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The role of banana weevil *Cosmopolites sordidus* in the epidemiology of the Panama disease *Fusarium oxysporum* f. sp. *cubense* race 1

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Avant-propos

Connaitre l'épidémiologie des maladies des cultures est essentiel pour développer une agriculture durable, qui en mobilisant des leviers écologiques de contrôle pourra assurer une production d'aliments sains. Au sein de l'épidémiologie des maladies, leur vection par des organismes intermédiaires (des animaux) représente un exemple particulier d'interactions biologiques qui mêle à la fois des processus liés à la biologie d'un organisme pathogène, de son hôte etcelle de son vecteur. Pour comprendre ces interactions complexes, cela demande souvent des approches multidisciplinaires relevant notamment de l'entomologie, de la phytopathologie et de l'écologie des interactions plantes-insectes. Seules des approches holistiques, intégrant l'ensembles des mécanismes impliqués dans les processus de dispersion via le vecteur puis dans la vection proprement dite avec la contamination d'une plante saine par la maladie. L'ambition de cette thèse s'inscrit clairement dans une approche multidisciplinaire de « décorticage » de ces processus.

A l'instar d'autres crises sanitaire, la culture de la banane subit actuellement un bouleversement avec l'épidémie d'une nouvelle souche de *Fusarium oxysporum* f. sp. *Cubense (Foc)* : la race 4 tropicale ; qui s'attaque notamment au seul groupe de bananiers cultivé pour l'exportation (le groupe Cavendish), mais aussi à de nombreuses variétés de bananiers essentielles pour les marchés locaux à travers le monde. Cette épidémie fait écho à celle de la maladie de Panama causée par Foc race 1 qui avait complétement transformé la production mondiale de la banane dans les années 1950-60 et qui avait justement abouti à un changement variétal : passage de la variété Gros Miche à Cavendish. Le présent travail vise à apporter une brique dans la connaissance de l'épidémiologie de cette maladie, en étudiant le rôle potentiel du charançon du bananier (*Cosmopolites sordidus*). Au-delà de tenter d'apporter de nouvelles connaissances sur l'épidémiologie de

Foc, ce sujet est singulier par le fait que le vecteur étudié est aussi le ravageur principal des bananiers. Cela rend la tâche particulièrement stimulante car l'épidémiologie du ravageur et de la maladie s'entremêlent et répondent à des enjeux majeurs de production au niveau mondial.

En tant qu'entomologiste, en position fixe à CORBANA depuis 2009, ce sujet m'a tout de suite passionné dès la lecture de la publication de Meldrum et al., 2013 qui ont suggéré le rôle du charançon du bananier, mais sans le démontrer et ouvrant de nombreuse pistes de recherches. Avec le support de mon institution, notamment Dr Jorge Sandoval (Directeur scientifique), j'ai porté ce sujet et construit avec l'unité Geco du CIRAD la recherche présentée dans ce manuscrit. Mon statut d'entomologiste à CORBANA fait qu'à côté de la réalisation de ce travail j'ai dû intervenir dans différents autres projets, expliquant que ma première inscription de thèse s'est faite en mai 2017, menant à un travail en un peu plus de 4 ans. Par ailleurs, comme beaucoup de doctorants, mon travail a été affecté par la crise du covid. Ainsi, la période de rédaction en France auprès de mes directeurs de thèse, qui été prévue à partir du premier semestre 2020, n'a pas pu se faire.

Le travail présenté dans ce manuscrit fait l'objet de valorisations scientifiques sous formes d'articles dans des revues à comité de lecture. Un article a été publié en 2021 dans la revue Pest Management Science, un autre est soumis à la revue Journal of Applied Entomology, et deux autres articles seront soumis par la suite (un d'ici fin 2021 et l'autre en 2022).

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Chapter 1 – General introduction



1. General issues of pests and diseases in Agriculture

1.1. Pests and diseases as the main biotic constraint of agriculture

Since the beginning of agriculture, plants have been affected by pests and diseases, which limit food production throughout the world. Crop losses due to these biotic agents can be minor damage, reducing the quality or appearance of the food. In other cases, severe damage can cause the total loss of plants or of their productive organs. In recent decades, climate change has favored the emergence or increase of pests and diseases. This can compromise the production of major crops, on which world food production depends (Tirado et al., 2010).

Insect pests and plant diseases can substantially reduce crop yield and their quality, and increase input costs (Hull, 2014). The Food and Agriculture Organization of the United Nations (FAO) estimates that losses associated with pests can reach up to 40% of agricultural production in the world (FAO 2021). In high demand crops such as bananas, it is estimated that losses can reach 100% when affected by primary pests. For instance, Fusarium wilt tropical race 4 (Foc TR4) represents an important risk to produce banana worldwide, and especially in developing countries.

One of the challenges of agriculture is producing enough food of good quality to meet the world's food needs. To achieve this goal, conventional agriculture has turned to synthetic pesticides (Tudi et al., 2021). Given the high dependence on these inputs and the environmental imbalance that they generate, it is estimated that the losses in the world, due to not using these tools, could reach 78% in the production of fruits, 54% in vegetables and 32 % in cereals (Zhang et al., 2011). In order to do not threat food security, it is necessary to implement strategies that reestablish the stability of agroecosystems and natural regulations processes. Agroecological management practices include changes

in soil properties, microclimate, plant diversity and associated beneficial fauna, cover crops, green manure, intercropping, agroforestry, biological control, resource and biodiversity conservation practices, or livestock integration (Altieri 1995, 2002; Arrignon 1987; Gliessman 1998).

In a meta-analysis of 21 studies Tonhasca and Byrne (1994) compared the suppression of pests in polycultures versus monocultures, finding that in polycultures the density of pests was reduced by 64%. Similarly, Letourneau et al. (2011) found a 44% increase in the abundance of natural enemies, a 54% increase in herbivore mortality and a 23% reduction in damage to crops in diversified farms. These studies show that the diversification schemes of the productive systems generally achieve positive results in the management of insect pests, which is reflected in the increase in natural enemies, the reduction in the abundance of pest herbivores and the reduction of damage to crops. Mixed culture systems can reduce the incidence of pathogens by decreasing the rate of disease development, as well as by modifying environmental conditions that make it less favorable to the spread of pathogens (Boudreau 2013).

1.2. Issues of pesticides applications

The application of pesticides has contributed positively to meet the food needs of humanity; however, it has brought with it serious health problems and environmental pollution. Far from being a sustainable solution, its increasing use i) did not manage to mitigate pests and diseases and ii) led to important issues for environment and human health. The emergence of resistant insects and diseases is constantly increasing, which means that farmers must have to change pesticide molecules, often more toxic and at higher doses in order to overcome the economic losses of crops.

1.2.1. Implications for human health

According to World Health Organization, each year, about 3,000,000 cases of pesticide poisoning and 220,000 deaths are reported in developing countries (Lah 2011). About 2.2 million people, mainly belonging to developing countries are at high risk of exposure to pesticides (Hicks 2013). Some peoples are more susceptible to the toxic effects of pesticide than others; including infants, young children, agricultural farm workers and pesticide applicators (Mahmood et al., 2016).

There are also questions with the new pesticide molecules in relation to the possible impact on human health, especially when they are applied at low and prolonged doses. Numerous studies show that these molecules can influence a wide range of vital functions, causing genetic and epigenetic alterations, endocrine imbalances, as well as alterations of the nervous system (Bianco 2018). Pesticides enter the human body through ingestion, inhalation, or penetration in the skin, but most people get affected via the intake of pesticide contaminated food (Spear 1991). For example, in Costa Rica there was an impact related to the use of the nematicide 1,2-Dibromo-3-chloropropane (DBCP, NemagónR, FumazoneR) on the loss of male fertility in 1978 (Ramirez and Ramirez 1980).

1.2.2. Environmental issues

When pesticides are applied to plants, they enter into their tissues (in the case of systemic pesticides), furthermore, a good part of it falls to the ground or drifts into the environment (van der Werf 1996). Once in the environment, pesticides can undergo microbiological, chemical or photodegradation processes (Luo et al., 2018; Su et al., 2016) to new chemical products and in this way affect non-target organisms along trophic chains.

Human and environmental issues are extremely diverse, for instance, application of fungicides can indirectly reduce birds and mammal populations by killing earthworms on which they feed. Granular forms of pesticides are disguised as food grains by birds. Organophosphate insecticides are highly toxic to birds and they are known to have poisoned raptors in the fields. Sublethal quantities of pesticides can affect the nervous system, causing behavioral changes (Mahmood et al., 2016). Schreck et al., (2008) reported the toxic effect of insecticides and fungicides on earthworms. These pesticides are fixed in the soil and cause a neurotoxic effect. Similarly (Lang and Cai 2009) pointed out that chlorothalonil and dinitrophenyl fungicides alter the functioning of bacteria in charge of nitrification and denitrification of soils.

1.3. Challenges of developing a clean agriculture that control efficiently pest and diseases

New management approaches are increasingly developed. It includes agroecology, which incorporates scientific, methodological, and technical bases for a "new agrarian revolution" on a world scale (Altieri 2009, Ferguson and Morales 2010). This management scheme is characterized by incorporating biodiverse, resilient, energy efficient and socially just production systems that constitute the basis of an energy and production strategy strongly linked to food sovereignty (Altieri 1995, Gliessman 1998; Ratnadass et al., 2012).

As a complement to these management schemes, there is also the Agroecological Crop Protection (ACP), which is a concept of crop protection based on ecological practices and principles (Deguine et al., 2017). It results from a systematic implementation of agricultural practices through systemic and participatory approaches, gives priority to

preventative measures instead of curative approaches and places emphasis on the protection and encouragement of beneficial organisms. It aims at promoting the ecological function within farming systems by directly or indirectly optimizing interactions between plants, animals, and microbial communities as well as the soil health. The challenge of ACP is to keep pests and diseases in balance within the agroecosystem, under thresholds causing damage that can put food production at risk. It includes a wide range of levers, as the conservation of natural enemies that regulate the populations of pest insects, the use of biopesticides (repellents, and microorganisms), trapping systems with allelochemicals, and all prophylactics measures for diseases control.

Parallel to this natural regulation of pests, it is necessary to maintain and conserve the microbiological diversity of biological control agents such as: bacteria, filamentous fungi, yeasts, algae and some free-living protozoa and nematodes, which stimulate growth or suppress colonization and pathogen infection (Lindow and Brandl 2003). In banana cultivation, González et al. 1996, Ruíz et al. 1997, Arango 2002, Ceballos 2012, pointed out that the diversity in the Musaceae phyllosphere is capable of regulating *Pseudocercospora fijiensis*.

1.4. Potential interactions between pest and diseases

Insects and diseases are the main biotic limitations in agricultural production systems in the world due to the direct and indirect damage they can cause to plants. This is aggravated when pests and diseases interact in the same agroecosystem or when diseases are dispersed by insects (Chakraborty and Newton 2011). In a changing climate, all living organisms, including interactions between insects, plants, and pathogenic microorganisms are succeptible to be altered by changes of temperatures, severity of

drought, rain, humidity, by cyclones and hurricanes (Cannon,1998; Chakraborty et al., 2000; Rosenzweig et al., 2001; Jones, 2016). These changes would likely increase the spread of viruses or bacteria or fungus (Trebicki *et al.*, 2010, 2016; Jones, 2016).

The density of the pest as well as the amount of pathogen inoculum are key to determine the impact of the pathogen on plants. Kluth et al. (2002) found that insects play an important role in the dispersal of plant pathogenic fungal spores when they feed on infected leaves. However, much is unknown about the mechanisms involved in these dissemination processes.

2. The role of insects in the transmission of fungal diseases

2.1. Insects as vectors of diseases

The transmission of pathogens in plants can occur by different pathways. Insects are likely to be particularly important in fungal diseases epidemics due to their great ability to disperse by walking or flying. This mobility might increase pathogen dispersal especially when their propagules are not airbone. Insects often interact with the aerial part of plants, explaining why it is one of the most studied pathways of vection of diseases to plants. Their great taxonomic and eating habits diversity open a wide range of contamination pathways. There are other more specialized groups of insects that feed on plant belowground, thus contributing to plant damages and to the epidemiology of soil borne diseases (Chakraborty 2021).

Insects can be involved in the dispersal of fungi, especially when they cause lesions in the epidermis of plants (during feeding or oviposition), creating entry “doors” for fungal invasion. When feeding on diseased plants, insects can acquire fungal propagules and then transmit them to healthy plants. Usually only the disease benefit from this

interaction (Chung et al., 2013). The role of insects in the transmission of phytopathogenic fungi was notably shown by Selikhovkin et al., (2018) that demonstrated that certain species of weevils (particularly *Hylastes attenuatus*) can transport pathogenic fungal spores *Ophiostoma* spp., *Leptographium* spp. and *Fusarium* spp. (Romón et al., 2007; Bezos et al., 2017).

2.2. Active and passive mechanisms of transmission of diseases

The spread of diseases in plants linked to the vection of insects is a frequent process in nature. It occurs when insects come into contact with the plants either for feeding on its tissues, for oviposition, or simply because it is attractive. Two types of disease transmission mechanism are involved:

i) Passive: when the insect is disseminating propagules by casual contact, production of feces on plant environment or by its death in the neighborhoods of the plant. Here, the contact with the plant does not involve any exploitation of the plant,

ii) Active: when the insect is disseminating propagules while exploiting the plant when feeding or oviposition on it (Willsey et al. 2017).

3. The banana plants

3.1. Botanical taxonomy, diversity and development

Bananas and plantains are monocotyledonous plants, originating from intra and interspecific crosses between *Musa acuminata* (genome A) and *Musa balbisiana* (genome B) that belong to the Musaceae family also includes the Asian and African genus *Ensete*.

These diploid species come from genomes A and B, respectively (Simmonds and Shepherd, 1955; Simmons, 1962). Triploid (AAA, AAB and ABB) have the highest economic value, followed by diploids (AA and AB) and then tetraploids (AAAA, AAAB and AABB). The main commercial cultivars are triploid notably because they are more vigorous and productive, while being highly sterile (absence of seeds), parthenocarpic, and asexually propagated (Simmonds and Shepherd, 1955; Simmonds, 1962; Ortiz and Vuylsteke, 1996).

In recent decades, the classification of bananas has undergone a new nomenclature, considering both the genetic and genomic aspects of bananas. Cultivated hybrid bananas are also classified into genomic groups and subgroups according to the relative contribution of their ancestral wild relatives. This classification proposed by Simmonds and Shepherd (1955) is currently the most common system for classifying edible bananas. Its genome-based system also allows the classification of tetraploid bananas, which have essentially been developed through genetic improvement for plant breeding purposes in different parts of the world.

Dessert bananas for exportation belong to the Cavendish sub group (AAA), the cultivar 'Grande Naine' being the most cultivated. Stover (1979) defined four stages of development in the "Grande Naine" banana: i) juvenile, which occurs before the leaves n°11, ii) adult pre-floral that occurs between leaves n°11-25, iii) floral initiation, that occurs between leaves n°26 and n°31, and iv) postfloral, ranging from leaves n°31 to n°43. Banana plants are usually managed as a perennial crop population, which is composed of a succession of shoots, offspring, young plants, in flowering, production, freshly harvested (Stover (1979), which means that there is a constant availability of food resources for *C. sordidus* (Figure 1).

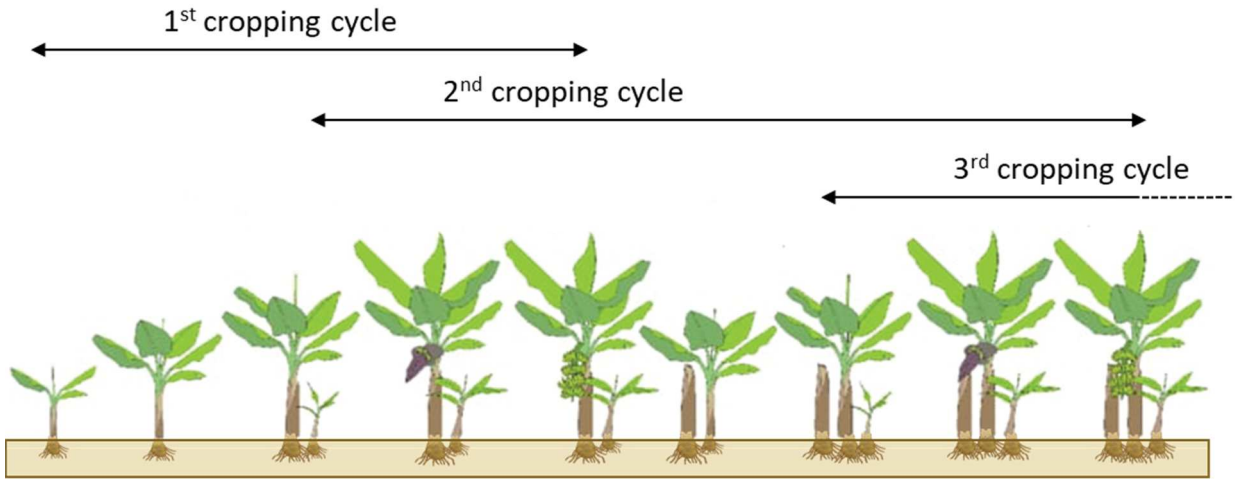


Figure 1. Illustration of the succession of stage for a given banana plant over cropping cycles. *Cosmopolites sordidus* and Foc can affect all these stages.

3.2. Importance of the bananas for developing countries

Bananas are at the fourth place of the most important crops worldwide after rice, wheat, and corn (Bakry et al., 2009, Churchill 2011) so they represent an important source of income for more than 135 producing countries. According to FAO, dessert bananas represent an annual production of more than 148 million tons, positioning it as one of the most important basic products according to FAOSTAT (2016).

For many developing countries, with economies based on agriculture, banana production is one of the greatest development opportunities since it represents an estimated value of 35.2 billion dollars in the tropical world. Furthermore, more than 80% of the fruits are produced for local consumption (Churchill 2011). Despite great genetic diversity, only a few triploids (AAA, AAB, ABB) are responsible for almost 75% of world banana production, which corresponds to Cavendish cultivars (~ 20 cultivars; 46%), bananas (~ 120 cultivars; 15%) and East African highland bananas (~ 150 cultivars; 39%) (Lescot 2017). Aside the exportation of bananas to countries at higher latitudes, banana

production is an important fruit for local markets and for the nutrition of population in producing countries (exploiting a much wider range of cultivars, especially in Asia and Africa).

4. Fusarium wilt and *Cosmopolites sordidus*: two major biotic issues for banana production

4.1. Fusarium wilt in banana plantations

Fusarium wilt, also known as Panama disease, is the most important lethal disease of banana plantations around the world (Ordoñez et al. 2015; Ploetz 2005). It is caused by *Fusarium oxysporum* f. sp. *Cubense* (E.F. Smith) W.C. Snyder & H.N. Hansen (hereafter referred as Foc). The pathogen is most likely originated from Southeast Asia but was first reported in Australia in 1876 (Ploetz and Pegg 2000; Ploetz et al., 2003). In the 1950s, the Race 1 strain of the fungus (Foc Race 1) devastated commercial production of the Gros Michel banana, the former main cultivar for exportation in Latin America and the Caribbean at this time (Stover R., 1962; Ploetz, 2005). As a solution to the crisis caused by Foc Race 1 was the very successful change towards resistant cultivars: banana plants from the Cavendish Subgroup (AAA), which contributed to the expansion of Cavendish production for both the domestic market and for export. However, resistance to Cavendish was also broken, giving way to Tropical Race 4 (TR4). Many cultivars grown in most banana producing countries are highly susceptible to this new strain, which has generated a global concern (Plotz, 2015). This strain is currently the main threat to international banana production, since it has already spread to Africa (Ordóñez et al., 2015) where bananas are a staple food. Recently it spread to Latin America where it has

been confirmed in Colombia and Peru, which are of great importance for the world export industry (Figure 2).

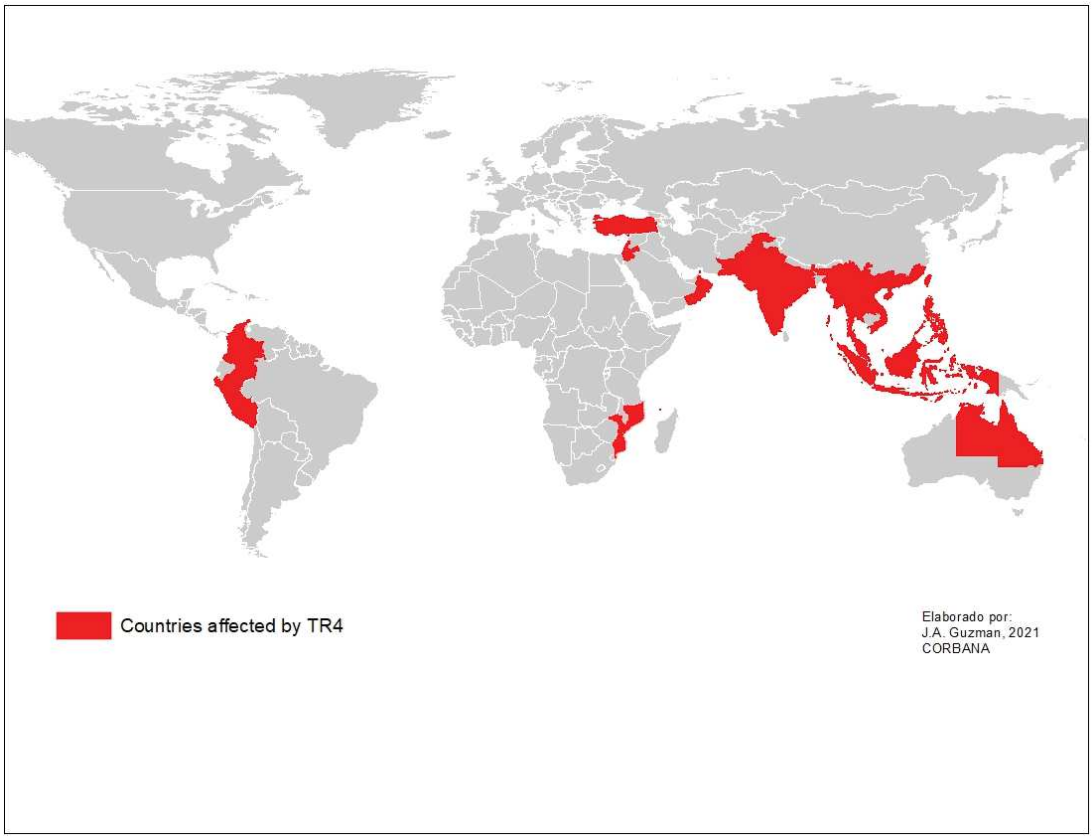


Figure 2. Distribution of Foc Tropical Race 4 in the world (CORBANA 2021).

4.1.1. Taxonomy and life cycle of Foc

The life cycle of *Foc* begins with the entry of the pathogen into the potential host via the root tip. Substances produced by the host, in the region behind the zone of elongation of the root meristem elicit a pathogen response which results in the germination of chlamydospores. The germinated hyphae then infect the lateral roots and progress to invade the xylem vessels. Further spread of most initial infections is usually stopped in the xylem by the vascular occluding responses of the host, which include the formation of gels, tyloses and the collapse of vessels.

In susceptible cultivars, some of these infections become established in the xylem and advance ahead of these defense mechanisms. Microconidia are formed in the xylem vessels and are spread through the vascular system of the plant, streaming to new sites where they germinate. Hyphae are then produced, and this begins the invasion at this new site, thus repeating the cycle. In resistant varieties, the initial pathogen-induced occlusion reaction is further enhanced by the production of phenolic compounds, which lignifies these obstructions and limits the pathogen to the infected vessels; no further colonization of the xylem vessels occurs (Beckman 1987; Beckman and Talboys 1981).

4.1.2. Current controlling strategies

There is a lot of literature that shows the effect of various pesticides to combat Panama disease, however most of them were tested *in vitro*. Products such as benzimidazole, strobilurins, carbendazim, benomyl, methyl bromide and methylation inhibitors, have shown good results in fighting the disease *in vitro;* however, many of them are highly toxic and when evaluated in the greenhouse or field, they do not control the disease (Herbert & Marx, 1990; Nel et al., 2007). So far, no fungicide strategy is available to control Fusarium wilt in field conditions.

In some Asian countries, some physical measures have been tested, as the burning of rice husks on infected soil and solarization, which only reduce the inoculum of the most superficial centimeters of the soil (Herbert & Marx, 1990, Ploetz, 2015). The use of resistant varieties or somaclones remains the most promising method (Siamak and Zheng 2018; Dita et al., 2018). However, plant breeding programs take a long time, and the varieties that are resistant in most cases do not have the quality traits required by the market, which makes their establishment in the market difficult (Ploetz, 2015). The

management of Panama disease should also rely on the improvement of soil health (Segura et al., 2021).

4.2. The banana weevil

4.2.1. Small history of *Cosmopolites sordidus*

The banana weevil is native from Southeast Asia, possibly from the Indo-Malaysian region comprising Malaysia, Java and Borneo (Gold et al. 2001). The insect was first described by Ernst Friedrich Germar in 1824 as *Calandra sordida*, and later redescribed as *Cosmopolites sordidus* by Louis Alexandre Auguste Chevrolat in 1885 (Gold et al. 2002). The insect was reported in 1900 in Indonesia, China, East Africa, Australia, and Brazil, in 1920 in New Guinea, southeast Africa, islands of the Pacific, islands of the Indian Ocean, Central America and the Caribbean (Gold et al. 1994) .

4.2.2. Taxonomy and life cycle of *Cosmopolites sordidus*

The black weevil *C. sordidus* (Germar) (Coleoptera, Curculionidae), presents a complete metamorphosis and goes through the stages of development of egg, larva, prepupa, pupa and adult. The egg is 2.5 mm long, white to slightly yellow; it is ovoid in shape and takes five to eight days to hatch (Coto and Saunders 2004, Muñoz 2006). The larva is 10 to 15 mm long, creamy-white in color, with a reddish-brown head, a thick body, curved in the shape of a “C” and apodal. During its development, the larvae go through six stages (sometimes seven), lasting 28 to 42 days (Coto and Saunders 2004). The prepupa lasts for one to four days (Messiaen 2002). The pupa is of the exarated type (Andrews and Caballero 1989); yellowish and dark; it appears in the galleries found on the periphery of the corm and lasts for five to eight days (Coto and Saunders 2004). The adult measures

between 15 and 20 mm long and four millimeters wide. The life strategy is known as "K", because the insect has a high longevity (up to 3-years in controlled conditions); being able to live from one to two years and a low fertility (Gold et al., 2001, Cubillo et al. 2001, Messiaen 2002). The female individually lays her eggs in holes that she makes in the corm, but she can also do so in the leaf sheaths or freshly overturned stems.

The adult is gregarious and presents negative phototropism, which is why they are not commonly observed in the field but are found in places with high relative humidity in the soil, or in the cavities that it makes with its beak, in stem depressions, rhizomes or in harvest residues (Castrillón 2003). In high populations, this insect can cause a deterioration in the root system, as well as a weakening in the mother plants, which could culminate in the rupture of the stem or the overturning of the productive unit, mainly when the fruits are filled or by the passage of wind currents. Additionally, the injuries caused by the larvae or even by the cavities made by the females to the ovipositor or when feeding can cause the entry of pathogens.

4.2.3. Dispersal

Adults are nocturnal and remain on the same plant for long periods of time, with only a few moving distances greater than 25 m during a six-month period (Gold et al., 2001). *Cosmopolites sordidus* is characterized by having functional wings, although it rarely flies (Merchán 2002, Dahlquist 2008). *Cosmopolites sordidus* is often dispersed at long distances by the movements of planting material.

4.2.4. Damages causes by *Cosmopolites sordidus*

The damage is caused by the larvae of *C. sordidus*, which make galleries in the corms of banana plants of all stages of development (Gold et al. 2001, Carballo 2001, Muñoz 2006). These damages cause the death of roots, after the ruptures of the vascular bundles (Montesdeoca 1998) (Figure 3). Damage to corm varies accordingly to the variety of banana, the stage of development of the larvae, the pest control strategy (pesticide application), and the age of the culture. In Uganda, weevil damage caused a 4% decrease in the first cycle and a 48% decrease in yields in the fourth harvest cycle (Gold 1999). Similarly, Rukazambuga et al. (1998) determined that the lesions caused by the weevil can lower plant height, reduce functional leaves, lower bunch weight, and delay the growth suckers. Likewise, in Colombia, weevil damage in a plantain crop of the Dominico Hartón variety (Musa AAB), presented a reduction in bunch weight from 4 to 20% for the first cycle; from 30 to 40% for the second cycle, and from 48 to 60% for the third cycle (Castrillón 2003).

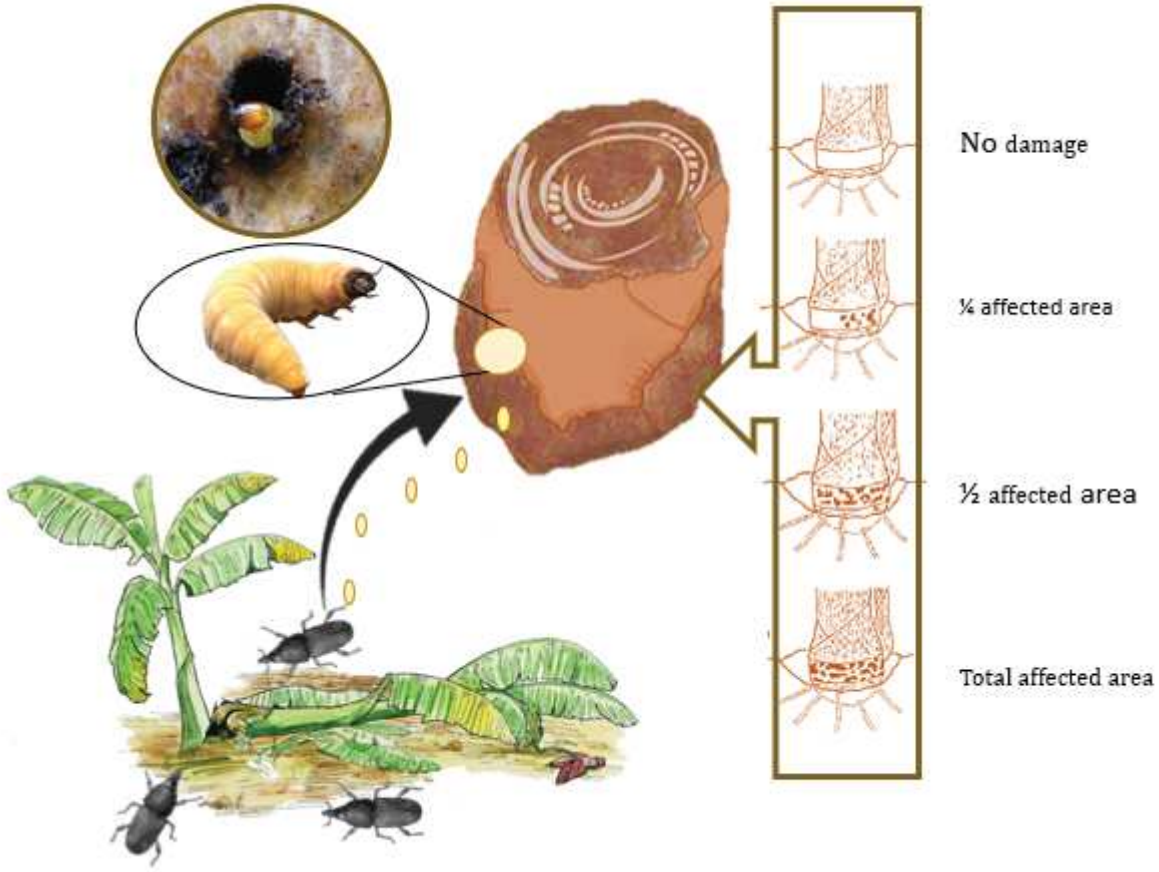


Figure 3. Damage of *Cosmopolites sodidus* in pseudostem in the corm of banana plant (adapted from FiBL 2011).

4.2.4. Current controlling strategies

In conventional farms in Latin America, scheduled applications of nematicides participate to control *C. sordidus* since these pesticides also have an insecticide effect. More recently in Costa Rica, new nematicide molecules have been incorporated with no insecticide effect, which has resulted in dramatic increase in weevil populations and consequently in damages to plants (Sponagel et al., 1995). Cultural control methods includes: i) farm sanitation (Masanza et al., 2005), ii) the use of pseudostem traps (Gold et al., 2002;

Mazanza et al. 2009), iii) biological control with entomopathogens (Nankinga & Moore, 2000; Treverrow et al., 1991) and myrmicine ants (Castineiras & Ponce, 1991), iv) planting of host plants with resistance (Kiggundu et al., 2003), v) the use of botanical pesticides such as neem extracts (Musabyimana et al., 2001), and vi) mass trapping with aggregation pheromone lures (Alpizar et al., 1999; Tinzaara et al., 2005).

Large scale control of *C. sordidus* is currently achieved by chemical methods, while cultural controls remain highly valuable in preventing the establishment of the pest. Cultural control methods are also the main available means of management of the pest by smallholder farmers and growers, while biological control methods such as arthropods and fungi integrated pest management strategies are also being developed (Braimah & van Emden, 1999). In French West indies, the mass trapping strategy was particularly developed, resulting in a drastic reduction of nematicide/insecticide applications (Rhino et al., 2010).

5. Scientific questions related to the putative role of *Cosmopolites sordidus* in the dispersion of Foc

Meldrum et al. (2013) determined that *C. sordidus* carries infective propagules of FocRT4 in the exoskeleton and concluded that it could be a vector of the disease. Heck et al. (2021) provided complementary evidence for the involvement of *C. sordidus* and the false weevil borer (*Metamasius hemipterus*) in the dispersal of *Fusarium oxysporum* f. sp. *cubense* (Foc) using a comparative epidemiology approach under field conditions. These authors also concluded on the putative role of *C. sordidus* in the dispersion of the disease. These studies were important in suggesting the importance of *C. sordidus* in dispersing Foc, but the proof still must be done.

The general objectives of this thesis are i) to test the hypothesis that *C. sordidus* can be an effective vector of Foc, and ii) to disentangle and quantify each step of the process of vection of *C. sordidus* from a diseased plant to a healthy one.

The Figure 4 illustrates the processes involved in this vection. It includes processes as diverse as dispersion of *C. sordidus*, which include a phase of attraction, inoculum acquisition and survival internally or externally on *C. sordidus*, and finally the infection of a healthy plant that could be achieved through passive or active ways. Meldrum et al., only reported an external putative vection of Foc on the insect, here we wanted to explore the internal vection of fungal propagules that might be an important trait of fungal dissemination, and also to demonstrate clearly insect transmission of Foc to healthy plant. This knowledge is rather important to reevaluate integrated management of Panama disease. Lastly, this work was done in Costa Rica with Foc R1, but this research is clearly aimed to improve Foc TR4 management.

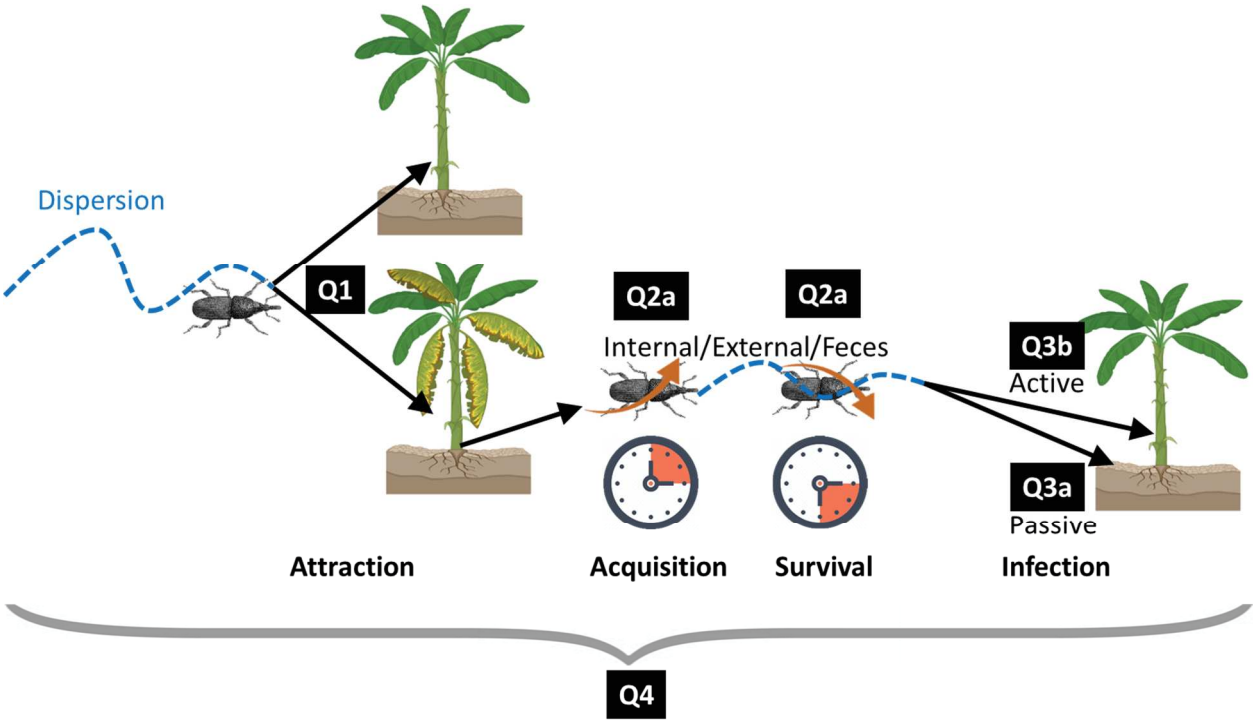


Figure 4. Illustration of the different processes involved in the vection of Foc by *Cosmopolites sordidus*. Black boxes figure out the different scientific questions addressed in this thesis.

The scientific questions addressed specifically in this thesis are related to key steps in the putative vection of Foc by *C. sordidus*, including:

Q1 - Scientific question 1: ***Is there a difference between Foc-free and Foc-contaminated plants in attracting* C. sordidus*?***

This question is important because it may affect the capacity of *C. sordidus* to be in contact with diseased plants and potentially increase their fungal contamination. A greater attraction of Foc contaminated plants would make *C. sordidus* a more efficient vector, especially in situations where the disease is at low incidence levels. Inversely, a reduced attraction of Foc contaminated plants would make *C. sordidus* a poorer vector. This

question was addressed here using laboratory experiments with olfactometers. It allowed the quantification of the relative attraction of Foc-infected and Foc-free banana plant tissues (corm and pseudostem) by adults of *C. sordidus (*male or females). This work is presented in the **Chapter 2** of this manuscript.

Q2 - Scientific question 2: ***Is C. sordidus potentially infected with propagules of Foc?***

This question is one key of the potential role of *C. sordidus* in the vection of Foc. Here, in order to explore further this aspect of fungus-insect interactions we have considered either external (at the surface of the exoskeleton) or internal (in the digestive tract) insect contamination with effective (alive) propagules. Effectively, this aspect was very important in order to evaluate not only the presence of fungal propagules, but mostly their effectiveness for potential plant contamination. We divided this question in two sub-questions related i) to time of contact between *C. sordidus* and infected plant material needed for the acquisition of efficient inoculum internally and externally of the insect, and inside its feces (Q2a), and ii) to the survival time of the inoculum of Foc in and on the insect (Q2b). Indeed, these aspects of fungal acquisition and inoculum survival are important traits in the putative role of this insect in the epidemiology of Fusarium wilt.

This question was addressed here using laboratory experiments with mesocosms. This work is presented in the **Chapter 3** of this manuscript.

Q3 - Scientific question 3: Are ***C. sordidus adults infected with Foc effective vectors of Foc to healthy plants?***

Here we have explored two processes that might be involved: i) a passive infection (i.e. only the presence of an infected insect in the surroundings of a healthy plant can lead to an infection of a healthy plant), and ii) an active infection (i.e. the insect has an exploitation

behavior through feeding or oviposition that creates “doors” for the pathogen to enter the plant). This question was then divided in two sub-question: Q3a- Is there a passive contamination of healthy plants by infected insects without any contact? Q3b- Is there an active contamination through *infected insects* that were in contact with healthy plants? These two questions were addressed through mesocosm experiments, presented in the Chapter 4 of this manuscript.

Q4 - Scientific question 4: ***What is the efficiency of C. sordidus to disperse Foc in field conditions?***

Here the objective was to address the integrated effect of i) the dispersal of *C. sordidus*, ii) the survival of the inoculum it is potentially holding, and iii) its infection efficiency in real field conditions. This question was addressed with a field experiment including an inoculation of adults of *C. sordidus* that were infected with Foc. Since it is a long-term experiment, only primary results are presented in Chapter 4 of this manuscript.

Chapter 2 - Effect of Foc in the attraction of banana plants



This chapter presents results that contribute to the understanding of the feeding preference of *Cosmopolites sordidus*. It addresses the scientific question Q1 presented in Chapter 1 section 5. Four-armed olfactometers were used to test whether *C. sordidus* is preferentially attracted by Foc-contaminated or Foc-free banana plants material. Understanding this feeding behavior is fundamental to elucidate the role of the insect in the dissemination of Panama disease. This paper is submitted for publication in ***Journal of Applied Entomology***.

Article 1 - Some like it Foc, preference of *Cosmopolites sordidus* for banana plants infected with *Fusarium oxysporum* f.sp. *cubense* race 1

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Abstract

The objective of this study is to understand the role of *C. sordidus* in the dispersal of *Fusarium oxysporum* f.sp. *cubense* race 1 (Foc) and more specifically whether *C. sordidus* is preferentially attracted by Foc-contaminated banana plants material than Foc-free ones. The attraction of *Cosmopolites sordidus* was studied using four-armed olfactometers that compared the relative attraction of healthy and Foc contaminated Gros Michel banana bulbs and pseudostems, as well as pure culture of Foc. The weevils were separated into males and females and were later taken to a dark room where they were placed in the central chamber of olfactometers. At 15 minutes, the orientation of each of the weevils was determined according to the food source placed in each of the chambers of the olfactometer. Insects were found to prefer Fusarium infected plant tissue. No specific preference associated with females or males was observed. This preferential attraction of *C. sordidus* for Foc contaminated plant material suggests that this insect could be involved in the dissemination of the disease, especially in early stages of the contamination of a field or farm when contaminated plants are scarce. This behaviour of the insect could thus have a positive feedback on the dissemination of Foc.

Keywords

Banana weevil, Panama disease, Food preference, Gross Michel Banana, Olfactometer

1. Introduction

To develop agroecological crop protection, a pest and disease management plan have to address the whole pathosystem (Deguine *et al.* 2017), in a global approach of the crop protection (Lewis *et al.* 1997). Understanding the links between pest and disease interactions is thus crucial in the development of integrated management options (Rapidel *et al.* 2015). As many cultivated crops, banana plants are threatening by a complex of pests and diseases damaging leafs, fruit, roots and corm (Jones 2009). The banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) is a widely distributed pest in Musas and Ensetes in most regions growing bananas (Stover & Simmonds; 1987; Masanza et al., 2018). The larva is the principal damage agent, causing tunnels in the corms, loss of roots, a weakening of the plants that causes plant topping during windstorm. For high populations, it can attack the pseudostems of living plants, causing the rupture and a reduction in suckers growth, a reduction of absorption of water and nutrients, and a delay in flowering (Reddy et al., 1995; Rukazambuga et al., 2002). Adults are free living, nocturnal and prefer humid environment (Carval et al., 2016; Luzano-Soria et al., 2020). They live in banana debris or fallen mats, and although they move freely within banana stands, few disperse more than 50m in 3 months (Gold et al. 2001; Abagale et al., 2018; Vinatier et al 2010). Both sexes are attracted to stressed or damaged plants and residual corm and freshly cut pseudostem (Treverrow & Bedding 1993; Gold et al. 1994).

Fusarium wilt is a destructive soilborne disease of banana caused by *Fusarium oxysporum* f. sp. c*ubense* (Foc). The pathogen affects the xylem of the plants producing the wilting and progressive death. In the 1950's a Foc race 1 outbreak devastated more than 50 000 ha of Gros Michel (AAA) plantations (Ploetz and Pegg, 2000; Thangavelu et l., 2020), which

led to a major shift of the entire banana production to Foc race 1 resistant Cavendish (AAA) banana varieties such as Williams, Grand Naine and Dwarf Cavendish. *Fusarium oxysporum* Tropical race 4 (Foc TR4) is a more virulent form of the pathogen and can cause disease in banana from the Cavendish group. The disease is distributed in most of the countries producers of bananas and unfortunately, it was reported in Colombia in August 2019 and recently in Peru in April 2021, which are a majors concern for the banana industries involved in export in the rest of countries in Latin America. It is of major importance to have a knowledge of how this disease spread, and, whether insects are involved in this dispersal.

The role of pest in spreading disease was already demonstrated in the case of the European grapevine moth, where the larva of *Lobesia botrana* Den. & Schiff. (Lepidotera: Tortricidae) interacts mutualistically with the fungal pathogen *Botrytis cinerea* as vector. Mondy et al., (1998) evidenced of similar interactions occurring below the soil where Clover root borers (*Hylastinus obscurus* Marsham; Coleoptera: Scolytidae) show a clear preference for red clover roots infected with fungal pathogens over healthy roots (Leath and Byers, 1973). The *C. sordidus* as a pathogen spreader, has been demonstrated in the case of *Xanthomonas campestris* that is a bacterial disease of banana plants (Were et al., 2015). Recently Guillén et al., (2021) demonstrated that *C. sordidus* that fed on banana corms infected by Foc could contain infective structures of this disease on the exoskeleton, in the digestive system, and even in excreta of the insect. Since the persistence of Foc propagules on and in *C. sordidus* is moderate (i.e. very low after two days), its efficiency in spreading Foc in the field depends on the frequency at which it is in contact with infected plants. It is therefore important to quantify whether *C. sordidus* is attracted to infected plants or if it avoided them, which would make it a good or a poor disperser.

Adults of *C. sordidus* are strongly attracted by banana plant (obligatory host plant), and by freshly cut or decomposing banana material (Budenberg et al., 1993; Braimah & van Emden, 1999; Sunil and Singh 2018). The stage of the plant alters its attractiveness, *C. sordidus* select preferentially corm from plants after than before flowering (Cerda et al., 1996). Very little is known on the chemical clues that attract *C. sordidus*, but it is likely that volatile organic compounds are at play. Sumani (1997) stated that both *C. sordidus* males and females are preferentially attracted by banana pseudostems than banana corm material, although oviposition is larger on the corm based on olfactory signal and chemoreception to locate to the host (Tinzaara, 2005). Fungi produce volatile compounds through their primary and secondary metabolic pathways (Korpi et al., 2009; Müller et al., 2013). These compounds in the soil surface, act like semiochemicals that intervene in the insect behaviour (Morath et al., 2012). In the case of Foc, volatile aldehyde production has been used to determine the isolates of Foc (Brandes 1919; Moore et al., 1991).

To better understand the role of *C. sordidus* in the dispersal of Foc, we used olfactometers to quantify the attractiveness of Foc colonies (isolated in petri boxes on Komada medium), Foc-free and Foc-infected banana materials on *C. sordidus*. We analysed whether the type of plant tissues (corm or pseudo-stem) had an influence on their attractiveness. We also tested whether males and females of *C. sordidus* are attracted similarly by the tested substrates. Statistical analyses were carried out at the individual scale using multinomial generalized linear models with the choice of the preferred substrate as a response variable. Finally, we discuss the implication of our findings in terms of epidemiology, both for *C. sordidus* and for Foc.

2. Materials and Methods

2.1. Experimental Conditions

To measure the relative attractiveness of Foc infected and Foc free banana planting material, we carried out 560 olfactometers experiments. All experiments were conducted at the Biological Control Center in the National Banana Corporation (CORBANA), La Rita, Pococí, Limón, Costa Rica. Olfactometers were in a dark room maintained at 24-26 °C and with a relative humidity ranging between 89% and 85%. Set up of olfactometers and observations were made under red light. The room was maintained in the complete dark after weevils' release.

2.2. Insects

Experiments were performed using weevils collected from San Pablo Farm, localized in Siquirres, Limón, Costa Rica. The insects were maintained in a chamber at 24 °C and 90% relative humidity. They were fed on banana corm and pseudostem from San Pablo Farm. Previously to the experiments, weevil sex was determined in females and males using the curvature of the last abdominal segment and the punctation on the rostrum (Gold et al., 2004). The insects used in the experiments were isolated in plastic containers of 1000 cm^3 without food for 12h before.

2.3. Corm and Pseudostem tissues

The experiments were conducted using banana corm and pseudostem material of flowering bananas plants (*Musa* AAA and cultivar Gross Michael) from CORBANA experimental Station in La Rita de Pococí, Limón, Costa Rica. Pieces of pseudostems were collected from plants with symptoms compatible with Foc, such as: yellowish leaves; older

leaves with evident wilting and rupture of pseudostem. Internally, the pieces of pseudostems were selected to have dark brown vascular bundles. Damage to the corms was between 35 and 50%, with discontinuous reddish lines due to the obstructed vascular bundles. Two types of corm and pseudosteam were used: (i) Infected with *Fusarium oxysorum* RT1 (Foc+) and (ii) healthy (Foc-). The material was chopped into pieces of 5 cm x 5 cm x 3 cm. In order to confirm the presence of Foc, pieces of vascular bundles of pseudo stem and corm were taken and placed in Komada medium. The colonies obtained were purified on PDA with Chloramphenicol. The molecular identification was carried out according to the methodology described by Guillén et al., 2021.

2.4. Olfactometers set-ups

In this study, we used four-arms olfactometers (Figure 1) to test simultaneously the attractiveness of Foc+ and Foc- banana material, pure colonies of Foc and a control (empty chamber). Each olfactometer consisted of a cylindrical central chamber 15 cm in diameter and 8 cm high made of high-resistance plastic which was connected by four outlets in opposite directions by tubes of 5 cm in diameter and 15 cm long to four arms in a peripheral chamber with the same dimensions of the central chamber described above. The middle chamber contained an opening, covered by a glass stopper, which allowed test insects to be added to the central chamber. We tested separately the two types of banana plant materials (corm and pseudostem) and the sex of weevils, leading to four combinations of set-up. We carried out seven replicates with 20 olfactometers per combination (type of plant material – sex of weevils), leading to the measure of 560 olfactometers (Figure 2).



Figure 1. Four arms olfactometer used in this study.

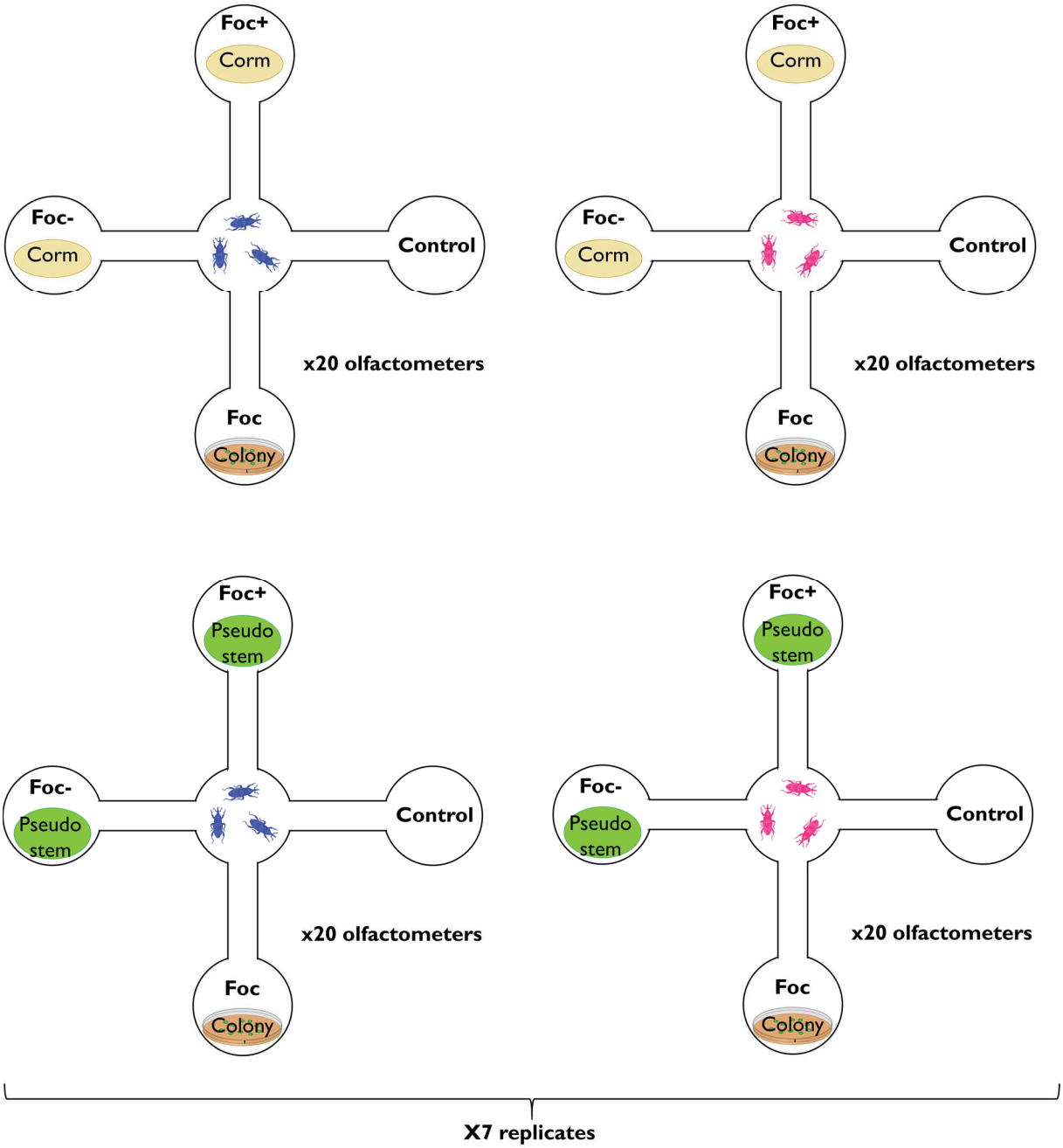


Figure 2. Illustration of the experimental design of the study. Blue and pink weevils illustrate the sex of the insects released in the central chamber of olfactometers. Foc+, Foc-, Foc colony and control represent the four treatments tested in the peripheral chambers. Corm and Pseudostem indicate the type of planting material tested in Foc+ and Foc- treatments.

Between each measure, all olfactometers were cleaned with alcohol at 70% followed by distilled water and dry with sterile paper. The location of the four treatments were randomly selected for the next experiment. The weevils were use only one time. In each olfactometer, three weevils (of the same sex) were deposited at the center. After 15 minutes in the complete dark, the location of each weevil was measured (selected treatment or absence of movement).

2.5. Statistical analysis

To compare the attractiveness of the different substrates tested in our experiments and to test whether the type of substrate, the sex of *C. sordidus* and the replicate altered this attractiveness, we used multinomial logit generalized linear models. These models were implemented with the 'vglm' function from the 'VGAM' R package (Yee 2010). In all models, the selected substrate by each *C. sordidus* individual was the qualitative response and the sex of *C. sordidus*, the type of substrate and the replicate were the predictor variables. We used likelihood ratio tests (LRTs) to select the best model by removing non-significant predictors in a backwards-stepwise process. The selection procedure was done until the models included only significant predictors (Zuur *et al.* 2011). The significance of the intercept of each type of substrate compared to the control treatment were tested on a selected model with all predictors significant**.** All statistical analyses were performed with R 4.0.2 (R Core Team 2020) and with an alpha level of 0.05.

3. Results

In total, the substrate selection of 1680 *C. sordidus* were observed in the seven replicates. There were no significant effects of the sex of *C. sordidus* nor of the replicate on selected substrates. There was a significant effect of the type of plant material proposed in the chambers of olfactometers (Table 1, Figure 3).

Table 1. Result from the multinomial logit generalized linear models of the effect of the replicate, the sex of weevils, and the type of plant material on the selected substrate by *C. sordidus*.

Predictors	**δAIC**	**Log-Likelihood**	**df**	**X2**	**p-value**
Repetition	5.199	-2305.2	4	2.8008	0.5917
Sex	3.480	-2307.5	4	4.5199	0.3402
Plant material type	21.299	-2322.1	4	29.299	**< 0.001**

With δAIC the variation of AIC (Akaike information criterion) explained by the predictor, df the degrees of freedom, X^2 the value of the chi-squared test.

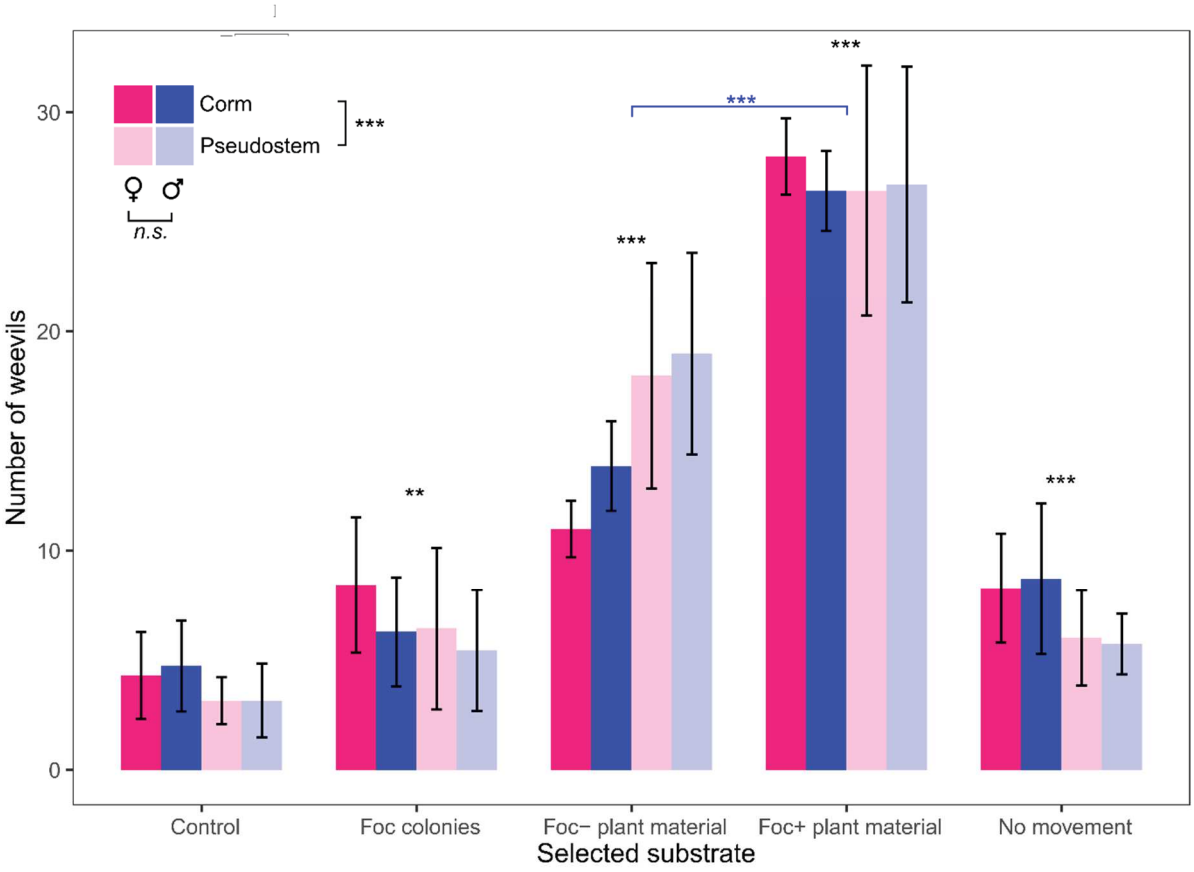


Figure 3. Number of *C. sordidus* per replicate (mean ± SD) according to their selected substrate. Each replicate included 60 individuals per sex and per type of planting material. Pink and blue colours represent females and males, respectively. Plain and clear colours represent the 'corm' and the 'pseudostem' plant material types, respectively. In the legend, stars show the significance of the sex and plant material predictors (see Table 1 for details). Above each group of bars, black stars show the significance of the intercept of each selected substrate compared to the control substrate (see Table 2 for details). Stars in blue shows the significance between Foc+ and Foc– selected substrates (tested separately).

The interaction between the plant material and the selected substrate was only significant in case of the intercept of the Foc- treatment (Table 2). In this later case, the 'pseudostem' was preferred to the 'corm' plant material. All intercepts of the different substrates selected by *C. sordidus* were significantly different compared to the 'control' substrate (Table 2).

Table 2. Intercepts of the different substrates compared to the control and the interaction terms due of the 'pseudostem' plant material type (compared to the 'corm'). These estimates were calculated from the select model only included the plant material type as predictor of the selected substrate.

Response levels	**Estimate (mean ± SD)**	**z value**	**p-value**
Foc+plant material	1.800 ± 0.136	13.233	**< 0.001**
Foc-plant material	1.016 ± 0.147	6.910	**< 0.001**
Foc isolated	0.4916 ± 0.160	3.074	**0.0021**
No movements	0.636 ± 0.156	4.082	**< 0.001**
Pseudostem: Foc+plant material	0.335 ± 0.209	1.599	0.1098
Pseudostem: Foc-plant material	0.757 ± 0.219	3.447	**< 0.001**
Pseudostem: Foc isolated	0.143 ± 0.246	0.582	0.5603
Pseudostem: No movements	-0.0135 ± 0.243	-0.055	0.9559

With ":" indicating the interaction between two factors.

The chambers with plant materials were most frequently selected (25.8 ± 7.9% and 44.8 ± 6.5 % for Foc- and Foc+ plant materials, respectively) than chambers with the Foc colonies isolated in petri plates (11.0 ± 5.1 %), the control substrate (6.4 ± 3.0 %), and the insects that did not moved (12.0 ± 4.5 %). When comparing separately Foc- and Foc+, the intercept of Foc+ plant material (0.78 ± 0.09, i.e. almost 80% higher) was significantly different from those of the Foc- plant material ($p<0.001$).

4. Discussion

Significant effect of Foc+ vs Foc-

Our results demonstrate that corms or pseudostems infected with Foc are more attractive for *C. sordidus* than the same type of plant material not infected by Foc. Raman and Suryanarayanan (2017) commented that many insects visit plants infected by fungus either for feeding on died tissues or attracted by the volatiles produced by the plant infected by the fungi. The fact that fresh pseudostems are more attractive to *C. sordidus* than fermented and other part of the plants (Hord and Flippin 1956; Delattre 1980) suggests that the preference for Foc+ plant material is specific to Foc. Plant pathogens may induce local or systemic emissions of volatiles or non-volatile secondary metabolites that insects may exploit for locating or discriminating between host plants (Kruess 2002; Ako et al., 2003; Jallow et al., 2008).

The results obtained suggest that the volatiles emitted by the corms and pseudostems of plants infected by Foc positively interfere with the feeding behavior of weevils, as indicated by Kariyat et al., (2013), Pashalidou et al., (2015), and Conchou et al. (2019). These authors mentioned that the chemical interaction between insects and plants is a complex mechanism that allows many species to recognize and select the most

appropriate feeding time, oviposit, or fulfil some basic need of the host in the host. Complementary, Holighaus and Rohlfs (2016) mentioned that many fungi can generate volatile allelochemical compounds that attract insects either for feeding or egg-laying, which is likely to explain what we observed here.

Here, we did not observed a significant difference of preference between *C. sordidus* males and females. It potentially suggests that the preferential attraction for Foc+ plant materials was not linked to oviposition behaviours, but rather to feeding behaviour. It is thus not providing clues to support the 'preference-performance hypothesis' in the case of *C. sordidus*. This hypothesis, that predicts that females are laying eggs preferentially on habitats that maximize their fitness, is however not always verified (Menacer et al., 2021). This result coincides with other observations that the sex of *C. sordidus* influenced their preference for host plant varieties (Budenberg et al., 1993a; Alpizar et al., 1999; Tinzaara et al., 2011).

From an evolutionary point of view, it is not clear what the weevils gain with this reciprocal interaction since on the one hand their aggregation could be favored and consequently an increase in mating and on the other hand it may happen that diseased bulbs are a better substrate for the development of larvae. Conversely, from this interaction, the disease clearly gains in greater dispersal capacity, having one more source of dispersal.

Implication for disease dispersion

In return, our results have important potential feedbacks on the epidemiology of Foc. Since it was proven that *C. sordidus* feeding on diseased tissues are carriers of viable structures of the pathogen (Guillén et al., 2021), the preference of *C. sordidus* for diseased plants means that it increases its probability to transport Foc propagules. This effect

would be particularly important when the outbreak is just beginning with only one or few contaminated plants in a plot. In this case, it means that even a small population of *C. sordidus* is likely to rapidly become a carrier of Foc propagule and contaminate healthy banana at bigger distances than the disease can do alone. In terms of management, it has a strong implication in sanitation procedure when a new spot of the disease is located in a plot. It suggests that aside the destruction of the inoculum of Foc, *C. sordidus* should be controlled in order to limit its potential dispersal inside the field or even between fields. For instance, a mass trapping strategy (using pitfall trap synergized with sordidin, the specific aggregation pheromone for *C. sordidus*, (Beauhaire *et al.* 1995)) all over the contaminated fields could be deployed extremely rapidly after the detection of Foc symptoms.

On the other hand, in areas with Foc presence, changes should be made in some agricultural practices such as removal of banana residues, the cutting of the corms and pseudostem as well as the dedaughtering since insects are going to be attracted to these residues for feeding and therefore serve as sources of inoculum for the pathogen.

The use of effective agroecological strategies to reduce weevil populations is fundamental in a Foc management strategy since this insect is a vector of the disease and the damage caused by the larvae facilitates the entry and subsequent infection of the pathogen.

Perspectives

Our results, together with the proof that *C. sordidus* can carry propagules of Foc Race 1 internally, externally and in the excreta (Guillén et al. 2021), confirm that *C. sordidus* has probably a key role in the dispersion of this disease. The next step in disentangling this complex interaction will be to measure the ability of *C. sordidus* that carry Foc propagules

to contaminate healthy banana plants. At last, an evaluation of the overall role of *C. sordidus* in dispersing the disease could be quantified in the field by comparing the dynamic of dispersion of Foc with and without *C. sordidus* in plot artificially infected. Our results illustrate the importance of managing together pest and disease in each agrosystems since Foc and *C. sordidus* mutually interact.

Chapter 3 - Internal and external acquisition of effective inoculum of Foc by *Cosmopolites sordidus*



This chapter answered the hypothesis posed by Meldrum et al., 2013 on the role of the black weevil *Cosmopolites sordidus* as a vector of Foc. It addresses the scientific questions Q2a and Q2b as presented in the Chapter 1 section 5. Based on greenhouse experiments with Gros Michel banana plants contaminated by Foc race 1 it was tested i) whether the insects are able to carry infective propagules of the pathogen internally and externally, ii) the acquisition time of effective propagules and iii) the survival of this inoculum in order to better evaluate the potential insectborne infection of healthy plants. This work was published in Pest Management Science in 2021.

Article 2- Can the banana weevil *Cosmopolites sordidus* be a vector of *Fusarium oxysporum* f.sp. *cubense* race 1? Unravelling the internal and external acquisition of effective inoculum

César Guillén Sánchez, Philippe Tixier, Ana Tapia Fernández, Ana María Conejo Barboza, Jorge A. Sandoval Fernández, Luc de Lapeyre de Bellaire

Abstract

Background: With the undergoing world outbreak of *Fusarium* wilt of bananas, it is essential to unravel all the possible process of dissemination of this disease. The host-pest interactions of the banana weevil with banana plants make this insect an important potential vector. This study, carried in controlled conditions, explores the interaction between the banana weevil and *Fusarium oxysporum* f.sp. *cubense* Race 1 (FOC), with a focus on the external and internal transport of viable fungal propagules.

Result: Viable inoculum of FOC was detected very rapidly on external teguments and in the digestive tract of the insect, i.e. at the lowest time studied of 5 min after contact with infected pseudostems. Maximal inoculum acquisition occurred after 1 h contact with an inoculum source. External inoculum was higher than the inoculum present in the digestive tract, but external and internal inoculum had the same dynamics. After a contact of an infected source, external and internal inoculum decreased exponentially within 50 h, but weevils remained infested for a long time, as long as 2 or 3 days that would be enough for inoculum dispersal. Viable inoculum was also detected in feces. Foc strains

isolated were pathogenic when inoculated to banana plants of the Gros Michel variety but did not provoke any symptom on Cavendish banana plants

Conclusion: These results demonstrate that the infective structures of Foc remain externally viable in the digestive system and the excreta of the banana weevil. Such excreta are capable of infecting healthy banana plants of Gros Michel.

Keywords

Insect mediated dispersion, Acquisition dynamics, Foc, Gros Michel cultivar, Pathogenicity

1. Introduction

Insects play an important role in the spread of plant diseases when they come into contact with diseased tissues, either during feeding, oviposition, or through involuntary contact with diseased organs or viable reproductive structures of pathogens (Purcell & Almeida 2005; Agrios 2008; Mauck *et al.* 2012; Heck 2018). Insect mobility can increase the spread of pathogens, however the speed and manner in which insects disperse pathogens and their functional role in disease epidemics will depend on the type of contamination mechanism, which can be either external or internal (Hatcher 1995; Tiberi *et al.* 2016).

However, the transmission of fungal diseases by insects remains poorly documented as compared to insect transmission of viral diseases (Nault 1997; Gray & Banerjee 1999; Mitchell 2004; Whitfield *et al.* 2015; Perilla-Henao & Casteel 2016; Eigenbrode *et al.* 2018; Heck 2018). Among the few examples studied, the case of the role of the weevil *Sitona hispidulus* in the dispersal of Fusarium wilt of alfalfa root rot (*Fusarium oxysporum* f.sp. medicaginis) has been well documented (Dickason *et al.* 1968; Leath & Hower 1993; Kalb *et al.* 1994). It has been shown that *S. hispidulus* plays an active role in *Fusarium* wilt of alfalfa through feeding activity of larvae, the fungus been present in excised heads of the insect (Leath & Hower 1993). In the case of western corn rootworm (*Diabrotica virgifera*; Coleoptera: Chrysomelidae), it has been shown that larvae feed on corn roots, thus facilitating root colonization by the plant-pathogenic fungus *Fusarium verticillioides* (Kurtz *et al.* 2010; Franco *et al.* 2017). Indeed, such interactions between root-feeding insects and plant pathogens have been reviewed recently by Willsey et al. (2017) stating that root feeding insects might play a role in plant fungal diseases through feeding injuries that facilitate pathogen infection or being vectors of fungal pathogens. The putative role of insects as vectors of fungal diseases might rely on the adhesion of fungal inoculum

(spores or mycelia) on their teguments during insect-plants contacts, as in the case of clover root borers (Jin *et al.* 1992). Insects might also carry fungal inoculum internally in their digestive tube and then excrete viable fungal propagules (Goldberg & Stanghellini 1990; Hyder *et al.* 2009).

Fusarium wilt of bananas is one of the most important diseases of this crop worldwide, and it is caused by the fungus *Fusarium oxysporum* f.sp*. cubense* (Foc) (Stover 1962; Ploetz & Pegg 1997; Gordon 2017). This pathogen belongs to the Eumicota class (Leslie & Summerell 2006) and it has three types of asexual reproduction structures: macroconidia, microconidia and chlamydospores (Smith 2007). The Fusarium wilt of bananas is divided into four races where race 1 and race 4 are the most impactful for the banana industry worldwide (Ghag *et al.* 2015). The race 1 was responsible of the devastation of more than 40,000 hectares of banana Gros Michel (AAA) between 1890 and 1950 (Stover 1962) which led to the varietal conversion to bananas from the Cavendish Subgroup (AAA) in the 1960s, the banana industry being still highly dependent on this banana Subgroup (Stover 1986; Shivas *et al.* 1995; Ploetz & Pegg 1997). More recently, the race 4 highly pathogenic on bananas from Cavendish Subgroup emerged in Asia and had a quick and severe development in Asia over the last 10 years. First cases were recently reported in Africa and South America, which are world major production areas of Cavendish bananas (Dita *et al.* 2018; Garcia-Batista 2019).

Understanding the dispersal of *Fusarium* wilt of bananas is a key for the development of management strategies. The first step of the dispersal is the introduction of the pathogen in a new area. Then the second step of the dispersal is the epidemics of the disease at a field or farm level. Dita et al., (2013) highlighted that *Fusarium* wilt of bananas can be dispersed i) by farmers that transport infected planting material, ii) by abiotic processes

through soil adhering to footwear, vehicles, agricultural machinery, by erosion, and by contaminated water used for irrigation, and iii) by animals, such as vertebrates, that can transport contaminated soil. All these mechanisms of fungal dispersion might explain the long-distance dispersal and are taken into consideration for biosecurity measures. Recently, it has been shown that *Cosmopolites sordidus* might potentially disperse FOC spores on external teguments (Meldrum *et al.* 2013). Then, this insect might play a role at local scale in the epidemiology of *Fusarium* wilt of bananas.

The role of this pest could potentially be very important since it is tightly associated to banana plant for feeding and for oviposition. Furthermore, the weevil is a cosmopolitan insect considered as the most limiting pest of banana cultivation in most tropical and subtropical countries (Gold *et al.* 2001). *Cosmopolites sordidus* damages are caused by their larvae that mainly feed from the rhizome of banana plants. This causes a significant reduction in yield due to poor absorption of water and nutrients as well as a reduction in the longevity of the plantations. In the literature, it is mentioned that *C. sordidus* could play a role in the dissemination of several bacterial banana diseases caused by *Ralstonia solanacearum*, *Erwinia carotovora*, *Xanthomonas campestris* pv. *Musacearum* (Were *et al.* 2015). Although the dispersal capacities of *C. sordidus* are moderate, i.e. measured up to 9 meters per day (Vinatier *et al.* 2010), it has a clear potential to spread the disease within and between fields. However, the potential role of *C. sordidus* in epidemiology of *Fusarium* wilt of bananas at field level remains unclear and requires further studies. In their study, Meldrum et al., (2013) considered a potential external dispersal but they did not consider an internal acquisition. Furthermore, the dynamic of acquisition and permanence of the disease remains unknown while it is a key factor in the potential role of *C. sordidus* in the dispersion of this pathogen.

This study aims at better understanding the potential role of *C. sordidus* as an external or internal inoculum carrier. Here, the capacity of *C. sordidus* to acquire *F. oxysporum* f.sp. *cubense* race 1 externally and internally was measured in controlled experiments. We quantified and followed the dynamic of acquisition of inoculum. Importantly, we verified the viability of external and internal inoculum in order to better understand their epidemiological importance. Lastly, we discussed the implications of these fungi-insect interactions in terms of spatial epidemiology of Foc.

2. Materials and methods

Our study was based on three experiments (summarized in **Fig. 1**) that aimed at measuring i) the external and internal acquisition of Foc by *C. sordidus* over time, ii) the external and internal survival of Foc on *C. sordidus* over time, and iii) the presence of Foc in excreta of *C. sordidus* fed from Foc-infected banana pseudostem.

2.1. Experimental design

Experiment 1. External and internal acquisition of Foc by *C. sordidus* over time

The objective of this experiment was to measure in controlled conditions the time required for the insects to acquire Foc both externally and internally after contact with a food source infected by the pathogen. It consisted in releasing *C. sordidus*, from a Foc-free rearing and previously isolated for 48 hours without feeding before the experiment, in 252 cm3 plastic boxes containing a 70 cm^3 pieces of pseudostems collected from Gros Michel banana plants. Each replicate included twelve boxes, ten containing a pseudostem collected from a Foc-infected plant and two containing a pseudostem collected from a healthy plant. The Foc-infested pseudostems were collected from plants showing typical

external and internal symptoms of *Fusarium* wilt, while the healthy plant was brought from a Foc-free experimental area. In each box, 20 insects were released simultaneously. Then, at 0, 5, 15, 45, 90, 180, 360, 720, 1440 and 2880 minutes after release, two insects were removed, one to evaluate the external Foc inoculum and one to evaluate the internal Foc inoculum. In total, each replicate included 200 and 40 insects that fed from infected (100 for external and 100 for internal inoculum study) and non-infected pseudostem, respectively (**Fig. 1**).

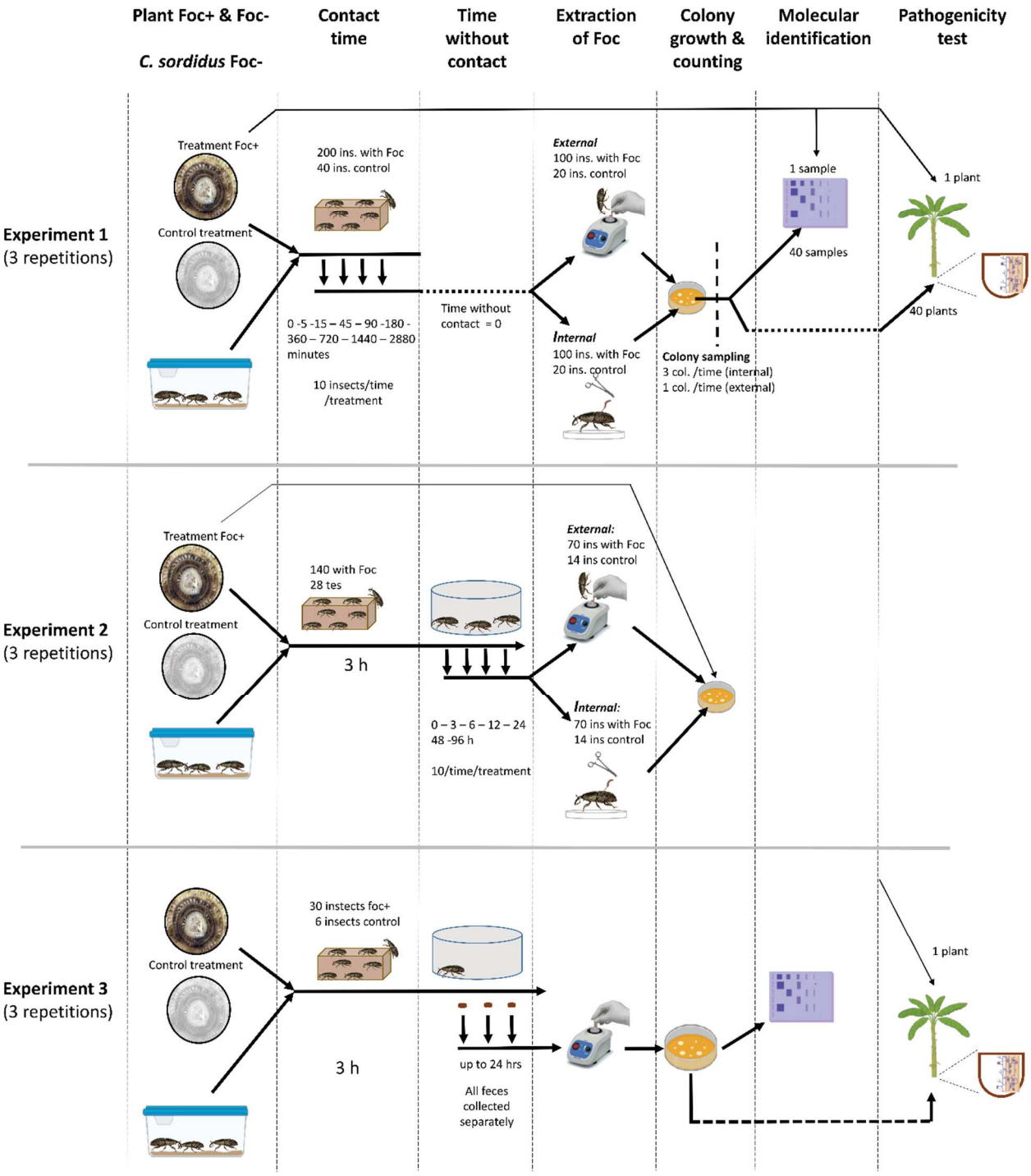


Figure 1. Description of the various experiments carried in this study.

For external inoculum assessment, each insect was placed in a 1 ml Eppendorf tube with 300 µl of distilled water and stirred in a Vortex for 1 minute at high speed. A 100 µl sample was taken, which was seeded in a Petri plate of Komada medium K2 and incubated for five days in constant light. The Komada medium consisted in a mixture of 900 ml of a basal medium and 100mL of an antibiotic solution. The composition of the basal medium was: K2HPO4, 1 g; KCl, 0.5 g; MgSO4.7H2O, 0.5 g; FeNaEDTA, 0.01 g; L-asparagine 2 g; galactose 10 g; Agar 16 g; distilled water 900 ml. After autoclaving, the basal medium was mixed with 100 mL of antibiotic solution filtered through 0,2 µm before mixing with the autoclaved basal medium containing the following agents: PCNB (pentachloronitrobenzene, 75% WP), 0.9 g; oxgall, 0.5 g; Na2B4O7 0.10H2O 0.5 g; streptomycin sulfate 0.3 g. The medium was adjusted to pH 3.8 with phosphoric acid 10% after being autoclaved.

For the internal inoculum analysis, each insect was disinfected twice with NaOH at 1000 mg/l during 5 minutes and washed three times in sterile water. Then, with microdissection equipment, the digestive tract was extracted and placed in an Eppendorf tube with 300 µl of sterile water for its subsequent maceration with sterile glass beads in a vortex during 1 minute at a high revolution. Then, 100 µl of the macerate was deposited in Petri dishes with Komada medium and incubated for five days at 26 ° C under constant light.

On each plate, the colonies of Foc were counted on the sixth day. Each colony of Foc was characterized by its salmon pigmentation and the form of the hairy growth of the mycelium that is an important characteristic of Foc on the Komada medium (Sun *et al.* 1978). The identification of *Fusarium* genus was confirmed by microscopic observation of

the reproduction structures of the fungus: uni or bi-cellular oval microconidia, presence of sickle-shaped macroconidia, globose-shaped chlamydospores.

For each repetition of the experiment, 30 *Fusarium* strains isolated from the digestive tract of the insect and 10 *Fusarium* strains isolated externally from the insects were randomly selected. These strains were subjected to a molecular identification and to a pathogenicity test in order to confirm their status as Foc race 1 isolates.

Experiment 2. External and internal inoculum survival of Foc on *C. sordidus* over time

The objective of this experiment was to measure the persistence of Foc externally and internally in *C. sordidus* after a contact with Foc-infested banana pseudostems. Similarly, to Experiment 1, it consisted in releasing *C. sordidus*, from a Foc-free rearing and previously isolated for 48 hours without feeding before the experiment, in 252 cm3 plastic boxes containing a 70 cm3 piece of pseudostems collected from Gros Michel banana plants. Each replicate included twelve boxes, ten containing a Foc-infested pseudostem and two containing healthy pseudostem. After 3 hours, insects were transferred to sterile boxes. Then, after 0, 180, 360, 720, 1440, 2880 and 5760 minutes, two insects were removed, one to evaluate the external Foc inoculum and one to evaluate the internal Foc inoculum. The assessment of external and internal Foc inoculum was performed with the same methodology as in Experiment 1. In total, each replicate included 200 and 40 insects that fed from infected and non-infected pseudostem, respectively. This experiment was completely replicated three times leading to the analyse of 760 insects.

For each repetition of the experiment, 30 *Fusarium* strains isolated from the digestive tract of the insect and 10 *Fusarium* strains isolated externally from the insects were randomly selected. These strains were subjected to a molecular identification test in order to confirm their status as Foc race 1 isolates.

Experiment 3. Presence of Foc in excreta of *C. sordidus* fed from Foc-infected banana pseudostem

The objective of this experiment was to quantify the presence of Foc in the excreta of *C. sordidus* that fed from Foc-infested pseudostems. Thirty insects were placed in sterile plastic containers after being in contact with Foc-infected pseudostems for 3 hours. Additionally, six insects were placed in the boxes with healthy pieces of pseudostem as control. After the feeding time had elapsed, the insects were moved individually (one insect per box) to sterile boxes to recover the excreta. Then, at 6, 12, 24 and 48 hours, the excreta were removed (when present) in Eppendrof tubes with 200 µl of sterile water. The samples were placed in Vortex for 1 minute and 100 µl was taken and seeded in Petri plates with Komada medium as described in the Experiment 1. The colonies were characterized as described above to determine their Foc race 1 status: morphology of colonies, molecular identification, and pathogenicity test.

2.2. Weevil rearing

In a commercial Cavendish banana plantation resistant to Foc race 1, located in the canton of Siquirres, Limón province, Costa Rica, a total of twenty wedge traps were placed every two weeks on freshly harvested plants. One week later, the wedge traps were reviewed,

and the insects trapped were collected in sterile plastic boxes. The insects were transferred to the Entomology Laboratory at the Biological Control Lab in CORBANA located in La Rita de Pococí, Limón province, Costa Rica. In order to verify the absence of Foc, 10% of the population was sampled. An external wash of the integument was done to each insect and external FOC inoculum was detected as described in experiment 1 (external inoculum).

2.3. Sources of pseudostem

The plants contaminated by Foc came from an experimental area located in Rita de Pococí, Limón province, Costa Rica. These plants belong to the cv Gros Michel, susceptible to Foc race 1. All selected plants showed typical symptoms of Panama disease (**Fig. 2**): yellow foliage, light brown to reddish-brown vascular bundles in the corm or leaf veins. In order to confirm the presence of Foc, pieces of vascular bundles were taken and placed in Komada medium. The colonies obtained were purified on PDA with Chloramphenicol before final identification as described above.

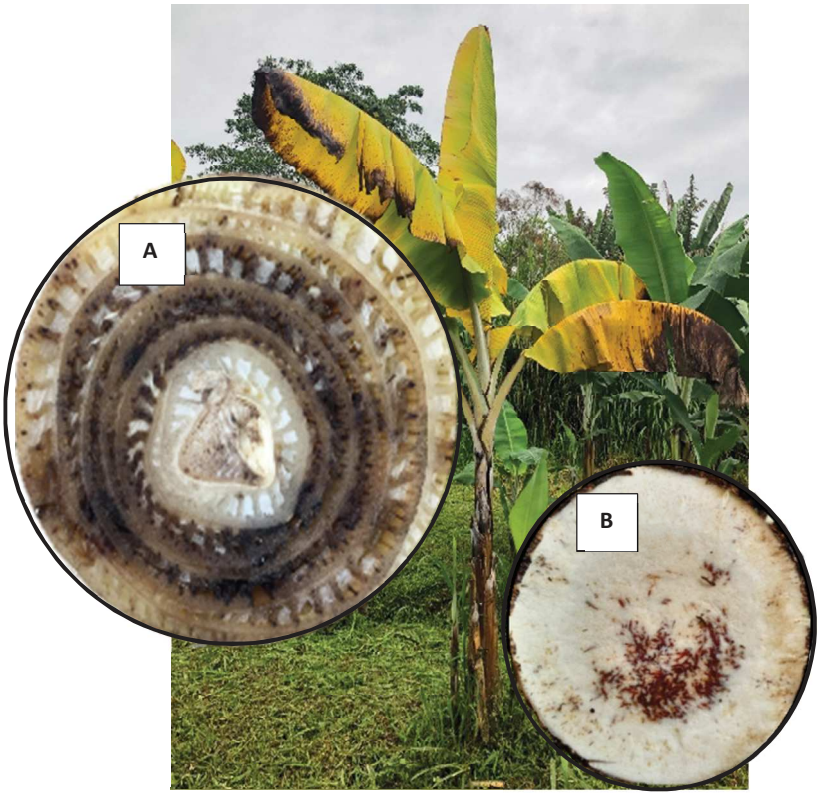


Figure 2. Typical symptoms of *Fusarium* wilt shown on banana plants selected for the provision of infected pseudostems.

2.4. Molecular analysis of strains isolated from insects

DNA isolation from vegetable tissue (pseudostem), animal tissue (insect), excreta of insect, and strains isolated from each sample type.

The samples (50-100 mg) were placed in a 2 ml tube with a per of small steel balls and ground in a mixer (Mill MM 400 RETSCH) for 5 min before addition of 1ml lysis buffer (1 M Tris, 0.5 M EDTA, 1% (w/v) SDS, pH set to 8.0 with HCL), 2µl of Proteinase K and 7 ul β-mercaptoethanol. The lysis mixture was incubated at 55°C for 45 min, mixed by turning the tubes every 10 min and incubated again at 65°C for10 min. Subsequently, 300 ul chloroform-isoamyl alcohol (24:1) was added before vortex agitation carried out under

cool conditions (-20°C) for 2 min. Then the samples were centrifuged at 13000 × g for 10 min. The aqueous phase was transferred to a new tube containing 400 ul Polyethylene Glycol (PEG 30%) and 200 ul 5 M NaCl, then kept at 8°C overnight. Samples were centrifuged at 13000 × g for 10 min to recover pellets, supernatants were discarded. Pellets were washed with 200 ul 70% ethanol twice, air-dried and resuspended in nuclease-free water.

PCR conditions

Four PCR were performed with different primers in order to confirm the taxonomic position of *Fusarium* strains isolated from insects (**Table 1**). In the first PCR, the status of the genus *Fusarium* was confirmed through specific ITS primers (Wei *et al.* 2012). With the second PCR, the FOC status of strains was confirmed using specific primers designed in SIX genes (Carvalhais *et al.* 2019). In a third PCR, possible FOC R1 or FOC TR4 status of strains was tested using specific primers originally designed for FOC TR4 (Lin *et al.* 2009), which also match for R1 isolates from Latin America (Magdama *et al.* 2019). Foc strains used in all experiments collected in banana plots on experimental farms of CORBANA were positive to these primers while belonging to race 1 (Conejo Barboza, personal communication). To rule out FOC TR4, a fourth PCR was performed with a specific primer from a putative virulence gene very specific for the identification of FOC TR4 strains (Li *et al.* 2013).

For all amplifications, DreamTaq PCR Master Mix (Thermo Scientific™) was used in 20 uL reactions according to the manufacturer's recommendations. The primer and DNA template concentration in the final PCR reaction were 0.5 mM and 20 ng. uL^{-1}, respectively. The following cycling conditions were used for all PCR: 94 °C for 1 min, 30

cycles of 94 °C for 30 s, and annealing temperature were primer-set dependent (see **Table 1**) for 30 s and 72 °C for 90 s, and finally extension 72 °C for 10 min.

Table 1. Primer sequences used in this study in order to confirm the taxonomic status of fungal strains isolated from *Cosmopolites sordidus*

Target taxa	**Primer**	**Target region***	**Band size (bp)**	**Anneal temperature (°C)**	**Reference**
Belongs to *Fusarium* genus	ITS-Fu-f / ITS-Fu-r	ITS	389	56.6	Wei *et al.* 2012
Belongs to *Fusarium oxysporum* fsp *cubense*	SIX9-Foc-F SIX9-Foc-R	SIX gene	260	58	Carvalhais *et al*. 2019
Possible race 1 or TR4	Foc-1-F Foc-2-R	OPA02 $_{404}$ (RAPD)	242	68	Lin *et al*. 2009; Magdama et al., 2019
Specific to tropical race 4	W2987-F W2987-R	Gene coding for an hypothetical protein	452	60	Li *et al*. 2013

* ITS= Internal transcribed spacer, RAPD= Random amplified polymorphic DNA, IGS= Intergenic Spacer Region of the rDNA, and SIX= secreted in xylem.

2.5. Pathogenicity tests

Each *Fusarium* strain was grown in triplicate for 10 days in PDA medium with 1% chlorphenicol. For the preparation of inoculum, 20 ml of distilled water was added to Petri plates to remove fungal propagules. The content was filtered in a 250 mL beaker with a sterile gauze in order to separate the mycelium from the spores. The concentration of spore suspensions was adjusted to $1x10^{-6}$ cfu / ml with a Neubauer chamber. Inoculations of *Fusarium* strains were performed by pouring a 200 ml spore suspension directly into the plots of banana plants of 50 cm high from which laterally roots were previously cut according to the methodology described by García-Bastidas et al., (2019). The inoculated plants were kept in the greenhouse for 10 weeks until they were harvested for internal symptoms assessment through longitudinal cut of the corm.

Disease severity was visually assessed following a 1-7 scale where 0 = No discoloration or damage in the corm, 1 = isolated points in corm damage between 1 and 5%, 2= damage >5 and ≤10%, 3= >10 and ≤20%, 4 = >20 and ≤35%, 5= >35 and 50%, 6=> 50 and ≤75% and 7 >75% (**Fig. 3**).

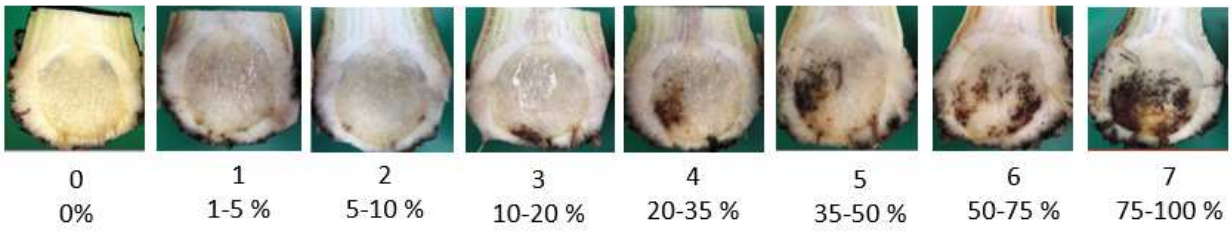


Figure 3. Disease severity scale used for evaluation of symptoms after artificial inoculation. This scale relies on internal symptoms on corms of banana plants ten weeks after inoculation with *Fusarium oxysporum* f.sp. *cubense* race 1. The severity scale ranges from small reddish brown spots, to severe rot.

2.6. Statistical analysis

In order to analyze and to represent the number of colonies measured externally and internally in relation with the time of contact between insects and Foc-infected pseudo-stem, we fitted a Gompertz model. The Gompertz model is particularly suitable to fit to sigmoid type growth that includes asymmetry and that follow non-linear patterns (Tjørve & Tjørve 2017).

Eq. 1. $$y = a\, exp^{\left(-\exp\left(\frac{\mu\, e}{a\,(\lambda - t)}\right)+1\right)}$$

The Gompertz equation (Eq. 1) represents the growth of y as a function of time t. It includes three parameters a, μ and λ representing population at equilibrium, the population growth rate, and the time of the inflexion of the growth, respectively. The Gompertz models were fitted as non-linear mixed effects models using the 'nlmer' function from the lme4 package (Bates *et al.* 2015), including the box identification number as a random factor altering the three parameters of the model. In this analysis, the number of colonies and the time of contact were log transformed.

Similarly, to analyze and to represent the decrease of the number of colonies measured externally and internally in insects as a relation of the time after been in contact with Foc-infected pseudo-stem, we used exponential models (Eq.2).

Eq. 2. $$y = a\, exp^{(b\, t)}$$

This equation represents the dynamic of y as a function of time t. It includes two parameters a and b, representing the initial population and the growth rate, respectively. This model was also fitted using the 'nlmer' function and included the box identification number as a random factor altering the two parameters of the model.

All these analyses only included the data from treatments with insects in contact with Foc-infected (excluding the controls). All analysis was performed using the R software 4.0.2 (R Core Team 2020). For all fitted models, we verified graphically that the residuals were following a normal distribution.

3. Results

3.1. External and internal dynamics of fungal acquisition by the insect fed from infected material

From the total of colonies obtained in the Komada medium after external isolates and from the digestive system in *C. sordidus*, it was determined that more than 85% were morphologically compatible with Foc (**Fig. 4**). The rest of colonies were undoubtedly contaminants. We did not observe any colonies of Foc (either externally or internally) on insects that were in contact with healthy pseudostem.

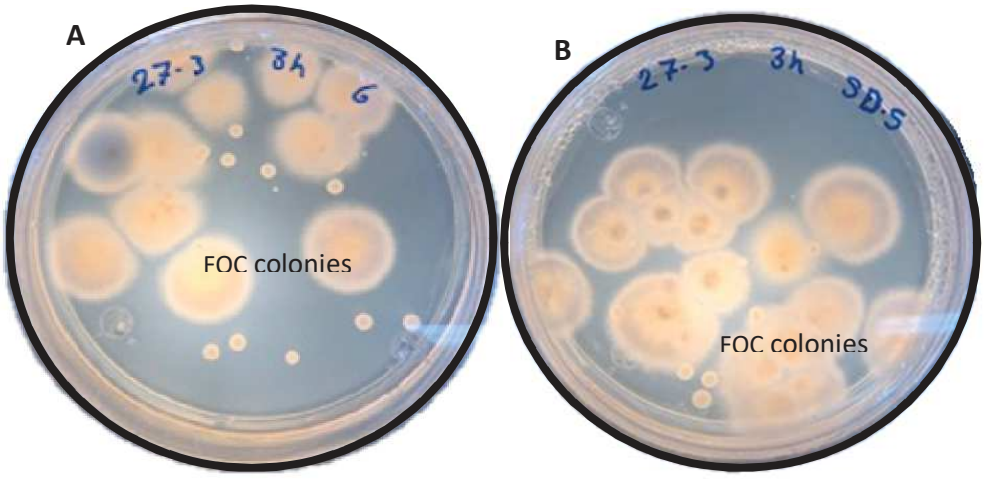


Figure 4. Example of typical fungal colonies recovered on Komada medium seven days after (A) External or (B) internal isolements from the weevils. These pictures show typical colonies of *Fusarium oxysporum* f. *cubense* sp (Foc Race 1).

There was a good fit of the Gompertz model to the dynamics of the external inoculum as a response to the time of contact between *C. sordidus* and Foc-infected pseudostem (a= 4.353 ± 0.147 (SD), μ= 1.118 ± 0.152 (SD), λ= 0.388 ± 0.232 (SD), AIC= 712.9, log-Likelihood= -349.3 deviance= 698.9, residual degrees of freedom= 273). As early as 5 minutes of contact with Foc-infected pseudostem, fungal inoculum could be detected on the external teguments of *C. sordidus*. The 90% of the maximal inoculum level was reached after an average contact time of 154 minutes (within the 78-301 minutes predicted confidence interval) with Foc-infected pseudostem (**Fig. 5A**). The three replicates showed the same trend although one replicate showed lower inoculum levels.

There was a similar trend for the dynamics of the internal inoculum in the digestive tract of *C. sordidus*, but the number of colonies was much lower as compared with those measured externally (**Fig. 5B**). The level of inoculum measured was also more variable between the different insects for a same sampling time. The Gompertz model (a= 1.422 ± 0.131 (SD), μ= 0.678 ± 0.229 (SD), λ= 1.705 ± 0.412 (SD), AIC= 777.4, log-Likelihood= -381.7, deviance= 763.4, residual degrees of freedom= 273) highlights that 90% of the maximal inoculum was reached on average at 66 minutes (within the 42-166 minutes predicted confidence interval). Alike the external inoculum, the internal inoculum of Foc in *C. sordidus* was detected very early since 5 minutes after the beginning of the contact with Foc-infected pseudostem some insects already exhibited inoculum.

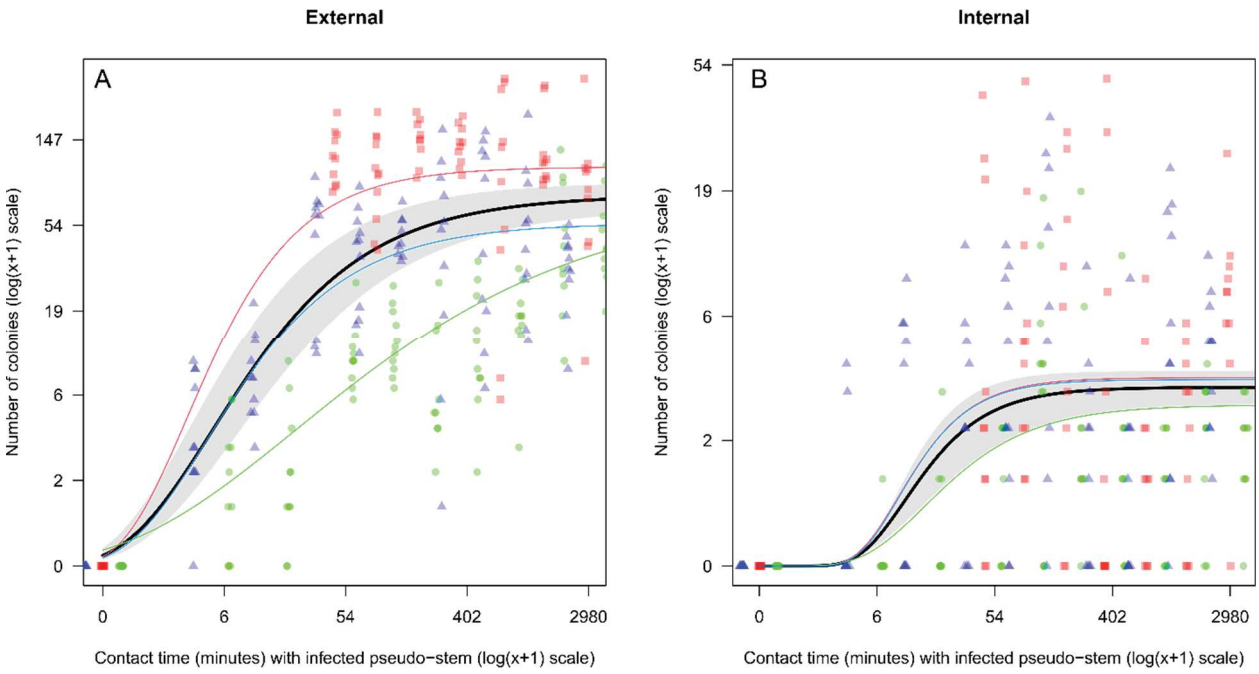


Figure 5. Dynamics of viable inoculum of *F. oxysporum* f.sp. *cubense* race 1 isolated externally (A) and internally (B) in *C. sordidus* as a function of the time of contact between banana weevils and Foc-infected pseudostem. The experiment was replicated 3 times and the color of the points indicates the replicate. The black curve and the grey area show the fit and the standard error of the Gompertz model (fitted with the 'box identification number' as a random factor, see section 2.6 for details). Colored curves show the fit for each replicate random factor. For an easier visualization, the contact time variable was slightly jittered.

3.2. External and internal survival of Foc on *C. sordidus*

It was observed that the dynamics of the external inoculum of Foc on the insect tegument showed a fast exponential decrease over time after insects were removed from Foc-infected pseudostems (a= 36.265 ± 3.514 (SD), b= -0.007 ± 0.008 (SD), AIC= 1620.8, log-Likelihood= -805.4, deviance= 1610.8, residual degrees of freedom= 205. At 54 hours the residual amount was very low and only 33% of the insects exhibited trace of external inoculum (**Fig. 6A**). The dynamics of the internal inoculum of Foc in the digestive tract of *C. sordidus* showed also a fast exponential decrease over time after insects were removed from Foc-infected pseudostems (a= 0.311 ± 0.051 (SD), b= -0.381 ± 0.066 (SD), , AIC= -270.4, log-Likelihood= 140.2, deviance= -280.4, residual degrees of freedom= 205). As in experiment 1, the amount of internal inoculum was lower than the inoculum found externally. There were always insects that did not show inoculum internally (30% at the initial time), but this proportion increased over time to reach 90% at 96 hours (**Fig. 6B**).

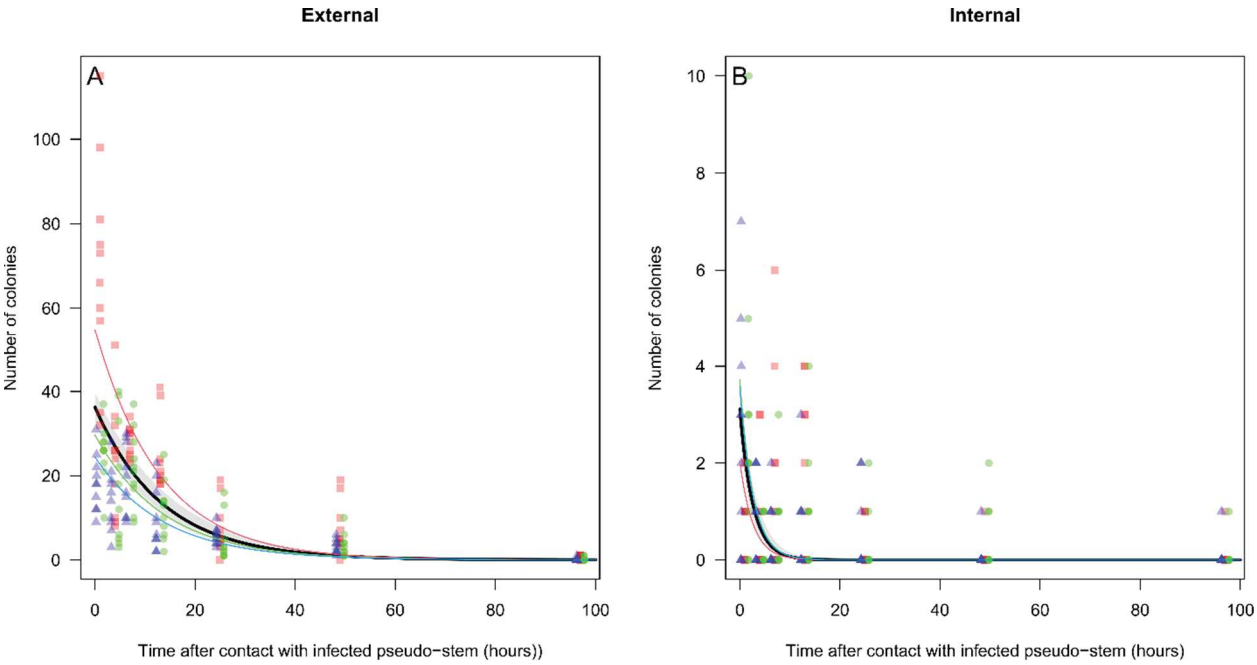


Figure 6. Decrease of viable inoculum of *F oxysporum* f.sp. *cubense* race 1 recovered externally (A) and internally (B) in *C. sordidus* as a function of the time after been removed from Foc-infected pseudostem. This experiment was replicated 3 times and the color of the points indicate the replicate. The black curve and the grey area show the fit and the standard error of the exponential models (fitted with the 'box identification number' as a random factor, see section 2.6 for details). Colored curves show the fit for each replicate (random factor). For an easier visualization, the contact time variable was slightly jittered.

3.3. Presence of Foc in the excreta of *C. sordidus*

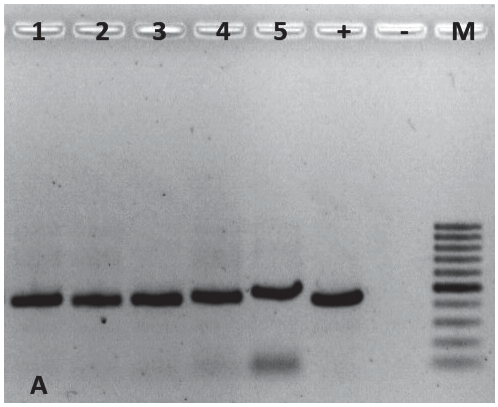
The quantity and quality of the excreta recovered from *C sordidus* was low. The analysis of the 90 excreta carried out after the insects fed from the diseased pseudostem during 3 hours showed that 12 excreta (13.3%) contained one or two Foc colonies and very few contaminants were observed growing on the Komada medium.

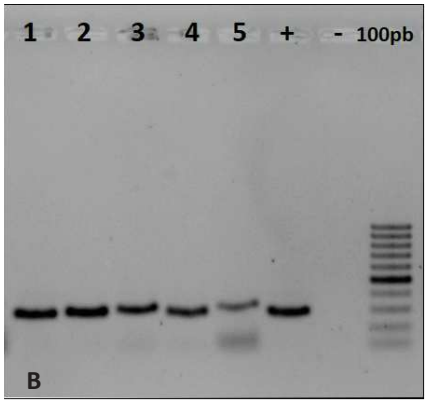
Molecular analysis of strains isolation

For each experiment developed in this investigation, molecular tools were used to confirm the identity of the Foc strains. For experiments 1 and 2 and feces analysis, 100% of the strains isolated were confirmed as belonging to the *Fusarium* genus (**Table 2**). It was also confirmed that they belonged to the FOC group and that they did not match with specific Race 4 (**Fig. 7**).

Table 2. Results of the 4 PCR analyses carried on each fungal strain isolated in the various experiments either from external teguments or digestive tract (experiments 1 & 2); either on feces. DNA was always extracted from pure cultures.

Experiment	Origin	% Positive amplification/(N° of strains)			
		Primer 1 (ITS-FU F&R)	Primer 2 SIX 9 F&R	Primer 3 FOC 1 and 2 F&R	Primer 4 W 2987 F&R
Exp 1	External teguments	100 (30)	100 (30)	100 (30)	0 (30)
	Digestive tract	100 (90)	100 (90)	100 (90)	0 (90)
Exp 2	External teguments	100 (30)	100 (30)	100 (30)	0 (30)
	Digestive tract	100 (90)	100 (90)	100 (90)	0 (90)
Feces		100 (12)	100 (12)	100 (12)	0 (12)





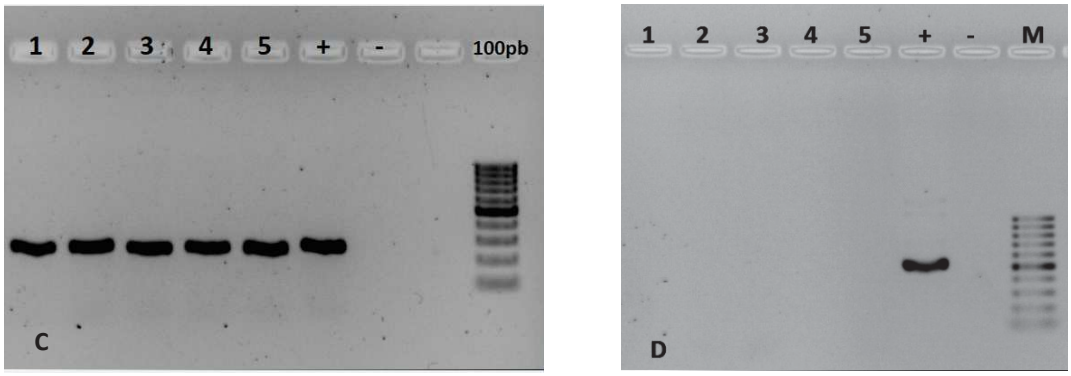


Figure 7. Agarose gel (1%) showing amplification products obtained with primers used in this study. **A.** *Fusarium* spp.:ITSFuF/R, **B.** *F. oxysporum* f.sp. *cubense* :FOC1/2, **C.** F. *oxysporum* f.sp. *cubense:* SIX9 Foc F/R y **D**. *F. oxysporum* f.sp. *cubense* tropical race 4: W2987F/R. Lane 1, culture sample T2R2H; lane 2, culture sample T2R3M; lane 3, culture sample T2R3H 4, culture sample T2R4M; lane 5, culture sample T2R4H 6, DNA positive control (from A to C: DNA from Foc R1 strain, and for D: DNA from Foc TR4 strain); lane 7, DNA negative control (water); and lane 8, Ladder used: GeneRuler DNA 100 bp Ready to Use Thermo Scientific™.

Pathogenicity test

We found that 100% of the Gros Michel banana plants inoculated with Foc strains isolated in experiment 1 showed symptoms of Panama Disease in the corm at 10 weeks after inoculation (**Fig. 8A-B**). Most of the plants had a disease index between 20 and 35% and only a low percentage to the plants showed severe damage (DI > 35%, disease severity note > 5 in the scale). No symptoms were observed in the corms of Cavendish plants

inoculated as in the Gros Michel control plants (**Fig. 8C**). The 12 strains isolated from feces were also pathogenic on Gros Michel plants but not on Cavendish plants.

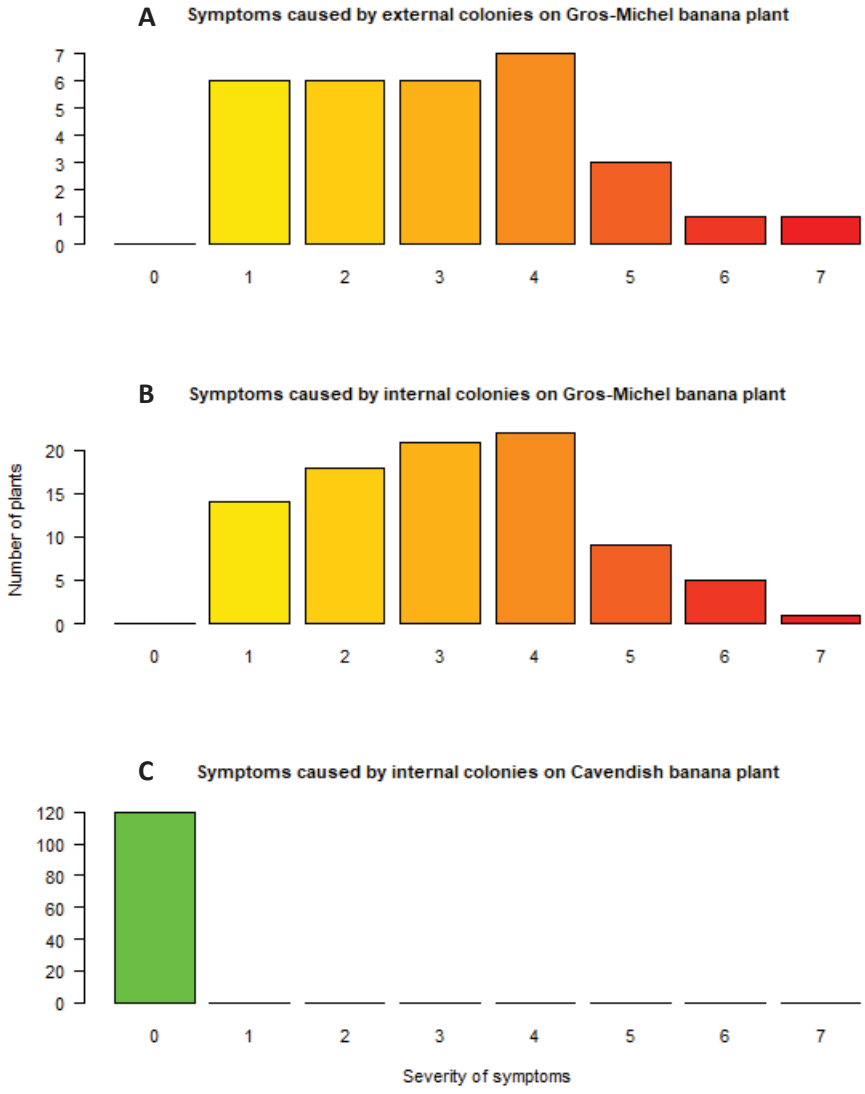


Figure 8. Disease severity on the corms of Gros Michel banana plants inoculated with different strains recovered from experiment 1. A total of 30 strains were isolated externally (A), and 90 strains were recovered internally (B). Graph (C) shows the disease severity on the corms of Cavendish banana plants isolated with these 120 isolates.

4. Discussion

4.1- *Cosmopolites sordidus* is confirmed as an effective vector of Foc on external teguments but also in its digestive tract

Cosmopolites sordidus was previously mentioned as a potential vector in the dissemination of pathogens that affect banana plants (Were *et al.* 2015) and Foc in particular (Meldrum *et al.* 2013). To date, only an external dissemination was suggested in the literature. Here, we confirm that *C. sordidus* can be a very effective carrier of infective Foc propagules on external teguments. For the first time, we demonstrate that *C. sordidus* can also transport Foc viable propagules in its digestive tract and feces. Meldrum et al (2013) could not isolate Foc isolates from internal sections of *C. sordidus* from insects collected at field level. Here the insects used starved before experiments and were fed exclusively on infected pseudostems, by another hand all the digestive tract was macerated which could explain the successful internal isolation as compared with former attempts. This new result is supported by the following facts : i) most isolations on Komada medium were quite pure with little contaminants either on teguments, digestive tract or feces of the insect; ii) isolates were morphologically identified as belonging to the *Fusarium* genus; iii) molecular identifications confirmed that these isolates belonged to the Foc group; iv) pathogenicity tests showed that these isolates were pathogenic to Gros Michel and not to Cavendish bananas confirming their race 1 status (Ploetz 2015b).

Internal inoculum was lower than external inoculum, probably because most propagules were killed in the digestive tract. Indeed, chlamydospores are more resistant than micro or macroconidia and might resist better through the digestive process (Ploetz 2015a). The females visit the corms and tissues of the banana plants where they lay their eggs inside holes that they dig with their jaws and rostrum. These lesions may allow the entry of

pathogens such as FOC, which is carried by the insect in its jaws or by the digestive contents that carry the eggs when they leave the female.

In our experiment 100% insects had Foc propagules on their teguments and we were also able to quantify this inoculum. During a survey on insects captured in Foc TR4-infected fields, Meldrum at al. (2013) only detected the fungus on 10% of the trapped insects and they did not quantify the inoculum level. Overall, the quantity of propagules transported externally and internally by *C. sordidus* were not extremely high. However, Foc has the capacity of causing a disease even at low inoculum levels (Pegg *et al.* 2019).

4.2. Importance of transport within the digestive tract

External inoculum viability might be altered by solar radiation, washing by rain or dew from plants and leaf litter, among other factors. However, the presence of inoculum inside the insect makes it less susceptible to external environmental conditions, which might facilitate its long-distance dispersal.

Such internal presence of fungi has also been reported in other biological models. Stanghellini et al. (1999) found chlamydospores of *Thielaviopsis basicola* in the intestinal tract of larvae and adults of shore flies (*Scatella stagnalis*) showing that these insects are involved in the dissemination of this disease to *Valerianella locusta*. Gardiner et al. (1990) and Jarvis et al. (1993) found that *Pythium* spp. can pass through the intestine of the fungus gnat larvae, which makes this insect plays an important role as vectors of this pathogen. Likewise, Gillespie and Menzies (1993) reported internal dissemination of *Fusarium oxysporum* f.sp. *radicisly copersici* by Fungus gnat in tomato plants and broad beans. These examples and what were found in this investigation demonstrate that

internal inoculum in the digestive tract of insects is an efficient and probably underestimated dissemination way for fungal plant pathogens. Here we have also shown that the excreta contain viable propagules of Foc but only in a smaller number of colonies regarding to what was found in the digestive tract. This may suggest that the digestive system of the weevil can destroy a great part of the viable propagules or that only a small part of fungal structures can survive in the alkaline environment of the digestive tract.

4.3. Implication of these results on the role of *C. sordidus* for Foc dispersal

Our results on the dynamics of acquisition and persistence of propagules, both externally and internally, provide a basis to estimate the role of *C. sordidus* in spreading Foc inside and between banana fields. Here we showed that either external or internal acquisition of the fungus by *C. sordidus* is very fast since inoculum could be always detected at the shorter time of 5 minutes and maximal inoculum acquisition occurred after 1 h contact with an inoculum source. If internal contamination was as fast as external it might be because insects starved before the experiments, so in natural conditions, internal contamination could depend on feeding activity of the weevils. Although the insects can get through long periods without feeding in the field, this research shows that the contamination of the insect with Foc would be rapidly effective. Weevils remain externally or internally infested for a long time, as long as 2 or 3 days that would be enough for inoculum dispersal. The rate of dissemination of the pathogen will depend on the displacement of the insect, which can be up to 9m per day (Vinatier *et al.* 2010) and up to 35 m in 3 days (Gold & Bagabe 1997), so it can be expected that the dissemination of the pathogen would be of this magnitude from a single event of contamination.

Another factor that could influence the spread of the Foc in the field is the size of the insect population; larger populations are likely to spread the disease faster. The number of plants infected with Foc, the severity and external damages to the plant on which the insects feed, are fundamental in the role of *C. sordidus* on the disease spread.

All this work shows that undoubtedly *C. sordidus* is an effective vector of viable inoculum of FOC and that is a good candidate to play an important role in the epidemics of this important disease of bananas. The next step will be to prove that *C. sordidus* can efficiently transmit the disease from infected plants to healthy ones.

5. Conclusion

Finally, our work participates to unravel the role of *C. sordidus* as an effective vector of Foc by different ways. Inoculum acquisition is enough fast and persistent for effective disease dispersal. It is evidenced for the first time that the infective structures of Foc are capable of remaining viable in the digestive system and being expelled in the excreta, the risk of infecting healthy plants or soils is very high.

Acknowledements

The authors would like to acknowledge to CORBANA for the support of this project. Grateful to the CORBANA Phytopathology colleagues for their comments, suggestions, and support. Thanks to Ing. Alfonso Vargas for his reviews and comments; and grateful with Ing. Miguel Gonzalez for his support. Finally, thanks to Marcos Hernandez, Julio Rodriguez, and Pedro Avila for the collaboration in the green house phase.

Chapter 4 - Role of *Cosmopolites sordidus* in the contamination of healthy banana plants by Foc



This chapter aims at testing whether *C. sordidus* infected with Foc can actively or passively infect Gros Michel banana plants. It addresses the scientific questions Q3a and Q3b presented in chapter 1 section 5. This work was presented in an article (article 3) to be submitted by the end of 2021. These results are complemented with a field experiment aimed at estimating the potential of released Foc-contaminated insects to infect healthy plants (addressing the scientific question Q4 presented in chapter 1 section 5). This work was presented in an article (article 4) to be submitted in 2022.

Article 3 - Disentangling the role of *Cosmopolites sordidus* in the effective transmission *Fusarium oxysporum* f.sp. *cubense* race 1 to banana plants

César Guillén Sánchez, Luc de Lapeyre de Bellaire, Philippe Tixier

1. Introduction

Insects are common vectors of many pathogens of crops worldwide (Willsey et al., 2017). Many groups of insects and arthropods can contain plant pathogens in their body but only few are considered as vectors to plant (Purcell 2005). In contrary, some insects with specialized eating behaviors can be very effective in transmitting a pathogen from one plant to another, including at long distances (Willsey et al., 2017). Insects when feeding on plants or during oviposition, cause wounds in plant tissues that can facilitate the invasion of infectious agents. Windels et al. (1976) reported contamination of corn seed by picnic beetles (*Glischrochilus quadrisignatus*) carrying *Fusarium oxysporum* internally and externally. Similarly, Were et al., (2015) indicate that *C. sordidus* is a potential vector of *Xanthomonas campestris* in banana plants when it visits the plants to feed or oviposit. Understanding whether an insect can be an effective vector or not is of major importance to better manage diseases.

In the case of fungal pathogens, there is a wide range of processes that are at play in the vection by insects following diverse processes of transmission of the pathogens. For

instance, the Dutch elm disease caused by the fungus *Ophiostoma ulmi* is spread by the Elm Bark Beetles (*Scolytus* spp.) that can be a carrier of spores just after haching (Santini and Facconi 2014; Webber 1990; Jürisoo et al., 2021). A similar case occurs with the blue stain fungus (*Ceratocystis ips*) which is spread among pine trees by the pine engraver (*Ips pini*) and other bark beetles. The pathogen for chestnut blight (*Endothia parasitica*) is also spread by the beetles of Scolytidae. Fruits can also be directly infected by insects, as the Plum curculio, *Conotrachelus nenuphar* belonging to family Curculionidae, that can infect fruits of peach cherry and plum with *Sclerotinia fructicola*, which is the fungal pathogen of brown rot (Sarwar and Sarwar 2018).

Fusarium wilt is the most important lethal disease of banana plantations around the world (Ordoñez et al. 2015; Ploetz 2005a) and is caused by *Fusarium oxysporum* f. sp. *cubense* (hereafter referred as Foc). This fungus can reach banana plantations and infect healthy plants in multiple ways: diseased plant material, water contaminated with particles of the fungus, soil contaminated mainly with Foc resistance structures, agricultural machinery and tools contaminated with crop residues or particles with the fungus, by the carriage of some vertebrates such as pigs and chickens. There is still a gap of knowledge on the efficiency of insects to spread this disease and their exact roles in the vection of Foc.

Meldrum et al., (2013) reported the presence of infective structures of Foc race 4 tropical adhered externally to the body of *C. sordidus* in banana plantations in Australia. Subsequently, Guillén et al. (2021) determined that infective propagules of Foc race 1 is present in the exoskeleton, digestive system and in the excreta of *C. sordidus* that were in contact with Foc infected plant material. Heck et al., (2021) found that, in banana plantations the higher the incidence *C. sordidus* damages, the greater the Foc incidence.

Although it is suspected that *C. sordidus* is related to dissemination of Foc, the proof it can be an effective vector has still to be demonstrated. Furthermore, the putative process of infection must be elucidated; in particular, it is unknown whether the behavior of *C. sordidus* in exploiting banana plant through feeding and oviposition is important or not.

Cosmopolites sordidus is an insect from the family of Curculonidea and considered one of the most important pests of banana, plantain and ensetes in the world Gold et al., (2001). The weevil attack crops established and cause death of suckers, affects plant snapping and toppling, lengthens maturation rates and leads to reduced yields. Females lay eggs on corms, roots, soil surface and pseudostem, preferring it last substratum (Abera et al., 2000). Adults can feed on fresh tissues of banana plants, and at respect some authors comment about host plant resistance studies in Africa and Latin America have provided inconsistent results (Ortiz et al., 1995). Moreover, it is unclear whether differential levels of damage among cultivars reflect differences in host plant location, timing of attack, acceptance (ovipositional rates) or larval survivorship.

According to these behaviors of exploitation of banana plants, active processes of vection, i.e. through the tight contact with the plant when the insect feed or lay eggs, are possible. It remains unknown whether such active processes of vection, passive processes of vection (i.e. though the presence of the insect in the surroundings of the plant but without tight and active contacts), both active and passive processes simultaneously, or none are at play.

In this study, we used mesocosm experiments i) to measure whether *C. sordidus* can be an effective vector of Foc to healthy banana plants, and ii) to disentangle whether the involved processes are active, passive, or both. A first experiment aimed at quantifying the active infection of banana plant by *C. sordidus* separately for females and males in

order to identify whether the oviposition is at play in the vection. The second experiment aimed at quantifying the passive infection of banana plant by *C. sordidus*. Together these results give new insights on the role of *C. sordidus* on the epidemiology of Foc. Finally, we discuss these results in terms of implication for the management of Foc and of gaps of knowledge to be addressed in future studies.

2. Materials and Methods

2.1. Experimental design

To determine the potential of *C. sordidus* as a vector of Foc, two experiments were established at the CORBANA research center, La Rita, Pococí, Limón, Costa Rica in order to measure the active (Experiment 1) and the passive (Experiment 2) mechanisms of infection of healthy plants (Gros Michel variety) with *Fusariun oxysporum* f.sp. *cubense* race 1 by *C. sordidus* previously in contact with corms infected with Foc. These experiments were carried out on individual plants in pot located in green-house conditions (Figure 1).

Experiment 1 – Quantification of the active infection of banana plant by Cosmopolites sordidus previously in contact with corms infected with Foc

This experiment included four treatments, two treatments with the inoculation of *C. sordidus* that were in contact with plant material infected by Foc (one treatment with males called Foc+m and one treatment with females called Foc+f) and two control treatments with the inoculation of *C. sordidus* that were only in contact with healthy plant material (one treatment with males called Foc-m and one treatment with females called

Foc-f). See sections §2.2, §2.3 and §2.4 for details on insects preparation. For all treatments, 10 individuals of *C. sordidus* were inoculated at the same time in the pot of a given plant. Each replicate of this experiment included 10 individual plants for Foc+m and Foc+f treatments and 5 individual plants for Foc-m and Foc-f treatments. In this experiment, the inoculated *C. sordidus* can move freely inside each pot and had the possibility to enter in contact with the plant. A net covering each pot stops them to escape from the pot. One week after the inoculation, all insects were manually removed. Ten weeks after insect removal, the damage score of each plant was measured (see below §2.5) and a molecular verification the strain of Foc was achieved (see below §2.6). This experiment was completely replicated three times leading to the inoculation of 900 insects on 90 plants.

Experiment 2 - Quantification of the passive infection of banana plant by Cosmopolites sordidus previously in contact with corms infected with Foc

This experiment included two treatments, one treatment with the inoculation of *C. sordidus* (5 males and 5 females) that were in contact with plant material infected by Foc (called Foc+) and one control treatments with the inoculation of *C. sordidus* (5 males and 5 females) that were only in contact with healthy plant material (called Foc-). See sections §2.2, §2.3 and §2.4 for details on insects preparation. In each replicate, there were 10 and 5 individual plants for Foc+ and Foc- treatments, respectively. In this experiment, plants and their bulbs were previously isolated by a sieve, preventing insects from coming into direct contact with the plant. A net covering each pot stops them to escape from the pot. One week after the inoculation, all insects were manually removed. Ten weeks after insect removal, the damage score of each plant was measured (see below §2.5). This experiment

was completely replicated three times leading to the inoculation of 450 insects on 45 plants.

2.2. *Cosmopolites sordidus* rearing

Experiments were performed using weevils collected from San Pablo Farm, localized in Siquirres, Limón, Costa Rica. The insects were maintained in a chamber at 24 °C and 90% relative humidity and feeding on banana corm and pseudostem Cavendish from San Pablo Farm. Previously to the experiments, weevil sex was determined in females and males using the curvature of the last abdominal segment and the punctuation on the rostrum (Gold et al., 2004).

2.3. Sources of pseudostem

The plants contaminated by Foc came from an experimental area located in Rita de Pococí, Limón province, Costa Rica. These plants belong to the cv Gros Michel, susceptible to Foc race 1. All selected plants showed typical symptoms of Fusarium wilt: yellow foliage, light brown to reddish-brown vascular bundles in the corm or leaf veins.

2.4. Insect preparation

The preparation of insects was done in 252cm^3 plastic boxes separately for males and females and for Foc+ and foc- treatments, respectively. In each box pieces of pseudostems from Gros Michel banana plants affected by Foc and from healthy Cavendish banana plant were deposited for Foc+ and Foc- treatments, respectively. The insects were placed to feed for 4 hours before inoculation.

2.5. Assessment of corm damages

The plants were harvested ten weeks after direct and indirect contact with insects. The corm was extracted from each plant, which was washed and cut longitudinally to visually determine the severity of the disease according to the scale indicated by Guillen et al., (2021): 0 = no discoloration or damage in the corm, 1 = isolated points in corm damage between 1 and 5%, 2 = damage > 5 and ≤ 10%, 3= > 10 and ≤ 20%, 4 = > 20 and ≤ 35%, 5= > 35 and 50%, 6= > 50 and ≤ 75% and 7 > 75%.

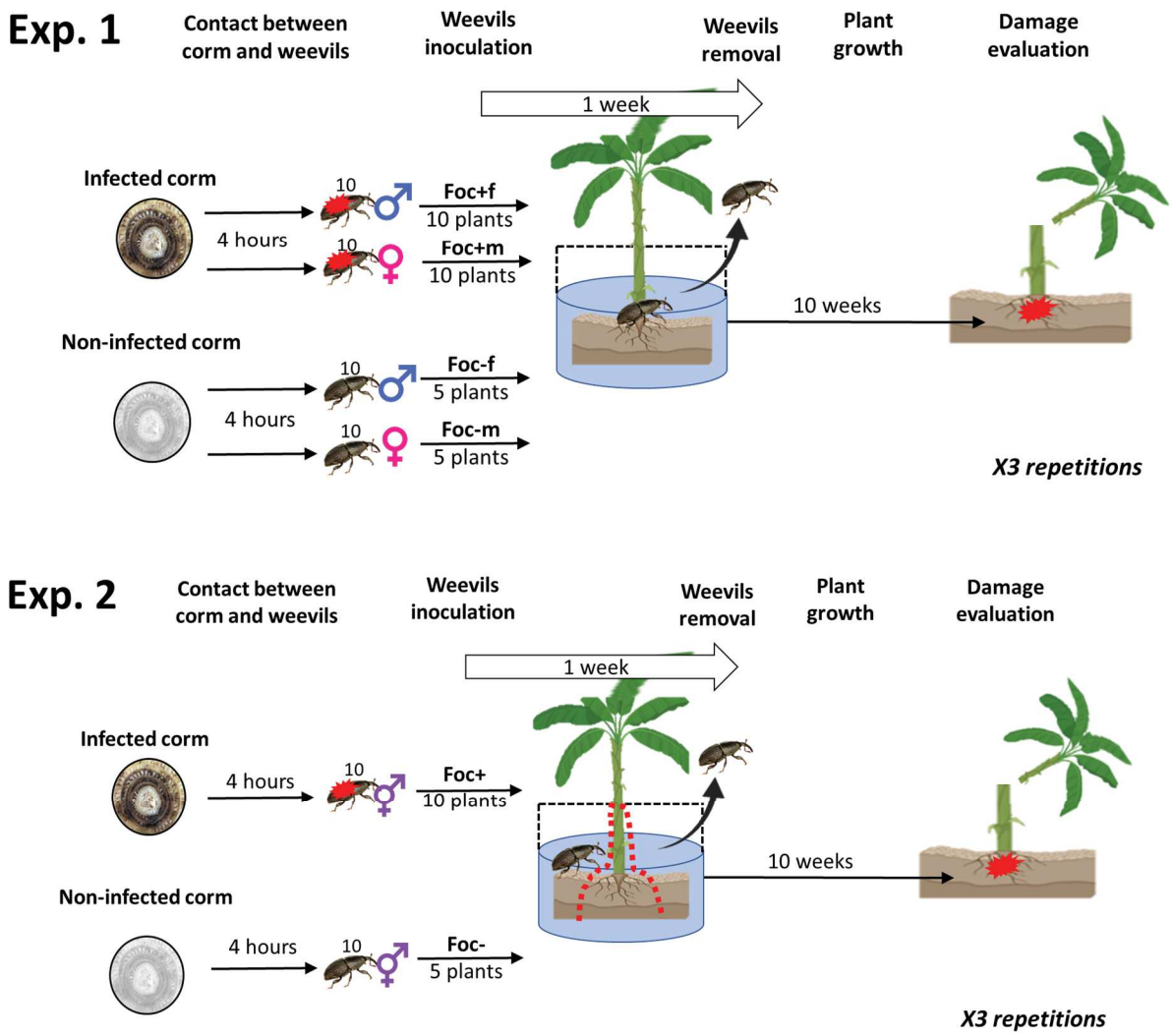


Figure 1. Illustration of the experimental design of this study. Experiment 1 aims at testing the infection of healthy plant including active infection process through the possible exploitation of the banana plant (feeding and oviposition). Experiment 2 aims at testing the importance of passive infection of a healthy banana plant in the case that no direct contact is possible between the insects and the plant.

3. Results

3.1 Quantification of the infection of banana plant by Foc in the case *Cosmopolites sordidus* can be in contact with the plant (Exp. 1)

There was a drastic (and highly significant) difference between treatments in which *C. sordidus* were in contact with Foc infected plant material (Foc+f and Foc+m) and control treatments (Foc-f and Foc-m). Indeed, all control treatments exhibited a damage score equals to zero regardless to the sex of *C. sordidus* (Figure 2). The mean damage score was 2.33 and 1.70 for Foc+f and Foc+m treatments, respectively. The maximal value observed was a damage score of 4 in both Foc+f and Foc+m (on a scale that goes up to 7). Although there was a trend for females of *C. sordidus* to lead to a greater damage score than males, the difference between these two treatments was not significant but close to significance ($p=0.065$). The proportion of damage score above zero was equals to 80.0% for females (Foc+f) and equals to 63.3% for males (Foc+m).

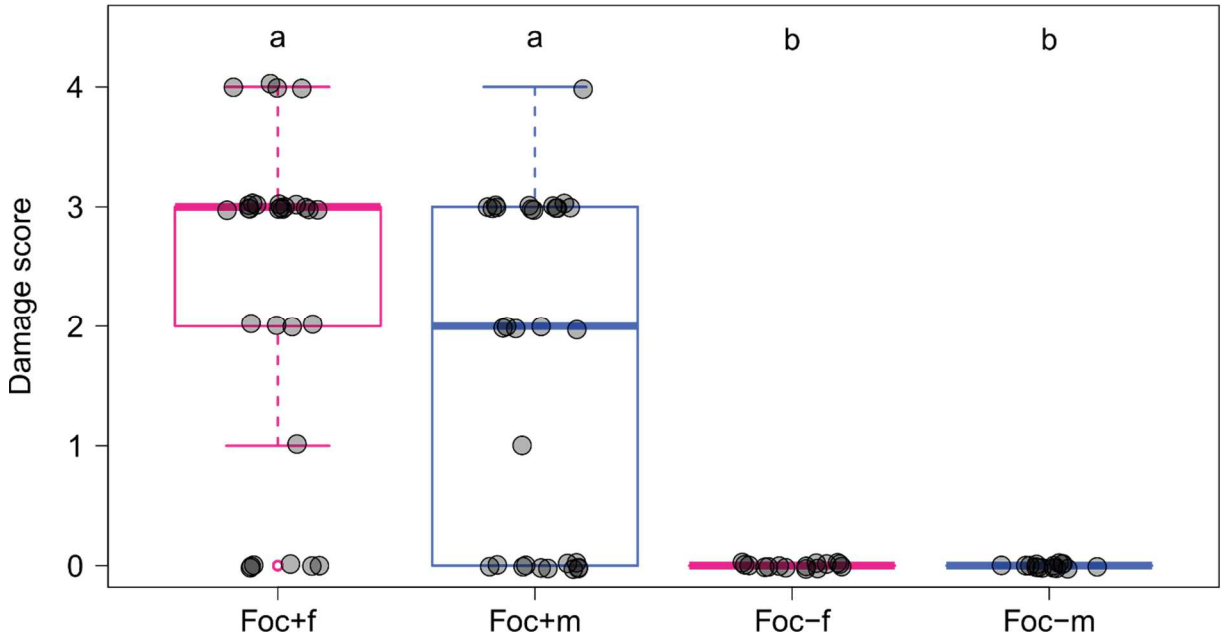


Figure 2. Distribution of the damage score (jittered for more readability) caused by Foc observed in the four treatments of the experiment 1. Red and blue boxplots show results for female and male, respectively. Foc+f and Foc+m are corresponding to treatments in which inoculated *Cosmopolites sordidus* were in contact with Foc infected plant material before inoculation. Foc-f and Foc-m are corresponding to control treatments. Letters in the upper part of the figures indicates the significance of the difference between with a Tuckey test based on a linear mixed model including the experiment number as a random factor.

3.2 Quantification of the infection of banana plant by Foc in the case *Cosmopolites sordidus* cannot be in contact with the plant (Exp. 2)

Similarly, to Experiment 1, the control treatment only led to damage scores equal to zero (Figure 3). In the Foc+ treatment, in which *C. sordidus* were in contact Foc infected plant material but without a contact with the plant, the mean value damage score was equal to

0.73 and the maximal score was equal to 3. In this treatment, 43.3% of the measures were above the note of zero, and 6.6% of the values were at 3.

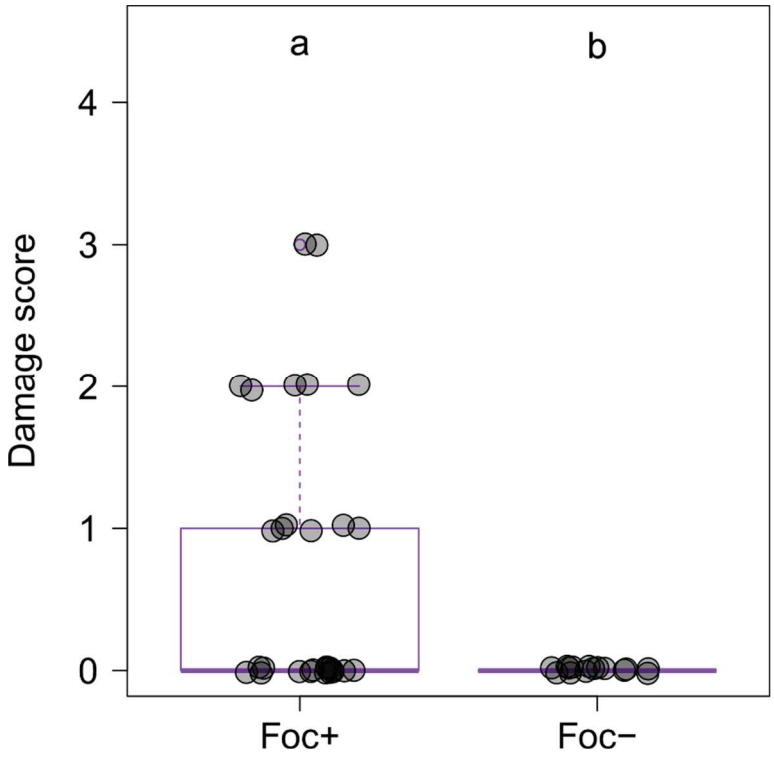


Figure 3. Distribution of the damage score (jittered for more readability) caused by Foc observed in the four treatments of the experiment 2. Foc+ and Foc- are corresponding to treatments in which the inoculated *Cosmopolites sordidus* that were in contact with Foc infected plant material and with healthy plant material, respectively. Letters in the upper part of the figures indicates the significance of the difference between with a Tuckey test based on a linear mixed model including the experiment number as a random factor.

4. Discussion

It was demonstrated that *C. sordidus*, when in contact with banana plants, was able to transmit the infective propagules of *Fusarium oxysporum* f.sp. *cubense* race 1 and infect healthy plants, thus proving that the insect is a vector of Fusarium wilt. Furthermore, it was determined that corm damage caused by Foc was significantly greater when the insect was in direct contact with the plants (active contamination) compared to when the insect was not in direct contact with the plants (passive contamination). This suggests that the mechanical damage caused by the insect when feeding or ovipositing significantly favors the entry of the pathogen. Treverrow et al. (1992) pointed out that weevils feed on corms and pseudostem, making possible mechanical damage that can facilitate Foc to penetrate the plant more rapidly and thus increase disease severity.

The presence of slightly greater damage caused by Foc when *C. sordidus* females that were in direct contact with banana plants may be due to mechanical damage caused by the insect's rostrum forming oviposition holes in the corms and pseudostems. Night, et al. (2011) comment that the primary site of an infestation is an orifice just below the collar, the junction between pseudostem and rhizome that are created by adult females with the rostrum to lay eggs.

The fact that the weevils are not in direct contact with the banana plants (passive contamination) was not sufficient to prevent the transmission of Foc. However, the severity of corm symptoms was lower compared to active transmission; some corms were not damaged by Foc which was scarce in the active transmission experiment. Guillén et al., 2021 reported the presence of infective propagules of Foc in the excreta of *C. sordidus* in smaller quantities than what can be found in the exoskeleton of the insect. This may partly explains the low severity of Foc on corms in the passive transmission experiment.

The determination that *C. sordidus* is both an active and passive vector of Foc opens a new line of research to design controlling strategies of the Panama disease. Foc management programs should thus consider an efficient control strategy of *C. sordidus*. For instance, this may be achieved with mass trapping, *i.e.* using pitfall traps synergized with pheromone (Rhino et al., 2010). Other types of trap, for instance relying on the use of fresh banana plant material (pseudostems or corm) would not be adequate due to the risk of contamination of Foc. Furthermore, while traps based on fresh plant material ar efficient, they are capturing less compared to pitfall traps with pheromones (Tinzaara et al., 2005).

Our results suggest that the tight contact between the insect and the plant is important in the process of infection. In order to minimize this contact, it would probably be important to reduce wounds in the corms and pseudostems caused at different step of the management of banana plant. For instance, sucker removal cause lesions that are attractive for *C. sordidus* (by aromatic compounds emitted by the plants) and that give it an access to the internal part of the plant. In future studies, it would be interesting to estimate the effect of such lesions on the banana plant on the efficiency of its infection by *C. sordidus*. The use of repellent plants for *C. sordidus* could also be a practice that could be evaluated in terms of reducing weevil arrival on banana. In this regard, Walangululu et al. (1993) reported that the legume *Tephrosia* spp. May be repellent on adults of *C. sordidus*. Similarly, applications of repellents and deterrents such as clove extract produce adverse effects on weevil eggs and larvae, contributing to a significant reduction in the impact of the pest (Bakaze et al., 2020).

Resistance to damage caused by *C. sordidus* is another important factor to consider when selecting a cultivar. Kiggundu et al. (2003) mentioned that the wild diploid banana

Calcutta 4, the cultivar Yangambi Km5 and the Cavendish group have a high level of resistance to the black weevil. In order to favor an integrated resistance to pest and diseases, it would be a good option to include this resistance to *C. sordidus in* future breeding and selection program of banana plant.

Chemical control has been a rapid and effective alternative to reduce *C. sordidus* populations worldwide; however, its excessive use has contributed to the generation of resistance to most commercially available molecules (Gold et al., 2001). On the other hand, weevil control has been directly related to nematode management, which is regulated by the application cycles of traditional nematicides (Robalino et al. 1983; Bujulu et al. 1986). However, one of the current limitations for chemical control of *C. sordidus* is the changes in the regulation of insecticide use in many countries, mainly in the European Union, which has increasingly limited the use of insecticides applied on foodstuffs.

Article 4 - Efficiency of *Cosmopolites sordidus* to disperse *Fusarium oxysporum* f.sp. *cubense* race 1 in field conditions

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This article is presenting preliminary results that will be completed by measures to be done up to mid-2022.

1. Introduction

Panamá disease is a disease caused by the fungus *Fusarium oxysporum* f.sp. *cubense* (Foc) and is considered one of the most destructive diseases of banana cultivation worldwide. This pathogen attacks the xylem of the plants causing a progressive wilting that culminates in plant death (Stover 1962). Foc was first reported in Australia, but it has spread to several countries around the world, causing economic losses due to the destruction it causes to banana crops and the phytosanitary measures that farms and countries have had to adopt to prevent its arrival or spread. However, once established in a plantation, its spread to other plants becomes eminent as it uses water, wind, soil, agricultural equipment, some cover plants, vertebrate animals, and insects as means of transport to neighboring plants (Dita et al., 2018).

Among insects, the black weevil *Cosmopolite sordidus* is one of the most important agents in the reduction of the disease due to the high populations in banana farms, as well as the damage that the larvae cause in the corms, leaving exposed wounds through which pathogens such as Foc can enter more easily. According to Gold, (2001), the black weevil

is the most abundant cosmopolitan pest in banana plantations in all regions of the world and its importance lies in the damage caused by the larvae in the corms, causing a deficiency in the root system, the absorption of nutrients and water, and even in high populations it can cause the fall of the production units. This insect is nocturnal, visiting banana plants of all stages of development, although Budenberg et al., 1993) mentions that they prefer plants that have just emitted the inflorescence, since this is the moment when the plant releases the greatest number of volatiles (Masanza et al., 2009). *C. sordidus* is not an active walker, however, Vinatier et al., (2010) demonstrated that this insect is able to move up to 9 m in one day, which implies that, in one week, they can move for a range of up to 30 meters.

The role of *C. sorididuss* as a disease disperser of fungal diseases has been poorly studied. Meldum et al. (2013) were the first to determine the presence of Foc TR4 in the exoskeleton of *C. sordidus*. More recently, Guillén et al., 2021 demonstrated that *C. sordidus* can be a carrier of viable Foc structures internally, externally, and in feces. Complementarily, Guillen et al. (Paper 3 of the thesis) showed that *C. sordidus* can be an effective vector of Foc in mesocosm conditions. These authors showed that both passive and active processes can be at play in the infection process of healthy plants. To date, there was no investigations that demonstrate that *C. sordidus* can be a vector of Foc in real field conditions.

In this study, we measured the capacity of *C. sordidus* to disperse Foc in field conditions. A planted a plot of banana plants (Gros Michel Cultivar that is susceptible to Foc race 1) in a context where no contamination of Foc was possible. At the center of this plot, we released 100 adults of *C. sordidus* that were previously in contact and feeding on Foc race 1 infected plant material. We then detected the appearance of the symptoms over the

time. The analysis of the dynamic of appearance of symptoms and their location in the plot give new insights on the effective role of *C. sordidus* in dispersing Foc. Finally, we discuss the implication of these results in terms of management of Foc.

2. Material and methods

Background of the experimental plot

The research was developed in a banana experimental plot area located at the CORBANA Research Center, in La Rita de Pococí, Limón, Costa Rica. The plot size was 1200 m^2, which was planted in December 2016 with banana seedlings of the Gros Michel variety from the CORBANA Tissue Culture Laboratory and using a double row planting arrangement. Prior to planting the seedlings, soil sampling was performed to ensure it was not contaminated by Foc. To this end, we used a Powersoil extraction kit and subsequently performed a Foc race 1 detection following the method described by Guillen et al. (2021). The plot was located in an area far from other banana plantations to avoid the arrival of weevils from neighboring plants. In addition, the plot was surrounded by two deep canals and barriers that prevented the entry of any walking organisms from neighboring areas or any flows of water coming from outside the field.

Release of C. sordidus in the experimental plot

The release of 100 *C. sordidus* was done in the center of the experimental plot (December 18th 2018), with a female/male ratio of 1:1. Prior to release, the insects were placed for 4 hours in plastic containers of 1000 cm3 with pieces of pseudostems infected with Foc. The

release was carried out in the end of the afternoon (5:00 pm), which is juist before the beginning of activity of these insects during the night.

Foc infected food source for the C. sordidus

Pieces of Foc-infected banana pseudostem were brought from CORBANA's Foc experimental area in La Rita de Pococí. To ensure the presence of Foc in the food source, the pathogen was isolated on Komada medium and subsequently identified by molecular methods using the SIX gene technique.

Identification of suspect plants for Foc

Once a week, all plants were evaluated to determine the presence of symptoms compatible with Panama Disease: yellowing and necrosis of leaves, broken leaves on the petiole with a skirt-like appearance, deformation, and openings in the pseudotallus, elongated and narrow leaves.

Identification of internal symptoms

Once the plant with external symptoms was identified, a longitudinal cut was made in the pseudostem, with the previously disinfected tool, at a height of 50-100 cm from the base of the plant and a portion of pseudostem of approximately 15 cm high x 10 cm wide x 3 cm deep was extracted, avoiding the extraction of samples from areas where there was advanced decomposition of the tissues. Subsequently, vascular bundles with brown coloration were taken and placed in pieces of sterile paper towel and then in sterile bags

to be transferred to CORBANA's Biological Control laboratory to cultivate the fungus in Komada medium.

The hole where the sample was taken was closed with the remaining material from where the sample was taken and then sealed with adhesive tape and applied with insecticide to prevent the arrival of other insects.

Isolation of Foc from vascular bundles

This procedure was performed with a scalpel and sterile forceps and a 2 cm long piece of vascular bundle was immersed in the sodium hypochlorite solution for 30 seconds and then washed with sterile distilled water and dried with sterile paper towel. The vascular bundle pieces were placed in Petri dishes duly identified with Komada medium and subsequently placed in a continuous light in a chamber at 27°C for 5 days.

Diagnosis of Foc from colonies obtained in culture medium

After 5 days, the Petri dishes were removed from the incubator and the salmon-stained colonies were isolated and purified on PDA medium for subsequent identification by molecular techniques according to Guillén et al. (2021).

Management of the infected plants by Foc

After molecular confirmation for Foc of each of the plants, were cut and buried 50 cm from the site. Subsequently, the site was disinfected with quaternary ammonium.

3. Results

The soil analysis confirmed that the field is free of Foc at the beginning of the experiment. Fifteen months after weevils inoculation, a first plant showing Fusarium wilt symptoms was found at a distance of 3 m from the place where the insects were released. It was a plant that had just emerged from inflorescence, 2 m tall. The external signs observed were a yellowing of the lower leaves, lower leaves with breaks in the petioles resulting in the appearance of a skirt and a successor with elongated and narrow leaves. No external signs were observed on the pseudostem.

Seventeen months after weevils inoculation, a second plant showing Fusarium wilt symptoms was observed 5 m away from the insect release site. It was a plant with a 3-week-old, 2.5 m tall cluster with yellow leaves, thickened pseudostem and a deformed candela leaf with a twist in the center. No symptoms were observed in the adjacent suckers.

And twenty-seven months after weevils inoculation, we found a third plant with various symptoms characteristic of Fusarium wilt was observed 20 m away from the insect release (Figure 1). This plant showed yellowing, folded leaves in the form of a skirt, opening in the pseudostem and elongated son, with thin and long leaves. It was a plant with a 3-week-old bunch. Unlike the other two plants, this third plant showed a sudden death, developing symptoms in an accelerated manner within three weeks.

The internal symptoms observed in the three pseudostems of the plants diagnosed as Foc-positive were characterized by the presence of reddish to brown vascular bundles (Figure 2). Diagnostics of the three sampled plants indicated that they were positive for Foc race 1 according to the methodology of Carvalhais et al. 2019 FOC using specific primers designed in SIX genes.

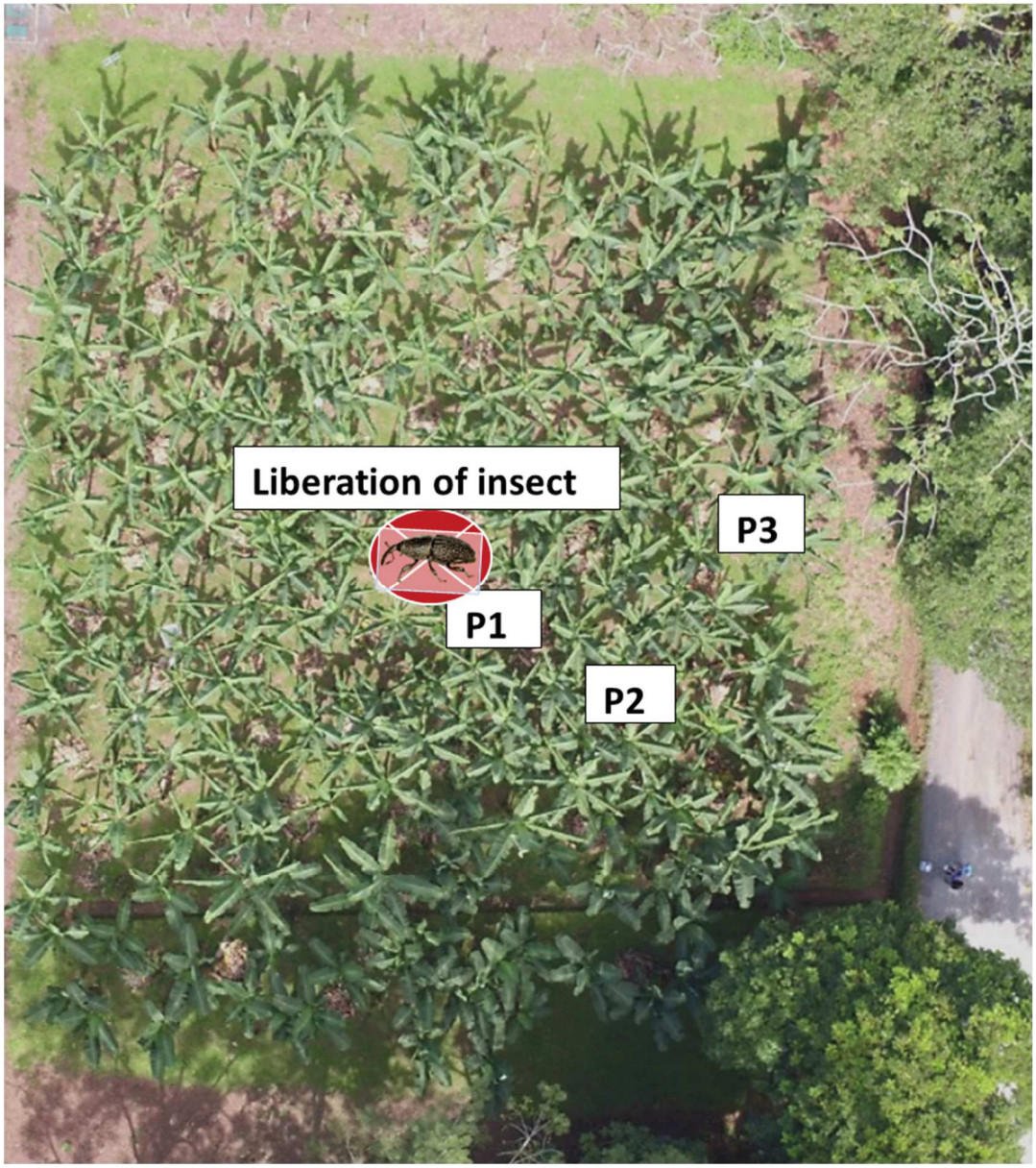

Liberation of insect
P3
P1
P2

Figure 1. Insect release in Gros Michel banana experimental area, La Rita Pococí, Limón, Costa Rica. P1, P2, and P3 show the location of the three plants that were detected positive to Foc.



Figure 2. Vascular bundles of reddish coloration in pseudstem of banana tree Gros Michel. La Rita, Pococí Limón, Costa Rica.

4. Discussion

Panamá disease is a polycyclic disease that causes wilting and death due to the obstruction of vascular bundles by preventing the transport of water and nutrients to the aerial parts (Li et al., 2017) as observed in the three plants affected by Foc in this research. The spread of this disease has been studied mainly when abiotic factors are involved, however, in this research the role of *C. sordidus* as a vector of Foc under field conditions is experienced for the first time. Indeed, even if this experiment did not include a control plot without infected-insects inoculation, the detection of Fusarium wilt symptoms was very fast as compared with other plots in the same area (personal data) and related to Foc-infected weevils release. Moreover, this observation is clearly in line with mesocosm experiments (article 3).

Although in the experimental area there were plants of all stages of development, it was clear to observe that the three plants infected by Foc corresponded to plants in post-flowering stage of development, which, according to Dita et al., (2018) is the moment where Foc symptoms are mostly perceived in plants grown under natural conditions since it is when the plant requires a greater expenditure of energy for the development of the bunch.

First symptoms of development were observed after one year and three months after the release of Foc-infected insects. This indicates that the disease development in adult plants is slower than three months usually reported for the appearance of first symptoms (Dita et al., 2018). This difference may be attributed to the fact that in our case the inoculum is probably relatively low compared to the one of other studied that reported a shorter time (eight weeks) for symptoms appearance (Guillén et al., 2021).

Having only three diseased plants 19 months after the release of Foc-infected insects in the plot does not ensure that they are the only diseased plants in the plot, in this regard, Hennesey et al., (2005) reports that many plants can be infected with Foc but do not exhibit symptoms of the disease. There are two possible scenarios: i) that the three diseased plants come from a primary infection, and ii) that some or all plants with symptoms are the result of a secondary infection. The fact that only 3% of the weevils managed to contaminate the banana plants with Foc in this field research is consistent with the limited mobility of the insect as indicated by Gold et al. (2001). In the case of only primary infection, the distances at which plant were detected positive to Foc is consistent with the existing knowledge on *C. sordidus* dispersal. Indeed, Vinatier et al., 2010 indicates that a low percentage of weevils can move up to 9 m in one day. This make possible a primary infection of the third plant located 20 meters in approximately two days. This duration is compatible with the persistence of internal and external inoculum of Foc by *C. sordidus* during several days as shown by Guillen et al. (2021). This field research demonstrates that *C. sordidus* is involved in the dissemination of Foc, proving experimentally the hypothesis proposed by Meldrum et al., 2013.

Chapter 5 - General discussion



5.1. The contribution of the study to understanding of Foc epidemiology

The contribution of Meldrum et al. (2013) was a first presumption for the putative role of *C. sordidus* in the epidemiology of Fusarium wilt. Similarly, the research of Heck et al. (2021) complemented the knowledge on the dispersal of *Fusarium oxysporum* f. sp. *cubense* using a comparative epidemiology approach in field conditions. This thesis aimed at giving proof of the role of *C. sordidus* as a vector of Foc and quantifying the different processes involved in this plant-insect interaction.

5.1.1. The positive feedback of Foc on *C. sordidus* attraction

The finding that Foc-contaminated plant materials attract more *C. sordidus* than non-contaminated ones has in return a strong implication on the potential of dispersion of Foc. In banana plantations, where the population of *C. sordidus* is high (estimated to be up to 15,600/ha in a field in Cameroon by Delattre (1980)), the preferential attraction of Foc-contaminated plants on *C. sordidus* would probably increase Foc expansion at the beginning of an epidemy as compared to banana fields free from weevils. By another hand, when banana plants and *C. sordidus* are both scarce in the environment, e.g. in natural forest or in mixed systems such agroforestry systems, this preferential attraction would be a strong advantage for the disease to spread. In mixed systems, the distance between bananas is often much greater than in banana monoculture and the vection of Foc via *C. sordidus* probably represents an important disease spreading process because direct plant-to-plant dispersal is limited in these systems. For *C. sordidus*, this preferential attraction could also favor mating when their populations are low. It could be seen as a message that complement their aggregation by the “sordidin” pheromone.

5.1.2. *Cosmopolites sordidus* as a carrier of infective propagules of Foc

The quantification of the capacity of *C. sordidus* to be a carrier of effective Foc propagules is a new information compared to the work of Meldrum et al. (2013). Aside the proof of the presence of effective propagules of Foc on the exoskeleton and in the digestive tube of *C. sordidus*, the understanding of the dynamics of acquisition of these propagules and their persistence over the time is crucial for the final capacity of dispersal of Foc. The relatively rapid acquisition of propagules by *C. sordidus* and their persistence for 48 hours (see Article 2) is compatible with the dispersal of the insect from a Foc-contaminated plant to a healthy one. This information shows that *Cosmopolites sordidus* might potentially play an important role if Fusarium wilt of banana epidemiology. The presence of fungal propagules and the demonstration that feces of *C. sordidus* can contain infective propagules is also a new unsuspected finding, Even if dispersal of fungus by feces was already reported in the dispersion of *Frankia* propagules by vertebrates (Chaia et al 2012). This internal presence of fungal propagules is probably due to the presence of chlamydospores that might resist better to digestive chemical processes than micro or macroconidia. Internal acquisition of chlamydospores might be beneficial for longer distance Foc dispersal (the longest survival time is reported at 100 hr in the digestive tract and not on the exoskeleton). This might be due to the fact that external contamination is probably linked to micro and microconidia that are easily dispersed from fungal hyphae conversely to chlamydospores that would be better acquired through insect feeding on infected tissues.

5.1.3. Active and passive infection capacities of *C. sordidus*

Our main result on the process of infection of a plant by Foc through the vection of *C. sordidus* is that both active and passive processes are at play. Although the passive

infection was less effective than the active one (when the insects come into direct contact with the plants), our study clearly demonstrated experimentally that passive infection is possible. Furthermore, our results suggest that the passive contamination could probably be achieved by feces that we demonstrated to contain viable propagules of Foc. In the active process, females and males feed on corms and pseudostems causing lesions that allow the entry of pathogens. Females were likely to cause more severe lesions in plant tissues with the rostrum at the moment of egg laying (Gold et al., 2001). This is confirming the importance of the active infection process.

5.1.4. Integrated role of *C. sordidus* in Foc dispersal

Alltogether, the results provided in this thesis combined with existing knowledge on Foc and *C. sordidus* epidemiology draw a clearer role of the possible vection in the context of a banana plantation. With a capacity to carry propagules for at least two days and with a dispersal capacity that can reach about 10 m per day (although it is rare), *C. sordidus* has the potential to disperse the disease up to more than 20 meters around an infected plant. Our field study (Article 4) is consistent with this potential of dispersion since we observed contamination at 20 meters from the releasing point of Foc-contaminated *C. sordidus*. Until September 2021, only three plants have been observed diseased by Foc in this field experiment, this implies that (to date) there has been an infection rate of 3%. Our hypothesis is that these infections were caused by a primary infection. It is likely that a source of inoculum that will be maintained a long time, as a diseased plant that will not be removed, will make possible repeated events of contamination, leading to the infection of a much higher number of plants.

5.1.5. The banana-*C. sordidus*-Foc interactions in an evolutionary perspective

The insect-plant-pathogen interactions have been studied by several authors (Futuyma and Agrawal 2009), demonstrating that insect, plants, and pathogens present close and even co-evolutionary links between them (Baldrian 2017). In some cases, these relationships can be beneficial to plants as pointed out by Garantonakis et al. (2018) who report that *Fusarium solani* reduces damage caused by *Nesidiocoris tenuis* by inducing a physiological change in plants and producing excess ethylene and jasmonic acid that protect plants from insect attack.

On the other hand, the fungus-insect relationship is one of the most studied events since it can occur in multiple directions: when insects and fungi usually interact in ways that are positive for both (mutualism), positive for one and neutral for the other (commensalism), positive for one and negative for the other (predation, parasitism), or negative for both (competition) (Biederman and Vega 2020). In the case of the relationship between *C. sordidus* and Foc, it could be thought as i) a commensalism interaction where the beneficial agent is the fungus, which uses the weevil to move quickly and directly to the banana plants, or as ii) a mutualistic interaction if *C. sordidus* can benefit from the fungus, e.g. by locating more easily its primary resource or other individuals for mating. Our results are likely to support a mutualistic relationship since we have demonstrated that Foc infected banana are more attractive for *C. sordidus* than healthy ones. This advantage for the insect would be important in the case of a scarce resource of banana. It is likely to be the case in the tropical forest from which banana, *C. sordidus* and Foc are originally in their centers of origin in South-East Asia. We can hypothesize that in their native context, at low density of banana and in ecologically rich soils the Banana-*C. sordidus*-Foc complex may have co-evolved in a balanced way that allow their coexistence. However, changes in plant management conditions, the

introduction of new varieties, physical and chemical soil management schemes have contributed to break this balance, causing Foc and weevils to increase exponentially, leading to a negative impact on banana plants.

In banana cultivation, the existing knowledge on mutualistic relationships - insects - pathogens - plant is very scarce, limited mainly to research on the banana bunchy top virus (BBTV), which uses the aphid *Pentalonia nigronervosa* as a vector, or the relationship between *C. sordidus* and wet rots caused by the bacteria *Erwinia* spp or *Ralstonia solanacearum* race 2. This thesis generated scientific knowledge aimed at answering many of the questions raised when the black weevil *C. sordidus* and Foc coexist in a susceptible banana plantation. Firstly, it was demonstrated that the weevils prefer to feed on Foc-infected tissues, a circumstance that represents a risk for the initiation of an epidemy of Foc since these plants will attract more insects that will come to feed and subsequently disperse to neighboring plants, carrying infective propagules.

5.2. Implication for management of Fusarium wilt of bananas

As previously discussed, weevils contribute directly and indirectly to the epidemiology of Panama disease. To date, the management and contingency plans for *Fusarium oxysporum* f.sp *cubense* do not establish a program for the prevention, management and control of this insect in banana farms that could be an important factor to minimize the risk of spreading of the disease. For instance, a pest management program based on mass trapping with pheromone traps and eventually with a chemical control would be appropriated. Since Foc infective propagules have been found in the insects, it is necessary that the captured insects are placed in closed containers containing disinfectants, or incinerated, and finally stored in a safe place far from a banana plantation. At the field

level, it was demonstrated that the insects were capable of infecting plants with Foc and that symptoms were observed after one year and three months for the first ones. In the Article 4 of this thesis, we showed that the location of diseased plant after releasing Foc-contaminated insect can be 20 m away from the place of insect release. This suggests that the control of *C. sordidus* should be carried out around a plant detected positive to Foc within a radius of at least 20 meters; the double (40 meters) would probably be a safer option.

Another point to consider in Foc *C. sordidus* management is that in plantations with Fusarium wilt, all work that generates wounds on plants such as pruning, propping, soil removal, hilling, leaf removal and harvesting should be eliminated, since they cause wounds to plant that produce volatiles that attract insects. Although few information is available on the efficiency of physical barriers to limit *C. sordidus* dispersion, it could be a good option to use such physical barriers (e.g. make with plastic that do not allow *C. sodidus* to climb) to minimize the movements of individuals between Foc-contaminated and Foc-free areas. Such a barrier approach could also be improved using repellents or insecticides applied at the base of the plants to prevent the arrival of females.

5.3. Perspectives

Weevils are widely distributed in all banana-growing areas of the world and represent a potential disease dispersal agent, so there is a need for further research to understand holistically the interactions of this insect with plant pathogens. In this research, it was elucidated that the weevil can contain infective structures of Foc viable for several days. However, it was not possible to know which infective structures were present either internally or externally in the insect (macro, micro conidia or chlamydospores) and in

what proportion. This information would be important to know since the infection and survival capacity of the pathogen depends to a great extent on these structures. In this regard, Pittaway et al. (1999) and Hennessy et al. (2005) pointed out that the pathogen persists mostly in the soil or in the host tissue by means of chlamydospores that can manage to survive for several years. Future studies should determine which structures predominate in the digestive system, excreta, and exoskeleton of insects. It is important to know if these Foc resistance structures, when ingested by the insect larvae, manage to remain viable and survive the processes once the insect completes its developmental stage from larva to pupa to adult. By another way, it would be also interesting to analyse whether female ovaries and eggs are also infected with Foc because larvae feeding on the corm might increase disease spreading in the plant.

The damage to the corm caused by weevil larvae is a variable that influences root health and plant vigor as well as serving as an entry point for pathogens. However, it would be important to determine experimentally how different degrees of corm damage influence the efficiency of the vection of Foc by *C. sordidus*. To go further in understanding the dispersal of Foc in field conditions, it could be cogent to use marked strains of Foc. For instance, strains transformed with a green fluorescent protein might allow a better detection of contaminated plants and removing all the risk of external contamination.

As a broader perspective, it could be important to study whether other species of arthropods are involved in the vection of Foc. Heck et al. (2021) suggested that *Metamasius hemipterus* could also be linked to Foc dispersal. In banana agrosystems, many other arthropods feed on banana tissue. However, they have far a less close relationship than *C. sordidus* with banana plants.

Finally, it could be particularly relevant to integrate of all these processes into a simulation tool, for instance based on the COSMOS model (Vinatier et al., 2009), an individual based model that simulate the dispersal of *C. sordidus*. Adding the Foc-insecte interactions in this model could help the quantification of the contribution of the different processes (time of acquisition of Foc by *C. sordidus*, its persistence on and inside the insect, the dispersal of the insect, the efficiency of vection) to the overall epidemiology of Fusarium wilt. It could also be helpful to better think strategies of control of the insect to minimize its vection.

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Résumé

Cette thèse vise à comprendre le rôle de *Cosmopolites sordidus*, le charançon du bananier qui est le principal ravageur des bananiers, dans l'épidémiologie de la Fusariose des bananiers causée par le champignon pathogène *Fusarium oxysporum* f. sp. *Cubense* (Foc), une maladie majeure des bananiers. L'originalité de ce travail réside dans le fait qu'il explore les relations entre un ravageur, un champignon pathogène et une plante (hôte de ces deux bioagresseurs). Cette thèse combine des approches expérimentales en milieu contrôlé et au champ, ce qui a permis de mieux décortiquer les interactions champignon-insecte et de déterminer l'importance relative de ces interactions dans l'épidémiologie de cette fusariose. Les étapes étudiées concernent i) l'étude de l'attractivité différentielle de plantes saines et malades, ii) la capacité de *C. sordidus* à acquérir et à transporter des propagules de Foc, de manière interne et externe, ii) la capacité de *C. sordidus* à infecter des plantes saines. Dans les expérimentations menées en milieux contrôlé, la présence de propagules transportées par *C. sordidus* et leur pouvoir pathogène ont été vérifiés par approche moléculaire et par tests de pathogénicité. Les résultats obtenus ont démontré que *C. sordidus* à la capacité de transporter des propagules infectieuses à la fois par voie externe et interne. De manière surprenante, les résultats ont également mis en évidence une attraction préférentielle de *C. sordidus* pour des plantes malades par rapport à des plantes saines. Ces résultats permettent de repenser de manière intégrée la protection des cultures, avec des perspectives pratiques en terme de stratégies à mettre en œuvre dans les premières phases d'une épidémie de Foc et plus globalement dans une approche multi-pest. Cela inclus l'utilisation de moyens de lutte contre le charançon dans des phases initiales contamination par Foc. De manière générale, ce travail permet également d'apporter des éléments de discussion sur la possible co-évolution du complexe insecte-maladie-plante.

Summary

This thesis aims to understand the role of *Cosmopolites sordidus*, the banana weevil, which is the main pest of banana, in the epidemiology of banana wilt caused by the fungal pathogen *Fusarium oxysporum* f. sp. Cubense (Foc), a major disease of banana. The originality of this work lies in the fact that it explores the relationships between a pest, a pathogenic fungus and a plant. This thesis combines experimental approaches in controlled environment and in the field, which allowed to better disentangle the fungus-insect interactions and to determine the relative importance of these interactions in the epidemiology of this Fusarium wilt. The steps of this work included i) the study of the differential attractiveness of healthy and diseased plants, ii) the ability of *C. sordidus* to acquire and transport Foc propagules, both internally and externally, ii) the ability of *C. sordidus* to infect healthy plants. In the experiments carried out in controlled environments, the presence of propagules transported by *C. sordidus* and their pathogenicity were verified by molecular approach and by pathogenicity tests. The results obtained showed that *C. sordidus* has the capacity to transport infectious propagules both externally and internally. Surprisingly, the results also showed a preferential attraction of *C. sordidus* to diseased plants compared to healthy ones. These results allow rethinking crop protection in an integrated way, with practical perspectives in terms of strategies to be implemented in the early phases of a Foc epidemic and more globally in a multi-pest approach. This includes the use of weevil control measures in the initial phases of Foc contamination. In a general way, this work also provides elements of discussion on the possible co-evolution of the insect-disease-plant complex.