, individual females produced between 12 and 409 surviving adult offspring over the experiment, with means of 145 ± 98 and 99 ± 60 offspring when laying on Grape and Corn respectively. The effect of diet treatment on the total number of offspring per female was the same as on the total

Table 2 Summary of results for generalized linear models for total number of eggs laid, egg-to-adult survival, total number of offspring, and egg-to-adult development time. Df = degree of freedom; Dev. = deviance; $P > |\chi^2|$ = *P*-value

	Total number of eggs laid			Egg-to-adult survival			Total number of offspring			Egg-to-adult development time		
Effect	**Df**	**Dev.**	**P > |χ²|**	**Df**	**Dev.**	**P > |χ²|**	**Df**	**Dev.**	**P > |χ²|**	**Df**	**Dev.**	**P > |χ²|**
Wolbachia (Wol)	1	136.71	**<0.0001**	1	0.605	**<0.0001**	1	9.05	**0.0026**	1	19.1	**0.0001**
Laying diet (LD)	1	670.46	**<0.0001**	1	0.041	0.105	1	723.91	**<0.0001**	1	5.5	**0.039**
Diet shift (S)	1	32.33	**<0.0001**	1	0.003	0.650	1	43.89	**<0.0001**	1	2.1	0.201
Wol × LD	1	0.20	0.651	1	0.001	0.763	1	1.70	0.192	1	6.2	**0.029**
Wol × S	1	0.07	0.791	1	0.045	0.090	1	13.92	**0.0002**	1	0.0	0.864
LD × S	1	2112.90	**<0.0001**	1	0.029	0.174	1	1526.86	**<0.0001**	1	21.5	**<0.0001**
Wol × LD × S	1	24.75	**<0.0001**	1	0.035	0.134	1	0.46	0.498	1	1.5	0.282

number of eggs laid (Table 2, Fig. 2C). A mean of 67 ± 23 offspring was produced in the G/C treatment, almost a third of those produced in the C/G treatment (182 ± 106 offspring). The numbers of offspring produced in treatments not involving a diet shift were 108 ± 75 and 127 ± 67 for G/G and C/C, respectively. *Wolbachia* had a significant effect only in the treatments involving a diet shift (C/G and G/C), where the numbers of offspring were increased by 14.1% and 23.1%, respectively.

Effect of diet treatment and *Wolbachia* status on developmental time

Across all treatments, the development time from egg to adult was 14.6 ± 1.1 days, with a minimum of 10 days and a maximum of 21 days. The laying diet, *Wolbachia* status and occurrence of a diet shift had an interactive effect on the development time (Table 2). The longest development time was observed on Corn when no diet shift occurred while the shortest times were observed on the G/C and G/G treatments (Fig. 2D). *Wolbachia* presence had a significant effect only on the G/C treatment, increasing the development time by almost half a day (W–: 14.3 ± 1.4 days, W+: 14.7 ± 1.2 days; F = 14.7674, p = 0.008). *Wolbachia* presence had no effect on egg-to-pupa development time (Table S4). The larval phase lasted about 8.2 ± 1.0 days and was significantly shorter in the G/C and G/G treatments, where larvae pupated from 3 to 7 hours earlier than in the other treatments (Fig. S2A). The pupal phase lasted 6.4 ± 0.8 days and even if *Wolbachia* presence had an overall effect on pupa-to-adult development time, the effect was not significant anymore within the diet treatment (Table S4, Fig. S2B). Diet treatment did not have a significant influence on pupa-to-adult development time (Table S4, Fig. S2B).

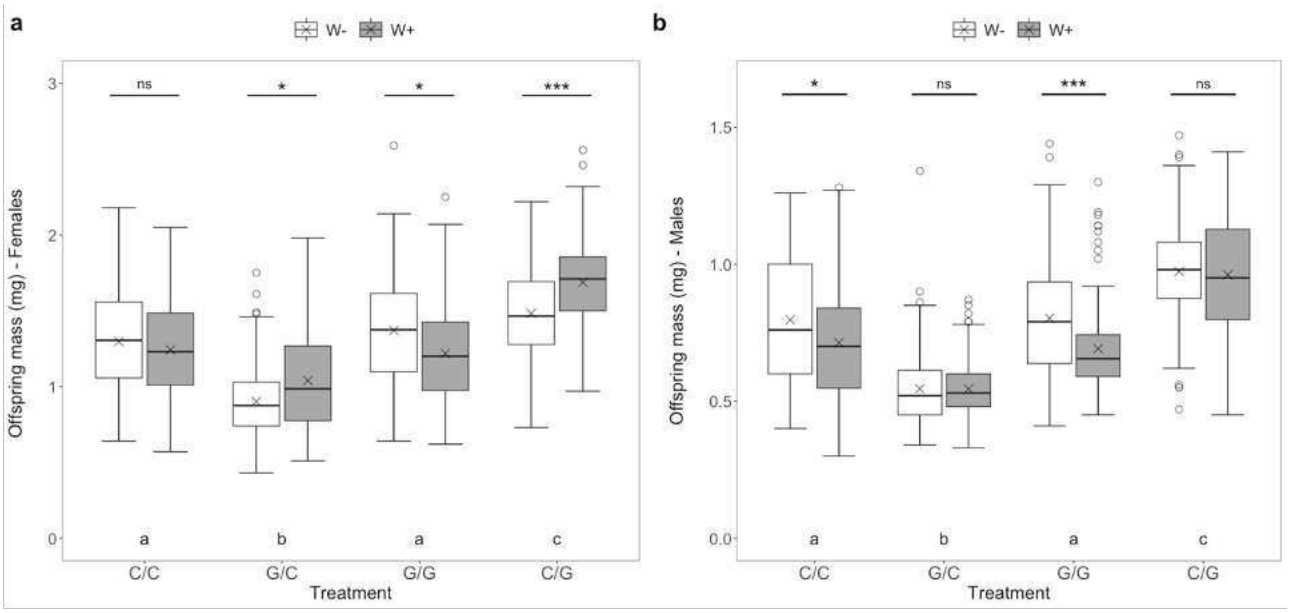

Fig. 3 Influence of diet treatment and *Wolbachia* presence (W+ or W-) on *Drosophila suzukii* offspring mass in mg with (A) females and (B) males. The treatments are the same as in Fig. 2. N = 120. Analysis of variance (ANOVA) was performed after square root transformation. Statistically significant differences are represented either by different letters (a, b, c, d; bottom of each panel) or by asterisks (*: $p < 0.05$, ***: $p < 0.001$; ns: non-significant; top of each panel)

Effect of diet treatment and *Wolbachia* status on offspring mass and sex-ratio

Overall, female offspring were almost twice as heavy as males, with a mean mass of 1.28 ± 0.40 mg vs. 0.75 ± 0.24 mg. The diet treatments had comparable effects on female and male mass, with the heaviest individuals obtained in the C/G treatment and the lightest ones from the G/C treatment. When there was no diet shift (C/C and G/G treatments), the adult masses were intermediate (Table 3, Fig. 3). *Wolbachia* presence had an effect on the mass of both sexes produced, but only when a diet shift occurred (Table 4). When there was no diet shift, the mass of W+ offspring was significantly lower than those of W– for both males and females, although this was not significant in females from the C/C treatment (Fig. 3). In contrast, under diet shifts, the offspring mass did not differ in relation to infection status for males (Fig. 3B), and the W+ offspring were significantly heavier than W– offspring for females (Fig. 3A).

The mean offspring sex-ratio (females/males) was 0.51 ± 0.06. *Wolbachia* had no effect on sex ratio (ANOVA, F = 1.099, $p = 0.2961$). The sex-ratio of offspring from G/C treatment was significantly higher (more females) than the sex-ratio of offspring from the G/G treatment (G/C: 0.53 ± 0.07, G/G: 0.49 ± 0.06, $p = 0.0258$). The sex-ratio did not differ between other treatments.

Table 3 Summary of results from generalized linear models for female and male offspring mass. For details, see legend of Table 3

	Offspring mass Female			Offspring mass Male		
Effect	**Df**	**Dev.**	***P*** **> |χ²|**	**Df**	**Dev.**	***P*** **> |χ²|**
Wolbachia (Wol)	1	0.048	0.132	1	0.93	**0.0001**
Laying diet (LD)	1	5.006	**<0.0001**	1	19.61	**<0.0001**
Diet shift (S)	1	0.015	0.409	1	0.09	0.238
Wol × LD	1	0.007	0.555	1	0.06	0.342
Wol × S	1	0.840	**<0.0001**	1	0.77	**0.0004**
LD × S	1	4.310	**<0.0001**	1	19.91	**<0.0001**
Wol × LD × S	1	0.046	0.142	1	0.00	0.896

DISCUSSION

Across their lifetime, polyphagous insects are exposed to various diets that may change across generations and phenology. Polyphagy at the population level is generally interpreted as an individual developing on a single plant species, yet being able to develop on several hosts (Singer 2008), so that the female has a range of host plants on which to oviposit. The larvae produced can thereby be exposed to diets varying in quality. Endosymbiotic partners may contribute to improve the performance of insects faced with such diet shifts, and could be especially critical for invasive species. Here, we show that the invasive fly *D. suzukii* performs better in terms of survival when it hosts the *Wolbachia* strain *w*Suz, in particular if it is faced with diet shifts. Hosting this endosymbiont may, however, also result in costs, since a negative effect was observed on other life history traits, such as fecundity or offspring mass. As the fly is an invasive species, and because it is present year-round in temperate regions, the influence of *Wolbachia* presence on its survival likely provides an overall advantage to *D. suzukii*. This effect may provide an important element explaining the maintenance of *w*Suz in natural populations and the success of this pest fly species worldwide. Caution is needed however when interpreting our findings, as they are specific to a single *Wolbachia* genotype and one *D. suzukii* population. To ensure broader applicability, it is essential to evaluate our results with various *D. suzukii* genotypes and *Wolbachia* strains, given that numerous studies has revealed that the influence of *Wolbachia* is contingent on both its own genotype and that of its host (Fry et al. 2004; Capobianco et al. 2018; Serga et al. 2021; Strunov et al. 2022).

Wolbachia presence and the tradeoff between fecundity and offspring survival

In all treatments, *Wolbachia*-infected females laid fewer eggs than non-infected ones, although this tendency was not significant for flies on Corn without diet shift. This apparent fitness cost was counterbalanced by the large increase seen in offspring survival, specifically larval survival, across all treatments, which resulted in almost perfect compensation in the number of offspring when the diet was stable, and even overcompensation (i.e., more surviving offspring in total) under diet shift treatments. This effect of *Wolbachia* on overall offspring survival contrasts with some previous studies. Hamm et al. (2014) reported a negative effect of *Wolbachia* presence on progeny production while Mazzetto et al. (2015) reported an increase of 30-50% in progeny production by *Wolbachia*-infected flies. However, the diet history of the flies was not taken into account in these studies. Mazzetto et al. (2015) reared flies on various organic fruits, then transferring them to a standard diet, while Hamm et al. (2014) collected flies from an organic raspberry field and then reared them on blueberry. For both, no antibiotic was used on the fruits they used as diet and they only had a few populations, which may have led to genetic confounding effects. In our study, larval survival included both hatch rate and direct larval survival, which means *Wolbachia* could have acted either on one parameter or the other or both. *Wolbachia* is already known to act on hatch rate but its effects are inconclusive. Saeed et al. (2018) showed that *Wolbachia* maintained a high egg hatch rate in *D. suzukii* even at increased temperature while in Cattel et al. (2018) infected flies had a lower hatch rate than uninfected flies.

Saeed et al. (2018) concluded that *Wolbachia* presence does not offer any fitness benefit linked to nutritional tolerance. However, their study mainly focused on survival of eggs and subsequent development time on a yeast-deprived diet, which may have been insufficient to detect any *Wolbachia*-associated benefit. Our results suggest that the influence of *Wolbachia* presence may differ depending on the diet history (i.e., the maintenance of a stable diet or occurrence of a diet shift). The increases we observed in offspring survival also provide support for the hypothesis that *Wolbachia* plays a mutualistic role for *D. suzukii.* Increasing host survival certainly is beneficial for the bacteria since this effect likely contributes to its higher prevalence in the population (Newton and Rice 2020).

The mechanisms at play in this nutritional mutualism were not addressed directly in this study. As in other insect-*Wolbachia* nutritional mutualisms (Nikoh et al. 2014), *Wolbachia* may produce and supply nutrients that contribute to *D. suzukii* fitness (Newton and Rice 2020). Indeed, studies of other insect species have confirmed that *Wolbachia* genomes can possess pathways for B vitamins, either

complete (e.g., riboflavin B2) or partial (e.g., thiamine B1, pyridoxine B6, biotin B7, and folate B9) (Nikoh et al. 2014), as well as pathways for nucleotide and heme syntheses (Newton and Rice 2020). For example, *Wolbachia* supplements biotin and riboflavin to bedbugs (Moriyama et al. 2015; Balvín et al. 2018) and planthoppers (Ju et al. 2020). The bacteria also influence iron metabolism in the parasitoid wasp *Asobara tabida*, the mosquito *Aedes aegypti* and the flies *Drosophila simulans* (Kremer et al. 2009) and *D. melanogaster* (Brownlie et al. 2009). Supplementation seems of particular importance for insect species that feed on single diets (e.g., blood, plant-sap, and grain), as some important nutrients (mostly amino acids and vitamins) are missing from these diets (Ju et al. 2020). Similar effects may also be useful for polyphagous insects developing using diets of varying quality. In our diets, riboflavin and tyrosine differed quantitatively, even if we cannot assess statistical significance. If *w*Suz have a role in riboflavin and tyrosine metabolism, we would have expected W– flies to struggle in Grape diet, resulting in a lower survival or mass. It was not the case (Fig. 2, 3), either because *w*Suz does not play a role in riboflavin or tyrosine metabolism, or because our diet was not lacking these nutrients and just had a reduced amount, limiting the potential beneficial impact of *Wolbachia*. The diets were suboptimal in their P:C ratio for *D. suzukii* (Silva-Soares et al. 2017; Young et al. 2018) and differed mostly in starch content (Table S1). The role of starch reserves, which likely determine the energetic status of flies, in this nutritional mutualism requires further investigation to identify the mechanisms involved.

The possible contributions of other bacteria, such as *Acetobacter*, to the observed differences also cannot at present be completely excluded. Acetic acid bacteria are important components of the microbiota associated with *Drosophila* (Winans et al. 2017; McMullen et al. 2021), and a role in *D. suzukii* gut response to diet modification has been suggested (Vacchini et al. 2016). Also, *Wolbachia* presence can itself modulate the relative titers of these bacteria, although the mechanisms involved are not known (Simhadri et al. 2017). We compared the microbiota of W– flies and W– flies with restored microbiota after being in contact with faeces of W+ flies, but some bacteria species may not be transferred by this mechanism and future research should explore these aspects. It is also known that cultivating insects for numerous generations on the same diet stabilizes the gut-microbiome (Sugio et al. 2015; Bing et al. 2018). Overall, we assume that the differences observed in this study are most likely directly linked with *Wolbachia* presence rather than other bacteria such as *Acetobacter*.

Finally, our data indicate that experiencing stressful conditions can increase the beneficial effects of *Wolbachia* presence. In the current study, the stress was induced by the diet shift, through exposure to a

new nutritional environment. In other studies on the effect of *Wolbachia* in a nutritional context, benefits are seen in nutrient-deprived situations. For example, *Wolbachia* presence increased *D. melanogaster* fecundity under iron-restricted diets (Brownlie et al. 2009). Similarly, *w*Cle conferred fitness advantages to the host *Cimex lectularius* when fed on B-vitamin-deprived food (Nikoh et al. 2014). Considering other microbes, Bing et al. (2018) showed that the general microbiota of *D. suzukii* is advantageous in the context of a nutrient-poor diet, while having no influence or even deleterious effects when the fly was cultivated on a nutrient-rich diet. Thermal stress is also known to have differential effects on *Wolbachia* and the host insect (Strunov et al. 2013; Saeed et al. 2018). As *Wolbachia* can be both mutualistic and parasitic with the same host (Zug and Hammerstein 2015), studies of its effects should include stressful conditions that can impact the entire holobiont.

Diet affects reproductive traits

As expected, fecundity was affected by the laying diet treatment applied. However, the direction of the effect observed was unexpected. When the diet was stable, the maximum number of laid eggs and surviving offspring were observed on Corn rather than Grape, with the latter containing grape powder, a fruit that *D. suzukii* can use for its development (e.g., Shu et al. 2022). *Drosophila suzukii* is known to favor a low protein diet on which to oviposit (Silva-Soares et al. 2017; Young et al. 2018). This is related to the particular behavior of *D. suzukii* of ovipositing in ripening and ripe fruit, stages at which the fruit reach their maximum sugar content (Burrack et al. 2013) and lowest protein content (Silva-Soares et al. 2017). Sugars subsequently decrease and proteins increase as the fruit starts decaying (Silva-Soares et al. 2017). The overall protein content of our two diets did not differ. Both diets had anti-microbial agent (Table S1), which could have led to low microbial proliferation and disturbance in microbiota between Corn and Grape, but it did not seem to influence our results. However, as with proteins, the sugar content influences the number of eggs laid (Burrack et al. 2013). Therefore, females may have relied on sugar concentrations to drive their reproductive behavior in our experiments, thereby preferring to oviposit on the Corn diet, which had lower sugars content even if it had the same P:C ratio of 1:10.

Another factor which may have influenced oviposition is the penetration force required by the diet. Burrack et al. (2013) found that the optimal force required by *D. suzukii* is around 30 cN, which corresponds to the Grape diet. Our direct observations of oviposition suggest that the penetration force required on the Corn diet may have been inferior and therefore suboptimal, even though the females laid more eggs on Corn. Conversely, Silva-Soaeres et al. (2017) reported no difference in the proportions of

eggs laid by females on diet patches differing in agar concentration. These observations might explain why, in our experiment, the difference observed between Corn and Grape diets was small, about 40 eggs and 19 offspring. In their study of *D. suzukii*, Olazcuaga et al. (2021) described positive fitness changes across generations when a population was exposed to a new diet after 12 generations and more. As our populations were reared on Grape for about ten generations, it is therefore possible that the differences we observed between Grape and Corn could disappear completely over a longer period.

Notably, diet shift had a strong directional effect on fecundity. Females originating from Grape that were forced to lay eggs on Corn laid the minimum amount of eggs while, in the inverse sequence, females originating from Corn and forced to lay eggs on Grape laid the maximum amount of eggs. In the short term, the Grape diet possible allowed better performance of *D. suzukii* than the Corn diet. In the current study we only tested two artificial diets, but Olazcuaga et al. (2021) used eight fruit and obtained similar results: depending on the diet on which the population were reared, fitness would increase or decrease depending on the new diet, but this effect would disappear after >12 generations on the same diet. In the natural environment, this feature has clear relevance to the multitrophic context in which *D. suzukii* develops. Depending on the context (i.e., availability of different food sources), the species' high polyphagy could either be an advantage or a disadvantage. For instance, after several generations laying eggs on the same fruit (e.g., during a fruit harvest season), females would have to find new fruit to oviposit on, either increasing or decreasing their abundance. This is a general pattern in polyphagous insect pests that depend on dynamic cropping systems (Kennedy and Storer 2000). It would also be of importance in temperate climates when fruit resources are scarce during winter and alternative resources provide nutritional support (Stockton et al. 2019).

Offspring survival was not affected by any diet treatment. The slight differences in diet composition may not have been sufficient to induce a difference in larval mortality (see Young et al. 2018). However, the diet treatment did affect surviving offspring mass and development time. When the diet was stable, offspring mass did not differ between Corn and Grape, though the development time in Corn was longer than in Grape. However, the differences in development time were small and may not have been sufficient to induce differences in offspring mass. These findings are in agreement with Silva-Soares et al. (2017), who found a faster development and larger offspring when *D. suzukii* was raised in intermediate protein concentrations, and also with Jaramillo et al. (2015), who reported faster development on a low sugar diet (blueberry) than on a sugar-rich diet (corn-molasses based) and no difference on phenotypic traits (e.g.,

wing length). Our results differ however from those of Young et al. (2018) who observed longer egg-to-adult development time and/or smaller adult mass when flies developed in high sugar environments. One possible explanation relies on egg density, which can lead to overcrowding. Overcrowding generally induces longer development time and smaller adult size (Da Silva et al. 2019) and can promote cannibalism in some insects (Richardson et al. 2010), including *D. suzukii* (Da Silva et al. 2019). We did not control for egg density, but the replacement of the diet every two days contributed to avoid reaching densities that can be detrimental. As seen in real fruit (Da Silva et al. 2019), when larval density is higher, thanks to the large quantity of resource in the experimental Petri dishes larvae pupated around the Petri dish edges in order to avoid competition.

When a diet shift was enforced the development time was shorter on Corn and offspring were lighter, while on Grape the development time was longer and offspring were heavier. Fellous and Xuéreb (2017) found the same pattern when comparing the larval performance of *D. suzukii* on diets differing in their protein / sugar concentrations, with the shortest larval development time on high protein, low sugar diets. On the other hand, Shu et al. (2022) found larval development time to be shorter and adult mass to be greater on high protein, low sugar diets. However, the latter study took place on actual fruits, which might have impacted larval development differently to artificial diets, especially because of the absence of antimicrobial agents (Young et al. 2018). Generally, it might be expected that, the longer the development time, the heavier the offspring will be as they can benefit from the diet over a longer period. However, this is not always the case as insects are also able to increase their foraging rate, becoming heavier in a shorter time (Da Silva et al. 2019). Faster development without effects on body size can also indicate a higher quality diet (Edgar 2006). In this study, the diet shift induced modifications in both development time and in offspring mass and it is plausible that the diet shift affected both the foraging rate of the larvae and their nutrient intake. Even if both Corn and Grape diets had the same P:C ratio and mainly differed in starch content, the slight differences in nutrients between them may have induced the same consequences observed in studies with diet differing in their P:C ratio. The role and even importance of starch in the performance of *Drosophila* flies remains undetermined and future studies should investigate its role in modulating fitness during diet changes.

CONCLUSIONS

In our study, *Wolbachia* presence improved the survival of *D. suzukii* in the earlier stages of development. The beneficial effects on offspring performance were particularly clear when females

experienced a diet shift. Our results unveil an unappreciated role of *Wolbachia* in polyphagous insects: the bacteria support survival of the fly and this effect is especially important during a diet shift. These findings suggest that *Wolbachia* could supplement the diet of its host when it is developing on a low-quality resource, but this hypothesis requires further investigation. Such a role could also explain the maintenance of the *w*Suz strain in *D. suzukii* globally. Depending on specific host fruit availability and quality, *Wolbachia* presence could be more or less advantageous to *D. suzukii*. *Wolbachia* presence is also associated with cost that can offset the benefits, such as costs related to the energy needed to host the endosymbiont (Clavé et al. 2022). Further studies are required to investigate how the cost-benefice balance is modulated in a multitrophic context, depending on the host plants. Abiotic variables (e.g., temperature, humidity) also need to be taken into account as it has been shown that the effect of *Wolbachia* can vary with temperature (Saeed et al. 2018). An integrative approach is needed to properly understand the role *Wolbachia* plays in polyphagous insects. If *Wolbachia* has a mutualistic role, its presence would benefit the fly during invasion, helping with establishment and maintenance of populations in heterogeneous environments. A better understanding of these dynamics is crucial to identify and apply efficient management strategies to limit the incidence of this invasive species on crops.

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SUPPLEMENTARY MATERIAL

Characterization method of the bacterial and fungi communities in *Wolbachia*-uninfected *D. suzukii* flies before and after microbiota restoration with *Wolbachia*-infected fly's faeces

Material and Methods

To restore W− flies' microbiota, we followed the method by Strunov et al. (2022). We restored the microbiota of the W− flies with W+ faeces to see if the microbiota can be enriched by species that are present in the W+ flies (and therefore faeces), indicating a possible effect of the antibiotic during the curing process of the lines. We did not test directly the difference between W+ flies and W− flies with restored microbiota because (i) we expected them to be the same after microbiota restoration and (ii) the over-representation of *Wolbachia* in W+ lines might hide the potential presence of other taxa in the PCR runs. Briefly, 15 W+ males were transferred in bottles containing fresh standard cornmeal medium to let them contaminate the medium with their faeces. Males were removed after 3 days and about 20 W− flies were transferred into each 'faeces contaminated' vial. After 5 days of egg laying, flies were removed. Emerged F1 W− flies were transferred into a new 'faeces contaminated vial'. These operations were repeated until F3 W− flies emerged. The microbiota was then considered restored.

To compare W− *D. suzukii* flies before and after the restoration of their microbiota with the faeces of W+ flies, six females from each treatment were placed in ethanol to eliminate their cuticular microbiota. Each female was then placed individually in 2 ml tubes with a 5 mm stainless steel bead. The tubes were left at −80°C for 15 min and then grinded in a tissue-lyzer (Retsch) at 20 Hz for 2 min. DNA from each sample was then extracted using Zymobiomics Magbead DNA Kit (Zymo). In each tube, 550 µL of lysis buffer was added and mixed well. This buffer was then transferred to the tubes provided in the kit containing the ZR Bashing Beads and vortexed for 40 minutes.

The sequencing library was prepared according to Illumina 16S Metagenomic Sequencing Library guidelines ('16S Metagenomic Sequencing Library Preparation', n°15044223 Rev.B) but using Invitrogen's Platinum II Hotstart PCR Mastermix. 16S rRNA gene was amplified with 16S V3-V4 gene primers 341F (5'-*TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CA*G CCT ACG GGN GGC WGC AG-3') and 805R (5'-*TCT CGT GGG CTC GGA GAT GTG TAT AAG AGA CA*G GAC TAC HVG GGT ATC TAA TCC-3') for bacteria, as previously described (Klindworth et al. 2013); and with ITS3-ITS4 primers 3F (5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GCA TCG ATG AAG AAC GCA GC-3') and 4R (5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GTC CTC CGC TTA TTG ATA TGC-3') for fungi, as

previously described (White et al. 1990). Primers were synthesized with overhang adapters (in italics) for index attachment and Illumina sequencing adapters. For each sample, consisting of one individual, triplicates were performed consisting of three PCR reactions containing 200nM of each primer, 12.5 µL Invitrogen Platinum II Hotstart PCR masterMix and 2µL of DNA template for 16S amplification or 4µL for ITS2 amplification, resulting in a final volume of 25 µL. Reaction conditions were 94°C for 2 min followed by 35 cycles of 98°C for 5 sec and 55°C for 15 sec. A negative PCR control was performed under the same conditions, except the addition of DNA. Each amplification product was checked on agarose gel to verify that only specific amplification was present. All replicates were then pooled by sample before purification. The second amplification step, adding multiplexing indices and Illumina sequencing adapters, was performed by Microsynth AG (Balgach, Switzerland), as well as PE sequencing of the library on Illumina NovaSeq instrument for 500 cycles (2 x 250 bp). The produced paired-end reads which passed Illumina's chastity filter were subject to de-multiplexing and trimming of Illumina adaptor residuals using Illumina's bcl2fastq software version v2.21. The quality of the reads was checked with the software FastQC version 0.11.9 and sequencing reads that fell below an average Q-score of 24 or had any uncalled bases (N) were removed from further analysis. The locus specific V34 and ITS2 primers were trimmed from the sequencing reads with the software Cutadapt v4.0 (Martin 2011). Paired-end reads were discarded if the primer could not be trimmed. Trimmed forward and reverse reads of each paired-end read were merged *in-silico* to reform the sequenced molecule using the software USEARCH version 11.0.667. Merged reads that contained ambiguous bases or were outliers regarding the expected amplicon size distribution were also discarded, although the sizes were allowed to be more flexible for the ITS2 system than for the V34 system. Samples that resulted in less than 5000 merged reads were discarded, to not distort the statistical analysis. The remaining merged reads were denoised using the UNOISE algorithm implemented in USEARCH to form zero-radius OTUs (zOTUs), also named amplicon sequence variants (ASVs), discarding singletons and chimeras in the process (Edgar 2010, 2016b). The resulting OTU abundance table was then filtered for possible barcode bleed-in contaminations using the UNCROSS algorithm (Edgar 2018). OUT sequences were compared to the reference sequences of the RDP 16S (Cole et al. 2014) and UNITE databases provided by https://www.drive5.com/usearch/manual/sintax_downloads.html, and taxonomies were predicted and confidences were calculated using the SINTAX algorithm implemented in USEARCH (Edgar 2016a). The metagenome was visualized via krona charts (Ondov et al. 2011). Alpha diversity was estimated using the Richness (Observed), Simpson and Shannon indices. Rarefaction analysis was carried out to estimate the coverage of the captured metagenome in contrast to the potential metagenome. Alpha diversity calculations and the

rarefaction analysis were performed with the R software packages phyloseq v1.44.0 (McMurdie & Holmes) and vegan v2.6-4.

Results

16S gene bacterial sequences. Between 91,464 and 177,598 raw reads were obtained per sample (mean ± sd: 128,509 ± 28,994). After filtering and trimming, the final number of merged pairs per sample ranged from 35,393 to 107,118 (mean ± sd: 73,670 ± 22,769). Taxonomic identification at the genus level was retained, as taxonomic assignment at the species level is unreliable (see details in the "16SOTUs_unfiltered" tab of the « sup_file1.xlsx » file). The vast majority of sequences belong to the genus *Acetobacter* (between 89.7 and 100% per sample, see details in tab « 16Sfiltered_Aggregated_taxonomy » of the file « sup_file1.xlsx »). Several alpha diversity indices were calculated (see details in the "16Salpha" tab of the « sup_file1.xlsx » file) and showed no significant difference between individuals with restored microbiota versus W− flies that were not exposed to faeces of W+ flies (Wilcoxon tests, $p = 0.96$).

ITS2 fungi sequences. Between 62,296 and 156,170 raw reads were obtained per sample (mean ± sd: 96,297 ± 25,853). After filtering and trimming, the final number of merged pairs per sample ranged from 8,768 to 65,740 (mean ± sd: 23,270 ± 14,415). One sample, designated W_ur_1, with an excessively low number of reads (2,035) was excluded from the dataset. In all samples, the majority of sequences belonged to the genus *Saccharomyces*, ranging from 27.8 to 99.5%. Note that sample B5E, with the lowest proportion of *Saccharomyces* sequences, is also the sample with the highest proportion (21.3%) of unassigned sequences (see details in tab « 16Sfiltered_Aggregated_taxonomy » of the file « sup_file1.xlsx »). A second genus, *Wallemia*, was present in all but one sample (W_ur_6). Its frequency was much lower, ranging from 1.6 to 30.6%. No significant differences were found between the two groups, with or without the microbiota restoration treatment (Wilcoxon tests, $p = 0.12$).

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File S1: Data about the bacterial and fungi communities of *Wolbachia*-uninfected *D. suzukii* flies before and after microbiota restoration with *Wolbachia*-infected fly's faeces

Sup Mat S2 contains six tabs:

16SOTUs_unfiltered: Unfiltered OTU table with raw reads number per sample per OTU, taxonomic assignment, confidence index in assignment by rank and FASTA sequences

16S_alpha: Alpha diversity indices calculated for each sample

16Sfiltered_Aggregated_taxonomy: Aggregate relative abundance by genus

ITSOTUs_unfiltered: unfiltered OTU table with raw reads number per sample per OTU, taxonomic assignment, confidence index in assignment by rank and fasta sequences

ITS_alpha: alpha diversity indices calculated for each sample

ITSfiltered_Aggregated_taxonomy: Aggregate relative abundance by genus

Table S1 Ingredients and macronutritional analysis of the two diets. Analyses were made as described by Foray et al. (2012). Statistical differences were assessed using the Wilcoxon-Mann-Whitney test. Bold: significant difference between the two diets. Notice that some p-values are close to significance (i.e., carbohydrates and energy), likely to be a function of the sample sizes (N = 5) used in the analyses.

	Corn diet	Grape diet	Unit	*P value*
Ingredients (/ 1 L)				
Yellow cornmeal	33	20	g	
Inactive dry yeast	17	20	g	
Agar	12		g	
Sucrose	50		g	
Grape Agar Powder FlyStuff®*		80	g	
Nipagin in 15 ml 95% ethanol	4	4	g	
Energy (/ 100 g)	58.82 ± 12.47	75.29 ± 11.21	Kcal	0.056
	246.10 ± 52.17	315.00 ± 46.90	Kj	0.056
Carbohydrates	13.19 ± 2.76	17.16 ± 2.31	g	0.056
Free sugars	8.76 ± 1.80	10.79 ± 1.81	g	0.095
Starch	4.43 ± 1.05	6.37 ± 1.17	g	**0.032**
Lipids	0.61 ± 0.14	0.66 ± 0.21	g	0.691
Proteins	0.15 ± 0.11	0.17 ± 0.09	g	0.841

*Ingredients: Agar, Grape Powder, Sucrose, Tegosept

Table S2 Amino acid and micronutrient analyses of the two diets. Analyses carried out by Eurofins©. One sample per diet was analysed and the numbers after "±" correspond to the level of uncertainty of the method used to quantify these nutrients. The difference was calculated when the difference in the amounts of nutrient between the two diets was higher than that level of uncertainty.

Quantity per 100 g	Corn diet	Grape diet	Unit	Difference
Amino acids				
Alanine	77.3 ± 11.0	79.7 ± 11.0	mg	
Arginine	66.4 ± 9.0	50.5 ± 7.0	mg	
Aspartic acid	88.1 ± 12.0	85.0 ± 12.0	mg	
Cystéine + Cystine	< 6	< 6	mg	
Glutamic acid	143.0 ± 20.0	132.0 ± 18.0	mg	
Glycine	42.9 ± 6.0	43.3 ± 6.0	mg	
Histidine	21.5 ± 3.0	24.5 ± 3.0	mg	
Hydroxyproline	< 200	< 200	mg	
Isoleucine	41.0 ± 6.0	39.4 ± 6.0	mg	
Leucine	85.7 ± 12.0	71.6 ± 10.0	mg	
Lysine	54.8 ± 8	59.0 ± 8.0	mg	
Methionine	< 24	< 24	mg	
Ornithine	< 50	< 50	mg	
Phénylalanine	46.7 ± 7.0	45.2 ± 6.0	mg	
Proline	53.7 ± 8.0	43.4 ± 6.0	mg	
Serine	51.3 ± 7.0	48.5 ± 7.0	mg	
Threonine	39.9 ± 6.0	39.5 ± 6.0	mg	
Tryptophan	10.4 ± 1.0	10.2 ± 1.0	mg	
Tyrosine	40.2 ± 6.0	28.6 ± 4.0	mg	29 %
Valine	51.9 ± 7.0	52.1 ± 7.0	mg	
Water-soluble vitamins				
B1 vitamin - thiamine	28.0 ± 4.0	30.0 ± 5.0	µg	
B2 vitamin - riboflavin	33.2 ± 5.0	22.9 ± 4.0	µg	31 %
B6 vitamin - pyridoxine	35.8 ± 5.0	33.8 ± 5.0	µg	
B9 vitamin - folates, folic acid	18.8 ± 5.6	17.1 ± 5.13	µg	
Trace elements				
Iron - Fe	390.0 ± 117.0	360.0 ± 108.0	µg	

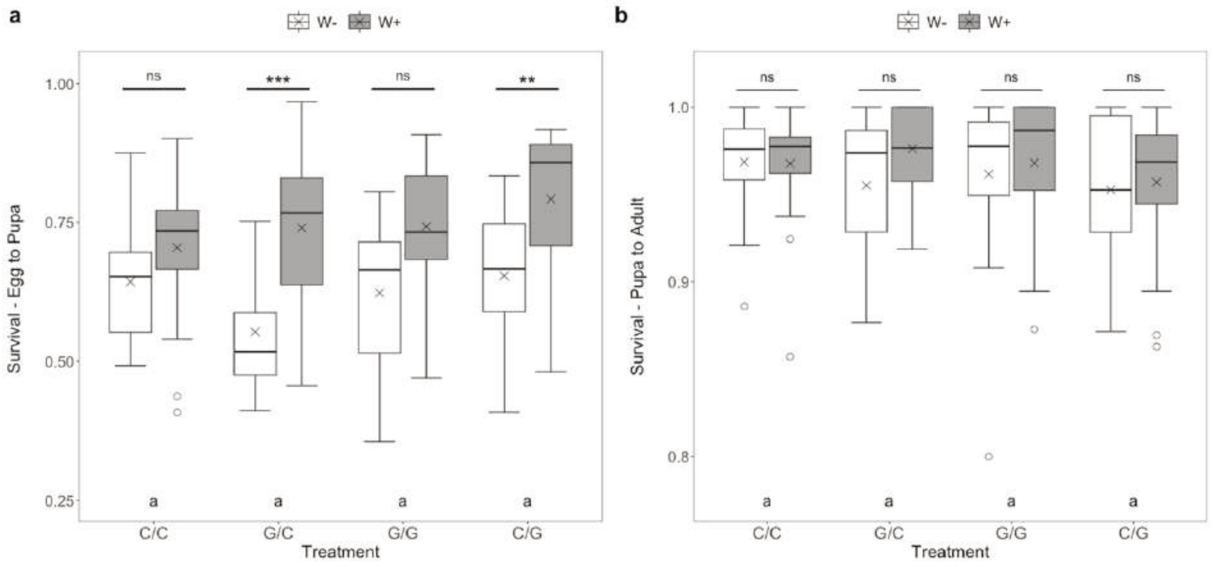

Fig. S1 Influence of diet treatment and *Wolbachia* infectious status (W+ or W-) on *Drosophila suzukii* survival with (A) Egg to pupa, and (B) Pupa to adult survival. The treatments are the same as in Fig 1. Analysis of variance (ANOVA) was performed after square root transformation. Statistically significant differences are represented either by different letters or by stars (**: $p < 0.01$, ***: $p < 0.001$; ns: non-significant)

Table S3 Summary of results for generalized linear models for egg-to-pupa and pupa-to-adult survival. Df = degree of freedom; Dev. = deviance; $P > |\chi^2|$ = P-value

	Survival Egg to pupa			**Survival Pupa to Adult**		
Effect	**Df**	**Dev.**	**P > |χ²|**	**Df**	**Dev.**	**P > |χ²|**
Wolbachia (Wol)	1	0.546	**<0.0001**	1	0.022	0.177
Laying diet (LD)	1	0.051	0.054	1	0.011	0.335
Diet shift (S)	1	0.012	0.343	1	0.004	0.540
Wol × LD	1	0.003	0.631	1	0.005	0.524
Wol × S	1	0.049	0.061	1	0.004	0.581
LD × S	1	0.031	0.132	1	0.029	0.122
Wol × LD × S	1	0.016	0.280	1	0.017	0.232

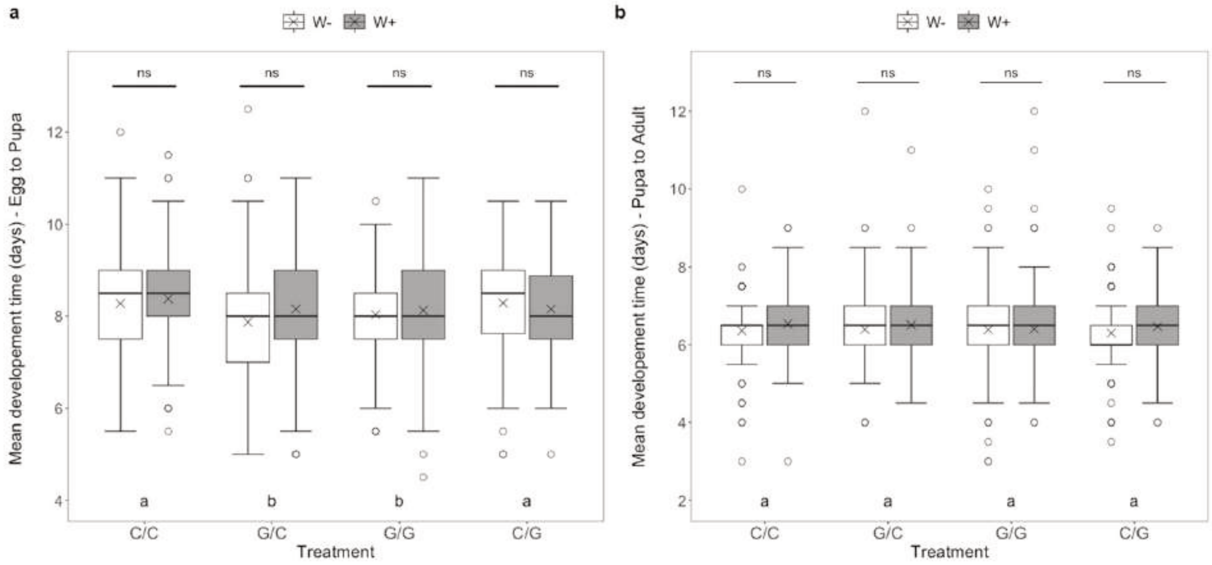

Fig. S2 Influence of diet treatment and *Wolbachia* infectious status (W+ or W-) on *Drosophila suzukii* mean development time in days with (A) Egg to pupa, and (B) Pupa to adult mean development time. The treatments are the same as in Fig. 1. Analysis of variance (ANOVA) was performed after square root transformation. Statistically significant differences are represented either by different letters or by stars (ns: non-significant)

Table S4 Summary of results for generalized linear models for egg-to-pupa and pupa-to-adult development time. Df = degree of freedom; Dev. = deviance; $P > |\chi^2|$ = P-value

	Development time Egg to pupa			**Development time Pupa to Adult**		
Effect	**Df**	**Dev.**	$P > \|\chi^2\|$	**Df**	**Dev.**	$P > \|\chi^2\|$
Wolbachia (Wol)	1	2.1	0.151	1	7.6	**0.0004**
Laying diet (LD)	1	1.0	0.324	1	1.8	0.088
Diet shift (S)	1	1.6	0.209	1	0.0	0.844
Wol × LD	1	3.8	0.052	1	0.4	0.447
Wol × S	1	0.5	0.483	1	0.3	0.477
LD × S	1	23.5	**<0.0001**	1	0.0	0.900
Wol × LD × S	1	5.2	**0.024**	1	1.4	0.130

Chapter 9

Trade-off between thermal and trophic resources

Chapter 9 • Objectives and main outcomes

This final chapter integrates the data obtained throughout my thesis. In turn, I explored the fruit resources used by *D. suzukii* from autumn to spring and the drivers of its infestation. I documented the effect of these fruit on *D. suzukii* fitness under winter-/spring-like temperature regimes. I, further, studied the response of *D. suzukii* to different thermal acclimation regimes, examining changes in its thermal preference relating to these temperatures and to its physiological status, and also the adaptive value of possessing such a plastic response to acclimation temperature. Finally, I explored the role of *Wolbachia* infection in facilitating both polyphagy and temperature acclimation in *D. suzukii*. In this chapter, I set out to integrate these findings and explore the extent to which females can make the 'correct' choice in terms of performance for their progeny, by presenting them with an interaction between temperature and nutritional quality. This chapter examines whether temperature, nutritional quality or both drive the choice of fruit in which to oviposit, and whether *Wolbachia* presence plays any influence in this choice.

Wolbachia-infected and uninfected flies and four fruit purees were used (*Mahonia japonica*, *Ribes nigrum*, *Rubus idaeus* and *Viscum album*), that were known to be suitable for *D. suzukii* and on which the performance was predicted to differ. First, *D. suzukii*'s performance was measured on each fruit in no-choice experiments. Then, diet- and temperature-based oviposition choices were evaluated. Finally, the existence of any trade-off between nutritional and temperature preference was investigated by presenting females with combinations of sub-optimal fruit at optimal temperature and *vice versa*.

The performance of *D. suzukii* differed depending on both the temperature and the fruit on which oviposition took place. Females did not always choose the best oviposition site in terms of nutrition but prioritised optimal temperature for larval development. *Wolbachia* presence enhanced fly performance in terms of emergence rate, development time and longevity, and also influenced oviposition choices.

This is the first study demonstrating that temperature is the primary criteria used to select oviposition sites, ahead of fruit nutritional quality. This study highlights some of the mechanisms underlying the invasive success of this insect, shaped by its thermal and nutritional preferences in selecting oviposition sites.

Contributions

Sylvain Pincebourde, Vincent Foray and I conceptualised the study.

Wendy Destierdt performed the experiment, analysed the data, produced the graphics and tables and wrote the original draft while she was under my supervision.

Evaluating the trade-off between thermal and food preferences in an invasive polyphagous insect to better understand its success

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ABSTRACT

Insects, as ectotherms, utilise microhabitats where the temperature can vary significantly compared to the surrounding air. In phytophagous insects, the oviposition site determines the microclimate to which the larvae are exposed, influencing their development. The selection of a microhabitat based on its temperature can interact with the choice of nutritional resources. In polyphagous insects, the behavioural thermoregulation is theoretically less constrained by food choice but the nutritional quality often varies between host plants. Consequently, an oviposition choice that is thermally optimal can be nutritionally suboptimal, and vice versa, particularly for larvae that cannot change their microhabitat. In this context, our study aimed to evaluate the existence of a trade-off between thermal and nutritional preferences in the choice of oviposition sites in an invasive polyphagous species, *Drosophila suzukii*, while also considering the influence of *Wolbachia* infection. We tested the performance of this species, from egg-to-adult, on four different fruit purees and analysed oviposition preferences based on the temperature and on the nutritional quality of these fruits, according to the 'mother knows best' hypothesis. The results showed that *D. suzukii* exhibited varying performance depending on the temperature and the substrate on which the eggs were laid. Females did not always choose the best oviposition site in terms of nutrition but prioritised optimal temperature for larval development, partially in line with the theory. The presence of *Wolbachia* enhanced performance in terms of emergence rate, development time and survival, and also influenced oviposition choices. This study highlights the mechanisms underlying the invasive success of this insect, shaped by its thermal and nutrition preferences in selecting oviposition sites.

Keywords: *Drosophila suzukii*, polyphagy, trade-off, mother knows best hypothesis, oviposition choice, *Wolbachia*.

INTRODUCTION

Insects, as ectotherms, depend directly on the surrounding ambient temperature (Woods *et al*., 2015). Temperature influences physiological functions (*e.g*., reproduction, development, metabolism, locomotion, survival) (Deutsch *et al*., 2008; Paaijmans *et al*., 2013; Sinclair *et al*., 2016) and, consequently, geographical distribution at large (Baffoe *et al*., 2012) and fine scales (Pincebourde and Woods *et al*., 2020). In phytophagous insects, the eggs are subjected to the thermal environment in which they are laid, as well as the subsequent larval stages (Dillon *et al*., 2009). To improve their fitness, females may choose to oviposit in microclimates where temperatures are favourable for their offspring (Feder *et al*., 2000). They should protect the eggs from extreme temperatures that could be deleterious or lethal and provide optimal conditions for their development. Females appear to influence the success of egg hatching and development by selecting oviposition sites with different temperatures (Stillwell and Fox, 2005). For instance, females of the apple leaf miner oviposit throughout the tree canopy to ensure that a portion of the offspring survive and develop (bet-hedging strategy; Hopper, 1999) (Pincebourde *et al*., 2007). The consequences of the female's choices on larval performance (*i.e*., development rate, survival) can be amplified when larvae are unable to move between microhabitats (Scheirs *et al*., 2000; García-Robledo *et al*., 2010; Poyet *et al*., 2015).

In polyphagous insects (*i.e*., generalist) that oviposit in fruit, the offspring's performance is not only influenced by the selection of the fruit microclimate but also by its nutritional quality (Janz, 2002). Polyphagy allows insects to exploit a wide range of hosts, reducing the time searching for food, giving a greater choice of enemy-free space and enabling the use of resources throughout the year (Ali and Agrawal, 2012; Poyet *et al*., 2015). Polyphagy increases the chances of survival and reproduction (Singer, 2008) but can be costly as the nutritional value varies between host plants (Janz, 2003; Poyet *et al*., 2015). Oviposition strategies involve complex trade-offs, including nutritional resource quality, the probability of finding a suitable resource, and the limited mobility of offspring (Scheirs *et al*., 2000; Janz, 2002; García-Robledo *et al*., 2010). This has led to two hypotheses regarding the influence of natural selection on female oviposition site preferences and larval performance: the 'mother knows best' and the 'optimal bad motherhood' (Mayhew, 2001). According to the former, females should select oviposition sites suitable for their offspring based on habitat quality (Scheirs *et al*., 2000; Mayhew, 2001). However, host plants that maximise larval development and survival do not necessarily maximise adult longevity. This situation can lead to parent-offspring conflicts (Scheirs *et al*., 2000), where the correlation between larval

and adult female preferences for a host plant can be weak (Olazcuaga *et al*., 2019) or non-existent (Martin *et al*., 2005). Consequently, females may sometimes ignore suitable plants (Wiklund, 1975), or choose to oviposit on less suitable ones for larvae, thereby compromising larval survival (Van den Berg, 2006). This observation arises from the 'optimal bad motherhood' hypothesis (Mayhew, 2001). However, studies based on these hypotheses only consider the nutritional aspect of the fruit, neglecting other abiotic factors such as temperature.

Biotic interactions can also improve the performance of polyphagous insects when faced with poor-quality host plants. Notably, close associations with microorganisms can enable insects to feed efficiently on a wide variety of plants (Sugio *et al*., 2015; Giron *et al*., 2018). They can broaden the range of suitable host plants for their hosts (Benhamou *et al*., 2021) by improving the host's ability to exploit suboptimal diets, providing digestive enzymes, producing vitamins, detoxifying plant defence compounds (Dillon and Dillon, 2004; Engel and Moran, 2013; Ashra and Nair, 2022). Among these associations, interactions with bacteria of the genus *Wolbachia* are one of the most recognized and widespread among insects (Bourtzis, 2008; Zug and Hammerstein, 2015). Substantial evidence shows that some *Wolbachia* strains can be beneficial for insects as either facultative (Teixeira *et al*., 2008) or obligate mutualists (Hosokawa *et al*., 2010), including nutritional benefits (Hosokawa *et al*., 2010; Zug and Hammerstein, 2015; Lindsey *et al*., 2023).

The interplay between temperature, nutrition and symbiont infection to shape oviposition choice has never been studied. *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) provides a good model to tackle this challenge, as a polyphagous species (Poyet *et al*., 2015; Kenis *et al*., 2016) that shows important behavioural thermoregulation abilities (Deconninck et *al*., *in prep* - Chapter 5) and is infected by *Wolbachia* (Cordaux *et al*., 2008). This fly is native to South-East Asia (Walsh *et al*., 2011) and became invasive in Europe and North America in the late 2000s, and more recently, in Africa (Walsh *et al*., 2011; Asplen *et al*., 2015; Kwadha *et al*., 2021). Unlike most *Drosophila* species that typically oviposit on overripe or decaying fruits, *D. suzukii* females have a serrated ovipositor allowing them to oviposit in fresh and ripening fruits, providing a considerable adaptive advantage and a major challenge for fruit growers (Atallah *et al*., 2014; Lee *et al*., 2015; Poyet *et al*., 2015). It causes substantial economic losses in the agriculture sector, particularly affecting a wide range of cultivated (Lee *et al*., 2015) and wild fruits (Poyet *et al*., 2015; Kenis *et al*., 2016). The broad dietary spectrum of *D. suzukii*, along with the extensive variety of host plants available year-round, ensures a constant supply of resources contributing to its persistence

and invasive success (Poyet *et al*., 2015; Olazcuaga *et al*., 2021; Deconninck *et al*., 2024a,b,c - Chapters 1, 2, 3). However, its polyphagous nature exposes the fly to diet varying in quality, which results in various effects on its performance (Poyet *et al*., 2015; Olazcuaga *et al*., 2019).

In terms of thermal adaptability, *D. suzukii* can thrive across a wide range of temperatures (Raynaud-Berton *et al.*, 2024), allowing it to sustain viable populations year-round (Rossi-Stacconi *et al*., 2016; Santoiemma *et al*., 2019b; Delbac *et al*., 2020). The species exhibits resilience to climatic variations by enduring low temperatures in winter and achieving high rates of development and reproduction during warmer months (Kirschbaum *et al*., 2020; Little *et al*., 2020). The *Wolbachia* strain *w*Suz was reported in *D. suzukii* by Cordaux *et al*. (2008) and influences its performance. Benefits included increased female fecundity (Mazzetto *et al.,* 2015), enhanced thermal tolerance (Saeed *et al*., 2018) and improved fitness after a diet shift (Deconninck *et al*., 2024d - Chapter 8). To date, no studies have yet explored how *D. suzukii* choose oviposition sites under varying nutritional and temperature conditions, nor have they examined if *w*Suz influences this choice.

This study aims to evaluate the oviposition choice of females *D. suzukii* when faced with varying food resources and temperature regimes, and to determine if *Wolbachia* influences this choice. We used two fly lines sharing the same genetic background but differing in the presence or absence of *Wolbachia*. Four fruit purees were tested: two winter fruits (*i.e*., *Viscum album* and *Mahonia japonica*) and two summer fruits (*i.e*., *Ribes nigrum* and *Rubus idaeus*), that were known to be used by *D. suzukii* and on which the performance was expected to differ (Poyet *et al*., 2015; Kenis *et al*., 2016; Olazcuaga *et al*., 2019, 2021). To assess the existence of a trade-off between thermal and trophic choices, we followed four steps (Fig. 1). The first step aimed at assessing *D. suzukii*'s performance on each fruit in no-choice experiments. We predicted differences in performance (*i.e*., fecundity, development time, survival, mass) between the four fruits puree. Specifically, we expected higher egg-laying on *Ribes nigrum* compared to other fruits (Olazcuaga *et al*., 2019), but better overall performance in *Rubus idaeus* and poorer performance on *Ribes nigrum* (Olazcuaga *et al*., 2019, 2021). The second step evaluated diet-based oviposition choices and the influence of ambient temperature on fruit choice. We hypothesised that females choose oviposition sites based on optimal nutritional value whatever the ambient temperature, favouring *Rubus idaeus*, in line with the 'mother knows best' hypothesis (Mayhew, 2001). The third step investigated temperature-based oviposition choices on each fruit. We predicted that females select oviposition sites based on the most favourable temperature for larval development, with a preference for warmer temperatures (Raynaud-

Berton *et al*., 2024). The final step investigated the existence of the trade-off by confronting females to a combination of sub-optimal fruit at optimal temperature and *vice versa*. A trade-off between fruit and temperature was expected, with either the optimal fruit or temperature favoured by the female. For all these steps, we hypothesised that the presence of *Wolbachia* could influence both performance (Deconninck *et al*., 2024d - Chapter 8) and the females' oviposition choices.

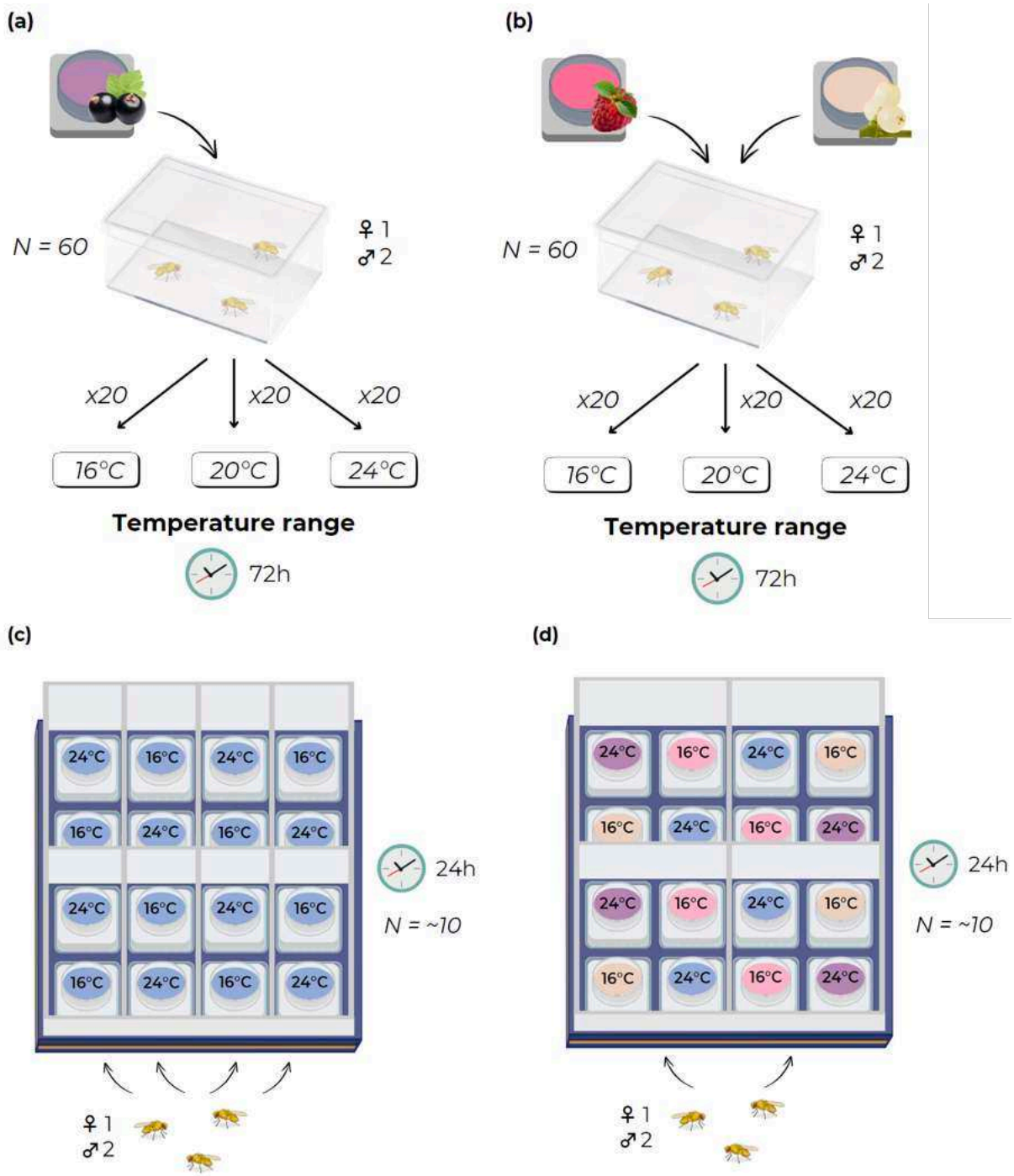

Fig. 1 Experimental design for (a) 'Step 1: Performance in no choice experiments', (b) 'Step 2: Diet-based oviposition choice', (c) 'Step 3: Temperature-based oviposition choice', and (d) 'Step 4: Trade-off between temperature and diet-based oviposition choices'.

MATERIALS AND METHODS

Field sampling and laboratory maintenance

Drosophila suzukii flies' stock was the same as in Deconninck *et al.* (2024d - Chapter 8). Briefly, they were collected from infested raspberries in 2020 in Rennes, France (48°7′2.158″N, 1°40′40.053″W) by ECOBIO Laboratory (Rennes). Two lines differing by the presence (W+) or absence (W−) of *Wolbachia* were obtained with tetracycline treatment (method by Hague *et al.*, 2020). The W− fly microbiota was restored to reduce confounding effects from the antibiotic treatment (method by Strunov *et al*., 2022). Before and after experiments, *Wolbachia* presence in the two lines was assessed by diagnostic PCR using specific PCR assays with *Wolbachia*-specific primers targeting the wsp gene (81F/691R) (method by Braig *et al.*, 1998). The flies were reared in 100 ml plastic bottles containing ~30 ml of standard cornmeal diet and incubated at 20°C, with 12:12 h light-dark cycle, and 75% relative humidity (STRADER, EV1300, Angers, France). At least 30 bottles with 50–200 flies per line were used for continuous maintenance.

Artificial fruit media

The influence of fruit on *D. suzukii* performance was evaluated using fresh media made from four different fruits. We used fruit purees instead of whole fruits to isolate the effects of fruit nutritional composition on preference and performance from variables such as skin thickness, shape, colour and size. These fruits ripen at various times of the year, so using purees allowed us to test them concurrently in the laboratory. The purees were made from ripe fruits collected in the wild or originating from growers: *V. album* and *M. japonica* were collected in Tours, France (47°23′24.171″N, 0°41′20.136″E), *Ribes nigrum* originated from Ajou, France (Le Domaine du Framboisier, 48°58′44.196″N, 0°46′57.67″E) and organic *Rubus idaeus* fruits originated from Serbia and were bought from Maison Thiriet. The fruit puree was prepared using a recipe adapted from Olazcuaga *et al*. (2019): each fruit (600 g) was mixed with agar (15 g) dissolved in warm water (1 L), inactive yeast (65 g), and nipagin (4 g) dissolved in 70% ethanol (10 ml). Mistletoe purees were coloured in pink with red food colourant to minimise the influence of colour on egg-laying choice (Deconninck *et al*., 2024d - Chapter 8). To preserve their flavours and colours, the purees were stored at 4°C until use, the following day.

Thermal landscape

To study the thermal and food preferences for oviposition sites by females, we developed a 'thermal landscape' system (TLS; **Fig. 2**). It consisted of 16 aluminium plates that were warmed (24°C) or cooled (16°C) independently by Peltier elements and on which 3D-printed egg-laying cylinders containing fruit puree were placed. The temperature selection was made using temperature controllers. A water-cooling system ran continuously below the Peltier elements to prevent overheating of the system. Arenas enclosing two or four plates were created with separation grids and covered with mesh to isolate replicates and allowed the flies to move freely.

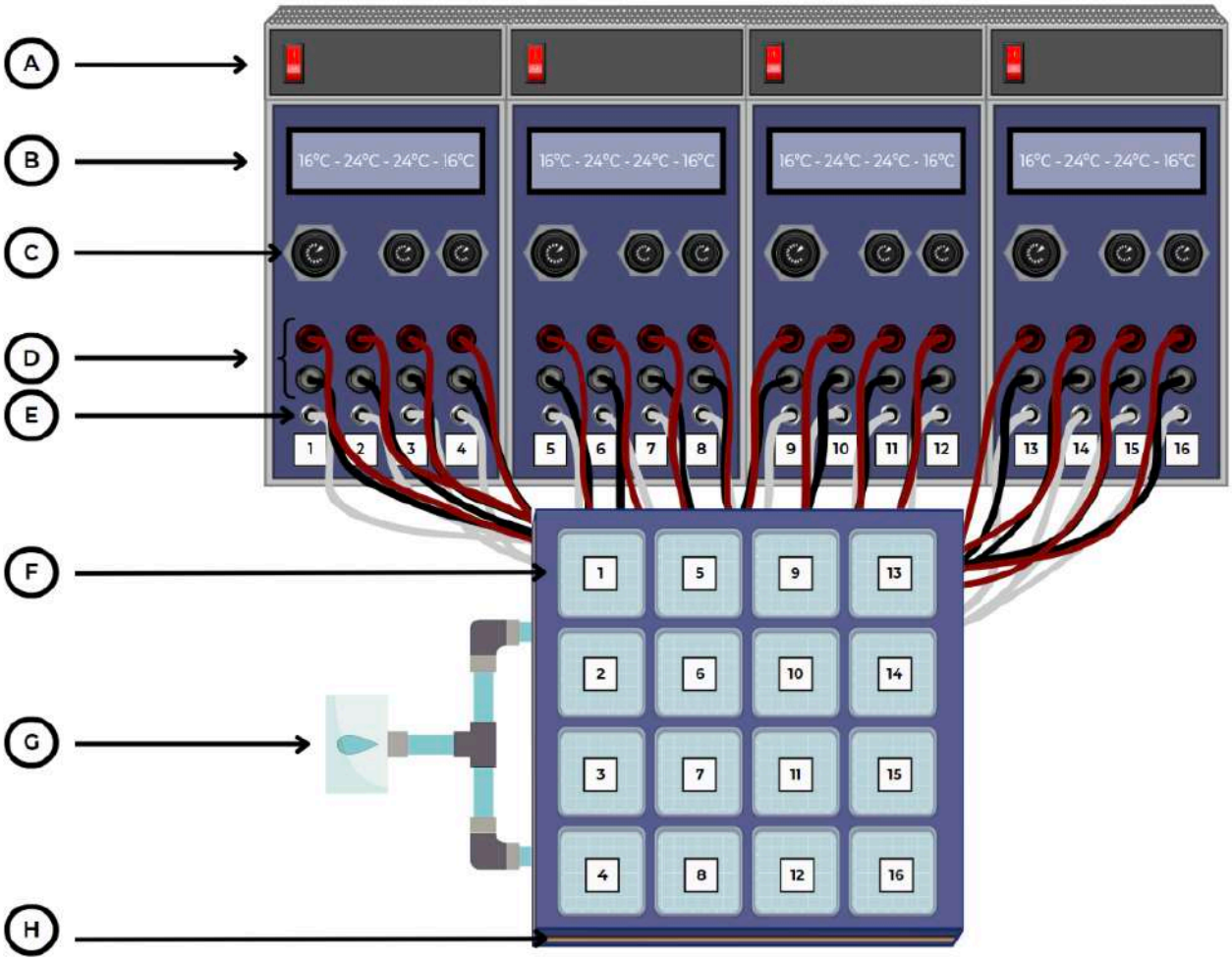

Fig. 2 Thermal landscape system (TLS) that allows the measurement of the trophic and thermal preference of females. Numbers from 1 to 16 indicate the number of Peltiers and aluminium plates associated with each temperature controller. Letters from A to G represent the different elements of the thermal landscape: A. Power supply, B. Temperature controllers, C. Setting buttons, D. Electric cables supplying power to each Peltier, E. Temperature probes, F. Aluminium plates, G. Water-cooling system, H. Peltier.

Prior to the experiment, temperatures were calibrated using egg-laying cylinders filled with fruit puree and thermocouples placed within each Peltier module (see **Fig. S1**). Absorbent paper was placed in each egg-laying cylinder to prevent condensation. The tested temperatures (16 and 24°C) were selected because they offer an important contrast in development time (Raynaud-Berton *et al*., 2024), so that we could expect an influence on the female oviposition choice.

Step 1: Performance in no choice experiments

This step aimed to evaluate the performance (*i.e.*, fecundity, development time, survival, mass) of *D. suzukii* on each fruit puree in no choice environment. We tested both W+ and W– flies across the four fruit purees (*M. japonica*, *R. nigrum*, *R. idaeus* and *V. album*) and three temperature regimes (16, 20 and 24°C), resulting in twenty-four combinations. Egg laying boxes were prepared for the experiments. Each box, made of crystal polystyrene and measuring 60x45x50 mm, was drilled to accommodate a 6x30 mm 3D-printed egg laying cylinder containing 5 ml of fruit puree. In each box, one female and two males, aged between 10 and 15 days, were introduced. Each treatment had 20 replicates, with each replicate consisting of one female. The boxes were incubated at 16, 20 or 24°C with a 12:12 h light-dark cycle and 75% relative humidity (STRADER, EV1300, Angers, France). After three days, eggs were counted under a stereomicroscope. Developmental progress was monitored daily, tracking the number of pupae and emerging adult flies. Survival rates and developmental times were calculated for each stage: egg-to-pupa, pupa-to-adult and egg-to-adult. Upon emergence, adult flies were sexed and weighed using a laboratory precision balance (0.01 mg; QUINTIX125D-1S; Sartorius, Göttingen, Germany).

Step 2: Diet-based oviposition choice

This step aimed to assess the female's nutritional choice for oviposition and was based on offspring performance in the previous step. The oviposition preference of W– and W+ females was tested in polystyrene boxes (90x60x50 mm) with two egg-laying cylinders containing 5 ml of fruit purees: either winter fruits (*i.e.*, *V. album* vs *M. japonica*), summer fruits (*i.e.*, *R. idaeus* vs *R. nigrum*) or one winter and one summer fruit in which *D. suzukii* performed best (*i.e.*, *Viscum album* vs *Rubus idaeus*). The boxes were randomly placed within climatic chambers set at 16, 20 or 24°C with a 12:12 h light-dark cycle and 75% relative humidity (STRADER, EV1300, Angers, France). We tested both W+ and W– flies. Each treatment had 20 replicates, with each replicate consisting of one female. Oviposition preference was measured after 3 days by counting the number of eggs laid in each cylinder under a stereomicroscope.

Step 3: Temperature-based oviposition choice

This step aimed to investigate the influence of temperature on *D. suzukii* oviposition preferences using a single fruit puree (*i.e.*, temperature choice but no nutritional choice). 3D-printed egg-laying cylinders containing 5 ml of fruit puree from a single fruit (*i.e.*, *M. japonica*, *R. ideaus*, *R. nigrum* or *V. album*) were placed on each module of the TLS. The TLS was divided into eight sections, with each section exposing one female and two males of standard-reared flies to two fruit purees maintained at 16°C and 24°C, for 24 hours. After 24 hours, the flies were removed and the number of eggs laid in each fruit puree counted. We tested both W– and W+ flies. Each treatment had at least 10 replicates, with each replicate consisting of one female.

Step 4: Trade-off between temperature and diet-based oviposition choices

This step aimed to investigate the existence of a trade-off in the oviposition choice. We assessed the influence of temperature and diet on *D. suzukii* oviposition preferences using the four distinct fruit purees together. The TLS was divided into four sections, each section exposing one female and two males of standard-reared flies to four egg-laying cylinders. Among the four egg-laying cylinders, the nutritionally-optimal diets, *V. album* and *R. idaeus* purees, were maintained at suboptimal temperature (16°C) while the suboptimal diets, *M. japonica* and *R. nigrum* purees, were maintained at optimal temperature (24°C). This set-up was based on offspring performance in the previous steps. After 24 hours, the flies were removed and the number of eggs laid in each fruit puree / temperature compartment was counted. We tested both W+ and W– flies. Each treatment comprised 20 replicates, with each replicate consisting of one female.

Statistical analyses

All statistical analyses were performed using R version 4.1.3 (R Core Team 2024). The explanatory variables included the fruit puree, the temperature and *Wolbachia* infectious status. The total numbers of eggs laid was compared among treatments using generalised linear models (GLM) with a Quasi-Poisson distribution. Mean development time, survival between each stage (*i.e.*, egg-to-pupa, pupa-to-adult and egg-to-adult) and offspring mass were analysed using analysis of variance (ANOVA). When ANOVA was significant, differences between means were identified using Tukey's HSD tests. Before analysis, a log transformation was applied to achieve normality and homoscedasticity of error variance for mean development time. Data on female oviposition preference between two fruit purees, data on the influence

of temperature on females' oviposition preference using a single fruit puree, and data on the influence of temperature and diet on female oviposition preferences using the four distinct fruit purees were transformed into proportions and analysed using chi-square tests. Pairwise comparisons were conducted with a pairwise chi-square test to pinpoint specific differences between diets, providing insight into which diet preferences varied significantly across test conditions.

RESULTS

Step 1: Performance in no choice experiments

Fecundity - The total number of eggs laid per female was influenced by fruit puree ($p < 0.0001$), temperature ($p < 0.0001$), *Wolbachia* infection ($p < 0.0001$) and their interactions (Fig. 3a, Table S1a). The total number of eggs laid per female increased with temperature, except on *M. japonica*. At 16°C, the number of eggs laid per female was higher on *V. album* and *M. japonica* than on *R. nigrum* and *R. idaeus*. At 20°C, the number of eggs laid on *V. album* was higher than on *M. japonica*, *R. nigrum* and *R. idaeus*. The number of eggs laid was more contrasted at 24°C. The number of eggs laid on *V. album* (23.00 ± 14.32) was higher than at 16°C and 20°C, *R. nigrum* and *R. idaeus* were intermediate (15.20 ± 12.19; 9.50 ± 8.50) and *M. japonica* had fewer eggs (6.20 ± 5.15). Overall, W– females laid more eggs compared to W+ females (7.79 ± 9.29 and 5.43 ± 6.94, respectively; $p < 0.0001$; Fig. S3a, Table S1a), although it depended on temperature and fruit puree. For instance, at 16°C, the mean number of eggs laid by W– females on *M. japonica* was six times higher than the number laid by W+ females (W–: 6.35 ± 5.36; W+: 1.65 ± 1.90; $p = 0.0015$). For *V. album*, at 20°C, the number of eggs laid by W– females were twice as high as the number laid by W+ females (W–: 12.55 ± 8.09; W+: 5.10 ± 4.06; $p = 0.0004$) and one and a half time higher at 24°C (W–: 23.00 ± 14.32 and W+: 14.15 ± 9.77; $p = 0.0030$).

Survival - Egg-to-adult survival (*i.e.*, emergence rate) of offspring was influenced by fruit puree ($p < 0.0001$), temperature ($p < 0.0001$), *Wolbachia* infection ($p < 0.0001$) and their interaction (Fig. 3b, Table S1b). Regarding temperature, *D. suzukii* emergence rates were globally higher at 24°C compared to 16°C ($p = 0.0017$), but similar between 20 and 24°C ($p = 0.8442$). Emergence rates also varied according to fruit puree. *Drosophila suzukii* had lower survival rates at any temperatures on *R. nigrum* compared to the other fruit purees, except at 16°C where survival rates were also low in *M. japonica*. In addition, W+ offspring had a higher emergence rate compared to W– offspring (0.73 ± 0.45 and 0.61 ± 0.49, respectively; $p < 0.0001$), depending on both temperature and fruit puree (Fig. 3b, Table S1b). For

example, the presence of *Wolbachia* in W+ individuals that developed on *R. nigrum* at 24°C showed a drastic increase in emergence rate compared to W– individuals (W–: 0.34 ± 0.48; W+: 0.80 ± 0.40; $p <$

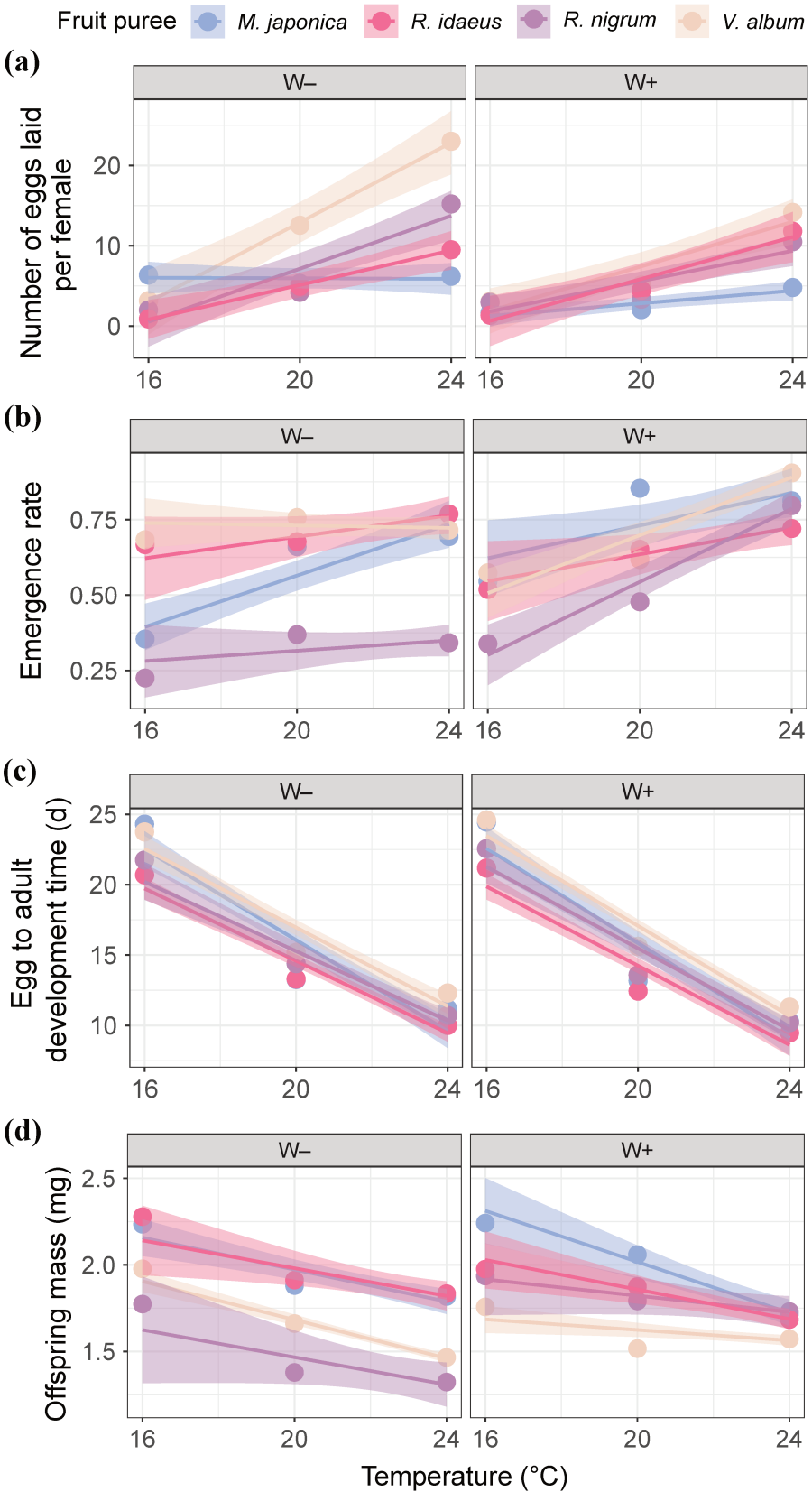

Fig. 3 Performance of *D. suzukii* (Step 1). (a) Mean number of eggs laid, (b) Emergence rate, (c) Egg-to-adult mean development time and (d) Offspring mass (mg) on each fruit puree (*i.e.*, *V. album*, *M. japonica*, *R. nigrum*, *R. idaeus*) and at each temperature (*i.e.*, 16, 20 and 24°C) for *Wolbachia*-infected (W+) and -uninfected individuals (W–). (a: GLM with Quasi-Poisson distribution; b,c,d: ANOVA).

0.0001). Similar results were obtained on *M. japonica* at 16°C (W–: 0.35 ± 0.48; W+: 0.55 ± 0.51; p = 0.0481).

Development time - Egg-to-adult mean development time was influenced by fruit puree (p < 0.0001), temperature (p < 0.0001), *Wolbachia* infection (p = 0.0019) and their interaction (Fig. 3c, Table S1c). Overall, the mean development time of *D. suzukii* decreased with temperature (p < 0.0001). At all temperatures, the mean development time was the longest on *V. album*, followed by *M. japonica*, while it was the shortest on both *R. nigrum* and *R. idaeus*. Finally, W+ individuals exhibited a shorter mean development time compared to W– individuals (14.52 ± 5.31 and 15.03 ± 4.98 d, respectively; p = 0.0019) depending on temperature and fruit purees (Fig. 3c, Table S1c). For instance, at 20°C, W+ individuals exhibited a development time one day shorter compared to W– individuals on *R. idaeus* (W–: 13.30 ± 1.36 d; W+: 12.44 ± 1.01 d; p = 0.0418), *V. album* (W–: 12.31 ± 0.79 d; W+: 11.30 ± 0.62 d; p = 0.0037) and *M. japonica* (W–: 11.18 ± 0.95 d; W+: 10.28 ± 1.02 d; p = 0.0066) at 24°C.

Mass - Offspring mass was influenced by fruit puree (p < 0.0001), temperature (p < 0.0001), *Wolbachia* infection (p < 0.0001), sex (p < 0.0001) and their interactions (Fig. 3d, Table S1d). Females exhibited a higher mass than males (1.65 ± 0.37 and 1.32 ± 0.29 mg, respectively; p < 0.0001), depending on temperature and fruit puree, so only the data for females were retained for the analysis. The mass of individuals decreased with temperature (16°C: 2.11 ± 0.30 mg; 20°C: 1.72 ± 0.30 mg; 24°C: 1.56 ± 0.35 mg; p < 0.0001), depending on fruit puree. At 24°C, the heaviest individuals developed on *M. japonica* and *R. idaeus* (1.82 ± 0.31 and 1.83 ± 0.40 mg, respectively; p = 0.9868), while the lightest developed on *V. album* and *R. nigrum* (1.47 ± 0.20 and 1.32 ± 0.47 mg, respectively; p = 0.0082). Finally, there was no overall significant difference in mass between W+ and W– individuals (1.69 ± 0.32 and 1.65 ± 0.37 mg, respectively; p = 0.3433), but it depended on temperature and fruit puree (Fig. S3d, Table S1d). W– individuals were lighter than W+ ones on *R. nigrum* at 20°C (p < 0.0001) and at 24°C (p < 0.0001), while it was the contrary on *V. album*: W– were heavier than W+ at 16°C (p = 0.0196) and at 20°C (p = 0.0175), but not at 24°C, where W+ were heavier (p = 0.0010). On *R. idaeus*, W– individuals were heavier than W+ ones at 24°C (p = 0.0010).

Step 2: Diet-based oviposition choices

Whatever the combination of tested-fruits, the oviposition choice by females changed according to *Wolbachia* infection (Fig. 4, Table S2a,b,c). When females were exposed to *V. album* and *M. japonica* purees, most eggs were laid on *V. album* at all temperatures (0.68 ± 0.39 and 0.32 ± 0.39, respectively),

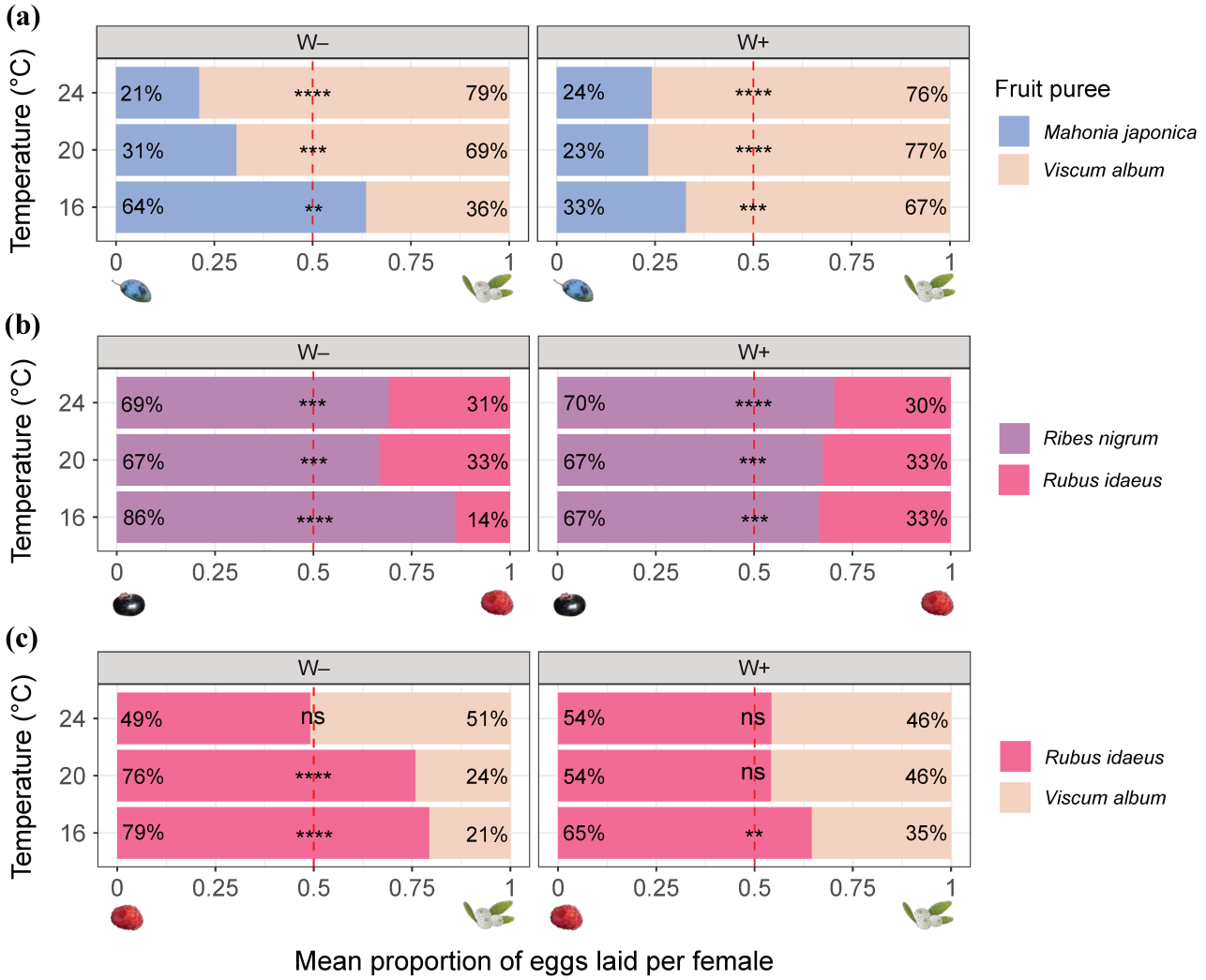

Fig. 4 Comparison of the mean proportion of eggs laid by *Wolbachia*-infected (W+) and -uninfected (W–) *D. suzukii* female in a choice environment between (a) *V. album* and *M. japonica*, (b) *R. nigrum* and *R. idaeus* and (c) *V. album* and *R. idaeus* at each temperature (*i.e.*, 16, 20 and 24°C) (Step 2). The dotted red line indicates the non-choice in oviposition site selection. Significance of differences are represented by asterisks (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$, ns: non-significant) (Chi-square test). Pictures are used to represent each fruit.

except at 16°C where W– females laid more eggs on *M. japonica* ($p = 0.0136$; Fig. 4a, Table S2a). When exposed to *R. nigrum* and *R. idaeus* purees, females laid significantly more eggs on *R. nigrum* at any temperature (0.71 ± 0.38 and 0.29 ± 0.36, respectively), regardless of the females' infection status (Fig. 4b, Table S2b). Finally, when exposed to *V. album* and *R. idaeus* purees, both W– and W+ females laid more eggs on *R. idaeus* at 16°C (W–: 0.79 ± 0.34 versus 0.21 ± 0.34, respectively; W+: 0.65 ± 0.42 versus 0.35 ± 0.42, respectively; Fig. 4c, Table S2c). At 20°C, W– females laid more eggs on *R. idaeus* than on *V. album* (0.76 ± 0.34 versus 0.24 ± 0.34, respectively; $p < 0.0001$), while W+ females showed no preference

between *V. album* or *R. idaeus* (0.46 ± 0.39 versus 0.54 ± 0.39, respectively; $p = 0.798$). This pattern was also observed at 24°C, where both W– and W+ females laid a comparable number of eggs on *V. album* and *R. idaeus* (W–: $p = 0.863$; W+: $p = 0.388$).

Step 3: Temperature-based oviposition choices

Although some eggs were laid at 16°C by females (*e.g.*, five and 12 eggs by W– and W+ females in *V. album*, respectively; one egg by W– females on *R. idaeus*), females laid consistently more eggs at 24°C ($p < 0.0001$; Table 1).

Table 1 Summary pairwise chi-square test of the mean proportion of eggs laid by W– and W+ *D. suzukii* females, during the temperature-based oviposition choice on the same fruit puree (*V. album*, *M. japonica*, *R. nigrum* or *R. idaeus*) (Step 3). Under null-hypothesis, the expected proportion was 50-50.

Step 3 - Temperature-based oviposition choice								
	Proportion of eggs laid by W– females				Proportion of eggs laid by W+ females			
Fruit puree	16°C	24°C	statistic	*p*. adj	16°C	24°C	statistic	*p*. adj
V. album	18.8	81.2	39.0	**8.62e-10**	18.9	81.1	38.8	**9.36e-10**
M. japonica	26	74	23.0	**3.18e-6**	0	0	-	-
R. nigrum	2.5	97.5	90.2	**4.20e-21**	20	80	36	**3.94e-9**
R. idaeus	2.08	97.9	91.8	**1.88e-21**	0	100	100	**3.04e-23**

p.adj: adjusted *p*-value. Significant *p*-values ($p < 0.05$) are in bold.

Step 4: Trade-off between temperature and diet-based oviposition choices.

Females' oviposition preference among fruit purees exposed to different temperatures varied significantly (Fig. 5, Table S3). Both W– and W+ females showed a significantly higher preference for suboptimal fruit purees exposed at 24°C (*i.e.*, *M. japonica* and *R. nigrum*), compared to optimal fruit purees exposed at 16°C. Females laid significantly more eggs than expected by chance on *M. japonica* and *R. nigrum* (0.36 ± 0.48 and 0.59 ± 0.49, respectively) compared to *V. album* and *R. idaeus* (0.05 ± 0.22 and 0.00 ± 0.00, respectively), regardless of the females' infection status. However, W– females laid more eggs on *R. nigrum* than on *M. japonica* (0.86 ± 0.32 and 0.14 ± 0.32, respectively) and W+ females conversely (0.3 ± 0.48 and 0.6 ± 0.52, respectively). Additionally, oviposition in fruit purees exposed at

16°C was very low for *V. album* (W–: 0.005 ± 0.02; W+: 0.1 ± 0.32) and null for *R. idaeus* (0.00 ± 0.00), regardless of the infection status of the females.

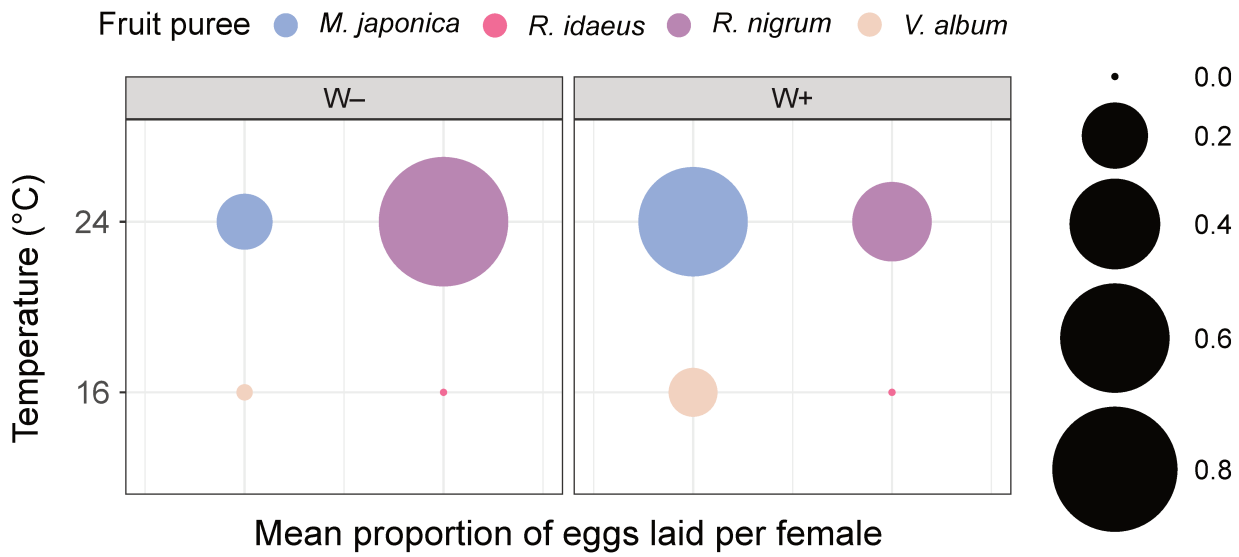

Fig. 5 Comparison of the mean proportion of eggs laid by both *Wolbachia*-infected (W+) and -uninfected (W–) *D. suzukii* females in a choice situation, with fruit purees crossed with temperature: *V. album* and *R. idaeus* at 16°C, and *M. japonica* and *R. nigrum* at 24°C (Step 4). A proportion of 0.25 corresponds to a non-choice of females (Chi-square test).

DISCUSSION

Understanding how *D. suzukii* selects oviposition sites when faced with fruits of varying quality and temperatures is essential for gaining insight into its invasive success. The aim of this study was to assess the existence of a trade-off between thermal and trophic preferences in oviposition choice, using the invasive fruit fly *D. suzukii*. Here, we discuss (i) the influence of temperature and fruit on the performance of *D. suzukii*, (ii) whether *D. suzukii* females select the optimal fruit and / or the optimal temperature for the performance of their offspring, (iii) the existence of a trade-off between thermal and trophic preferences, and (iv) how the presence of *Wolbachia* influence performance and oviposition choice.

Influence of temperature and fruit on *D. suzukii* performance

The performance of *D. suzukii* varied both with temperature and with fruit puree. Temperature is a key factor affecting the life history traits of insects (Deutsch *et al.*, 2008). In *D. suzukii*, exposure of immature stages (*i.e.*, egg-to-adult) to a constant and elevated temperature increased fecundity and

survival up to a specific temperature, while the mean development time from egg-to-adult and mass decreased with temperature (Tochen *et al*., 2014; Schlesener *et al*., 2020; Larges *et al*., under review). As expected, *D. suzukii* performed better overall at 24°C, with increased fecundity, higher emergence rate and faster development compared to 16 and 20°C. *Drosophila suzukii* was able to complete its life cycle on all fruit purees tested (*i.e.*, *V. album*, *M. japonica*, *R. nigrum*, *R. idaeus*). Previous studies showed that *D. suzukii* was able to develop on these fruits both *in natura* and in experimental conditions (Walsh *et al*., 2011; Poyet *et al*., 2015; Kenis *et al*., 2016; Olazcuaga *et al*., 2019, 2021), including on fruit purees (Olazcuaga *et al*., 2019, 2021). Overall, temperature induced larger performance changes than fruit purees.

Regarding fecundity, the number of eggs laid per female on each fruit puree increased with temperature, except for *M. japonica*. At 16°C, fecundity was the highest on *M. japonica* and *V. album*. At 20 and 24°C, fecundity was the highest on *V. album* and the lowest on *M. japonica*. Olazcuaga *et al*. (2019) found that fecundity was higher on *R. nigrum* compared to *R. idaeus,* however, even if we found the same trend at 24°C, the difference was not significant.

Overall, *R. nigrum* had the lowest adult emergence rate compared to *V. album*, *M. japonica* and *R. idaeus* which was consistent with Olazcuaga *et al*. (2019) and Larges *et al*. (under review). The low emergence rate on *R. nigrum* could be due to higher larval densities resulting from the important number of eggs initially laid (Olazcuaga *et al*., 2019; Wang *et al*., 2019). However, as the fecundity in *V. album* was comparable and did not impact the emergence rate, it is unlikely that competition induced mortality. The low emergence rate on *R. nigrum* would rather be attributed to the fruit's nutritional composition (Young *et al*., 2018).

Overall, the development time was the longest in *V. album*, intermediate in *M. japonica*, and the shortest in *R. nigrum* and *R. idaeus*. This aligns with Poyet *et al*. (2015), who found that *V. album* induced a longer development time compared to *R. idaeus* (19.94 ± 0.11 d and 17.76 ± 0.12 d, respectively). Shorter development limits the exposure to inter and intraspecific competition (Matavelli *et al*., 2015; Berezza Da Silva *et al*., 2019) and reduces the risk of predation (Dahl and Peckarsky, 2003). The development times in *R. nigrum* and *R. idaeus* were comparable; however, at all temperatures, individuals developing in *R. nigrum* had a lower mass compared to those in *R. idaeus*. This is indicative of a difference in diet quality, with *R. idaeus* being better in terms of nutrient quantity / quality (Edgar, 2006; Jaramillo *et al.*, 2015) than *R. nigrum*. *Ribes nigrum* berries contain important concentrations of

phenolics, flavonoids and tannins (Paunović *et al*., 2022). Toxicity of *R. nigrum* berries and leaves were investigated in the grain aphid (*Sitobion avenae*) and the cowpea weevil (*Callosobruchus maculatus*) in which they impaired fecundity, development and survival (Oparaeke and Daria, 2005; Chrzanowski *et al*., 2012). These toxic properties could also explain the lower emergence rate observed on *R. nigrum* (Ulmer *et al*., 2020). Finally, individuals emerging from *M. japonica* and *R. idaeus* were the heaviest, followed by *V. album* (intermediate) and *R. nigrum* (the lightest).

Drosophila suzukii is a polyphagous species that exploits a wide range of host plants. However, the differences in nutritional composition between host plants can directly impact larval performance. The fruit P:C ratio influences the performance of insects (Rodrigues *et al*., 2015; Lihoreau *et al*., 2016), and especially *D. suzukii* (Young *et al*., 2018). A low P:C ratio (*i.e.*, high carbohydrate - low protein content) improves *D. suzukii* larval survival and emergence rates (Silva-Soares *et al*., 2017; Rendon *et al.*, 2018; Larges *et al*., under review). For instance, in Larges et al. (under review), survival was better on *V. album*, which had a low P:C ratio (0.44 ± 0.19) compared to other fruits with higher P:C ratio. Based on data from Ciqual (2020), *R. nigrum* has an estimated P:C ratio of 0.31, compared to 0.57 for *R. idaeus*. This P:C ratio might have been too low to allow proper development (Silva-Soares *et al*., 2017). *Ribes nigrum* fruit also has a high phosphorus content, which may be responsible for the higher oviposition rate in this fruit (Olazcuaga *et al*., 2019), even if the larval performance is reduced later.

Do *D. suzukii* females make the right oviposition choice?

We investigated the oviposition preference of *D. suzukii* to determine its preferred fruit puree, as well as its preferred temperature. We hypothesised that females would select oviposition sites that offer optimal nutritional value to enhance the performance of their offspring, aligning with the 'mother knows best' hypothesis (Mayhew, 2001). Based on the performance results of *D. suzukii* on each fruit puree and existing literature (Poyet *et al*., 2015; Kenis *et al*., 2016; Olazcuaga *et al*., 2019, 2021; Larges *et al.*, under review), we suggested that *D. suzukii* females should ideally select *V. album* and *R. idaeus* to maximise the performance of their offspring. However, under our experimental conditions, the oviposition choice of females did not always align with the 'mother knows best' hypothesis. Females predominantly laid their eggs on *R. nigrum* rather than *R. idaeus*. Although *D. suzukii* exhibited similar fecundity rates and development times on both fruit purees, this choice appeared suboptimal since *R. idaeus* provided better outcomes in terms of emergence rate and individual mass. Olazcuaga *et al*. (2019) reported similar

findings and suggested that females might be more attracted to *R. nigrum* due to its higher phosphorus content compared to other fruits.

The colour contrast between our fruit purees could have influenced the oviposition choice, as *D. suzukii* is usually more attracted by darker fruit (Little *et al*., 2020; Bühlmann and Gossner, 2022). However, the females had 72 hours to lay their eggs and chose the lighter *V. album* puree over the darker *M. japonica*, which makes it unlikely that the colour contrast influenced the female's choice. The "mother knows best" hypothesis could also not be verified. While some studies found a positive correlation between female oviposition choice and offspring performance in various species (Gripenberg *et al*., 2010), in *D. suzukii* studies are inconclusive with contrasting results. For instance, the correlation was found in Young *et al*. (2018), but not in other studies (Poyet *et al*., 2015; Olazcuaga *et al*., 2019; Larges *et al*., under review). During the oviposition selection process, *D. suzukii* females are influenced by numerous olfactory, visual (*e.g.*, colour, size, shape) and tactile (*e.g.*, texture, hardness, firmness) cues, as well as the presence of specific compounds (*e.g.*, P:C ratio, phosphorus) or pH levels (Silva-Soares *et al*., 2017; Little *et al*., 2017, 2019, 2020; Olazcuaga *et al*., 2019; Akutsu and Matsuo, 2022; Bühlmann and Gossner, 2022). In our study, we controlled for the influence of fruit size and colour, but other factors could have played a role and should be further investigated in other studies.

We also hypothesised that in a choice situation, *D. suzukii* females should select fruit purees at a temperature that optimises its offspring performance. The temperature of the oviposition site can provide reliable cues about the thermal condition for larval development, as larvae, with their limited mobility, develop near the oviposition site (Silva-Soares *et al*., 2017; Reyes-Ramirez *et al.*, 2023). Therefore, females might be sensitive to temperature when choosing a site for their offspring. In some species, females lay eggs in microhabitats where the temperature is optimal for development (Notter-Hausmann and Dorn, 2010). For instance, in the peach moth *Grapholita* (*Cydia*) *molesta*, females preferred to lay their eggs at 30°C, a temperature that reduced the development time of eggs, larvae and pupae, maximises larval and pupal survival, and resulted in higher pupal mass (Notter-Hausmann and Dorn, 2010). However, there are exceptions where females choose less favourable sites for their offspring's development. For instance, Rausher (1979) found that female butterflies of three Papilionidae species preferred sunny habitats for laying eggs, even though shaded habitats were better for juvenile survival and development. Stoeckli *et al*. (2008) observed that the codling moth, *Cydia pomonella*, infested the southern and eastern sides of trees more than the northern sides, with females laying eggs in the warmer areas that reduce egg survival (Kührt

et al., 2006). Our results showed that *D. suzukii* preferentially laid eggs at 24°C, supporting the 'mother knows best' hypothesis concerning temperature oviposition choice in this species.

Trade-off between temperature and fruit for oviposition choice

Drosophila suzukii is a polyphagous species with oviposition choices influenced by various factors, including the nutritional quality and temperature. The 'mother knows best' and 'optimal bad motherhood' hypotheses focus on the trade-off between the larvae's nutritional needs and the oviposition choices of adult females, without considering abiotic factors such as temperature. In the previous steps, we observed that, in *D. suzukii*, the 'mother knows best' hypothesis was not always supported in diet-based oviposition choice but was in temperature-based oviposition choice. In the last step of our study, we highlighted the presence of a trade-off in *D. suzukii* oviposition choice between temperature and fruit puree. Females preferentially laid their eggs on *R. nigrum* and *M. japonica*, fruit purees with lower larval performance but exposed to the optimal temperature of 24°C. In contrast, *V. album* and *R. idaeus*, which offered better larval performance but were maintained at a suboptimal temperature of 16°C, received few or no eggs. These results suggest that while the 'mother knows best' hypothesis may not hold for nutritional aspects in *D. suzukii*, it could apply to temperature. Females appeared to prefer laying eggs on fruits at an optimal temperature, even if nutritional quality was lower, indicating a significant influence of fruit temperature on oviposition choice. Nutritionally, our results aligned with the findings of Scheirs *et al*. (2000) and Olazcuaga *et al*. (2019), which demonstrated that the correlation between adult female preferences and larval needs can be weak. As temperature induced larger performance changes than our fruit purees, our results suggest that the 'mother knows best' hypothesis might stand in this multifactorial context.

This choice also reflects a trade-off between fecundity and larval survival. On *R. nigrum*, although females lay a high number of eggs, larval survival is low due to poor nutritional quality. This aligns with an *r*-strategy, where high egg production is offset by reduced survival of the larvae (Taylor and Condra, 1980). On the contrary, the low number of eggs laid on *V. album* at 16°C, where nutritional conditions were better but the temperature was sub-optimal, illustrated a *K*-strategy (Taylor and Condra, 1980). In this strategy, females produce fewer eggs, but prioritise more favourable conditions to maximise the survival chances of the larvae. Larges *et al*. (under review) also proposed a similar trade-off, where *D. suzukii* females laid more eggs in *Aucuba Japonica*, a fruit that increased the offspring' energy reserves and prolonged adult survival, but induced a low emergence rate, consistent with a *K*-strategy.

Another hypothesis that could explain this behaviour is the bet-hedging strategy (Hopper, 1999). Females can enhance the hatching and developmental success of their eggs by selecting oviposition sites with varying temperatures (Stillwell and Fox, 2005). This strategy, observed in the apple leafminer, involves spreading eggs across different microclimatic environments within the canopy to maximise the survival chances of some offspring, regardless of fluctuating conditions that cannot be predicted by the females (Pincebourde *et al*., 2007). Similarly, although fewer eggs were laid at 16°C compared to 24°C, the distribution of *D. suzukii* eggs across puree fruits at both temperatures could be a form of bet-hedging, ensuring that a portion of the offspring survives even if conditions become unfavourable.

Wolbachia influence on performance and oviposition choice

Our results indicate that *Wolbachia* influenced both performance and oviposition choice in *D. suzukii*. Overall, W+ females laid fewer eggs compared to W– females. However, this reduction in fecundity was counterbalanced by increased survival rates, greater mass and shorter development time in W+ offspring. Our results are in accordance with Deconninck *et al*. (2024d - Chapter 8), who found similar effects of *Wolbachia* after a diet shift. Other studies have reported contrasted effects of *Wolbachia* in *D. suzukii*: reduced fecundity (Hamm *et al*., 2014), more offspring (Mazzetto *et al*., 2015), or even no effect on fecundity or survival (Saeed *et al*., 2018).

To our knowledge, the influence of *Wolbachia* on female oviposition choice in *D. suzukii* was never explored. Deconninck *et al*. (2024d - Chapter 8) showed that *Wolbachia* enhanced performance in nutritionally-variable environments, suggesting an impact on oviposition choice. Our results revealed effects in choices between *V. album* and *M. japonica* at 16°C, and between *R. idaeus* and *V. album* at 20°C. On the contrary, *Wolbachia* had globally no effect on temperature-based oviposition choice, except for *R. nigrum*, where W+ females laid a higher proportion of eggs at 16°C compared to W– females at the same temperature. The presence of *Wolbachia* may influence oviposition choice by favouring some fruit purees, which could improve distribution in neo-populations or enhance offspring survival. This hypothesis could explain why W+ females laid more eggs in *V. album* than in *M. japonica* at 16°C, where offspring from *V. album* exhibited better survival rates. The influence of *Wolbachia* in oviposition choices could be explained by two hypotheses. If *Wolbachia* has a mutualistic effect, as suggested by Deconninck *et al*. (2024d - Chapter 8), then *D. suzukii* should maximise its prevalence. This would favour a bet-hedging strategy, resulting in faster development and better overall performance at 24°C. Conversely, if *Wolbachia* has a neutral or even negative effect, potentially due to the cost of hosting the symbiont (Clavé

et al., 2022), *D. suzukii* might attempt to eliminate it by laying more eggs in toxic fruits like *V. album* or by limiting its proliferation by choosing cooler temperatures. Even if the role of *Wolbachia* in oviposition preference has been explored in other species, with contrasting effects (Wiwatanaratanabutr *et al*., 2010; Abrun *et al*., 2021), it would be worth exploring it in different nutritional and thermal contexts.

To conclude, our study highlights the influence of temperature on the oviposition choice in *D. suzukii*, even surpassing the nutritional quality of the substrate. This finding raises intriguing questions about other polyphagous insects: Do they exhibit a similar trade-off between temperature and nutritional quality when selecting oviposition sites? Comparative studies could reveal similar patterns or fundamental differences in the egg-laying strategies of different species. Moreover, we showed for the first time that infection by *Wolbachia* influences the oviposition choice in *D. suzukii*. *Wolbachia* is present in many insects, it could be interesting to examine if this endosymbiotic bacteria has similar effects in the choice of nutritional resources in other insects and to assess the ecological value of this influence. This could open some fascinating avenues of research on how host-symbiont interaction influences oviposition behaviour. For instance, does *Wolbachia* change the choice of its host, or does the host change its behaviour as a result of the infection? These questions need further exploration to better understand the impact of these interactions on the evolutionary dynamics of insect populations, particularly invasive species.

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Supplementary material

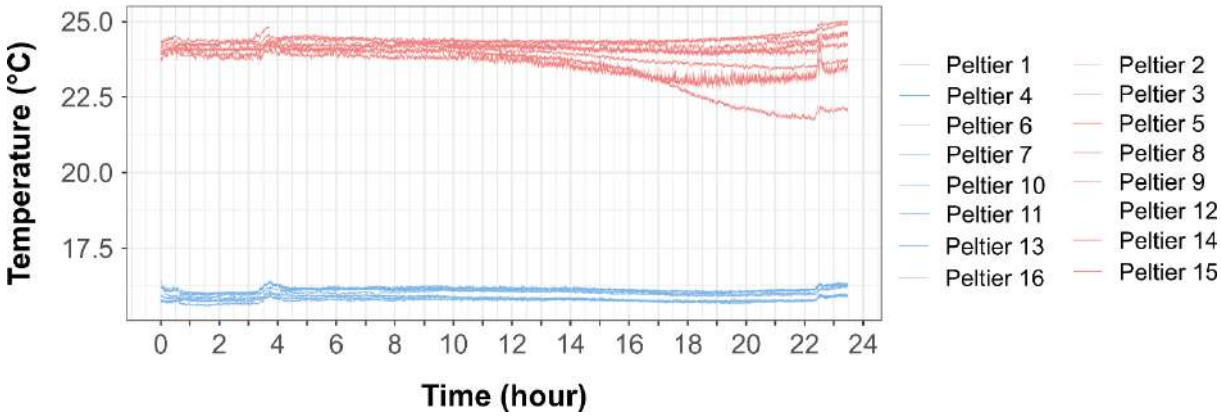

Fig. S1 Temperature calibration for Peltier units 1 to 16 took approximately 24 hours. Blue: Peltier unit set at 16°C, red: Peltier unit set to 24°C.

Table S1 Summary of results for (a) generalised linear model with a Quasi-Poisson distribution for total number of eggs laid per female, (b) ANOVA on emergence rate, (c) ANOVA on log-transformed egg-to-adult development time and (d) ANOVA on offspring mass (mg).

(a) Total number of eggs laid per female				
	Sum Sq	Df	F value	*p*-value
Fruit puree (FP)	324.30	3	22.9803	**7.215e-14**
Temperature (Temp)	1068.41	2	113.5625	**<2.2e-16**
Wolbachia (Wol)	100.29	1	21.3193	**5.066e-06**
Temp × FP	178.38	6	6.3202	**2.124e-06**
Wol × FP	70.94	3	5.0269	**0.0019**
Wol × Temp	14.31	2	1.5214	0.2195
Wol × Temp × FP	44.15	6	1.5643	0.1558
(b) Emergence rate				
	LR Chisq	Df		*p*-value
Fruit puree (FP)	169.738	3		**<2.2e-16**
Temperature (Temp)	96.408	2		**<2.2e-16**
Wolbachia (Wol)	57.097	1		**4.148e-14**
Temp × FP	12;371	6		0.05418
Wol × FP	53.349	3		**1.545e-11**
Wol × Temp	33.372	2		**5.667e-08**
Wol × Temp × FP	29.142	6		**5.720e-05**
(c) Mean egg-to-adult development time				
	Sum Sq	Df	F value	*p*-value
Fruit puree (FP)	1.4425	3	60.4409	**<2.2e-16**
Temperature (Temp)	30.6898	2	1928.8436	**<2.2e-16**
Wolbachia (Wol)	0.0779	1	9.7924	**0.0019**
Temp × FP	0.2424	6	5.0773	**5.329e-05**
Wol × FP	0.0089	3	0.3712	0.7738
Wol × Temp	0.1081	2	6.7935	**0.0013**
Wol × Temp × FP	0.0520	6	1.0900	0.3680
(d) Offspring mass (mg)				
	Sum Sq	Df	F value	*p*-value
Sex	60.158	1	842.9533	**<2.2e-16**
Fruit puree (FP)	23.712	3	110.7506	**<2.2e-16**
Temperature (Temp)	17.193	2	120.4587	**<2.2e-16**
Wolbachia (Wol)	2.074	1	29.0668	**7.807e-08**

Sex × FP	1.614	3	7.5402	**5.131e-05**
Sex × Temp	0.541	2	3,7896	**0.0228**
Sex × Wol	0.124	1	1.7368	0.1877
Temp × FP	1.365	6	3.1881	**0.0041**
Temp × Wol	0.494	2	3.4600	**0.0316**
Wol × FP	10.807	3	50.4782	**<2.2e-16**
Sex × FP × Temp	0.545	6	1.2724	0.2666
Sex × FP × Wol	0.825	3	3.8553	**0.0092**
Sex × Temp × Wol	0.236	2	1.6554	0.1913
FP × Temp × Wol	2.872	6	6.7080	**4.781e-07**
Sexe × FP × Temp × Wol	0.240	6	0.5614	0.7613

Sum Sq = Sum of Squares; LR Chisq = Likelihood Ratio Chi-Square; Df = Degrees of freedom; F value = F-statistic. Significant *p*-values ($p < 0.05$) are in bold.

Table S2 Summary of pairwise chi-square test for *Wolbachia*-infected (W+) and -uninfected (W–) *D. suzukii* females' diet-based oviposition choice (step 2) (a) *V. album* versus *M. japonica*, (b) *R. nigrum* versus *R. idaeus* and (c) *V. album* versus *R. idaeus*. A percentage of 50% in each fruit would correspond to a non-choice of females.

	W–				W+			
(a)	% of eggs laid in *V. album*	% of eggs laid in *M. japonica*	statistic	*p*.adj	% of eggs laid in *V. album*	% of eggs laid in *M. japonica*	statistic	*p*.adj
16°C	36.5	63.5	7.32	**0.0136**	67.2	32.8	11.8	**0.00116**
20°C	69.4	30.6	15.1	**0.0002**	76.8	23.2	28.7	**1.73e-07**
24°C	78.9	21.1	33.3	**1.58e-08**	75.9	24.1	26.8	**4.42e-07**
(b)	% of eggs laid in *R. nigrum*	% of eggs laid in *R. idaeus*	statistic	*p*.adj	% of eggs laid in *R. nigrum*	% of eggs laid in *R. idaeus*	statistic	*p*.adj
16°C	86.1	13.9	52.1	**1.04e-12**	66.7	33.3	11.1	**0.00172**
20°C	66.7	33.3	11.2	**0.0016**	67.4	32.6	12.1	**0.0010**
24°C	69.2	30.8	14.8	**0.0002**	70.5	29.5	16.8	**8.52e-05**
(c)	% of eggs laid in *V. album*	% of eggs laid in *R. idaeus*	statistic	*p*.adj	% of eggs laid in *V. album*	% of eggs laid in *R. idaeus*	statistic	*p*.adj
16°C	20.6	79.4	34.6	**7.96e-10**	35.4	64.6	8.48	**0.0072**
20°C	24.1	75.9	26.9	**4.32e-07**	45.8	54.2	0.711	0.798
24°C	50.9	49.1	0.0297	1	45.7	54.3	0.746	0.776

p.adj = Adjusted *p*-value. Significant *p*-values ($p < 0.05$) are in bold.

Table S3 Summary of pairwise chi-square test of the mean proportion number of eggs laid per both *Wolbachia*-infected (W+) and -uninfected (W–) *D. suzukii* females in temperature and diet-based oviposition choices (step 4). A percentage of 25% in each condition would correspond to a non-choice of females.

Step 4 - Temperature and diet-based oviposition choices						
	Proportion of eggs laid by W– females			Proportion of eggs laid by W+ females		
Fruit puree × Temperature	% of eggs	statistic	*p*. adj	% of eggs	statistic	*p*. adj
V. album × 16°C	0.5	32.1	**5.96e-8**	10.0	12.0	**0.0021**
M. japonica × 24°C	13.6	6.9	**0.0347**	60.0	65.3	2.53e-15
R. nigrum × 24°C	85.9	198.0	**2.64e-44**	30.0	1.33	0.9920
R. idaeus × 16°C	0	33.3	**3.10e-8**	0	33.3	**3.10e-8**

p.adj: adjusted *p*-value. Significant *p*-values ($p < 0.05$) are in bold.

DISCUSSION

Part 3

Polyphagy, temperature and resource selection

This Part 3 of my thesis highlights the importance of multifactorial approaches to studying organism performance or behaviour. In particular, the data presented in these three chapters confirm that temperature, nutritional quality and symbiont infection interact to shape *D. suzukii* oviposition choices and, therefore, fitness.

Experimental studies are necessary to understand physiological and ecological processes, but often are performed under controlled conditions that can have very limited ecological relevance. Rearing temperature, for instance, influenced both the performance of *D. suzukii* in different fruit, but also changed the ranking of these fruit in terms of performance, as well as changing the oviposition choice of females. Combining field observations and field and laboratory experiments is, therefore, an essential part of understanding both the drivers of fruit infestation in the natural environment and also in exploring the existence of trade-offs between life history traits associated with environmental conditions.

The preference-performance hypothesis states that females should preferentially oviposit in substrates that maximise the fitness of their offspring (Gripenberg et al. 2010). This hypothesis is primarily based on the nutritional quality of the considered substrates. However, the data obtained in this study suggest that temperature is even more important than nutrient quality in driving offspring performance in *D. suzukii*. However, this was not considered in the preference-performance hypothesis. Our data confirm that temperature was more important than nutritional quality in determining *D. suzukii* oviposition choice, confirming that the preference-performance hypothesis does not apply to *D. suzukii* based on nutritional choices (see also Scheirs et al. 2000; Olazcuaga et al. 2019). It appears that, in a multifactorial context (including both nutritional quality and temperature), *D. suzukii* is able to make a choice that favours the best temperature for their offspring's development. Further research is now required on the influence of other fruit properties (e.g., odour, colour, shape, texture) on oviposition choice, in combination with different temperature conditions, to increase understanding of the range of trade-offs that may be involved in maximising fitness, both in *D. suzukii* and in multi-species studies.

Finally, Part 3 generated new insights into the influence of *Wolbachia* infection on *D. suzukii*. While hosting *Wolbachia* appeared to reduce fecundity, this cost was counterbalanced by an overall higher survival of progeny. *Wolbachia* also influenced *D. suzukii* oviposition choice under some temperature conditions. The interaction between temperature and *Wolbachia* effects would need further research.

General Discussion

The objectives of my thesis were to explore with great detail the remarkable abilities of the highly invasive and commercially important fruit fly, *D. suzukii*, with a focus on its trophic and thermal ecology. In **Part 1**, I first studied the continuum of resources available to the fly during the harsh season from autumn to spring outside the commercial crop season. The results obtained showed that *D. suzukii* was able to use fallen apples, mistletoe and various ornamental plants. These back-up resources could constitute a reservoir for *D. suzukii* and catalyse the development of the fly's population in spring in advance of infesting early fruiting crops. The work in **Part 1** also demonstrated that, while environmental factors driving *D. suzukii* infestations varied between host plants, plant architecture and temperature were consistently important. These two variables are key components interacting to produce temperature (microclimate) mosaic at the scale of the host plant and therefore could be linked to the behavioural thermoregulation abilities then explored in **Part 2**. The results from this second part showed that *D. suzukii* thermal preference (major trait of behavioural thermoregulation) is highly plastic, which could help protect the fly from harmful conditions and result in improved fitness. A large inter-individual variability exists however, which seems to get its roots in the metabolic physiology of the fly. In the final part of my thesis (**Part 3**), I examined the relationship between trophic resources and temperature, and the existence of potential trade-offs influencing *D. suzukii* development. The results obtained suggested that the influence of temperature is more important than that of fruit quality in determining the overall performance of the fly. I also examined the role of *D. suzukii*'s *Wolbachia* strain, *w*Suz, on some of the traits identified throughout the thesis, concluding that it could provide nutritional benefits to the fly. My data demonstrate that *w*Suz infection helps *D. suzukii* to deal with diet shifts, increases larval survival during development and can influence the female oviposition choice. The conceptual figure originally presented in the Introduction, updated in the light of these findings, provides an overview of my thesis (Figure 1).

In this Discussion, I further explore and integrate the findings of the different studies performed within my thesis and highlight new research questions that would be valuable to develop.

> "The important thing is not to stop asking questions. Curiosity has its own reason for being. You cannot help but be in awe of the mysteries of eternity, life or the wonderful structure of reality. It is enough to try to grasp just a little of this mystery every day. This sacred curiosity should never be lost." Albert Einstein

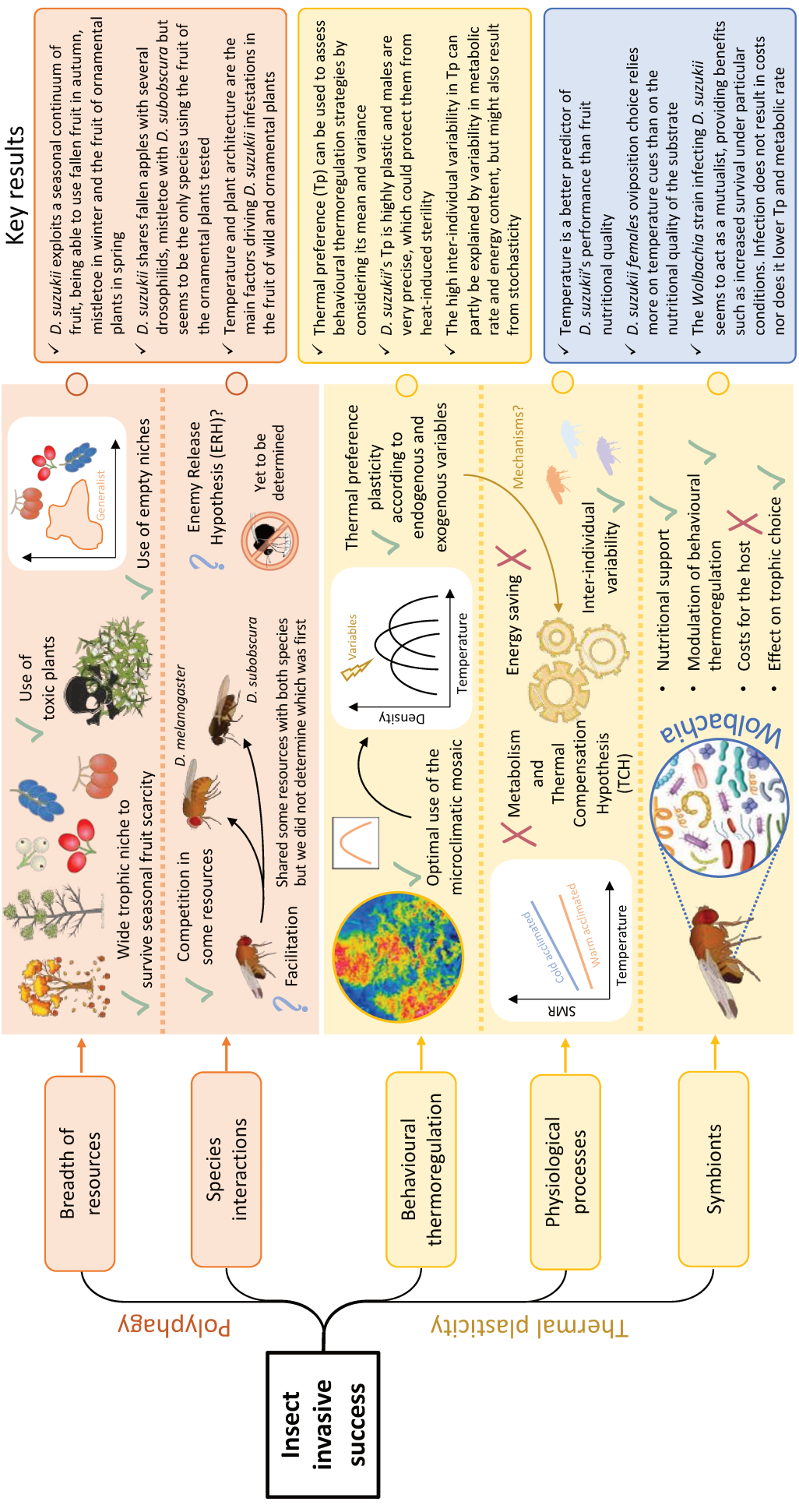

Figure 1 Updated conceptual figure of the thesis.

This Discussion chapter is presented in three sections. The first considers the trophic niche of *D. suzukii* and its implications, including in the context of climate change. The second addresses behavioural thermoregulation in *D. suzukii* and in insects in general. The third and final section focuses on the role of *Wolbachia* in the biology of *D. suzukii*.

1. TROPHIC NICHE AND ECOLOGICAL IMPLICATIONS

In this section, I address subjects relating mainly to Part 1. In particular, I discuss how the polyphagy of *D. suzukii* helps the fly to cope with resource seasonality and, in turn, how climate change could impact the overwintering of the fly in temperate regions. Then, I emphasise the particular ability of *D. suzukii* to use fruit that are toxic to other consumers, highlighting that, while this might reduce the pressure from competitors and predators, it also necessitates detoxification capacities. Finally, I consider the potential cascading effects *D. suzukii* could have on native communities, including insects, plants and vertebrate seed dispersers.

1.1. Toxic fruit, advantage and detoxification

Drosophila suzukii has the special ability to use toxic fruit. This could provide the fly with several advantages, including the use of empty niches free from competitors, release from natural enemies and even defence from parasitoids. To perform in these toxic fruit, *D. suzukii* must possess detoxification abilities that would be worth exploring.

1.1.1. Toxic fruit

The numerous resources used by *D. suzukii* include toxic fruit such as those of *Atropa belladonna, Aucuba japonica* and *Viscum album* (Poyet et al. 2015, 2017; Panel et al. 2018; Bühlmann and Gossner 2022; Deconninck et al. 2024a - Chapter 2: Deconninck et al. under review a - Chapter 3). *Atropa belladonna* fruit contain high concentrations of atropine, an entomotoxic alkaloid (Poyet et al. 2017), but oviposition and development in these fruit provide *D. suzukii* larvae with protection against parasitoids (Poyet et al. 2017). In Chapters 2 and 3, *D. suzukii* co-occurred in *V. album* fruit with *D. subobscura* and it was numerically dominant. These fruit contain lectins, in particular agglutinin, which exhibit anti-nutritive effects that have been demonstrated to impair development and survival in Lepidoptera larvae (Gaidamashvili et al. 2012). In Chapters 3 and 7, *D. suzukii* was also able to develop in *A. japonica*, an

ornamental plant whose fruit contain aucubin, an iridoid glycoside which has been shown to exhibit deterrence (Biere et al. 2004) and toxicity (Vivekanandhan et al. 2022) properties against insects.

The exceptional resistance of D. suzukii to these toxic plants is striking. Generally, specialisation in phytophagous insects is thought to assist in the evolution of adaptations to overcome specific plant defences (Ehrlich and Raven 1964). Consequently, polyphagous insects are thought to be more impacted by plant toxicity than specialists (Ali and Agrawal 2012). However, the ability of D. suzukii to use ripe undamaged fruit thanks to its serrated ovipositor may limit its exposure to toxins (Bühlmann and Gossner 2022), as fleshy fruit are generally less toxic because they evolved to encourage consumption by vertebrates (Lei et al. 2021). However, given the range and extreme toxicity of fruit that D. suzukii can utilise, the fly appears to show particularly well developed resistance.

1.1.2. Detoxification

In order to develop on toxin-containing fruit, *D. suzukii* must possess suitable detoxification or sequestering abilities, but these are currently unknown. Improved knowledge of these abilities would assist the development of pest management strategies, which can involve the use of plant-derived biomolecules to deter pests (e.g., Dam et al. 2019; Hussain et al. 2023). Insects have evolved various defence mechanisms against plant protective chemicals, including sequestration, behavioural avoidance and enzymatic degradation. Furthermore, recent omics analyses have also revealed that a number of insects possess specific gut microorganisms with abilities to degrade phytotoxins and pesticides (Itoh et al. 2018; Kline and Joshi 2024). For instance, the coffee berry borer, *Hypothenemus hampei,* is able to feed exclusively on coffee beans, which are highly toxic because of the presence of caffeine, due to the presence of the gut bacteria, *Pseudomonadales fulva*, which can degrade this alkaloid (Ceja-Navarro et al. 2015). *Drosophila suzukii* also provides a good model to study the contribution of microorganisms to detoxification processes. While most research on mutualistic insect-microorganism associations has focused on nutrition and protection against natural enemies, the role of microbiota in plant allelochemical detoxification requires investigation (Douglas 2009, 2015).

Bacterial symbionts typically have much more diverse genes and pathways for toxin degradation than insects, as well as much shorter life cycles, which can promote their resistance to toxicants (Kline and Joshi 2024). Acquiring these symbionts could also provide resistance to the insect partners (Kline and Joshi 2024). Although the *D. suzukii* microbiota is overall dominated by *Acetobacter* (see Deconninck et al. 2024c - Chapter 8), the fly could acquire symbionts when developing on toxic fruit that could help it

overcome the negative effects of toxic plant compounds. On the other hand, toxic compounds with antimicrobial effects could alter the microbial communities in *D. suzukii* gut, in turn increasing the titre of *Wolbachia*, as it was found in *D. melanogaster* fed on moringa fruit (Schaffer et al. 2024). Potential effects of varying *Wolbachia* titre in *D. suzukii* are discussed in section 3.2.3. Moreover, *Wolbachia* can increase the resistance to insecticides by inducing higher expression of detoxification genes (Ye et al. 2024) but the effect is species dependent and in some cases *Wolbachia* induce a higher susceptibility to insecticides (Liu and Guo 2019). Study of the gut microbiota of *D. suzukii* that are able to develop in toxic fruit is required to improve our knowledge of the detoxification abilities of the fly and the presence and contribution of microbial symbionts.

1.1.3. Competitive advantage of toxicity resistance

Experimental studies appear to indicate that *D. suzukii* is a poor competitor, its emergence rate and development time being severely impacted by the presence of competitors such as *D. melanogaster* or *Zaprionus indianus* (Shaw et al. 2018; Shrader et al. 2020). Moreover, *D. suzukii* avoids the presence of competitors when ovipositing (Shaw et al. 2018; Kidera and Takahashi 2020). Using toxic fruit may provide an advantage to *D. suzukii*, in an analogous fashion to using ripening and ripe fruit. Both fruit are less used by other insects and represent an empty niche, exempt from competitors. Use of such fruit could contribute to the invasive success of *D. suzukii,* as successful invaders are commonly those with niche requirements that differ most from native species present in the recipient community (Stachowicz and Tilman 2005).

The use of commonly empty niches could also contribute to enemy release (Colautti et al. 2004). Natural enemies of *D. suzukii* mainly comprise macroarthropods (Ulmer et al. 2021) and parasitoids (Chabert et al. 2012). The Enemy Release Hypothesis (ERH) suggests that the establishment of a new invading species can be facilitated by a lack of natural competitors and enemies for regulation of the population (Williamson and Fitter 1996; Keane and Crawley 2002) or that, even if potentially appropriate native species are present, they are generally naïve and unable to recognise/detect and then predate/parasitise the invading species. *Drosophila suzukii* already benefits from the ERH as it is susceptible to very few parasitoids in its invaded compared to its native range (Wang et al. 2021). Additionally, using fruit that are usually not infested by other *Drosophila* species could reduce the risk of parasitism as a lower density of prey is mostly associated with low parasitism (Umbanhowar et al. 2003;

Gunton and Pöyry 2015). Further investigation is required to compare, for instance, parasitism rates in fruit commonly infested by other drosophilids and those that are only infested by *D. suzukii*.

Finally, the use of toxic fruit may provide a means by which *D. suzukii* can defend itself against parasitoids. In an experimental study, in the presence of parasitoids, *D. suzukii* females preferred to lay eggs in a toxic substrate containing atropine, the alkaloid present in *Atropa belladonna* fruit, conferring greater resistance to their offspring against parasitoids (Poyet et al. 2017). Studying these defensive mechanisms with other toxic plants should be explored, such as the dynamics of both *D. suzukii* and associated parasitoids with the development of *D. suzukii* in toxic plants, especially in winter.

1.2. Resource seasonality and phenology

Drosophila suzukii has the ability to develop in multiple plant species thanks to its polyphagy and this allows the fly to cope with resource seasonality, shifting from one resource to the other according to availability. At the same time, low temperature acts on both *D. suzukii* development and plant phenology which can limit the fly population, especially in winter in temperate regions. However, in some areas that the fly has invaded, it may not be limited by cold temperatures and with ongoing climate change, the overwintering requirement may also be shortened in other regions.

1.2.1. Annual resource cycle

The polyphagy of *D. suzukii* is impressive. The fly is known to develop in at least 80 plant species belonging to 19 families (Poyet et al. 2015; Kenis et al. 2016). These include both cultivated and wild fruit bearing species (Poyet et al. 2015; Kenis et al. 2016). Female flies preferentially oviposit in maturing (Poyet et al. 2014) or mature fruit (Walsh et al. 2011) but can also use decaying fruit (Kienzle et al. 2020) and even unripe fruit (Arnó et al. 2016). When fruit resources are scarce, they can also utilise other substrates such as mushrooms, flowers or even bird guano (Mitsui et al. 2010; Stockton et al. 2019). The use of ephemeral resource patches (Butterworth et al. 2023), including fallen fruit (Deconninck et al. 2024b - Chapter 1), is clearly advantageous to *D. suzukii*. This trophic niche flexibility gives *D. suzukii* the ability to develop all year round and complete multiple generations per year, even in many seasonal temperate environments.

During winter, *D. suzukii* overwinters as adults (Kanzawa 1939; Mitsui et al. 2010), and a small proportion of larvae and pupae can survive, especially if insulated (Kaçar et al. 2016). Cold-acclimation generates cold-tolerant females which show reversible reproductive dormancy that ends spontaneously

when temperatures rise (Shearer et al. 2016; Grassi et al. 2018; Panel et al. 2018). Further research is required to understand any determinants of dormancy termination, such as how warm the temperature should remain above any thermal threshold to reactivate egg production. As *D. suzukii* is a synovigenic species, female nutrition may also influence the process of reactivation.

In other invaded regions, females may not require dormancy. This could be the case in some recently invaded regions in Africa or South America. For instance, in the subtropical Indian Ocean island of La Réunion, temperatures in the coldest months are above 14°C (DIROI 2021), negating the requirement for any temperature-driven dormancy. Exploring D. suzukii population dynamics in such regions would complement the type of thermal seasonality studies undertaken in this thesis. Such regions may experience other environmentally- (e.g., desiccation) or resource-driven (e.g., fruit availability) seasonality which will require further investigation to broaden knowledge of the fly's responses and trophic niche, which may differ markedly from those that have mostly been studied in Europe and North America.

1.2.2. Climate change

Temperature consistently affected *D. suzukii* infestation in all host plants studied in Part 1. With ongoing global change, in particular trends of temperature increase and warmer winters (Marshall et al. 2020), fly infestations are likely to increase in future. As autumn phenology shifts towards later, and spring towards earlier, onset dates (Marshall et al. 2020), infestations may persist later in autumn, leading to larger overwintering populations as well as the initiation of population expansion earlier in spring. Warmer temperatures could also reduce development time and increase emergence success. In *D. suzukii*, under fluctuating conditions, an increase from a mean temperature of 5 to 6°C can theoretically increase the egg-to-adult viability by 10% and allow completion of larval development (Raynaud-Berton et al. 2024). In other insect species, winter warming is predicted to have a negative impact by increasing metabolism which would accelerate the depletion in energy reserves leading to starvation and death (Williams et al. 2012). This is unlikely to happen in *D. suzukii* as adult flies can use a wide range of feeding resources in autumn and winter that would maintain their energy reserves (Stockton et al. 2019).

Fruit maturation may also be impacted by climate change. Plant phenology has advanced in recent years, in some cases by several weeks, with median advances of 4-5 d per degree Celsius of temperature increase (Menzel et al. 2006; Bertin 2008). While precise details will vary, elevated temperature and CO_2 are overall likely to enhance plant growth and reproduction, alter the production of secondary

metabolites and change the nutritional quality of plants (Jamieson et al. 2012; Ramirez and Kallarackal 2015). Some plant species may produce more fruit as a result of climate change (Springate and Kover 2014; Alatalo et al. 2021; but see Zi et al. 2022). However, unlike some other plant-insect interactions under climate change, *D. suzukii* is unlikely to face ecological mismatches as the fly is able to switch between any readily available host plant thanks to its polyphagy (DeLucia et al. 2012; Yadav et al. 2023). With greater and longer fruit resource availability, *D. suzukii* populations may increase in future, as has been noted and predicted for other pest outbreaks (Jamieson et al. 2012). Furthermore, as climate change will also alter cultural practices, inducing earlier cultivation of crops (Menzel et al. 2020), this may also contribute to increasing infestation rates and crop fruit damage.

Our national-scale study of mistletoe infestation (Deconninck et al. 2024a - Chapter 2), gives some pointers towards the effect of climate change on *D. suzukii* infestations. Future research should expand this study to more frequent sampling over a longer temporal scale, with additional sampling from January to June in the locations, and expanding the European geographical coverage to include locations (e.g. in Italy or Spain) with milder winters. This would allow testing in the field the hypotheses that warmer winters will induce earlier infestations and higher infestation rates.

On the other hand, climate change in summer may be more challenging for *D. suzukii* than winter warming. Temperature increase in Mediterranean areas was already found to negatively impact *D. suzukii* populations (Ulmer et al. 2022). In summer, the combination of elevated temperature with decrease in water availability and atmospheric humidity can negatively impact both fruit bearing plants and *D. suzukii* (Renner and Zohner 2018), but this also requires further investigation.

1.3. Cascading effects

Drosophila suzukii, as an invasive species, arrives in native ecosystems that are generally assumed to be in an equilibrium or stable state. By entering these native communities, *D. suzukii* can lead to cascading effects through multiple ecosystem components (Figure 2).

1.3.1. On plant reproduction and seed dispersal

Infestation of ripening fruit by *D. suzukii* may cause premature fruit abscission while the plant seeds are still immature (Walsh et al. 2011; Poyet et al. 2014). Larval development is known to cause fruit abscission in other insect-fruit systems. For instance, infestation by the plum curculio, *Conotrachelus nenuphar*, induces premature fall of plums and apples (Levine and Hall 1977). Similarly, infestation by

the mango seed weevil, *Sternochetus mangiferae*, increases fruit drop during early fruit development (Follett et al. 2002). If this also occurs with *D. suzukii* infestation, many plant species will be at risk of having their reproduction pattern impacted, with potential increase in competition (for space, light, nutrients) between plant parents and offspring (Poyet et al. 2015).

In addition to fruit abscission, fruit that are infested by *D. suzukii* also experience accelerated decay, reducing their attractiveness to frugivorous species such as birds and mammals (Herrera 1982; Manzur and Courtney 1984; Dixon et al. 1997; Garcia et al. 1999). Animal- and bird-mediated dispersal allows many plants to reproduce and colonise new habitats, with birds being the main vectors for fleshy fruit in Europe (Herrera 1982; Garcia et al. 2010; Rey et al. 2014). Poyet et al. (2015) discuss potential shifts in the functional composition of forest communities, with plants infested by *D. suzukii* being disadvantaged compared to those reproducing through vegetative organs or seeds that are not attacked by the fly. In ecosystems invaded by *D. suzukii*, patterns of plant distribution may shift towards greater dominance of plants relying on anemochorous and ectozoochorous processes rather than endozoochory (Poyet et al. 2015). The effect of *D. suzukii* infestation on plant reproduction and dispersal requires further research that could, for instance, compare naturally damaged plants with plants protected from *D. suzukii* (Hendrix 1988).

1.3.2. On invertebrate

By infesting unripe and ripe fruit, *D. suzukii* acts as a pioneer species further facilitating infestation by both other drosophilids and microorganisms (Poyet et al. 2014; Rombaut et al. 2017). By piercing the fruit skin with its serrated ovipositor, females create an entry point for other drosophilids that do not possess this ability, such as *D. melanogaster* in grapes (Rombaut et al. 2017) or *D. subobscura* in *Prunus serotina* (Poyet et al. 2014). Oviposition also inoculates yeasts into the fruit that contribute to acceleration of the decaying process while supporting larval development (Cini et al. 2012; Hamby and Becher 2016).

It is likely that the facilitation of other insect species occurs in several host plants of *D. suzukii,* as we found in mistletoe (Deconninck et al. 2024a - Chapter 2), although this requires further investigation. Overall, the population of decomposers is likely to increase as a result of *D. suzukii* infestations of previously healthy fruit. Fruit that are infested and fall on the ground because of premature abscission are less likely to be eaten by birds (Roche et al. 2023). The resulting increase in the quantity of decomposing matter is, in turn, likely to impact the nutrients fluxes in the wider ecosystem (Weisser and Siemann 2008; Poyet et al. 2015).

The presence of larger numbers of drosophilids may also lead to increased populations of their natural enemies, including parasitoids, with unpredictable outcomes, particularly for other native prey/host species (Fleury et al. 2004). Conversely, some predator populations such as macroarthropods (Wolf et al. 2018; Englert and Herz 2019; Ulmer et al. 2021), birds (Carroll et al. 2023) or bats (Dekeukeleire et al. 2020) could be supported.

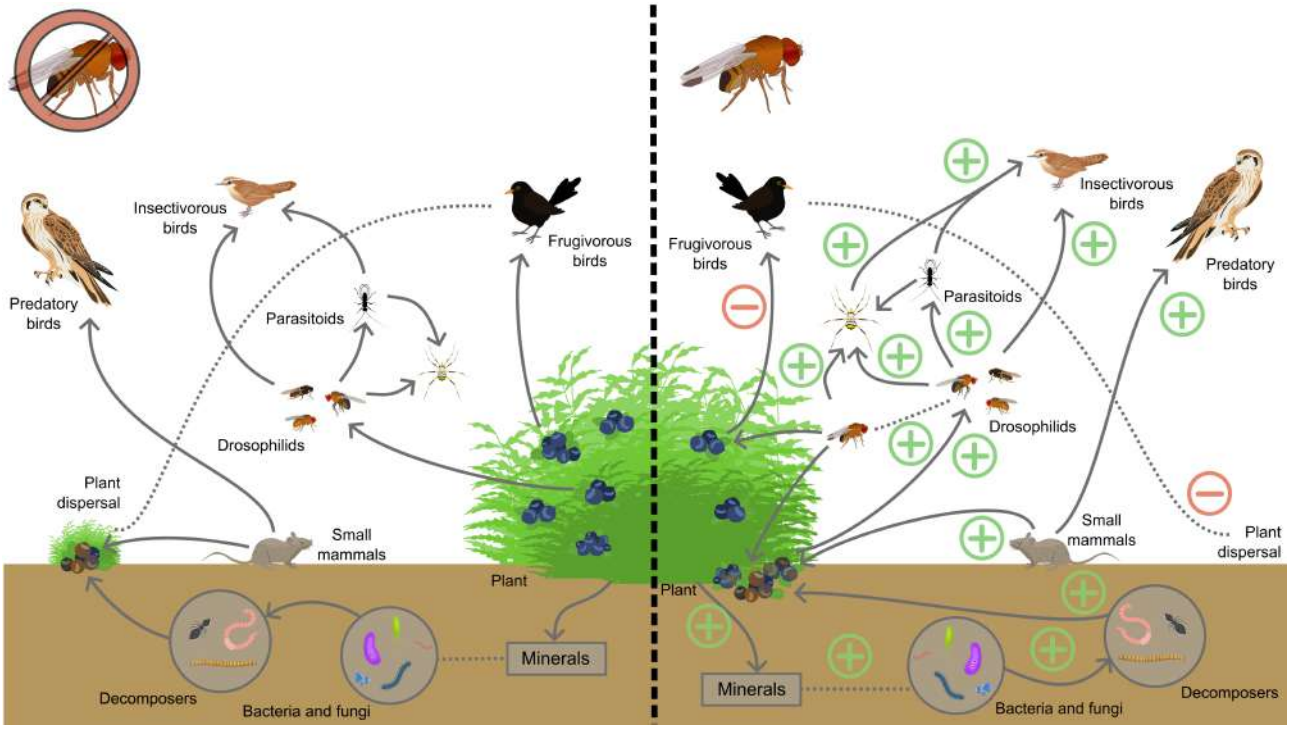

Figure 2 Hypothetical cascading effects of *D. suzukii* infestations on plant reproduction and on insects and seed/propagule dispersing communities. The left panel illustrates a native ecosystem equilibrium, and the right panel illustrates the same ecosystem after invasion of *D. suzukii*, indicating negative (red) or positive (green) impacts on communities.

1.3.3. On seed dispersers

The potential impact of *D. suzukii* on dispersers could influence both plant reproduction and the dispersing organisms. By damaging healthy fruit, *D. suzukii* is likely to reduce the resources available to dispersers, including birds and mammals. Research has started to address this subject. For instance, Roche et al. (2023) reported that relative abundances of the eastern towhee (*Pipilo erythrophthalmus*) and hooded warbler (*Setophaga citrina*) were negatively correlated with *D. suzukii* abundance and with fruit infestation rate, respectively. This is concerning as, after the breeding season, many songbirds face intense energetic demands to build the fat deposits needed for successful migration (Cyr et al. 2008; Roche et al. 2023). Consequently, they rely on the availability of abundant fruit resources at this time of the year

(Thompson and Wilson 1979; Parrish 1997). However, the autumn period also coincides with *D. suzukii* peak abundance and infestation (Arnó et al. 2016; Pelton et al. 2016; Wang et al. 2016). Some of the bird species included in Roche et al.'s (2023) study were not correlated with *D. suzukii* abundance or infestation, with other factors (vegetation cover and date) having greater impact. Roche et al. (2023) suggested that year-round residents may be more at risk from *D. suzukii* presence than migratory species, but this requires further investigation. Many bird species utilise unharvested fallen fruit in orchards (Bouvier et al. 2020) and the infestation of *D. suzukii* (as shown in Deconninck et al. 2024b - Chapter 1) could also impact these communities.

The presence of *D. suzukii* and the increased abundance of fallen fruit on the ground due to infestation may also directly benefit species such as insectivorous birds and small mammals, respectively. Studies of fruit flies (Tephritidae) have revealed that fruit infestation positively influenced rodent foraging (Wilson et al. 2012). This was suggested to be due to the ease of consuming softer decaying fruit, contrary to firm fruit that require more chewing and may be more difficult to digest (Sallabanks and Courtney 1992). Decaying fruit with stronger odours may also be easier to locate for foraging mammals (Wilson et al. 2012). Network and ecosystemic approaches should be considered in future studies of the effects of *D. suzukii* in invaded communities, considering consequences on host plant reproduction and communities of parasitoids, predators and dispersers using trophic networks that are invaded and non-invaded by *D. suzukii*.

2. THERMAL PREFERENCE AND INSECT DISTRIBUTION

In this section, I discuss and develop questions that remain open around the thermal preference of ectotherms. In particular, I emphasise the need for improved knowledge of the range of temperatures experienced by organisms at microclimatic scale in their natural environments and for the development of approaches to measure thermal preference in semi-natural conditions. I develop why studying behavioural thermoregulation strategies can improve the assessment of species vulnerability to climate change. I also encourage integrative studies including both behavioural thermoregulation strategies of organisms and other traits of organisms, such as thermal limits, metabolisms or desiccation resistance, to better understand mechanisms that allow them to cope with environmental variability. In support of this, I briefly present some preliminary results from a comparative study between insect species collected in Spain during an international collaboration project with Cameron Ghalambor (Professor, NTNU, Norway).

2.1. Thermal preference in natural conditions

Thermal preference of arthropods is often measured using thermal gradients in the laboratory, which limits the generalisation of the results at broader ecological scales (Kinzner et al. 2019). While the framework we designed and applied in Chapter 6 allows the prediction of the potential behavioural thermoregulation strategies of any species, assessments of these responses in natural conditions are not available. To do so, two complementary steps are required: (i) knowledge of the temperatures to which organisms are exposed in the natural environment (i.e. at microhabitat scale) and (ii) analyses of whether the responses of organisms in nature are comparable to those obtained in the laboratory.

2.1.1. Exploitable range of temperatures

In the natural environment, insects are exposed to varying atmospheric temperatures when flying, when in contact with surfaces and to internal substrate temperature during development. Wide variations in temperature can occur within and between microhabitats resulting from, for instance, exposure to solar radiations, surface/substrate colour and hydration, or size of the substrate (Pincebourde et al. 2007; Saudreau et al. 2009). In studies of thermal preference, determination of the actual range of temperatures that insects can be exposed to and exploit is vital, but remains an under-researched area.

In Chapters 2 and 3, *D. suzukii* emerged from fruit on the ground. Infestation of these fruit close to the soil surface is likely to buffer air temperature, even more when covered with leaf litter or snow (Pauli et al. 2013; Wallingford et al. 2018). This is consistent with the winter ecology of *D. suzukii,* finding shelters in microhabitats such as woodland and hedgerows (Rossi-Stacconi et al. 2016; Tait et al. 2021; Ulmer et al. 2024). Adult flies probably use the buffering environments provided by leaf litter, tree bark or man-made structure (Wallingford et al. 2018). Better characterising the temperature heterogeneity of habitats seems vital to improve our understanding of insect ecology and behaviour.

Furthermore, it would be particularly relevant to record the variation of temperature in various microhabitat according to different climatic parameters and microhabitat traits. For instance, records of the temperature at the surface and within the fruit of *D. suzukii*'s host plants, as well as in fruit of different size or colour and under different local conditions of exposure to solar radiation. Appropriate frequencies and durations of such temperature records combined with measurement of surrounding air temperature would allow the modelling of fruit temperature over different timescales from the daily to the seasonal. Such models could be used to assess the range of temperatures to which adults and larvae are

exposed to, as it was already done in apple fruit (Saudreau et al. 2011). These approaches allow the assessment of thermoregulation behaviour. In the codling moth, *Cydia pomonella*, they revealed an heterogeneity of temperature inside apple fruit which was exploited by larvae that preferred the warmer parts of the apple (Kührt et al. 2005).

As an example of the potential of this type of microclimatic study, within the DroThermal project (https://www.drothermal.cnrs.fr/) we have started recording microclimatic temperatures in different fruit. We have also performed a preliminary experiment using fallen apple fruit to assess the potential warming resulting from microbial proliferation during the decay process that could potentially contribute to thermal buffering in winter. In this simple experiment, six apples were scarified, to mimic a natural fall from the tree and decay, and were placed in experimental cages in a grassy garden with thermocouples positioned in the healthy and decaying parts of the apples and also recording surrounding environmental temperature. The results of this preliminary study indicated that the apples were almost always warmer than ambient air and that differences between the decaying and healthy parts were low (Figure 3). Therefore, the use of fallen fruit on the ground could protect insects, including the Drosophilidae

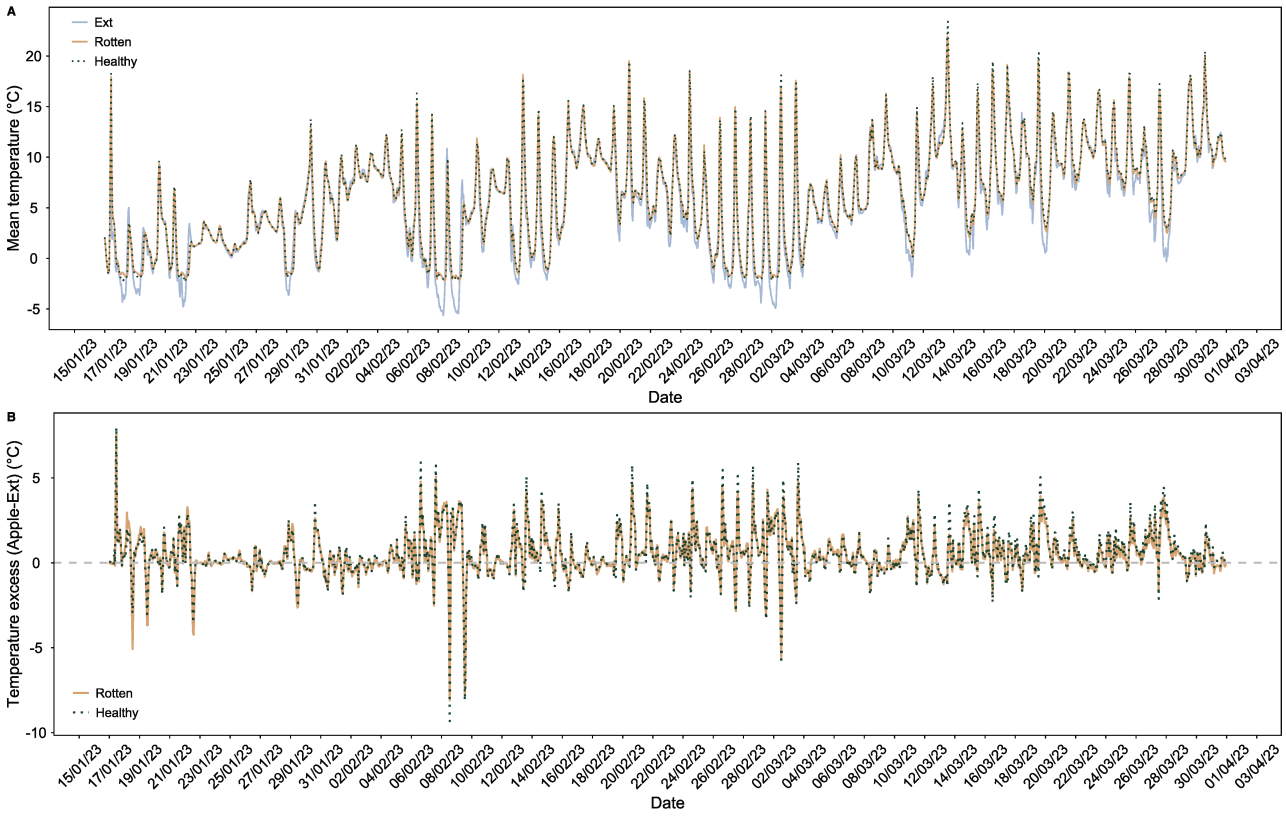

Figure 3 Variation of minimum (Temp_min), mean (Temp_mean) and maximum (Temp_max) temperatures (°C) in experimentally damaged fallen apples between January and March 2023. Surrounding temperature (Ext) is shown in blue, decaying apple in brown (Rotten) and healthy apple in green (Healthy).

community described in Chapter 1 (Deconninck et al. 2024b) in apples, from potentially harmful cold temperatures in winter, allowing their development. While records of atmospheric temperature would not have support possible development, microclimatic records can lead to different conclusions.

2.1.2. Thermal preference under (semi-)natural conditions

Experimental assessment of thermal preference in the laboratory places insects into artificial systems and removes them from the influence of most environmental variables, including solar radiation or wind exposure. Such factors can strongly influence the temperature of surfaces (Saudreau et al. 2009) as well as the body temperature of insects by impacting heat transfer processes (e.g., convection, radiation, conduction) (Pincebourde et al. 2007, 2021). Consideration of the influence of more natural conditions in the measurement of thermal preference would increase the ecological relevance of the data obtained.

To study the thermal preference of insects under natural or semi-natural conditions, a key requirement is to be able to recognise and follow individual insects. In *D. suzukii*, several techniques have been developed to achieve this (Klick et al. 2015, 2016; Clymans et al. 2020). The most reliable and least damaging approach is considered to be the use of fluorescent dust (Clymans et al. 2020). In principle, flies could be reared under different temperature regimes (as done in the studies presented in Part 2 of this thesis), then emerging adults marked with the dust and released into a large cage/enclosure containing a host plant bearing fruit. The temperature landscape throughout the enclosure could be documented at fine scale using multiple thermocouples and classic and thermal cameras used to film the experiment. Flies could be followed using specialist software such as AnimalTA (Chiara and Kim 2023) to document their thermal history and any final positions selected.

Comparing results between laboratory and semi-natural thermal preference studies would provide important insights into the relevance of thermal preferences measured for *D. suzukii* in the laboratory. In *D. nigrosparsa*, thermal preference measured in the laboratory differed strongly from that in the field, with the two approaches leading to different conclusions about preferred and experienced temperatures (Kinzner et al. 2019). These results were mainly a consequence of behaviour perturbation induced by competition, predation pressure and other non-thermal stressors (Kinzner et al. 2019). Working in semi-natural conditions with competitor and predator exclusion allows better control of these parameters.

2.2. Behavioural thermoregulation in insects

While critical temperatures (CTmin and CTmax) are commonly used in studies of species

distribution patterns or to predict responses to climate change (Huey et al. 2012), thermal preferences (Tp) are rarely considered (Sinclair et al. 2016). However, the behavioural thermoregulation abilities of species could provide an important element of responses to environmental variations, including reducing the susceptibility to climate change or facilitating range expansion. Synergies between climatic features, such as temperature and relative humidity, could favour particular behavioural thermoregulation strategies (e.g., precision at optimum or at extreme, as discussed in Chapter 4). Furthermore, Tp might be correlated to other traits such as CTmin, CTmax, respiration rates or desiccation resistance, and more importantly this level of correlation could differ largely between species, subjects that require further investigation.

2.2.1. The crucial role of behavioural strategy in climate change responses

Global change results in increases in temperature and occurrence of extreme events like drought, fire or floods (Pecl et al. 2017). Most organisms are impacted, either adapting, migrating or becoming extinct (Gienapp et al. 2008; Pecl et al. 2017). Steep declines in insect populations around the world have been reported (Wagner et al. 2021) while, conversely, many invasive pest species are expanding their geographic ranges, in turn threatening biodiversity and agriculture (Skendžić et al. 2021; Clarke and McGeoch 2023). Insects, as ectotherms, are particularly susceptible to climate change because their body temperature generally varies with environmental temperature (Woods et al. 2015). Correlations exist between climatic variables and insect population changes (Halsch et al. 2021; Forister et al. 2018), but the mechanisms underlying these correlations generally remain unclear The physiological sensitivity and tolerance of organisms are admitted to be key factors (Huey et al. 2012; Sunday et al. 2014; Seebacher et al. 2015), but most approaches do not incorporate water loss, another critical physiological parameter responding to temperature increase (see section 2.2.3), neither behavioural thermoregulation abilities of organisms.. Better understanding of environmental drivers and physiological responses are required to enable better predictions about future insect population trajectories.

In Chapter 4, we developed a conceptual framework allowing the determination of behavioural thermoregulation strategies, including 'precision at extreme' or 'precision at optimum' for instance, that also allow the determination of how precise an organism can be to select a temperature. Results on the insect model *D. melanogaster* (Deconninck et al. under review b - Chapter 4) and on the invasive pest *D. suzukii* (Deconninck et al. in prep a - Chapter 5), indicated a dominant 'precision at extreme strategy', consistent with previous studies stating that behavioural thermoregulation is primarily used to escape

overheating and improve survival (Leith et al. 2024). Studying the behavioural strategy of ectotherms can also give powerful insights on the vulnerability of species to climate change. While recent studies alerted on the probable overestimation of ectotherms thermal limits because the ones for survival are above the ones for reproduction (van Heerwaarden and Sgrò 2021; Parratt et al. 2021; Grandela et al. 2024), studies on behavioural thermoregulation abilities of species can give more insights on the resulting vulnerability to climate change. In Chapter 5, our data suggest that while *D. suzukii* males might have low thermal limits for reproduction (Ørsted et al. 2024), as other *Drosophila* species (Jørgensen et al. 2006), they have a highly precise thermoregulation behaviour and actively select relatively low temperatures, which would protect them from heat-induced sterility. Critical thermal limits alone are clearly not sufficient to assess organisms' vulnerability to climate change and integrative approaches with determination of the local thermal heterogeneity available to organisms and of their thermoregulation ability are required.

2.2.2. The missing link between Tp and and other thermal biology metrics

In ectotherms, Tp vary with numerous physiological and environmental factors including body mass (Shepherd et al. 2008; Cabon et al. 2023), sex and reproductive status (MacLean et al. 2019; Deconninck et al. under review b - Chapter 4; Deconninck et al. in prep a - Chapter 5), age and life stage (Bowler and Terblanche 2008; Kingsolver et al. 2011; Colinet et al. 2013; Pincebourde and Casas 2015; Lockwood et al. 2018; Deconninck et al. under review b - Chapter 4; Deconninck et al. in prep a - Chapter 5), acclimation (Rajpurohit and Schmidt 2016; MacLean et al. 2019; Mascaro et al. 2019; Deconninck et al. under review b - Chapter 4; Deconninck et al. in prep a - Chapter 5), water status (Rozen-Rechels et al. 2019), circadian rhythm (Goda et al. 2014), diet quality or quantity (Hardison and Eliason 2024), altitude/latitude (Hodgson and Schwanz 2019), seasonality (Hadamova and Gvozdik 2011; Ortega et al. 2016), pathogens/disease (Beukema et al. 2021), the presence of symbionts such as *Wolbachia* (Arnold et al. 2019; Truitt et al. 2019; Hague et al. 2021; Deconninck et al. in prep a - Chapter 5), social behaviours (Stapley 2006) and the presence of prey/predator cues (Smolinsky and Gvozdik 2012). Globally, however, the plasticity level of Tp remains understudied and poorly conceptualised (Gvozdik 2012) and particularly how physiological traits are linked to Tp (Killen 2014).

Studying correlations (covariations, links) between traits allow prediction of their potential evolutionary trajectories (Goulet et al. 2017; Castañeda et al. 2019), as traits that covary cannot evolve freely, with selection working upon them as a single unit (syndrome; Sih et al. 2004). Multivariate approaches that consider variations within and among traits are required (Castañeda et al. 2019). Some

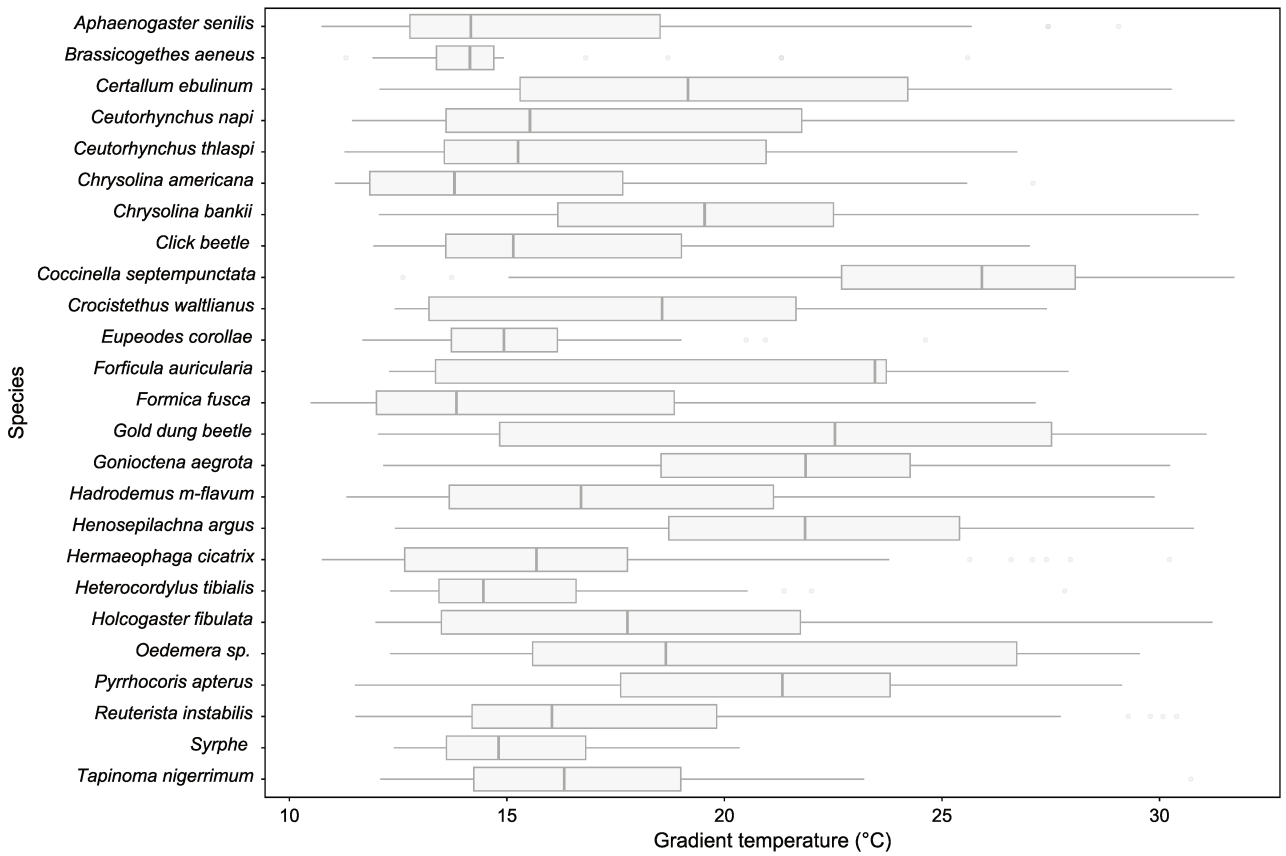

Figure 4 Distribution of insects in the thermal gradient reflecting their thermal preference.

have been used recently to estimate the (co)variation among thermal traits but mainly focused on a single trait such as desiccation resistance or upper thermal limits (van Heerwaarden and Sgrò 2014; Blackburn et al. 2014; Hangartner and Hoffmann 2016). Studies that investigated correlations between Tp and other thermal traits mainly focused on optimal performance temperature (Bauwens et al. 1995; Artacho et al. 2013; Goulet et al. 2017), on critical thermal limit (Goulet et al. 2017) and particularly upper limit (Castañeda et al. 2019) and on metabolic rate (Killen 2014; Norin et al. 2024). The presence/absence of correlations seems to be trait and species dependent. In the delicate skink, *Lampropholis delicata*, Tp was not correlated to thermal traits of the TPC (Goulet et al. 2017). Similarly, in *D. subobscura*, Tp was not correlated to CTmax (Castañeda et al. 2019). In the common minnow, *Phoxinus phoxinus*, the standard metabolic rate (SMR) was negatively correlated with Tp (Killen 2014) while in Chapter 6 we did not find any correlation between Tp and SMR in *D. suzukii*. Data are clearly missing on correlations between thermal traits and, again, integrative approaches are needed, considering different methods, species, taxonomic group of ectotherms and also the influence of different endogenous and exogenous factors on these potential correlations that are overlooked (Goulet et al. 2017). Underlying the physiological mechanisms involved in the variation of thermal trait across sympatric species is crucial to predict climate

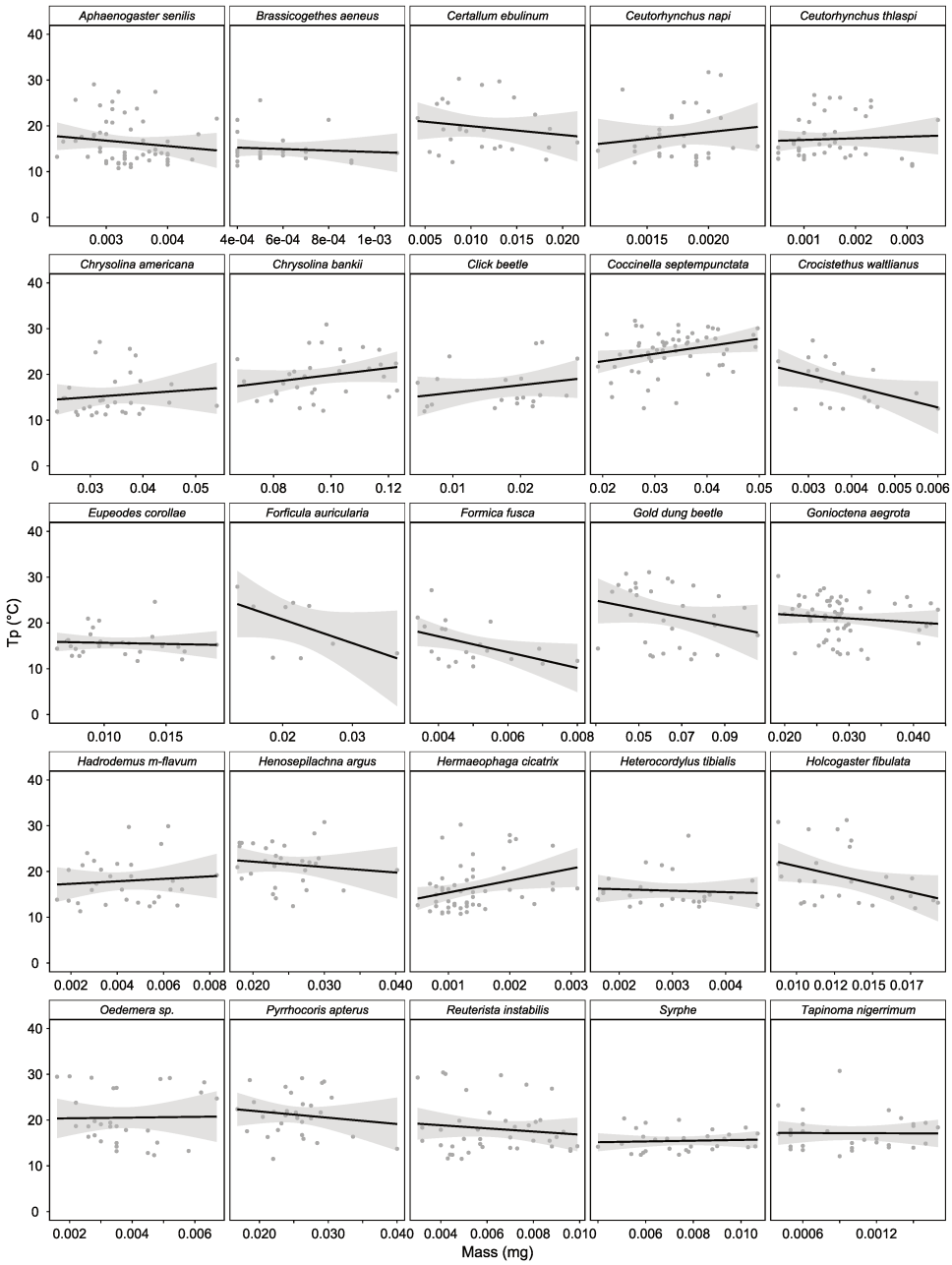

Figure 5 Correlation between insect thermal preference (Tp, °C) and mass (mg) according to species. orders (Coleoptera, Dermaptera, Diptera, Hemiptera, Hymenoptera) (Appendix 2).

change effects on ecosystems at both species and community levels (Pörtner and Farrell 2008). In the next section, I present some preliminary data where I started to take up this challenge.

2.2.3. Preliminary data from Spain

Knowledge of how physiological traits vary geographically is essential for understanding the process of adaptation and is critical to explain why some insect species are more vulnerable to climate change while others benefit from it (Huey et al. 2012). To date, researchers have not investigated how thermal limits, metabolism, water loss and behavioural thermoregulation strategies change together in response to

temperature across insects occupying different climatic niches. As an illustration of a practical initial approach to addressing such questions, in spring 2024 I collaborated with Cameron Ghalambor's team (from Norwegian University of Science and Technology, NTNU) working on the project 'Physiological basis of insect community responses to climate change' (https://prosjektbanken.forskningsradet.no/en/project/FORISS/326233). The aim was to study the trade-off between CTmax, metabolism, desiccation resistance and Tp in a range of insect species, with differing climatic niches, functional characteristics and taxonomy to better assess the link between these physiological traits and if they drive the vulnerability of species to climate change.

Insect collection took place from March 15 to April 15 at low elevation (600 m a.s.l.) in Parque Regional del Sureste, southeast of Madrid, Spain, and from April 15 to May 15 at high elevation (1200-1400 m) around the Biological Station Ventorrillo, northwest of Madrid, Spain. Each afternoon, ~30-40 insects from different taxonomic groups were collected and identified using relevant keys. Environmental variables recorded included temperature and humidity using weather stations and locally placed data-loggers, as well as information on the insects' habitat (e.g., host plant, soil type) and microhabitats (e.g., shade, sun) and insect colour. Insects were acclimated at 20°C for 18 h before Tp measurement the following morning. The thermal gradient described in Chapter 5 was used across a range of 8 to 35°C to measure individual Tp. After Tp measurement, insects were weighted on a laboratory precision balance (0.1 mg). Tp and body mass can be correlated in some species (Shepherd et al. 2008; Alfaro et al. 2013), as the ability and need to thermoregulate can depend on body mass and closely associate surface/volume ratio of individuals (Shepherd et al. 2008). In total, the Tp of 942 insects was measured during the two months of fieldwork, 485 from the low elevation area and 457 from the high elevation. The full dataset included 42 species from five different insect orders (Appendix 2). Only species with sample size ≥ 15 were kept for further analyses (25 species). Some of these species were also used in parallel experiments to measure CTmax, desiccation resistance and metabolism. The distributions of species across the thermal gradient varied widely between insect species (Figure 4), Some species were distributed across the gradient (e.g., *Pyrrhocoris apterus*) while others showed much narrower preferences (e.g., *Eupeodes corollae*). Correlations between Tp and insect mass differed between species (Figure 5), with examples of negative (e.g., *Crocistethus waltianus*), positive (e.g., *Coccinella septempunctata*), or no correlation (e.g., *Eupeodes corollae*).

These preliminary data already demonstrate that some species seem to be thermal specialists while others are generalists when considering their Tp range. The thermal specialists all have relatively low Tp. Further analyses will search for correlation between Tp and temperatures recorded from the weather stations or hobo at different temporal scales (e.g., temperature recorded at sampling time, mean temperature of sampling day, mean temperature of the week before sampling, etc). Also, future analyses will determine the behavioural strategy of every species to highlight the variability in thermoregulatory abilities at community scale. One of the most interesting parts will also be to search for correlations between all recorded traits including CTmax, desiccation resistance and metabolism. Species that are more specialist with narrow range of Tp might be more sensitive to desiccation and / or have lower CTmax than thermal generalists. The difference observed between species could also be due to genetic differences and quantitative genetics could provide insights about the phenotypic variance of Tp and its link with other thermal traits (as performed in Castañeda et al. (2019) for instance).

3. ROLE OF *WOLBACHIA* IN *D. SUZUKII* BIOLOGY

The role of *D. suzukii*'s *Wolbachia* strain, *w*Suz, has been investigated in various studies, including in this thesis. Most studies to date have been inconclusive about whether the bacteria acts as a mutualist, a parasite or is neutral, although evidence such as that reported here in Chapters 6 (metabolism lowering), 8 and 9 (emergence success increase) provide stronger support for a mutualistic role. The costs and benefits associated with *Wolbachia* infection may not be black and white, for instance only becoming apparent under certain (combinations of) conditions that are difficult to identify, as is the case for symbionts that do not manipulate reproduction but are maintained at intermediate to high frequency (Zug and Hammerstein 2015; McPherson et al. 2023). I consider what is known more widely about the symbiosis and important unresolved issues, including the prevalence of *w*Suz in natural populations, the known effects of *w*Suz on *D. suzukii* and areas of future research that will be important in advancing understanding in this field.

3.1. Prevalence in natural populations

The prevalence of *w*Suz in *D. suzukii* populations is known to be highly variable both between geographical regions and between generations (Hamm et al. 2014; Cattel et al. 2016a; McPherson et al. 2023). Assessments of regional prevalence are only available over around the last ten years, which limits understanding of *w*Suz dynamics and role in populations.

3.1.1. Geographical differences

In field populations, *w*Suz maternal transmission is imperfect with *D. suzukii* females producing 5–10% infected progeny on average (Hamm et al. 2014). The prevalence of the bacteria varies widely between populations and also across continents. In the United-States, infection frequencies from California and New York ranged between 7 and 23% with an outlier frequency of 58% (Hamm et al. 2014). In Europe, the prevalence of *w*Suz in *D. suzukii* populations from eight countries varied between 0 and 100%, with most values ranging between 17 and 85% and a mean of 46% (Cattel et al. 2016a). In contrast with some other *Wolbachia* strains, *w*Suz does not induce cytoplasmic incompatibility (CI) nor manipulate *D. suzukii* reproduction (Hamm et al. 2014; Cattel et al. 2016a). The imperfect maternal transmission and the absence of CI combined with a (a priori) stable infection frequency suggested that *w*Suz gives a fitness advantage to its host (Hamm et al. 2014; Cattel et al. 2016a). It was estimated that this selective advantage should be around 20% according to the prevalence in the United-States (Hamm et al. 2014). Based on the estimation approach used in that study, the selective advantage of *w*Suz in European populations would be around 55%. Since the 2014-2016 studies of *w*Suz prevalence in American and European natural populations of *D. suzukii*, no new assessment was performed.

3.1.2. Variation between generations

Hamm et al. (2014) suggested that the differences in *w*Suz infection frequencies between eastern and western American populations could be due to founder effects. If this is the case, they predicted that the then low prevalence in the eastern populations would increase over time, after a few generations, to reach the higher prevalence of the western populations, based on the apparent selective advantage of hosting *Wolbachia*. However, the evolution of *w*Suz prevalence in natural populations has yet to be researched in any region. At a shorter time-scale, McPherson et al. (2023) reported that *w*Suz prevalence in a given population would change by over 30% in a single generation, decreasing in early summer and increasing as summer ended. This highlights the potential interplay between environmental conditions and the costs and benefits of infection mentioned above. Better understanding of the dynamics of *w*Suz infection in natural populations over both short and long time-scales is required in order to decipher the role of *Wolbachia* in *D. suzukii*.

3.1.3. Recent assessment in France

As part of the ongoing DroThermal project, one task was to assess the prevalence of *Wolbachia* in natural populations of *D. suzukii* from different parts of France across seasons (Laurence Mouton and Hélène Henri, unpublished data). Between November 2021 and May 2023, 20 females and 20 males were collected in each of Amiens, Lyon, Rennes and Tours in November, March and May and the prevalence of *Wolbachia* was assessed using PCR. Results were surprising as the mean prevalence of *Wolbachia* was very low (~6.1%) compared to the first assessment in 2016 (~46%; Cattel et al. 2016a). No seasonal or regional patterns were significant in the data obtained (Kruskal-Wallis test, p = 0.164), even if the prevalence, ranging from 4.2% in Amiens to 8.4% in Lyon, seems to decrease with latitude (Figure 6). These results, and especially the low prevalence values, are not consistent with the above-mentioned expected selective advantage of hosting *Wolbachia* for *D. suzukii*, and there is no obvious explanation for the considerable decline in prevalence between 2016 and 2021. Further similar studies are required across other parts of the species' invaded and natural ranges to explore the drivers of infection prevalence.

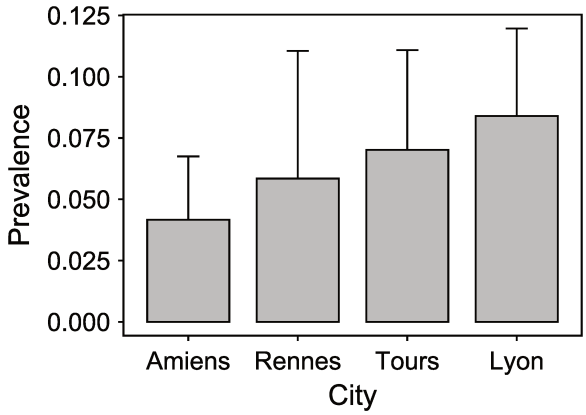

Figure 6 Mean prevalence of *Wolbachia* in natural populations of *D. suzukii* sampled (20 males and 20 females) in Amiens, Rennes, Tours and Lyon (France) between November 2021 and May 2023. There was no difference between cities (Kruskal-Wallis test, p = 0.164). Raw (unpublished) data provided by Laurence Mouton and Hélène Henri, LBBE laboratory, Lyon (France).

3.2. *Wolbachia w*Suz: Mutualist, parasite or neutral?

Most data obtained on the role of *w*Suz in *D. suzukii* so far indicate overall positive effects resulting from the bacterial infection, as also apparent in Chapters 6, 8 and 9 of my thesis. As noted above, the strength of these effects appears to be related to the physiological state of the fly and/or the environmental conditions to which it is subjected.

3.2.1. Influence on life history traits

Mutualistic symbionts can act on nutritional supply, pathogen protection or tolerance to abiotic stressors to increase their host fecundity and/or longevity (Zug and Hammerstein 2015). In *D. suzukii*, most of these effects have been explored. Female fecundity is either reduced or not impacted by *w*Suz infection (Hamm et al. 2014; Deconninck et al. 2024c - Chapter 8), but there is an increase in egg-to-adult survival resulting in overall higher emergence rates (Mazzetto et al. 2015; Deconninck et al. 2024c - Chapter 8). Adult longevity does not appear to be influenced by *w*Suz infection (Mazzetto et al. 2015) and infected individuals do not show increased starvation tolerance or desiccation resistance (Hamm et al. 2014) but do show increased thermal tolerance (Saeed et al. 2018). Infection by *w*Suz protected *D. suzukii* against two RNA viruses, *Drosophila* C virus and Flock House virus, reducing the virus titres and increasing host survival (Cattel et al. 2016b). Finally, investigation of the potential nutritional role of *w*Suz was explored in this thesis (Chapters 8 and 9), identifying that the benefits of *w*Suz on offspring fitness (survival, mass, development time) existed and were amplified after a diet shift (Deconninck et al. 2024c - Chapter 8; Destierdt et al. in prep - Chapter 9). These findings suggest that *Wolbachia* infection contributes to the effectiveness with which the polyphagous *D. suzukii* adapts to the nutritional variability of the different host fruit that it uses.

Generally, symbiont infections are associated with both costs and benefits (Evans et al. 2009; Clavé et al. 2022). However, the results reported in this thesis seem to indicate that the cost of hosting *Wolbachia* is low or undetectable for *D. suzukii*. Infection by *Wolbachia* did not impact the total energy content of flies (Deconninck et al. in prep b - Chapter 6) and either had no effect on metabolic rate or led to some reduction (Deconninck et al. in prep b - Chapter 6). Reductions in metabolic rate could either be advantageous, as lower metabolic rate is generally associated with increased longevity (Mołoń et al. 2020), or disadvantageous, as in ectotherms, a lower standard metabolic rate would likely reduce metabolic scope and hence levels of activity achievable.

3.2.2. Influence on behaviour

Wolbachia infection also influenced the behaviour of *D. suzukii* (Deconninck et al. in prep a - Chapter 5). Infected individuals had lower thermal preference than uninfected. One result of this could be to slow *Wolbachia* proliferation in tissues (Truitt et al. 2019). Reduction of Tp induced by *Wolbachia*, as found here for *D. suzukii*, has previously been reported in *D. melanogaster*, with the magnitude of the

reduction ranging from 2 to 8°C depending on the *Wolbachia* strain (Truitt et al. 2019). However, such effects may not be universal as a later study did not find any effect of *Wolbachia* strain *w*MelCS on Tp in *D. melanogaster* (Strunov et al. 2023). Authors argued that factors such as humidity, diet and design of the gradient device can influence Tp measurements and alert on the necessity of a careful experimental design and cautious interpretations of Tp measurements together with a thorough description of the methods and equipment used to conduct behavioural studies (Strunov et al. 2023).

In this thesis (Chapter 9), *Wolbachia* infection also influenced oviposition choice of females in some circumstances. The oviposition preference of infected and uninfected females between two fruit changed according to the temperature, especially at low temperature. Infected females also laid more eggs at low temperature than uninfected ones did. Influencing oviposition choice could be interpreted as a bet-hedging strategy (Hopper 1999), whereby *Wolbachia* induces behavioural changes in *D. suzukii* to maximise its prevalence (Destierdt et al. in prep - Chapter 9).

3.2.3. Influence depending on conditions

In my thesis, in most cases when *Wolbachia* had a detectable effect, this occurred under certain conditions, for instance, under the challenge of a diet shift which could induce nutritional stress (Deconninck et al. 2024c - Chapter 8; Destierdt et al. in prep - Chapter 9), or of different temperature regimes (Deconninck et al. 2024c - Chapter 8; Destierdt et al. in prep - Chapter 9). This may indicate a direct effect of temperature on the titre of *w*Suz in *D. suzukii*, as an increase in temperature would accelerate the proliferation of the bacteria, although studies are yet to address this subject. Furthermore, because the effects of *Wolbachia* vary in time and space, it is challenging to anticipate the dynamics of its prevalence in nature. Experimental studies are reaching their limits and a high resolution monitoring would be crucial to better identify the role and the selective advantage of hosting *Wolbachia*.

Apparent discrepancies in the findings of different studies of the role of *w*Suz in *D. suzukii* could result from various factors including the influence of *Wolbachia* titre in flies and of both host or *w*Suz genetic background. *Wolbachia* titre can vary greatly between individuals (Ahantarig et al. 2008; Unckless et al. 2009; Ponton et al. 2015). For instance, in *Drosophila innubila*, variation in relative *Wolbachia* density among wild flies was about 20 000-fold (Unckless et al. 2009). In *D. suzukii*, the density of *w*Suz has been rarely investigated. Cattel et al. (2016b) found higher relative density of *w*Suz in two Japanese fly lines compared to two French lines, but no differences between lines of the same geographical origin. The magnitude of the effects of infection on host life-history traits and protection against pathogens or abiotic

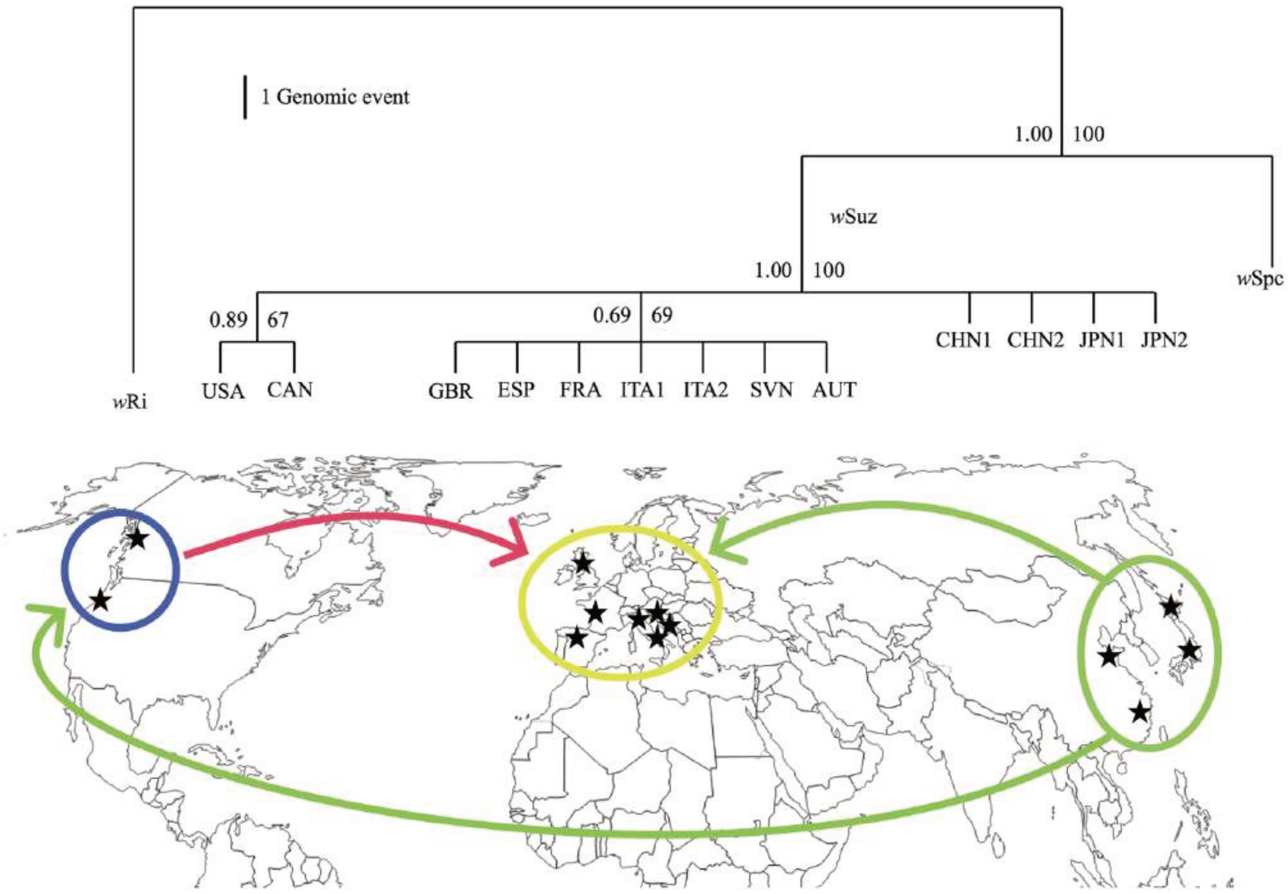

Figure 7 Genetic groups of *w*Suz are consistent with *D. suzukii* genetic groups and invasion pathways. Cladogram of *w*Ri, *w*Spc and *w*Suz *Wolbachia* strains from Kaur et al. (2017). On the left and on the right of each node are the Bayesian posterior probability and the percentage bootstrap support from TNT-based parsimony analysis, respectively. Black stars on the map represent each sampled population of *D. suzukii* used to produce the cladogram. Circles represent *D. suzukii* genetic groups and arrows the introduction pathways (based on Fraimout et al. 2017).

stresses has been correlated with within-host *Wolbachia* density (Ponton et al. 2015). For instance, across different *Wolbachia* strains, the level of antiviral protection is strongly correlated to the density of the bacteria in host tissues (Martinez et al. 2014). In *D. suzukii*, correlations between *w*Suz density and effects on fitness have been investigated for some traits (e.g., antiviral protection, fecundity) but none have been found to date (Hamm et al. 2014; Cattel et al. 2016b). Further research is required to explore the relationship between within-host *w*Suz density and effects of the infection on *D. suzukii* fitness. Differences in bacterial density could also contribute to explaining the temporal and spatial variations observed in *w*Suz prevalence, such as between American and European populations, as the transmission

fidelity of maternally transmitted endosymbionts impacts their population-level dynamics (Unckless et al. 2009), an area that again requires further research.

3.2.4. Host and symbiont genetic background

The genetic backgrounds of both *D. suzukii* populations and *w*Suz could also influence variations in *w*Suz prevalence (Cattel et al. 2016a) and also observed effects on life history traits (Kaur et al. 2017). Intra-strain genetic polymorphism of *w*Suz has been detected in different geographical populations of *D. suzukii*, especially between North America, Europe and Asia (Kaur et al. 2017) (Figure 7). The pattern of variation was consistent with the invasion pathways of the fly (see Introduction section 5.2.2), suggesting that it resulted from founding individuals carrying different *w*Suz variants when invading Europe and North America (Kaur et al. 2017). Environmental constraints could also have induced genomic changes in *w*Suz because of adaptation in new environments, meaning that another scenario could be that the geographical diversity of *w*Suz has resulted from bottlenecks and differential selective pressures in the two continents (Kaur et al. 2017). On the host side, there is also a certain degree of genetic diversity between *D. suzukii* populations, including between Europe and America (Adrion et al. 2014; Fraimout et al. 2017). As *Wolbachia*-induced phenotypes depend on both the genetic background of the host (Dean 2006) and of the symbiont (Sakamoto et al. 2005), and on the association of both host and symbiont genetic backgrounds (Iturbe-Ormaetxe and O'Neill 2007), the variable effects of *w*Suz reported in different studies could be underlain by a combination of *w*Suz polymorphism in populations and the genetic diversity of *D. suzukii*.

4. CONCLUSION

The significance of my thesis lies in its integrative approach, combining disciplines such as trophic ecology, thermal ecology, ecophysiology, insect-plant-symbiont interactions, community ecology and behavioural ecology in the context of biological invasions. This approach provides a broader perspective on complex phenomena such as the establishment and spread of an invasive species.

The data obtained clearly indicate the extraordinary adaptive plasticity of *D. suzukii*, which very likely contributed to its invasive success. Its broad polyphagy allows *D. suzukii* to exploit fruit resources available all year round, some of which are entirely free of competitors, while others are toxic and may be used to repel parasitoids. Its remarkable plasticity in behavioural thermoregulation enables the fly to precisely select temperatures that favour its development (or other physiological processes) and to avoid

(sub)lethal temperatures. Infection by *Wolbachia* offers benefits to *D. suzukii* under particular conditions and may have contributed to its range expansion during the early years of its invasion. Taken together, the results of my thesis help to unravel what makes an invasive species so successful (or to 'crack the code', see Appendix 1).

This work was collaborative and involved researchers from several laboratories, as well as passionate undergraduate and Master's students, who contributed their skills, knowledge, expertise, curiosity, and perspectives. This is also one of the strengths of my thesis: building a network that maximises the value of these three years of research.

> "Without the curiosity of the mind, what would we be? This is the beauty and nobility of science: an endless desire to push the boundaries of knowledge, to seek the secrets of matter and life without preconceived ideas of the possible consequences." Marie Curie

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Appendix

Appendix 1 • Cracking the code of Drosophilidae invasive success

Cracking the code of Drosophilidae invasion success

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ABSTRACT

Biological invasions, including that of insect pests, have continuously escalated in recent decades with ongoing climate change. Once, non-native species become invasive, it can deeply impact natural ecosystem stability, agroecosystem sustainability and result in severe economic losses. Invasive populations trigger cascading consequences for communities and ecosystems through the disruption of local species interaction networks. Amongst recent invasions, that of *Drosophila suzukii* is one of the most successful, yet not an isolated case. *Drosophila suzukii* remains an example among the 31 Drosophilidae species including species from the genus *Drosophila*, *Scaptomyza* and *Zaprionus*, reported invasive since 1900. Their specific features and capacities allowed them to overcome environmental barriers which result in the spread and establishment of populations. Our review aims to leverage the factors contributing to the invasion success of Drosophilidae, based on the considerable literature on this family. We first consider the selective forces experienced by pioneer individuals and how they manage the foundation of reproducing populations. Then we discuss the role of abiotic and biotic factors in shaping the invasion success. A nuanced understanding of how these constraints interact is essential for predicting and managing the proliferation of invasive Drosophilidae as well as other non-native species. We highlight 16 traits that facilitate the ability of a species to turn into an invasive, and emphasise future perspectives to fill crucial knowledge gaps to extensively succeed in cracking the code of Drosophilidae invasion success.

Keywords: Climate change, insects, invasion ecology, life history traits, phenotypic plasticity

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1. INTRODUCTION

Numbers of biological invasions have continuously escalated in recent decades with no sign of abatement (Seebens *et al*., 2017), especially of insects (Bonnamour *et al.*, 2021) and, notably, pest and disease agent species (Neupane *et al*., 2024). Furthermore, factors such as climatic change, habitat degradation and land use intensification threaten native biodiversity and increase opportunities for non-native species to establish (Pysek *et al*., 2020). There is growing evidence that biodiversity, in particular in cold and temperate regions, will be increasingly threatened by the synergy between climate change effects and biological invasions (Butchart *et al*., 2010). Ongoing climate change may also catalyse modifications in the status of some introduced populations from naturalised to invasive (Renault *et al*., 2018), with cascading consequences for communities and ecosystems through the disruption of local species interaction networks, and the propagation of pathogens via spillover effects (Vilcinskas *et al.*, 2019). In this context, non-native insect herbivores and predators are predicted to have the greatest impacts on biodiversity, partly because warming increases metabolic and feeding rates of these ectothermic species (Harvey *et al*., 2020).

Non-native species (also often referred to as exotic, non-indigenous or alien), once they become invasive, can deeply impact natural ecosystem stability, agroecosystem sustainability and result in severe economic losses (Diagne *et al*., 2021; Renault *et al*., 2022). The average annual cost was estimated at ~US$ 165 billion in 2020 (Renault *et al*., 2022). Without prompt and urgent action, the number and impacts of biological invasions will continue to rise, degrading natural capital and impeding sustainable development (Henry *et al*., 2023). Given the increasing impacts caused by invasive insects worldwide, comprehensive knowledge of the mechanisms underlying successful invasions is crucial. In Europe, amongst invasive species, insects are the second most represented taxa after plants (DAISIE, 2009). Many of these invasive insects originate from other continents (Roques *et al*., 2009) and their invasion routes are often related to the globalisation of human-mediated transport routes (Levine & D'Antonio, 2003). Three phases are commonly recognised in the invasion process: introduction, establishment and proliferation (Levine, 2008; Daly *et al.*, 2023). National and international human transportation increases the connectivity between distant regions and the probability of introduction of non-native species. Global warming further strengthens the success of establishment and invasion by reducing or removing the environmental barriers to the establishment of species after their arrival (Skendžić *et al*., 2021). The undeniably significant ecological, economic and societal consequences of invasions have catalysed research on

strategies to control invaders (Olson, 2006; Pimentel, 2011). Identifying effective means of control or mitigation requires detailed knowledge of the species concerned, including the biotic and abiotic factors associated with invasion success. This review aims to decipher to what extent deep fundamental knowledge of species is required to reveal the key factors and their interactions determining invasion success. We illustrate this challenge by focusing on the Drosophilidae, an insect family including some of the most studied invasive species with important economic impacts.

Among recent insect invasions affecting Europe, that of *Drosophila suzukii* has arguably been one of the most successful. This species, native to south-east Asia and Japan (Walsh *et al*., 2011), arrived in Europe and North America in the late 2000s (Walsh *et al*., 2011; Cini *et al*., 2012; Asplen *et al*., 2015). Since then, the species has spread widely across the globe, reaching South America in 2013 (Deprá *et al*., 2014), northern Africa and the Comores in 2017 (Hassani *et al*., 2020), Kenya in 2019 (Kwadha *et al*., 2020) and, most recently, South Africa in 2023 (IPCC, 2024). It appears to be only a matter of time before it reaches other parts of Africa and Australia (dos Santos *et al*., 2017; Ørsted & Ørsted, 2018) and further expands in northern latitudes as a consequence of climate warming (Ulmer *et al*., 2022). Several non-mutually exclusive hypotheses have been suggested to explain the spectacular invasion success of this agricultural pest species. The first is its ability to use vacant niches, with *D. suzukii* adapting perfectly to the western agricultural environment and facing very few competitors for fruit, or specialised predators and parasitoids (Chabert *et al*., 2012). Second, the species has a high capacity to tolerate a range of climatic conditions thanks to its ecophysiological and life history plasticity, enabling both rapid development and high reproductive potential (Gibert *et al*., 2016; Kirschbaum *et al*., 2020; Little *et al*., 2020). Third, *D. suzukii* can disperse widely, either by active flight, passive wind-assisted movement in the air column or anthropogenic assistance through association with the fruit trade (Cini *et al*., 2014). Finally, it can colonise both natural and anthropogenic habitats such as forests, hedgerows, fruit crops and urban ecosystems (Ulmer *et al*., 2022, 2024; Deconninck *et al*., 2024b), increasing its dispersal potential and broadening its ecological niche. Furthermore, *D. suzukii* shows a remarkable level of polyphagy (Poyet *et al*., 2015), both in cultivated and wild fruit, including those of invasive plants (Poyet *et al*., 2014). As a consequence of these traits, effective and sustainable control protocols remain to be identified and research is ongoing (Ulmer *et al*., 2020, 2021; Wang *et al*., 2021; Hogg *et al*., 2022; Fellin *et al*., 2023; Krüger *et al*., 2024). The example of *D. suzukii* illustrates the potential for invasion success to be

determined by multiple interacting factors, highlighting the need for fundamental and multidisciplinary research integrating different aspects of the species' biology and ecology.

Drosophila suzukii is only one example of the at least 31 Drosophilidae species that have been reported to be invasive since 1900 (Table 1), amongst which nine are currently considered cosmopolitan due to the scale of their global establishment. All these species, including representatives of the genera *Drosophila*, *Scaptomyza* and *Zaprionus*, can differ markedly in their behavioural, ecophysiological, trophic and seasonal ecology, implying that invasion success or failure is not linked to a single key feature but rather to a combination of traits and their interplay with the environmental conditions of the recipient ecosystems. Drosophilidae species are ideal models for studying the biology of invasion due to their great adaptability and rapid invasion success (Gibert *et al*., 2016). They have various feeding strategies: most are detritivorous, utilising bacteria, yeasts, micro- and macro-fungi, and feeding on decaying fruit (or sometimes dead animals), while others feed on healthy fruit. They have specific ecological and physiological features as well as plastic capacities that allow them to overcome environmental stochasticity and barriers, enter new areas and become established. Their wide distributions and interactions with diverse environments allow them to provide valuable models and tools for investigating the biological and ecological factors influencing the establishment and spread of invasive species and their impacts on species assembly rules in communities (Deconninck *et al.*, 2024b). The considerable literature on Drosophilidae can also serve as a valuable resource, offering insights into the factors contributing to their invasion success.

In this review, we leverage this knowledge to comprehensively explore the genetic, physiological and ecological determinants of invasion success of Drosophilidae species, with the aim of identifying broader principles applicable to invasive species dynamics more widely. We examine the global abiotic and biotic constraints that invading Drosophilidae can encounter during the invasion process. First, we consider invasion dynamics, comparing natural and human-facilitated routes of invasion. Arriving individuals experience selective forces which may be specific to each local site of introduction. Second, we discuss the role of abiotic factors, such as temperature, desiccation stress and the potential synergy between stressors, for the invasion success in new areas. Third, we discuss the role of biotic factors, including trophic niche, competition, predation, facilitation and symbionts in shaping invasion success. A nuanced understanding of how these constraints interact is essential for predicting and, ultimately, managing the proliferation of invasive Drosophilidae and other non-native species arriving in new environments. This review provides a

road map of the various processes that must be integrated to achieve a comprehensive understanding of successful biological invasions. Finally, we consider perspectives for addressing crucial knowledge gaps and opening new research avenues in the light of information collated in this review.

2. ROLE OF SPECIES TRAITS IN INVASION DYNAMICS

The invasion process of any non-native species beyond its native range consists of three main stages: (i) the movement of individuals from their native range to a new area; (ii) the foundation of a reproducing population by pioneer individuals; and (iii) the further spread of the introduced population to different locations, as well as possible development of local adaptations to different ecological habitats within the invaded area (Daly *et al.*, 2023). Each of these steps is critical in a successful invasion. Here, we summarise general aspects of these invasion steps before focusing on how they related to invasive Drosophilidae species traits.

2.1. Transportation and the introduction pathways

The anthropogenic dimension in biological invasions is unquestionable, with the geographic expansion of many invasive species tracking major routes of human travel and trade (the phenomenon of hitch-hiking; Verdasca *et al*., 2021; Lanner *et al.*, 2022). The propagation success of Drosophilidae species strongly depends on their association with human activities. The transport and dispersal of species such as *D. simulans* and *D. melanogaster* has been greatly facilitated by their close association with human activities such as in agrosystems or urban structures (Keller, 2007; Ulmer *et al.*, 2024). Indeed, *D. melanogaster* was found to be more abundant in fruit stores and domestic dwellings than in natural habitats (Ulmer *et al.*, 2024). Therefore, for species that have arrived recently in new areas, proximity to humans can be a significant positive factor promoting invasion success (e.g., overwintering in synanthropic habitats such as dwellings or stores, or the exploitation of fruit cultivated by humans). While *D. melanogaster* and *D. simulans* have dispersed naturally over a long period (see below), human activities, particularly unintentional transportation through trade, have significantly accelerated their global spread (David & Capy, 1988). Similarly, while adults of the invasive *Zaprionus indianus* can disperse long-distance using air currents, the transportation of infested commercial fruit is also recognized as a significant factor in their spread (Bragard *et al*., 2022), as is also the case for *D. suzukii* (Cini *et al*., 2014).

Drosophila melanogaster and *D. simulans* also demonstrate remarkable ability to disperse in different climatic regions without human intervention. Their ability to thrive in both tropical and temperate regions points to a particular sequence of selection regimes across the invasion process. The existence of a distinct Far Eastern genotype of *D. melanogaster* with unique morphological and physiological traits suggests a journey that may have begun long before the last glacial period, highlighting the long-lasting impact of independent evolution over time (David & Capy, 1988). Considerable variation exists in natural dispersal rates and distribution changes, both within and between species. An illustrative example is that of *D. subobscura*, which exhibited rapid north-south dispersal of ~850 km/year in the Americas during the first decade after introduction. In contrast, its east-west dispersal was only 85 km/year. This provides a classic example of accelerated dispersal in favourable environments (Williamson, 2009). The combination of such natural dispersal coupled with unintentional anthropogenic assistance has fuelled the successful global invasion of these Drosophilidae (David & Capy, 1988; Tidon *et al*., 2003).

2.2. The foundation of reproducing populations

A non-native species can only persist in its new range if pioneer individuals are able to form a reproducing population that will ensure the production of future generations. However, establishing a viable population with a small number of individuals can be challenging. A strong reduction in population size (*i.e.*, a demographic bottleneck) resulting from the introduction process can have important deleterious consequences increasing the risk of extinction (Fauvergue *et al*., 2012).

Small populations, such as those of recent introductions, can suffer from low-density Allee effects, which describe the way in which some components of individual fitness can decrease in low-density populations due to a reduction of positive interactions between conspecifics. For instance, in allogamous sexual species, individuals may not find mates when the population density is too low. Various traits related to reproduction (e.g., mate finding) or survival (e.g., foraging efficiency) may also be affected when population density is low (Berec *et al.,* 2007). For instance, in *D. melanogaster*, increased adult densities on substrates prior to larval development resulted in higher larval survival and larger emerging flies, likely due to reduced fungal growth on the substrate (Wertheim *et al*., 2002). However, this effect varies depending on the species or type of substrate, as it has not been observed in *D. subobscura* on *Sorbus* fruit (Hoffmeister & Rohlfs, 2001).

Inbreeding and its negative consequences on individual fitness (*i.e.*, inbreeding depression) associated with the maintenance of deleterious alleles through genetic drift (*i.e.*, drift load) are well-known phenomena that can severely affect small populations and lead to their extinction (Bijlsma *et al*., 2000). In this regard, invasive drosophilid species might have an advantage, as widespread representatives suffer less from inbreeding depression than do specifically tropical species when population size declines (Bechsgaard *et al*., 2013).

2.3. Genetic diversity and adaptive capacities of invasive populations during the expansion phase

The fact that many species have successfully invaded new areas and rapidly adapted to new ecological conditions, despite demographic bottlenecks that potentially reduce their genetic diversity, has long been considered a paradox (Allendorf & Lundquist, 2003). More recently, however, it has been argued that the many cases of successful invasions should not be considered a paradox (Estoup *et al.*, 2016; Schrieber & Lachmuth, 2017). Rather, the loss of genetic diversity may not be significant or it may only concern neutral genetic markers and not ecologically relevant quantitative traits (Reed & Frankman, 2007). In some cases, even if introduced populations do show lower genetic diversity compared to their source populations, they may not face any significant adaptive challenge in the invaded habitat (Reed & Frankman, 2007). Bottlenecks of intermediate intensity can also purge the deleterious alleles that cause inbreeding depression in the introduced population (Estoup *et al.*, 2016).

In *D. suzukii* and *D. subobscura*, reconstruction of invasion routes has clearly highlighted the occurrence of moderate to severe bottlenecks during their invasions (Pascual *et al*., 2007; Adrion *et al*., 2014; Fraimout *et al*., 2017). However, these studies also showed that the genetic diversity of introduced populations is only slightly reduced compared to the original populations (Pascual *et al*., 2007; Adrion *et al*., 2014). This could be a result of the large effective size and genetic diversity of the original populations, which allow the retention of a substantial amount of genetic variation even with a small number of founding individuals (Pascual *et al*., 2007; Adrion *et al.*, 2014). Additionally, in Drosophilidae, introduction events rarely occur only once (Yassin *et al*., 2009; Lavrinienko *et al.*, 2016), and admixture between introduced populations (Fraimout *et al*., 2017) likely contributes to maintaining genetic diversity and adaptive capacity in these populations. The high rate of population growth of invasive species can also mitigate the impact of bottlenecks on genetic variability (Nei *et al*., 1975).

Ultimately, the genetic paradox of invasive species assumes that the mutation rate is low and constant during the invasion process. However, numerous examples indicate that the activity of transposable elements (TE) can be significantly increased in response to environmental disturbances, generating genetic variation and facilitating adaptation (Oliver & Greene, 2009). The role of TEs in the adaptive capacity of invasive species represents an important area for investigation (Stapley *et al*., 2015). For instance, in *D. melanogaster*, several TEs were positively selected during the colonisation of the North American continent (Gonzalez *et al*., 2008), and one of these TEs (pogo) was associated with increased resistance to a natural xenobiotic and a synthetic insecticide (Mateo *et al*., 2014). Similarly, a recent study revealed 15 potentially adaptive TE insertions in *D. suzukii*, six of which were associated with its recent invasion (Mérel *et al.*, 2021)

3. ROLE OF ABIOTIC FACTORS IN NICHE COMPATIBILITY

When an invading species expands its distribution, it encounters new abiotic environments that may be more or less compatible with its requirements, *i.e.* its ecological niche. Abiotic stressors in the invaded area may thus limit the establishment and further spread of the non-native species. Adaptation to stressful environments is a key factor influencing invasion success (Sexton *et al*., 2002; Lee *et al*., 2003; Piiroinen *et al*., 2013). Parameters such as temperature and humidity may exert strong selective pressure on non-native populations in the recipient ecosystems resulting in the evolution of tolerance traits. For instance, because *D. subobscura* populations were regularly constrained to live at the margin of their specific niche when invading low-latitude regions of North America, they faced strong selective pressure that led to better tolerance to desiccation and starvation (Gilchrist *et al*., 2008). Depending on the species, other abiotic factors such as available oxygen concentration may also provide selective pressure on introduced Drosophilidae populations (Pincebourde & Casas, 2016). By assessing how abiotic stressors shape the ecological niches of invading Drosophilidae species, we can better anticipate their establishment and spread in new environments, thereby providing information to improve strategies for conservation and ecosystem management.

3.1. Thermal stress and plasticity

Temperature is a key environmental factor that drives all physiological processes by acting on biochemical rates, with consequences from cell to community levels (Colinet *et al.,* 2015; Ma *et al.*, 2021). Temperature dictates growth, reproduction and survival, and it is often the primary environmental factor

determining the geographical distributions of insects (Kimura 1988; Hoffmann & Sgrò 2011; Kellerman *et al*., 2009), including *Drosophila* species at regional (Deconninck *et al*., 2024b) and global (Ulmer *et al.*, 2022) scales. It has been suggested that populations originating from more thermally variable environments (e.g., temperate species) are more likely to become invasive than those from more stable areas (e.g., the tropics) because they can better endure thermal stresses (Lee & Gelembiuk, 2008). However, this does not appear to be a robust rule in the Drosophilidae. For instance, while members of the genus *Zaprionus*, which are of tropical origin, were generally assumed to be unable to colonise temperate regions (Araripe *et al*., 2004), recent records of establishment in Europe (Georges *et al*., 2024; Raspi *et al*., 2014) suggest that even representatives of Afrotropical origin are able to survive the harsh winter conditions experienced in their exotic range. A climatic niche shift may have taken place enabling population adaptation to colder climates (da Mata *et al*., 2010). Such observations suggest that, with climate change, environmental conditions in southern Europe may become favourable for the establishment of new invasive species from tropical regions. In terms of pre-adaptation to more variable climates it is notable that, while species native to temperate environments may have theoretical invasion advantages (Kellerman e*t al.*, 2009), the successful establishment of invasive drosophilids in diverse climatic zones indicates that their great adaptability and plastic responses to temperature variation and stress are crucial to their invasion success.

The ability to tolerate thermal stress through plastic responses (*i.e.*, by acclimation) and/or adapt to abiotic stressors is critical in determining the range limits of invasive drosophilid populations (Gilchrist *et al.*, 2008). Enhanced tolerance to temperature extremes (both cold and heat) can be acquired through plastic responses such as rapid cold or heat hardening (in the short term) and to more gradual acclimation responses (Colinet & Hoffmann, 2012). Drosophilid species are notorious for exhibiting remarkable capacities for acclimation to thermal stresses through plastic thermal responses (Hoffmann *et al*., 2003b; Enriquez & Colinet, 2019), highlighting the pivotal role of acclimation in facilitating invasion success. *Drosophila kikkawai* is a non-pest species whose ability to significantly increase tolerance of colder and drier conditions through plasticity facilitated its invasion potential (Parkash *et al.*, 2021). Acclimatory responses differ between the cold and warm ends of the temperature gradient (Colinet *et al.*, 2013). Studies indicated that drosophilid species exhibit greater thermal plasticity and acclimation ability to cold temperatures compared to heat. This difference in thermal plasticity is attributed to physiological and phylogenetic constraints that make heat acclimation less effective than cold acclimation (Colinet *et al.*,

2013; Overgaard *et al.*, 2011; Kellermann *et al.*,2012a,b), a limitation that might be crucial for predicting the future distribution and managing the impact of these invasive species.

In addition to physiological plasticity in response to thermal stress, behavioural plasticity can also play an important role in invasion success. Drosophilid species inhabiting different climatic environments can have species-specific behavioural responses to temperature as part of their life history strategies (Ito & Awasaki, 2022). For instance, optimal temperature for daily locomotor activity and sleep of different species approximately matches the temperatures they frequently encounter in their habitats (Ito & Awasaki, 2022). Invasive species can shift their daily time of activity when they progress into novel thermal environments. For example, thermoregulatory behaviours enable invasive populations of *D. subobscura* to compensate for seasonal and latitudinal variation in climate (Huey & Pascual, 2009). This behavioural flexibility was not sufficient, however, to fully compensate for the overall decrease in body temperature at higher northern latitudes (Huey & Pascual, 2009). Furthermore, thermoregulatory behaviours may confer the ability to find and exploit microclimatic niches in the invaded area that provide conditions similar to those of the native area, despite the overall shift in larger scale regional climate conditions. This aspect of microclimatic niche similarity between native and invasive ranges may explain why invasive ants display a smaller (macro)climatic niche shift than non-invasive alien ants (Bates *et al.*, 2020). While this hypothesis remains to be investigated for invasive Drosophilidae, several key traits linked to behavioural thermoregulation (e.g., thermal preference) have been assessed in various fruit fly species. Overall, Drosophilidae are capable of selecting a relatively large thermal range (Dillon *et al.*, 2009; Kinzner *et al*., 2019; MacLean *et al.*, 2019) indicating that a portion of the population is likely to encounter and satisfy microclimatic requirements.

3.2. Winter and overwintering

Overwintering is a crucial phase of the life cycle for many insect species and this phase can act as an important bottleneck for invading populations. For instance, *Drosophila suzukii* can overwinter either as winter-acclimated dormant adults (in reproductive quiescence) or as juvenile stages developing gradually during winter, suggesting a bet-hedging strategy (Raynaud-Berton *et al*., 2024). The use of mixed strategies for overwintering may contribute to this species' invasive success in several ways. Overwintering as both dormant adults and juvenile stages may reduce the threat from different environmental conditions (Raynaud-Berton et *al.*, 2024). For instance, if winter conditions are milder, the juvenile stages may have a better chance of surviving and emerging when conditions become favourable in spring, while adults may

become active and exhaust their energy stores before suitable breeding sites become available. In colder winters, overwintering females of *D. suzuki*i and *D. melanogaster* retain their reproductive capacity, enabling them to relaunch populations in spring if they successfully survive the winter (Panel *et al*., 2020; Cloutier *et al*., 2022; Hoffmann *et al*., 2003a). The ability to overwinter at different developmental stages suggests that *D. suzukii* is poised to opportunistically take advantage of any early warm spells as the winter comes to an end, facilitating rapid population increase when conditions become suitable for reproduction. Such adaptability and plasticity have been suggested to have played a crucial role in *D. suzukii*'s rapid range expansion and ability to thrive in diverse environments (Little *et al*., 2020).

Drosophilid species can display a variety of overwintering strategies. For instance, in six Eurasian species of the *D. obscura* group, two overwinter without diapause (*D. subobscura*, *D. guanche*), two in pupal diapause (*D. alpina*, *D. subsilvestris*) and two in reproductive diapause (*D. bifasciata*, *D. obscura*) (Goto *et al*., 1999). Species with diapause exhibited higher cold resistance and their distribution extended further into colder northern areas. Again, the fact that there is more than one effective strategy for surviving winter in this closely related species group suggests that the flexibility in overwintering strategies may contribute to their invasive success, as they can exploit different ecological niches and survive diverse and changing environmental conditions. The diversity of overwintering strategies across species may also suggest rapid radiation at an early phase of adaptation to temperate climates (Goto *et al*., 1999). It has been suggested that the *D. subobscura* subgroup exhibits an ancestral trait in climatic adaptations (lack of diapause), similar to tropical species in the *D. melanogaster* group, which also lack a true diapause mechanism (Saunders *et al*., 1989), although it is also possible that the *D. subobscura* subgroup lost the diapause mechanism during its colonisation of temperate regions. Kimura (1988) reported a case in *D. triauraria* where subtropical populations of this species were assumed to have lost winter reproductive dormancy following secondary colonisation of subtropical areas. Clear shifts in dormancy incidence also exist in *D. melanogaster* and they vary predictably with latitude (Schmidt *et al*., 2005). Hence, it seems that plasticity in overwintering strategies, including an increased incidence of dormancy in cold-invaded areas or loss in milder invaded areas, may be an integral life-history component of invasive drosophilid populations.

3.3. Summer and heat stress

Heat stress may act as a critical limiting factor influencing the ability of drosophilid species to establish and sustain populations in new and potentially hostile climates. Like any ectotherm,

drosophilids have specific thermal limits beyond which their survival, development and reproduction are severely compromised (Hoffmann, 2010; Hangartner *et al*., 2015; MacLean *et al*., 2019; Colinet & Kustre, 2024). Population growth and successful range expansion can be affected by summer temperatures and thus heat tolerance (Xue & Ma, 2020; Alruiz *et al*., 2022). Capacity to tolerate heat stress may be advantageous for invasive species with the potential to show rapid expansion in the African and Australian subtropics, as predicted for *D. suzukii* (dos Santos *et al.*, 2017). Heat stress during summer thus poses a significant challenge to the invasion success of drosophilid species by directly affecting their physiological functions and reproductive fitness (Green *et al.*, 2019; van Heerwaarden & Sgrò, 2021; Grandela *et al*., 2024). High summer temperatures can affect populations directly by inducing cellular damage and reducing metabolic efficiency (Krebs & Loeschcke, 1994; Sørensen *et al*., 2012), and indirectly by impairing gamete production and embryo development (Araripe *et al*., 2004; Van Heerwaarden & Sgro, 2021; Grandela *et al.*, 2024). The negative effects of summer heat stress can, however, act in a more subtle way, with damage and mortality accumulating exponentially over time as the population experiences repeated heat events - a dynamic system well captured for *D. melanogaster* populations (Rezende *et al*., 2020). However, there is no information currently available to indicate whether, in invaded ecosystems, invasive species possess greater tolerance to summer heat or adaptive mechanisms than native relatives. Based on the finding that upper thermal limits of Drosophilidae are strongly constrained phylogenetically (Kellerman *et al.*, 2012b), it is unlikely that invasive species possess inherently greater thermal tolerance or adaptive mechanisms compared to native species. However, invasive species may counteract the effect of heat stress and their physiological limits by plasticity in behaviour, as mentioned above (see also Ulmer *et al.*, 2024), thereby allowing them to exploit new environments more effectively. Pupae of *D. suzukii* survive winter more effectively when buried in the soil (Kaçar *et al.,* 2016), and such a protective effect may also occur during hot summer conditions. Nevertheless, we lack the data needed to compare native and invasive drosophilid species. For instance, the thermal range of activity is narrower and the circadian rhythm of locomotor activity is less plastic in *D. suzukii* than in *D. melanogaster* (Plantamp *et al*., 2019) suggesting that the picture is much more complex than expected from single trait approaches.

3.4. Water availability

Theoretically, invasion success should be favoured for species that can tolerate greater variability in water availability, in particular those tolerant of drier locations. Although tolerance to low water

availability and desiccation stress is clearly not limited to invasive species, the success of various drosophilid species in exploiting novel dryer areas can be attributed, at least in part, to their high physiological and morphological plasticity and adaptability to varying environmental conditions, including desiccation (Little *et al*., 2020). As noted, invasive Drosophilidae are commonly found in environments facing a wide range of climatic conditions, including varying water availability. *Drosophila suzukii*, with its near-global distribution, showcases high tolerance to varying water availability and water stress. For instance, atmospheric humidity has to be below 20% to impair its survival and reproduction (Tochen *et al.*, 2016). A similar example is given by the tropical invasive drosophilid, *Zaprionus indianus*, which now occurs from humid subtropical to cold semi-arid climates (Bragard *et al.*, 2022) and has substantial capability to increase (> 2-fold) its tolerance to various stresses including desiccation. Likewise, *D. ananassae*, a tropical dimorphic species, has successfully invaded cold and dry montane localities of the Western Himalaya in recent years, with its success related to the greater desiccation tolerance of a dark morph. The body colour morphs differ substantially in their physiological capacity for water conservation under experimental conditions as well as desiccation acclimation potential, consistent with their distributions in dry or wet habitats (Chahal *et al.*, 2013; Parkash *et al.*, 2014). Dark phenotypes of *D. suzukii* also show greater desiccation tolerance than paler ones (Fanning *et al.*, 2019).

Drosophilid species may display other adaptive traits limiting direct exposure to desiccation stress, even in dry climatic areas. For instance, their larval stages often develop within fruit or decaying organic materials, which buffer direct exposure to atmospheric conditions (Fekete *et al.*, 2016). Larvae of *D. melanogaster* perceive and react to humidity, selecting damp substrates at pre-pupation stages and drier ones to pupate (Godoy-Herrera *et al*., 1989). Practically, the measurement of relative humidity at such fine spatial scales remains technically challenging. Adult stages exposed to the open air can also react behaviourally, for instance by actively searching for and selecting wetter microenvironments, such as in leaf litter cavities. Indeed, litter cover and dead wood debris have been identified as major ecological indicators of the presence of *D. suzukii* (Ulmer *et al.*, 2024). At the landscape scale, water elements (ponds, rivers) are expected to attract insects, including invasive drosophilids, during hot periods as they provide cool and moist microclimates (Maceda-Veiga *et al.*, 2021). This has been verified in urban areas, where the proportion of *D. suzukii* increases along riversides in spring and summer (Ulmer *et al.*, 2024). However, varying associations between fly density and distance to water sources were found between years and sites in forest environments (Maceda-Veiga *et al.*, 2021). Further research on the behaviour of flies in

relation to humidity gradients is required to shed light on their ability to buffer negative impacts from desiccation stress on their fitness.

3.5. Synergies between multiple stressors in heterogeneous habitats

Temperature and humidity are probably the two most important drivers of physiological and ecological rates. However, because species live in heterogeneous and complex microenvironments (Ma *et al.*, 2021), other abiotic factors (e.g., oxygen availability) can both play a role directly and interact with temperature and humidity to impact invading species (Galic *et al.*, 2018; Sarà *et al.*, 2018). Interactions between temperature and desiccation stresses are clearly well documented. High temperatures contribute to increased evaporation and lower water availability, leading to desiccation stress, with small organisms such as insects being particularly vulnerable. High temperature is, thus, often associated with drying conditions, in turn resulting in multi-stress conditions that can synergistically affect insect physiology and behaviour (Hoffmann *et al*., 2003b). In *D. suzukii*, laboratory data showed that exposure to relative humidity below 20% prevents flies from surviving and reproducing (Tochen *et al*., 2016). However, such low humidity conditions are very unlikely to be encountered in natural situations in most temperate and tropical regions. Even in the driest field habitats, shaded microhabitats may provide refuges to enable persistence in otherwise unsuitable areas (Tochen *et al.*, 2016). Desiccating conditions in combination with thermal stress are often associated with reduced survival probability compared to thermal stress alone, as noted in both *D. melanogaster* (Bubliy *et al*., 2012) and *D. suzukii* (Enriquez & Colinet, 2017). However, desiccation stress does not seem to interact with low temperature to affect survival (Enriquez & Colinet, 2017), probably because the vapour pressure deficit remains limited under low temperatures (Sinclair *et al*., 2024). Finally, exposure to wind could act in synergy with both temperature and humidity, further increasing the stress exposure of invasive Drosophilidae. Supporting this, wind, along with temperature and rainfall, were the main abiotic forces shaping the genome evolution of *D. melanogaster* (Boagerts-Márquez *et al.*, 2021).

Physiological plasticity and flexibility should be a key aspect of tolerance to multiple stressors. The tropical drosophilid, *Z. indianus*, has substantial capability to increase its tolerance to various stresses including desiccation, heat exposure and starvation after hardening/acclimation and also shows cross-tolerance between these stressors (Kalra *et al*., 2017). Such flexibility in abiotic stresses tolerance likely accounts for its invasion potential (Kalra *et al.*, 2017). In general, overall plasticity to multiple stressors does not simply result from additive plasticity to individual environmental factors. For instance, the non-

linearity of the physical interaction between temperature and water vapour pressure deficit (the driving force of evapotranspiration) implies that the plastic response to warm and dry environments is a complex mixture of physiological responses to both variables (Sinclair *et al.*, 2024).

The larval stages of Drosophilidae develop in microenvironments (e.g., fruit, decomposing materials) that can display contrasting abiotic conditions. Living plant tissues influence the thermal, hydric and gaseous conditions experienced throughout larval development (Pincebourde & Casas, 2016). Drosophilids developing within unripe fruit can be subjected to conditions that are warmer than ambient external conditions (Saudreau *et al.*, 2009). For instance, fallen fruit on the ground expose the larvae to the soil microclimate (Deconninck *et al.*, 2024b; see the seasonal buffering effects in the Suppl. Mat.). The soil microclimate often buffers atmospheric extremes when in the shade (Geiger, 1959), particularly when soil moisture levels are high (Greiser *et al.*, 2024) or, conversely, amplifies ambient conditions when exposed to solar radiation (Feder *et al.*, 1997). These contrasting thermal conditions can interact with the gaseous environment within the fruit to generate strong physiological impacts on larvae. For example, low oxygen levels within fruit tissues can lower the physiological threshold to high temperature tolerance, thereby causing thermal death at temperatures lower than those expected on the basis of knowledge of the critical temperature alone (the so-called oxygen and capacity limitation of thermal tolerance hypothesis), although this effect can be observed in terrestrial insects only at very low oxygen levels (well below 2 kPa; Verberk *et al.*, 2016). Unfortunately, there is limited knowledge of the temperature and gas composition patterns experienced within such fruit resources, and it is therefore unclear whether and to what extent invasive drosophilids are better adapted to such peculiar micro-environments.

Finally, drosophilids live and move across mosaics of micro-environments that can present flies with a wide range of abiotic conditions. Their behaviour is key to selecting the most appropriate micro-environment both for their own performance (survival, fecundity) and the success of their progeny (developmental rate, survival). These behavioural abilities must be integrated with spatial and temporal mapping of abiotic conditions at relatively fine scales, as illustrated with temperature by Pincebourde & Woods (2020). Such integrative approaches remain to be applied to other variables such as water vapour partial pressure and oxygen levels, for example, and behavioural choice strategies at these fine scales remain to be investigated for most Drosophilidae.

4. ROLE OF BIOTIC FACTORS IN RESOURCE USE AND NICHE ACCESSIBILITY

Besides facing new combinations of abiotic conditions, invasive drosophilid species need to access the resources of recipient ecosystems (e.g., fruit) and adapt to new biotic interactions and local resistances in order to successfully establish. Here we consider different strategies through the lens of biotic interactions in terms of (i) adaptation to new trophic conditions, (ii) competition, (iii) predation, (iv) facilitation and (v) symbiosis.

4.1. Adaptation to new trophic conditions

Invasion success in drosophilid species is largely influenced by trophic niche width or adaptability (Poyet *et al*., 2015). The invading species may utilise a completely distinct host range in the area being invaded compared with that of its native range. For instance, the success of *D. melanogaster* was, in part, a result of transitioning from a natural to a synanthropic habitat (Lachaise & Silvain, 2004). Proliferation and spread can occur only if trophic preferences and requirements (the trophic dimension of the 'species invasiveness'), match with the quality, quantity and availability of resources (the trophic dimension of 'ecosystem invasibility') (Alpert *et al.*, 2000; Richardson & Pyšek, 2006). The trophic dimension of drosophilid species invasiveness is, thus, based on a set of life history traits related to their physiology, morphology and behaviour, that enable them to detect, access and use the resources of the recipient ecosystem.

The degree of polyphagy impacts a species' invasiveness and, consequently, the trajectory and the success of non-native drosophilids. Polyphagy could be considered as a form of pre-adaptation leading towards the evolution of a cosmopolitan invader, allowing a species to benefit from a diverse range of substrates/hosts, whatever the geographic area invaded (Lachaise *et al*., 1988; Mack, 2003). Most drosophilid species display high degrees of polyphagy, which could underpin the frequency and success with which many of them have become invasive. For instance, invasive populations of *D. simulans, Z. indianus, Z. tuberculatus* and *D. melanogaster* are notably abundant in South America due, in part, to their ability to exploit a diverse range of plant species (Bragard *et al*., 2022; Ribeiro *et al*., 2023; Viana *et al*., 2024). Urban commercial fruit and vegetable markets, characterised by consistent and cosmopolitan resource availability favour generalist/polyphagous drosophilids, such as *D. melanogaster* (Ulmer *et al.*, 2024). These species can readily locate suitable breeding sites, leading to the development and maintenance of larger populations in such urban settings (Ribeiro *et al*., 2023).

The advantages of polyphagy include a lower time requirement for searching for food, greater success in locating suitable substrates within and between years, nutrient complementation, attenuation

of possible toxic effects of some substrates and higher fecundity (Zhang *et al*., 2014; Poyet *et al*., 2015). There are also costs or disadvantages of polyphagy, such as nutritional composition/quality varying between substrates, and intergenerational impacts on offspring performance and fitness (e.g., development rate, survival) (Janz, 2003; Poyet *et al*., 2015). *Drosophila suzukii*, like other polyphagous insects, can also make oviposition mistakes by laying eggs on host plants that are unsuitable for the development of its larvae, such as the dead-end trap plant *Pyracantha coccinea* (Ulmer *et al*., 2020). Nevertheless, very few drosophilids are strictly monophagous or show narrow specialisation to a specific substrate, with a few exceptions (e.g., *D. nigrospiracula*, *D. mettleri*, *D. mojavensis*, *D. pachea*, a group of desert-adapted species; or *D. sechellia* and *D. mauritiana*, insular specialist species), none of which are invasive (Barker & Starmer, 1982; Lachaise *et al*., 1988).

In their invaded range, drosophilids must perform well in the key steps summarised as "host habitat finding, host finding, host recognition and acceptance, and host suitability" by Kogan (1977). To access this resource, some species developed specific organs, generally associated with specific behaviour (Cavey *et al*., 2023). For instance, *D. suzukii* and *Scaptomyza flava* evolved a serrated ovipositor allowing them to insert eggs in harder substrates that are inaccessible to other species, unripe fruit for the former (Atallah *et al*., 2014) and fresh leaves for the latter (Peláez *et al*., 2022). These convergent adaptations allow these fly species to be the first to access and benefit from the resource, avoiding most competitors (Clemente *et al*., 2018), even before native species (Rombaut *et al*., 2017). These two examples echo the empty niche hypothesis (Stachowicz & Tilman, 2005) which is often used to explain invasion success. In regions invaded by non-native species, particular ecological niches (in the current context, trophic) are little or unoccupied by native species due to their different evolutionary history. Potential competitors among native drosophilids do not possess the same levels of behavioural, morphological and physiological adaptive traits required to readily recognize and consume unripe fruit or fresh leaves and thereby outcompete the arriving non-native species. Thus, the establishment success of non-native populations strongly relies on niche differentiation from native populations. This explains the higher performance and competition success of the invasive *D. suzukii* over the native *D. subobscura* in unripe fruit (Poyet *et al*., 2014; Deconninck *et al*., 2024a) and the reverse in rotting fruit (Deconninck *et al*., 2024b)

Most plants evolved defences against insects, in particular through the production of toxic compounds (Bruce, 2015; Hilker & Fatouros, 2015). Some invasive and polyphagous insect species are able to overcome plant defences in their invasive range and develop in fruit or other plant substrates that

are unsuitable for (or not used by) native species. *Drosophila suzukii* provides a striking example of this ability, being able to develop in fruit that are highly toxic to most fauna, such as those of *Atropa belladonna* (Poyet *et al*., 2015) or *Viscum album* (Deconninck *et al*., 2024a). It is also able to use the secondary metabolites contained in *A. belladonna* berries and pass them transgenerationally as a means of increasing resistance to parasitoid attacks (Poyet *et al*., 2017). Similarly, the invasive *D. busckii* shows tolerance towards high concentrations of dimethyl disulfide, a neurotoxic compound widespread in nature (emitted by bacteria, fungi, plants and animals) that is highly toxic to most drosophilid species (Mahadevan *et al*., 2024). Even if such biochemical strategies can be used by species that are not invasive (e.g., *Drosophila sechellia* is restricted to The Seychelles infest *Morinda citrifolia* fruit that are toxic to other drosophilids), they are clearly advantageous and can make an important contribution to invasion success.

These beneficial traits of invasive drosophilids (detoxification capacity, serrated ovipositor, detection of immature fruit) already existed in their native range (Atallah *et al*., 2014), where the species are in equilibrium with the combination of functional traits characterising the local communities with which they coevolved. However, in their invaded range, these traits are original (not present in the newly colonised area) and become new weapons ("Novel Weapon Hypothesis"; Callaway & Ridenour, 2004), disturbing community equilibrium (Callaway *et al*., 2008). Original traits using such weapons that give advantages to invasive over native species were originally recognised and are well-known in plants (Callaway & Aschehoug, 2000), but examples in insects also exist (Vilcinskas *et al*., 2013).

4.2. Overcome inter- and intraspecific competition

Interspecific and intraspecific competition are widespread among insects, particularly for trophic resources and reproduction sites. Interspecific competition is commonly regarded as one of the main drivers of distribution and abundance of phytophagous insects (Denno *et al*., 1995; Kaplan & Denno, 2007), including invasive drosophilids (Bühlmann & Gossner, 2022), although its importance relative to other ecological factors remains debated (Facon *et al*., 2021).

In the context of biological invasions, limited attention has been paid to the role of competition compared to those of other biotic and abiotic factors such as the existence of ecological niches not exploited by native species (empty niche hypothesis; Herbold & Moyle, 1986) or the lack of natural enemies in the invaded area (enemy release hypothesis; Williamson & Fitter, 1996; Keane & Crawley, 2002). It is generally predicted that communities are more likely to be invaded when large amounts of

unused resources are available (Davis *et al*., 2000), when introduced species have better acquisition efficiency for a given resource than do native species, or when, at comparable acquisition efficiency, the non-native species has lower requirements for this resource than the native species (Shea & Chesson, 2002). For instance, *D. suzukii,* with its specialised serrated ovipositor, faces little competition among the drosophilids for oviposition resource due to its ability to puncture the surface of ripening fruits. This ability is likely to reduce competition with other drosophilid flies that cannot exploit these hosts at the same early stage of maturity. However, while the serrated ovipositor of species like *D. suzukii* and *S. flava* may facilitate their ability to exploit new ecological niches and contribute to their invasion success, this morphological adaptation alone does not automatically underlie invasiveness, as evidenced by species like *D. subpulchrella* and *D. biarmipes*, which possess similar ovipositors but are not invasive (Atallah *et al*., 2014).

Food substrates such as fruit are ephemeral resources, creating the conditions for intra- and interspecific competition (Rohlfs & Hoffmeister, 2004). The need to invest in strategies related to competition was shown to reduce the performance of various drosophilid life history traits (e.g., survival, growth, fecundity) in experimental situations where access to resources was limited (Henry & Colinet, 2018; Henry *et al*., 2020). As a consequence, invasive drosophilids generally adopt temporal partitioning in the use of a given resource with native species to escape or reduce competition between members of the fruit fly community (Deconninck et *al.*, 2024b).

Direct demonstration of the role of competitive ability in the success of invasions is rare. De Paiva Mendonça *et al*. (2023) studied the co-occurrence and relationships between two invasive drosophilids, *D. suzukii* and *Z. indianus*, in both the laboratory and the field in Brazil. In the field, *Z. indianus* was by far the most prevalent species (>80% in collected fruit). Interspecific competition bioassays investigated the responses of both species to increasing levels of inter- and intraspecific competition and showed interesting differences in life history traits between the species, suggesting better competitive ability in *Z. indianus*, consistent with its prevalence in the field. In laboratory trials with ripe strawberries, there was a significant and positive interaction, indicating facilitation between damage caused by *D. suzukii* and infestation by *Z. indianus* (Bernardi *et al*., 2017). Shrader *et al*. (2020) also reported that both competition from *Z. indianus* and grape variety could limit *D. suzukii* numbers.

Intraspecific competition is likely to take place in the field as females usually oviposit more than one egg in the same substrate. For instance, *D. suzukii* can lay up to 50 eggs in individual blueberries in

experimental conditions (Kienzle *et al.*, 2020) and up to 88 eggs in 10 mL of a medium made from blackberry puree (Olazcuaga *et al.*, 2019), even if in the field the number of eggs per fruit is lower, for instance with a mean of ~5 eggs in raspberries (Wallingford *et al*., 2016). Laboratory experiments have shown that pupation is affected by intraspecific competition in *D. suzukii* (Bezerra Da Silva *et al*., 2019). Larvae exposed to intraspecific competition pupated further away from their host fruit relative to singly-developed individuals, and detached pupation was associated with survival and fitness gains. In two other invasive species, *D. ananassae* and *D. nasuta*, experiments on larval crowding over several generations showed that the evolution of competitive ability was achieved through a combination of a reduced duration of the larval stage, more rapid attainment of minimum critical size for pupation, greater efficiency of food conversion to biomass, increased pupation depth and, perhaps, greater urea/ammonia tolerance (Nagarajan *et al*., 2016). In contrast, in *D. melanogaster*, the evolution of competitive ability seems to be the outcome of a balance between mutually antagonistic traits such as increased larval feeding and foraging behaviour, greater tolerance to nitrogenous wastes and reduced efficiency of conversion of food to biomass. Together, such results suggest that increased competitive ability can be attained through the evolution of different suites of traits. Intraspecific competition was also assessed in *D. suzukii* and *Z. indianus* by de Paiva Mendonça *et al.* (2023). For these two species, development time, pupal survival, adult emergence and fecundity were all negatively affected by larval density. Under laboratory conditions, *D. suzukii* larvae achieved a higher developmental success on damaged blueberries but suffered less from density-dependent intraspecific competition in healthy fruit (Kienzle *et al*., 2020). Therefore, while invasive drosophilids experience intraspecific competition, their strategies to overcome it may also have contributed to their invasive success.

De Paiva Mendonça *et al*. (2023) emphasised that co-occurring fruit flies can use different strategies when exposed to intra- or interspecific competition and that their responses (e.g., life-history parameters) to these conditions may help to explain the different distribution patterns observed on local and global scales. In addition, although climate warming is likely to intensify competition in both established and novel communities, longer growing seasons, more severe environmental stress and increased climatic variability may buffer against competition intensification (Lancaster *et al.*, 2017). A better understanding of competitive interactions between invasive and native species, as well as between the invasive species themselves, will help to better predict their distributions at various scales and to develop better pest control strategies.

4.3. Avoid and resist natural enemies

The biotic resistance of the recipient ecosystem can significantly influence the ultimate outcome of invasions (Daly *et al*., 2023). A non-native drosophilid species arriving in a novel area will encounter new local communities and interact with species that may hinder its long-term establishment in the recipient ecosystem. Natural enemies of drosophilid flies can be generalist or specialist and target specific or multiple life stages (*i.e.*, eggs, larvae, pupae and/or adults) (Symondson *et al*., 2002). Native predators and parasitoids are among the most important contributors to biotic resistance towards drosophilid invasions (Gibert *et al*., 2010; Schmidt *et al*., 2019; Ulmer *et al*., 2021), but interactions with other entomopathogenic organisms (e.g., pathogenic fungi, bacteria, nematodes, viruses) in the newly-colonised range may also influence invasion success (Woltz *et al.*, 2015; Wang *et al.*, 2021). These regulating taxa are grouped under the general term 'natural enemies' (Keane & Crawley, 2002). They can affect invasive drosophilid through a consumptive effect by which a predator directly affects the prey population by directly killing the individuals, and/or by a non-consumptive effect, by which their presence of the predator or enemy influences the performance of some of the *Drosophila*'s traits such as reproduction or oviposition activity (Ulmer *et al*., 2021). Laboratory experiments showed that predator body size and mobility were the main traits involved in *D. suzukii* predation or disruption of oviposition (Ulmer *et al*., 2021). The Enemy Release Hypothesis (ERH) suggests that the establishment of a new invading species can be facilitated by a lack of natural competitors and enemies for regulation of the population (Williamson & Fitter, 1996; Keane & Crawley, 2002) or that, even if potentially appropriate native species are present, they are generally naïve and unable to recognize/detect and then predate/parasitise the new species. The ERH is one of many hypotheses erected to explain the abundance and/or impact of invading species (see Colautti *et al*., 2004 for review). Once again, *D. suzukii* provides a pertinent example of how the ERH affects invasion success: in its native range, it is susceptible to at least 19 larval parasitoids while, in its invaded area it is susceptible to much fewer (Wang *et al*., 2021).

Strategies to counter predation and parasitism include avoidance, camouflage, physical resistance and protective biochemical responses. One or more of these strategies are used by different drosophilid species and, in both native and invaded ranges, their effective use leads to improved survival. The avoidance strategy involves the use of behaviours induced by the presence of natural enemies, and depends on the type of predator (de la Flor *et al*., 2017). Flies are able to visually detect predators, retain innate defensive behaviours and increase their exploratory activity (de la Flor *et al.*, 2017). Olfaction can also be

important, as illustrated by Ebrahim *et al*.'s (2015) demonstration that larval and adult *D. melanogaster* avoid sites 'smelling of' *Leptopilina* wasps, their primary parasitoid enemy. Based on specific volatile organic compound cues emitted by the wasps, the fly develops a dedicated circuit (*i.e.*, olfactory sensory neuron, OSN) to detect this enemy. Polyphagy and the use of fruit of a wide range of colours can also be incorporated in an enemy avoidance strategy by disorienting those attracted by a specific range of colours (Vargas *et al*., 1991).

Drosophilids have developed other strategies to reduce predation impacts. For example, it was hypothesised that the relatively thick and hard cuticle (about 20 μm) of pupae protects them from predator attacks (Borne *et al*., 2021). Borne *et al*. (2021) investigated the efficacy of pupal adhesion in reducing predation and found that attached pupae were 30% less predated than detached ones. The former were less efficiently predated by ants in the laboratory, although predation rate varied widely between ant species (Borne *et al*., 2021; Ulmer *et al*., 2021). The presence of ants, through their smell or their production of formic acid, can even stimulate the oviposition by *D. suzukii* (Ulmer *et al*., 2021). Invasive *D. suzukii* and *D. simulans* both benefit from the adhesion resistance strategy (Borne *et al*., 2021). The depth of *D. suzukii* pupation in fruit or soil is also likely to influence their predation and parasitism rate (Kanzawa 1939; Woltz & Lee, 2017).

Another resistance strategy is encapsulation, which is an immune response to large parasites, such as endoparasitoids (Carton & Nappi, 2001). For instance, the parasitoid community of frugivorous Drosophilidae in France primarily comprises five species, three larval and two pupal parasitoids (Fleury *et al*., 2009). In *D. suzukii* invasive range, only the generalist pupal parasitoids develop successfully, whereas the much more specialised larval parasitoids mostly fail to parasitize this species' larvae (Chabert *et al*., 2012). The low parasitic efficiency of larval parasitoids of the wasp genus *Leptopilina* is associated with a high capacity for immune resistance in *D. suzukii* (Poyet *et al*., 2013). These authors showed that the numbers of circulating haemocytes (linked to the immune response) in *D. suzukii* larvae were the highest ever recorded in larvae of the *D. melanogaster* group. Globally, however, the role of the immune system in the success of invasion has been poorly investigated, and commonalities in immune responses among invasive drosophilid species warrant further study.

Finally, the activation of specific biochemical pathways can be used in resistance strategies, either directly leading to the death of parasitoids or reducing their impacts, for instance by taking advantage of plant molecules (Poyet *et al*., 2017). For example, *D. suzukii* adjusts its oviposition behaviour in the

presence of parasitoids, preferentially ovipositing on media containing atropine (an entomotoxic alkaloid, Poyet *et al*., 2017) and by doing so, they provide prophylactic medication to their offspring. Polyphagous drosophilids, such as *D. suzukii*, may have more opportunities than oligo- or monophagous species to medicate in this way as they may encounter numerous toxic plants (Poyet *et al*., 2015), sequester their secondary metabolites, and use them to reduce the impact of enemies. Besides *D. suzukii*, *D. melanogaster* is also able to tolerate plant alkaloids (Fogleman, 2000) and performs auto-medication by laying eggs in an alcohol-containing food source to protect the developing larvae against parasitoids (Milan *et al*., 2012; Kacsoh *et al*., 2013). In other insect orders, pupae have also evolved chemical defences either to chemically hide from predators (Mizuno *et al.*, 2018) or become toxic (Deyrup *et al*., 2014). Such strategies may exist in drosophilid species but have yet to be widely explored. Overall, medication strategies in insects have rarely been reported, and may provide a promising line of future research in other invasive drosophilids and more generally in all species subject to parasitism.

4.4. Taking advantage of facilitation

There is growing research interest in understanding the impact of positive interactions between species, including facilitation, on community structure (Stachowicz, 2001; Fridley *et al*., 2007; Cavieres, 2021; Koffel *et al.*, 2021). Facilitation is defined as a positive interaction between two species that results in an increase in the density or biomass of at least one of the species (Rodriguez, 2006). A large body of work demonstrated that the presence of invasive species can facilitate infestations of native species, with sometimes wide-ranging impacts (Rodriguez, 2006). Within the Drosophilidae community, the introduction of *D. suzukii* could promote other fruit flies by increasing the amount of oviposition substrate available. Through this species' ability to pierce the skin of ripening fruit, an opening is created that allows access to the flesh for other species of fruit flies not possessing that ability, such as *D. subobscura* or *Z. indianus* (Poyet *et al*., 2014; Bernardi, 2017). The opening also allows pathogenic microorganisms to access the fruit, causing premature decay (Walsh *et al*., 2011) and thereby creating resources for fruit fly species that develop preferentially in rotten fruits (Poyet *et al*., 2014). For example, in grapes, where *D. suzukii* facilitates the infestation by *D. melanogaster* in this way (Rombaut *et al.*, 2017).

Conversely, the question of whether and how native species can facilitate invasive species has received less attention (Fridley *et al*., 2007; Gribben *et al*., 2020). The facilitation of invasive Drosophilidae by native species can occur at different trophic levels, via the host plant or via the activity of

other insects (including other drosophilids) or microorganisms. Invasive species can benefit from the presence of favourable host plant species (native or invasive) already present in their introduced ranges. For instance, exotic ornamental plants present in parks and gardens can be very good hosts for non-native species of Drosophilidae on which they are already pre-adapted. This is the case for *Aucuba japonica* (Thunb.), *Mahonia japonica* (Thunb.) and *Elaeagnus* ×*submacrophylla* (Servett), three widely planted exotic plants native to Asia, which host *D. suzukii* in its invaded distribution (Poyet *et al*., 2015; Deconninck *et al.*, under review). Invasive plant species can also act as favourable hosts for the development of invasive Drosophilidae. For example, the native American tree *Prunus serotina* is invasive to Europe and provides a suitable reservoir for *D. suzukii* and *D. subobscura* (Poyet *et al*., 2014).

Facilitation can also occur when an invasive drosophilid benefits from a native or other invasive insect species. For example, *Chymomyza amoena* oviposits in the galleries made by larvae of the moth *Cydia pomonella* in apples (Deconninck *et al*., 2024b). This invasive drosophilid also benefits from seed-boring insect parasites that damage and modify the fruit of several broad-leaved tree species, providing opportunities for the flies to oviposit (Band *et al*., 2005). Similarly, it has been observed that both *Z. indianus* and *Z. tuberculatus* can benefit from figs already infested by the invasive Mediterranean fruit fly, *Ceratitis capitata* (Bragard *et al.*, 2022 ; Geroges *et al.*, 2024). The presence of *C. capitata* larvae may facilitate the invasion of *Zaprionus* spp. by breaking down the fruit tissue, making it more suitable for oviposition and larval development. This interaction allows *Zaprionus* spp. to exploit a niche created by the native flies, thereby enhancing its ability to establish and spread in new environments. *Zaprionus indianus* also benefits from fruit infested by *D. suzukii* (Bernardi *et al.*, 2017), producing more offspring (de Paiva Mendonça *et al.*, 2023).

Finally, yeasts can act as facilitators for invasive species. For example, the yeast *Hanseniaspora uvarum,* which proliferates on both unripe and ripe raspberries, creates favourable conditions for *D. suzukii* development, suppressing an antagonist grey mould (*Botrytis cinerea*) and producing less ethanol (Chakraborty *et al*., 2022). Consequently, *D. suzukii* females lay more eggs on raspberries when *H. uvarum* is present (Chakraborty *et al*., 2022).

4.5. A little help from symbionts

Microbial symbionts are pervasive in insects and encompass a diverse array of bacteria, fungi and viruses that colonise the insect cuticle, gut and cells (Douglas, 2015). These symbionts have the ability to influence various aspects of the insect phenotype, ranging from physiology to behaviour (reviewed by

Feldhaar, 2011), thereby holding a central position in shaping the ecology and evolutionary trajectory of their hosts. Recent studies have increasingly emphasised the role of these microbes in enhancing the invasion success of their insect hosts (Lu *et al*., 2016; Liu *et al*., 2022). Throughout the introduction-naturalisation-invasion process, microbial symbionts can play crucial roles, influencing propagule pressure, resource acquisition, resistance to pathogens, tolerance to abiotic stressors and the competitive capacity of insects. These effects may stem from symbionts already present in the native population or arise from others acquired during the invasion process via horizontal transfer from native insects or directly from the environment (Wooding *et al*., 2013). Conversely, the diversity of the microbiome can be negatively impacted by the invasion process, particularly through the occurrence of bottlenecks, which may result in reduced fitness of invasive populations (Ørsted *et al.*, 2022).

Some symbionts have evolved mechanisms to manipulate the reproduction of their host to favour their own transmission. The best-known example is the bacterial symbiont *Wolbachia*, which has been described in various invasive *Drosophila* species including *D. suzukii, D. simulans* and *D. immigrans*. *Wolbachia* can spread by providing a fitness advantage to infected host individuals compared to uninfected ones (Kriesner & Hoffmann, 2018). For instance, in *D. simulans* and *D. suzukii*, *Wolbachia* infection is associated with increased fecundity and/or offspring survival (Mazzetto *et al*., 2015; Cao *et al*., 2019; Deconninck *et al*., 2024c). Such symbiont-induced effects could increase the propagule pressure of their host and, consequently, the likelihood of invasive events.

There are many examples within drosophilid species of diverse bacterial symbionts enhancing resistance to natural enemies such as viruses, fungi and parasitoids. Here, again, *Wolbachia* provides a well-known example and has been associated with resistance to viruses in *D. melanogaster* and *D. suzukii* (Hedges *et al*., 2008; Teixeira *et al*., 2008; Martinez *et al.*, 2014; Cattel *et al*., 2016a; Cogni *et al*., 2021) and towards entomopathogenic fungi in *D. melanogaster* (Panteleev *et al*., 2007). Another bacteria, *Spiroplasma,* is associated with parasitoid resistance in *D. hydei* (Xie *et al*., 2010).

The acquisition of symbionts can also increase tolerance to abiotic stresses which may, in turn, enhance the invasive potential of species. The gut microbiota is involved in heat tolerance in *D. subobscura* (Jaramillo & Castañeda, 2021) and cold tolerance in *D. melanogaster* (Henry & Colinet, 2018; see Corbin *et al.*, 2017 for review). Symbionts can also be beneficial to their host under nutrient-deficient conditions. For example, under iron stress, *D. melanogaster* females infected with *Wolbachia* have a higher fecundity than uninfected females (Brownlie *et al*., 2009). Recent studies have shown that the gut microbiota can

affect food choice and foraging decisions under laboratory conditions in *D. melanogaster* and can allow fruit flies to tolerate unfavourable nutritional situations (Yamada *et al*., 2015; Leitão-Gonçalves *et al*., 2017; Henriques *et al.*, 2020). Thus, symbiotic microbiota could impact the ability of their host flies to adapt to various available food sources and, more generally, to diverse abiotic and biotic conditions.

The microbiota associated with invasive species can also affect their competitive interactions with native species. For example, in the case of the invasive ladybird *Harmonia axyridis*, an associated microsporidium is benign, but is a lethal pathogen towards native ladybird species (Vilcinskas *et al*., 2013). To date, such effects have not been documented in invasive drosophilid species, but is further area worthy of research, given that many invasive species share the same or overlapping trophic niches with native counterparts.

Despite the multiple potential impacts microbiota may have on invasive success, no symbiont has yet been directly linked to the invasion of any drosophilid species. Some studies have noted differences in the microbiota associated with invasive and native populations. For example, Martinez-Sañudo *et al*. (2018) reported alterations in the microbiota of *D. suzukii* in introduced populations compared to their native counterparts. However, the precise roles of microbiota in their host's adaptation to a new environment remain a challenge to disentangle from the influence of the environment in shaping the microbiota.

5. FUTURE PERSPECTIVES

Understanding the invasive success of members of the Drosophilidae is crucial, as their presence not only impacts agricultural systems but also natural ecosystems through new interactions that can disrupt ecosystem functioning (Poyet *et al*., 2014; Tonina *et al*., 2018; Bühlmann & Gossner, 2022). Better evaluation of the traits underlying invasion success is required to grasp the full extent of the influence of these invasive species (Poyet *et al.*, 2015; Bühlmann & Gossner, 2022; Greenleaf *et al*., 2023). Our review allows us to crack the code of Drosophilidae invasions only partially. Among the traits or processes that clearly facilitate the ability to turn into an invasive species we can highlight: (1) the association with human activities, (2) the ability to counteract the allee effect, (3) being already a widespread species to limit inbreeding depression, (4) the maintenance of enough genetic diversity despite the bottleneck, (5) the occurrence of several introduction events, (6) the activity of transposable elements, (7) great adaptability and plastic responses to temperature variation and stress, (8) high level of behavioural plasticity, (9) the use of mixed strategies for overwintering, (10) tolerance to desiccation, (11) a broad

trophic niche width or adaptability, (12) the ability (pre-adaptation) to exploit empty niches, (13) a high degree of polyphagy, (14) strategies to attenuate the strength of competition, (15) the lack of natural enemies in the invaded area, and (16) the presence of microbial symbionts that can amplify any of the 15 previous traits. Not all these traits are mandatory to become invasive but a species should verify most of them.

We emphasise two categories of perspectives for filling crucial knowledge gaps and opening new research avenues in the light of the information collated in this review. First, key knowledge is still missing or incomplete, even for basic traits such as the ability to tolerate heat and cold, limiting our capacity to totally crack the code of invasion success in Drosophilidae. Here, we recommend the following actions:

- Acquire knowledge on the **traits that improve survival and even reproduction during the transportation routes**. Transportation via human means can take time, (e.g., several months for a species travelling in a container on a cargos), allowing a species to already develop several generations, providing that the conditions are adequate, thereby increasing the propagule size. However, the abiotic conditions within containers transported via marine or terrestrial routes are rarely recorded (Marquez *et al.*, 2012).
- Develop **knowledge of stress tolerance traits** across invasive and non-invasive Drosophilidae, using comparative approaches applying comparable methodologies between species to alleviate the challenge of comparing traits that have been measured differently in different studies (Ørsted *et al*., 2022). We further highlight that, although physiological traits related to temperature have received research attention, we still lack information on important areas such as behavioural thermoregulation or the capacity for behavioural adjustments including shifts in daily activity rhythms or in the ability to detect and exploit particular microclimates. In addition, we lack information and the technical ability to measure the precise microclimatic conditions experienced by larvae within their food resources.
- Investigate the **physiological and ecological correlations involved in the immune responses** of native versus invasive species. The role of the immune system in the success of invasions has been poorly investigated to date. Complex interactions between immune responses and physiological tolerance to stressors including heat and cold are known (Ferguson & Sinclair, 2020). Comparative analyses of these interactions between native and invasive species will offer novel insights into the determinants of invasion success.

- Better document **the ability to medicate**. Medication strategies in insects have rarely been reported and this appears to be an important area lacking research, both in terms of its potential influence on invasion success or more widely.
- Resolve **the puzzle of mutualistic symbionts**. While numerous examples illustrate the extent to which symbionts provide various benefits across drosophilid species, key knowledge gaps exist in the dynamics of these symbionts in natural populations and the drivers of invasion success. A key limitation here is that the prevalence of most symbionts in native and invading populations remains to be quantified (Cattel *et al*., 2016b).

Second, the outcomes of our review pave the way for new research avenues:

- There is a clear **need for the use of integrative approaches in understanding the success of invasions**. The pillars of such integrative approaches should interlink the genetic and genomic processes along the invasion route, the physiological and behavioural determinants of tolerance/resistance to multiple stressors, the roles of neurobiology and sensory ecology, chemical ecology and the biotic drivers of population establishment and development (see Fig x). Nonetheless, we also recognise the sheer complexity of the mechanisms that can contribute to invasion success. Neurobiology and sensory ecology of species is expected to play a role in distribution spreading (Szabo *et al.*, 2020) such as in hematophagous bugs for instance (Lazzari *et al*., 2013; De la Vega & Schilman, 2017).
- Trait databases for insects need to be developed, including the identification of invasive potential. There are ongoing initiatives (Insect trait database by Franck Courchamp), but functional and multi-trait-based approaches of Drosophilidae' distribution and ecology are rare (but see Kellermann *et al*., 2012a; Parratt *et al*., 2021) and do not target invasive species in particular. Moreover, most of them focus only on the gut microbiome of *Drosophila* species (Kang & Douglas, 2020) or are restricted to a single or a low number of traits such as wing size or colour (Ulmer *et al.*, 2024).

This review highlights the diversity of factors that promote invasion. The Drosophilidae family has been chosen as a study model, whose physiological traits and invasion strategies are likely to be found in many invasive species. Limiting biological invasions is a major challenge for our modern society that cannot be met without an integrative approach and in-depth knowledge of the multiple factors involved in invasion processes.

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Appendix 2 • Insect species collected in Spain

Table Insect species collected in Spain with mean mass ± sd (mg) and mean thermal preference ± sd (°C).

Site	Order	Species	N	Mass	± sd	Tp	± sd
Cercedilla	Coleoptera	*Ampedus sanguineus*	1	0.0381	–	17.79	–
		Black dung beetle	9	0.0199	0.0048	19.93	4.65
		Ceutorhynchus thlaspi	14	0.0024	0.0007	16.99	5.22
		Chrysolina americana	30	0.0342	0.0067	15.38	4.70
		Chrysolina bankii	2	0.0754	0.0113	20.66	3.76
		Click beetle	20	0.0169	0.0074	17.15	4.75
		Coccinella septempunctata	26	0.0347	0.0077	26.01	3.66
		Danacea sp.	3	0.0016	0.0007	18.32	3.64
		Gold dung beetle	30	0.0633	0.0184	21.81	6.50
		Gonioctena aegrota	32	0.0286	0.0071	22.39	3.92
		Gonioctena olivacea	5	0.0073	0.0025	17.79	4.17
		Hermaeophaga cicatrix	30	0.0012	0.0003	15.33	4.19
		Lachnaia tristigma	6	0.0365	0.0053	15.44	5.89
		Long horn beetle big	1	0.0102	–	29.77	–
		Oedemera sp.	30	0.0037	0.0013	20.52	5.91
	Dermaptera	*Forficula auricularia*	5	0.0257	0.0067	15.45	4.80
		Forficula sp	2	0.0278	0.0117	17.39	4.54
	Diptera	Drosophilidae	12	0.0002	0	14.49	3.17
		Syrphe	29	0.0075	0.0019	15.44	2.34
		Tephritis sp.	6	0.0026	0.0007	13.59	3.08
	Hemiptera	*Cinara cedri*	10	0.0020	0.0004	22.55	2.71
		Hadrodemus m-flavum	30	0.0043	0.0017	17.93	5.03
		Heterocordylus tibialis	25	0.0029	0.0008	15.82	3.72
		Holcogaster fibulata	27	0.0134	0.0032	18.58	6.24
		Nymph	3	0.0011	0.0006	14.28	5.33
		Reuterista instabilis	10	0.0057	0.0020	18.32	6.43
	Hymenoptera	*Aphaenogaster senilis*	30	0.0031	0.0004	15.70	4.98
		Formica cf. *rufa*	9	0.0036	0.0013	20.06	5.52
		Formica fusca	20	0.0049	0.0012	15.44	4.55
Perales	Coleoptera	*Brassicogethes aeneus*	25	0.0006	0.0002	14.98	3.35

		Certallum ebulinum	26	0.0112	±	0.0047	19.71	± 5.44
		Ceutorhynchus napi	30	0.0018	±	0.0003	17.94	± 5.72
		Ceutorhynchus thlaspi	30	0.0011	±	0.0004	17.15	± 4.39
		Chrysolina bankii	30	0.0981	±	0.0148	19.56	± 4.83
		Coccinella septempunctata	30	0.0320	±	0.0073	24.19	± 4.85
		Gonioctena aegrota	30	0.0289	±	0.0045	19.70	± 4.19
		Gonioctena aegrota subsp	3	0.0277	±	0.0011	19.98	± 2.56
		Henosepilachna argus	30	0.0246	±	0.0050	21.61	± 4.46
		Hermaeophaga cicatrix	21	0.0017	±	0.0008	17.91	± 5.84
	Dermaptera	*Forficula auricularia*	4	0.0178	±	0.0038	24.84	± 2.09
	Diptera	Diptera	9	0.0045	±	0.0020	15.77	± 2.81
		Drosophilidae	33	0.0001	±	0.0000	14.54	± 3.84
		Eupeodes corollae	27	0.0110	±	0.0035	15.61	± 2.89
		Platipalpus	2	0.0007	±	0.0001	15.34	± 1.45
	Hemiptera	*Crocistethus waltlianus*	19	0.0038	±	0.0009	18.07	± 4.85
		Eurydema ornata	14	0.0310	±	0.0087	18.64	± 5.10
		Myzus persicae	5	0.0009	±	0.0005	16.75	± –
		Pyrrhocoris apterus	30	0.0253	±	0.0048	21.15	± 4.66
		Reuterista instabilis	30	0.0065	±	0.0020	17.98	± 5.34
	Hymenoptera	*Aphaenogaster senilis*	27	0.0036	±	0.0006	17.17	± 4.74
		Tapinoma nigerrimum	30	0.0010	±	0.0004	17.13	± 3.89
Total			**942**	**0.0162**	±	**0.0218**	**18.34**	± **5.40**

Traduction

en français

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Résumé

L'augmentation des cas d'invasions biologiques dans le monde et les dégâts engendrés amènent à se poser des questions sur les facteurs qui favorisent leur succès afin de pouvoir anticiper les invasions futures. Dans cette thèse, je me suis basée sur la drosophile à ailes tachetées, *Drosophila suzukii*. La rapidité de son invasion et les pertes économiques importantes qu'elle engendre en pondant ses œufs dans des fruits mûrs, en font un modèle de choix. J'ai exploré certains mécanismes qui ont pu contribuer à son succès d'invasion. En particulier, je me suis intéressée à sa polyphagie, aux ressources alternatives qu'elle utilise en l'absence de fruits cultivés, ainsi qu'aux facteurs locaux, paysagers et climatiques qui favorisent ses infestations. Je me suis également intéressée à ses capacités de thermorégulation comportementale et à la plasticité de sa préférence thermique afin de mieux comprendre la facilité de *D. suzukii* à exploiter des microhabitats qui lui sont favorables. Enfin, j'ai étudié l'effet de l'interaction entre la ressource alimentaire, la température et la présence de symbiotes sur la performance de *D. suzukii* et sur ses choix de ponte. Cette thèse a mis en évidence la grande plasticité de *D. suzukii* face à des changements dans son environnement. Celle-ci a pu constituer une forme de pré-adaptation qui a favorisé son invasion mondiale. Elle ouvre la voie à des recherches appliquées pour la protection des cultures, mais aussi à de nombreuses recherches fondamentales autour de la sélection de la ressource, des stratégies de thermorégulation chez les ectothermes ou encore des approches intégratives pour comprendre le succès de l'invasion.

Mots clés: Ecologie trophique, Interaction plantes-insectes, Invasion biologique, Thermorégulation comportementale, *Wolbachia*

Succès invasif de *Drosophila suzukii*: Rôle de la polyphagie et de la plasticité thermique

Thèse par Gwenaëlle Deconninck

Introduction générale

1. CONTEXTE ET DÉFIS À RELEVER

À l'échelle mondiale, la biodiversité est actuellement confrontée à deux principales menaces : le changement climatique et les invasions biologiques (Ward et Master 2007). Le nombre d'espèces invasives, en particulier de plantes et d'insectes, a considérablement augmenté (Roques et al. 2009 ; Bonnamour et al. 2021). La mondialisation en facilitant les échanges transfrontaliers a favorisé la dispersion de ces espèces via le transport de marchandises ou humain (Levine et D'Antonio 2003 ; Fenn-Moltu et al. 2023b), tandis que le changement climatique facilite leur établissement (Dukes et Mooney 1999 ; Ward et Master 2007). Les espèces invasives peuvent impacter la plupart des services écosystémiques et ainsi causer des pertes économiques importantes (Bradshaw et al. 2016 ; Henry et al. 2023). Elles perturbent également le fonctionnement des écosystèmes et peuvent impacter la biodiversité (Kenis et al. 2009 ; Tobin 2015 ; Yadav et al. 2023).

De nombreuses études se sont penchées sur le processus d'invasion et sur ce qui fait la réussite d'une espèce envahissante, en particulier dans le contexte de la prévention ou de la gestion des invasions (Tait et al. 2021 ; Zhao et al. 2023). Pour réussir, les espèces doivent passer par toutes les étapes du continuum introduction-naturalisation-invasion (Chapple et al. 2012 ; Daly et al. 2023). Globalement, les insectes invasifs qui réussissent ont souvent plus de voies d'introduction, occupent plus d'habitats, ont une plus grande fécondité et un nombre de générations plus élevé, une durée de vie plus courte et un développement plus rapide de l'œuf à l'adulte (Zhao et al. 2023). Les capacités d'adaptation des espèces sont également déterminants dans le succès de l'invasion (Alpert et al. 2000 ; Richardson et Pyšek 2006 ; Lee et Gelembiuk 2008).

Drosophila suzukii (Matsumura) est reconnue comme l'une des 100 espèces d'insectes invasives les plus sérieuses au monde (Zhao et al. 2023). Originaire d'Asie, elle a réussi à envahir tous les continents, à l'exception de l'Antarctique, en moins de 20 ans (Walsh et al. 2011 ; Fraimout et Monnet 2018). Cette mouche coche toutes les cases d'une espèce invasive réussie. Elle a tiré parti de multiples voies

d'introduction (Fraimout et al. 2017), exploite aussi bien des habitats agricoles, naturels ou urbains (Poyet et al. 2015 ; Urbaneja-Bernat et al. 2020 ; Ulmer et al. 2024), a une grande fécondité combinée à une longue durée de vie adulte (Kanzawa 1939) et est capable de produire plusieurs générations par an (Kanzawa 1939 ; Schlesener et al. 2020) grâce à son développement rapide (Schlesener et al. 2020 ; Raynaud-Berton et al. 2024). *Drosophila suzukii* possède également plusieurs stratégies adaptatives comme la polyphagie, l'utilisation d'une niche souvent vacante (i.e., des fruits mûrs / sains ; Atallah et al. 2014), et de bonnes capacités d'acclimatation (Raynaud-Berton et al. 2024). *Drosophila suzukii* constitue un modèle idéal pour étudier les mécanismes sous-jacents au succès de l'invasion. Bien que *D. suzukii* ait été largement étudiée, des questions clés étaient sans réponse ou non résolues au début de cette thèse.

2. OBJECTIFS DE LA THÈSE

Dans le contexte de l'invasion biologique, la recherche fondamentale est essentielle. Acquérir des connaissances en biologie, en écophysiologie, en écologie des communautés et en écologie trophique contribue à prédire l'expansion des espèces, leur potentielle perturbation sur les écosystèmes et leurs impacts économiques, et souvent à trouver des solutions préventives ou curatives pouvant être incluses dans les programmes de lutte intégrée contre les ravageurs. *Drosophila suzukii* était prédisposée à devenir le ravageur envahissant qu'elle est devenue, en raison de sa polyphagie extrême, de sa large niche thermique et de sa proximité avec les humains, des caractéristiques connues pour favoriser les invasions (Zhao et al. 2023). Dans ma thèse, j'ai voulu approfondir les capacités remarquables de *D. suzukii*, en mettant l'accent sur son écologie trophique et thermique (Figure conceptuelle). La plupart des études sur *D. suzukii* se sont concentrées sur son impact sur les cultures à haute valeur économique, tandis que l'utilisation d'autres fruits en dehors de la saison de culture a été beaucoup moins étudiée. La question des fruits utilisés par *D. suzukii* en hiver et la manière dont ces fruits soutiennent les populations restait ouverte. C'est ce que j'étudie dans la première partie de ma thèse. Du côté thermique, *D. suzukii* a la capacité de se disperser et de sélectionner des micro-habitats qui la protègent des conditions nuisibles, y compris des températures chaudes ou froides. Cette capacité est probablement liée à d'importantes aptitudes de thermorégulation comportementale qui n'avaient pas été évaluées auparavant. La deuxième partie de ma thèse se concentre sur ce trait. De plus, les questions liées aux ressources trophiques et aux effets de la température sont liées puisque le cycle de vie de *D. suzukii* se déroule principalement dans des fruits dont la température varie selon les conditions environnementales. La dernière partie de ma thèse examine les relations entre la température et les ressources trophiques et s'intéresse à l'existence d'un compromis entre

les choix trophiques et thermiques. Enfin, le rôle de la souche de *Wolbachia* de *D. suzukii*, *wSuz*, est encore débattu. D'autres souches étant connues pour fournir des nutriments à leur hôte dans des conditions de carence nutritionnelle ou pour influencer la préférence thermique de leur hôte, je m'attendais à ce qu'elle influence à la fois la thermorégulation comportementale et les choix trophiques de *D. suzukii*. C'est pourquoi j'ai étudié le rôle de *Wolbachia* dans les parties expérimentales de ma thèse (c'est-à-dire la deuxième et la troisième parties).

2.1. Polyphagie

La première partie de ma thèse est divisée en trois chapitres dans lesquels je m'intéresse aux plantes non cultivées utilisées par *D. suzukii* en automne, en hiver et au début du printemps, qui sont des périodes difficiles pour la mouche. J'ai examiné l'influence des variables locales, climatiques et paysagères sur les infestations de *D. suzukii* sur plusieurs espèces de plantes à fruits charnus. Je me suis également intéressé à la place de *D. suzukii* dans la communauté des Drosophilidae dans ces fruits.

Le premier chapitre porte sur l'utilisation des fruits tombés au sol, en particulier les pommes (*Malus domestica*). Après la récolte, la plupart des fruits endommagés restent au sol et ces fruits peuvent constituer un réservoir pour les Drosophilidae (Bal et al. 2017 ; Leach et al. 2018). Même si *D. suzukii* pond préférentiellement sur des fruits sains et en cours de maturation (Atallah et al. 2014), elle peut également utiliser des fruits en décomposition (Lee et al. 2011 ; Cai et al. 2019). Les pommes sont une culture fruitière majeure dans le monde et une proportion considérable est endommagée et commercialement perdue (Jeannequin et al. 2015). *Drosophila suzukii* a déjà été signalée infestant des pommes dans quelques études (Walsh et al. 2011 ; Weydert et Mandrin 2013 ; Asplen et al. 2015 ; Briem et al. 2015 ; Bal et al. 2017). D'autres études ont également montré que *D. suzukii* était attirée par des sous-produits de la pomme (par exemple, le vinaigre de cidre ; Lasa et al. 2020) ou était capable de se développer dans des substrats contenant des pommes (Wallingford et al. 2018 ; Stockton et al. 2019 ; Jiménez-Padilla et al. 2020). Nous nous attendions à ce que les pommes tombées au sol constituent une ressource de secours pour la mouche en automne. Nous nous attendions également à ce que d'autres Drosophilidae utilisent cette ressource et nous voulions connaître la place de *D. suzukii* dans cette communauté. Nous avons cherché à démêler les rôles relatifs des facteurs microclimatiques, paysagers et locaux qui influencent la diversité de la communauté des Drosophilidae dans les pommes en décomposition au fil des saisons. Ce chapitre a été publié dans *Agricultural and Forest Entomology* en janvier 2024 (Deconninck et al. 2024b).

Le deuxième chapitre concerne l'utilisation des fruits du gui (*Viscum album*) entre la fin de l'hiver et la fin du printemps. Le gui constitue également un important réservoir de fruits qui mûrissent lentement de l'hiver à la fin du printemps. La capacité de *Drosophila suzukii* à infester *V. album* avait déjà été évaluée par des suivis sur le terrain (Briem et al. 2016 ; Panel et al. 2018 ; Delbac et al. 2020a) et lors d'expériences en laboratoire (Poyet et al. 2015 ; Briem et al. 2016 ; Wolf et al. 2020). Cependant, l'influence des facteurs environnementaux n'a pas été explorée, bien qu'ils soient censés agir sur le taux d'infestation des fruits. Nous nous attendions à ce que la température soit un important prédicteur de l'infestation de *V. album*, mais aussi à ce que la proximité d'un habitat naturel comme les bois, qui constituent des abris pour la mouche (Briem et al. 2018 ; Rossi-Stacconi et al. 2016 ; Ulmer et al. 2022), jouent un rôle. L'étude était nationale, avec la participation de sept laboratoires français pour l'échantillonnage. Ce chapitre a été publié dans *Arthropod-Plant Interactions* en mai 2024 (Deconninck et al. 2024a).

Le troisième chapitre porte sur l'utilisation des plantes ornementales exotiques et du gui. Cette étude a été réalisée à l'échelle régionale, dans le nord de la France. Il existe peu d'espèces végétales susceptibles d'être infestées par *D. suzukii* pendant l'hiver et le début du printemps. Nous avons étudié les plus infestées : *Aucuba japonica*, *Elaeagnus* ×*submacrophylla*, *Mahonia spp.* et *V. album* (Poyet et al. 2015). Ces plantes se trouvent couramment dans les parcs et jardins et pourraient être les catalyseurs des populations de *D. suzukii* au printemps à la fin de l'hiver, avant la disponibilité des premières cultures fruitières telles que les cerises, les fraises ou les framboises. Nous nous sommes intéressés aux rôles relatifs des facteurs climatiques, paysagers et locaux influençant les taux d'infestation de ces plantes hôtes par *D. suzukii*. Ce chapitre a été soumis à *Ecological Entomology* en juillet 2024 (Deconninck et al. 2024 under review a).

2.2. Plasticité thermique

Dans la deuxième partie de ma thèse, je me suis intéressé aux capacités de thermorégulation comportementale de *D. suzukii* et à leur valeur adaptative potentielle. Cette partie est divisée en trois chapitres.

Le premier chapitre vise à concevoir un nouveau cadre conceptuel pour étudier la plasticité des préférences thermiques chez les ectothermes et, plus généralement, les capacités de thermorégulation comportementale. Pour ce faire, j'ai analysé un ensemble de données produit en 2017 par Nicolas Meyer, un stagiaire co-encadré par Sylvain Pincebourde et Hervé Colinet. Son stage visait à quantifier la plasticité de la préférence thermique de *D. melanogaster*. Étant donné que *D. suzukii* et *D. melanogaster* partagent

plusieurs caractéristiques biologiques et sont proches phylogénétiquement (voir section 4.1.1), et parce que *D. melanogaster* est une espèce modèle bien étudiée, ce premier chapitre était un point de départ idéal. La préférence thermique a été étudiée à travers les stades de vie et différentes générations en considérant l'influence de différentes températures d'acclimatation. Plutôt que d'analyser les données de manière "classique", j'ai étudié la relation entre la moyenne de la préférence thermique et sa variance. Cela nous a permis de formuler plusieurs hypothèses sur les stratégies de thermorégulation comportementale qui pourraient coexister chez les ectothermes. Ce chapitre a été soumis à *The American Naturalist* en septembre 2024 (Deconninck et al. under review b).

Le deuxième chapitre concerne l'(hyper)plasticité de la préférence thermique de *D. suzukii*. *Drosophila suzukii* est capable de sélectionner activement des abris en fonction de la saison, du rythme circadien et des conditions environnementales en général (Tait et al. 2020a,b). Par conséquent, nous nous attendions à ce que sa préférence thermique soit hautement plastique. J'ai étudié l'influence de la température d'acclimatation sur la préférence thermique de *D. suzukii* ai cours des différents stades de vie et au fil de plusieurs générations, et j'ai considéré d'autres variables telles que le sexe, l'âge ou le statut reproductif. J'ai utilisé le cadre conceptuel mentionné ci-dessus pour examiner les capacités de thermorégulation comportementale de *D. suzukii*. Ce chapitre sera soumis à *Proceedings of the Royal Society series B* en 2024 (Deconninck et al. in prep a).

Le troisième chapitre vise à savoir si la plasticité de la préférence thermique de *D. suzukii* est adaptative. J'ai mené plusieurs expériences pour répondre à cette question à travers le prisme de la bioénergétique de la mouche. Tout d'abord, j'ai examiné l'influence de la température d'acclimatation sur le métabolisme de *D. suzukii* et sur sa sensibilité thermique. Je l'ai fait pour trois générations distinctes et j'ai également étudié l'influence de *Wolbachia*. J'ai mesuré le contenu énergétique (c'est-à-dire les glucides, protéines, lipides) des mouches provenant des différents traitements d'acclimatation. Ensuite, j'ai étudié s'il existait une relation entre la préférence thermique et le métabolisme. La question était la suivante : si une mouche choisit une température (dans le gradient thermique), est-ce lié à son métabolisme ? Nous nous attendions à ce qu'une mouche choisisse une basse température pour ralentir un taux métabolique de base élevé, par exemple. Ce chapitre sera soumis en 2025 (Deconninck et al. in prep b).

2.3. Approche intégrative

La troisième et dernière partie de ma thèse concerne l'interaction entre les choix thermique et trophiques, en prenant également en compte l'influence de *Wolbachia*. Elle est divisée en trois chapitres.

Le premier chapitre porte sur l'influence de la température sur la performance de *D. suzukii* dans les fruits d'hiver. Dans la première partie de ma thèse, j'ai étudié l'infestation de ces fruits d'hiver dans des conditions de terrain de janvier à juin. Bien que nous ayons obtenu des informations sur l'utilisation phénologique de ces fruits, ainsi que sur l'influence de plusieurs facteurs climatiques, paysagers et locaux sur leur infestation (voir Partie 1), la performance de *D. suzukii* dans les fruits d'hiver dans des conditions de températures réalistes d'hiver/début de printemps était encore inconnue. Panel et al. (2018) ont comparé la performance dans ces fruits sous température de laboratoire (~20°C) et ont trouvé des différences en termes de temps de développement, de fécondité et de survie. Sous des températures d'hiver/début de printemps, nous nous attendions à des différences dans la performance de la mouche en raison de besoins nutritionnels différents en conditions froides (Rendon et al. 2019). Ce chapitre a été soumis à *Insect Science* en juillet 2024 (Larges and Deconninck et al. 2024 under review).

Le deuxième chapitre porte sur le rôle potentiel de *Wolbachia* dans la polyphagie. La polyphagie peut être coûteuse pour la mouche en raison de la qualité nutritionnelle variable des fruits de différentes espèces de plantes, ce qui peut impacter la fitness générale (Janz 2003). Ce coût peut être compensé par une femelle qui pond des œufs dans différents fruits (stratégie de 'bet-hedging'), mais *Wolbachia* pourrait aussi jouer un rôle à cet égard. Comme d'autres souches de *Wolbachia* ont déjà été reconnues comme mutualistes nutritionnels (e.g., Hosokawa et al. 2010), nous avons émis l'hypothèse (après une discussion avec Aurélien Vigneron) que *wSuz* pourrait fournir des nutriments à *D. suzukii* en contexte de carence. Ce chapitre a été publié dans *Journal of Pest Science* en janvier 2024 (Deconninck et al. 2024c).

Dans le dernier chapitre, nous étudions l'existence d'un compromis entre le choix trophique et le choix thermique. Sur le terrain, *D. suzukii* fait face à deux contraintes lorsqu'elle choisit une ressource pour pondre. Premièrement, la qualité nutritionnelle de la ressource doit maximiser la performance de la descendance, selon l'hypothèse de la performance-préférence (Gripenberg et al. 2010). Selon cette même hypothèse, nous nous attendions également à ce que la température de la ressource soit importante lors du choix du fruit, puisque la température détermine également la performance de la descendance (Raynaud-Berton et al. 2024). Nous avons examiné l'existence d'un compromis entre la température et la qualité nutritionnelle de la ressource et étudié le rôle de *Wolbachia* dans ce compromis. Ce chapitre sera soumis d'ici la fin de l'année 2024 (Destierdt and Deconninck et al. in prep).

INTRODUCTION

Partie 1
Ressources trophiques au fil des saisons

En hiver, les conditions climatiques difficiles peuvent engendre des goulets d'étranglement pour les populations. Dans cette première partie, je me suis intéressé aux ressources utilisées par *D. suzukii* entre l'automne et le printemps, en dehors de la saison des fruits cultivés. L'étendue de la niche trophique de *D. suzukii* a déjà été étudiée, dont l'utilisation des fruits sauvages et ornementaux (Poyet et al. 2015 ; Kenis et al. 2016), mais pas les facteurs régissant l'infestation de ces fruits de l'automne au printemps.

Dans le premier chapitre de la Partie 1 (Chapitre 1), j'ai étudié l'utilisation des fruits au sol par *D. suzukii* de l'automne à l'hiver. Ces fruits peuvent jouer un rôle important en tant que substrats pour l'alimentation et la reproduction des communautés d'arthropodes (Alamiri 2000 ; Rohlfs et Hoffmeister 2004 ; Mészárosné Póss et al. 2022). Ils peuvent également servir de réservoir pour les insectes nuisibles lorsque les fruits cultivés se font rares, offrant un refuge saisonnier et pouvant potentiellement initier de futures épidémies de ravageurs (Liquido 1991). Ce chapitre a exploré l'utilisation des pommes tombées au sol par *D. suzukii*, une ressource présente en quantités importantes après la récolte (Jeannequin et al. 2015) et qui peut rester au sol plusieurs mois jusqu'à décomposition complète (Myczko et al. 2013 ; Stockton et al. 2019 ; Folwarczny et al. 2022).

Dans le Chapitre 2, je me suis intéressée au gui dont les fruits sont connus pour être utilisés par *D. suzukii* (Briem et al. 2016 ; Panel et al. 2018) et qui constitue un important réservoir de fruits en hiver et au printemps (Thomas et al. 2023). Le gui est l'un des rares fruits disponibles durant cette période, mais ses dynamiques d'infestation n'avaient pas encore été explorées. L'étude a été menée à l'échelle nationale en France afin de mieux comprendre les dynamiques biogéographiques des infestations de *D. suzukii*.

Dans le Chapitre 3, j'ai poursuivi l'exploration du continuum des ressources disponibles pour *D. suzukii* de l'hiver au printemps (Grassi et al. 2018). Les fruits connus pour être utilisés incluent diverses plantes ornementales de jardins et parcs, ainsi que des espèces sauvages (Poyet et al. 2015 ; Kenis et al. 2016). Ces fruits pourraient initier les populations de *D. suzukii* au début de printemps, pourtant leur rôle en tant que sites de ponte potentiels a été peu documenté. Dans ce chapitre, les infestations dans les plantes ornementales *Aucuba japonica, Elaeagnus ×submacrophylla, Mahonia aquifolium* et *M. japonica* agg., ainsi que dans le gui *V. album*, ont été étudiées. L'étude a été réalisée à une échelle plus réduite que celle décrite dans le Chapitre 2, en utilisant une approche paysagère multi-ressources régionale et une substitution espace-temps (SFTS ; Pickett 1989) permettant de reconstituer les dynamiques temporelles.

DISCUSSION

Partie 1
Ressources trophiques au fil des saisons

Les trois chapitres de la Partie 1 ont mis en évidence l'utilisation saisonnière des ressources par *D. suzukii* ainsi que les facteurs locaux, paysagers et climatiques qui influencent ses infestations.

En automne, *D. suzukii* est capable d'utiliser les ressources éphémères (ERP ; Butterworth et al. 2022) fournies par les pommes tombées au sol qui commencent leur décomposition, ce qui est cohérent avec l'écologie de *D. suzukii* (Kienzle et al. 2021). L'utilisation d'autres ERP par *D. suzukii* reste à explorer, mais il est probable que d'autres fruits au sol, tels que les prunes, les poires ou, plus tard dans la saison, les kakis, soient infestés et entretiennent les populations dans une période où les fruits frais sont rares.

En hiver et au printemps, les mesures microclimatiques récentes sont plus adaptées pour prédire les infestations de *D. suzukii* que les mesures régionales, reflétant probablement l'effet de la température sur la dormance reproductive des femelles (Colinet et Kustre 2024). Des recherches supplémentaires sont nécessaires pour identifier les facteurs déclenchant la fin de cette dormance (Numata et Shintani 2023).

Dans les trois chapitres, les principaux facteurs influençant les infestations variaient selon les plantes hôtes. Toutefois, deux facteurs ont été constamment identifiés : la température locale des jours précédents et l'architecture des plantes. La température impacte le développement et le succès de l'émergence des mouches (Raynaud-Berton et al. 2024) et, par conséquent, la taille de la population disponible pour infester les plantes hôtes environnantes. Le rôle du climat (et du paysage) sur les performances de vol et la dispersion passive de *D. suzukii* mérite d'être étudié pour distinguer les effets sur la taille des populations et la capacité à atteindre les ressources. L'architecture des plantes peut également fournir une protection contre les conditions environnementales défavorables. Des études sur le rôle de l'architecture des plantes dans la persistance de *D. suzukii* en hiver sont nécessaires.

Enfin, la Partie 1 a soulevé des questions sur les stratégies de gestion des ravageurs et leurs conséquences écologiques. Pour réduire les infestations de *D. suzukii* dans les cultures, il serait possible de récolter les fruits au sol, d'éradiquer le gui ou d'élaguer les plantes ornementales. Cependant, même si ces plantes et leurs fruits entretiennent les populations de *D. Suzukii*, elles fournissent aussi des ressources importantes pour d'autres arthropodes, oiseaux, petits mammifères, etc. (Watson 2001 ; Myczko et al. 2013 ; Thomas et al. 2023). Pour limiter les impacts sur les espèces natives, les stratégies de gestion devraient donc se concentrer sur les zones proches des productions agricoles, avec un suivi des plantes hôtes potentielles environnantes tout en limitant la plantation de plantes ornementales.

INTRODUCTION

Partie 2

Plasticité de la préférence thermique et mécanismes physiologiques

La capacité à sélectionner des microhabitats favorables pourrait favoriser certaines espèces au cours du processus d'invasion. Pourtant, les capacités de thermorégulation comportementale des espèces invasives ont rarement été explorées. Dans la Partie 2, mon objectif était de caractériser la plasticité de la préférence thermique (Tp) de *D. suzukii* et sa valeur adaptative potentielle.

Le premier chapitre de la Partie 2 (Chapitre 4) décrit une nouvelle méthode pour étudier la Tp chez les ectothermes. La plupart des études s'intéressent à la moyenne de la Tp (e.g., Labra et al. 2009 ; Takeuchi et al. 2009), puisqu'elle représente la température ciblée par une population (Camacho et Rusch 2017). Seulement, la moyenne ne dit rien des capacités de thermorégulation d'une espèce. En m'appuyant sur des exemples de la littérature, j'ai développé un nouveau cadre conceptuel avec des hypothèses concernant les stratégies de thermorégulation comportementale. J'y ai également intégré le niveau de plasticité de la Tp. J'ai ensuite éprouvé ce cadre conceptuel avec l'organisme modèle *D. melanogaster*.

Dans le Chapitre 5, j'ai utilisé ce nouveau cadre pour étudier la plasticité de la Tp chez *D. suzukii*. Puisque *D. suzukii* présente plusieurs adaptations saisonnières (Tait et al. 2021a,b), une flexibilité d'acclimatation (Raynaud-Berton et al. 2024), exploite différents habitats (e.g., cultures, forêts, zones urbaines ; Ulmer et al. 2024), et envahit aussi bien des zones tempérées que tropicales (Walsh et al. 2011), nous nous attendions à des capacités de thermorégulation comportementale (+Tp) hautement plastiques.

Dans le Chapitre 6, j'ai étudié la variabilité inter-individuelle de la Tp en intégrant quatre étapes distinctes. Premièrement, j'ai examiné la répétabilité individuelle de la Tp de *D. suzukii* à court terme. J'ai ensuite quantifié les capacités d'acclimatation de *D. suzukii* en mesurant le métabolisme des mouches élevés dans différents régimes de température à différentes températures. Puis j'ai mesuré le contenu énergétique des mouches se développant aux mêmes températures d'acclimatation. J'ai également exploré l'existence d'une relation entre la Tp et le métabolisme. Enfin, j'ai intégré ces étapes en calculant la variabilité inter-individuelle de chacun de ces traits pour les comparer.

Dans les Chapitres 5 et 6, j'ai intégré la présence ou l'absence de la bactérie *Wolbachia* comme variable afin d'étudier son rôle, encore non élucidé, chez *D. suzukii*. Chez l'espèce apparentée *D. melanogaster*, certaines souches de *Wolbachia* diminuent la Tp (Truitt et al. 2019), bien que ce ne soit pas le cas dans toutes les études (Strunov et al. 2023). Les symbiotes peuvent également induire un coût et entraîner des impacts sur le contenu énergétique ou le métabolisme de l'hôte (Clavé et al. 2022).

DISCUSSION

Partie 2

Plasticité de la préférence thermique et mécanismes physiologiques

Cette deuxième partie explore la plasticité de la préférence thermique (Tp), ses conséquences sur le comportement de thermorégulation, ainsi que ses liens avec le métabolisme et la bioénergétique.

La Tp fournit des informations cruciales sur la capacité des ectothermes à thermoréguler de manière comportementale, et donc à survivre et/ou se reproduire dans des environnements hétérogènes (Leith et al. 2024). Nous avons développé un cadre conceptuel permettant de déterminer les stratégies de thermorégulation comportementale (TC) en intégrant la moyenne de la Tp et sa variance, correspondant respectivement au besoin thermique et à la précision de la TC. En testant ce cadre sur *D. melanogaster*, nous avons pu déterminer qu'une stratégie de "précision à l'extrême" dominait à tous les stades de vie, en accord avec des études récentes affirmant que la TC était principalement utilisée pour éviter la surchauffe et améliorer la survie (Leith et al., 2024). Il est probable que l'amplitude des réponses de la Tp à diverses variables, tant endogènes qu'exogènes, module les réponses au réchauffement climatique.

Nous avons appliqué notre cadre en utilisant *D. suzukii* et en induisant des réponses plastiques de la Tp avec un plus grand nombre de variables qu'avec *D. melanogaster*. Une fois encore, la stratégie de "précision à l'extrême" était la principale stratégie de TC. De manière surprenante, l'infection par *Wolbachia* a inversé cette stratégie, ce qui pourrait être dû soit au fait que *D. suzukii* choisisse des températures limitant la prolifération de *Wolbachia* (comme suggéré chez *D. melanogaster* par Truitt et al. 2019), soit au fait que *Wolbachia* manipule la stratégie de TC de la mouche pour son propre bénéfice. Ces hypothèses nécessitent des études complémentaires. Nos données ont également démontré que les mâles de *D. suzukii* étaient très précis dans le choix de températures basses qui les protègeraient contre la stérilité, ce qui suggère qu'ils pourraient être moins à risque face au réchauffement climatique que d'autres stades de vie, contrairement à ce qu'ont avancé d'autres études (van Heerwaarden et Sgrò, 2021 ; Ørsted et al. 2024). Les limites thermiques critiques ne sont pas suffisantes pour évaluer la vulnérabilité des organismes au changement climatique et nécessitent des approches intégratives incluant la détermination de l'hétérogénéité thermique locale disponible et de leur capacité de thermorégulation.

Enfin, nous avons exploré les mécanismes physiologiques liés à la la variabilité interindividuelle de la Tp et avons détecté une apparente stochasticité dans les choix individuels. Cette sélection stochastique, combinée à des capacités thermorégulatrices très précises chez *D. suzukii*, pourrait être un élément crucial de son succès d'invasion, associé à sa polyphagie exceptionnelle, facilitant potentiellement sa dispersion.

INTRODUCTION

Partie 3
Polyphagie, température et sélection de la ressource

Cette troisième partie intègre les deux premières parties de ma thèse en abordant l'interaction tripartite entre les ressources trophiques, la température et l'infection par *Wolbachia*.

Dans le premier chapitre de la Partie 3 (Chapitre 7), j'ai étudié l'influence de températures froides sur les performances de *D. suzukii* dans les fruits d'hiver. La plupart des études sur les performances de *D. suzukii* dans des fruits sauvages utilisent une température expérimentale d'environ 20-25°C (Poyet et al. 2015 ; Panel et al. 2018), ce qui peut limiter la pertinence écologique pour les périodes d'hiver et de printemps étant donné que la température influence à la fois ses traits d'histoire de vie (Raynaud-Berton et al. 2024) et ses besoins nutritionnels (Rendon et al. 2019). J'ai donc étudié l'effet de températures fluctuantes, simulant les conditions hivernales et printanières, sur plusieurs traits d'histoire de vie de *D. suzukii* développés dans les fruits d'hiver *A. japonica, E. ×submacrophylla* et *V. album* (cf. Chapitre 3).

Dans le Chapitre 8, je me suis intéressée au rôle *Wolbachia*. La plupart des études disponibles sur la souche de *Wolbachia* de *D. suzukii* semblent indiquer qu'elle joue un rôle mutualiste plutôt que parasitaire (Saeed et al. 2018 ; Mazzetto et al. 2018 ; également soutenu par les résultats de la Partie 2), ce chapitre examine l'existence d'un rôle nutritionnel. En tant qu'espèce polyphage, *D. suzukii* fait face à d'importantes variations dans la qualité nutritionnelle de ses ressources que *Wolbachia* pourrait aider à surmonter. J'ai utilisé des mouches infectées et non infectées, deux substrats alimentaires de qualité nutritionnelle différente, et j'ai mesuré plusieurs traits d'histoire de vie pour évaluer l'effet de *Wolbachia*.

Dans le Chapitre 9, j'intègre l'influence de la température, des fruits d'hiver et de *Wolbachia*, et les lie avec les préférences d'oviposition de *D. suzukii*. Plusieurs études ont exploré l'influence de la qualité nutritionnelle sur le choix de ponte et sur les performances en tenant compte du fruit utilisé (Cai et al. 2019), du ratio protéines:sucres (Silva-Soares et al. 2017), de la présence de dommages (Kienzle et Rohlfs 2021) ou de microbes (Sato et al. 2021). Cependant, aucune n'a encore étudié l'influence combinée de la température et de la qualité nutritionnelle sur le choix de ponte de *D. suzukii*. De même, bien que *Wolbachia* influence le choix de l'hôte chez certaines espèces (Wiwatanaratanabutr et al. 2010 ; Abrun et al. 2021), son rôle dans la ponte n'a pas été étudié chez *D. suzukii*. Ce chapitre a examiné l'influence de la température et de la qualité nutritionnelle sur le choix de ponte de *D. suzukii,* chez des femelles infectées ou non par *Wolbachia*. J'ai également évalué si l'hypothèse de préférence-performance (Gripenberg et al. 2010) pouvait être vérifiée chez *D. suzukii* dans ces conditions.

DISCUSSION

Partie 3

Polyphagie, température et sélection de la ressource

Cette Partie 3 souligne l'importance des approches multifactoriales pour étudier la performance ou le comportement des organismes. En particulier, les données présentées dans ces trois chapitres confirment que la température, la qualité nutritionnelle et la présence de symbiotes interagissent pour influencer les choix de ponte de *D. suzukii* et, par conséquent, sa fitness.

Les études expérimentales sont nécessaires pour comprendre les processus physiologiques et écologiques, mais elles sont souvent réalisées dans des conditions contrôlées qui peuvent limiter leur pertinence écologique. Par exemple, la température a influencé à la fois la performance de *D. suzukii* dans différents fruits, mais a également modifié le classement de ces fruits en termes de performance, ainsi que le choix de ponte des femelles. Associer les observations sur le terrain à des expériences en laboratoire est essentiel pour comprendre les facteurs influençant l'infestation des fruits dans la nature, ainsi que pour explorer les compromis entre les traits d'histoire de vie associés aux conditions environnementales.

L'hypothèse de préférence-performance stipule que les femelles devraient préférentiellement pondre dans des substrats qui maximisent la fitness de leurs descendants (Gripenberg et al. 2010). Cette hypothèse repose principalement sur la qualité nutritionnelle des substrats sans considérer la température. Pourtant, les données obtenues dans cette étude suggèrent que la température est plus importante que la qualité nutritionnelle pour déterminer la performance des descendants chez *D. suzukii*. L'hypothèse de préférence-performance ne s'applique pas à *D. suzukii* sur la base des choix nutritionnels (voir aussi Scheirs et al. 2000 ; Olazcuaga et al. 2019) mais dans un contexte multifactoriel (incluant la qualité nutritionnelle et la température), *D. suzukii* est capable de faire un choix qui favorise le développement de ses descendants. Des recherches supplémentaires sont nécessaires pour étudier l'influence d'autres propriétés des fruits (e.g., odeur, couleur, forme, texture) sur le choix de ponte, associées à différentes conditions de température, pour améliorer la compréhension des compromis impliqués dans la maximisation de la fitness, tant chez *D. suzukii* que dans le cadre d'études multi-espèces.

Enfin, la Partie 3 a généré de nouvelles perspectives sur l'influence de l'infection par *Wolbachia* sur *D. suzukii*. Bien que la présence de *Wolbachia* semble réduire la fécondité, ce coût est compensé par une survie globale plus élevée des descendants. *Wolbachia* a également influencé le choix de ponte de *D. suzukii* dans certaines conditions de température. L'interaction entre la température et les effets de *Wolbachia* nécessiterait des recherches supplémentaires.

Discussion générale

Les objectifs de ma thèse étaient d'explorer les capacités remarquables de *D. suzukii*, espèce invasive et d'importance commerciale, en mettant l'accent sur son écologie trophique et thermique.

Dans la **Partie 1**, j'ai commencé par étudier le continuum de ressources disponibles pour la mouche, de l'automne au printemps, période pauvre en resources puisqu'en dehors de la période des cultures commerciales. Les résultats obtenus ont montré que *D. suzukii* était capable d'exploiter les pommes tombées au sol, le gui et diverses plantes ornementales. Ces ressources 'de secours' pourraient constituer un réservoir pour *D. suzukii* et catalyser le développement de ses populations au printemps, prêtes à infester en nombre les cultures de fruits précoce. Les travaux de la Partie 1 ont également démontré que, bien que les facteurs environnementaux influençant les infestations de *D. suzukii* varient entre les plantes hôtes, l'architecture des plantes et la température jouent invariablement un rôle. Ces deux variables sont des éléments clés qui interagissent pour produire une mosaïque de températures (microclimat) à l'échelle de la plante hôte et pourraient donc être liées aux capacités de thermorégulation comportementale explorées dans la Partie 2.

Les résultats de la **Partie 2** ont montré que la préférence thermique de *D. suzukii* (un trait majeur de la thermorégulation comportementale) est très plastique, ce qui pourrait aider à protéger la mouche des conditions défavorables et améliorer sa fitness. Il existe cependant une grande variabilité interindividuelle dans la préférence thermique, qui semble trouver son origine dans des mécanismes physiologiques.

Dans la dernière partie de ma thèse (**Partie 3**), j'ai examiné la relation entre les ressources trophiques et la température, ainsi que l'existence de compromis potentiels influençant le développement de *D. suzukii*. Les résultats obtenus suggèrent que l'influence de la température est plus importante que celle de la qualité des fruits pour déterminer la performance générale de la mouche.

J'ai également examiné le rôle de la souche de *Wolbachia* de *D. suzukii*, *wSuz*, sur certains des traits identifiés tout au long de la thèse, concluant qu'elle pourrait fournir des bénéfices nutritionnels à la mouche. Mes données démontrent que l'infection par *wSuz* aide *D. suzukii* à s'adapter aux changements de régime alimentaire, augmente la survie des larves pendant le développement et peut influencer le choix de l'oviposition par les femelles.

La figure conceptuelle présentée initialement dans l'introduction, mise à jour à la lumière de ces résultats, fournit une vue d'ensemble de ma thèse (Figure 1).

Dans cette discussion, j'explore et intègre plus en profondeur les résultats des différentes études réalisées dans le cadre de ma thèse, et je mets en lumière de nouvelles questions de recherche qui seraient intéressantes à développer.

> « L'important est de ne jamais cesser de poser des questions. La curiosité a sa propre raison d'être. On ne peut s'empêcher d'être en admiration devant les mystères de l'éternité, de la vie ou de la merveilleuse structure de la réalité. Il suffit d'essayer de saisir un peu de ce mystère chaque jour. Cette curiosité sacrée ne devrait jamais être perdue. » Albert Einstein

Ce chapitre de discussion est présenté en trois sections. La première traite de la niche trophique de *D. suzukii* et de ses implications, y compris dans le contexte du changement climatique. La deuxième aborde la thermorégulation comportementale de *D. suzukii* et des insectes en général. La troisième et dernière section se concentre sur le rôle de *Wolbachia* dans la biologie de *D. suzukii*. Dans cette partie traduction, ne sont présentées que les introductions des sections et sous-sections.

1. NICHE TROPHIQUE ET IMPLICATIONS ÉCOLOGIQUES

Dans cette section, je traite principalement des sujets abordés dans la première partie. En particulier, j'examine comment la polyphagie de *D. suzukii* aide cette mouche à faire face à la saisonnalité des ressources, et comment le changement climatique pourrait influencer son hivernage dans les régions tempérées. Ensuite, je mets en avant la capacité particulière de *D. suzukii* à utiliser des fruits toxiques pour d'autres consommateurs, soulignant que, bien que cela puisse réduire la pression des compétiteurs et des prédateurs, cela nécessite des capacités de détoxification. Enfin, j'évalue les effets en cascade potentiels que *D. suzukii* pourrait engendrer sur les communautés locales, en particulier les insectes, les plantes et les disperseurs de graines vertébrés.

1.1. Fruits toxiques, avantage et détoxification

Drosophila suzukii possède la capacité particulière d'exploiter des fruits toxiques. Cela pourrait conférer à la mouche plusieurs avantages, tels que l'occupation de 'niches vides' sans compétiteurs ('empty niche hyptohesis'), non utilisées par les ennemis naturels ('enemy release hypothesis) et potentiellement une défense contre les parasitoïdes. Pour se développer dans ces fruits toxiques, *D. suzukii* doit probablement posséder des capacités de détoxification qui mériteraient d'être étudiées.

1.2. Saisonnalité des ressources et phénologie

Drosophila suzukii peut se développer sur plusieurs espèces végétales grâce à sa polyphagie, ce qui lui permet de faire face à la saisonnalité des ressources, en passant d'une ressource à une autre selon leur disponibilité. En parallèle, la température basse agit sur le développement de *D. suzukii* ainsi que sur la phénologie des plantes, ce qui peut limiter la population de la mouche, notamment en hiver dans les régions tempérées. Cependant, dans certaines zones envahies par la mouche, elle pourrait ne pas être limitée par les températures froides, et avec le changement climatique en cours, les besoins en hivernage pourraient également être réduits dans d'autres régions.

1.3. Effets en cascade

En tant qu'espèce invasive, *D. suzukii* pénètre dans des écosystèmes natifs qui sont généralement considérés comme étant dans un état d'équilibre ou stable. En s'intégrant dans ces communautés locales, *D. suzukii* peut entraîner des effets en cascade sur divers composants des écosystèmes.

2. PRÉFÉRENCE THERMIQUE ET DISTRIBUTION DES INSECTES

Dans cette section, je développe les questions encore ouvertes autour de la préférence thermique des ectothermes. En particulier, je souligne la nécessité d'améliorer nos connaissances sur les plages de températures auxquelles sont exposés les organismes à l'échelle microclimatique dans leur environnement naturel et de développer des approches pour mesurer la préférence thermique dans des conditions semi-naturelles. J'explique pourquoi l'étude des stratégies de thermorégulation comportementale peut améliorer l'évaluation de la vulnérabilité des espèces face au changement climatique. J'encourage également le développement d'études intégratives qui prendraient en compte à la fois les stratégies de thermorégulation comportementale ainsi que d'autres traits des organismes, tels que les limites thermiques, les métabolismes ou la résistance à la dessiccation, afin de mieux comprendre les mécanismes qui leur permettent de faire face à la variabilité environnementale. En ce sens, je présente brièvement des résultats préliminaires d'une étude comparative menée entre des espèces d'insectes collectées en Espagne dans le cadre d'un projet de collaboration internationale avec Cameron Ghalambor (Professeur, NTNU, Norvège).

2.1. Préférence thermique en conditions naturelles

La préférence thermique des arthropodes est souvent mesurée à l'aide de gradients thermiques en laboratoire, ce qui limite la généralisation des résultats à des échelles écologiques plus larges (Kinzner et al. 2019). Bien que le cadre conceptuel que nous avons conçu et appliqué dans le chapitre 6 permette de prédire les stratégies potentielles de thermorégulation comportementale de n'importe quelle espèce, les évaluations de ces réponses en conditions naturelles restent rares. Pour ce faire, deux étapes complémentaires sont nécessaires : (i) acquérir des connaissances sur les températures auxquelles les organismes sont exposés dans leur environnement naturel (c'est-à-dire à l'échelle du microhabitat) et (ii) analyser si les réponses des organismes dans la nature sont comparables à celles obtenues en laboratoire.

2.2. Thermorégulation comportementale chez les insectes

Alors que les températures critiques (CTmin et CTmax) sont couramment utilisées dans les études sur les schémas de distribution des espèces ou pour prédire leurs réponses au changement climatique (Huey et al. 2012), les préférences thermiques (Tp) sont rarement prises en compte (Sinclair et al. 2016). Cependant, les capacités de thermorégulation comportementale des espèces pourraient constituer un élément important des réponses aux variations environnementales, en réduisant la vulnérabilité au changement climatique ou en facilitant l'expansion des aires de répartition. Les synergies entre les caractéristiques climatiques, telles que la température et l'humidité relative, pourraient favoriser certaines stratégies de thermorégulation comportementale (par exemple, précision à l'optimum ou aux extrêmes, comme discuté dans le chapitre 4). De plus, la Tp pourrait être corrélée à d'autres traits, tels que CTmin, CTmax, les taux de respiration ou la résistance à la dessiccation, et plus important encore, ce niveau de corrélation pourrait largement varier entre les espèces, des sujets qui nécessitent des recherches supplémentaires.

3. RÔLE DE *WOLBACHIA* DANS LA BIOLOGIE DE *D. SUZUKII*

Le rôle de la souche *Wolbachia* de *D. suzukii*, *wSuz*, a été étudié dans divers travaux, y compris dans cette thèse. La plupart des études à ce jour n'ont pas pu conclure pour déterminer si la bactérie agit comme mutualiste, parasite ou est neutre, bien que des preuves comme celles rapportées dans les chapitres 6 (réduction du métabolisme), 8 et 9 (augmentation du succès d'émergence) soutiennent davantage un rôle mutualiste. Les coûts et avantages associés à l'infection par *Wolbachia* peuvent ne pas être clairement

définis, ne se manifestant que sous certaines conditions difficiles à identifier, comme c'est le cas pour les symbiotes qui ne manipulent pas la reproduction et sont maintenus à des fréquences intermédiaires à élevées (Zug et Hammerstein 2015 ; McPherson et al. 2023). J'examine ce que l'on sait plus largement sur cette symbiose et les questions importantes qui restent sans réponse, notamment la prévalence de *wSuz* dans les populations naturelles, les effets connus de *wSuz* sur *D. suzukii* et les axes de recherche à développer pour pour mieux comprendre ce sujet.

3.1. Prévalence dans les populations naturelles

La prévalence de *wSuz* dans les populations de *D. suzukii* est connue pour être très variable, à la fois entre les régions géographiques et entre les générations (Hamm et al. 2014 ; Cattel et al. 2016a ; McPherson et al. 2023). Les évaluations de la prévalence régionale remontent à environ 10 ans, ce qui limite la compréhension de la dynamique de *wSuz* et de son rôle dans les populations.

3.2. *Wolbachia wSuz* : Mutualiste, parasite ou neutre ?

La plupart des données obtenues jusqu'à présent sur le rôle de *wSuz* dans *D. suzukii* indiquent des effets globalement positifs résultant de l'infection bactérienne, comme cela apparaît également dans les chapitres 6, 8 et 9 de ma thèse. Comme indiqué précédemment, la force de ces effets semble être liée à l'état physiologique de la mouche et/ou aux conditions environnementales auxquelles elle est exposée.

4. CONCLUSION

L'importance de ma thèse réside dans son approche intégrative, combinant des disciplines telles que l'écologie trophique, l'écologie thermique, l'écophysiologie, les interactions insecte-plante-symbiote, l'écologie des communautés et l'écologie comportementale dans le contexte des invasions biologiques. Cette approche offre une perspective plus large sur des phénomènes complexes, tels que l'établissement et la propagation d'une espèce envahissante.

Les données obtenues indiquent clairement l'extraordinaire plasticité adaptative de *Drosophila suzukii*, qui a très probablement contribué à son succès invasif. Sa large polyphagie permet à *D. suzukii* d'exploiter des fruits disponibles tout au long de l'année, dont certaines sont entièrement exemptes de compétition, tandis que d'autres sont toxiques et pourraient être utilisées pour éloigner les parasitoïdes. Sa remarquable plasticité de thermorégulation comportementale permet à la mouche de sélectionner

précisément des températures favorables à son développement (ou à d'autres processus physiologiques) et d'éviter des températures (sub)létales. L'infection par *Wolbachia* offre des avantages à *D. suzukii* dans des conditions particulières et pourrait avoir contribué à l'expansion de son aire de répartition lors des premières années de son invasion. Ensemble, les résultats de ma thèse contribuent à percer le secret de ce qui rend une espèce envahissante si performante (voir Annexe 1).

Ce travail a été collaboratif et a impliqué des chercheurs de plusieurs laboratoires, ainsi que des étudiants passionnés de Licence et de Master, qui ont apporté leurs compétences, connaissances, expertises, curiosité et perspectives. C'est aussi l'une des forces de ma thèse : créer un réseau qui maximise la valeur de ces trois années de recherche.

Gwenaëlle DECONNINCK

Invasive success of the fruit fly *Drosophila suzukii*: Role of polyphagy and thermal plasticity

Résumé

L'augmentation des cas d'invasions biologiques dans le monde et les dégâts engendrés amènent à se poser des questions sur les facteurs qui favorisent leur succès afin de pouvoir anticiper les invasions futures. Dans cette thèse, je me suis basée sur la drosophile à ailes tachetées, *Drosophila suzukii*. La rapidité de son invasion et les pertes économiques importantes qu'elle engendre en pondant ses œufs dans des fruits mûrs, en font un modèle de choix. J'ai exploré certains mécanismes qui ont pu contribuer à son succès d'invasion. En particulier, je me suis intéressée à sa polyphagie, aux ressources alternatives qu'elle utilise en l'absence de fruits cultivés, ainsi qu'aux facteurs locaux, paysagers et climatiques qui favorisent ses infestations. Je me suis également intéressée à ses capacités de thermorégulation comportementale et à la plasticité de sa préférence thermique afin de mieux comprendre la facilité de *D. suzukii* à exploiter des microhabitats qui lui sont favorables. Enfin, j'ai étudié l'effet de l'interaction entre la ressource alimentaire, la température et la présence de symbiotes sur la performance de *D. suzukii* et sur ses choix de ponte. Cette thèse a mis en évidence la grande plasticité de *D. suzukii* face à des changements dans son environnement. Celle-ci a pu constituer une forme de pré-adaptation qui a favorisé son invasion mondiale. Elle ouvre la voie à des recherches appliquées pour la protection des cultures, mais aussi à de nombreuses recherches fondamentales autour de la sélection de la ressource, des stratégies de thermorégulation chez les ectothermes ou encore des approches intégratives pour comprendre le succès de l'invasion.

Mots clés: Ecologie trophique, Interaction plantes-insectes, Invasion biologique, Thermorégulation comportementale, *Wolbachia*

Summary

Globally, the apparently inexorable increase of biological invasions and their resulting damage raise questions about the factors that influence invasion success. In this thesis, I considered the spotted wing drosophila, *Drosophila suzukii*, which provides an exemplar as a highly successful invasive species. It is an ideal model because of the rapidity of its invasion and the important economic losses resulting from its ability to oviposit in healthy fruit. I explored the mechanisms that could contribute to its rapid global invasion. In particular, I considered its polyphagy, the alternative resources it can use in seasons when cultivated fruit are not available, as well as the local, landscape-scale and climatic factors that influence its infestations. I also investigated its behavioural thermoregulation capacities and the plasticity of its thermal preference, traits that allow a better understanding of the ability of *D. suzukii* to exploit favourable microhabitats. Finally, I explored the effect of the interaction between food resource, temperature and the presence of the bacterial symbiont *Wolbachia* on the performance of *D. suzukii* and its oviposition choices. My thesis highlights the great plasticity of *D. suzukii* when confronted with environmental changes. This could represent a form of pre-adaptation facilitating the fly's rapid global spread. My work opens research avenues in applied research around crop protection by controlling the wild plants used by *D. suzukii*, and also in fundamental research around resource selection and the use of integrated approaches to understand invasion success.

Keywords: Behavioural thermoregulation, Biological invasions, Plant-insect interactions, Trophic ecology, *Wolbachia*