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Importance des interactions multi-trophiques dans les agrosystèmes pour la mise au point d'une lutte biologique contre une espèce invasive

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Importance des interactions multi-trophiques dans les agrosystèmes pour la mise au point d'une lutte biologique contre une espèce invasive

Résumé

Les interactions multi-trophiques jouent un rôle clé dans la structuration des agrosystèmes et régissent la dynamique des populations qui les composent. Dans le cadre de la lutte biologique, les populations de ravageurs sont contrôlées par ces interactions. Quand une espèce exotique envahit l'agrosystème, cela déstabilise l'équilibre préexistant et peut, via des interactions directes ou indirectes, modifier le contrôle biologique des espèces autochtones. D'autre part, l'efficacité des auxiliaires envisagés pour lutter contre l'espèce invasive va aussi dépendre des interactions au sein de l'agrosystème. En France, depuis 2008, l'agrosystème constitué par la culture de tomates sous serre est perturbé par l'arrivée d'une espèce invasive originaire d'Amérique latine : *Tuta absoluta* (Gelechiidae). La chenille mineuse provoque de graves défoliations des plants de tomate et attaque aussi les fruits. Les agents de lutte majoritairement utilisés en tomates sous serre en France sont un prédateur omnivore, *Macrolophus pygmaeus* (Miridae), initialement utilisé contre diverses espèces de ravageurs, notamment les aleurodes, ainsi que le parasitoïde oophage *Trichogramma achaeae*. Le prédateur *M. pygmaeus* s'est avéré capable de consommer les œufs et, plus rarement, les jeunes stades larvaires de *T. absoluta*. L'objectif de cette thèse fut (i) d'étudier la perturbation des interactions liée à l'invasion, (ii) de rechercher de nouveaux auxiliaires autochtones, puis (iii) d'évaluer l'efficacité de ces auxiliaires lorsqu'ils sont intégrés dans l'agrosystème.

Nous avons montré que de nouvelles interactions sont apparues suite à l'invasion, notamment la compétition apparente entre les aleurodes et *T. absoluta* en présence de *M. pygmaeus*. Dans un second temps, nous avons recherché de nouveaux auxiliaires, en se focalisant d'abord sur les trichogrammes, *Trichogramma achaeae* étant déjà commercialisé contre *T. absoluta* en France. Une souche de trichogramme plus efficace et ayant la capacité de se mettre en diapause ou en quiescence était recherchée. Les expérimentations de laboratoire et de terrain n'ont pas permis de mettre en évidence un trichogramme plus intéressant que celui déjà commercialisé. De plus, nous avons observé une prédation intra-guilde de *M. pygmaeus* sur les trichogrammes juvéniles i.e. œufs parasités, et nous avons montré que *T. absoluta* n'était pas un hôte approprié pour ces parasitoïdes. Même si les œufs de *T. absoluta* permettent la production de générations filles, l'impact de ces générations en serre dans des conditions réalistes était négligeable. Les trichogrammes ne s'installant pas sur la culture, les études concernant les parasitoïdes oophages se sont arrêtées là et nous nous sommes intéressés aux parasitoïdes larvaires. En partenariat avec une équipe espagnole (IRTA), la biologie de deux parasitoïdes larvaires a été étudiée et a souligné l'intérêt de *Stenomesius japonicus* (Eulophidae) en tant que potentiel agent de lutte biologique contre *T. absoluta*. Malgré les interactions négatives subies par le parasitoïde (compétition pour la ressource et cléptoparasitisme), des expérimentations sous serre en conditions réalistes ont montré que le prédateur et le parasitoïde larvaire pouvaient coexister pendant plusieurs mois. L'effet de l'addition dans l'agrosystème d'une proie alternative pour le prédateur omnivore sur l'exclusion du parasitoïde a ensuite été évalué. Cette étude a prouvé, elle aussi, que le parasitoïde était capable de se maintenir dans la culture, même en présence d'autres proies pour le prédateur.

Ce travail de thèse a donné des pistes prometteuses pour le contrôle biologique de *T. absoluta* en Europe grâce à des espèces autochtones. Il a clarifié les interactions multi-trophiques en jeu dans l'agrosystème étudié et a mis en évidence le rôle des interactions dans le succès ou l'échec des programmes de lutte biologique.

Mot clés : interactions multitrophiques, lutte biologique, *Tuta absoluta*, prédateur, parasitoide, competition interspecifique.

Importance of multi-trophic interactions in agro-ecosystems for the development of biological control programs against an invasive species

Abstract

Multi-trophic interactions play a key role in structuring agro-ecosystems and regulating arthropod population dynamics. In the context of biological control, such interactions are crucial because trophic links are used to maintain pest populations at low levels in crops. When an alien pest invades an agrosystem, it often destabilizes pre-existing multi-trophic interactions and it could disturb, via direct or indirect interactions, the biological control of indigenous pest species. In addition, the effectiveness of potential natural enemies to control invasive species also depends on the interactions within the agro-ecosystem. Since 2008, in Europe, the tomato agro-ecosystem is disrupted by the invasive South American tomato leafminer, *Tuta absoluta* (Gelechiidae). This leafminer causes severe defoliation of tomato plants and also attacks fruits. The indigenous biocontrol agents mainly used in France to control this pest are the omnivorous predator, *Macrolophus pygmaeus* (Miridae) and the oophagous parasitoid *Trichogramma achaeae* (Trichogrammatidae). The predator was initially used to control various prey, mainly whiteflies, but also feeds on eggs of *T. absoluta*. The objective of the PhD was (i) to study the possible disruptions of biotic interactions owing to the recent invasion by *T. absoluta* in Europe, (ii) to identify local natural enemies attacking *T. absoluta*, (iii) to evaluate their effectiveness against *T. absoluta* when integrated into the agro-ecosystem.

We showed that new interactions occurred after the arrival of *T. absoluta*, mainly apparent competition between whiteflies and *T. absoluta*, in presence of *M. pygmaeus*. We recorded possible new biocontrol agents and focused first on *Trichogramma* parasitoids, as *Trichogramma achaeae* was promptly commercialized against *T. absoluta* in France. More effective *Trichogramma* strains and ones having the ability to diapause or quiescence were researched. Laboratory and field experiments failed to detect promising *Trichogramma* strains. In addition, intraguild predation of *M. pygmaeus* on parasitoid juveniles i.e. parasitized eggs, was observed and we demonstrated that *T. absoluta* was not a suitable host for *Trichogramma* parasitoids. The impact of *T. absoluta*-developed parasitoid generations on the pest was negligible under greenhouse conditions. Therefore, further studies switched on parasitoids of larval stages of *T. absoluta*. In partnership with IRTA (Spain), we demonstrated the importance of *Stenomesius japonicus* (Eulophidae) as a possible biological control agent against *T. absoluta*. The parasitoid was able to reproduce on *T. absoluta* over several generations under laboratory and greenhouse conditions. Laboratory studies showed that, in addition to the resource competition, the parasitoid would suffer kleptoparasitism from the predator *M. pygmaeus* in the tomato agro-ecosystem. Such interactions would increase the risk of the larval parasitoid exclusion of the tomato crops. However, further greenhouse experiments demonstrated that the predator and the larval parasitoid can coexist for several months. Moreover, the presence of an alternative prey for the omnivorous predator (whitefly) in the agro-ecosystem did not prompt larval parasitoid exclusion, and the parasitoid remained in the crop even in the presence of other prey for the predator.

Taken as whole, this work provided promising ways for developing sustainable biological control of *T. absoluta* in Europe using indigenous natural enemy. We highlighted the importance of multitrophic interactions in the studied agro-ecosystem and clarified the role of interactions in the success or failure of biological control programs.

Keywords: multitrophic interactions, biological control, *Tuta absoluta*, predator, parasitoid, interspecific competition.

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Introduction générale

Introduction générale

Les interactions entre les espèces qui composent un écosystème ont un rôle majeur dans la composition et l'évolution des systèmes naturels. En plus des interactions herbivore-plante et prédateur-proie, il est rapidement apparu évident que de nombreuses interactions impliquent plus de deux espèces et/ou plus de deux niveaux trophiques, et que, par conséquent, une approche multi-trophique des réseaux trophiques se révélerait plus réaliste (e.g. Price *et al.* 1980, Hawkins 1984). Ces interactions peuvent être directes ou indirectes et donc plus ou moins évidentes (Wootton 1994).

La compréhension de ces interactions est essentielle pour l'agriculture, particulièrement dans le cadre du développement d'une protection biologique des cultures. Longtemps, la principale raison de rechercher des moyens non-chimiques de contrôler les ravageurs des cultures était l'inquiétude au sujet des risques liés à l'utilisation des pesticides sur l'environnement et la santé humaine (e.g. Metcalf 1980). Maintenant, s'ajoute l'augmentation des résistances aux pesticides et la difficulté à développer de nouvelles molécules efficaces. Cela mène progressivement à une demande de la part du milieu agricole, de trouver des moyens alternatifs aux pesticides (e.g. Lumdsen et Vaughn 1993). L'un des moyens efficaces et alternatifs aux pesticides est le contrôle biologique, défini comme « l'utilisation d'ennemis naturels pour le contrôle d'espèces nuisibles, de maladies ou d'adventices ». La lutte biologique utilise au profit de l'homme les relations naturelles entre deux espèces, les méthodes de lutte biologique exploitent donc les mécanismes de régulation naturelle des populations. C'est pourquoi une bonne connaissance des interactions qui régissent un agrosystème est un point clé pour la mise en place d'une protection biologique efficace.

La complexité des interactions rend difficile la prévision des effets de la suppression (extinction) ou de l'ajout d'espèces (réintroduction, arrivée d'espèces invasives) dans les écosystèmes, ce que l'on tente pourtant de faire en lutte biologique. De plus, l'augmentation des échanges commerciaux d'un pays à l'autre, mais aussi d'un continent à l'autre, a induit une augmentation sensible du nombre d'espèces invasives. Ainsi, avant d'engager des programmes de lutte biologique à grande échelle contre une espèce invasive, doit-on se poser la question des interactions engendrées/modifiées par l'introduction, dans un premier temps de l'espèce invasive, puis par la suite de l'ajout de nouveaux auxiliaires, qui peuvent perturber l'équilibre des réseaux trophiques déjà en place.

Les systèmes sous serre sont ceux où la lutte biologique est la plus utilisée, probablement parce que les spécificités (unité isolée, vide sanitaire hors saison) de ce type de culture en facilitent la mise en place (Van Lenteren 1988). Cependant on connaît tout de même environ 140 espèces

d'insectes et d'acariens qui sont des ravageurs des cultures sous serre (Heinz *et al.* 2004). On assiste donc à la mise en place simultanée de différents programmes de lutte biologique contre plusieurs ravageurs, qui sont en général composés d'espèces spécialistes et généralistes. Or, en France depuis 2008, l'agrosystème constitué par la culture de tomates sous serre est perturbé par l'arrivée d'une espèce invasive originaire d'Amérique latine : *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) (Desneux *et al.* 2010). Ce lépidoptère de la famille des Gelechidae a une chenille mineuse qui provoque de graves défoliations des plants de tomates. Il est très probable que cette espèce et son cortège d'ennemis naturels perturbent les interactions qui régissent l'écosystème en place et donc la lutte biologique contre d'autres espèces ravageuses autochtones. Il apparaît donc pertinent de s'intéresser aux potentielles modifications des interactions du réseau trophique de l'agrosystème tomates sous serre. D'autre part, la lutte ciblée contre l'espèce invasive va nécessiter la recherche d'agents de lutte, préférentiellement autochtones, puis entraîner leur introduction dans l'agrosystème afin de compléter le contrôle, éventuellement déjà effectué, par les prédateurs généralistes déjà présents dans la culture pour réguler d'autres espèces. Cela va engendrer des interactions multi-trophiques nouvelles et moduler l'efficacité des auxiliaires choisis.

Le travail de thèse présenté ici a eu pour but de fournir des informations cruciales à la mise en place de la lutte biologique contre *T. absoluta* et de contribuer à l'étude de l'importance du rôle tenu par les interactions multi-trophiques dans le succès d'un programme de lutte biologique. Par contre, cette thèse n'a pas eu pour vocation de mettre en place un programme de lutte biologique prêt à être appliqué par les agriculteurs, et par conséquent nous ne nous sommes pas intéressés à des problématiques telles que les méthodes d'élevage en masse des nouveaux auxiliaires trouvés, ou telles que les doses de lâcher optimales. En ce qui concerne les interactions, la thèse fut centrée sur les interactions entre insectes via un insecte ; les interactions entre la plante et les insectes, ou entre les insectes via la plante, bien qu'ayant elles aussi un rôle important, n'ont pas été étudiées.

La première partie du travail a consisté à étudier l'impact de l'espèce invasive sur le contrôle du principal ravageur sous serre de tomates : les aleurodes. Dans la seconde partie nous avons tenté de détecter de nouveaux auxiliaires contre *T. absoluta*, puis la troisième et dernière partie a consisté à étudier l'impact de l'insertion de ces nouveaux auxiliaires dans l'agrosystème.

Chapitre 1 : Synthèse bibliographique

I) Le contexte : la lutte biologique

I.1) Bref historique de la lutte biologique

L'un des plus vieux cas de lutte biologique connus date de 300 ans ap. J-C en Chine (Huang et Yang 1987), il s'agit probablement de la plus vieille utilisation connue d'un insecte pour en contrôler un autre dans le cadre de la protection des cultures. Il s'agissait de l'utilisation de colonies de fourmis oecophylles pour protéger des agrumes. Cependant, le contrôle biologique concerne aussi les vertébrés dont l'utilisation en lutte biologique a probablement commencé avec la domestication et l'utilisation des chats contre les rongeurs bien avant J-C en Egypte (Baldwin 1975).

Van Lenteren (2002) récapitule les pré-requis qui ont été nécessaires pour le développement et l'expansion du contrôle biologique. Il s'agit (i) de l'acceptation générale du fait que les insectes ne proviennent pas de générations spontanées (documenté par F. Redi en 1668), (ii) de la compréhension des processus de prédation (documenté dans la littérature chinoise il y a environ 2500 ans), (iii) de l'interprétation correcte du comportement des insectes parasitoïdes (documenté par van Leeuwenhoek en 1700), (iv) de la reconnaissance des processus d'infection par les pathogènes (documenté par Kirby en 1826) et enfin (v) de l'évolution de l'idée d'utiliser les ennemis naturels pour le contrôle des ravageurs au 18ème siècle. En Europe, Réaumur fut le premier à proposer une technique de contrôle biologique, déjà en 1734 il recommandait de lâcher des chrysopes pour lutter contre des pucerons. En 1800, le grand-père de Charles Darwin, Erasmus Darwin, écrivait sur le rôle des parasitoïdes et des prédateurs dans le maintien des populations de ravageurs à de bas niveaux. Après cela, le nombre de travaux sur le sujet s'est accru en développant la même idée.

Par la suite, certains cas de lutte biologique sont devenus célèbres, comme le cas de la myxomatose pour lutter contre les lapins européens en Australie, qui a particulièrement bien fonctionné dans un premier temps, puis qui a perdu de sa virulence, remplacé ensuite par le « Rabbit Haemorrhagic Disease » (Fenner and Fantini 1999). Chez les arthropodes, le cas de lutte biologique contre la cochenille des agrumes en Amérique du Nord est l'un des plus célèbres. En effet, l'introduction de la coccinelle *Rodolia cardinalis* (Muslant) par Koebele en 1988 en Californie pour limiter les ravages de la cochenille des agrumes australienne *Icerya purchasi* est un des premiers succès de la lutte biologique classique (Iranzo *et al.* 2000).

En ce qui concerne la France et l'Europe, Boisgiraud en 1840 démontra en pratique le contrôle biologique grâce à des lâchers du coléoptère prédateur *Calosoma sycophanta* Linnaeus

pour lutter contre le Lépidoptère *Lymantria dispar* Linnaeus sur peuplier. Ensuite, on tenta pour la première fois d'introduire un auxiliaire exotique en Europe pour lutter contre le phylloxera *Viteus vitifoliae* Fitch en 1873, en introduisant l'acarien prédateur *Tyroglyphus phylloxerae* Riley & Plancon, mais cela ne fonctionna pas. Le premier succès de lutte biologique classique date de 1897, quand des Portugais ont importé et établi *R. carinalis* contre *Icerya purchasi* Maskell, qui avait précédemment était introduite en Amérique du Nord avec succès pour lutter contre le même ravageur, comme décrit précédemment. Actuellement, la lutte biologique, et notamment la vente d'agents de lutte biologique, est utilisée sur beaucoup de cultures. Les ennemis naturels sont produits en masse et relâchés pour contrôler des ravageurs en vergers de pommiers et d'oliviers, dans les vignobles ainsi que dans le maïs, mais la plus importante diversité d'auxiliaires est utilisée sous serre. Ces 25 dernières années, environ 80 espèces d'ennemis naturels ont été évaluées pour une utilisation sur culture protégée (van Lenteren *et al.* 1997).

Encadré 1: Les aleurodes (Homoptera, Aleyrodidae)

Les aleurodes sont parmi les ravageurs des cultures les plus importants dans le monde, les espèces causant des dégâts en culture sous serre sont *Bemisia tabaci* Gennadius et *Trialeurodes vaporariorum* Westwood. Les dégâts sont causés par les nymphes et les imagos se nourrissant du phloème, mais aussi par le miellat produit par les stades larvaires qui contamine les feuilles et les fruits. Cela favorise le développement de fumagine qui va empêcher la photosynthèse (Byrne et Bellows 1991). D'autre part *B. tabaci* est un important vecteur de virus, notamment du TYLCV (Tomato Yellow Leaf Curl Virus), pour la tomate (Oliveira *et al.* 2001).


B. tabaci


T. Vaporariorum entouré d'œufs de la même espèce

Le contrôle biologique contre les aleurodes a commencé au Etats-Unis avec *Encarsia formosa* Gahan (Speyer 1927), ce parasitoïde est maintenant utilisé partout en Europe. Depuis, de nombreuses espèces ont été reconnues comme des prédateurs d'aleurodes mais très peu sont commercialisés (Gerling *et al.* 2001). Les ennemis naturels les plus fréquents sont *E. formosa*, *Eretmocerus mundus* Mercet et *Er. eremicus* Rose & Zolnerowich, ainsi que la punaise prédatrice *Macrolophus pygmaeus* (Rambur) (souvent mal identifiée en tant que *Macrolophus caliginosus* Wagner) et l'acarien prédateur *Amblyseius swirskii* Athias-Henriot (Nomikou *et al.* 2002, Cock *et al.* 2010).

Les cycles de vie de ces espèces sont assez proches, à 25°C, le stade œuf dure 8-10 jours chez *B. tabaci* et 5-6 jours chez *T. vaporariorum*. Il y a 4 stades larvaires chez ces deux espèces, en totalité le stade larvaire dure 10-15 jours chez *B. tabaci* et 15-19 jours chez *T. vaprariorum* (Xie *et al.* 2011, Bonato *et al.* 2007).

Le premier succès de lutte biologique sous serre fut en 1926, quand des aleurodes noirs, donc parasités, ont été trouvés chez un producteur de tomates anglais (Encadré 1). Par la suite le parasitoïde responsable, *Encarsia formosa* Gahan, fut découvert, et le contrôle biologique avéré (Speyer 1927, Hussey *et al.* 1958). A partir de là, la commercialisation d'agents de lutte biologique commença en Europe.

I.2) Les différentes luttes biologiques

Différents types de lutte biologique se sont développés depuis les premières tentatives de lutte biologique classique, ceux-ci peuvent être classés de différentes manières. Le classement suivant est généralement celui retenu (Heinz *et al.* 2004) :

- *La lutte biologique classique ou par importation* : Elle consiste en l'importation et l'installation d'une espèce exotique d'ennemi naturel dans l'écosystème cible, en vue d'un contrôle permanent. Elle est en général utilisée quand les espèces autochtones ont une efficacité insuffisante, notamment quand le ravageur est lui-même une espèce exotique et que les auxiliaires autochtones ne sont pas adaptés à ce nouvel arrivant. Cette méthode, nécessitant l'installation de l'ennemi naturel, est plus adaptée à des agrosystèmes non perturbés, i.e. cultures pérennes, comme les vergers. De nombreux ennemis naturels ont été importés en Europe et permettent toujours le contrôle de ravageurs dans les vergers de citronniers et pommiers par exemple (Greathead 1976).
- *La lutte biologique par augmentation* : Il s'agit du lâcher d'espèces autochtones afin de favoriser/accélérer leur installation, en particulier en milieu perturbé, ou d'augmenter le nombre d'individus dans la culture. S'il s'agit uniquement de lâchers en début de culture, le contrôle reposant ensuite sur la reproduction naturelle de l'auxiliaire dans le milieu, on parle de *lâchers saisonniers inoculatifs*. Si, au contraire, il s'agit de lâchers de quantités importantes et répétées d'individus qui ne vont pas ou peu se reproduire sur la culture, on parle de *lâchers inondatifs*. L'utilisation des Miridae, e.g. *Macrolophus pygmaeus* ou *Nesidiocoris tenuis*, pour lutter contre les aleurodes sous serre de tomates, est un exemple de lutte biologique inoculative. Au contraire, l'utilisation de trichogrammes se fait majoritairement par lutte biologique inondative.
- *La lutte biologique par conservation* : Elle consiste à favoriser les ennemis naturels en établissant des conditions biotiques et abiotiques qui leur permettent de se développer. Les plantes permettant/favorisant l'installation d'ennemis naturels dans l'agrosystème en leur

fournissant des proies alternatives, du nectar ou encore un site de ponte, sont un exemple de lutte biologique par conservation (Parolin *et al.* 2012).

I.3) Lutte biologique classique ou ennemis naturels autochtones ?

En termes de lutte biologique, la lutte biologique classique a longtemps été considérée comme la plus efficace pour lutter contre les espèces invasives, et de nombreux exemples viennent appuyer cette opinion (e.g. Caltagirone et Huffaker 1980, Clausen 1978). Cependant, de plus en plus de chercheurs partagent l'idée que les risques engendrés par l'introduction d'espèces exotiques sont extrêmement importants et ont souligné l'importance d'effectuer une évaluation des risques détaillée avant d'effectuer de nouveaux lâchers d'ennemis naturels exotiques (e.g. Howarth 1991, Simberloff et Stiling 1996, Folette et Duan 1999, Strong et Pemberton 2000, Wajnberg *et al.* 2001). Cette idée s'est répandue essentiellement après le malheureusement célèbre cas de la coccinelle asiatique *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) qui fut introduite en Europe et aux Etats-Unis dans le cadre de la lutte biologique contre les pucerons (Koch 2003). Lâchée à partir de 1916 aux Etats-Unis, elle s'est montrée invasive à partir de 1980. Malgré une efficacité reconnue dans le contrôle de plusieurs espèces de pucerons dans différentes cultures, les effets négatifs liés au caractère extrêmement invasif de certaines souches sont devenus prédominants, notamment le remplacement des espèces de coccinelles autochtones et l'entrée dans les habitations de certaines régions d'Amérique du Nord et d'Europe (Koch 2003). Actuellement, on considère que l'utilisation des espèces d'ennemis naturels doit être privilégiée par rapport aux ennemis naturels exotiques dans le cadre de la mise en place d'un programme de lutte biologique contre une espèce invasive.

Les ennemis naturels autochtones attaquant une espèce introduite sont considérés comme un composant essentiel de la résistance des écosystèmes aux invasions (Elton 1958, Levine *et al.* 2004, voir aussi section *Les invasions biologiques*). La prédation réduit le taux de croissance de la population de l'espèce invasive et, par conséquent, réduit l'impact de la compétition pour la ressource exercé par l'espèce invasive sur les espèces natives analogues (Robinson et Wellborn 1988, Baltz et Moyle 1993, Trowbridge 1995, Byers 2003). Cependant, lorsqu'un prédateur autochtone consomme une espèce exotique, les pertes en population invasive sont reconverties en augmentation de la biomasse de prédateurs. Donc la prédation sur une espèce exotique peut indirectement nuire aux proies originelles du prédateur (Roemer *et al.* 2001, Rand et Louda 2004). Ainsi, même l'utilisation d'ennemis naturels autochtones contre un ravageur invasif ne permet pas d'éviter tous les effets négatifs, en termes de dynamique des populations, liés à l'invasion du ravageur exotique.

II) Les interactions au sein des agro-écosystèmes

Les interactions biotiques entre espèces ont un rôle majeur dans la composition et l'évolution des systèmes naturels (Paine 1980, Wootton 1994). Les différentes interactions trophiques interspécifiques, sur lesquelles on se focalisera ici, se décomposent en deux catégories : les interactions directes et les interactions indirectes (Settle et Wilson 1990).

II.1) Les interactions interspécifiques directes

Les interactions directes regroupent toutes les interactions ne nécessitant pas l'intermédiaire d'une troisième espèce. On y retrouve les différentes formes d'interactions positives : la symbiose (++), le mutualisme (++), le commensalisme (+0) ainsi que les interactions négatives : la prédation (+-), la compétition par interférence (--), l'amensalisme (-0) (Wootton 1994).

Plus particulièrement, les relations proie-prédateur déterminent le fonctionnement et l'organisation des réseaux trophiques et constituent depuis longtemps un sujet d'étude privilégié pour les écologistes (Godfray *et al.* 1994). Les prédateurs influent sur la dynamique prédateur-proie et donc sur les populations de proies. Ils contribuent par ce biais à maintenir l'équilibre biologique des écosystèmes. Les interactions proie-prédateur constituent les interactions de base utilisées en lutte biologique. Elles furent les premières étudiées, ce n'est qu'après, que les biologistes ont porté leur attention sur les interactions indirectes, réalisant leur rôle essentiel dans la structuration des écosystèmes (Janssen *et al.* 1998).

II.1.1) Les interactions interspécifiques

Prédation et réponse fonctionnelle et numérique

Holling (1959) proposait de séparer la réponse fonctionnelle au niveau de l'individu de la réponse numérique au niveau de la population. Lorsque la densité de proie s'accroît, un prédateur peut augmenter son taux de consommation de proies (réponse fonctionnelle), puis de reproduction (réponse numérique). Ainsi, la densité de prédateurs est également dépendante de celle de leurs hôtes (Holt et Lawton 1994). Depuis cette proposition, on a ajouté l'agrégation comme facteur augmentant la dynamique des populations, en plus de la reproduction (Barbault 2008).

Holling a divisé la réponse fonctionnelle en trois types (Holling 1965). La première est une fonction linéaire de la densité de proie jusqu'à une certaine valeur à partir de laquelle le nombre de

proies consommé par individu et par jour reste constant. On l'a observé chez des crustacés (organismes filtrants) et dans certaines gammes de valeurs chez certains oiseaux (Korpimäki et Norrdahl 1991), mais elle reste rarement représentative de la réalité. La réponse de type 2 est caractérisée par un taux de consommation décroissant à mesure qu'augmente la densité de proies, c'est la plus fréquente chez les arthropodes. Enfin la réponse de type 3, représentée par une sigmoïde, est caractéristique des vertébrés mais a aussi été observée chez certains parasitoïdes. En théorie, c'est la seule réponse numérique qui peut avoir un effet régulateur sur la population de proies (Barbault 2008). Dans le cas de prédateurs généralistes, la réponse à la densité d'une proie peut aussi dépendre de la densité de ses autres proies. Quand la consommation d'une proie dépend de la fréquence de celle-ci par rapport aux autres, on parle du phénomène de bascule ou « switching ».

En ce qui concerne la réponse numérique, plusieurs mécanismes sont en jeu, elle dépend du comportement du prédateur, de la fécondité et de la durée du cycle de vie du prédateur, mais aussi de la qualité nutritive des proies. Il y a plusieurs exemples dans la littérature où l'abondance d'un prédateur a augmenté à la suite d'un accroissement de la densité de sa proie, comme dans le cas de la musaraigne étudié par Holling qui se nourrit de cocons de tenthrèdes (Holling 1959); et de nombreux cas ont aussi été observés dans le cadre de la lutte biologique contre les insectes ravageurs (DeBach 1964). D'autre part, lorsque les proies ont une distribution groupée, les ennemis naturels peuvent éventuellement exercer un choix et se concentrer sur les parcelles riches en proies, c'est la réponse agrégative (Hassel et Wilson 1997). Cette réponse est aussi une forme de réponse numérique positive du prédateur. Un deuxième type de réponse numérique est le cas nul, dans lequel la densité des prédateurs n'est pas affectée par les variations du nombre de proies. Holling (1959) a constaté lors de son étude qu'une autre espèce de musaraigne n'a pas augmentée en nombre quand la densité des cocons de tenthrèdes s'accroissait. Troisièmement, il existe un certain nombre de cas où l'on a observé que le nombre de prédateurs a diminué à des densités de proies très élevées. Cela a été observé chez des insectes aphidiphages, un diptère (Hafez 1961), une coccinelle (Pschorn-Walcher et Zwolfer 1956) et certaines espèces de syrphes (Hafez 1961, Hughes 1963). On ne sait pas si les réponses numériques négatives sont répandues, cependant elles sont plus susceptibles de se produire avec des proies qui forment des agrégats de haute densité (comme les pucerons et les insectes sociaux).

Conséquences en termes de dynamique des populations : Un prédateur/parasitoïde n'aura un effet régulateur sur sa population d'hôtes que s'il est capable de répondre numériquement à un accroissement de la densité d'hôtes avec une réponse de Holling de type 3. La réponse numérique varie suivant que le prédateur/parasitoïde est un spécialiste ou un généraliste. Un spécialiste

répondra numériquement à une variation de la densité de sa proie et limitera les pics de pullulation, tandis que chez un généraliste la réponse numérique à une espèce de proie sera absente ou faible en particulier en cas de présence de nombreuses autres espèces de proies présentes dans l'écosystème, la dynamique des populations de celui-ci reposant sur plusieurs espèces (Holling 1959, Snyder et Ives 2001).

En lutte biologique : En termes de lutte biologique, chez les prédateurs, la réponse fonctionnelle atteint un plateau avec la satiété et peut être modifiée par la présence de proies alternatives, rendant la lutte contre le ravageur ciblé plus aléatoire (Sabelis 1992, Greenstone et Pfannenstiel 2005), au contraire des parasitoïdes dont l'effet sur la population d'hôtes ne dépend pas seulement de la consommation pour se nourrir, mais aussi de la fécondité. De nombreux cas d'effet positif des prédateurs généralistes sur le contrôle biologique ont cependant été observés (Symondson *et al.* 2002). D'après Symondson *et al.* (2002), ils ont permis un contrôle efficace des populations de ravageurs dans 75% des expériences de plein champ. Snyder et Ives (2003) indiquent qu'ils auraient également une action sur la stabilité dans le contrôle des populations de ravageurs. En effet, un large régime alimentaire permet au prédateur généraliste de se maintenir dans la culture en l'absence de la proie cible en consommant d'autres proies ou encore, dans le cas des prédateurs omnivores, en utilisant une source de nourriture alternative (Symondson *et al.* 2002). Cela permet en particulier que le prédateur soit présent dans la culture avant l'arrivée du ravageur et donc d'effectuer un contrôle dès le début de l'infestation (Snyder et Ives 2001 et 2003). Cette faculté est, par exemple, utilisée en culture de tomates où certains Miradae omnivores sont relâchés en début de culture voire en pépinière (Calvo *et al.* 2012). De plus, chez les généralistes, le comportement de prédation peut varier selon les préférences (voir Meyling *et al.* 2003, Cheli *et al.* 2006, Reitz *et al.* 2006, pour des exemples de préférences chez des agents de lutte biologique), qui peuvent être en faveur ou en défaveur de la lutte biologique. Un comportement de préférence particulier, le « switching », est considéré comme ayant un effet stabilisateur sur les populations de proies (Roughgarden et Feldman 1975, Kimbrell et Holt 2005). Ce comportement a été observé chez divers agents de lutte biologique (e.g. Enkegaard *et al.* 2001, Chow *et al.* 2008, Saha *et al.* 2010) et peut être intéressant si les deux proies sont des ravageurs. A l'inverse, un spécialiste ne dépend que d'une seule ressource pour se maintenir dans le milieu et son cycle de développement est souvent lié à celui de son hôte, ce qui peut conduire à son extinction en cas de disparition de la proie (Snyder et Ives 2003).

Cependant, à cause de leur degré de spécificité moindre, les prédateurs généralistes peuvent générer de nombreuses interactions directes et indirectes dans un écosystème, dont il n'est pas toujours évident de prévoir les conséquences sur le milieu. Malgré tout, certains prédateurs

généralistes se sont révélés plus efficaces que des parasitoïdes (Greenstone et Pfannenstiel 2005) et de nombreux prédateurs, permettant un bon contrôle des ravageurs, sont commercialisés à l'heure actuelle.

Le cas particulier des parasitoïdes

Bien que les parasitoïdes ne soient qu'un type particulier de prédateurs, certaines interactions entretenues avec leur hôte leurs sont propres.

- *Parasitisme :* Le comportement de parasitisme peut évoluer différemment en fonction de la disponibilité en hôtes. La distribution du parasitisme peut être : directement, inversement dépendante ou indépendante de la densité d'hôtes (Godfray 1994). Dans le premier cas, les parasitoïdes sont capables de répondre à des différences dans la disponibilité d'hôtes entre patchs par une agrégation dans les patchs de fortes densités d'hôtes, ce qui se traduit par une augmentation du parasitisme avec cette dernière. Ce phénomène a été reconnu par de nombreux auteurs comme facteur favorisant la persistance des populations et la stabilité des systèmes hôte-parasitoïde (Hassell *et al.* 1991). Dans le cas d'une dépendance inversement liée à la densité d'hôtes, le parasitisme décroît avec leur abondance. Cela peut s'expliquer par différents mécanismes : absence de réponse agrégative, abandon du patch à intervalles constants afin d'éviter le super-parasitisme, décélération de la réponse fonctionnelle du parasitoïde causée par exemple par le comportement de manipulation de l'hôte ou encore interférences entre parasitoïdes (Umbanhowar *et al.* 2003). Cependant, une réponse agrégative directe à une forte densité d'hôtes n'implique pas toujours un parasitisme, lui aussi croissant avec cette densité (Hemachandra *et al.* 2007).

Les parasitoïdes, même s'ils sont parfois capables d'attaquer plusieurs espèces, sont plutôt spécialisés car le degré d'adéquation entre la proie et le parasitoïde doit être extrêmement élevé. L'hôte doit fournir une ressource nutritive de qualité et de quantité suffisante pour assurer le développement de tous les stades juvéniles du parasitoïde, les défenses de l'hôte doivent être maitrisées (Vinson et Iwantsch 1980), en particulier pour les endoparasitoïdes koinobiontes, qui se développent à l'intérieur de leur hôte tout en le maintenant vivant pendant un certain temps (Pennacchio et Strand 2006). Au contraire, les ectoparasitoïdes idiobiontes ont un degré de spécificité moindre et vont, par conséquent, souvent être les premiers parasitoïdes à attaquer des espèces invasives.

- *Host-feeding :* Il existe deux grands types d'ovogénèses chez les parasitoïdes : la proovigénie si la majorité des œufs est mûre et prête à être pondue à l'émergence, et la synovigénie lorsque l'ovogénèse se poursuit tout au long de la vie de la femelle. Dans ce second cas, la femelle a souvent besoin de ressources supplémentaires pour compléter le développement des œufs, qu'elle

trouve dans l'hémolymphe et les tissus de l'hôte. Le phénomène de nourrissage sur l'hôte ou « host-feeding » est très répandu chez de nombreux parasitoïdes utilisés en lutte biologique (Jervis et Kidd 1991) et a été mis en évidence chez de nombreux Eulophidae. Il dépend principalement de l'état physiologique interne du parasitoïde et de la taille/qualité de l'hôte (Bernardo *et al.* 2006), il semblerait que certains parasitoïdes larvaires utilisent préférentiellement les stades les plus jeunes, impropres au parasitisme car trop petits, pour le « host-feeding » (pour une revue des cas de partitionnement « host-feeding »/oviposition en fonction du stade larvaire, voir Kidd et Jervis, 1991). Dans le cas d'une telle ségrégation, on observerait une synergie entre le « host-feeding » et le parasitisme sur le contrôle d'une espèce ravageuse. Les parasitoïdes synovigéniques ont une capacité de recherche importante et sont capables de différer leur ponte en fonction de la densité d'hôtes, alors que les proovigéniques peuvent parasiter un nombre important d'hôtes dès l'émergence. Le « host-feeding » a souvent été sous-estimé comme facteur de mortalité de l'hôte, mais il peut dans certains cas participer de façon non négligeable au contrôle (Jervis et Kidd 1986 1991, Bernardo *et al.* 2006). Dans l'étude de Bernardo *et al.* (2006), 14% de la mortalité observée était due au « host-feeding ». Dans le cadre de la lutte biologique contre *T. absoluta*, Luna *et al.* (2010) ont constaté qu'une partie des hôtes paralysés par *Dineulophus phtorimaeae* (Hymenoptera : Eulophidae) était utilisée pour le nourrissage du parasitoïde. Un effet bénéfique du « host-feeding » a également été démontré par Woong *et al.* (2009) et Zappala et al. (2012).

- *Piqure mortelle de l'hôte sans « host-feeding » :* Chez de nombreux parasitoïdes d'insectes mineurs, on observe également un comportement de piqûre de l'hôte sans oviposition, et pouvant entraîner une forte mortalité chez l'hôte (Bernardo *et al.* 2006). Ces derniers ont démontré qu'une femelle *Pnigalio soemius* Walker (Hymenoptera : Eulophidae) pouvait tuer près de 89 hôtes pendant sa vie par ce phénomène, ce qui représentait 38% de la mortalité totale observée. Woong *et al.* (2009) ont également pu mettre en évidence une mortalité additionnelle par un comportement semblable chez *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae), pour lequel ce phénomène a perduré jusqu'à la mort du parasitoïde. Patel *et al.* (2003) ont, quant à eux, étudié la mortalité engendrée par *Diglyphus intermedius* Girault (Hymenoptera : Eulophidae) en fonction de la densité d'hôtes présentée aux parasitoïdes. La proportion d'hôtes tués sans oviposition a augmenté avec la densité d'hôtes et la fréquence des hôtes de petites tailles rencontrés. Par ailleurs, le pourcentage de mortalité par piqûre avec rejet s'est révélé supérieur au pourcentage de « host-feeding » qui est resté constant. L'hypothèse la plus évidente pour expliquer cela est le rejet de l'hôte par la femelle parasitoïde après l'avoir paralysé (Casas 1989), mais d'autres explications ont ensuite été proposées. D'après Patel *et al.* (2003), le rejet d'une certaine proportion d'hôtes pourrait être un mécanisme de gestion de la densité d'hôtes par feuille visant à garantir la survie des larves de

parasitoïdes en évitant la destruction du feuillage par les larves non parasitées. Si les larves du parasitoïde sont mobiles, une autre explication pourrait être que la femelle tue plus d'hôtes afin de fournir une ressource supplémentaire à sa progéniture, au cas où l'hôte parasité ne lui suffirait pas (Lauziere *et al.* 1999).

Conséquences en termes de dynamique des populations : En théorie, les parasitoïdes vont provoquer des dynamiques de populations cycliques et vont répondre rapidement aux changements de dynamique des populations de leur hôte (May *et al.* 1981).

En termes de lutte biologique : Les parasitoïdes sont très utilisés en lutte biologique et on considère en général qu'ils sont plus à même de contrôler les pics des populations de ravageurs que les généralistes, à cause de leur cycle de vie court et de leurs spécificités (Hassel 1980, Hassell et May 1986, Berryman 1992, Murdoch 1994, Turchin *et al*. 1999). Leur spécialisation présente l'intérêt qu'ils ne sont pas *distraits* par d'éventuelles proies alternatives non ravageuses (Snyder and Ives 2003).

II.1.2) Les interactions intraspécifiques

La prédation intra-guilde

La prédation intra-guilde a lieu lorsqu'un ennemi naturel (le prédateur intra-guilde) attaque une autre espèce d'ennemis appartenant au même niveau trophique (la proie intra-guilde) et en compétition pour la même proie/hôte (Rosenheim *et al.* 1995). D'après Brodeur et Rosenheim (2000), la relation prédateur-parasitoïde favorise la prédation intra-guilde. Dans ce cas de figure elle est unidirectionnelle, le parasitoïde étant la proie, mais elle peut aussi se produire entre deux prédateurs généralistes se faisant subir de la prédation intra-guilde réciproquement, en général en s'attaquant aux stades juvéniles de l'espèce compétitrice (e.g. Moreno-Ripoll *et al.* 2012, Persons et Rypstra 2001).

Conséquences sur la dynamique des populations : Quand la prédation intra-guilde se produit dans un système, deux prédictions principales ressortent des modèles mathématiques comprenant trois espèces (le prédateur intra-guilde, le prédateur intermédiaire, la proie commune) : (1) la coexistence entre les deux espèces d'ennemis naturels est possible seulement si le compétiteur intermédiaire est le compétiteur supérieur en termes d'utilisation de la ressource commune (Holt et Polis 1997) et (2) la coexistence est possible seulement à un niveau intermédiaire de productivité de la ressource (Holt et Polis 1997, Diehl et Feissel 2000, Mylius *et al.* 2001, Borer *et al.* 2003).

En lutte biologique : La prédation intra-guilde a été très largement étudiée au sein des agrosystèmes. D'après la théorie, la prédation intra-guilde peut perturber la lutte biologique et

conduire à un niveau de contrôle des ravageurs moins important (Rosenheim *et al*. 1995) et cela a été observé en pratique sur certaines cultures (Rosenheim 2005, Finke et Denno 2003).

Certains comportements du prédateur peuvent aussi aggraver l'impact de la prédation intra-guilde, Meisner *et al.* (2011) ont démontré que la coccinelle *H. axyridis* attaquait préférentiellement les larves parasitées par *Aphidius ervi* Haliday (Hymenoptera: Braconidae) plutôt que les larves saines. Mais en général, c'est le contraire qui est observé, surtout lors de prédation intra-guilde entre deux prédateurs généralistes, on observe que le prédateur intra-guilde a une préférence pour sa proie phytophage et ne s'attaque au prédateur que quand la proie commune est rare (Dinter 1998). Il a, de plus, été montré que la proie intra-guilde permet souvent une réponse fonctionnelle faible, voire nulle (Onzo *et al.* 2005). Cela pourrait expliquer la préférence des prédateurs intra-guilde pour la proie phytophage.

D'autres comportements vont venir modifier l'impact de la prédation intra-guilde sur la dynamique des populations. On note en particulier le comportement du prédateur intermédiaire, qui peut chercher à éviter le prédateur intra-guilde, et par conséquent, risquer de passer moins de temps à attaquer l'espèce herbivore mais aussi à se disperser (Wagner et Wise 1996, Prasad et Snyder 2006). Ce comportement, même s'il peut être efficace pour réduire la prédation intra-guilde, a un effet négatif sur le contrôle biologique.

D'après Rosenheim et Harmon (2006), les conséquences de la prédation intra-guilde diffèrent selon la proie intra-guilde considérée : un autre prédateur ou un parasitoïde. En théorie, la prédation intra-guilde se produisant sur un parasitoïde risquerait moins de perturber le contrôle biologique que lorsqu'elle se produit sur un autre prédateur. Dans la pratique les conséquences sur le contrôle biologique sont très variables selon les systèmes étudiés (Janssen *et al.* 2006, Rosenheim et Harmon 2006). Celles-ci n'entraînent pas systématiquement une augmentation de la densité de la proie commune (Janssen *et al.* 2006, Rosenheim et Harmon 2006, Snyder 2009), cela a été observé dans différents systèmes, par exemple lorsque la prédation intra-guilde est exercée par des coccinelles sur des parasitoïdes de pucerons, des coccinelles sur des parasitoïdes d'aleurodes, des punaises prédatrices sur des parasitoïdes, etc. (e.g. Heinz et Nelson 1996, Snyder et Ives 2003, Bilu et Coll 2007, Messelink *et al.* 2013).

La compétition par interférences

La compétition interspécifique est habituellement considérée comme regroupant deux types d'interactions : la compétition par exploitation et la compétition par interférence. La première fait partie des interactions indirectes puisqu'elle se produit via la ressource partagée, alors qu'au contraire la seconde ne nécessite pas l'intervention d'une espèce tiers. Les deux ont un rôle

primordial dans la détermination de l'abondance et de la distribution des espèces dans les écosystèmes. Dans l'étude de Gause (1932), l'interaction entre les deux micro-organismes est une interférence par un effet allélopathique de l'un sur l'autre. On considère en général que la compétition par interférence regroupe tout ce qui peut, chez une espèce donnée, interférer avec l'accès à la ressource de son compétiteur, comme l'allélopathie, l'évitement, les agressions (Case et Gilpin 1974, Vance 1984, Denno *et al.* 1995), le cléptoparasitisme (vol d'une ressource récoltée/attaquée par une autre espèce [Brockmann et Barnard 1979]), la prédation intra-guilde est aussi considérée comme de l'interférence. Autrement dit, la compétition par interférence est une réduction de l'accès à la ressource d'une espèce, engendrée par la présence d'une autre espèce dans le système. Celle-ci a été observée chez beaucoup d'espèces de mammifères (e.g. Linnell et Strand 2000), d'oiseaux (e.g. Maniscalco *et al.* 2001, Vahl *et al.* 2005) ou d'arthropodes (e.g. Denno *et al.* 1995, Vahl *et al.* 2005), par exemple.

Conséquences sur la dynamique des populations : L'interférence a pour effet une réduction de la fitness d'une espèce et par conséquent une réduction du taux de croissance de sa population. Elle joue un rôle essentiel dans les régulations d'espèces, qui peut parfois être prédominant par rapport à la compétition pour la ressource (Eccard et Ylonen 2002), voire pouvant mener à l'exclusion d'un des deux compétiteurs (Tannerfeldt *et al.* 2002).

En lutte biologique : Les études se sont principalement focalisées sur les comportements d'évitement d'un compétiteur, mais elles restent encore peu nombreuses. La majorité des travaux portent sur l'évitement d'un prédateur intra-guilde par un prédateur intermédiaire (e.g. Persons et Rypstra 2001, Nakashima *et al.* 2004, Magalhaes *et al.* 2005, Cakmak *et al.* 2006, Nakashima *et al.* 2006, Wilson *et al.* 2010, Meisner *et al.* 2011). En fait, la majorité des études sur la compétition par interférence en lutte biologique porte sur la compétition intraspécifique où l'on a aussi pu observer des comportements de combat pour des sites de ponte chez des parasitoïdes, par exemple (e.g. Batchelor *et al.* 2005, Perez-Lachaud *et al.* 2002). De plus, rien n'a été observé en termes de cléptoparasitisme chez des agents de contrôle biologique semble-t-il.

II.2) Les interactions interspécifiques indirectes

Les effets indirects apparaissent quand l'impact d'une espèce sur une autre espèce requiert la présence d'une troisième espèce. En théorie, toutes les interactions directes peuvent être observées de manière indirecte (Wootton 1994) (Fig. 1). En général, le terme « apparent » est rajouté. Dans une première partie, les interactions entre proies via un prédateur commun seront développées sous forme d'une review. En ce qui concerne la compétition pour la ressource (--), elle se découpe en

deux interactions, la compétition par interférence, interaction indirecte présentée précédemment, et la compétition par exploitation. La compétition par exploitation (utilisation d'une ressource sans interaction directe), sera présentée dans la section suivante.

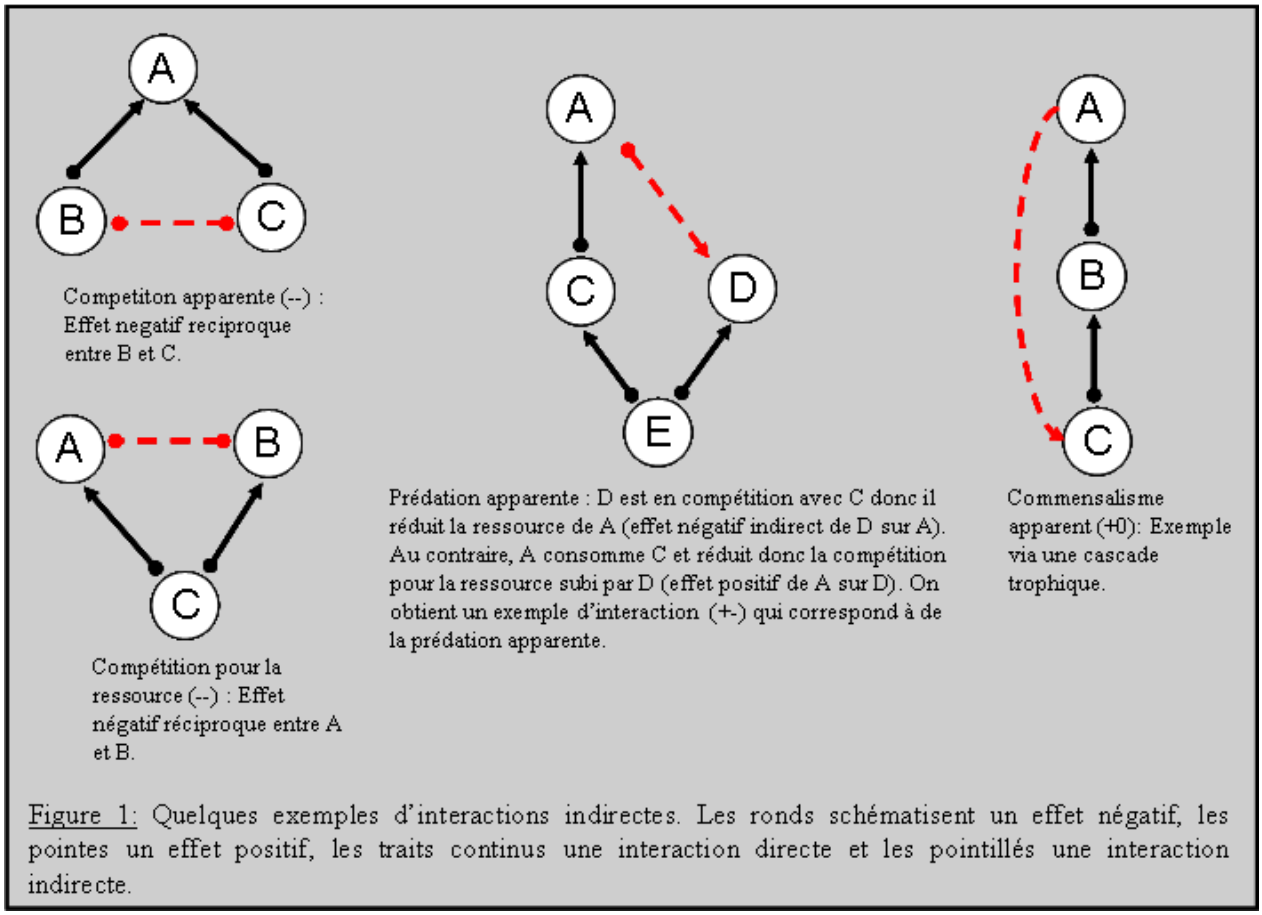


Figure 1: Quelques exemples d'interactions indirectes. Les ronds schématisent un effet négatif, les pointes un effet positif, les traits continus une interaction directe et les pointillés une interaction indirecte.

II.2.1) La compétition apparente

Présenté en tant que *Article 1*. En préparation, invité dans *Pest Management Science.*

Article 1

Natural enemy-mediated interactions among prey species: current theories and prospects for biological control in agro-ecosystems

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Natural enemy-mediated interactions among prey species: current theories and prospects for biological control in agro-ecosystems

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Abstract:

Understanding how arthropod pests and their natural enemies interact in complex agro-ecosystems is essential for pest management. Theory predicts that prey sharing a predator, such as a biological control agent, can also indirectly reduce each other's density at equilibrium. From this premise, we discuss the complexity of indirect interactions between pests in agro-ecosystems. We especially suggest that each crop and species has traits that may modify the strength or nature of interactions. Crop management practices, coupled with the physiological, behaviour, and life-history traits of interacting species, can influence indirect interactions between prey and affect pest population dynamics. These factors could be taken into account when developing biological control programs. In this review, (1) describe the theory of apparent competition (2) outline factors that affect the nature of enemy mediated indirect interactions in the field (3) identify practices that may facilitate the use of enemy mediated interactions for biological control (4) discuss the consequences of higher-order predation for biological control in light of enemy mediated interactions.. We argue that theoretical predictions must be combined with knowledge of specific agro-ecosystem traits in order to best understand the nature of the interactions in multi-species food webs and design appropriate interventions to facilitate biological control.

Keywords: apparent competition, apparent amensalism, apparent mutualism, generalist predator, indirect interactions, pest control, crop protection, commensalism, shared predator.

1 INTRODUCTION

Arthropod species are known to directly interact with others through predation or competition, but also indirectly through the action of other species (Andrewaetha and Birch, 1954). The complexity of multispecies interactions in communities can make it difficult to detect indirect interactions or to distinguish between the effects of direct and indirect components (Connell, 1983; Bender et al., 1984; Messelink et al., 2012). A thorough exposition of the contributions of direct and indirect interactions on abundance of co-occurring species has been firstly accomplished by Miller (1994) in an old field plant community. In his study, Miller (1994) demonstrated that indirect effects were positive (facilitative) and negatively correlated with the direct effects, which thus acted to mitigate the usually larger competitive effects. The indirect effects found in this study were larger in magnitude than suggested by theoretical studies. The role of indirect interactions has also been highlighted in other ecosystems (Wotton, 1994) and the relevance of these interactions in biological control programs has been rapidly highlighted (Ehler, 1996; Janssen et al., 1998). Indirect interactions among species do not only occur between trophic levels (i.e. bottom up and top down effect through trophic cascade, see for example: Paine, 1980; Silander and Antonovics, 1982; Rosenheim, 1998; Polis et al., 2000; Schmidt-Entling and Siegenthaler, 2009) but also within a trophic level via resource competition (Tilman, 1982) or shared predation (Holt, 1977, 1984). In Holt (1977)'s theoretical model, prey species do not directly but negatively interact with each other through a shared predator. These negative indirect interactions have been termed predator mediated apparent competition by Holt (1977) (Fig.1).

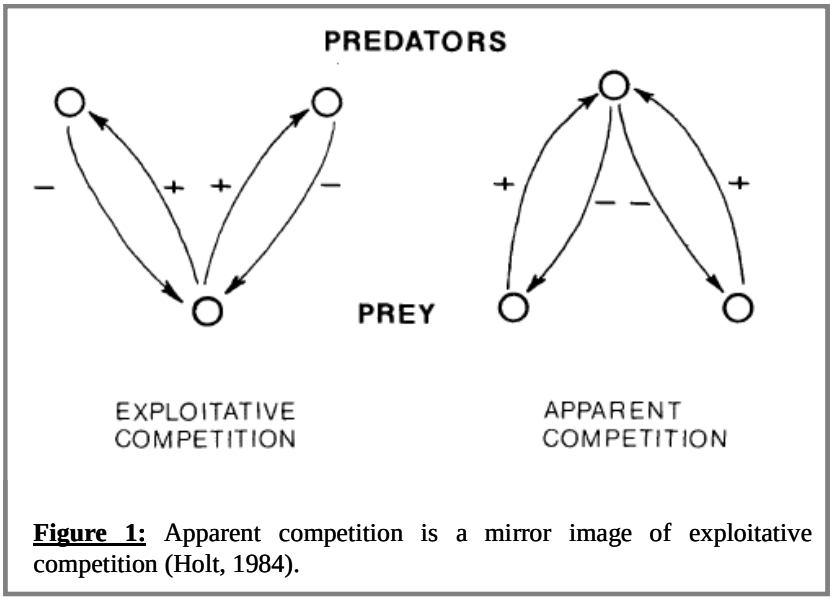


Figure 1: Apparent competition is a mirror image of exploitative competition (Holt, 1984).

In this review we focus on agricultural arthropod communities, intending to show that a better understanding of factors driving pest population dynamics is essential for optimizing pest control. In agricultural systems, biological control of pest species based on occurring or released natural enemies is often based on a mixture of both generalist predators and specialist predators, including parasitoids. These predators can then feed on shared or non-shared prey.

The trophic structure of arthropod communities is often complex, with a huge abundance of parameters influencing indirect interactions (van Veen et al., 2006). Therefore we aim to outline the complexity of indirect interactions in agro-ecosystems, suggesting that each agro-ecosystem has its own traits (e.g. perennial or annual crops, species characteristics, etc.) that may modify the strength of species interactions. These specificities are crucial and could be taken into account to manage effective biological control programs (Holt and Lawton, 1994; Chaneton and Bonsall, 2000). In this review we (1) highlight the occurrence of natural enemy-mediated interactions in the fields, (2) outline the principal factors that may modulate enemy-mediated interactions, (3) propose some possibilities to use enemy-mediated interaction in biological control and (4) discuss the consequences of intraguild predation and cannibalism on biological control at the light of enemy-mediated interactions.

2 THEORY OF APPARENT COMPETITION

2.1 Ecosystem modelling

For the last decades, many models have been formulated and explored that represent a one predator-two-prey system. A strong focus has been given to simple models, based on the Lotka-Volterra model, in order to study effects between prey that indirectly interact through a shared predator (e.g. Holt, 1977 and subsequent works; Wootton, 1994; Harmon and Andow, 2004). These mathematical models represent the basic components of predation in the functional and the numerical responses, which have been identified as the main factors driving apparent competition (Holt, 1977; Janssen et al., 1998). Indeed, they directly link the dynamics of predator and prey densities together: the former represents the consumption rate of the predator as a function of prey densities; the latter corresponds to a change in the predator number according to a change of the prey densities (Holling, 1959a, 1959b).

Although the functional and numerical response may be simply represented, they may be modified to account for numerous factors that affect interactions in natural ecosystems (Abrams, 1987a, 1987b; Strauss, 1991). For example, the theoretical expression of the functional or the numerical responses can take into agro-ecosystem traits such as predator and prey behavior, as well

as temporal and spatial structure (Holt and Lawton, 1994). To account for this complexity, a rich variety of models have been developed in the theoretical literature (Harmon and Andow, 2004). There is no consensus on the best way to model agro-ecosystem traits, but some key principles can predict the occurrence of apparent competition (Abrams, 1987c).

2.2 Roots of apparent competition

Holt (1977) showed how apparent competition occurs in a one-predator–two-prey system, using generalized differential equations. Let N_1 and N_2 denote the populations of two different prey, and P their shared predator. F_1, F_2 and F_P are functions representing their respective dynamics. His model reads:

$$\begin{cases} \frac{dN_1}{dt} = F_1(N_1, N_2, P), \\ \frac{dN_2}{dt} = F_2(N_1, N_2, P), \\ \frac{dP}{dt} = F_P(N_1, N_2)P. \end{cases} \quad (1)$$

Apparent competition can occur in such a one-predator-two-prey system under some specific assumptions (Holt, 1977). 1) The predator per-capita growth rate depends on prey densities, so that the predator is food-limited. 2) All species of the system must reach a stable equilibrium; this equilibrium depends on the prey characteristics because of the first assumption. 3) Prey have a positive effect on the predator's numerical response, meaning that an increase in prey density induces an increase in predator density. As a consequence, if one prey increases its equilibrium density, the predator population intensifies its pressure on each of the prey, which results in apparent competition. In model (1), at equilibrium, this outcome is equivalent to:

$$\frac{dN_2}{dN_1} = -\left(\frac{\frac{\partial F_P}{\partial N_1}}{\frac{\partial F_P}{\partial N_2}} \right), \quad (2)$$

with positive $\frac{\partial F_P}{\partial N_1}$ and $\frac{\partial F_P}{\partial N_2}$ because of the third hypothesis stated above. This leads to $\frac{dN_2}{dN_1} < 0$: an increase in N_1 density at equilibrium implies that N_2 equilibrium density should decrease. Holt (1977) has extended this result and showed that, for n prey, at least one term $\frac{\partial N_j}{\partial N_i}$

(with $i = 1,...,n$ and $j = 1,...,n$, $i \neq j$) must be negative. This means that at least one of the prey must suffer from the increase in another prey density.

The outcome of apparent competition depends on the prey demographic characteristics which can be quantified by the ability to withstand predator pressure, or the ability to subsist at a high density of the shared predator (Holt and Lawton, 1993; Scott Mills et al., 1993). Indeed, the prey species that supports the highest predator densitywill negatively affect the other prey (Holt et al., 1994). This may lead to apparent competition exclusion, which occurs in models according to the P^* rule (Holt et al., 1994), largely inspired by the Tilman's R^* rule of resource competition (Tilman, 1982). In the context of apparent competition, the prey that withstands the highest predator density at equilibrium has the highest P^* and can exclude the other prey which suffers at such a high predator density. This rule can be illustrated with a simple one-predator–two prey system (Holt et al., 1994) (Fig. 2):

$$\begin{cases} \dfrac{dN_1}{dt} = r_1 N_1 - a_1 N_1 P, \\ \dfrac{dN_2}{dt} = r_2 N_2 - a_2 N_2 P, \\ \dfrac{dP}{dt} = F_P(N_1, N_2) P. \end{cases} \qquad (3)$$

The prey populations grow exponentially, with r_1 and r_2 their respective intrinsic growth rate. The functional response of the predator is linear with a_1 and a_2 the attack rates of the predator for its respective prey. F_P represents generalized dynamics of P that ensure the stability of the system. If P only interacts with and forages for N_1, it reaches $P_1^* = \dfrac{r_1}{a_1}$ at equilibrium, whereas if it only forages for N_2, it reaches $P_2^* = \dfrac{r_2}{a_2}$ at equilibrium. If $P_1^* > P_2^*$, the predator benefits from the presence of N_1 and its density exceeds the one withstood by N_2. Because of apparent competition, N_2 is then excluded (Fig. 2A).

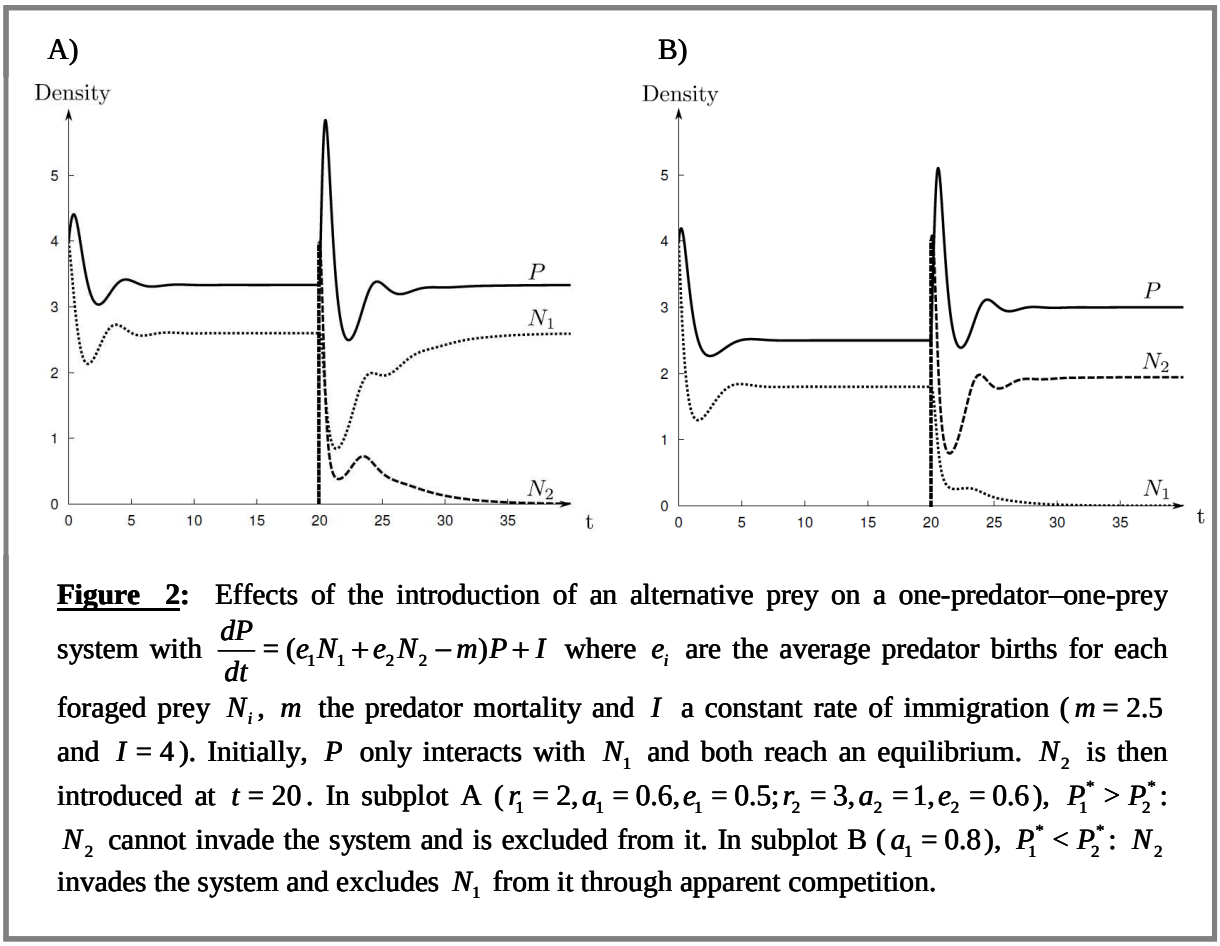


Figure 2: Effects of the introduction of an alternative prey on a one-predator–one-prey system with $\frac{dP}{dt} = (e_1N_1 + e_2N_2 - m)P + I$ where e_i are the average predator births for each foraged prey N_i, m the predator mortality and I a constant rate of immigration ($m = 2.5$ and $I = 4$). Initially, P only interacts with N_1 and both reach an equilibrium. N_2 is then introduced at $t = 20$. In subplot A ($r_1 = 2, a_1 = 0.6, e_1 = 0.5; r_2 = 3, a_2 = 1, e_2 = 0.6$), $P_1^* > P_2^*$: N_2 cannot invade the system and is excluded from it. In subplot B ($a_1 = 0.8$), $P_1^* < P_2^*$: N_2 invades the system and excludes N_1 from it through apparent competition.

Since both prey play a symmetric role in the equations, similar outcomes occur when N_1 and N_2 are commuted (Fig. 2B). If both prey are identical, they both experience apparent competition. When both prey grow logistically, with K_1 and K_2 their respective carrying capacity, they can coexist for a small range of parameters (Fig. 3). Regarding these parameters, e.g. the ratio between P_1^* and P_2^* and their carrying capacity, prey always experience apparent competition. However, these negative indirect effects can lead to the exclusion of one prey for some larger ranges of parameters.

Environmental changes that increase the birth rate, decrease the death rate, or increase the carrying capacity of a prey species will benefit the predator and enhance its pressure on both prey (Kotler and Holt, 1989). Specifically, the predator consumes more prey and experiences a larger numerical response, thus increasing predation pressure on the other prey. On the other hand, agro-ecosystem characteristics such as predator interference, satiation, distraction, emigration or additional seasonal mortality may penalize predator growth reducing the numerical response and thus apparent competition (Abrams and Matsuda, 1996; Teixeira Alves et al., submitted). Indeed, in such situations the predator reaches an equilibrium density below the ones withstood by both prey

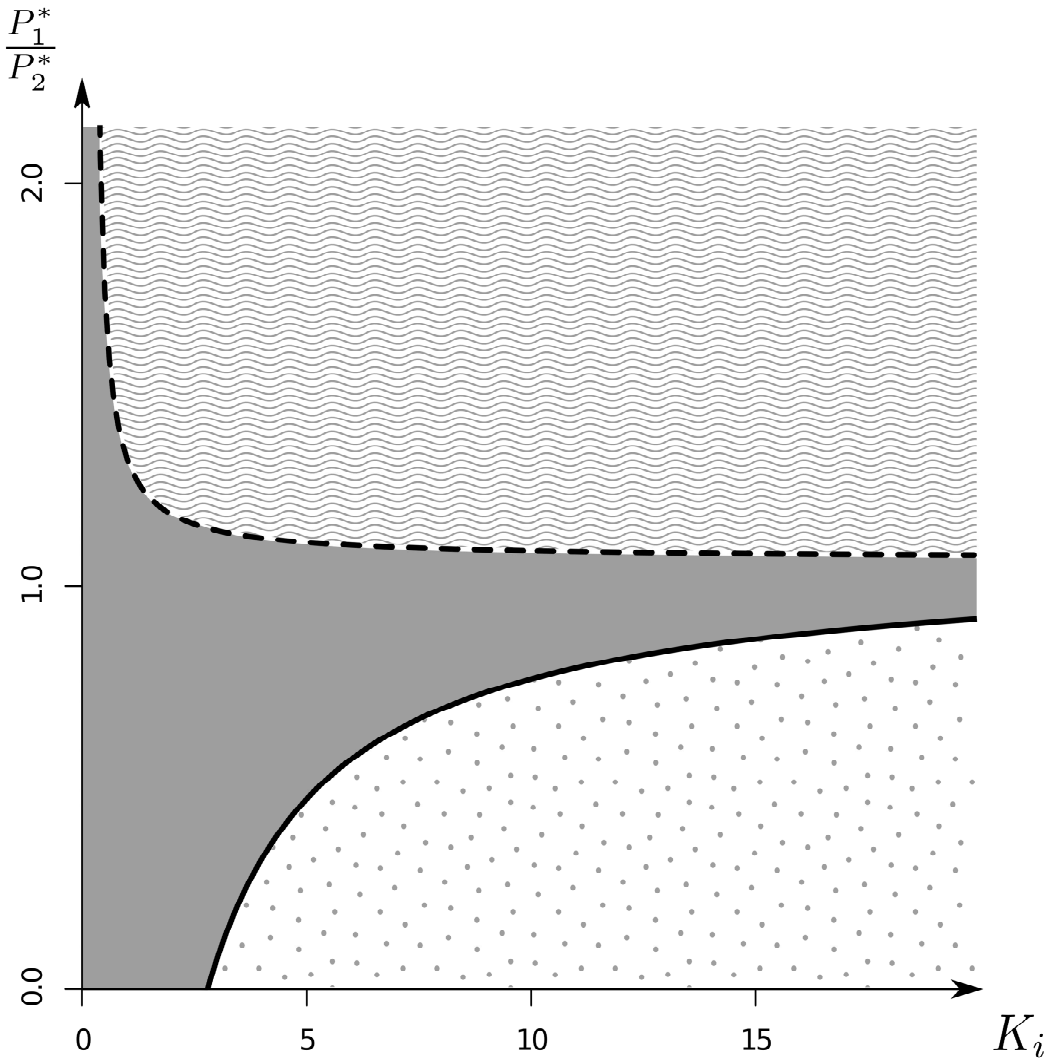


Figure 3: The ratio $\frac{P_1^*}{P_2^*}$ as a function of the carrying capacities of the prey ($m = 3, a_1 = 0.1, a_2 = 1.5$). N_1 is excluded from the system if the ratio $\frac{P_1^*}{P_2^*}$ is under the condition represented by the black line (dotted region). N_2 is excluded from the system if the ratio $\frac{P_1^*}{P_2^*}$ is above the condition represented by the dotted black line (waved region). Between both condition, prey coexist and experience apparent competition (gray region).

when alone, so each can benefit from the presence of an alternative prey. This is called apparent mutualism.

The functional response has an important influence on apparent competition as it defines the predator foraging behavior and is closely linked to the numerical response (Harmon and Andow, 2004). On the one hand, a linear functional response generates apparent competition: predator

consumption proportionally increases with prey density, and combined with the numerical response increases the predator density and predation pressure on the prey (Abrams, 1984). On the other hand, many functional responses, such as the Holling type II, model predator satiation (Skalski and Gilliam, 2001). In this case, as prey density increases, the probability that the predator forages for any given prey decreases. Consequently, prey diversity increases predator satiation, and apparent competition is relaxed (Krivan and Eisner, 2006). Such decelerating functional responses may also generate population cycles that reduce the apparent competition. Indeed, the population fluctuations imply that, when cycling, the changes of the prey densities are more important than the changes of the predator foraging (Abrams et al., 1998).

2.3 Variable indirect effects caused by agro-ecosystem characteristics

More complex mathematical models that account for variability among species (especially predator and prey behaviours) and environments (including spatial or temporal structure) have been developed that predict a variety of indirect effects. In such models, natural agro-ecosystem characteristics can be represented by the simultaneous use of different theoretical mechanisms. For example, Krivan and Eisner (2006) have explored the influence of both the exponential and logistic prey growth and predator foraging behavior on the indirect interactions between prey. They conclude that prey that have exponential growth and are preyed upon by inflexible predators with fixed preferences for resources experience strong apparent competition; whereas, prey with logistic growth that are preyed upon by adaptive predators are more likely to coexist. Similarly, Abrams and Matsuda (1996) have analyzed the predator satiation through the use of the Holling type II functional response and predator switching coupled with predator interference. They suggested that both predator behaviors may induce positive or negative indirect effects, depending on the strength of satiation, or on the degree of interference or of the trade-off between its prey. In a similar way, Teixeira Alves et al. (submitted) showed that indirect effects between prey can range from non-reciprocal interactions and unilateral interactions to positive reciprocal interactions, depending on the distraction and the density-dependence of their shared predator. These examples show how species characteristics can profoundly influence the occurrence of indirect interactions in natural ecosystems. .

2.4 Current developments in food web modelling

The apparent competition literature has focused on one-predator-two-prey systems (Abrams et al., 1998), but less effort has been devoted to simple models representing multiple species, because of their difficult analysis (Wootton, 1994, 2002). Recent studies have tried to take into account the

influence of environmental conditions in meta-communities (Leibold et al., 2004) and complex food webs (Jordán and Scheuring, 2002 and subsequent works; Christensen and Walters, 2004; Montoya et al., 2009). In particular, signed digraphs have been designed to represent complex networks with direct and indirect interactions (Liu et al., 2010). These models allow a quantification of the sign and the strength of interactions between species. However, approaches are still developing. Current models lack characteristics real ecosystems and of the interaction accuracy between prey species (Liu et al., 2010; Livi et al., 2011), but the problem of modelling of complex food webs deserves ecologists' attention. Complex models can demonstrate how direct and indirect effects stabilize natural ecological networks, especially food webs (Libralato et al., 2006; Montoya et al., 2009; Liu et al., 2010).

3 ASYMMETRIC INDIRECT INTERACTIONS

Even though apparent competition is one of the most studied enemy-mediated interaction in the theoretical literature, the role of this reciprocal interaction in natural ecosystems remain unclear. Indeed, depending on the temporal or spatial scale, the behavior of the prey or predator species, the quality of the prey species (which affects the numerical response of the predator), or prey population size, predator-mediated interactions can take different forms. This way, predator behavior is more likely to mitigate the strength of the interactions (imbalanced apparent competition) but predator biology and prey population size can cause asymmetric interactions (+/-,0). Temporal or spatial scales do not depend on the agrosystems but on the study context chosen by ecologist who should carefully interpret their results at the light of these two scales. Therefore these last will be developed in another paragraph (see section 5). Many experiments revealing asymmetrical indirect interactions have not been focused on biological control; nevertheless, these studies point to the mechanisms behind asymmetrical interactions, which is relevant to predicting outcomes of biocontrol. These mechanisms of asymmetric indirect interactions are not only relevant to biological control, but they also apply to interactions between native species and invasive pests that share common predators.

Prey populations that interact through shared predators may have reciprocal or asymmetrical effects on each other, and the effects may be either positive or negative (Fig. 4).

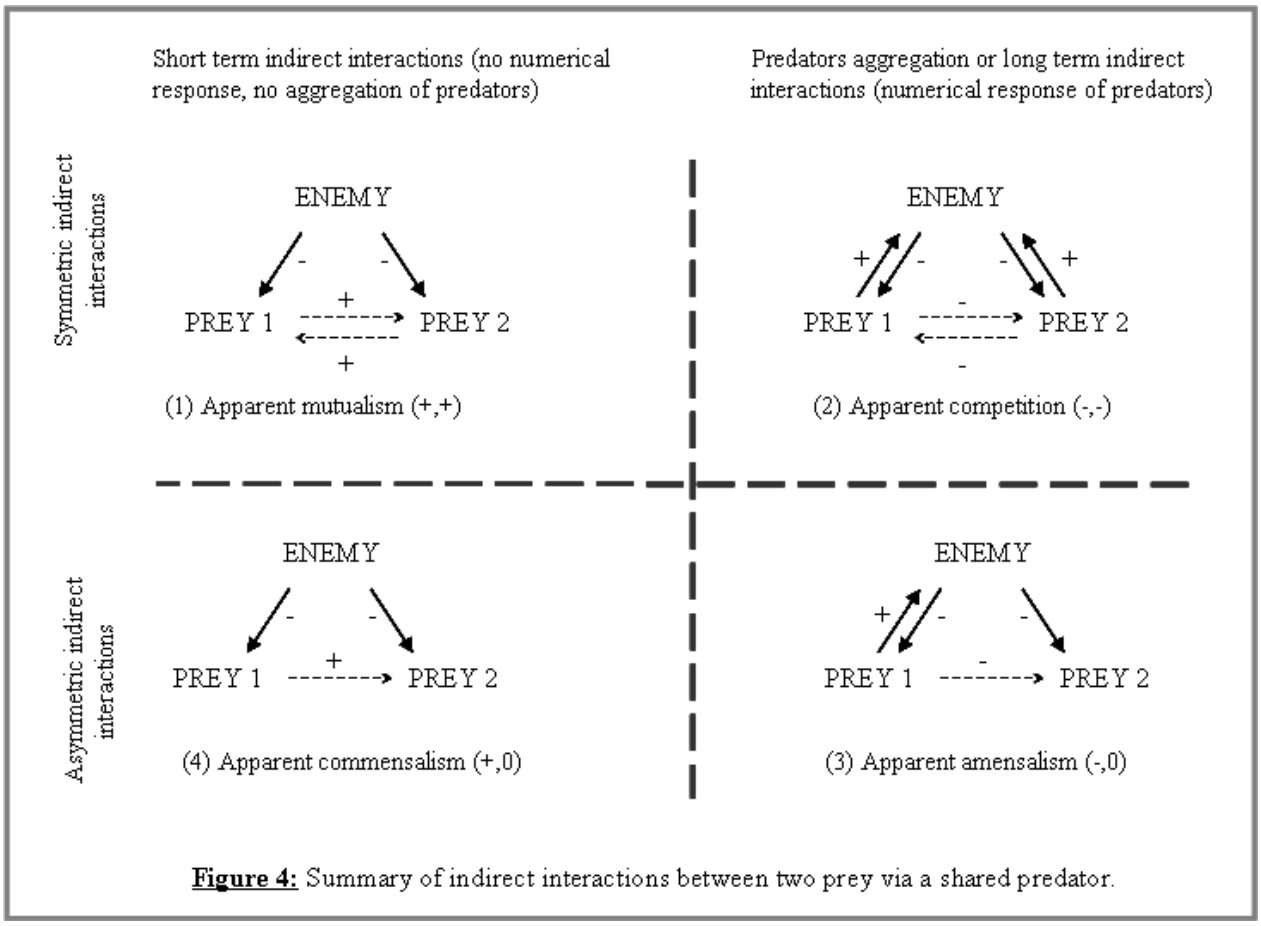


Figure 4: Summary of indirect interactions between two prey via a shared predator.

Apparent competition (-,-) and apparent mutualism (+,+) are two-way reciprocal indirect interactions between species that share a predator. Apparent amensalism (-,0) and apparent commensalism (+,0) are asymmetric indirect interactions that occur when one species affects the other through a shared predator. Negative indirect interactions typically occur when alternate prey increase the numerical response of a predator, resulting in increased consumption of the target prey. Positive indirect interactions typically occur in the absence of a numerical response when alternate prey reduce encounter rates with target prey. Mechanisms for asymmetric indirect interactions may include large differences in population sizes of the two prey, predator preferences, differences between the suitability of the prey (i.e. differences in the numerical response caused by the prey quality) (Meisner at al., 2007), among others.

Empirical studies frequently failed to detect asymmetric interactions since they do not fully test the reciprocity of the interactions between a primary prey and an alternate one (Chaneton and Bonsall, 2000; van Veen et al., 2006). For example, Muller and Godfray (1997) manipulated grass aphid densities and measured the effects on out planted nettle aphid densities, but they did not test for reciprocal effects of nettle aphids on grass aphids. Nevertheless, the potential asymmetry of these indirect interactions has important implications for biological control because it is important

to know whether alternate prey will negatively affect the target prey population or not. Furthermore, asymmetric indirect interactions may also occur between native species and invasive pests that share common predators. In the absence of effective control, abundant pests may supplement predator populations that could asymmetrically impact native species (Kenis et al., 2009; Noonburg and Byers, 2005). Here, we focus on two mechanisms that are likely to cause asymmetrical interactions: the predator numerical response and the prey population sizes. We address temporal, spatial, and behavioural mechanisms in separate sections. For example, certain practices are likely to increase the effectiveness of biological control by using a banker plant system before pests arrive, which can promote the introduction of alternate prey less profitable to the predator than the pest (Huang et al., 2011).

3.1 Role of predator numerical response

As described above, one of the conditions for apparent competition is the capacity of both prey species to allow a numerical response of the predator. However, differences in the numerical response could occur depending on the quality of the prey (e.g. Foglar, 1990; Hamdan, 2006; Seagraves, 2010) and lead to asymmetric enemy-mediated interactions. If one prey allows a numerical response of the shared predator and the other one does not, the latter species will have no effect on the former (i.e. apparent amensalism). For example, the field study of Pons et al. (2009) suggests that some heteropteran predators do not respond numerically to *Therioaphis trifolii* and *Aphis craccivora* on alfalfa, suggesting that these species will not reciprocally interact with other prey via heteropterans.

Higher equilibrium densities of predators through apparent competition may not merely be caused by increased prey availability alone but may also result from diet mixing. Predatory mites control thrips but not whiteflies when each prey is alone. However, previous experiments showed that juveniles of the predatory mite *A. swirskii* survive and develop better on a mixed diet of thrips and whiteflies than on a single diet of either of these species. These effects of a mixed diet were suggested to be responsible for strong increases in predator densities in greenhouses in which both thrips and whiteflies were present, and consequently, for lower densities of whiteflies in the presence of thrips (Messelink et al., 2008). Thus, thrips may promote control of whiteflies because diet mixing increases the predator numerical response to whiteflies without generating a reciprocal effect on thrips. So far, this aspect of mixed diets has been ignored in theoretical models about apparent competition but could be important for biological control.

3.2 Role of differences in population sizes

Apparent commensalism occurs when one prey reduces the predation on the other prey in an asymmetrical fashion, often with negative consequences for biological control. Short term apparent amensalism was observed on crested wheatgrass where lacewing larvae were found to less effectively reduce population growth rates of the Russian wheat aphid (*Diuraphis noxia*) in the presence of bird cherry-oat aphids (*Rhopalosiphum padi*) than when *D. noxia* was presented alone. However, the effect appeared to be asymmetrical, in that *D. noxia* had no effect on predator consumption of *R. padi* (Bergeson and Messina, 1997). This is an example where abundance (i.e. increased encounter) or availability (prey exposure to predation) of one prey species are the best explanations for asymmetry. However, the outcome of interactions at larger spatial and temporal scales could be markedly different.

Asymmetric interactions driven by differences in abundance across prey species appear to strongly influence the structure of aphid-parasitoid (Muller et al., 1999) and leaf miner-parasitoid communities (Valladares et al., 2001), where symmetrical links in quantitative food webs are rare but asymmetric links from common to rare prey species are more frequent. Note that food webs are essentially observational, and experiments to test their predictions are recommended (Tack et al., 2011). However, if relative prey abundance frequently generates asymmetric indirect interactions, conservation biological control may require significant investment. Otherwise, small populations of alternate prey may have little effect on a large pest population; instead, the alternate prey population may be subject to extinction through apparent amensalism.

4 ENEMY-MEDIATED PREY EXCLUSION

The potential for prey coexistence may change through the presence of shared generalist predators. Apparent competition affects population dynamics in the same way as competition for a shared limiting resource. Exclusion is a possible outcome if (1) predation intensity is very high (overexploitation) or if (2) there are no refuges for the prey species that supports the smallest predator population or if (3) the less competitive prey suffers other detrimental interactions (e.g. resource competition) (Holt, 1984). Thus an imbalanced apparent competition could lead to exclusion, as well as to apparent amensalism.

Another reason that apparent competition could theoretically lead to prey exclusion is a destabilisation of the ecosystem because the introduction of a second prey species increases the total availability of prey for a predator. Called the paradox of enrichment (Rosenzweig, 1971), increasing the carrying capacity of one prey increases predator-prey oscillations and leads to possible

extinction. However this phenomenon is rarely observed in ecosystems when both prey species are edible. Two hypotheses could explain this observation: (1) the predator population density has a negative effect on its own per capita population growth rate via some mechanism other than prey depletion or (2) a dynamical class of prey individuals are invulnerable, or have greatly reduced vulnerability to predators (Abrams and Waters, 1996).

As demonstrated in previous section, asymmetrical (±, 0) indirect interactions may be more common than reciprocal interactions in nature (Chaneton and Bonsall, 2000), and this asymmetry could cause declines in one of the two prey species. The presence of a prey species that supports high predator densities encourages the overexploitation, and potential eradication, of alternative prey. Exclusion via apparent amensalism (0,-) has been demonstrated empirically in a long-term population study in which prey species were not allowed to compete directly for resources (Bonsall and Hassell, 1997). This experiment proves that exclusion can occur exclusively due to enemy-mediated interaction.

DeCesare et al. (2010) have synthesised the results of experiments and the role of apparent competition in the context of conservation ecology. In this review they outline the role of asymmetric interactions in endangered species conservation. For instance, apparent competition had been implicated in extinction of the Macquarie Island parakeet *Cyanoramphus erythrotis* (Taylor, 1979). But in the context of biological control, just a few studies have tested the potential for apparent competition to locally exclude a pest in the field. Indeed, exclusions in natural agro-ecosystem are rare because prey species generally dispose of ecological niches.

Several studies suggest prey exclusion by shared predators occurs in the field. Müller et al. (1999) noticed that two species of aphids that were abundant in adjacent sites were rare in their study site, despite their host plant being common and other aphid species abundant. They reasoned that this absence might be due to stochastic colonization failures, unsuitable host plants, predators (generalist or aphid-specialist) or to the lack of ant attendance. Results showed that in absence of aphids specialist predators, the two rare species developed well. Because many aphid predators have been shown to display long-range attraction to aphid densities and a tendency to remain or lay more eggs in areas of high prey abundance, they suggested that diffuse apparent competition may be responsible for the aphids' absence. In another study, parasitoid-mediated asymmetric apparent amensalism between two plant hopper species excluded experimental populations of one species more frequently than control populations not exposed to apparent amensalism (Cronin, 2007). However the hypothesis that apparent competition can lead to pest exclusion in agro-ecosystems has never been clearly demonstrated and deserves more attention from experimental field ecologists.

5 SHORT TERM VERSUS LONG TERM EFFECT

In general, in enemy-mediated interactions, short term interactions occur faster than one predator life cycle, and long term interactions occur over more than one predator life cycle. Given the long generation times of many predators relative to their prey, the original concept of apparent competition that focused on the numerical response of food-limited predators may not explain many observations about the way predators can influence the structure of communities. In theory, both short- and long-term effects of shared natural enemies can lead to positive and negative predator mediated interactions.

5.1 Short term interactions

Positive enemy-mediated interactions: various mechanisms

Interactions on the short-term usually occur within a single generation through satiation or switching behavior of the shared natural enemy (Murdoch, 1969; Abrams and Matsuda, 1996). Many studies have shown reduced predation rates on a target pest in the presence of alternative prey (short-term apparent mutualism) (Madsen et al., 2004; Koss and Snyder, 2005; Symondson et al., 2006; Xu et al., 2006; Desneux and O'Neil, 2008). This can happen if predators settle in patches independently of the prey density (i.e. no aggregation) and have a time-limited functional response, then we expect short term apparent mutualism because each prey species will dilute the number of encounters with the alternate prey. Van Maanen et al. (2012) indeed show that predators aggregate in such a patch, which allows prey to temporary escape from predators that disperse slower than their prey. Hence, this study shows that apparent mutualism may also depend on the spatial scale and predator clustering.

Apparent commensalism may occur at a short term scale due to differences in prey population sizes causing a non-reciprocal dilution effect if the predators do not aggregate on high density prey patch (e.g. Bergeson and Messina, 1997; van Nouhuys and Kraft, 2012).

Negative enemy-mediated interactions via predator aggregation

On a short time scale, alternate prey can alter the behavior of predators (increased activities, aggregation, see also behavior section) (Holt and Kotler, 1987). Apparent competition could be observed not only in the long term with predators that undergo a numerical response, but also in the short term, if the predator is an optimal forager and prey are limited. This is because predators will aggregate to and stay longer in a patch where there are more prey, ultimately exploiting both prey

species more than if each had been alone at a lower density. For example, Evans and Toler (2007) tested the aggregative response of two ladybirds, a native and an exotic one, to aphid density and to an alternative prey density. They demonstrated that both species respond to aphid density, but only the exotic one respond to the alternative prey density. This study outlines that aggregative responses could differ between predator species for the same prey species.

5.2 Long term interactions

Negative enemy-mediated interactions via numerical response

Even if short term interactions result in apparent mutualism, food-limited predators should eventually cause apparent competition between shared prey over the long term due to a numerical response (Karban et al., 1994; Bonsall and Hassel, 1997; Hanna et al., 1997; Messelink et al., 2008, 2010). In one study with a generalist predatory mite, supplemental pollen added in a spatially separate location from whitefly prey was expected to cause short term apparent mutualism (Nomikou et al., 2010). However, mites increased predation on whiteflies in the presence of pollen. This result was due to a numerical response of the predators during the 60 day experiment, suggesting that the reproductive rate of the predator relative to that of the prey might be more important than other factors, such as spatial segregation of resources. However even if the combination of pollen and prey can give similar results as apparent competition, apparent competition is strictly between prey and not between an organism and a food source. In another example, Liu et al. (2006) observed more effective control of mites in apple orchards in the presence of both a predator and alternate prey than in the presence of a predator alone, which they attribute to a numerical response. The effects of alternate prey on predator densities were detectable within a week of predator introductions, but differences in prey densities between treatments took more than a month to observe.

Positive enemy-mediated interactions: various mechanisms

Examples of short term apparent mutualism are relatively common (see references above), but they are often observed in studies where alternate prey are introduced into a controlled system with a limited number of predators, or where the behavior of individual predators is measured. In such experiment the main limitation is that the time-scale of the experiments does not allow for a numerical response of the predators. For example, long term mutualism has been considered in the experiment of Symondson et al. (2006), who demonstrated a positive effect of the alternative prey on the pest when no numerical response of the predator was possible. This experiment demonstrates the possibility of *long term* mutualism because of the long lifecycle of the predator (for most

carabid beetles ≥ 1 year) compared to the pest. But this is not strictly long term, which is usually used for more than one predator life cycle.

If predators experience a density dependent per-capita growth rate and satiation or switching behavior, this could also lead to long-term apparent mutualism (Abrams and Matsuda, 1996). However, empirical evidence for this is limited (Tack et al., 2011). These predictions suggest that understanding the behavior (switching and preference) and biology (life cycle duration, satiation level) of key predators may be critical for managing prey populations to facilitate biological control.

Long-term apparent mutualism may also occur when population densities of one prey show cycles, resulting in repeated satiation of the shared predators and repeated reduced predation on the other prey (Abrams et al., 1998; Brassil, 2006).

6 SPATIAL SCALE EFFECT

The way predators perceive the spatial distribution of shared prey can affect the predicted outcome for indirect interactions (Holt, 1984). Experimental and observational studies of the effects of alternative food sources on biological control have occurred at a variety of scales, ranging from Petri dishes to landscape level manipulations, and, the spatial scale of the manipulation indeed often appears to affect the nature of the indirect interactions.

At the broadest scale, landscape diversity at a 1.5 km radius has been shown to positively impact biological control of soybean aphids (Gardiner et al., 2009), although this result was not linked to the availability of alternative food sources. A recent meta-analysis concludes that generalist natural enemies respond to landscape diversity positively, but pest populations show no corresponding changes, and biological control is rarely actually measured (Chaplin-Kramer et al., 2011). Studies at landscape scales suggest that apparent competition could improve biological control, but much more work is necessary to understand the nature and mechanisms of the observed effects.

Studies of apparent competition conducted at multiple scales repeatedly demonstrate the scale-dependent nature of indirect interactions. Ostman and Ives (2003) found predator aggregation to one prey species in field studies, consistent with short term apparent amensalism. However, they found short term apparent commensalism in cage studies, where predators spent time on plants with pea aphids and neglected plants with potato leaf hoppers. In a different short term experiment, big-eyed bugs consumed aphids and beetle eggs proportionately in Petri dish arenas, resulting in lower aphid consumption in the presence of the alternative prey. However no disruption of aphid consumption was observed at the plant scale due to a strong predator preference for aphids (Koss et al., 2004).

Tack et al. (2011) found that adding alternative leafminer prey had varied and sometimes surprising effects on population dynamics, depending upon the temporal and spatial scale considered. In the short term, prey additions had no effect on parasitism rates, ruling out a dilution effect, and suggesting that parasitoids either aggregate or display a type I functional response at the densities studied. Over the long term, however, increasing the target prey in one season resulted in increased survivorship of alternate prey the following season, suggesting apparent mutualism was occurring at the scale of the tree. At the landscape scale, however, there was a negative relationship between the density of one prey species and the survival of another prey species the following season, suggesting apparent competition (Tack et al., 2011). At the level of the tree, interference between parasitoids or learned preference for the parental host might explain the patterns of apparent mutualism, effects which might be diminished over a larger spatial scale. All of these studies illustrate that both the spatial and temporal scale can strongly affect the nature of the overall indirect interactions observed. We discuss the potential for temporal manipulations to facilitate biological control in cropping systems in section 9.2.

7 SPECIES SPECIFICITIES, THE BEHAVIORAL FACTOR

Predator and prey behavior can modify the strength, the direction, and the reciprocity of indirect interactions. For example, many studies have shown that the preference of a predator for a prey above another could modify interactions (Murdoch, 1969). On the prey side, antipredator behavior, habitat selection, activity level all have the potential to influence indirect interactions.

7.1 Predator behavior

Predator preference could arise from (1) prey preference depending on species characteristics (2) prey preference depending upon prey availability. This last will provoke switching behavior which also acts strongly on the stability of interactions. Changes in the way that two species interact through the presence of a third species are named functional indirect interactions by Janssen et al. (1998). This would be the case if the second prey modifies the foraging behavior of the predator against the first prey. Predator choice can occur at different stages in the foraging process: at the prey location stage due to semiochemical cues, after observation, or after handling.

The potential for apparent competition to enhance biological control can be reduced if predators prefer the alternate prey. Preferences have been demonstrated for multiple hemipteran predators (Desneux and O'Neil, 2008; Rosenheim et al., 1993; Eubanks and Denno, 2000). For example the predatory bug *Anthocoris nemorum* (L.) and *A. nemoralis* have been showed to prefer

the green peach aphid (*Myzus persicae)* among 5 species of aphids (Meyling et al., 2003). Reitz et al. (2006) showed that *Orius insidiosus* prefers the western flower thrips (*Frankliniella occidentalis)* more than the Florida flower thrips (*F. bispinosa)*. Preferences for host have also been shown for many parasitoids (see for example Yokomi and Tang, 1995 or Van Driesche et al., 2003). Preference commonly occurs and negative enemy-mediated interaction may become asymmetrical or of a different strength for the two species depending on this preference.

Preference according to prey densities: Switching behavior

A predator is said to 'switch' if its relative attack rate on a prey species increases faster than does that prey's relative abundance (Murdoch and Oaten, 1975). Predator switching has traditionally been argued both to have a stabilizing effect on predator–prey interactions and to permit the co-existence of strongly competing prey species (Roughgarden and Feldman, 1975). Kimbrell and Holt (2005) showed the impact of individual variation and evolution of switching on predator–prey dynamics. Their results help reinforce the emerging perspective that variability among individuals may be an essential feature promoting persistence and stability in many natural populations. Many predators are known to have a switching behavior (Sundell et al., 2003) and it had been widely study in biological control context (Enkegaard et al., 2001; Chow et al., 2008, 2010; Saha et al., 2010).

Preference according to prey nutritive values

The nutritive quality of the prey can affect predator preference (Greenstone, 1979; Meyling et al., 2003; Mayntz et al., 2005; Kuusk and Ekbom, 2010). When two prey have different nutritive values, Mayntz et al. (2005) showed experimentally that invertebrate predators can forage selectively for protein and lipids to redress specific nutritional imbalances. Some predators are able to "learn" which one is the best and an increasing prey preference could appear during the development time. It at been shown for the coccinellid *Hippodamia convergens* (Schade and Sengonca, 1998). On the contrary, spiders winter-active predators may select smaller spiders as prey because of better nutritional value for winter-active predators than herbivorous or detritophagous dipterans and collembolans (Korenko and Pekar, 2010).

Preference according to prey activities

The behavior of prey (i.e. mobility and activity) and the size of individual prey or patches of aggregated prey (Venzon et al., 2002) can influence predator foraging behavior. Foraging behavior depends on prey mobility: a very mobile prey could increase predator aggressiveness. Consequently

when one prey is mobile and the other one is not, the mobile prey could increase predation on the immobile prey. For example, aphids, by serving as preferred prey, reduced the focal predator activity, and weakened fly egg predation. However, egg predation was restored when both aphids and ground beetle predator were present together, because aphids triggered greater foraging activity, and thus increased incidental predation of fly eggs (Prasad and Snyder, 2006a). However, even if it has been observed on various systems (Rickers and Scheu, 2005) this is not always the case. Gavish-Regev et al. (2009) observed positive prey-prey interactions: more aphids were eaten in the low-density alternate prey treatment than in the high-density alternate prey treatment. They propose three explanations: 1) spiders built larger webs in response to prey scarcity, 2) spiders directly compete for prey and 3) an increasing in spider foraging behavior in response to hunger or competition.

Preference according plant response to prey attack

Plants respond to insect herbivory with the production of volatiles that attract carnivorous enemies of the herbivores, a phenomenon called indirect defence or 'plants crying for help' (Dicke, 2009). Depending on herbivores feeding modes, plants may release volatile organic compounds (VOCs) or secrete extrafloral nectar (EFN). VOCs primarily attract predatory mites and parasitic wasps (Heyl, 2008). However, the response of predators and parasitoids is highly variable depending on the pest and plant species. For example, adult *Bemisia tabaci* induced a plant response that attracts its parasitoid *Encarsia formosa* from bean leaves but not cotton (Inbar and Gerling, 2007). Plant semiochemical response could affect enemy-mediated interactions by altering with predator behavior. For example, it has been demonstrated that mite predators are attracted by cassava leaves infested by mites depending on the mite species (Gnanvossou et al., 2002). If a prey species which does not attract predators, is submitted to apparent competition with another prey which does provoke an attractive plant response for the predator, the first species will experience additional negative effects.

7.2 Prey behavior

Prey can display various behaviors (avoidance and counter-attack) to avoid predation, including seeking physical refuge (Krivan, 1998; Magalhaes, 2007; Chen, 2009; Cressmann, 2009), fleeing a patch occupied by predators at high densities (Holt and Kotler, 1987; Holt and Lawton, 1993) and prey can also adopt complex strategies by developing defence responses. In each case, the prey strategy leads to a decrease of the number of available prey encountered by the predator. As a consequence, the predator reduces its pressure on the prey that benefits from such adaptive

behavior, relaxing apparent competition. In some cases, prey behavior is the most important factor that mediating indirect interactions among prey that share a predator. This could arise via defence behavior but also via natural behavior as the prey mobility, as an example the dropping behaviour of aphids (Losey and Denno, 1998).

Behavioral defence

Prey are able to develop antipredator behavior (Villagra et al., 2002) and also to adapt this behavior to the switching behavior of the predator. To minimize costs, prey should invest in antipredator behavior only when dangerous predators are around. To distinguish these from harmless predators, prey may use cues related to predation on conspecifics, such as odours released by a predator that has recently eaten conspecific prey or alarm pheromones released by attacked prey. For example Venzon et al. (2000) studied refuge use by the omnivorous thrips, *Frankliniella occidentalis*, in response to odours associated with a generalist predatory bug, *Orius laevigatus*, fed either with conspecific thrips or with other prey. The refuge used by thrips larvae is the web produced by its competitor, the two-spotted spider mite, *Tetranychus urticae*, where thrips larvae experience lower predation risk because the predatory bug is hindered by the web. Thrips larvae moved into this refuge when odours associated with predatory bugs that had previously fed on thrips were present, whereas odours from predatory bugs that had fed on other prey had less effect.

Prey mobility

Eubanks and Denno (2000) suggest that prey mobility is the primary mechanism of prey selection by big-eyed bugs. Prey selection by big-eyed bugs does not therefore correspond with predictions of optimal foraging models based on caloric consumption or models of insect diet selection based on nutrient availability. Big-eyed bugs preferentially attacked pea aphids, the nutritionally inferior prey, in two choice experiments. Big-eyed bugs apparently do not assess the nutritional quality of their prey but instead focus their attacks selectively on mobile prey. The impact of prey movement has a different effect if the two prey are mixed or not. If they are, the predator could be attracted by the movement of one prey and then consuming the more sedentary one which is exposed to more successful attacks (Reitz et al., 2006).

Plastic defence

Some prey may be harder to eat for biological control agent because plastic traits. For example the hardness of the cuticle may affect the preference of sucking predators, which will have to sting the prey through the cuticle. However, behavioral and plastic defences can be costly for

prey (Abrahams and Dill, 1989). Consequently, some prey living under fluctuating predation risk develop reversible defences and adjust their defences to the current predation risk. Even if the plasticity of the phenotype of some prey (e.g. degree of whiteflies nymphal setosity) is known (Guershon and Gerling, 2001) and the effect of this plasticity on predation is also known (Guershon and Gerling, 1999), effect of plastic defences of pest had never been studied. This temporal physical adaptation to predator pressure deserves more attention because it could reduce predation on the target pest which is consequently transferred to the alternate prey. Because this variability can be difficult to anticipate, it may be advantageous to use more than on biological control agent against one pest.

8 IMPLICATIONS OF SHARED RESOURCES BETWEEN PREY FOR ENEMY-MEDIATED INTERACTIONS

As stated earlier, theory predicts that when an alternative prey species maintains a larger predator population than a target pest species, it is expected to exclude the pest through apparent competition. When both prey species are also competing for resources, however, the theoretical predictions can become more complex. The current theory assumes that the carrying capacity of each prey is independent, which may not be the case when they share the same host plant.

8.1 Resource competition

If predation is symmetrical on both species, then the better apparent competitor will also reduce resources (e.g. crops) to a lower level in equilibrium models (Holt et al., 1994), suggesting that introducing alternative prey onto a crop plant would only be beneficial if they could maintain novel predator populations that would otherwise not persist. However exploitative competition only occurs at high pest densities, which is undesirable in crops and in biological control. Therefore the impact of resource competition in agrosystem on apparent competition will be almost nonexistent. Instead, plant defence–mediated interactions would be more likely to influence apparent competition, either positively or negatively.

8.2 Host plant response

Prey species that share a host plant can interact by altering plant chemistry. For example, negative indirect plant-mediated interactions may arise between pests that induce similar plant defences, either through the SA (salicylic acid)-dependent or the JA (jasmonic acid)-dependent pathways (De Vos, 2006). However the antagonistic relationship between the JA and SA pathways

(Felton and Korth, 2000; Kunkel and Brooks, 2002; Becker and Spoel, 2006) could lead to indirect positive interactions between two pests when each induces a different pathway (Preston et al., 1999; Rodriguez-Saona et al., 2005). In general, necrotrophic pathogens and chewing insects such as caterpillars trigger the JA pathway, while biotrophic pathogens and phloem-feeding insects such as aphids induce the SA pathway. Soler et al. (2011) shows that a leaf chewer and phloem feeder asymmetrically interact, not via competition as would be expected from interspecific herbivores, but instead via facilitation; the phloem feeder attenuated JA-related plant defences, thus facilitating the growth and development of the leaf chewers. However, if both pests have the same feeding behavior they may interact negatively via the plant response.

Evidence suggests that some pathogens suppress induced defences by interfering with signalling pathways (Mouttet et al., 2011); however, such evidence is scarce for herbivores. Nevertheless, Sarmento et al. (2011) found that the invasive spider mite *Tetranychus evansi* suppresses the induction of the SA and JA signalling routes involved in induced plant defences in tomato. Plant-mediated interactions could either contradict or augment enemy-mediated interaction, depending on the feeding behavior. Prey species may also interact through non-defence modifications of the host plant. For example, corn rootworm damage results in smaller corn plants and a 98% reduction in corn borer parasitism (White and Andow, 2006). Host plants can also directly affect enemy-mediated interaction strength. For instance, domatia in camphor trees are used by some herbivores which are thought to support predator populations that control other herbivore species (Kasai et al., 2005; Yamamura, 2007). Host plants also provide food, such as nectar and pollen, which can generate a numerical response among omnivorous predators that may facilitate control of herbivores.

9 USING NEGATIVE ENEMY-MEDIATED INTERACTIONS IN CROPPING SYSTEMS

Two prey species may act as apparent competitors, whether they compete for resources or not. When it comes to biological pest control, it is often preferable to avoid introducing alternative pests onto economically valuable crops, but instead introduce alternative prey that cause no damage to the crop through practices like mulching, intercropping (Xu et al., 2011; Song et al., 2011; Bickerton and Hamilton, 2012), using banker plants, growing cover crops (Olson et al., 2012) or even maintaining landscape level diversity (hedges: Huang et al., 2011; Pineda and Marcos-Garcia, 2008; Xiao et al., 2011). At the same time, some of these methods may take valuable land out of production (Landis et al., 2000), there have the potential to attract additional pests (Karban, 1997; Norris and Kogan, 2005; Winkler et al., 2010), or they may draw predators away from crops,

reducing biological control (Birkhofer et al., 2008). Therefore these methods should be used carefully.

9.1 Increasing predator populations in the field

Within fields, a few examples suggest that alternative prey may increase consumption of target prey. Ostman (2004) found that alternative prey density in crops correlated with an increased abundance of natural enemies and resulted in higher predation rates on the soil, consistent with apparent competition mediated biological control. In another study, strips of legumes and gramines intercropped with winter wheat increased early season parasitoid abundance and later parasitism rates of aphids on wheat during one of two years; however, during the other year, aphid densities were much higher in strip plot fields than in controls (Langer and Hance, 2004), which highlights the uncertain outcome of this practice. Mollot et al. (2012) demonstrate the beneficial effect of a cover crop for ants in a banana field. These results are consistent with the idea that predators can build up their densities on alternative prey in one area and spill over to consume target prey in another.

Another way to introduce food sources for the alternate prey within an agricultural field involves external subsidies, such as mulch. Such subsidies may increase the availability of detritivores to omnivorous predator populations, resulting in improved herbivore control (Polis and Strong, 1996). However, these measures do not always improve herbivore suppression, and the outcome depends on characteristics of the agrosystem such as predator species, pest species, and crop. For example, external subsidies may disrupt biological control if predators prefer the alternate prey, especially when the pest and the alternate prey are spatially separated, such as collembolan prey in detrital subsidies and aphids at the top of crops (Birkhover et al., 2008). External subsidies frequently increase alternate prey and even predator densities but fail to improve biological control, often because predation is limited by intraguild predation, cannibalism, or predator interference (Halaj and Wise, 2002; Mathews et al., 2004; Oelbermann et al., 2008).

9.2 Early season growth of predator populations

Alternative prey can be useful to augment the predator population just before the arrival of the pest, in the early season. Temporal dissociation can result in high predator density relative to the density of the invading prey, preventing a pest outbreak. For example, corn leaf aphids arrive on sorghum early in the season, supporting populations of coccinellids that control economically damaging greenbugs later in the season (Kring and Gilstrap, 1986; Michels and Matis, 2008). When the pest does not permit a strong functional response of the predator, predator population growth

before pest arrival could augment the effectiveness of biological control. Yoo and O'Neil (2009) demonstrated that, *O. insidiosus* had an aggregative or a reproductive response to thrips densities but no numerical response to soybean aphid density. Consequently, the thrips helps to promote high predator densities early in the season that may exert significant mortality on soybean aphid populations.

Artificially introducing alternative prey early in the season could be a method to increase biological control by amplifying the natural enemy, using apparent amensalism interaction (Liu et al., 2006). In greenhouses, two methods of temporal asymmetry are used for biological control. First, the "alternative host and parasitoid in first" principle calls for intentional introduction of a non-pest prey on a non-target plant culture to maintain parasitoid populations a pest-free glasshouse where a target crop is or will be grown. If a target pest is accidentally introduced, the parasitoid populations drift from the reservoirs and attack populations of the target pest (Stary, 1993). The second method involves introducing and artificially feeding a generalist predator on a target crop. This last technique is widely used in tomato greenhouses, where mirid bugs (*Macrolophus pygmaeus* or *Nesidiocoris tenuis*) are released on the crop and maintained on commercialised and sterilized moth (*Ephestia kuehniella*) eggs. This method uses an indirect negative interaction between alternative food and pests through the numerical response of mirid bugs to enhance biological control.

9.3 Using alternate prey to maintain predator populations during a non-pest period

Even if two prey do not co-occur simultaneously in the system, they can interact indirectly at a temporal scale (Fig. 5). Some predators require the presence of alternative prey in order to survive or molt during times when the target prey are absent (Negloh et al., 2009). Therefore temporal separation of alternate prey could be an important tool for biological control. Particularly in annual crops where the habitat is totally destroyed annually, the availability of non-pest prey in the non-cropping period can maintain the predator population from one year to another. Even in perennial crops, the pest may not available during some seasons, so predators will need an alternative prey species to be maintained year round.

Many generalist predators are able to consume prey on plants as well as in the soil layer. This broad diet permits their survivorship in the field during the non-cropping period, when the soil is bare, and will augment predator effectiveness in the beginning of the crop. Therefore soil and tillage practice will modify the availability of alternative prey for generalist predators (Pereira et al., 2010). Eitzinger and Traugott (2011) examined the feeding behavior of predatory beetle larvae during autumn and winter in arable land. They focused on *Cantharis* spp. and *Nebria brevicollis* larvae,

two abundant generalist predator taxa in European arable land during the cold period of the year. The results provide evidence that autumn- and winter-active predatory beetle larvae are strongly linked to the detrital food chain by feeding on earthworm and collembolan prey. This kind of observations is also done in rice where the usefulness of ground invertebrate community has been shown for spiders (Settle et al., 1996; Sigsgaard, 2002).

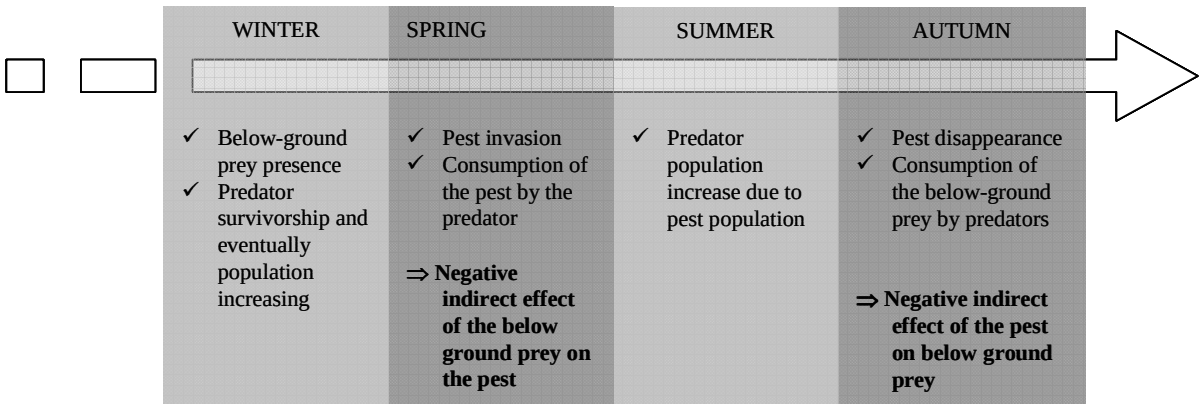


Figure 5: Apparent competition (-,-) at a scale of a year in annual crop.

When agrosystem are not destroyed, as in perennial crop like orchards, an absence of pests could also occur during certain crop stages or seasons (Pfannenstiel et al., 2010). In pistachio biological control in Turkey, the phenomenon of apparent competition is useful to biological control. The leaf-curling aphid, a pest of almond trees, is an alternative prey for the predatory bug *Anthocoris minki* when pistachio psylla are not available on pistachio trees in early spring (Yanik and Unlu, 2011). Similarly, alternate winter hosts on *Prunus* and *Rubus* plants are important for parasitoid control of grape leaf hoppers that diapause during California winters (Doutt and Nakata, 1973; Murphy et al., 1996, 1998).

10 ENEMY-MEDIATED NEGATIVE INTERACTIONS INVOLVING BIOCONTROL AGENTS AS PREY

10. 1 Indirect interactions between a biocontrol agent and a pest

Cannibalism is defined as predation of conspecifics, hyperpredation is typically defined as predators eating other predators without sharing prey, and intraguild predation (IGP) is defined as predation of interspecific competitors, often called intermediate predators. Hereafter the term *higher-order predation* will be used to refer to both intraguild predation and hyperpredation (Rosenheim, 1998; Symondson et al., 2002). These three interactions could be involved in higher

enemy-mediated interactions when the higher-order predator feeds on an interspecific species and an herbivore species (Polis et al., 1989; Rosenheim et al., 1995; Holt and Polis, 1997).

Impact on biological control

The abundance of the targeted pest may decrease, at short term scale, cannibalism or higher-order predation through a dilution effect (apparent commensalism) and increase, at long term scale, the number of higher-order predators (apparent amensalism or apparent competition) (Fig. 6).

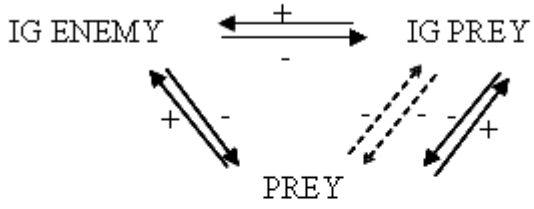


Figure 6: Indirect interaction involving a predator as prey in higher-order predation (via intraguild (IG) enemy). Shown is an example of apparent competition between the shared prey and the intraguild prey (IG prey).

Higher-order predation and cannibalism can reduce the impact of predation on prey species in the community (Finke and Denno, 2003; Claessen et al., 2004; Messelink et al., 2012) because of intermediate predator suppression (Rosenheim et al., 1993; Finke and Denno, 2003) or intermediate predator adaptive behaviors to avoid attacks (Lima, 1987; Lima and Dill, 1990). However the potential risk of higher-order predation disrupting biological control appears to be low in many cases (Janssen et al., 2006). In some cases, the intermediate predator could allow the survival of the top predator species during times of prey scarcity in the agro-ecosystem. This could be beneficial for biological control. For example, cannibalism and higher-order predation may maintain predator populations if the larvae of intermediate predator hatch before preferred prey are present (Batzer and Wissinger, 1996), which could benefit biological control on the condition that higher-order predation drastically decreases when pests are available. Otherwise, higher order predation can reduce apparent competition and disrupt biological control.

Higher-order predation

An intermediate predator and a shared prey species may experience apparent competition or apparent amensalism when the higher order predator experiences a strong numerical response. Moreover, if the higher-order predator and the intermediate predator share the resource, the intermediate predator will also suffer resource competition with the higher-order predator, which could lead to the exclusion of the intermediate predator. However, most higher-order predators feed on intermediate predators only when other prey are not available (Dinter, 1998), which considerably decreases the negative impact of intraguild predation on biological control. Though, there is a lack of long term field experiment that followed population dynamic in biological control situation to study the real effect of the higher-order predator functional response on higher-order predation. Using laboratory experiments, Onzo et al. (2005) determined that intraguild predation occurs between *Typhlodromalus manihoti* and *Eusehis fustis*, two phytoseiid mite predators of the cassava green mite *Mononychellus tanajoa* on cassava in Africa, and that the level of intraguild predation is affected by the availability of the primary prey, *M. tanajoa*. On a diet of intraguild prey alone, the two predator species survived for several days but failed to reproduce. Addition of abundant levels of *M. tanajoa* increased survival and reproduction of the two predator species and reduced intraguild predation to very low levels. In this case there is apparent amensalism between the IGP prey and the pest, as IGP allowed the survivorship when the prey is not available, but drastically decreases when preys appear. However this is not always the case: it had been show for ladybeetle species that aphid addition in the system did not alter the likelihood of IGP occurring, in lab experiment (Snyder et al., 2004; Chacon and Heimpel, 2010).

Cannibalism

Most generalist predators are cannibalistic, and as a consequence, even systems with only one predator and one prey include two prey types: conspecifics and heterospecifics. For most of cannibalistic species, cannibalism decrease with the abundance of heterospecific prey (as for intraguild predation above). The effect of prey availability on cannibalism is particularly well-documented in spider species (Frank et al., 2010; Langellotto and Denno, 2006; Rickers and Scheu, 2005; Rickers et al., 2006), where results consistently show that availability of alternative prey strongly reduces cannibalism between adult spiders and juveniles as well as among juveniles. This is a typical apparent amensalism at long time scales between young predator instars that allow the species to survive when prey is scarce.

10.2 Hyperpredator/hyperparasitoid-mediated indirect interactions between two biocontrol agents

Most empirical evidence for apparent competition concerns herbivores with a shared predator or parasitoids. Apparent competition may also occur on a higher trophic level between predators or parasitoids through hyperpredation or hyperparsitism. Existence of such interactions has been rarely studied, even though many top predators are known to eat several predator species. Systems with two intraguild prey and a hyperpredator are frequently encountered in greenhouses as well (Messelink et al., 2012). Dinter (1998) has shown that the carabid *Pterostichus melanarius* eats two ergonids, *Erigone atra* and *Oedothorax apicatus* and that it prefers *E. atra* over *O. apicatus*, potentially because of greater walking activity of *O. apicatus* females. These results suggest that apparent competition could occur between predators, and the strength of the interaction could be modified by the hyperpredator behavior.

Van Nouhuys and Hanski (2000) have studied the indirect interaction between two parasitoids via a shared hyperparasitoid. They experimentally added a second host species for the hyperparasitoids with which the natural parasitoids did not compete for resources. After the one time addition of the second parasitoid, the natural parasitoid species population declined in the treatment. Unfortunately the reciprocity has not been tested, so we can say that there is at least an apparent amensalism, and that apparent competition is likely to exist in this system. Moreover these interactions could be more complicated in hyperpredation situation because the two predators of the inferior level could interact as well via intraguild predation.

11 CONCLUSION

An understanding of the ways pests and natural enemies interact in complex agro-ecosystems is essential to the development of environment-friendly agriculture (Bunemann et al., 2006; Desneux et al., 2007; Lu et al., 2012). Ecologists and agricultural advisers should be able to recognize patterns of species interactions that could be important for pest control. By reviewing articles about natural enemy mediated interactions between prey, we have demonstrated that such interactions are frequent in agro-ecosystems and have a strong effect on pest dynamics. Most interactions appear asymmetric, but the reciprocity of the interaction is also rarely tested. Moreover in agrosystems, contrary to natural environments, the crop management techniques (e.g. mixed crops, banker plants, cover crop, etc.) will influence the type and impact of predator-mediated indirect interactions. Table 1 summarizes the characteristics and corresponding practices that could be carried out to positively affect enemy-mediated prey interactions in agro-ecosystems.

Nevertheless, natural factors, such as predator and prey behavior and existence of resource competition are also structuring forces. We argue here that agrosystem characteristics and species traits can strongly affect indirect interactions. Knowledge of this specificity for describing community dynamics and to propose adapted biological control programmes to each situation is inevitable. We outline relevant factors whose structuring forces could be identified by tests experimentally conducted in the field and supplemented by theoretical models.

Table 1: Summary of crop characteristics, potential human interventions, and consequent effects on enemy-mediated interactions to enhance biological control.

	Crop characteristics	Human possible interventions	Effects on enemy-mediated interactions
Plant characteristics	Presence of plant-provided food	Cultivar choice	Enhance predator survival and numerical response
	Presence of pest refuges		Preserve food sources for the predator
	Plant resistance to herbivores	Predator species choice depending to the feeding mode in case of artificial releases	Enhance the predator efficiency
Predator characteristics	Behavior (prey preference)	Predator species choice in case of artificial release	Enhance predator efficiency
	Numerical response	Artificial food addition	Enhance predator density
	Higher-order predator	Predator species choice in case of artificial release	Limit negative interaction between biocontrol agents
Prey characteristics	Nutritious value	Banker plants attracting high quality prey	Enhance numerical predator response
	Behavior	No possibilities	
Agrosystem characteristics	Plant diversity	Banker plant	Enhance prey diversity for the predator
		Intercropping	Enhance prey diversity for the predator
		Mixed crop	Enhance prey diversity for the predator
	Soil fauna	Soil practice: no-tillage practice	Enhance prey diversity for the predator
	Perennial crop	Intercropping	Enhance prey diversity for the predator
	Annual crop	Cover crop	Enhance prey diversity for the predator

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II.2.2) La compétition par exploitation et le problème de coexistence des espèces

Bien que la compétition par exploitation intègre toutes les compétitions indirectes pour tous types de ressources (e.g. la nourriture, mais aussi l'espace, les sites de reproduction, etc.), nous nous focaliserons ici sur la compétition pour la nourriture qui sera celle étudiée au cours de ces travaux de thèse.

II.2.2.1) La compétition par exploitation

La compétition pour la ressource a été démontrée par Gause (1934) sur des protozoaires dans un premier temps, puis Park (1954 et 1962) a prouvé que celle-ci dépendait des conditions environnementales. En effet l'existence/l'intensité de la compétition pour la ressource est extrêmement variable selon les conditions, mais cependant un principe est communément retenu depuis l'expansion des travaux sur la compétition, appelé *principe de Gause*, ou *principe d'exclusion compétitive*. Ce postulat établit que deux espèces ne peuvent pas coexister dans un écosystème si elles partagent une ressource commune limitante. Le terme *limitante* signifie ici que l'exploitation de la ressource en diminue la disponibilité et donc limite de ce fait l'accroissement populationnel. Cependant, dans la nature des espèces compétitrices coexistent alors qu'elles partagent une ressource commune. Cela implique l'existence de mécanismes qui vont permettre cette coexistence.

Pour qu'il y ait coexistence, il faut que la compétition intraspécifique soit supérieure à la compétition interspécifique. Actuellement, quatre phénomènes sont reconnus comme favorisant la coexistence à l'échelle d'un écosystème (nous n'aborderons pas ici les théories de coexistence qui concernent les métapopulations, qui ont un intérêt évident en termes d'évolution mais moindre pour le contrôle biologique, en particulier sous serre) : (1) le comportement d'agrégations intraspécifiques (Atkinson et Shorrocks 1981, Hanski 1981, Ives 1988), (2) la diversité du régime alimentaire (Miller 1967, Schoener 1974, Belovsky 1986, Hassell and May 1986) , (3) lorsque la ressource est ségréguée/structurée spatialement ou temporellement (MacArthur 1972, Raventos *et al.* 2010), (4) lorsqu'il y a des compromis entre les traits d'histoires de vie, comme des différences de longévité ou de fécondité (Tilman 1994, Vincent *et al.* 1996, Bonsall *et al*. 2004b).

Il est généralement admis que le terme de compétiteur supérieur correspond à l'espèce qui est capable de supporter les plus bas niveaux de proies et donc à terme va exclure l'autre (Tilman 1987). Une espèce peut être le compétiteur supérieur dans certaines conditions et ne plus l'être dans

d’autres, une espèce ne peut donc se voir attribuer le statut de compétiteur supérieur que dans des conditions précises.

Conséquences en termes de dynamique des populations : La compétition est reconnue comme étant l’un des mécanismes majeurs structurant les écosystèmes. Au cours de l’évolution, elle est à l’origine de répartition des espèces au sein des différents écosystèmes que nous observons actuellement, ceci est parfois appelé « le fantôme de la compétition » (Connell 1980).

En lutte biologique : La problématique se pose surtout en termes de coexistence des auxiliaires de culture sur un même ravageur, cela concerne la lutte biologique classique, la lutte biologique par inoculation et celle par inondation qui reposent sur l’installation de l’auxiliaire dans la culture et, par conséquent, sur la dynamique des populations qui s’établit par la suite. Plusieurs espèces de Braconidae ont été introduites successivement à Hawaï pour contrôler la mouche des fruits *Dacus dorsalis* Hendel (Diptera: Tephritidae) et on a assisté à une disparition et un remplacement successif de chaque espèce par compétition interspécifique (Godfray *et al.* 1994). De même, Murdoch *et al.* (1996) ont mis en évidence le remplacement du parasitoïde *Aphytis lignanensis* Compère (Hymenoptera: Aphelinidae) par *A. melinus* DeBach dans certaines régions de Californie, en raison de sa capacité à se reproduire sur de plus petits hôtes.

II.2.2.2) La coexistence entre espèces compétitrices pour la ressource en nourriture

Bien que la coexistence repose sur des différences qui peuvent se situer au niveau de tous les axes qui composent une niche écologique (e.g. réduction de la prédation, réduction de la compétition, besoins climatiques, etc.), ici, comme dans la partie précedente, nous nous focaliserons sur la ressource en nourriture qui est au cœur de la structuration des réseaux trophiques qui nous intéressent dans ce travail de thèse.

L’agrégation

Lorsque la ressource commune est répartie en patchs, l'hypothèse d'un mélange complet des individus des espèces compétitrices ne s’applique plus. Les individus dans le même patch interagissent, mais pas ceux dans différents patchs. Si les espèces qui utilisent le même type de ressources s’agrègent sur les patchs mais diffèrent dans leur répartition sur ces derniers, cela va réduire les interactions interspécifiques par rapport aux interactions intraspécifiques, la coexistence des espèces en sera donc facilitée (Hassell et May 1986, Godfray *et al.* 1994). Cette idée a été

formalisée comme le «modèle d'agrégation de la coexistence» (e.g. Shorrocks *et al.* 1979, Atkinson et Shorrocks 1981, Hanski 1981, De Jong 1982, Ives et May 1985, Shorrocks et Rosewell 1986, 1987 et 1988, Ives 1991). Des expériences en laboratoire, et en cage sur le terrain, sur des mouches à viande ont montré que l'augmentation des subdivisions de la ressource augmente l'intensité de la compétition intraspécifique (Kneidel 1985, Hanski 1987).

On distingue deux grands types de réponses agrégatives : celle dépendante et celle indépendante de la densité d'hôtes. Les deux types de réponses facilitent la coexistence entre ennemis naturels partageant une même proie, du moment que ces derniers ont des réponses comportementales différentes. Cependant, l'agrégation indépendante de la densité de l'hôte aurait un potentiel plus important (Ives *et al.* 1993).

Le concept de niche écologique et de ségrégation de la ressource

Le concept de niche écologique fut proposé par Grinell (1924), il a d'abord eu le sens d'habitat. Actuellement, la définition la plus utilisée considère qu'une niche écologique est l'ensemble des conditions abiotiques et biotiques dans lesquelles vit une espèce (Hutchinson 1957). Elle est caractérisée par trois composantes majeures (ou dimensions) : l'habitat (distribution spatiale, influence des paramètres physiques et biologiques du milieu), les ressources alimentaires (régime alimentaire, niveau trophique) et l'espace temps (variations saisonnières et nycthémérales de l'utilisation des habitats et des ressources). La niche écologique est propre à chaque espèce et représente l'ensemble des conditions et des ressources dans laquelle celle-ci vit et se perpétue. L'une des principales lois en écologie est que chaque espèce a sa propre niche écologique (Grinnell 1924). En d'autres termes, deux espèces ne peuvent occuper la même niche et coexister. Si deux espèces partagent une même niche, elles entrent en compétition et l'une d'entre elles va disparaître (Gause 1934), à moins que des mécanismes réduisant la compétition interspécifique entrent en jeu conduisant ainsi à la formation d'une association interspécifique. Ces différents mécanismes sont principalement tous ceux qui aboutissent à la ségrégation de la ressource (pour la compétition pour une ressource alimentaire : modes de recherche alimentaire différents, stades de proies/hôtes attaqués différents, etc) mais aussi la prédation (Jeffries et Lawton 1984, Chase et Leibold 2003). Au contraire, si deux compétiteurs partagent une niche seulement en partie alors que la ressource est présente sur l'intégralité de l'aire de distribution des deux compétiteurs, c'est la différence entre les niches écologiques des deux compétiteurs (zone de non recouvrement) qui va induire une ségrégation de la ressource, le compétiteur inférieur pouvant utiliser la ressource là où l'autre

espèce n'est pas. Ceci a été montré dans l'étude de Qvarnström (2009) où l'un des deux compétiteurs profitait de l'incapacité de l'autre espèce à supporter des températures élevées.

La ségrégation (ou structuration) de la ressource

Une caractéristique générale qui se dégage des études théoriques est que la structuration de la ressource commune (Miller 1967, Schoener 1974) et l'acquisition, par conséquent différente, de cette ressource structurée est cruciale pour la promotion de la coexistence d'espèces compétitrices (e.g. van Baalen et Sabelis 1995, Brown *et al.* 1997, Wilson *et al.* 1999). Par exemple, Wilson *et al.* (1999) ont démontré, en théorie, comment la coexistence des gastéropodes sur les microalgues peut survenir grâce à des comportements alimentaires différents entre les «diggers» (~ramasseurs) qui utilisent des patchs fournissant un faible niveau de ressource, au contraire des « grazers» (~brouteurs) qui utilisent des patchs fournissant un haut niveau de ressources. De même, Brown *et al.* (1997) ont montré comment les différences dans l'activité de la recherche de nourriture peuvent favoriser la coexistence entre les gerbilles et les alouettes. Les gerbilles sont de meilleures utilisatrices de la ressource (graines) sous les buissons que dans des micro-habitats ouverts, à l'inverse des alouettes. Bien qu'il s'agit là d'un mécanisme par lequel les ressources sont réparties et la coexistence promue, Brown *et al.* (1997) démontrent également que dans un patch, les gerbilles agissaient comme des "Ramasseuses de miettes" en enlevant toutes les graines tandis que les alouettes agissaient en tant que "Écumeuses de la crème" en prenant seulement une partie de la ressource. Il semble donc que la coexistence repose parfois sur des mélanges complexes de mécanismes, ici la séparation de la ressource via le comportement, et le comportement lui-même permettant une utilisation différente de la ressource sans ségrégation (mode d'utilisation de la ressource).

Même si le mécanisme prédominant, amenant à la ségrégation de la ressource, semble être la ségrégation spatiale, la ségrégation temporelle peut aussi jouer un rôle, voire être complémentaire (Schoener 1974, Baker et Ross 1981). Deux cas de ségrégation temporelle sont à noter : (1) les deux espèces partageant la ressource ne sont pas présentes, du tout ou partiellement, au même moment ; (2) la ressource est structurée en stades de développement différents, les stades attaqués par les deux compétiteurs sont distincts. Chez les arthropodes, Butt et Tahir (2010) ont pu observer une évolution de la structure d'une communauté d'araignées dans une culture de riz avec la phénologie de la culture et la saison. De plus, il apparait que la ségrégation spatiale joue un rôle prédominant dans la réduction de la compétition par interférence (Carothers et Jaksic 1984).

D’autre part, en fonction de leurs stratégies alimentaires et de leurs limitations physiques, les ennemis naturels ont tendance à se spécialiser sur différents stades de développement de la même proie (Brooks et Dodson 1965). Une telle spécialisation peut être considérée comme une forme de partitionnement de la ressource, ce qui en théorie pourrait permettre la coexistence des espèces en compétition (Schoener 1974). Cependant, les différents stades biologiques d’une même proie sont liés en termes de dynamique de population. Même si un prédateur exploite un seul stade, cela va réduire l'abondance des stades suivants. Le prédateur attaquant les premiers stades sera donc favorisé à court terme par rapport au prédateur spécialisé sur les stades suivants. A long terme, le prédateur s’attaquant aux stades les plus âgés va réduire le nombre d’individus adultes et donc la quantité de proie disponible au cycle suivant pour l’autre prédateur. Il a pourtant été démontré qu’il pouvait y avoir coexistence dans un modèle structuré par l’âge, mais les populations s’influencent quand même (Murdoch *et al*. 2003). Par ailleurs, il a été démontré que la coexistence de deux parasitoïdes d’insectes est impossible à moins que celui attaquant les derniers stades puisse aussi attaquer les proies déjà attaquées par son compétiteur (Briggs 1993), auquel cas les deux ennemis naturels sont engagés dans une interaction de prédation intra-guilde.

Le régime alimentaire

Chez des espèces plus ou moins généralistes, voire omnivores, la totalité de la gamme du régime alimentaire ne se recoupe pas forcément. Si deux espèces compétitrices partagent une ressource commune, mais ont chacune une ressource inaccessible à l’autre, cela peut réduire la compétition interspécifique et favoriser la coexistence (Goulson *et al.* 2008). Ceci peut être considéré comme un trait d’histoire de vie. Les traits d’histoires de vie dans leur ensemble seront présentés par la suite.

Un exemple de coexistence entre un spécialiste et un généraliste a été donné par Geervliet *et al.* (2000) qui a démontré la coexistence entre le parasitoïde généraliste *Cotesia glomerata* Linnaeus (Hymenoptera: Braconidae) et le spécialiste *C. rubecula* Marshall, sur des Pieridae. Dans une expérience de plein-champ, les deux parasitoïdes ont été soumis à différentes espèces de *Pieris* sp. (Lepidoptera: Pieridae) et *C. rubecula* a parasité préférentiellement *P. rapae* Linnaeus, tandis que *C. glomerata* s’est attaqué principalement à *P. brassicae* Linnaeus.

Les traits d’histoire de vie et la ségrégation de la ressource

Tilman en 1994 fut l’un des premiers à considérer que la coexistence nécessite l’existence de compromis sur des paramètres comme la compétitivité, la capacité de dispersion, la longévité ou la

fécondité. Ensuite, les écologistes se sont de plus en plus intéressés à cela, montrant qu'en plus des caractéristiques des écosystèmes (e.g. la productivité, la complexité, la stabilité...), les traits des organismes (e.g. la taille du corps, la capacité de dispersion, la fécondité, la période de reproduction...) ont une forte influence sur la coexistence des espèces (Tokeshi 1999). Il semble en effet que les espèces avec certains traits d'histoire de vie sont plus susceptibles de coexister que d'autres. Les différents traits d'histoire de vie peuvent favoriser la spécialisation sur des ressources, et par ce biais favoriser la coexistence. Par exemple, les différences morphologiques, telle que la taille du corps parmi un ensemble d'espèces étroitement apparentées, constituent souvent la raison de l'utilisation de différents types de ressources, qui à son tour facilite la coexistence entre ces espèces (Diamond 1973, Blondel 1995, Brown 1999). De même, des périodes de reproduction différentes au sein d'un groupe d'espèces, i.e ségrégation temporelle, peuvent réduire l'occurrence ou l'ampleur de la compétition pour la ressource (Mosquin 1971, Poole 1979). La dispersion est un autre trait important pouvant influer sur la coexistence des espèces. Si une espèce essaye de coloniser de nouveaux habitats, le succès et le potentiel d'adaptation locale dépendront en grande partie de sa dispersion (Gomulkiewicz et Holt 1995, Kawecki 1995, Holt 1996, Holt et Gomulkiewicz 1997, Kirkpatrick et Barton 1997).

Bonsall *et al.* (2002) a démontré que la durée de vie moyenne de deux parasitoïdes était sensiblement égale, mais que l'un d'eux présentait une plus grande variance de longévité, ce qui a pu permettre à un petit nombre d'individus de coloniser de nouveaux patchs et donc d'acquérir un avantage temporel, leur permettant de surmonter la compétition avec l'autre parasitoïde (le compétiteur supérieur). Ici la longévité résulte en une ségrégation spatiale de la ressource qui va favoriser la coexistence.

De même, dans l'étude de Luna *et al.* (2010) sur deux parasitoïdes de *T. absoluta*, certains traits biologiques de *Dineulophus phtorimaeae* de Santis (Hymenoptera: Eulophidae) (stades de l'hôte parasités plus restreints, fécondité plus faible) ont favorisé la coexistence avec *Pseudoapanteles dignus* Muesebeck (Hymenoptera: Braconidae), malgré une compétitivité supérieure du premier parasitoïde.

III) Un ravageur invasif dans le continent Afro-Eurasien : *Tuta absoluta*

III.1) Les invasions biologiques

La publication en 1958 du livre de Charles Elton a été le point de départ de l'étude systématique des invasions biologiques. Beaucoup de travaux ont été effectués ces dernières années sur toutes les facettes des invasions biologiques (e.g. Richardson et Pyšek 2006, Blackburn *et al.* 2009, Davis 2009) et notre connaissance sur l'occurrence des invasions a été nettement améliorée. Trois questions principales sous-tendent la plupart des travaux effectués en écologie des invasions : Quelles espèces envahissent, quels habitats envahissent-elles, et comment pouvons-nous gérer ces invasions (Pyšek *et al*. 2010) ? Le processus d'invasion peut être conceptualisé en référence au continuum émigration-introduction-établissement-expansion/prolifération, qui suppose qu'une espèce exotique doit passer plusieurs barrières pour pouvoir s'installer et éventuellement devenir invasive (Williamson et Fitter 1996a, Richardson *et al.* 2000). Seulement une fraction des espèces introduites, volontairement ou accidentellement (voir Hulme 2009 pour de plus amples informations), parvient à s'installer/envahir le nouvel écosystème (Williamson et Fitter 1996a, Caley *et al.* 2008).

Le succès d'une invasion biologique dépend des traits d'histoire de vie de l'espèce exotique qui vont lui permettre ou non, de s'adapter aux caractéristiques du nouvel écosystème, de s'y reproduire, de s'y disperser et de survivre à la compétition avec les espèces autochtones (Pyšek et Richardson 2007, Van Kleunen *et al.* 2010), mais aussi de la similitude entre le climat de la zone d'origine et celui de la zone envahie. Les traits contribuants au succès des groupes taxonomiques envahissants ne sont pas universels et doivent correspondre aux spécificités de la communauté envahie, aux conditions géographiques et à un ensemble de facteurs externes incluant la pression de propagule (Richardson et Pyšek 2006). Des effets stochastiques, qui dépendent de l'inoculum initial, du temps de résidence, i.e. du temps depuis l'introduction, de la pression de propagules, i.e. le nombre d'introductions, (Williamson et Fitter 1996b, Lockwood *et al.* 2009) et de leur distribution géographique, co-déterminent si une espèce invasive va devenir envahissante ou non.

La soustraction aux ennemis naturels est un autre mécanisme important favorisant le caractère envahissant d'une espèce exotique. Une espèce introduite dans un environnement ne possédant pas d'ennemis naturels susceptibles de l'attaquer va, en plus de ne plus subir de prédation, pouvoir dépenser moins d'énergie à sa défense et l'allouer à sa croissance et sa dispersion. Au contraire, les interactions avec les autres espèces de l'écosystème envahi vont jouer un rôle clé dans le succès de l'invasion et l'aspect envahissant ou non de l'espèce (Levine 2000, Seabloom *et al.* 2003), via des

interactions directes ou indirectes (voir section *Interactions multi-trophiques*). Par exemple la compétition pour la ressource et/ou la compétition apparente vont potentiellement limiter le caractère envahissant d'une espèce exotique (Case 1990, Levine *et al.* 2004). C'est ce qu'on appelle la résistance biotique d'un écosystème.

III.2) Le cas de *T. absoluta*

III.2.1) L'invasion

Les espèces invasives ont un coût, pour les Etats-Unis par exemple, on estime le nombre d'espèces d'invasives à 50000 espèces avec un coût pour l'économie de 120 billions de dollars par an (Pimentel *et al.* 2005), en partie lié aux espèces invasives en agriculture. Les échanges commerciaux entre les pays, la perturbation des écosystèmes naturels ainsi que les introductions intentionnelles d'espèces exotiques, ont provoqué une augmentation très importante du nombre d'évènements d'invasion dans le monde ces 200 dernières années (Ruiz *et al.* 2000). *Tuta absoluta*, ravageur de la tomate, invasif en Europe, a probablement profité de la mondialisation des échanges commerciaux (Bright 1999, Hulme 2009) pour se disperser, cependant la/les route(s) d'invasion précises ne sont pas encore connues. Arrivée en 2006 en Espagne, l'espèce a été retrouvée pour la première fois en France deux ans plus tard (Desneux *et al.* 2010) et est désormais classée sur la liste A2 de l'OEPP (espèce de quarantaine présente dans cette zone, mais non largement distribuée et officiellement contrôlée). De plus, il est possible qu'elle envahisse d'autres régions représentant des zones climatiques favorables ou produisant beaucoup de tomates sous serre, notamment la Chine et les Etats-Unis (Desneux *et al.* 2011) (Fig. 2). Ces derniers se préparent d'ailleurs à une potentielle invasion et mènent des prospections en vue d'une détection précoce du ravageur (USDA 2011, Lee et Brambila 2012).

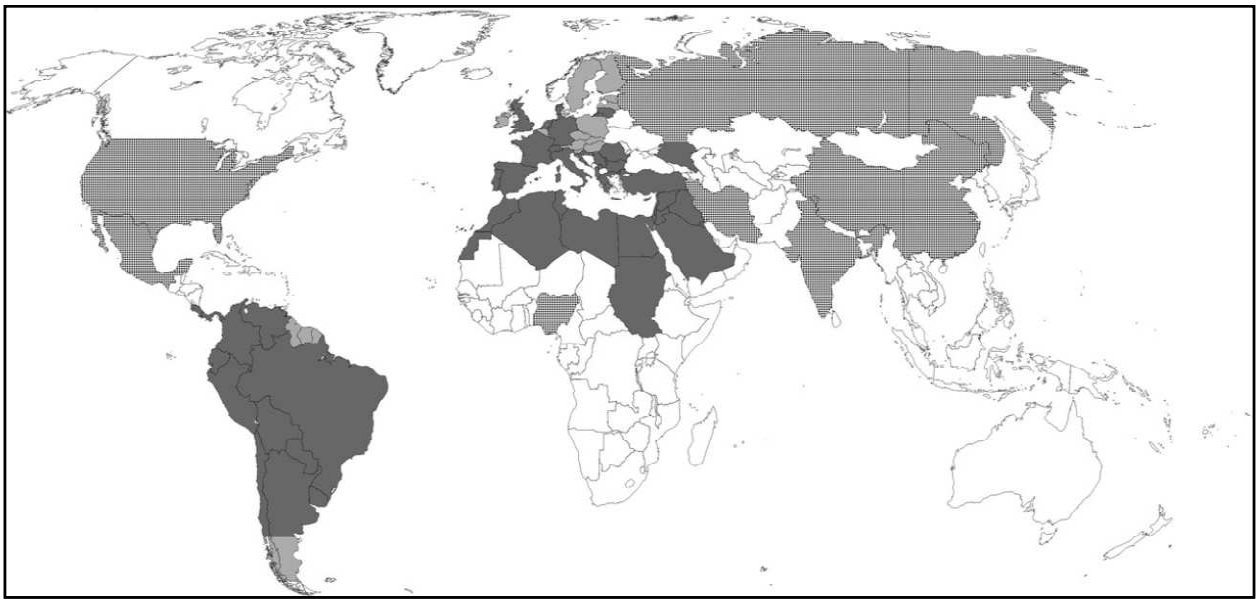


Figure 2 : Pays infestés et risquant d'être infestés par *T. absoluta*: (i) Gris foncé, présence avérée; gris clair, présence probable pour cause de haute proximité géographique avec les pays infestés, gris quadrillé, pays producteurs de tomates risquant d'être infestés (Desneux et al. 2011).

III.2.2) La Biologie de *T. absoluta*

T. absoluta est un Lépidoptère de couleur gris/argenté, qui mesure 6-7 mm de longueur, avec des antennes filiformes. Les femelles sont plus grosses que les mâles, elles peuvent s'accoupler jusqu'à six fois pendant leur vie. La durée de vie des adultes s'étale de 6-7 jours pour les mâles, à 10-15 jours pour les femelles. La longévité des individus varie peu avec la température, bien que les fortes températures favorisent le ravageur (Decoin 2011). Le pic d'oviposition a lieu 7 jours après le premier accouplement (76% des œufs pondus pendant cette période) et une femelle peut pondre un maximum de 260 œufs au cours de sa vie (Uchôa-Fernandes *et al.* 1995). Les adultes pondent souvent sous les feuilles et plus rarement sur les tiges et les fruits. L'activité des adultes est surtout crépusculaire (Desneux *et al.* 2010).

L'hôte principal du ravageur est la tomate (*Lycopersicon esculentum* Miller), mais il attaque un grand nombre d'espèces de Solanaceae cultivées comme l'aubergine, le poivron, la pomme-de-terre ou le tabac, et non cultivées comme la morelle noire ou le pétunia (Desneux *et al.* 2010).

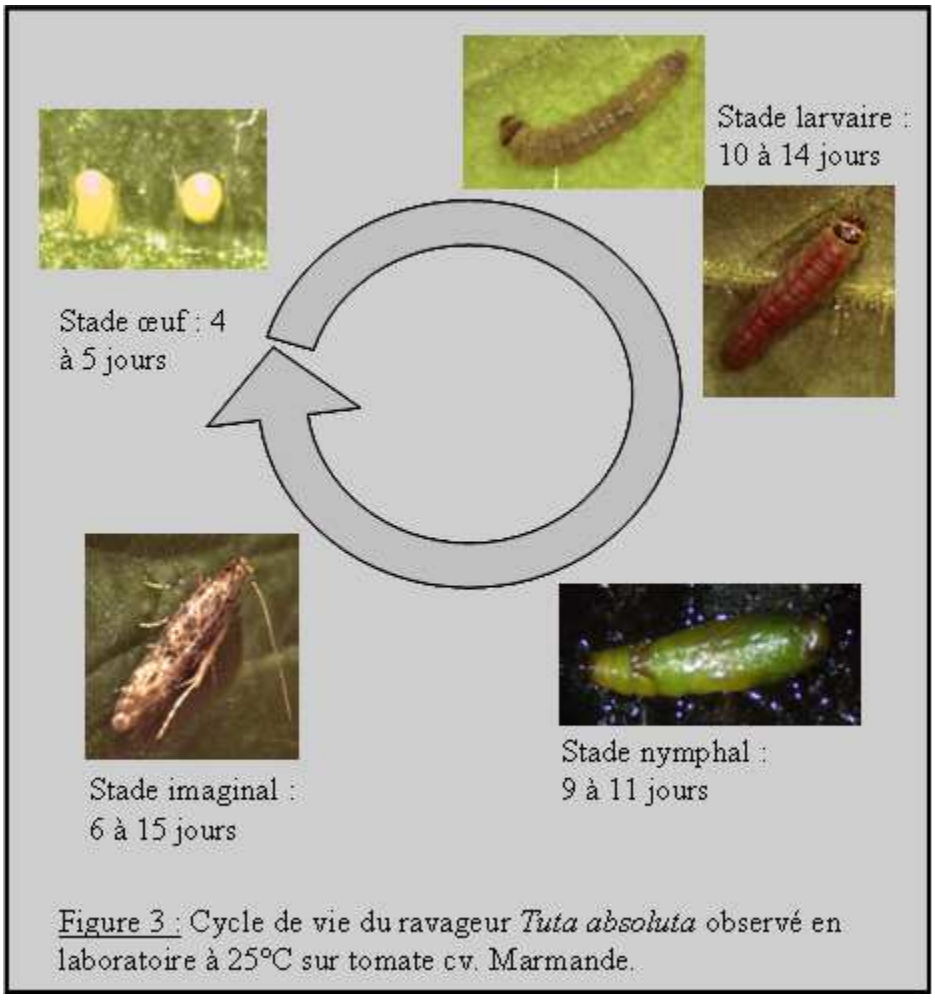


Figure 3 : Cycle de vie du ravageur *Tuta absoluta* observé en laboratoire à 25°C sur tomate cv. Marmande.

Le cycle de *T. absoluta* comprend quatre stades de développement : œuf, 4 stades larvaires, pupe, et imago (Desneux *et al.* 2010) (Fig. 3). Après l'éclosion, les jeunes larves pénètrent dans les organes végétaux (principalement les feuilles) et se nourrissent en développant des mines dans le mésophylle, causant une baisse de la photosynthèse et du développement de la plante (Desneux *et al.* 2010). Les chenilles sont de couleur crème/jaunâtre, avec une capsule céphalique marron foncé, puis deviennent verdâtres et ensuite rose clair au dernier stade. La métamorphose a lieu dans le sol ou dans la feuille, avec création d'un cocon dans ce dernier cas. Il s'agit d'une espèce multivoltine pouvant réaliser jusqu'à 12 générations/an en Amérique du Sud, et dont la durée du cycle de développement dépend des conditions environnementales, notamment de la température. Le développement est d'environ 21 à 24 jours (Barrientos *et al.* 1998) à une température de 26-27°C, et de 33 à 40 jours (Barrientos *et al.* 1998) à 20-21°C.



Figure 4 : Mines de larves de *T. absoluta* dans une feuille de tomate.

La chenille mineuse se nourrit en creusant des mines dans le mésophylle de la feuille (Fig. 4) mais elle peut aussi causer des dégâts sur les fruits, les tiges et l'apex de la plante. Si aucun moyen de lutte biologique est mis en place, les dégâts causés par la chenille peuvent atteindre 100% de la production (Apablaza 1992, López 1991).

III.3) La lutte biologique contre *T. absoluta*

III.3.1) En Amérique du Sud

En ce qui concerne l'Amérique du Sud, très peu de travaux sur la lutte, en particulier biologique, sont disponibles dans la littérature internationale. Des prospections ont cependant permis de mettre en évidence du parasitisme et de la prédation en plein champ. Une large diversité de parasitoïdes oophages et larvaires, regroupant une cinquantaine d'espèces (Desneux *et al.* 2010), a été observée. Certaines espèces apparaissent restreintes à une zone géographique alors que d'autres, sont réparties sur tout le continent Sud-Américain.

Les Encyrtidae, les Eupelmidae, les Trichogrammatidae sont rapportés parmi les parasitoïdes d'œufs, mais les trichogrammes restent la principale espèce utilisée en lutte biologique. Ils ont été l'objet de programmes d'élevage en masse, *T. nerudai* Pintureau, *T. bactrae* Nagaraja, *T. pretiosum* Riley et *T. exiguum* Pinto & Platner ont été élevés avec succès (voir Desneux *et al.* 2010 pour une revue exhaustive).

D'autre part, des parasitoïdes larvaires appartenant aux familles des Bethylidae, Braconidae, Eulophidae, Ichneumonidae et Tachinidae ont aussi été détectés. Les espèces les plus fréquemment rencontrées en culture de tomates appartiennent aux familles des Braconidae et des Eulophidae. En Argentine par exemple, plusieurs espèces ont été rapportées, *Pseudapanteles dignus* (Braconidae) et *Dineulophus phthorimaeae* (Eulophidae) étant les plus fréquemment rencontrés (Berta et Colomo 2000). Le parasitisme peut atteindre jusqu'à 70% (Sanchez *et al.* 2009). D'autres espèces

potentiellement importantes pour la lutte biologique contre *T. absoluta* sont *Neochrysocharis formosa* Westwood (Hymenoptera : Eulophidae) et *Closterocerus formosus* Westwood (Hymenoptera : Eulophidae), qui font actuellement l'objet de recherches en vue de leur utilisation dans des programmes de lutte biologique (Luna *et al.* 2010 et 2011).

Une diversité de prédateurs importante, d'environ 44 espèces (Miranda *et al.* 1998), a aussi été observée. Parmi d'autres, l'acarien *Pyemotes* sp. (Acarina : Pymotidae) qui se nourrit de larves, de pupes et d'adultes, la punaise *Podisus nigrispinus* Dallas (Heteroptera: Pentatomidae) qui se nourrit d'œufs et de larves, ont été étudiés en vue de développer des programmes de lutte biologique. Cependant, les prédateurs restent encore sous-exploités alors qu'ils seraient responsables à eux seuls de 80% des mortalités larvaires de *T. absoluta* (Desneux *et al.* 2010). De plus, jusqu'à récemment, aucune étude n'avait démontré l'utilité d'espèces de Miridae en Amérique du Sud, alors qu'au contraire leur efficacité dans le contrôle de *T. absoluta* sous serre a été remarqué en Europe rapidement après l'invasion (Urbaneja *et al.* 2012 et voir section *En Europe* ci-dessous). Mais Bueno *et al.* ont montré dans un article publié en 2013, que trois espèces de Miridae, i.e. *Campyloneuropsis infumatus* Carvalho, *Engytatus varians* Distant et *Macrolophus basicornis* Stal, attaquent *T. absoluta* au champ. Ils ont aussi prouvé leur efficacité au laboratoire, qui était supérieure à celle de *Geocoris punctipes* (Say) (Hemiptera: Geocoridae) et *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) précédemment relevés comme des prédateurs de *T. absoluta* (Lins et al. 2011, Bueno *et al.* 2012 et 2013).

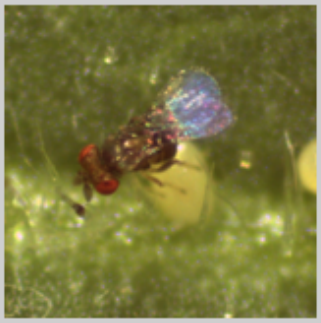
Cependant, la lutte chimique reste le moyen de contrôle le plus utilisé pour limiter les populations de *T. absoluta* (Lietti *et al.* 2005) en Amérique du Sud, et aucun programme de lutte biologique satisfaisant n'est en place à l'heure actuelle.

III.3.2) En Europe

La majorité des infestations et dégâts ayant lieu sous serre, la prophylaxie et une bonne gestion du ravageur, avec une surveillance et une détection précoce dans la culture, restent les meilleurs moyens de les contrôler (Decoin 2011). Ces dernières s'effectuent à l'aide de pièges à phéromones (Svatos *et al.* 1996, Michereff *et al.* 2000) ou de pièges à eau, et le piégeage massif permet également de réduire fortement la pression du ravageur. Dans un premier temps, des moyens de lutte chimique ont été utilisés, notamment le Chlorantraniliprole (commercialisé sous le nom de Rynaxypyr® par Dupond), mais ensuite un accent important a été mis sur la protection biologique. Celle-ci étant déjà bien développée contre d'autres ravageurs en serre de tomates, l'utilisation d'insecticides constituait un risque de déstabilisation de la lutte biologique. De plus, la lutte

chimique se heurte à de multiples problèmes : (1) la capacité de *T. absoluta* à développer des résistances aux insecticides, celles-ci ayant déjà été observées pour de nombreuses molécules e.g abamectine, deltamethrine et spinosad (Siqueira *et al.* 2000, 2001, Lietti *et al.* 2005, Reyes *et al.* 2011) ; (2) les larves sont protégées des insecticides à l'intérieur des galeries ; (3) les produits comme le spinosad et l'indoxacarbe, qui ne sont pas inoffensifs pour les auxiliaires (Biondi *et al.* 2012) et induisent des effets subléthaux sur la physiologie et le comportement de ces derniers (Desneux *et al.* 2007).

Encadré 2: Les trichogrammes (Hymenoptera, Trichogrammatidae)



Le trichogramme est un micro-hyménoptère chalcidien de la famille des Trichogrammatidae. On recense aujourd'hui près de 200 espèces appartenant au genre *Trichogramma*. Ces dernières sont naturellement présentes sur tous les continents et diverses espèces sont utilisées intensivement en lutte biologique par inondation dans plus de 50 pays (Smith 1996). Leurs hôtes principaux sont les lépidoptères, mais on peut les retrouver parasitant d'autres familles comme les chrysopes (Mansfield et Mills 2002) ou les coléoptères (Polaszek *et al.* 2002).

Ils sont particulièrement connus en Europe comme étant le premier succès de lutte biologique en plein champ. *T. brassicae* est en effet utilisé avec succès contre la pyrale du maïs depuis maintenant 20 ans (Pintureau 2009). Leur renommée en lutte biologique vient en particulier de la facilité à les élever. En effet, ceux-ci sont élevés en masse sur des espèces de lépidoptère que l'on est aussi capable d'élever en masse comme *Ephestia kuehniella* Zeller ou *Sitotroga cerealella* Olivier (Thomson *et al.* 2001). De plus, la plupart des populations (communément appelées «souches» pour les trichogrammes) sont capables de se mettre en quiescence ou en diapause ce qui permet le stockage d'œufs parasités et évite ainsi la production en flux tendu des parasitoïdes (Garcia *et al.* 2002).

Ce parasitoïde a un cycle de développement de durée variable en fonction de la température et de l'espèce, cela peut aller de 8 jours à 30°C à 15 jours à 21°C (Foerster et Foerster 2009). Le parasitisme a la particularité de varier énormément au sein d'une même espèce (Chassain et Bouletreau 1991) ce qui explique l'utilisation fréquente du terme souche, usuellement utilisé pour les champignons, à la place du terme population. Certaines espèces sont capables de parasiter jusqu'à 40 œufs en 24h sur *E. kuehniella* en conditions d'élevage.

Par ailleurs, différents agents de lutte sont déjà employés avec succès pour contrôler le ravageur. Le parasitoïde oophage *Trichogramma achaeae* Nagaraja and Nagarkatti (Hymenoptera : Trichogrammatidae) est le seul parasitoïde commercialisé en Europe contre *T. absoluta* à l'heure actuelle, et permet une réduction significative des dégâts. Cabello *et al.* (2012) ont obtenu une baisse de 91% des symptômes dus au ravageur sous serre grâce à cet auxiliaire. L'utilisation des

trichogrammes en lâchers inondatifs entraîne cependant des coûts importants et ne constitue pas une stratégie de lutte durable (Desneux *et al.* 2010) (Encadré 2).

Deux espèces de punaises prédatrices : *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) et *Nesidiocoris tenuis* Reuter (Heteroptera: Miridae) montrent également de bons résultats et ont une prédation active des œufs et larves de *T. absoluta* (Urbaneja *et al.* 2009) (Encadré 3). Leur efficacité a aussi été démontrée en présence d'aleurodes dans les serres (Calvo *et al.* 2012, Bompard *et al.* 2013). Séguret *et al.* (2011) ont pu montrer qu'une association *M. pygmaeus*/*T. achaeae* se révélait concluante, de même avec *N. tenuis* malgré l'observation de prédation intra-guilde (Cabello *et al.* 2012). D'autres espèces de prédateurs, appartenant par exemple au genre *Dicyphus*, ont aussi été rapportées comme consommant des œufs et des larves de stade L1 de *T. absoluta* (Urbaneja *et al.* 2012) mais cette espèce n'est pas commercialisée à l'heure actuelle, au contraire de *N. tenuis* et *M. pygmaeus*.

Encadré 3: *Macrolophus pygmaeus* (Heteroptera, Miridae)



M. pygmaeus est une punaise prédatrice omnivore capable de se nourrir sur les plantes hôtes (pollen, sève et nectar) et sur les proies qui s'y trouvent telles que les aleurodes, pucerons, acariens, thrips, et certains lépidoptères (Fauvel *et al.* 1987). Il est présent naturellement dans les cultures sous serre dans le sud de l'Europe, et est commercialisé comme agent de lutte biologique pour le contrôle des aleurodes en culture sous serre (Bonato *et al.* 2006). Tous les stades (larves et adultes) de *M. pygmaeus* ont une action prédatrice. La prédation est deux à trois fois plus importante entre le premier et le dernier stade larvaire (Fauvel *et al.* 1987).

Ce prédateur a un cycle de développement de durée très variable en fonction de la température, de la nature et de l'abondance des proies. Nourrit sur *T. vaporariorum*, son cycle est d'environ 30 jours à 25-30°C (Fauvel *et al.* 1987). Il pond ses œufs à l'intérieur des tiges et des nervures des feuilles de la plante hôte. La durée du stade œuf est de 11 jours à 25°C. Après éclosion se succèdent cinq stades larvaires, d'une durée totale de 19 jours à 25°C. Les adultes peuvent vivre plus de 3 mois à 25°C, et encore plus longtemps à plus faible température (Fauvel *et al.* 1987). Perdikis et Lykouressis (2002) indiquent une fécondité de 147 œufs/femelle à 25°C sur plants de tomates infestés de *T. vaporariorum*. Dans les mêmes conditions, Hamdan (2006) parle de 32 larves émergées/femelle nourrit sur œufs de *T. vaporariorum*.

Enfin, comme en Amérique du Sud, certaines espèces de parasitoïdes appartenant à la famille des Eulophidae et des Braconidae ont été détectées en serre, en particulier des espèces du genre *Stenomesius* (Fig. 5) et *Necremnus* pour les Eulophidae, ce dernier étant le plus fréquemment retrouvé, et *Bracon* pour les Braconidae (Urbaneja *et al.* 2012, Zappala *et al*. 2012).

Certaines recherches ont aussi été menées du côté de la microbiologie et ont montré l'efficacité, par exemple de *Metarhizium anisopliae* Metsch qui a révélé une bonne efficacité sur le stade œuf (Pires *et al.* 2009).

D'une manière plus générale, d'autres méthodes de lutte non-chimique ont fait leurs preuves, notamment le Bt (*Bacillus thuringiensis*) (Giustolin *et al.* 2001, Molla *et al.* 2011) qui connait cependant certaines limites car, comme les insecticides conventionnels, il n'atteint pas les larves à l'intérieur des galeries, nécessitant donc des traitements répétés. D'autre part, la lutte par l'utilisation de phéromones pour la confusion sexuelle a aussi fait ses preuves (Cocco *et al.* 2013) bien qu'il ait été démontré que les femelles de *T. absoluta* soient capables de deutérotoquie, mais, sans accouplement, la fécondité chute considérablement (Megido *et al.* 2012), de plus cela n'a pas été vérifié sur d'autres souches du ravageur que celle utilisée dans l'étude.

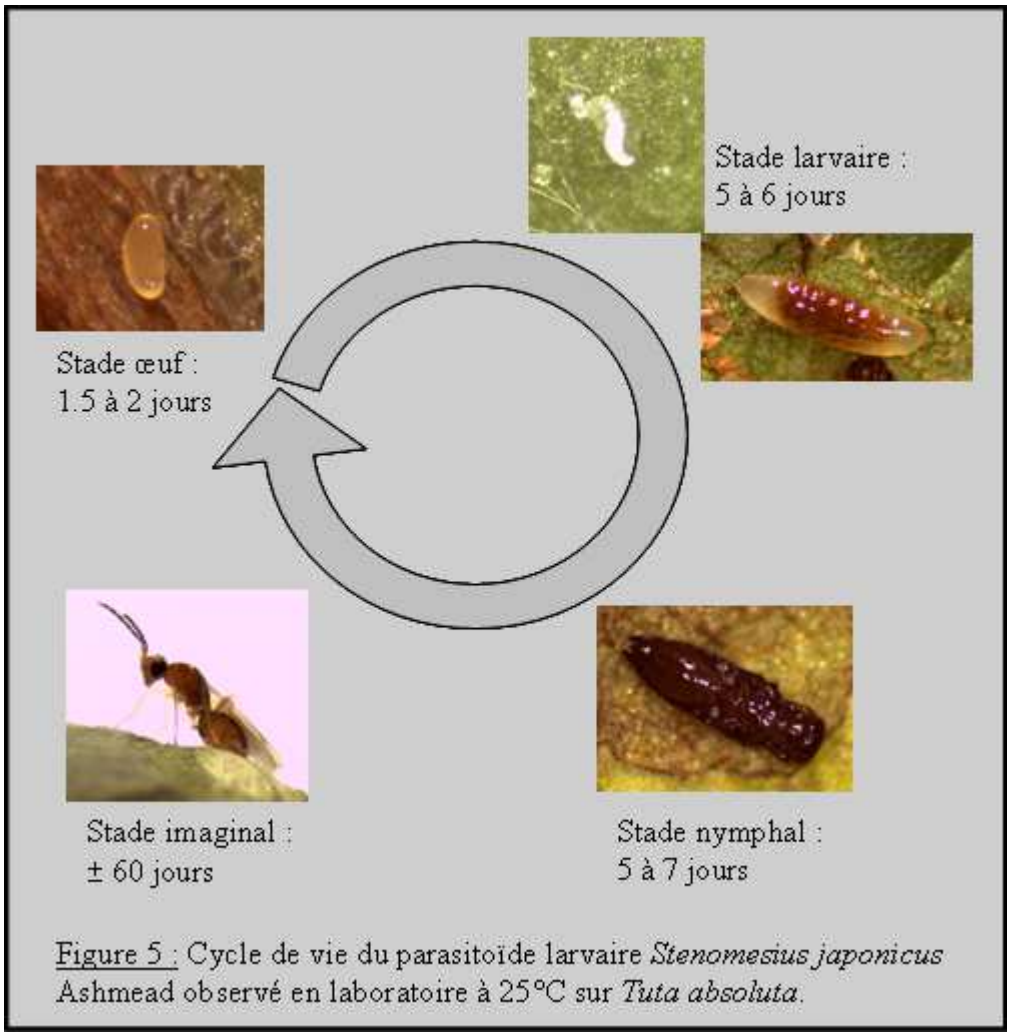


Figure 5 : Cycle de vie du parasitoïde larvaire *Stenomesius japonicus* Ashmead observé en laboratoire à 25°C sur *Tuta absoluta*.

III.3.3) Revue des nouveaux auxiliaires sur *T. absoluta* en Europe 2013

Présenté en tant que *Article 2*

Article 2

Western Palaearctic natural enemies of the Neotropical moth, *Tuta absoluta*, and their use in pest control strategies

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The composition of worldwide biotic communities has greatly changed in recent years due to the collapse of natural barriers to wild species movements mainly in relation to human activities (Liebhold and Tobin 2008). Among the newly-introduced species some can become invasive, with subsequent significant economic impacts. The success or failure of a biological invasion may depend on the species' life history parameters, on its response to climatic conditions, on the competition with native species and the impact of natural enemies (Grabenweger et al. 2010). This last factor is recognized as crucial in the invasion mechanism, as stated by the so called *Enemy Release Hypothesis* the success of an invader, in terms of distribution and abundance, is related to the absence or low efficacy of natural control in the new territories (Keane and Crawley 2002). Indeed, it is assumed that natural enemies in the newly invaded areas, need time to get adapted and to control the exotic species effectively. This may be due to the fact that native antagonists need to adjust their behavior and/or physiology to be able to successfully develop on the exotic prey/host. For this reasons natural enemy complexes on invaders may perform low percentage predation/parasitism (Cornell and Hawkins 1993). However, several examples of successful biological control using natural enemies that have not coevolved with the pest, the so called *New species association*, are also known (Hokkanen and Pimentel 1984; O'Connell et al. 2012).

In this framework, gaining knowledge on indigenous natural enemies that get adapted to the new hosts and understanding their role in limiting the alien species, is essential for establishing the basis of suitable and sustainable control strategies of exotic pests. This applies also to one of the latest invasive species arrived in the western Palaearctic region: the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). This moth is native to South America where it is considered a key pest of tomato (Luna et al. 2012; Guedes and Picanço 2012) and where it remained confined until 2006, when it was recorded for the first time for Western Palaearctic, in Spain (Desneux et al. 2010; Tropea Garzia et al. 2012). Afterwards, it rapidly spread throughout the Mediterranean basin, in Europe, North Africa and Middle East (Desneux et al. 2011). *Tuta absoluta*, for its capacity to develop very quickly on tomato cultivations and to spread rapidly in new areas causing

economically relevant damage, is considered a typical invasive species (Desneux et al. 2010; Caparros Megido et al. 2012).

Although chemical control has been the first strategy adopted in the newly invaded areas, alternative control measures are being investigated (Cagnotti et al. 2012; Cocco et al. 2012). This is mainly due the development of resistance to chemicals by the pest (Haddi et al. 2012; Gontijo et al. 2013) and to the side effects of pesticides on beneficials as well (Arnó and Gabarra 2011; Biondi et al. 2012). On the other hand, various predators and parasitoids spontaneously attack *T. absoluta* in tomato crops in Europe. Some of these, mainly native Miridae, have been already employed in Integrated Pest Management (IPM) (Mollá et al. 2011; Cabello et al. 2012; Zappalà et al. 2012b; Chailleux et al. 2013). However, several screenings for effective natural enemy species in the invaded area are still ongoing (Chailleux et al. 2012; Urbaneja et al. 2012).

Almost sixty species of generalist natural enemies have been reported developing on *T. absoluta* in the Western Palaearctic region so far. These have been sampled both on open field and protected susceptible crops as well as on wild flora and/or using infested sentinel tomato plants. Here we take into account all the available data aiming at giving a comprehensive picture of the composition of the species that spontaneously provide biological control services and their current role in *T. absoluta* control programmes.

Indigenous predators

Thirteen arthropod Western Palaearctic species were recorded preying on the South American tomato leafminer in the last few years (Table 1). They mainly belong to the Hemiptera order (ten species) and in particular to the families Miridae, Anthocoridae and Nabidae in decreasing order of numerousness. These predators, including zoophytophagous bugs, usually colonize and establish in organic and IPM crops as they are able to build up their populations before pests arrive exploiting alternative preys and host plants as alternative food sources (Perdikis et al. 2007; Desneux and O'Neil 2008; Ingegno et al. 2008).

The most widely spread species are those of the subfamily of the Dicyphinae, with *Nesidiocoris tenuis* (Reuter) spontaneously recovered in eleven countries almost all year round both in protected and open field tomato crops, and *Macrolophus pygmaeus* (Rambur) which was observed preying on *T. absoluta* eggs and young instar larvae in three countries. Guenaoui et al. (2011) recorded *M. caliginosus* Wagner [= *M. melanotoma* (Costa)], but considering that the discrimination between the closely related predatory species *M. melanotoma* and *M. pygmaues*, based exclusively on morphological, is rather uncertain, the record of *M. caliginosus* is likely to be due to a misidentification. Indeed, the classification history of the two species shows a great number of misconceptions that have left their identity in confusion and highlight the need for a

comprehensive taxonomical treatment (Martinez-Cascales et al. 2006). Other four Dicyphinae species (*Dicyphus* sp., *D. errans*, *D. maroccanus* and *D. tamanini*) were sampled from infested tomato plants in Algeria, France, Italy and Spain. Anthocoridae bugs belonging to the *Orius* genus were found feeding on in Jordanian open field and protected tomato crops infested by *T. absoluta.*

Species of the *Nabis* genus were found occasionally in Iran and in Spain. Whereas, two species of predatory mites (*Amblyseius swirskii* Athias-Henriot and *A. cucumeris* Oudemans) were also reported preying on *T. absoluta* eggs and first instar larvae in Spain. One unidentified species of Hymenoptera Sphecidae was found in Spain feeding on larval instars of the moth (Table 1).

Table 1. Overview of predators recovered on *Tuta absoluta* in Western Palaearctic area.

Order Family	Species	Known distribution[1]	*T. absoluta* instars	Country(ies)	Sampling method(s)	Season(s)	Reference(s)
Mesostigmata: Phytoseiidae	*Amblyseius swirskii* Athias - Henriot	Western Palaearctic	Eggs and L1	Spain	Protected crop (aubergine) sampling	Summer	Mollá et al. 2010
	Amblyseius cucumeris Oudemans	Cosmopolitan	Eggs and L1	Spain	Protected crop (aubergine) sampling	Summer	Mollá et al. 2010
Hemiptera: Miridae	*Dicyphus* sp.		Eggs and young larvae	France, Italy	Open field and protected crop sampling	Summer	Biondi et al. 2013a ; Zappalà et al. unpublished data
	Dicyphus errans (Wolff)	Western Palaearctic	Eggs and L1	Algeria, Italy	Open field and protected crop sampling	Spring-autumn in the open field; all year round in greenhouses	Boualem et al. 2012; Tavella et al. in preparation
	Dicyphus marrocannus Wagner	Europe	Eggs and young larvae	Spain	Open field and protected crop sampling	Summer	Mollà et al. 2010
	Dicyphus tamaninii Wagner	Western Palaearctic	Eggs and young larvae	Algeria,	Not specified	Not specified	Guenaoui et al. 2011
	Macrolophus pygmaeus (Rambur)	Western Palaearctic	Eggs and young larvae	Algeria, France, Italy, Spain	Open field and protected crop sampling	Spring, summer, autumn	Arnò et al. 2009; Biondi et al. 2013a; Boualem et al. 2012; Guenaoui et al. 2011; Mollà et al. 2010; Tavella et al. in preparation
	Nesidiocoris tenuis (=*Cyrtopeltis tenuis*) (Reuter)	Cosmopolitan	Eggs and young larvae	Algeria, Cyprus, Egypt, France, Jordan, Iran, Israel, Italy, Morocco, Spain, Turkey	Open field and protected crops sampling	Spring, summer, autumn, winter	Al-Jboory et al. 2012; Arnò et al. 2009; Biondi et al. 2013a; Boualem et al. 2012, R. Bouharroud pers. comm.; El-Arnauty and Kortam 2012; Guenaoui et al. 2011; Martinou and Stavrinides unpublished data; Rizzo et al. 2011; Kiliç, personal communication, Shaltiel-Harpaz and Gerling, unpublished data
Hemiptera Anthocoridae	*Orius* sp.		Not specified	Jordan	Open field and protected crop sampling	January-April	Al-Jboory et al. 2012
	Orius albidipennis (Reuter)	Western Palaearctic	Not specified	Jordan	Open field and protected crop sampling	January-April	Al-Jboory et al. 2012

Hemiptera Nabidae	*Nabis* sp.		Eggs and young larvae	Iran	Open field crop sampling	Summer	H. Madadi pers. Comm.
	Nabis pseudoferus ibericus Remane	Western Palaearctic	Not specified	Spain	Not specified	Not specified	Mollá et al. 2010
Hymenoptera Sphecidae	Undetermined species		Larvae	Spain	Not specified	Not specified	Mollá et al. 2008

[1]Kerzhner and Josifov 1999.

Indigenous parasitoids

A quite large number of parasitoids species (45) was recorded developing on all the young instars of the moth in the newly invaded areas (Table 2). Overall, the most abundant parasitoid family was the Eulophidae one with twenty-five recovered species. *Neochrysocharis formosa* (Westwood) [= *Closterocerus formosus* (Westwood)] was one of the most widely spread, being recovered in 4 countries (Italy, Algeria, Spain, France). So far, this is the only species recovered on *T. absoluta* both in Europe and in South America, where it was mentioned as a potential biocontrol agent based on its wide host range (Noyes 2013) and previous use in other crops (Luna et al. 2012). Another species belonging to the same genus, *Closterocerus clarus* (Szelenyi) was recovered on *T. absoluta* young larvae in Turkey. Six species belonging to the genus *Necremnus* were recovered on *T. absoluta* in Algeria, Egypt, France, Italy, Spain and Tunisia. Two entities were identified as *N.* sp. near *artynes* and *N.* sp. near *tidius* as the taxonomy of this species group is currently under revision (Ferracini et al. 2012; Zappalà et al. 2012a). *Necremnus artynes* (Walker) and *Necremnus tidius* (Walker) are generalist solitary ectoparasitoids of lepidopteran leafminers (Noyes 2013). Urbaneja et al. (2012) found another *Necremnus* species, *N. metalarus* Walker, developing on *T. absoluta*-infested tomato plants in Spain. The ectoparasitoids of Diptera, Lepidoptera and Coleoptera leafminers larvae, *Pnigalio incompletus* (Bouček) and *P. cristatus* (Ratzeburg), often associated due to their shared hosts (Noyes 2013), emerged from parasitized *T. absoluta* larvae in both Italy and Turkey. While, wasps identified as *P. soemius* species complex were recovered only in Italy (Table 2). This is a Palaearctic complex of generalist parasitoids, with an intense predatory behavior both as larva and as adult (Bernardo et al. 2006).

Stenomesius near *japonicus* was recovered in France and in the North-East of Spain on *T. absoluta* 2nd and 3rd instar larvae and an unidentified species belonging to the same genus was found in Algeria. Specimens of *Sympiesis* sp. near *flavopicta* and of *Hemiptarsenus ornatus* Nees emerged from larvae collected in Israelian tomato open field tomato crops. Whereas, another *Hemiptarsenus* species, *H. zilahisebessi* Erdös, as well as *Digliphus iseae* Walker were found in association with *T. absoluta* in Algeria. Specimens classified as *Elachertus inunctus* species group

emerged from artificially infested sentinel plants in Italy and other identified as *Baryscapus bruchophagi* (Gahan) were found in Turkey. Finally, five other Eulophid species, not identified at the specific level (*Chrisocharis* sp., *Cirrospilus* sp., *Dyglyphus* sp., *Elachertus* sp. and *Sympiesis* sp.), were also found parasitizing spontaneously the new host (see Table 2 for details).

Almost 30% of the recovered species were Ichneumonoidea, more precisely four species belonged to the Ichneumonidae family and the remaining eight to the Braconidae one. Among the four Ichneumonids those belonging to the *Diadegma* genus (*Diadegma* sp., *D. pulchripes* (Kokujev) and *D. ledicola* Horstmann) were found parasitizing *T. absoluta* mature larvae and pupae in Italy. Whereas, the other Ichneumonid, *Hyposoter didymator* (Thunberg), was recorded only in Algeria on unspecified host instar stage. Among Braconid wasps the only species found on wild flora was *Agathis fuscipennis* Zetterstedt, recovered in Italy on infested *T. absoluta-Solanum nigrum* (Table 1). *Bracon* species were already reported as *T. absoluta* parasitoids in the pest native areas (Desneux et al. 2010) and several species belonging to this genus were found developing on the exotic pest in the newly invaded areas. Some of these were found in various countries, such as *B. hebetor* Say, a worldwide distributed and very polyphagous species (Yu and van Achterberg 2010), which was recovered on *T. absoluta* in Israel, Italy and Turkey. The Palaearctic species *B. nigricans* (Szépligeti) was recorded parasitizing *T. absoluta* mature larvae in France, Italy, Jordan and Spain. Whereas, *B. osculator* (Nees) and *B. didemie* Beyarslan were found only in Italy and in Turkey, respectively. At the same time, another two braconid species not identified at the specific level, *Aghatis* sp. and *Bracon* sp., emerged from parasitized larvae collected in Italy and Tunisia.

Two Pteromalid wasp species, *Haticoptera enea* (Walker) and *Pteromalus intermedius* (Walker), were found developing on the moth larvae in Italy and in Turkey, respectively. Moreover, two species of Calcidid wasps, *Brachymeria secundaria* (Ruschka) and *Hockeria unicolor* (Walker), were associated with *T. absoluta* in Turkey. Whereas only one species of Elasmid, *Elasmus* sp., was found in Italy. *Tuta absoluta* eggs were parasitized spontaneously by *Trichogramma achaeae* Nagaraja & Nagarkatti in France, by *T. euproctidis* (Girault) in Egypt and by various other *Trichogramma* not identified species in Algeria, France, Iran and Spain (Table 2).

Table 2. Parasitoids recovered on *Tuta absoluta* in Western Palaearctic countries.

OrderFamily	Species	Known distribution[2]	*T. absoluta* instars	Country(ies)	Sampling method(s)	Season(s)	Reference(s)
Hymenoptera Ichneumonidae	*Diadegma* sp.		Mature larvae-pupae	Italy	Open field crop sampling	Autumn	Zappalà et al. 2012a
	Diadegma ledicola Horstmann	Western Palaearctic	Mature larvae-pupae	Italy	Open field crop sampling	Summer, autumn	Ferracini et al. 2012
	Diadegma pulchripes (Kokujev)	Palaearctic	Mature larvae-pupae	Italy	Open field (potato) crop sampling, sentinel infested plant	Summer, autumn	Zappalà et al. 2012a
	Hyposoter didymator (Thunberg)	Australia, Western Palaearctic	Not specified	Algeria	Protected crop sampling	Spring	Boualem et al. 2012
Hymenoptera Braconidae	*Agathis* sp.		Larvae not specified	Italy	Open field crop sampling	Summer	Ferracini et al. 2012
	Agathis fuscipennis Zetterstedt	Western Palaearctic		Italy	Open field sampling of infested *Solanum nigrum*	September - October	Loni et al. 2011
	Bracon sp.		Mature larvae	Tunisia	Sentinel infested plants exposure	Spring, summer	Abbes et al. 2013
	Bracon (=*Habrobracon*) *didemie* Beyarslan	Turkey	Mature larvae	Turkey	Open field and protected crop sampling	Spring	Doganlar and Yigit 2011
	Bracon (=*Habrobracon*) *hebetor* Say	Cosmopolitan	Mature larvae	Israel, Italy, Turkey	Open field and protected crop sampling	Spring, Summer	Ferracini et al. 2012a; Doganlar and Yigit 2011; Shaltiel-Harpaz and Gerling, unpublished data
	Bracon (=*Habrobracon*) *nigricans* (=*concolorans, concolor, mongolicus*) Szépligeti	Palaearctic	Mature larvae	Egypt, France, Italy, Jordan, Spain	Open field and protected crop sampling, sentinel infested plants	Spring, Summer	Al-Jboory et al. 2012; Biondi et al. 2013a; El-Arnaouty unpublished data; Urbaneja et al. 2012; Zappalà et al. 2012a
	Bracon (=*Habrobracon*) sp. near *nigricans*		Mature larvae	Israel, Spain	Open field crop sampling; sentinel infested plants	Spring, Summer	Gabarra and Arnò 2010; Shaltiel-Harpaz and Gerling, unpublished data
	Bracon (=*Habrobracon*) *osculator* (Nees)	Palaearctic	Mature larvae	Italy	Open field and protected crop sampling, sentinel infested plants	Summer, autumn	Ferracini et al. 2012; Zappalà et al. 2012a
Hymenoptera: Chalcididae	*Brachymeria secundaria* (Ruschka)	Turkey	Larvae not specified	Turkey	Protected crop sampling	Spring	Doganlar and Yigit 2011
	Hockeria unicolor (Walker)	Turkey	Larvae not specified	Turkey	Protected crop sampling	Spring	Doganlar and Yigit 2011
Hymenoptera: Elasmidae	*Elasmus* sp.		Larvae not specified	Italy	Open field crop sampling, sentinel infested plants	Summer	Zappalà et al. 2012a
Hymenoptera Eulophidae	*Baryscapus bruchophagi* (Gahan)	Turkey	Not specified	Turkey	Protected crop sampling	Spring	Doganlar and Yigit 2011
	Chrysocharis sp.		Larvae not specified		Protected crop sampling, sentinel infested plants	Spring, summer, autumn	Zappalà et al. 2012a

Cirrospilus sp.		Larvae not specified	Algeria	Protected crop sampling	Spring	Guenaoui unpublished data
Closterocerus clarus (Szelenyi)	Turkey	L1	Turkey	Protected crop sampling	Spring	Doganlar and Yigit 2011
Diglyphus sp.		L2	Algeria	Protected crop sampling	Spring	Guenaoui, unpublished data
Diglyphus isaea Walker	Australian, Nearctic, Palaearctic, Oriental	Larvae not specified	Algeria	Protected crop sampling	Spring	Boualem et al. 2012
Elachertus sp.		Larvae not specified	Italy	Sentinel infested plants	Autumn	Zappalà et al. 2012a
Elachertus inunctus species group		Larvae not specified	Italy	Sentinel infested plants	Spring	Zappalà et al. 2012a
Hemiptarsenus ornatus (Nees)	Palaearctic, Oriental	Larvae not specified	Israel	Open field crop sampling	Not specified	Shaltiel-Harpaz and Gerling unpublished data
Hemiptarsenus zilahisebessi Erdös	Palaearctic	L2	Algeria	Protected crop sampling	Not specified	Guenaoui unpublished data
Necremnus sp.		Larvae not specified	Italy	Open field crop sampling	Spring	Zappalà et al. 2012a
Necremnus artynes (Walker)	Palaearctic and Nearctic (USA)	L2-L3	Algeria, Egypt, Spain, France	Open field and protected crop (tomato, aubergine) sampling; *Solanum nigrum*, sentinel infested plants	Spring, summer	Boualem et al. 2012; Mollà et al. 2010; Delvare et al. 2011; Gabarra and Arnò 2010; Guenaoui unpublished data, El-Arnauty unpublished data, Kolai et al. 2011, Rizzo et al. 2011
Necremnus near *artynes*		L1-L2-L3	Italy, France, Tunisia	Open field and protected crop sampling, sentinel infested plants	Spring, summer	Abbes et al. 2013; Biondi et al. 2013a; Ferracini et al. 2012; Zappalà et al. 2012a
Necremnus metalarus Walker	Western Palaearctic and Nearctic (USA)	L2-L3	Spain	Open field and protected crop sampling	Not specified	Urbaneja et al. 2012
Necremnus tidius (Walker)	Palaearctic and Neartic (USA)	Not specified	Italy	Not specified	Not specified	Riciputi 2011
Necremnus near *tidius*		L1-L2	Italy	Open field and protected crop sampling	Spring, summer	Ferracini et al. 2012; Zappalà et al. 2012a
Neochrysocharis sp.			Algeria	Protected crop sampling	Spring	Boualem et al. 2012
Neochrysocharis formosa (Westwood) (=*Closterocerus formosus*)	Cosmopolitan	L1-L2-L3	Algeria, France, Italy, Spain	Open field and protected crop sampling	Spring, summer	Biondi et al. in press; Ferracini et al., 2012; Guenaoui unpublished data; Lara et al. 2010; Zappalà et al. 2012a
Pnigalio (=*Ratzeburgiola*) *cristatus* (Ratzeburg)	Palaearctic	L1-L2	Italy, Turkey	Open field and protected crop sampling, sentinel infested plant	Spring, summer, autumn	Doganlar and Yigit 2011; Ferracini et al. 2012a; Zappalà et al. 2012a
Pnigalio sp. *soemius* complex		L1-L2	Italy	Open field and protected crop sampling	Summer, autumn	Ferracini et al. 2012; Zappalà et al. 2012a
Pnigalio incompletus (Boucek) (=*Ratzeburgiola incompleta*)	Western Palaearctic	Not specified	Italy, Turkey	Protected crop sampling	Spring	Doganlar and Yigit 2011; Zappalà et al. 2012a
Stenomesius sp.		L2- L3	Algeria	Protected crop sampling	Spring	Guenaoui unpublished data
Stenomesius sp. near *japonicus*		L2- L3	France, Spain	Open field and protected crop sampling, sentinel infested plant	Spring, summer	Biondi et al. in press; Gabarra and Arnò 2010

	Sympiesis sp.		Not specified	Algeria, Italy	Protected crop sampling, sentinel infested plants	Spring	Boualem et al. 2012 ; Zappalà et al. 2012a
	Sympiesis sp. near *flavopicta*		Not specified	Israel	Open field crops sampling	Not specified	Shaltiel-Harpaz and Gerling unpublished data
Hymenoptera: Pteromalidae	*Halticoptera aenea* (Walker)	Nearctic, Palaearctic	Larvae not specified	Italy	Sentinel infested plants	Spring	Zappalà et al. 2012a
	Pteromalus intermedius (Walker)	Turkey	Larvae not specified	Turkey	Protected crop sampling	Spring	Doganlar and Yigit 2011
Hymenoptera: Trichogrammatidae	*Trichogramma* spp.		Eggs	Algeria, France, Iran, Spain	Protected crop sampling, sentinel infested plants	Spring, summer, autumn	Boualem et al. 2012; Biondi et al.2013a; Gabarra and Arnò 2010 ; H. Madadi pers. Comm.; Zappalà et al. 2012a
	Trichogramma achaeae Nagaraja & Nagarkatti	Nearctic, Neotropical, Oriental, Palaearctic	Eggs	France	Protected crop sampling	Summer	Biondi et al. 2013a
	Trichogramma euproctidis (Girault)	Nearctic, Neotropical, Palaearctic	Eggs	Egypt	Open field crop sampling	Autumn	El-Arnaouty unpublished data

[2]Yu and Van Actherberg 2010; Noyes 2013.

Potential for use of indigenous natural enemies

Many experiments have been conducted in laboratory or semi-field conditions to assess the suitability of *T. absoluta* for various predator and parasitoid species. The pioneer study of Urbaneja et al. (2009) proved how *N. tenuis* and *M. pygmaues* adults do feed on eggs and young larvae of the moth. Similar results were obtained by Cabello et al. (2009) studying *N. pseudoferus ibericus* and by Arnò et al. (unpublished data) for the bugs *D. tamaninii*, *O. majusculus* and *O. laevigatus.*

Other studies aimed to assess the biology of predators (Mollà et al. 2013) and the biology and behavior of parasitoid species on the new host. In the case of parasitoids it has been shown that under laboratory conditions *N.* sp. near *artynes*, *N.* sp. near *tidius* and *B. nigricans* were able to reduce significantly *T. absoluta* population not only owing to the parasitization activity but also thanks to a non reproductive host-killing activity, namely host feeding and host stinging behaviors (Ferracini et al. 2012; Biondi et al. 2013b).

Besides the *environmental resistance* that all the recovered fortuitous natural enemies can spontaneously offer in the realistic field conditions there are several approaches that can be artificially implemented to enhance their role in regulating pest populations. Indeed, these indigenous natural enemies can be *augmented* and *conserved* in the cultivated environment. Inoculation of mass reared *N. tenuis* has been successful applied in tomato nurseries for the early installation of the predator population in the young crop (Calvo et al. 2012), or directly in greenhouse with the concomitant application of microbial pesticides (Desneux et al. 2010; Mollá et

al. 2011). However, on one hand, although *N. tenuis* is largely employed in biological and integrated *T. absoluta* control programs (Abbes and Chermiti 2012; Nannini et al. 2012; Trottin-Caudal et al. 2012), its use often prompts insecticide applications when predator density reachs high levels because of damages it inflicts to both plants and fruits (Calvo et al. 2009). On the other hand, *M. pygmaeus* has been recently demonstrated to be not able to build up its populations when feeding only on this prey (Mollà et al. 2013). Thus, higher levels of prey species diversity are required for effective inoculative programs of this predator species (Bompard et al. 2013).

Commercially available *T. acheae* individuals are used in inundative releases and these have been demonstrated to be efficient in pest control under greenhouse conditions (Cabello et al. 2012; Trottin-Caudal et al. 2012). Lower density can be combined with mirid predators obtaining similar control levels (Calvo et al. 2012). Similar control levels were obtained in Southern Spain with multiple releases of *N. artynes*, although the reduction did not limit fruit damage to the level reached by *N. tenuis* released in the nursery (Urbaneja et al. 2012); furthermore, contrary to those advocated by some companies in the past years, the Eulophid is not commercialized so far.

Future outlooks

Many of the entomophagous species recovered in these past few years have been in the past recorded as widely diffused on tomato crops also in those countries in which they are not been yet found in association with *T. absoluta*. Thus, it is likely that this species will be associated to *T. absoluta* in other countries very soon (Baniameri and Cheraghian 2012). For these further surveys in those areas with still few records of *T. absoluta* natural enemies are encouraged. On the other hand, other aspects of their biology and their ecology should be also further investigated. This is particularly true for those species with an uncertain taxonomy, since different biological and ecological traits can be highlighted among different cryptic species (Desneux et al. 2009). Furthermore, in order to set up potential commercial mass rearings and/or to commercialize natural enemies among countries, their taxonomy should be definitively clarified.

The overall increase of knowledge on the indigenous natural enemy complex would help all the habitat management strategies. These should be aimed at increasing the functional biodiversity within the crop and within the farm, such as rational weeds management for increasing the food and alternative preys/hosts for indigenous predators and parasitoids (Gardiner et al. 2009; Tena et al. 2013). The increase in the abundance and diversity of the natural enemy community could be also obtained by the use of the *banker plants* technique, i.e. a tri-trophic system which typically consists of a non-crop plant that is deliberately infested with a non-pest herbivore (Parolin et al. 2012). In

this context, increasing knowledge on the prey/host range of these generalist entomophagous is crucial. Indeed, many are the potential applications and in our case one of them is for example the installation in the tomato crop of *Parietaria officinalis* L. plants infested by *Cosmopterix pulchrimella* Chambers (Lepidoptera: Cosmopterigidae) [an alternative host of *N. artynes* (Ferracini et al. 2012)], thus enhancing the parasitoid population.

In order to reduce the cost of the multiple egg parasitoid releases (Cabello et al. 2012) and/or the plant damage of the released omnivorous predators (Calvo et al. 2009), further study aiming at setting economically sound of mass rearing protocols of other indigenous natural enemies have to be developed. These studies should aimed at rearing entomophagous species showing the least secondary effects for the plants i.e. avoiding phytofagy, and for other beneficials present in the crop, i.e avoiding intraguild predator.

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Chapitre 2 : Les interactions indirectes liées à l'invasion et la perturbation potentielle de la lutte biologique préexistante

Présenté en tant que *Article 3*, en préparation, invité dans *Journal of Pest Science*,

voir l'Annexe 1 pour une photo du dispositif expérimental.

Article 3

Predator-mediated apparent competition between pests fails to prevent yield

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Predator-mediated apparent competition between pests fails to prevent yield

Coline Jaworski, Anaïs Chailleux, Philippe Bearez, Nicolas Desneux

Abstract

Indirect interactions among pests (or resource), e.g. apparent competition, have been manipulated in various agro-ecosystems to strengthen the control of pests by generalist predators. However the nature and strength of biotic interactions within a network strongly depend on species, and the effects of alternative resource on biocontrol agents do not always result in an increased control of the target pest species. Therefore, it is necessary to characterize both the direct and indirect interactions that are likely to happen between co-occurring pest species and biocontrol agents for an efficient IPM program. We measured the strength of apparent competition between two major tomato pests *Tuta absoluta* and *Bemisia tabaci* preyed by a generalist predator, the mirid *Macrolophus pygmaeus*, through monitoring of pests and predator population dynamics in a factorial greenhouse experiment. We also assessed the levels of damages due to pests on plants and fruits. Our results showed an asymmetric apparent competition between the pests, with: (i) a numerical response of *M. pygmaeus* to *B. tabaci* alone; (ii) a lack of numerical response to *T. absoluta* alone; (iii) an increased numerical response in presence of both prey; (iv) a strong negative indirect impact of *T. absoluta* on *B. tabaci*; and (v) a weak negative indirect impact of *B. tabaci* on *T. absoluta*. Although there was an increased numerical response of the predator, the damages observed on plants and fruits were not reduced in the presence of both prey when compared to *T. absoluta* alone, notably because *M. pygmaeus* hardly controlled *T. absoluta* populations in the conditions of our experiment. Using *M. pygmaeus* alone for management of *T. absoluta* would likely not be sustainable in tomato crops, even if the predator development can be enhanced by other co-occurring pest species in the crop, e.g. whiteflies.

Keywords: indirect interaction; generalist predator; asymmetric interaction; *Tuta absoluta*; *Macrolophus pygmaeus*; *Bemisia tabaci*.

Introduction

In the past decades, there has been an increasing interest toward Integrated Pest Management (IPM) practices relying on the release of pests' natural enemies (so-called biocontrol agents), notably for pesticide reduction and biodiversity conservation. The success of these releases depends on the capacity of natural enemies to regulate pest populations, which is conditional to the biotic interactions that occur among species (Symondson et al. 2002; Van Veen et al. 2006). In ecosystems, any given species is likely to interact directly or indirectly with any other species, resulting in both short-term effects on species abundances and densities, and long-term effects on population dynamics (Wootton 1994; Abrams and Matsuda 1996; Tack et al. 2011; Bompard et al. 2013). Direct interactions, such as predation and symbiosis, result from a direct contact between species. Conversely, indirect interactions occur between organisms that can be separated in time or space and necessarily involve at least a third mediating organism (Wootton 1994; Mouttet et al. 2011; Bompard et al. 2013). Both direct and indirect biotic interactions are likely to strongly affect species population dynamics in agro-ecosystems (Bompard et al. 2013).

Generalist predators are known for their capacity to regulate, through predation, herbivore arthropod populations in various ecosystems (Symondson et al. 2002; Desneux et al. 2006; Lu et al. 2012); they are widely used as biocontrol agents. While attacking several pest species simultaneously, a generalist predator may induce predator-mediated indirect interactions between these pest species (Holt 1977). The nature and strength of predator-mediated indirect interactions depend on prey and predator characteristics, and on temporal and spatial scales (Holt and Lawton 1994; Harmon and Andow 2004; Van Veen et al. 2006; Tack et al. 2011). Species characteristics that may significantly affect enemy-mediated indirect interactions are, among others, prey capacity to escape predation (e.g. through refuge; Lind and Cresswell (2005)), predator preference for particular prey (Eubanks and Denno 2000), and predator foraging behavior (Oaten and Murdoch 1975; Holt and Kotler 1987; Abrams and Matsuda 1996; Evans and Toler 2007). Spatial (patchiness) and temporal heterogeneities in prey densities may also affect predator-mediated indirect interactions (Hambäck et al. 2006). These are predicted to be generally positive at time scales shorter than the predator generation time; the shared predation pressure on all available prey may result in increased prey population densities compared to prey densities in unique prey systems (Holt and Lawton 1994; Abrams and Matsuda 1996; Bompard et al. 2013). If both prey benefit the presence of the second prey (i.e. reciprocal positive indirect interactions: increased population densities of both prey), the overall predator-mediated interaction is *apparent mutualism*. If only one prey species benefits the presence of the others (i.e. increased population density of only one prey), this is referred as *apparent commensalism* (Holt and Lawton 1994; Abrams and Matsuda 1996). At

longer time scales, i.e. time scales longer than the predator generation time, predator-mediated indirect interactions may become negative, owing to a higher numerical response of the predator to increased prey availability in multi-prey systems in comparison with one-prey systems (Holt 1977; Holt and Lawton 1994). If both prey suffer the presence of the other prey (i.e. reciprocal negative indirect interaction: decreased population densities of both prey), the overall predator-mediated indirect interaction is *apparent competition*. If only one prey suffers the presence of the other (i.e. decreased population density of only one prey), this is *apparent amensalism* (Holt 1977; Holt and Lawton 1994). The theoretical conditions required for apparent competition are: (i) food limitation for the predator in unique prey systems (density-dependence); and (ii) a numerical response to the density of each prey in unique prey systems (Harmon and Andow 2004).

The knowledge on apparent competition has been used for improving biocontrol. At large, field scales, the maintaining of a high biodiversity level was shown to enhance the population development of pests' natural enemies, resulting in increased predation pressure on target pests (Settle et al. 1996; Huang et al. 2011). The presence of alternative prey may increase control of pest species by generalist predators under field conditions (Harwood et al. 2007; Bompard et al. 2013). Smaller-scale experiments showed that polyphagous predators benefited from a mixed diet, either through an alternative prey (Liu et al. 2006; Messelink et al. 2008; Messelink et al. 2010; Calvo et al. 2011) or an alternative plant resource (Evans et al. 1999; Van Rijn et al. 2002; Nomikou et al. 2010; Vandekerkhove and De Clercq 2010). Maintaining simultaneously low levels of several pest populations should theoretically favor the development of generalist predators through apparent competition (Symondson et al. 2002). However, due to the multiple factors (detailed above) influencing predator-mediated interactions, the presence of several pests does not always result in negative enemy-mediated indirect interactions (Prasad and Snyder 2006; Kuusk and Ekbom 2010; Tack et al. 2011). This is why the possibility to increase the biocontrol on two pests sharing a common natural enemy, through apparent competition, needs to be experimentally tested for each combination of pests and shared natural enemy. The recent and rapid invasion of Afro-Eurasia by the South American tomato pinworm *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) (Desneux et al. 2010, 2011a) highlighted the need to search for new IPM practices against this invasive alien tomato pest, or readjust the already existing biological control programs (Chailleux et al. 2012, 2013). In a previous work, we showed that *T. absoluta* did not threat the control against whiteflies by a generalist mirid predatory bug *Macrolophus pygmaeus* Rambur (Heteroptera: Miridae) (Bompard et al. 2013). Conversely we highlighted a negative predator-mediated indirect interaction of *T. absoluta* on the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), suggesting a possibility for apparent competition.

In this study we formally tested the possibility for apparent competition between *T. absoluta* and *B. tabaci* through a shared predator *M. pygmaeus*, and evaluated the possible effects on crop protection. *Tuta absoluta* is a major pest for tomato crops causing losses up to 100 % by mining in tomato leaves, stems and fruits (Urbaneja et al. 2009; Desneux et al. 2010). *Bemisia tabaci* (biotype Q) is a widespread European indigenous species (McKenzie et al. 2012; Parrella et al. 2012; Saleh et al. 2012) responsible for major damages in tomato crops; as a sap-feeding insect, it causes both direct and indirect (e.g. vectoring viruses) damages to plants (Oliveira et al. 2001; Jiao et al. 2012). *Macrolophus pygmaeus* is one of the most used biocontrol agent against *B. tabaci* and other whiteflies. This generalist predator can also feed on a wide variety of prey such as thrips, aphids, mites, and eggs and larvae of Lepidoptera (Fauvel et al. 1987), notably on those of *T. absoluta* (Urbaneja et al. 2009; Desneux et al. 2010), and could thus benefit the presence of multiple pest species in crops (Symondson et al. 2002).

In this context, our study aimed at shedding lights on two specific questions: (i) is *M. pygmaeus* able to efficiently regulate *T. absoluta* populations? and (ii) does apparent competition occurs between *T. absoluta* and *B. tabaci*, and if so, does it result in an enhanced pest populations' control and a reduction in yield losses? We conducted greenhouse experiments to measure reciprocal predator-mediated indirect interactions between prey by monitoring predator and pest population dynamics, and we quantified the levels of damages on both tomato plants and fruits.

Materials and Methods

Biological materials

The plants used in the experiments were tomato plants, *Solanum lycopersicum* L. cv. Marmande, grown in climatic chambers (23±1°C, 65±5% RH, 16L:8D). The prey *B. tabaci* and *T. absoluta* were reared on tobacco and tomato plants respectively, in separate cages, in a climatic chamber (23±1°C, 65±5% RH, 16L:8D). The predator *M. pygmaeus* was provided by Biotop (Valbonne, France), and reared on tomato leaves complemented with *Ephestia kuehniella* eggs under similar controlled conditions than the prey. All predators used in the experiments did not have any previous experience of predation on *B. tabaci* or *T. absoluta*. No pesticides were used during the study.

Experimental design

We evaluated possible reciprocal indirect interactions between the pests *T. absoluta* and *B. tabaci* in tomato crop in greenhouse, through a 2 x 2 factorial design, in which the predator *M. pygmaeus* was present in all treatments. The first two-level factor consisted of the presence or absence of *T. absoluta*. The second two-level factor consisted of the presence or absence of *B. tabaci*. These two factors were fully crossed which resulted in four possible combinations (all with predator): *T. absoluta* alone, *B. tabaci* alone, *T. absoluta* + *B. tabaci*, and no prey i.e. only predator.

Each of the four treatments was replicated four times in 40m² distinct compartments of an environment-controlled greenhouse, with identical climatic conditions (T=25°C; RH=60%). The greenhouse was located at Sophia Antipolis INRA center (South-Eastern France) and the study was carried out during summer 2011. To prevent insect transfer between treatments inside a given compartment, they were isolated from one another by fine mesh material, which formed tunnels (height: 2m, width: 1m, length: 5m). Tomato plants were grown on rock wool slabs and each tunnel contained 2 rows of 8 plants. Plants were automatically supplied with water and nutrients that prevented any abiotic stress. Plants were regularly vertically tied up. Prey were released on 4-week old plants (8-11 leaves/plant), at a rate of 20 adults and 2 adults per plant, for *B. tabaci* and *T. absoluta* respectively. Predators were released one week later at a rate of 2 females, 1 male and 4 nymphs (L4/L5) per plant.

Sampling

Population dynamics of the three insect species were monitored on four plants per tunnel during the 10 following weeks after the release of predators. Adults and nymphs of *M. pygmaeus*, adults of *B. tabaci*, and larvae of *T. absoluta* were counted on all leaves of each sampled plant. For each plant sampled, eight leaflets were collected for additional observations in the laboratory. Juveniles of *B. tabaci* (eggs and nymphs) as well as eggs of *T. absoluta* were counted using a binocular microscope. The number of leaves per plant as well as the overall level of leaf damage due to prey activity (percentage of damaged leaves) were also recorded. Mature tomato fruits were harvested once or twice a week, and indexed either as undamaged or as damaged when they showed one gallery of *T. absoluta* larvae or sooty mold due to *B. tabaci*. Both categories (undamaged vs. damaged) were weighed for each tunnel. At the end of the experiment, all fruits (mature or not) were harvested and weighed.

Statistical analyses

Data of insect population densities, as well as plant characteristics (growth, production and levels of damage) were not normally distributed. Thus we built generalized linear models using a Generalized Estimated Equation (GEE; library geepack) to test for the two-level factors presence /

absence of *T. absoluta* and presence / absence of *B. tabaci*. All models were implemented with a Poisson-law (link-function log), except for data of the number of leaves per plant; whose distribution followed a Gamma-law (link-function inverse). The function GEE enables adding a random factor to correct for repeated measures over time, which was either the tunnel number, or the plant number (for data of number of leaves per plant and level of leaf damage). All statistical analyses were performed using the software R version 2.14.1 (R Development Core Team).

To depict possible short-term from long-term predator-mediated indirect interactions between prey, three analyses with three different time periods (weeks 1-10, weeks 1-7 and weeks 8-10) were run on data of insect population densities. These periods were chosen according to the development time of the predator and its possible linked predatory activity (see Bompard et al. 2013). Temperature inside the greenhouse reached 1086 cumulated degree days (CDD) at week 7 and 1593 CDD on week 10. We assume the entire development cycle of the predator *M. pygmaeus* to be about 940 CDD (Bompard et al. 2013). Consequently one predator cycle was achieved at the end of the first period (week 7) for eggs laid by the adult predators introduced at the beginning of the experiment. At the end of the second period (week 10), the descendants of the introduced juvenile predators had achieved their development cycle. Predator-mediated interactions correspond to short-term and long-term interactions, over the first and second periods, respectively.

Plant growth was estimated by the increase in number of leaves per plant over time. Measures of tomato production were analyzed as the total cumulated production over weeks, and the ratio of cumulated mass of damaged fruits over total cumulated production.

Results

Insect population dynamics

Populations of adult and nymph predators showed similar dynamics so the data were pooled. The factor *B. tabaci* had a significantly positive impact on predator population's dynamic over all the period ($Chi^2_1 = 266$, $P < 0.001$) as well as over the last period ($Chi^2_1 = 342$, $P < 0.001$) (Fig. 1). The factor *T. absoluta* had no significant impact on predator population's dynamic. However the interaction between the two factors had a significantly positive impact on predator population's dynamic over weeks 8-10 ($Chi^2_1 = 6$, $P = 0.014$). Predator populations were ~2.5 times and ~1.8 times higher in the two-prey systems than with only *B. tabaci*, at week 9 and 10 respectively (Fig. 1), and ~165 times and ~175 times higher in the two-prey systems than with the prey *T. absoluta* only, at week 9 and 10 respectively.

Populations of eggs and nymphs of *B. tabaci* showed similar dynamics so all individuals were gathered in a unique pool of juveniles. Populations of adults of *B. tabaci* showed also a similar dynamic than *B. tabaci* juveniles (data not shown). The factor *T. absoluta* had a significant negative impact on *B. tabaci* juvenile population's dynamic over all the period ($Chi^2_1 = 7$, $P = 0.006$) and over weeks 8-10 ($Chi^2_1 = 30$, $P < 0.001$). This resulted in a 4.2-fold decrease at week 10

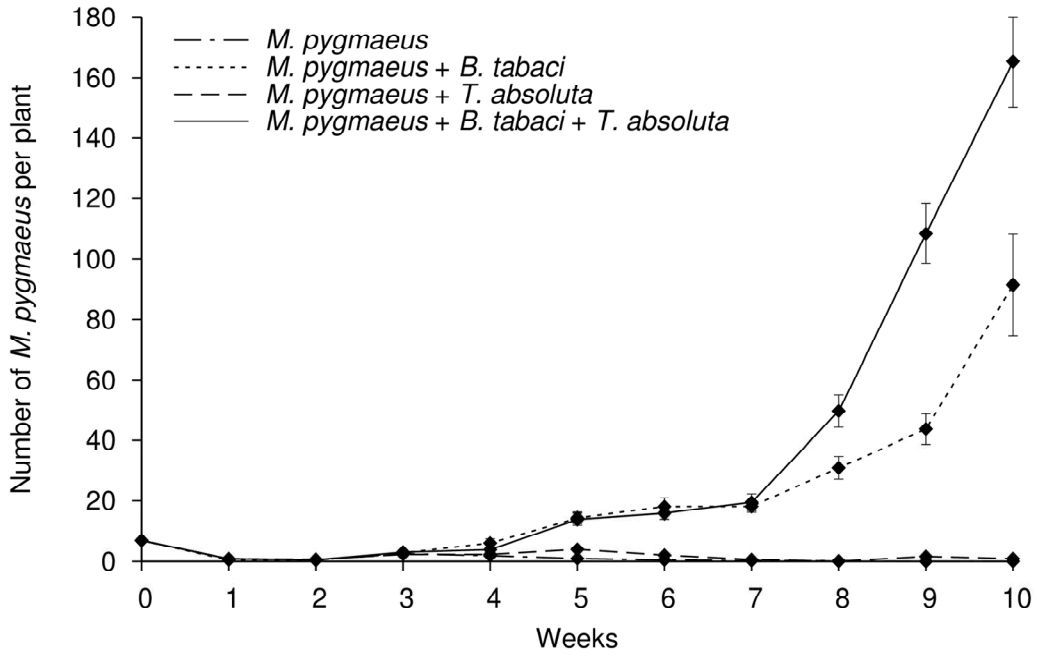


Figure 1: Population dynamic of the predator *M. pygmaeus*. Mean number (±SE) of individuals (adults and juveniles) per plant in no-prey system (control; mixed line), with *B. tabaci* only (dotted line), with *T. absoluta* only (dashed line), or with both prey (continued line).

when comparing population densities of *B. tabaci* juveniles in the one prey-system and population densities of *B. tabaci* juveniles in the two-prey system (Fig. 2).

Population's dynamic of larvae of *T. absoluta* was much more variable over time, with cyclic drops to very low densities (Fig. 3). The factor *B. tabaci* did not significantly affect *T. absoluta* population's dynamic over all the period, not on weeks 8-10. However, a significant negative impact of *B. tabaci* on *T. absoluta* larvae populations' dynamic was visible at weeks 9 and 10 only ($Chi^2_1 = 5$, $P = 0.023$) (Fig. 3).

Plant growth and fruit production

The presence of *T. absoluta* caused a small reduction in number of leaves per plant ($Chi^2_1 = 4.37$, $P = 0.036$; data not shown). *Bemisia tabaci* did not cause any reduction in plant growth. The factors *T. absoluta* or *B. tabaci* did not cause any significant reduction on cumulated tomato production

when considering all the period (Fig. 4a). However, when considering the harvest data set without the last point of harvest (week 10), *B. tabaci* induced a significant negative decrease in cumulated tomato production (Chi^2_1 = 5.94, *P* = 0.015), by 35% between control tunnels and tunnels containing the pest *B. tabaci* only at week 9. Conversely *T. absoluta* did not induce any significant

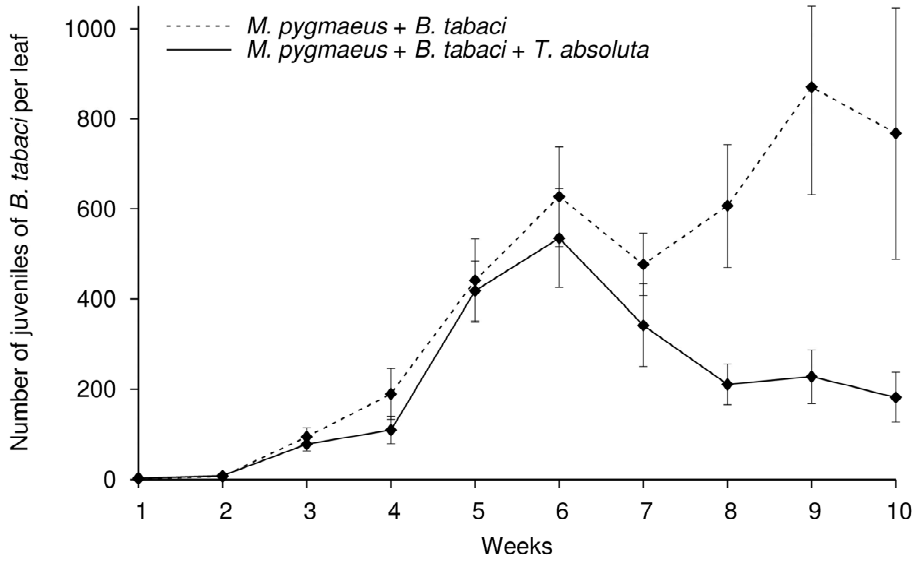


Figure 2: Population dynamic of the prey *B. tabaci*. Mean number (±SE) of juveniles (eggs and larvae) per leaf in one-prey system (dotted line), or in two-prey system (continued line).

impact and neither did the interaction between the factors *T. absoluta* and *B. tabaci*. Mean values of total cumulated production (week 10) did not differ significantly between the different pest-systems (Fig. 4a), though a tendency to be inferior in presence of the pest *T. absoluta*.

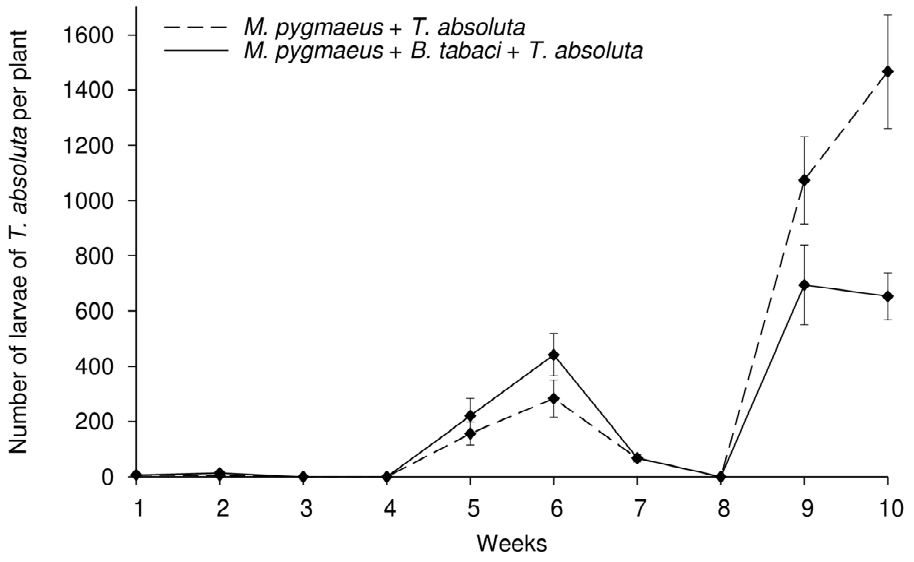


Figure 3: Population dynamic of the prey *T. absoluta*. Mean number (±SE) of larvae per plant in one-prey system (dashed line), or in two-prey system (continued line).

Ratio of damaged fruits and levels of leaf damage

The factor *T. absoluta* as well as the interaction between the factors *B. tabaci* and *T. absoluta* had a significant impact on the ratio of damaged fruits (Fig. 4b; $Chi^2_1 = 35.0$, $P < 0.001$ and $Chi^2_1 = 16403$, $P < 0.001$ respectively). Both factors *T. absoluta* and *B. tabaci*, as well as the interaction between factors had a significant impact on the levels of leaf damage (Fig. 4c; $Chi^2_1 = 4.46$, $P = 0.035$; $Chi^2_1 = 126.97$, $P < 0.001$; and $Chi^2_1 = 15.60$, $P < 0.001$ respectively). Overall, we observed increased damages to fruits and plants when there were two pests vs. only one pest.

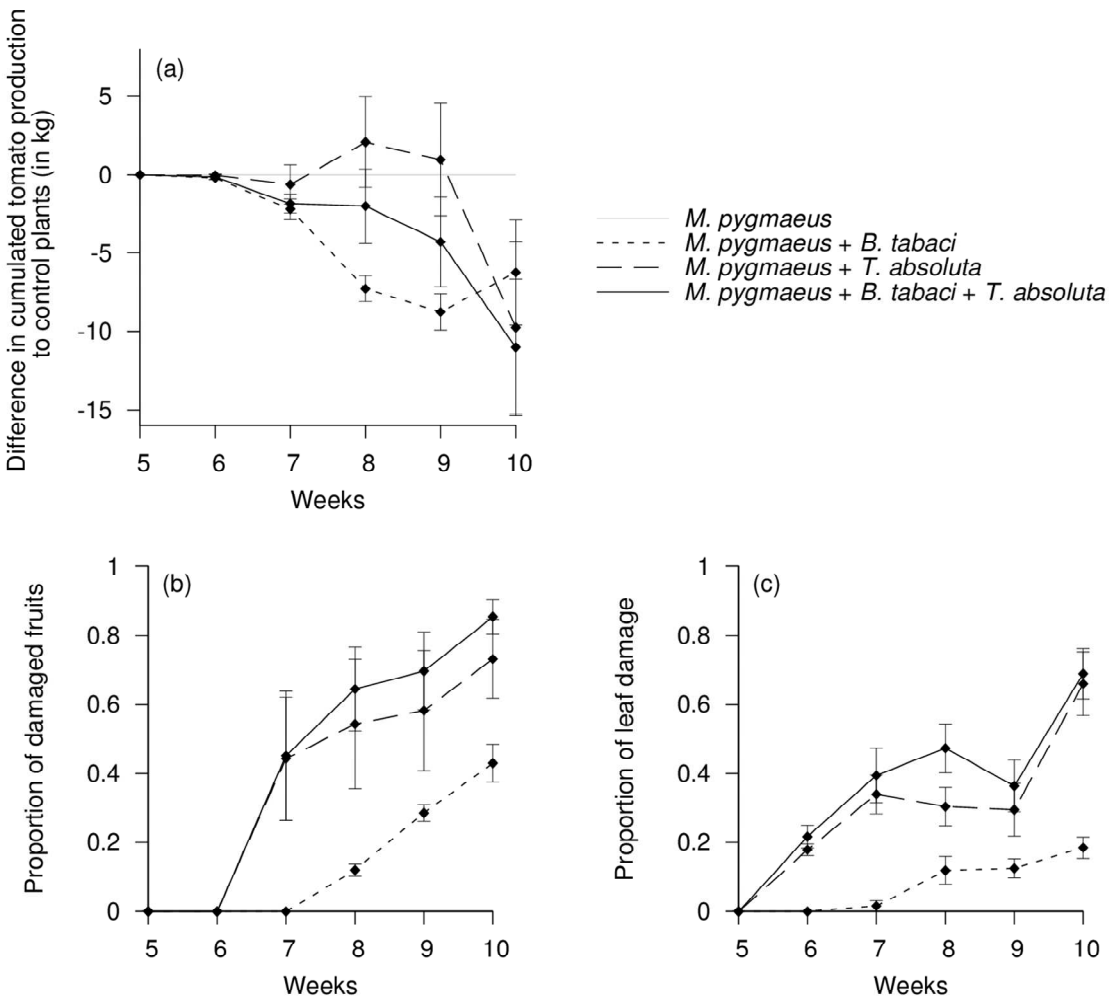


Figure 4: Tomato production and levels of damage on fruits and leaves, in free-prey systems (continued grey line), with the prey *B. tabaci* only (dotted line), with the prey *T. absoluta* only (dashed line), or in two-prey systems (continued black line). (a) Mean difference (± SE; N=4) in cumulated tomato production to cumulated production of control plants (freed from pests), in kg per groups of 16 plants (tunnels); (b) Ratio of mass of fruits damaged by pests over total cumulated production per 16 plants (mean number ± SE; N=4); (c) percentage of leaf damage due to pests (mean number ± SE; N=16).

Discussion

The population dynamics of the prey *B. tabaci* and *T. absoluta* and of the predator *M. pygmaeus* hinted an asymmetric apparent competition. We observed (i) an increased numerical response of the predator to a higher prey availability in the two-prey systems in comparison to the strength of the numerical response to the prey *B. tabaci* alone; (ii) a lack of numerical response of *M. pygmaeus* to the prey *T. absoluta* alone; (iii) a strong negative impact of *T. absoluta* on *B. tabaci*; and (iv) a weak negative impact of *B. tabaci* on *T. absoluta*. Moreover, the increased number of predators in the two-prey systems did not translate to reduced plants and fruits damages (caused mainly by *T. absoluta* larvae).

The enhancement of polyphagous predators' development by alternative food resources in crops has already been shown in various situations, with the presence of alternative prey (Settle et al. 1996; Oestman 2004; Liu et al. 2006; Harwood et al. 2007; Desneux and O'Neil 2008; Messelink et al. 2008; Messelink et al. 2010; Calvo et al. 2011; Huang et al. 2011) or plant materials (Evans et al. 1999; Van Rijn et al. 2002; Nomikou et al. 2010; Vandekerkhove and De Clercq 2010) leading, in some cases, to higher predator densities and an increase control of the target pest species. Our greenhouse experiment gives a new evidence for apparent competition between the co-occurring pests *B. tabaci* and *T. absoluta*. The increased numerical response of the shared predator *M. pygmaeus* to the co-occurrence of prey proves that it benefited the mixed diet, which has already been reported for other generalist arthropod predators (Eubanks and Denno 2000; Messelink et al. 2008). However, apparent competition between *B. tabaci* and *T. absoluta* was definitively asymmetric, owing to (i) differences in numerical responses of the predator to a given prey, (ii) predator preferences for prey and predator foraging behavior, (iii) differences in prey population sizes and dynamics (cycling) and/or (iv) co-occurring direct and indirect interactions between pests.

The lack of numerical response of the predator on *T. absoluta* alone was unforeseen. Laboratory preliminary experiments showed a functional and numerical response of *M. pygmaeus* when fed on *T. absoluta* eggs in small enclosures (Supplementary material Fig. S1, S2), though the numerical response observed was about four times weaker than when fed on *B. tabaci* nymphs (Desneux N, Chailleux A, data not shown). The sensitivity of the numerical response of *M. pygmaeus* to prey species has already been highlighted in previous studies (Foglar et al. 1990; Hamdan 2006). Interestingly a recent study reported relative poor nutritive quality of *T. absoluta* eggs for *M. pygmaeus* (Molla et al. submitted), which could explain the very low numerical response of *M. pygmaeus* on *T. absoluta* alone, and the subsequent asymmetric indirect interactions between *B. tabaci* and *T. absoluta* observed in our experiment.

Predator preference for one of the two prey is unlikely to be responsible for the asymmetry of predator-mediated indirect interactions, as a previous laboratory experiment demonstrated a preference of *M. pygmaeus* for *T. absoluta* eggs when encountering simultaneously *B. tabaci* nymphs, *T. absoluta* eggs and *T. absoluta* larvae, as well as *switching behavior* in this predator (Jaworski et al. submitted). If *T. absoluta* was systematically the most preferred prey, *M. pygmaeus* would increase its predation pressure over this prey, and consequently reduce the predation pressure over *B. tabaci*. This would result in predator-mediated indirect interactions in favor of *B. tabaci*, unlike what was observed on pest population dynamics in our greenhouse experiment. At larger, mesocosm scales, such a predation behavior could result in increased predator foraging time in high prey-density patches, so as to maximize food uptake (Murdoch et al. 2003). Nymphs of *B. tabaci* and *T. absoluta* eggs (the most preyed stage for *T. absoluta*) are rarely locally distributed in the same patches, as *B. tabaci* nymphs are encountered on old tomato leaves, whereas *T. absoluta* adults lay their eggs on fresh young tomato leaves (Jaworski C and Chailleux A, pers. obs.). In our experiment, tomato plants were grown and tied vertically, so that young tomato leaves were at the top of plants, and older leaves at lower levels. Combined with the switching predation behavior, such a spatially decoupled prey distribution could lead the predators to forage mostly in single prey type patches and move among prey type patches. Moreover, *B. tabaci* patches are generally denser (number of prey per leaf higher, see Figs. 2 and 3), thus if *M. pygmaeus* showed a pure switching predation behavior at a mesocosm scale, it would spend most of the time foraging in patches containing only *B. tabaci* nymphs at high densities (Murdoch 1969). This would result in simultaneous increased predation pressure over *B. tabaci* and decreased predation pressure over *T. absoluta*, and a subsequent asymmetric apparent competition.

In a broader context, prey population sizes and densities are likely to significantly impact enemy-mediated indirect interactions (Bergeson and Messina 1997; Müller et al. 1999; Valladares et al. 2001). In our greenhouse experiment, we observed cycling drops of *T. absoluta* populations to very low densities (Fig. 3), supposedly due to the absence of overlapping generations. This could explain the lack of numerical response of *M. pygmaeus* to *T. absoluta* alone, as predators may have suffered from the too variable quantity of available prey. Such large prey population fluctuations of only one prey species are likely to affect predator-mediated indirect interactions in an asymmetric way (Abrams et al. 1998; Brassil 2006).

The overall asymmetric interactions between *B. tabaci* and *T. absoluta* may hide several types of interactions. Such trophic systems where herbivorous share both a predator and a resource plant do not allow to discriminate the effects of direct interactions (resource competition), predator-mediated indirect interactions (apparent competition), and plant-mediated indirect interactions (but see Bompard et al. 2013). As leaf damages were very high at the end of the experiment (Figures 4),

it is likely that resource competition may have co-occurred with predator-mediated indirect interactions, which was the case in our previous greenhouse experiment (Bompard et al. 2013). Moreover, resource plants may induce indirect interactions between feeding pests, via induced defense pathways (Stout et al. 2006; Mouttet et al. 2011). Consequently the plant may favor one prey against the other through the activation of a given defense pathway and the inhibition of another defense pathway, resulting in plant-mediated indirect interactions (Preston et al. 1999; Rodriguez-Saona et al. 2005; Soler et al. 2012). Ultimately *B. tabaci* may interact on *T. absoluta* through the production of honeydew (Oliveira et al. 2001), which might be used as an alternative food resource by *T. absoluta* adults known to feed on honey. This would result in a facilitation of *B. tabaci* toward *T. absoluta* and increase the asymmetry of the overall apparent competition in favor of *T. absoluta*.

Although apparent competition occurred between *B. tabaci* and *T. absoluta*, the increased numerical response of the predator *M. pygmaeus* in the two-prey system did not translate to reduced yield losses: the levels of damage on leaves and fruits were higher in the two-prey systems than in the one-prey systems (Fig. 4). These damages were mainly due to *T. absoluta* larvae, which mined leaves and fruits, causing defoliation and strong losses in tomato production, as a fruit contaminated with a single mine cannot be commercialized anymore (because of necrosis induced in the fruit). It hints the low ability of *M. pygmaeus* to control *T. absoluta* populations in our experimental conditions. Our previous experiment showed a better control of *T. absoluta* by the predator *M. pygmaeus* in presence of *B. tabaci* (Bompard et al. 2013). In this earlier study, plants were coated in small tunnels, which resulted in a continuous biomass layer, and an average total smaller biomass quantity. The experimental conditions here were closer to conditions in tomato production greenhouses, with tomato plants vertically guided and insects contained in large tunnels. Total biomass quantity was thus higher and plant material less dense. This could have benefited pest populations, especially the Lepidoptera *T. absoluta*, through an increased resource availability as well as light. These vegetation conditions may have simultaneously negatively affected *M. pygmaeus* predation behavior. Moving from one plant to another was likely more difficult for the predators, notably because nymphs can not fly. Moreover, the tunnels were bigger, reducing the probability to encounter a prey, thus increasing the time the predator had to forage for finding prey. Along with the difficulties to settle predator populations (discussed earlier), these experimental conditions may explain the lower level of control of *T. absoluta* by *M. pygmaeus* than in our previous experiment (Bompard et al. 2013).

A delay in tomato production was observed in tunnels containing the prey *B. tabaci*, though similar levels of production were obtained at the end of ten weeks of experiment. This may result from differences in resource allocations by the plant toward fruit production vs. defense inductions

(Herms and Mattson 1992). Our experiment confirms that *T. absoluta* is a major pest for tomato crops (Desneux et al. 2010, 2011b). The predation of *M. pygmaeus* on *T. absoluta* remains insufficient to regulate *T. absoluta* populations and avoid serious damages on plants and production, at least in the conditions of our experiment. Although the beneficial impact of apparent competition on IPM practices is generally acknowledged, notably for through maintaining prey populations at low levels in some extent, this negative indirect interaction is not sufficient to prevent tomato plants infestation by *T. absoluta* when there is apparent competition with *B. tabaci* when sharing the predator *M. pygmaeus* (at least at prey densities tested in our study). In such context, this would be hazardous to use *M. pygmaeus* alone to control both *T. absoluta* and *B. tabaci* in an IPM approach, and the predator should be associated with other biocontrol agents, e.g. larval or oophagous parasitoids (Chailleux et al. 2012, 2013; Biondi et al. 2013), for biological control programs of *T. absoluta* on tomato.

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Supplementary material

Figure S1.

Functional response of the predator *M. pygmaeus* fed on eggs of *T. absoluta*. Mean number (±SE; N varied between 2 and 7) of eggs eaten per day for increasing numbers of eggs available. Experiments carried out in Petri dishes with a fixed number of fresh *T. absoluta* eggs provided to a female predator of *M. pygmaeus*.

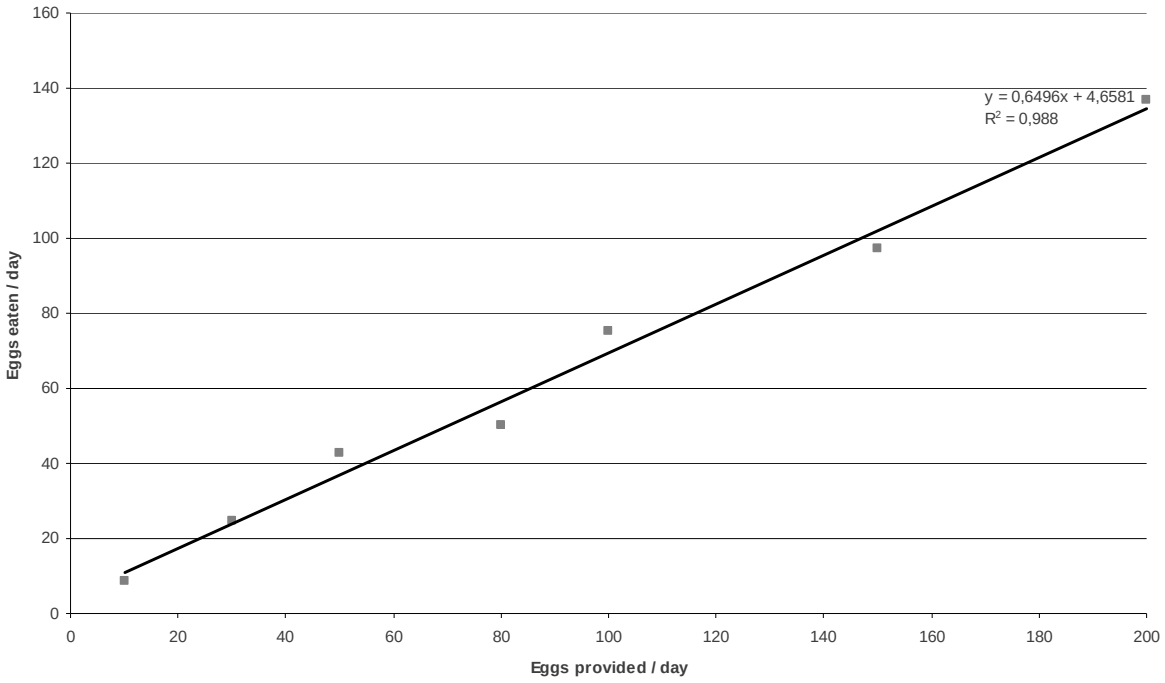


Figure S2.

Numerical response of the predator *M. pygmaeus* fed on eggs of *T. absoluta*. Mean number (±SE; N varied between 2 and 7) of offspring hatched (per day) for increasing numbers of eggs available. Experiments carried out in Petri dishes with a fixed number of fresh *T. absoluta* eggs provided to a female predator of *M. pygmaeus* during its entire lifetime.

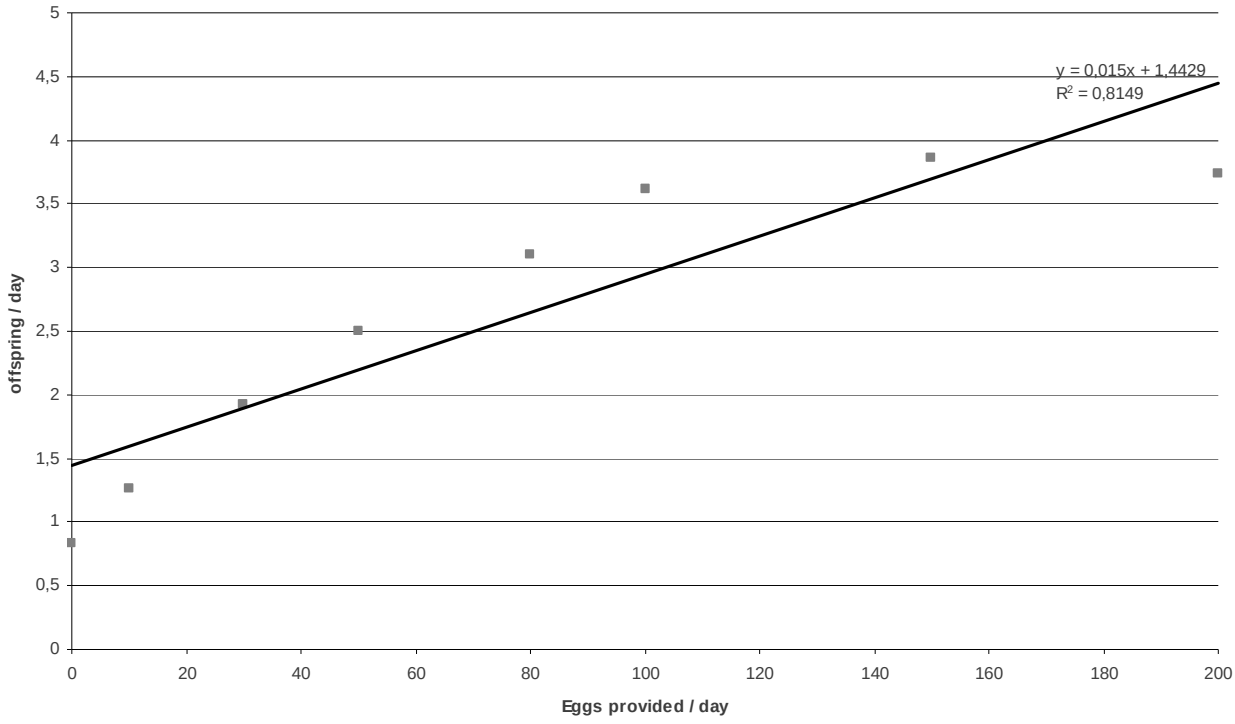
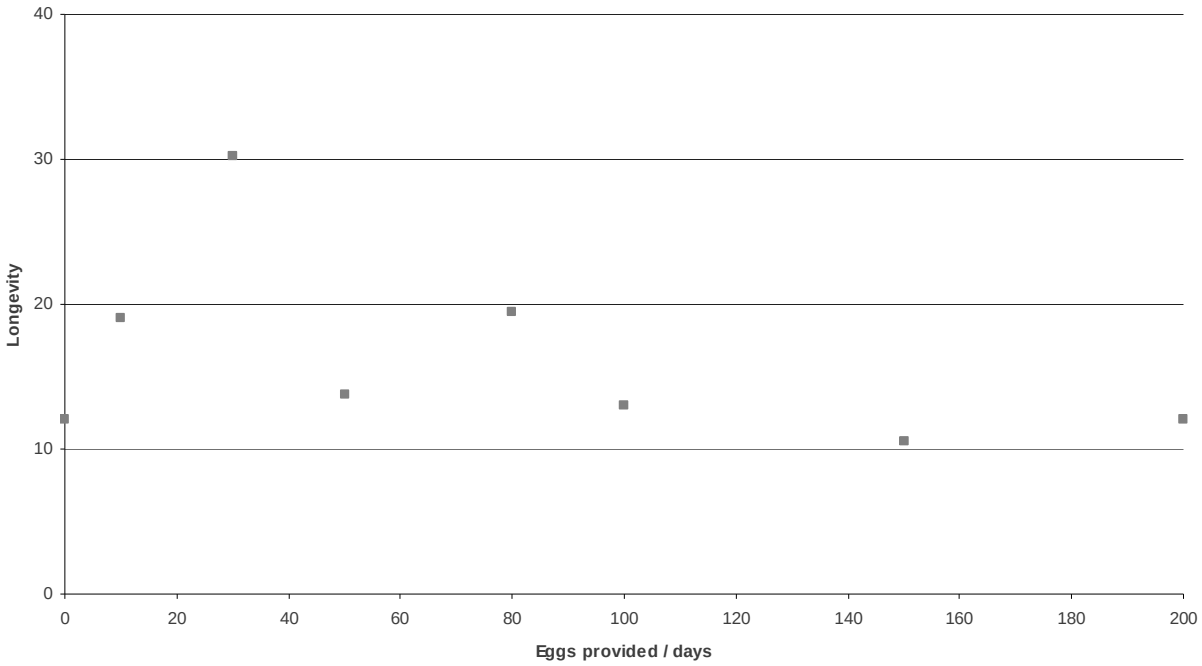


Figure S3.

Longevity of the predator *M. pygmaeus* fed on eggs of *T. absoluta*. Mean longevity (±SE; N varied between 2 and 7) for increasing numbers of eggs available. Experiments carried out in Petri dishes with a fixed number of fresh *T. absoluta* eggs provided to a female predator of *M. pygmaeus* during its entire lifetime.



Chapitre 3 : La recherche de nouveaux auxiliaires autochtones pour le contrôle biologique de *T. absoluta* en Europe

Présenté en tant que *Article 4*, publié dans PLoS ONE n°7,

voir annexe 2 et 4 pour des photos des dispositifs expérimentaux,

et *Article 5*, en préparation,

voir annexe 3 pour une photo du dispositif expérimental.

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Assessing European Egg Parasitoids as a Mean of Controlling the Invasive South American Tomato Pinworm *Tuta absoluta*

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Abstract

The South American tomato pinworm (*Tuta absoluta*) has recently invaded Europe and is rapidly spreading in the Afro-Eurasian continent where it is becoming a major pest on tomato crops. Laboratory tests were undertaken to evaluate the potential of 29 European strains of *Trichogramma* parasitoids to control *T. absoluta*. In addition to the host itself, the host plant (tomato) was used during the laboratory tests in order to increase the chance of selecting the best parasitoid strains. *Trichogramma* females were placed with *T. absoluta* eggs on a tomato leaflet in tubes. We compared the parasitism of *T. absoluta* by the various *Trichogramma* species tested to the *Trichogramma* species currently commercially available for the pest control in Europe, i.e. *Trichogramma achaeae*. Thereafter, the more promising strains were tested on a larger scale, in mesocosm (i.e. cages in greenhouses) and in greenhouse compartments to evaluate efficiency of laboratory selected strains under cropping conditions. The most efficient strain from the laboratory screening trials did not perform as efficiently under the greenhouse conditions. We discuss differences in parasitism levels among species and strains and among the different scales tested in the experiments, as well as implications of these results for further screening for biocontrol agents.

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Introduction

Some of the most serious arthropod pests in agricultural landscapes are invasive species [1–3]. In the USA, for example, introduced arthropod pests have been estimated to cause losses of around $20 billion each year [4]. For most of the invasive pests, chemical pesticides are the dominant pest management method, contributing to additional production costs and negative side effects on non-target organisms and human health [4–7]. The tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), is one of the most devastating tomato pests in South America [8]. This pest has recently invaded European and Mediterranean basin countries and in few years has become a major pest in both greenhouse and outdoor tomato crops [9,10]. Biological control is a key component of integrated pest management programs in tomato greenhouses in Europe, but its sustainability is threatened due to the extensive use of pesticides to control *T. absoluta* and possible well known associated side effects of these products on biocontrol agents [6,11,12].

The possible use of parasitic wasps of the *Trichogramma* genus (Hymenoptera: Trichogrammatidae) as biological control agents of *T. absoluta* is currently considered in Europe because of the natural parasitism of *T. absoluta* by various *Trichogramma* species reported in South America and in Europe (e.g. in Italy) [13,14] and the effective use of *Trichogramma pretiosum* Riley for inundative releases against *T. absoluta* in South American tomato crops [15–18]. *Trichogramma* parasitoids have already been successfully used in biological control of various lepidopteran agricultural pests [2,16,19]. They are easy to rear [20] and to release in open fields or protected crops. Every year, more than 32 million hectares are treated worldwide using *Trichogramma* spp. [21,22], mostly through seasonal inundative releases [19,23]. The success of the *Trichogramma* releases depends on the knowledge of the biological characteristics of the parasitoid species or strains used, and on their interactions with a specific host [24–26]. Selecting the *Trichogramma* species with the highest affinity for the target pest and for characteristics of the agro-ecosystem is crucial to the success of the biological control program [19,27]. *Trichogramma achaeae* Nagaraja and Nagarkatti, is currently available in some European and North African countries for inundative biological control of *T. absoluta* [28]. *T. achaeae* was shown to be efficient in pilot experiments and lowered *T. absoluta* infestation levels in experimental and commercial tomato greenhouses [9,29]. However, the efficiency of this parasitoid depends on the use of high quantities of parasitoids per release (as indicated by biocontrol companies; e.g. 250,000–1,000,000/ha per week [30]), the level of infestation by *T. absoluta*, and upon presence of other natural enemies on the crop. In addition, it is expensive to produce *T. achaeae* because the absence of diapause causes difficulty in storage and handling.

Identifying a more efficient *Trichogramma* species would allow establishing an optimized economically-sound biological control program against *T. absoluta*.

The aim of the present study was to compare the efficiency of 29 *Trichogramma* strains in parasitizing *T. absoluta* eggs on tomatoes on three different scales: laboratory, mesocosm (cages in greenhouse) and in greenhouse compartments. We tested European *Trichogramma* strains from 11 different species. They were selected because (i) they were representative of the biodiversity of the European *Trichogramma* species, (ii) they were collected on hosts similar (size and/or ecology) to *T. absoluta* or on hosts present on tomato plants, and (iii) they showed characteristics (e.g. diapause, thelytoky, etc.) which make mass rearing easier i.e. cost-effective industrial production. A strain of *T. pretiosum* was also tested to compare other strains to one from the area of origin of *T. absoluta*. *T. achaeae* was chosen as the control species as it is already commercially available. Only the most promising strains under laboratory conditions were tested on a larger scale to assess the effectiveness of these selected *Trichogramma* strains under cropping conditions.

Materials and Methods

Biological Materials

The plants used in the experiments were five-week old tomato plants, *Solanum lycopersicum* L. cv. Marmande. They were grown in climatic chambers (24±1°C, HR: 65%, photoperiod 16L:8D) and a nutrient solution was applied daily. A colony of *T. absoluta* was set up using greenhouse-collected individuals in July 2009 at INRA, Alenya, France (initial number of individuals = 190). *T. absoluta* were reared in growth chambers (25±1°C, RH 70±10%, 16L:8D). Adults were kept in cages (55×75×80 cm), containing tomato plants. Adult moths were fed on honey placed on one wall inside the cages. The eggs used in the screening were between zero and 12 h old. Parasitoids used for the experiments originated from collections in various countries (Table 1). Dr. B. Pintureau from the French National Institute for Agricultural Research in Lyon

Table 1. Year of collection, initial host and host plant, country of origin and Thelythoky status (females produced from unfertilized) of the 29 *Trichogramma* strains studied.

Species	Geographic origin	Host plant (family)	Host moth or butterfly (family)	Thelytoky	Year of Collection
T. achaeae	Canaries Island	Tomato (Solanaceae)	*Chrysodeixis chalcites* (Noctuidae)	no	2010
T. buesi	Southern France	Cabbage (Brassicaceae)	*Mamestra brassicae* (Noctuidae)	no	2009
T. cacoeciae 1	Southern France	Carnation (Caryophyllaceae)	*Epichoristodes acerbella* (Tortricidae)	yes	2002
T. cacoeciae 2 *	Northern France	Vine (Vitaceae)	*Lobesia botrana* (Tortricidae)	yes	1989
T. cordubensis 1	Spain	Blackthorn (Rosaceae)	*Iphiclides podalirius* (Papilionidae)	yes	1999
T. cordubensis 2	Egypt [a]	Olive tree (Oleaceae)	*Palpita unionalis* (Pyralidae)	yes	2005
T. cordubensis 3	Portugal	–	– (Noctuidae)	yes	1994
T. daumalae 1	Southern France	Apple tree (Rosaceae)	*Cydia pomonella* (Tortricidae)	no	2009
T. daumalae 2	Bulgaria	Apple tree (Rosaceae)	*Cydia pomonella* (Tortricidae)	no	1998
T. dendrolimi 1	China [a]	–	*Palpita unionalis* (Pyralidae)	no	1998
T. dendrolimli 2	Italy	Vine (Vitaceae)	*Lobesia botrana* (Tortricidae)	no	1991
T. euproctidis 1 *	Switzerland	–	–	no	–
T. euproctidis 2	Egypt [a]	Sugar cane (Poaceae)	*Chilo sacchariphagus* (Crambidae)	no	1999
T. euproctidis 3	Southern France	Carnation (Caryophyllaceae)	*Olethreutes arcuella* (Tortricidae)	no	2002
T. evanescens 1 *	Northern France	Vine (Vitaceae)	*Lobesia botrana* (Tortricidae)	no	1990
T. evanescens 2	Northern France	Cauliflower (Brassicaceae)	*Argyrotaenia sphaleropa* (Tortricidae)	no	2002
T. evanescens 3	Turkey	Maize (Poaceae)	*Ostrinia nubilalis* (Crambidae)	no	2003
T. evanescens 4	Southern France	Geranium (Geraniaceae)	*Cacyreus marshalli* (Lycaenidae)	no	2005
T. evanescens 5 *	Southern France	Tomato (Solanaceae)	*Chrysodeixis chalcites* (Noctuidae)	yes	1982
T. evanescens 6	Germany	Maize (Poaceae)	*Ostrinia nubilalis* (Crambidae)	no	2009
T. evanescens 7	Southern France	Vine (Vitaceae)	*Lobesia botrana* (Tortricidae)	no	1990
T. evanescens 8	Southern France	Cabbage (Brassicaceae)	*Ephestia kuehniella* (Pyralidae)	no	1998
T. evanescens 9	Southern France	Cabbage (Brassicaceae)	*Ephestia kuehniella* (Pyralidae)	yes	1998
T. evanescens 10	Southern France	Tomato (Solanaceae)	*Ephestia kuehniella* (Pyralidae)	yes	2010
T. oleae	Yugoslavia	Olive tree (Oleaceae)	*Glyphodes unionalis* (Pyralidae)	yes	1972
T. pretiosum	Uruguay	Vine (Vitaceae)	*Argyrotaenia sphaleropa* (Tortricidae)	no	1995
T. semblidis 1	Southern France	Rice (Poaceae)	*Ephestia kuehniella* (Pyralidae)	no	1997
T. semblidis 2	Northern France	Cabbage (Brassicaceae)	*Plutella xylostella* (Plutellidae)	no	2002
T. semblidis 3	Southern France	Apple tree (Rosaceae)	*Cydia pomonella* (Tortricidae)	yes	2009

Asterisks indicate the strains for which diapause or quiescence capacity has been identified in our laboratory.
[a]strain not collected in Europe but species is present in Europe.
doi:10.1371/journal.pone.0048068.t001

(France) identified all species before the experiments. Stock colonies of parasitoids were reared on UV-irradiated eggs of a substitute host, *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) (18±1°C, RH 70±10%, 12L:12D). Rearing was carried out in glass tubes (length: 4.5 cm; diameter: 0.7 cm) and the parasitoids were fed on honey. *Trichogramma* species were maintained for at least three generations at the temperature of 25°C on *E. kuehniella* eggs before experimentation. The parasitoids used in the screening were between 12 and 24 h old.

Laboratory Screening

The experiments were conducted in growth chambers at the temperature of 25°C (RH 70±10%, 16L:8D). Twenty-nine strains (among 11 species) were tested on *T. absoluta* eggs (designated as *strains* thereafter). Mated *Trichogramma* females were placed individually for 24 h with 30 *T. absoluta* eggs on a tomato leaflet (length: 7 cm ±0.5 SD) in a plastic tube sealed with a mesh to ensure ventilation (length: 14 cm × diameter: 4 cm), five drops of honey were deposited on the internal wall of the tube as food source for parasitoids. The 30 eggs per leaflet were obtained by releasing 15 *T. absoluta* (mixed males and females) on each leaflet in a tube overnight. Then, *T. absoluta* adults were removed and extra eggs were discarded (using a brush) to have 30 eggs per leaflet. The leaflet stem, sticking out of the tube, was planted into floral foam for watering. This design ensured that the leaflet stayed in good shape for the whole duration of the experiment. Between 10 and 15 replicates were conducted per parasitoid strain, and the replicates were carried out in a randomized order at different times. The tubes containing parasitized *T. absoluta* eggs were kept in the climatic chamber and maintained during five days. We counted the number of parasitized eggs (black eggs) and the number of aborted eggs (yellow non-hatched eggs). The proportion of females that parasitized at least one egg was also recorded.

In parallel to the tests using *T. absoluta* as host, experiments were also done using the rearing host *E. kuehniella* in order to compare biological characteristics of the various *Trichogramma*

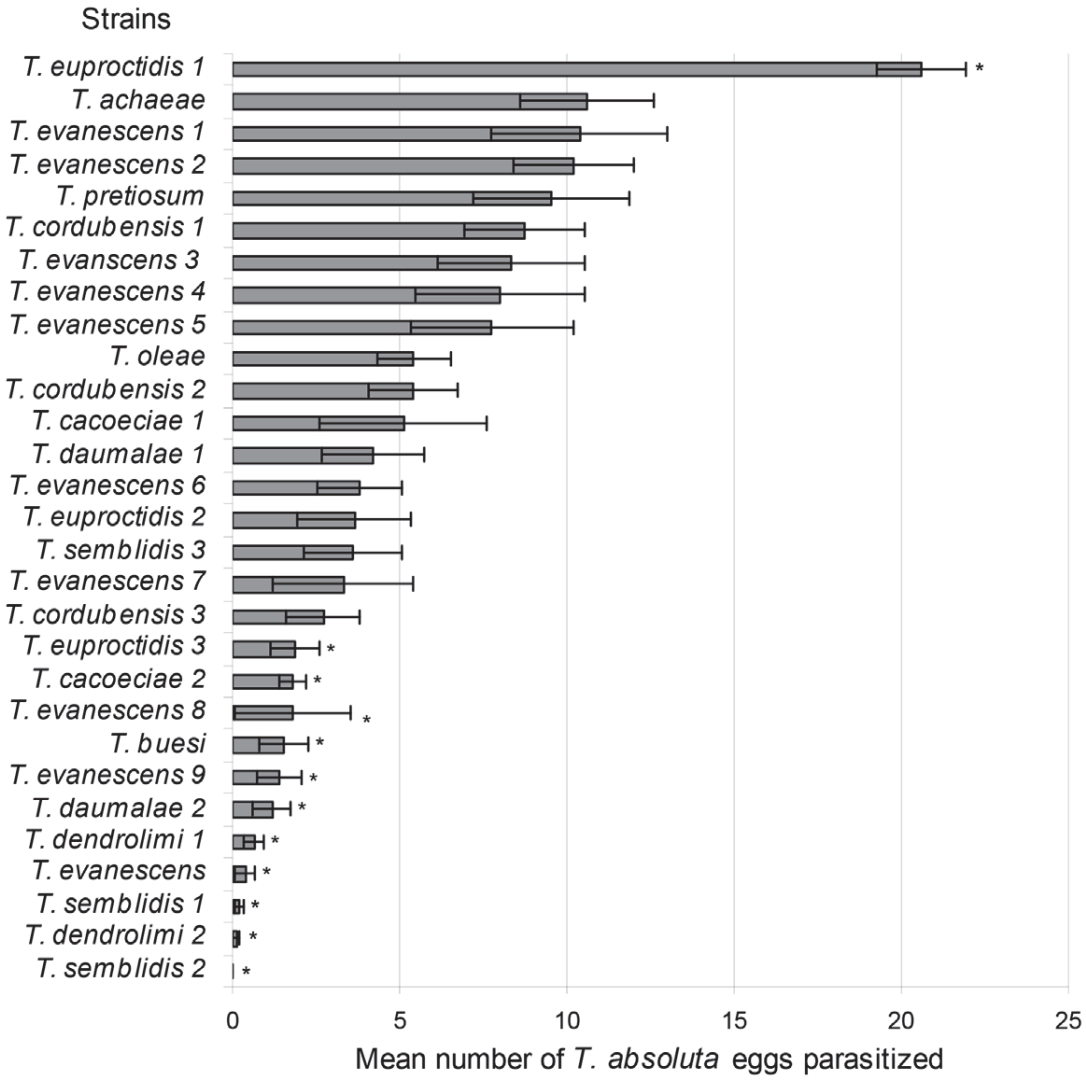


Figure 1. Parasitism of *Tuta absoluta* eggs under laboratory conditions. Mean (±SEM) number of parasitized *T. absoluta* eggs per *Trichogramma* strain in laboratory screening tubes on tomato leaflets**.** Strains with an asterisk are significantly different from *Trichogramma achaeae* at $P<0.05$ level (GLM analysis). One strain of *Trichogramma*, *Trichogramma euproctidis* 1, was significantly more efficient than *T. achaeae*.
doi:10.1371/journal.pone.0048068.g001

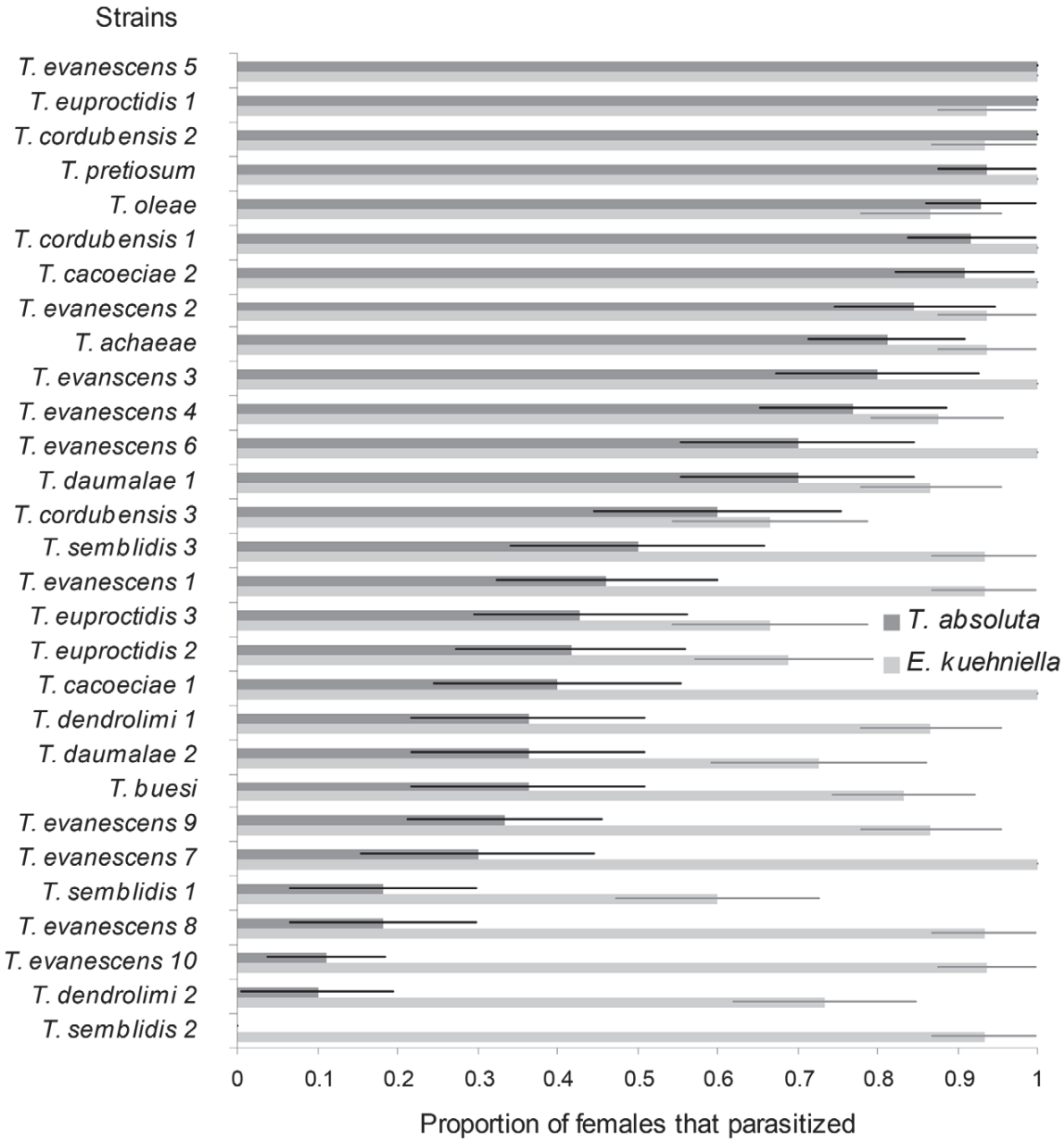


Figure 2. Acceptance of two hosts, *Tuta absoluta* and *Ephestia kuehniella*, by *Trichogramma* females. Data are presented as proportions (mean±SEM) of *Trichogramma* females that parasitized at least one egg on *T. absoluta* and on *E. kuehniella* in laboratory screening tubes.
doi:10.1371/journal.pone.0048068.g002

strains between the rearing and the targeted hosts. Mated parasitoid females were placed individually in glass tubes (containing honey as food) with 30–40 *E. kuehniella* eggs for 24 h in climatic chambers (25±1°C, RH 70±10%, 16L:8D). *E. kuehniella* eggs were glued on a strip of cardboard (3×10 mm) with 10% arabic gum. On each day of experiment, parasitoid strains were tested with the order of strains randomized (14–15 replicates per strain). The proportion of females that parasitized at least one *E. kuehniella* egg was recorded in the same way as tests using *T. absoluta* as tested host.

Cage Experiments

The experiments were conducted in cages placed in a greenhouse located in Valbonne (French Riviera, France). Two species were compared to *T. achaeae* in cages: *Trichogramma euproctidis* 1 which showed the highest parasitism of *T. absoluta* eggs in laboratory trial (see *Results section*), and *Trichogramma evanescens* 5 which showed a similar level of parasitism as *T. achaeae* but that can be easily reared because of its thelytoky. The cages were placed in a glass greenhouse with semi-controlled temperatures, and the temperature was recorded in the cages with data-loggers during the whole experiment (min < mean temperature < max: 16.3°C <25.3°C <33.7°C; min < mean RH < max, 27.8%

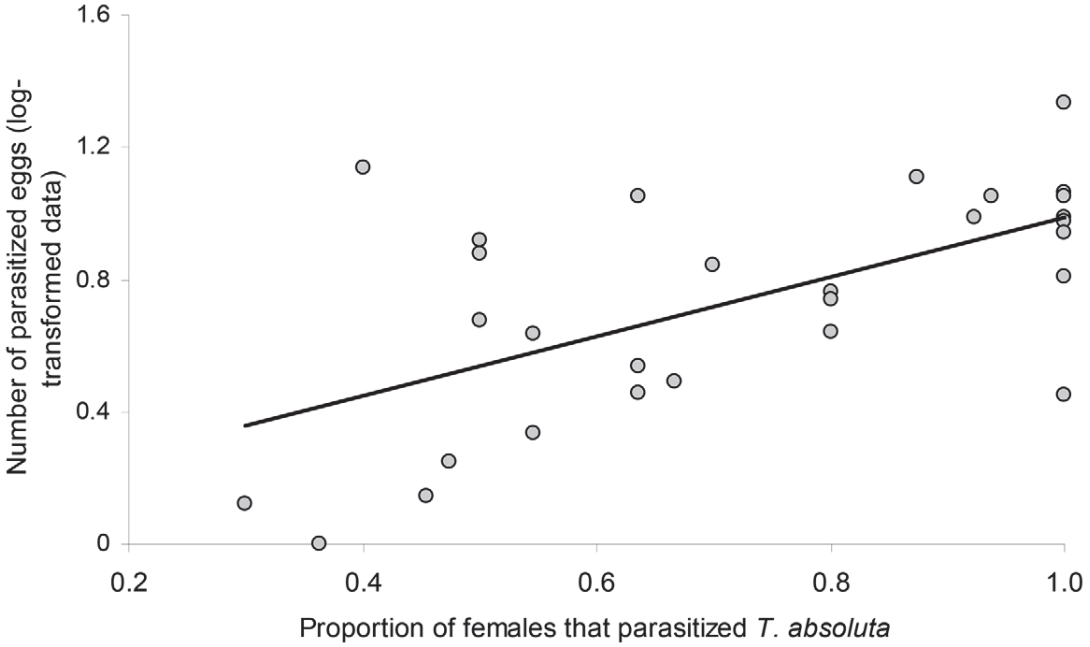


Figure 3. Preference-performance relationship. Data are presented as proportions of parasitoid females (for a given *Trichogramma* strain) attacking the host (*T. absoluta*) under laboratory conditions and the mean numbers of *T. absoluta* eggs parasitized (log-transformed data) (regression line: $y = 0.9001 \times - 0.0856$).
doi:10.1371/journal.pone.0048068.g003

<70.8% <98.3%; natural ambient light: May-June 2011). Eight tomato plants (development stage from seven to 10 leaves) were put into cages (145×70×80 cm) covered by an insect-proof mesh. Twenty-five two-day old *T. absoluta* adults (mixed males and females) were released in the cages simultaneously with one of the *Trichogramma* strains. To release the parasitoids, small cardboard strips containing about 400 parasitized eggs of *E. kuehniella* from which parasitoids were just beginning to emerge, were placed in an open tube (drops of honey were provided as food source). The experiment lasted three days which is nearly the mean longevity of *Trichogramma* adults in tomato greenhouse (Chailleux A. and Desneux N., unpublished data). Then leaflets were collected (20–30 leaflets collected on upper, medium and lower part of the plant) until 100 eggs were found. Eggs were kept on the leaflets and

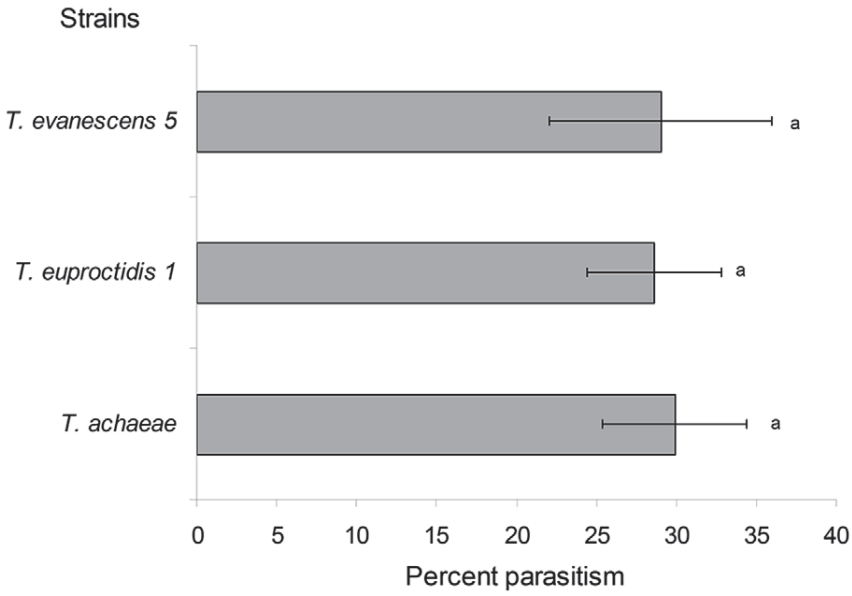


Figure 4. Parasitism of *Tuta absoluta* eggs in cages in greenhouse. Percentage (mean±SEM) of parasitized *T. absoluta* eggs per *Trichogramma* strain in cages in the greenhouse. Histograms bearing the same letter do not differ at $P<0.05$ (GLM analysis).
doi:10.1371/journal.pone.0048068.g004

placed into boxes in environmental cabinets (25°C, RH 70±10%, 16L:8D). Under these conditions, parasitized eggs become black in five days. The parasitized eggs were counted under a binocular microscope and the percentage of parasitism was calculated. We conducted seven to 12 randomized replicates for each *Trichogramma* strain (cage and order).

Greenhouse Experiments

The most promising strain, *T. euproctidis* 1 (see *Results section*), was tested under greenhouse conditions and compared to *T. achaeae*. Experiments were conducted in two 60 m^2 glass greenhouse compartments (min < mean temperature < max: 17.2°C <26.2°C <37.6°C; min < mean RH < max, 27.3% <71% <94.1%; natural ambient light: June-July 2011) located in Valbonne, French Riviera, France. Each greenhouse compartment contained three double rows of tomato plants under hydroponics cropping conditions. Forty adult moths (mixed males and females) were released simultaneously with 2,000 *Trichogramma* individuals. This situation mimicked a high level of infestation by the pest and a commercial release of *Trichogramma* for management of *T. absoluta* in tomato greenhouse (2,000 parasitoids for 25–100 m^2). Parasitoids were released using the same method used in cages (i.e. on cardboard strips) at one central point of the greenhouse. A sample of 50 eggs was collected randomly in each compartment three days after the release and kept for incubation as described in the previous section (cage experiments). Six replicates were conduced for each *Trichogramma* strain, three in each compartment of the greenhouse.

Statistical Analysis

All statistical analyses were performed using R software (R Development Core Team 2009) with the packages *multcomp* and *DTK*. For the laboratory experiments, the number of parasitized eggs (per female and per strain) were analysed using a generalized linear model based on Poisson distributed data with a log link function. Multiple comparisons were done using a Dunnett's post-hoc test (comparison to the reference species *T. achaeae*). In addition, the effect of *Trichogramma* species, along with host plant family, host moth family and year of collection (for each strain) was also tested using a similar generalized linear model. Differences between the proportions of females parasitizing *T. absoluta* eggs on tomato compared to *E. kuehniella* (control host, i.e. rearing host) were tested using a generalized linear model designed for modelling binomial data with a logit link function. The assessment of preference-performance relationship [31] may be an important factor in choosing biological control agents [32]. Therefore, a linear regression analysis was used to assess the relationship between the mean number of parasitized eggs (log-transformed data) and the proportion of females that accepted *T. absoluta* eggs as host for each *Trichogramma* strain (i.e. proportion of females stinging [aborted eggs] or parasitizing [black eggs] at least one egg of *T. absoluta*). Finally, for the cages and greenhouse experiments, the percent parasitism was analyzed using a generalized linear model designed for modelling binomial data.

Results

Screening of 29 Strains on *T. absoluta*

The mean number of eggs parasitized in tubes varied significantly depending on the strain ($F_{28,\ 338} = 10.907$, $P<0.001$) (Fig. 1). The level of parasitism was significantly linked to *Trichogramma* species ($F_{10,\ 335} = 8.296$, $P<0.001$). The characteristics of the habitat of origin also had a significant effect on parasitism of *T. absoluta* by the parasitoids tested (host moth family: $F_{6,\ 329} = 4.318$, $P<0.001$, and host plant family: $F_{5,324} = 7.328$, $P<0.001$); strains originally collected from Noctuidae, Plutellidae and Crambidae, as hosts, and from Solanaceae, Oleaceae and Vitaceae, as host plants, parasitized the most *T. absoluta* eggs. In contrast, the year of collection was not significant ($F_{1,323} = 0.146$, $P = 0.702$). Only the strain *T. euproctidis* 1 was significantly more efficient than *T. achaeae* ($Z = 3.379$, $P = 0.019$). Another relevant strain was *T. evanescens* 5 because of its thelytoky, and because it showed a similar level of parasitism to *T. achaeae* ($Z = -1.104$, $P = 0.999$). Moreover, *T. pretiosum* was not significantly different from *T. achaeae* ($Z = -0.428$, $P = 0.999$) although it came from the same area as *T. absoluta*. Altogether sixteen strains were not significantly different from *T. achaeae*.

The proportion of females that parasitized the host varied across the 29 strains tested: in three strains all females parasitized *T. absoluta*, and in one strain (*Trichogramma semblidis* 2) none of the females parasitized the host. The proportion of females that parasitized *T. absoluta* on tomatoes was significantly different than on *E. kuehniella* ($F_{1,\ 56} = 29.101$, $P<0.001$) (Fig. 2). For a majority of the strains tested, the proportion of females that parasitized at least one egg was lower on *T. absoluta* on tomatoes than on *E. kuehniella* on cardboard. The linear regression analysis between the mean numbers of *T. absoluta* parasitized eggs and the proportion of females that accepted *T. absoluta* eggs, i.e. preference-performance assessment [31,32], showed that strains parasitizing the most eggs also showed highest number of females accepting *T. absoluta* as host ($R^2 = 0.37$, $F_{1,\ 27} = 16.14$, $P<0.001$) (Fig. 3).

Cage Tests and Greenhouse Tests

Differences previously observed under laboratory conditions were no longer observed in cages (Fig. 4). The three strains (*T. achaeae*, *T. euproctidis* 1 and *T. evanescens* 5) showed similar efficiency against *T. absoluta* in cages; they all parasitized ~30% of the eggs ($F_{2,\ 25} = 0.019$, $P = 0.981$). In greenhouse compartments, both *Trichogramma* strains tested (*T. achaeae* and *T. euproctidis* 1) were able to parasitize *T. absoluta*. *T. achaeae* showed the highest efficiency: 65.9±7.77% (mean ± SEM) as opposed to 19.4±2.73% (mean ± SEM) for *T. euproctidis*. In this case, the difference was significant ($F_{1,\ 11} = 50.49$, $P<0.001$).

Discussion

Twenty-nine *Trichogramma* species-strains were tested under laboratory conditions and one strain of the *T. euproctidis* species (1) appeared promising (68.7% parasitism vs. 35.4% for parasitism for *T. achaeae* i.e. the species already commercialized in Europe and North Africa). Consequently this *T. euproctidis* strain was further tested on a larger scale i.e. in cages and greenhouses. However, the results of these later experiments did not corroborate the results obtained under laboratory conditions. Indeed the most efficient *Trichogramma* parasitoid against *T. absoluta* was still *T. achaeae*. Under cropping conditions, e.g. greenhouses, *T. euproctidis* was twofold less efficient against *T. absoluta* than *T. achaeae*. Our results showed that despite the fact that most *Trichogramma* strains did accept *T. absoluta* eggs on tomato leaflets, at least to some extent under laboratory conditions; the design was not sufficiently realistic to enable us to foresee their efficacy as a natural enemy of *T. absoluta* under real conditions.

The levels of parasitism of *T. absoluta* observed among the strains tested in the laboratory varied significantly. The low parasitism recorded for some strains may be attributed to two factors. First, in parasitoids, host specificity is mediated in part by host recognition and acceptance by the adult female parasitoid [25,32,33]. It has been shown that *Trichogramma* parasitoids prefer

hosts with relatively big eggs [34] but the eggs of *T. absoluta* are, by comparison, three times smaller than the eggs of *E. kuehniella* used in the rearing. Furthermore, we found a positive relationship between the number of parasitized eggs and the proportion of females accepting *T. absoluta* as host, demonstrating that parasitism levels were directly linked to the willingness of females to attack the host (as demonstrated in other parasitoid systems [25,32,35,36]). Second, low parasitism may result not only from rejection of *T. absoluta* eggs as host but rather from the poor capacity of some *Trichogramma* species/strains to cope with specific tomato plant characteristics. Various *Trichogramma* species have been reported to be highly susceptible to plant trichomes [37–41]. Although our study did not specifically assess the effect of trichomes on *Trichogramma* parasitoids, the experimental design was successful in identifying species having very little affinity for *T. absoluta*/tomato as host/host-plant complex.

Laboratory results also showed that the level of parasitism can differ greatly among strains of the same species. Variation within species of *Trichogramma* has already been encountered in other screenings [42–44]. Chassain and Bouletreau [45] studied the inter-strain variability of the main traits involved in *Trichogramma* parasitoid efficiency in host exploitation i.e. longevity, fecundity, progeny viability, progeny sex ratio and progeny allocation. They reported great differences among strains of the same species coming from different habitats, as well as between two different species coming from the same habitat. Consistent with these findings, our results showed that the characteristics of the original host and host plant of a given parasitoid strain, had an effect on its efficiency in parasitizing *T. absoluta* eggs, with strains originally collected from Solanaceae, Oleaceae and Vitaceae showing the best performance. Therefore, it is important to consider both initial host species and habitats when selecting strains of *Trichogramma* parasitoids for biological control programs.

The results from the cages/greenhouses did not match those from the laboratory; higher efficacy of *T. euproctidis* on *T. absoluta* disappeared when the scale of the experiments was increased. Differences between results under laboratory and greenhouse conditions may be due to both biotic and abiotic parameters. First, *Trichogramma* are known to be able to avoid plants bearing trichomes [46]. For some strains, females may have attacked *T. absoluta* eggs when constrained on tomato leaves in tubes in the laboratory but may have been able to avoid foraging on tomato leaves when released into cages or greenhouses. In addition, the oviposition pattern of hosts is a key factor for *Trichogramma* efficiency; *T. absoluta* does not lay egg masses but most of the time isolated eggs which thus increases the energy cost of foraging for hosts. Second, high temperatures, that are typical of greenhouses in summer, may impact differentially the various strains/species of *Trichogramma* [43,47]. Moezipour *et al.* [48] indicated that there is a significant difference in the functional response of *Trichogramma brassicae* when tested at 20 or 30°C, and previous studies have shown that temperature and relative humidity can affect biological traits in *Trichogramma* spp. [43,47,49].

On the other hand, we could assume that differences between parasitoid efficiency recorded under laboratory and greenhouse conditions may result from the time that the different strains have spent under rearing conditions (year of collection). Efficiency under cropping conditions of one *Trichogramma* strain could be modified by the length of time spent under the rearing conditions in the laboratory, i.e. in tubes on alternative hosts such as *E. kuehniella* eggs, and at optimal temperature and humidity i.e. 25°C and 70% HR [50]. As the life cycle of *Trichogramma* parasitoids is usually short (egg to adult in about 11 days), adaptation to rearing conditions (humidity, temperature, mass-rearing host, confined environment, etc.) may occur. On the contrary, previous adaptations to given field conditions could disappear after a long spell of rearing under optimal laboratory conditions. Despite this, the collection year did not affect *Trichogramma* efficiency when assessed under laboratory conditions (no significant *year of collection* factor, $P = 0.702$). However it may impact parasitism only at a larger experimental scale (e.g. in greenhouse) where, for example, the foraging and dispersal capacities are key components of the parasitoid efficacy [33,51]. Therefore, time spent in optimal rearing conditions may likely also partly explain some of the differences among strains tested in cages and greenhouse compartments.

We recorded the potential of various *Trichogramma* strains for biological control of *T. absoluta* in Europe. Nevertheless, our results did not identify that other *Trichogramma* strains showed better biocontrol traits than *T. achaeae*, i.e. higher fertility, high proportion of females/thelytoky and the capacity of diapause in cold storage in biocontrol company facilities. Further screening of *Trichogramma* parasitoids for potential management of *T. absoluta* would have to be based on the assessment of parasitoids collected on the targeted host in tomato crops under standard greenhouse cropping conditions in Europe. Doing this would increase the chances of assessing species that show greater likelihood of affinity within the cropping conditions. Colonies should be initiated with high number of field-collected individuals and new parasitoids should be added periodically. During laboratory screening, strains that showed very low parasitism levels were identified and removed; nonetheless, the efficacy of *Trichogramma* parasitoids under cropping conditions was not easily predictable from laboratory experiments. Studies could also be conducted directly in large cages (i.e. with multiple plants) in greenhouses when there are few strains to be tested. This way, all relevant criteria for strain selection could be taken into account and laboratory screening steps may be bypassed. Further studies would aim to (i) identify efficient parasitoids on *T. absoluta*, notably to prevent overuse of insecticides in tomato crops (and therefore ensure sustainability of current biological control and integrated pest management programs on this crop), and (ii) define new criteria that allow research and development programs at biocontrol companies to select accurately and quickly new *Trichogramma* strains (and more generally parasitoids) in the framework of biological control.

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Author Contributions

Conceived and designed the experiments: AC ND JS ET. Performed the experiments: AC. Analyzed the data: AC ND. Contributed reagents/materials/analysis tools: ND JS HDTK PM ET. Wrote the paper: AC ND.

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Article 5

Stenomesius japonicus (Asmead) and *Necremnus artynes* (Walker) (Eulophidae), two indigenous larval ectoparasitoids of the invasive pest *Tuta absoluta* (Lepidoptera) in Europe

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Abstract

During the last few years following the invasion of European tomato crops by the invasive South American tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), several indigenous larval parasitoid species were recorded as natural enemies of this pest. *Necremnus artynes* Ashmead and in a lesser extent *Stenomesius japonicus* Walker have been frequently reported attacking *T. absoluta* in tomato greenhouses in Europe and they are considered as possible biocontrol agents. Few biological data are available on these two species, notably when they parasitize *T. absoluta*. Therefore, the present study documents their biology when parasitizing *T. absoluta,* and provides scientific bases for potential development of biological control programs relying on larval ectoparasitoids in invaded areas. We demonstrate the ability of the two parasitoid species to reproduce on *T. absoluta* as host on the tomato plant. We characterize and detail biological traits hinting the importance of larvae killing behavior without actual parasitism i.e. host-feeding and host-stinging behavior. *Necremnus artynes* female parasitized 28.5 larvae and killed 26.2 larvae during their life, *S. japonicus* female was more efficient and parasitized 144.3 larvae and killed 90.1 larvae during their life. In a second step, we also explore the effect of the *T. absoluta* larvae instar on parasitism by *S. japonicus* and subsequent development of the parasitoid offspring. Our results support the hypothesis that the two species are good candidate for biological control programs targeting *T. absoluta* in Europe.

Keywords: host-feeding, host-stinging, parasitoid behaviour, indigenous biocontrol agent.

Introduction

Biological invasions are considered as a major threat to biodiversity and agriculture all over the world (Haack et al. 2010; Desneux et al. 2011; Paine et al. 2011; Ragsdale et al. 2011), often resulting in huge economic losses (Pimentel et al. 2000, 2005a). To control pest invasion, chemical pesticides are often used as a first measure to limit infestation and spread. However it implies additional production costs and negative side effects on non-target organisms, e.g. biological control agent against other pests, and on human health and environment (Desneux et al. 2007; Mack et al. 2000; Pimentel 2005b). The South American tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) invaded Spain in 2006 (Desneux et al. 2010). After arrival, it rapidly spread throughout the Eurasian continent and cause majors damage to crop and additional production cost (Desneux et al. 2010, 2011).

Biological control is a key component of integrated pest management programs in tomato greenhouses in Europe. The mirid bugs *Nesidiocoris tenuis* Reuter and *Macrolophus pygmaeus* Rambur (Heteroptera: Miridae) are the two predators most commonly used in tomato crops, notably for their ability to suppress whiteflies, leafminer and aphid populations (Albajes and Alomar 1999; Lykouressis et al. 2009; Castañé et al. 2004; Sanchez 2009; Castañé et al. 2011). Their efficiency as biocontrol agents of *T. absoluta* has been demonstrated in greenhouse experiments (Calvo et al. 2012 and Bompard et al. 2013 for *N. tenuis* and *M. pygmaeus*, respectively), but most of predation occurs on *T. absoluta* eggs; i.e. their effectiveness on the larvae stage of the pest is limited (Urbaneja et al. 2009; Arnó et al. 2009; Jaworski C, Bompard A and Desneux N, unpublished data). Egg parasitoids have showed some potential for reducing damages on greenhouse tomatoes and *Trichogramma achaeae* Nagaraja and Nagarkatti is currently available in Europe for inundative biological control of *T. absoluta* (Cabello et al. 2012; Chailleux et al. 2012; Desneux et al. 2010; Urbaneja et al. 2012). However, these parasitoids target pest eggs as mirid predators, plus they are costly and the possibility to use European *Trichogramma* species is yet limited (Chailleux et al. 2012, submitted). Overall, none of the natural enemies currently used on *T. absoluta* are able to prey significantly on larvae, the stage that actually cause the damages to tomato plants and fruits; farmers should therefore rely on insecticides when *T. absoluta* larvae density reaches the economic injury level. Integrating natural enemies able to predate the larval stages of *T. absoluta* in biological control programs is needed to provide sustainable biological control packages to farmers (Arnó and Gabarra 2011; Urbaneja et al. 2013).

Various larval parasitoids have been found parasitizing *T. absoluta* in Europe (Desneux et al. 2010; Urbaneja et al. 2012; Ferracini et al. 2012; Zappalà et al. 2012, in prep.; Gabarra et al., in prep.), notably species belonging to the Eulophidae, Braconidae and Ichneumonidae families. More specifically, the Eulophidae idiobiont ectoparasitoids *Necremnus artynes* Walker and *Stenomesius japonicus* Ashmead have occurred spontaneously in *T. absoluta*-infested tomato crops in various European Mediterranean basin countries (Desneux et al. 2010; Urbaneja et al. 2012; Zappalà et al. 2012; Zappalà et al., in prep.).

The present study aimed at characterizing and documenting various biological traits of these two endemic larval Eulophidae parasitoids when parasitizing *T. absoluta* larvae on tomato plants under laboratory conditions. The two larval parasitoids were chosen because they showed some potential against *T. absoluta* during pilot experiments and they occur frequently in the tomato production areas. First, biological traits were studied at the leaf level and then parasitism capacity was further documented at the plant level. Second, we choose the most promising species according to biological trait results to evaluate (i) the ability of the parasitoid to develop on the four *T. absoluta* larval instars and (ii) how the larval instar may affect the success of parasitoid offspring development. The general aim was to provide key data for further development of larval parasitoid-based biological control programs.

Materials and methods

Biological materials

The plants used in the experiments were 5-week old tomato plants, *Solanum lycopersicum* L. cv. Bodar. They were grown in climatic chambers (24 ± 1°C, HR: 65%, photoperiod 16L:8D) and a nutrient solution was applied daily. *Tuta absoluta* was reared on tomato plants in growth chambers (25 ± 1°C, RH 70 ± 10%, 16L:8D) inside cages (55×75×80 cm), and imago were fed on honey placed on one wall inside the cages. The parasitoids *Stenomesius* nr. *japonicus* and *Necremnus* sp. nr. *artynes* (named as *S. japonicus* and *N. artynes* thereafter, respectively) used for the experiments were collected in Northeast Spain in 2009 from tomato greenhouses and sentinel plants, (*S. japonicus* initial number of individuals = 11, *N. artynes* initial number of individuals = 80). The two species were identified by Dr. MJ Verdú (IVIA, Valencia, Spain) and a specialist of Hymenopteran taxonomy (A. Ribes, Lleida, Spain). Parasitoids colonies were reared in cages (45×45×55 cm) on tomato plants infested by *T. absoluta* (25 ± 2°C, RH 75 ± 10%, 16L:8D).

When using *T. absoluta* larvae during the experiments, the various larval instars were obtained from tomato infested plants grown in climatic chambers. The larvae were carefully extracted from their mine and deposited on the tomato leaf used in the experiments one hour before starting experiment to allow the larvae to dig new mines.

Experiment 1 - Parasitoid biological traits

Newly emerged adult parasitoids were kept together for mating, then each female was transferred to a transparent plastic box (d: 10cm; h: 5.5cm) covered with a mesh, containing a trifoliate infested with 10 *T. absoluta* late L2-early L3 instar larvae. These instars were demonstrated to be suitable for *N. artynes* and *S. japonicus* reproduction on previous works (Gabarra and Arnó, unpublished). Honey was added as a food source. The leaf stem was sticking out of the box and soak in water (see Biondi et al. 2012). Previous reports indicated that these two parasitoids parasitize the second and third instar (Desneux et al. 2010; Urbaneja et al. 2012). Females were checked and transferred to a new set-up three times a week, until their death. Each *T. absoluta* larvae was inspected under a binocular microscope and numbers of parasitized, dead and alive larvae were recorded separately. Number of parasitoid eggs per larva was also counted. Six to 7 replicates were conducted for each parasitoid species. To assess natural mortality three control cages with *T. absoluta* larvae but without parasitoids were carried out three times a week similarly to the replicas with parasitoids, and the number of naturally dead larvae were recorded.

Experiment 2 - Parasitism of Tuta absoluta larvae on tomato plants

Microcosms were created by placing a transparent plastic cylinder over a potted tomato plant (4-5 fully developed leaves). Cylinders (d: 20 cm, h: 30 cm) had a mesh top for ventilation. Two treatments were tested (i) plants with *T. absoluta* and *S. japonicus* and (ii) plants with *T. absoluta* and *N. artynes*. In each cylinder, 10 *T. absoluta* adults (0 to 2-days old) were released. After 12 days under controlled conditions (25 ± 2 °C, 70-80% R.H., 16 L: 8 D) three *S. japonicus* or three *N. artynes* mated females (24-72 h old), were released into the cylinder. This proportion of parasitoids and *T. absoluta* aimed at providing host in excess for the parasitoids. Eight days after the release, the parasitoid females were removed. In all the microcosms, three new tomato plants were added in order to increase plant availability for *T. absoluta* larvae. Containers were checked three times a week and number and sex of parasitoid adults were recorded. Six replicates were conducted per parasitoid species.

Experiment 3 - Influence of host larvae instars on parasitism, sex-ratio and adult size in Stenomesius japonicus

Based on results gained during Experiments 1 and 2 which hinted a better efficacy of *S. japonicus* against *T. absoluta* than *N. artynes* (see Results below), we explored the impact of *T. absoluta* larva instars on parasitism by *S. japonicus*. For this bioassay, the same transparent plastic boxes than in the Experiment 1 were used. Five larvae of each tested instar were placed per box. According to results from a pilot study on developmental timetable of *T. absoluta* larvae (Chailleux A. and Desneux N., unpublished data) four different batches were scheduled to made available the four different *T. absoluta* larva instars the day the experiment started (day 0). One mated *S. japonicus* female was introduced per box at day 0. After 24h, the females were removed and boxes were kept in climatic chambers for 15 days until emergence of parasitoid offspring. Offspring were counted, sex was recorded, and parasitoid size was measured with a micrometer under a binocular microscope.

Statistical analysis

All statistical analyses were performed using the R software (R Development Core Team 2009) with the *multcomp* and *agricolae* packages. The biological traits were compared using a generalized linear model (GLM), based on a gamma distribution using the identity link function for the longevity, and based on a Poisson distribution using a log link function for the fecundity, the number of killed and parasitized larvae and the number of eggs per larvae. The parasitism at the plant level was analyzed using a GLM based on a Poisson distribution for the number of parasitoid offspring, and using a GLM based on a binomial law using a logit link function for the sex-ratio (females/total). By the same way, for the *Influence of host larvae instars* experiment, the number of offspring produced per box was analyzed using a GLM based on Poisson distribution data. For the sex-ratio (females/total), we used a model designed for modelling binomial data. Parasitoid size was analyzed by a one-way ANOVA (data normally distributed). When statistically significant differences appear in the GLM or the ANOVA, means were separated using a Least Significant Difference post-hoc test (LSD test) for multiple comparisons.

Results

Experiment 1 - Parasitoid biological traits

Significant differences were observed between the longevity of the two species ($F_{1; 11} = 35.61$; $P < 0.001$). *Stenomesius japonicus* lived 62.4±10.2 days and *N. artynes* lived 15.2±2.3 days in our conditions and both species oviposited during their entire life. Because of this huge differences in longevity, the fecundity, killed larvae and parasitized larvae were analyzed in two ways: the total number (during the entire life of females) and per day. These three traits were significantly higher for *S. japonicus* (Fig. 1 A) when comparing the total value (fecundity: $F_{1; 11} = 42.46$; $P < 0.001$; killed larvae: $F_{1; 11} = 14.53$; $P = 0.003$; parasitized larvae: $F_{1; 11} = 36.14$; $P < 0.001$). However, when related to the longevity (Fig. 1 B), only the fecundity was still significantly higher for *S. japonicus* ($F_{1; 11} = 7.38$; $P = 0.020$), the parasitized larvae were marginally significant ($F_{1; 11} = 4.84$; $P = 0.050$) and, conversely, the killed larvae were significantly higher for *N. artynes* ($F_{1; 11} = 6.22$; $P = 0.030$). Finally the average number of eggs laid per *T. absoluta* larvae was almost the same for the two species, 1.68±0.09 for *S. japonicus* and 1.42±0.11 for *N. artynes* ($F_{1; 11} = 3.10$; $P = 0.106$) .

Experiment 2 - Parasitism of Tuta absoluta larvae on tomato plants

Stenomesius japonicus tended producing more offspring than *N. artynes* when tested on whole tomato plants, i.e. 55.6±11.1 vs. 34.8±7.2 for *N. artynes*, the difference was actually not significant, likely owing to the variance in the results ($F_{1; 10} = 2.36$; $P = 0.156$). Similar trends were found for offspring sex-ratios; the proportion of females tended to be lower for *S. japonicus* with a sex-ratio (females/total) of 0.43±0.07 compared to a sex-ratio of 0.57±0.08 for *N. artynes*. However this difference was not statistically significant ($F_{1; 10} = 1.57$; $P = 0.239$).

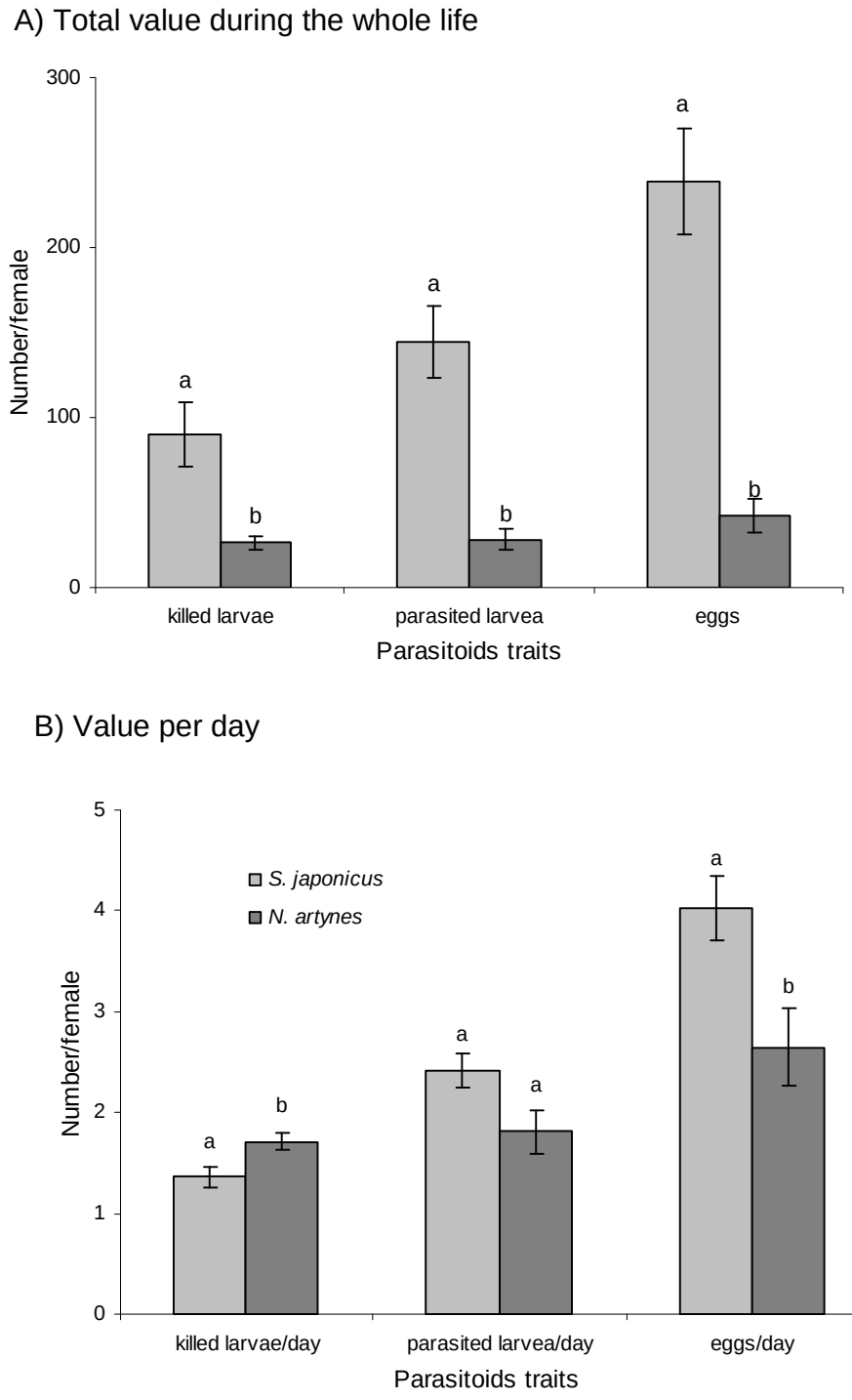


Figure 1: Mean total number (±SEM) of killed and parasitized *T. absoluta* larvae and parasitoid eggs per female for *Stenomesius japonicus* and *Necremnus artynes* under laboratory conditions during the whole life (A) and per day (B). Generalized linear model were carried out separately for each traits tested. The histograms bearing the same letter are not significantly different at $P < 0.05$.

Experiment 3 - Influence of host larvae instars on parasitism, sex-ratio and adult size in Stenomesius japonicus

Progeny per female parasitoid varied significantly with host instars ($F_{3; 64} = 6.05$; $P = 0.001$) (Fig. 2A). The highest offspring number was produced on third instar larvae whereas few parasitoids emerged from first and second instars. More offspring were produced on fourth instar larvae than on the first and second ones though both were only marginally significant (Z = 1.86, $P = 0.063$ and Z = 1.69, $P = 0.091$ respectively). The size of offspring produced also varied as function of host instars ($F_{2; 41} = 13.29$; $P < 0.001$) (Fig. 1B). Adults emerging from the third and fourth instars were about 0.5 mm longer than those emerging from first and second instars. Individual emerging from the fourth instar were marginally significantly taller than those emerging from the third instar (t = 1.76, $P = 0.085$). Sex-ratio varied significantly among host instars tested ($F_{2; 19} = 5.42$, $P = 0.014$), and was female-biased when hosts were older. Only females emerged when parasitoids developed in *T. absoluta* fourth instar larvae (Fig. 2C). Fifteen to 19 replicates were carried out for each instar.

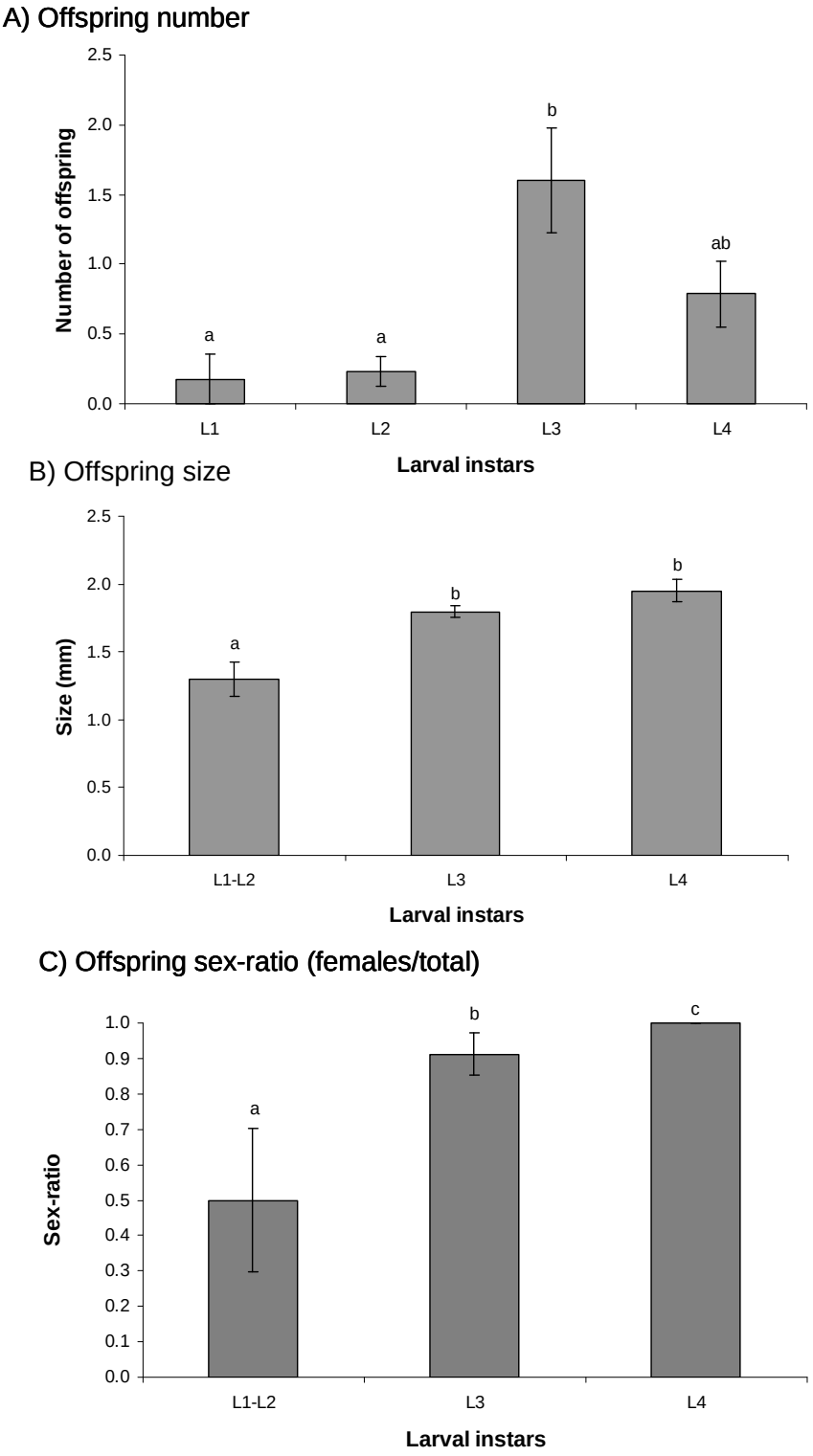


Figure 2: (A) Mean number (±SEM) of *Stenomesius japonicus* offspring/ female, (B) Sex-ratio and (C) Mean size (±SEM) of *S. japonicus* offspring produced on the various instars of *T. absoluta* larvae. Histograms bearing different letters are significantly different at $P > 0.05$ level (LSD post-hoc test).

Discussion

Our results demonstrated the ability of two indigenous European Eulophidae parasitoids, *S. japonicus* and *N. artynes*, to reproduce using the invasive species *T. absoluta* as a host under laboratory conditions (tests on tomato leaves and whole tomato plants). These are new association because these indigenous parasitoids never encountered the pests before; the later is an invasive alien pest species from South America. We recorded parasitoid key biological traits and detailed the impact of host larvae instar for the most promising species (*S. japonicus*).

No data are available in the literature about biological traits of *S. japonicus* parasitizing *T. absoluta* and a single study reported the biology of *N. near artynes* on *T. absoluta* larvae in laboratory (Ferracini et al. 2012). These authors reported that *N. near artynes* only accepts *T. absoluta* first and second instar larvae for ovipositing and feeding. These differences may be due to the existence of different cryptic species (as widely reported in parasitoids e.g. Sha et al. 2007; Desneux et al. 2009a; Gebiola et al. 2009) and/or to biological differences among the two *N. artynes* populations tested. Such differences among populations inside a given species have been largely documented in parasitoids (Chassain and Bouletreau 1991; Pizzol et al. 2010; Tabone et al. 2010; Chailleux et al. 2012).

Eulophidae have already been recorded has efficient parasitoids of *T. absoluta* in South America, especially *Dineulophus phthorimaeae* (Luna et al. 2010) and *Neochrysocharis formosa* (Luna et al. 2011). No biological data are currently available for *N. Formosa* and only few are for *D. phthorimaea* (Hymenoptera: Eulophidae); it is a solitary ectoparasitoid, adult life span is ~12 days for females and ~9 days for males (Luna et al. 2010), sex ratio is 0.5. The longevity of *S. japonicus* and *N. artynes* is higher than those of *D. phtorimaeae* when they develop on *T. absoluta* as host. The sex-ratio seems closed to what we observed, though we showed that the sex-ratio is biased depending on the larval instar. *D. phtorimaeae* parasitized less than one larvae per day (~0.1 larvae/day) in laboratory experiment, and the number of killed larvae without oviposition was almost equal to the number of parasitized larvae (Savino et al. 2012). Overall, the two parasitoids tested in our study showed higher efficiency than *D. phtorimaeae,* e.g. they parasitized > 1 larvae per day and they killed more *T. absoluta* larvae.

Our laboratory experiment stressed the important occurrence of non-parasitism parasitoid-induced death in *T. absoluta* larvae, i.e. parasitoids killed non-parasitized larvae, as an important component of larval parasitoid efficiency. Killed non-parasitized larvae, also

named non-reproductive killing (Byeon et al. 2009), grouped larvae killed for host-feeding and larvae killed for an unknown reason i.e. not for host-feeding and not for parasitizing, usually named sting larvae (Byeon et al. 2009; Bernardo et al. 2006). The number of *T. absoluta* non-parasitized larvae killed was high for both parasitoids, ~90 per *S. japonicus* female and ~26 per *N. arthynes* female. Host-feeding play an important role in biological control (Jervis et al. 1996) and it has been shown that Eulophidae parasitoids sometime kill more larvae than actually parasitize, the importance of host-feeding in parasitoid-host population dynamics was first noted by Johnson (1915) on an Eulophidae species and then other study reported this phenomenon (e.g. Foelkel et al. 2009; Musundire et al. 2012; Arnó et al. 2003). Several Eulophidae have been shown to switch oviposition behavior to host-feeding behavior in response to the size of hosts encountered, e.g. *Pnigalio minio* preferred to feed on second and early third *Phyllocnistis citrella* instar larvae and to oviposit on third instar (Duncan and Peña 2000).

In addition, it has been demonstrated that parasitoid females can kill host neither to oviposit nor to feed i.e. stinging behavior. Host-stinging behavior has been frequently observed in parasitoids of leafminers, particularly in the genera *Diglyphus, Sympiesis*, and *Pnigalio*. While most of the observations of host-stinging behavior of Eulophidae wasps were prevalently gained through laboratory tests, for instar for *Pnigalio soemius* (Bernardo et al. 2006), Casas (1989) observed frequent host larvae paralysis in the field without actual egg parasitoid and/or sign of host-feeding. Patel et al. (2003) suggested that the rejection of a portion of stung hosts may be a mechanism for managing the density of leaf-miner larvae on leaflets and thus avoiding offspring mortality in preventing premature plant death (that might comprise parasitoid offspring survival if host fall on the ground, e.g. increasing risk of intraguild predation). Excessive leaf-mining can cause desiccation and necrosis of leaflets and potentially decreasing survival of parasitoid larvae. Another possible reason for this behavior is that female parasitoids kill more host larvae to provide a food reserve for their developing offspring so the parasitoid larvae can switch from a larva to another when the resource on the first one gets depleted (Lauziere et al. 1999). Host-stinging behavior was observed in the laboratory for *S. japonicus* (Chailleux A and Gabarra R, unpublished data), and *N. artynes* likely also behaves this way. However the proportion of host-feeding and host-stinging was not assessed in the present study. These two behaviors can lead to underestimation of parasitoids efficiency in field study owing to sampling methods which generally consist in monitoring parasitized larvae. High levels of killed larvae without oviposition was also recorded on *T. absoluta* in laboratory for another parasitoids the Braconidae, *B. nigricans*, the

females killed ~87 larvae during their life, i.e. about 40 days (Biondi et al. 2013), and this result is similar to those recorded for *N. artynes* and *S. japonicus*.

The ability of the two parasitoids to reproduce on *T. absoluta*, confirmed with the experiment involving the use of whole tomato plants, stressed the possibility to use these parasitoids in inoculative biological control programs against *T. absoluta*. Eulophidae parasitoids have already been successfully used in biological control in Europe. In European greenhouse tomato crops, conservation of natural populations and inoculative releases of *Diglyphus isaeae* (Walker) are currently used to control leafminers (Gabarra and Besri 1999). In Spain, the Eulophidae *Citrostichus phyllocnistoides* (Narayanan) was used in classical biological control. Parasitism reached 60% and damages were reduced of 56% (Garcia-Mari et al. 2004). In Australia *S. japonicus* parasitized up to 75% of *Dialectica scalariella* (Zeller) larvae (James and Stevens 1992) in outdoor area. *Stenomesius japonicus* appeared to be the most promising species especially because of a higher longevity which induced a higher total fecundity. Moreover, the longevity is an essential criterion in inoculative biological control because it allows the survivorship of the parasitoids in the crop when no or few hosts are available.

Given these results, *S. japonicus* was chosen to explore the impact of the larval instar on the offspring production larval parasitoid on *T. absoluta*. Larval instars i.e. host size, are known to possibly impact larval parasitoids development, in some case the youngest host instars did not allow the development of the parasitoid (Fidgen et al. 2000; Karamaouna and Copland 2000). It had also been demonstrated that the proportion of offspring females increase with host size (Fidgen et al. 2000; Karamaouna and Copland 2000; Henry et al. 2006; Desneux et al. 2009b). Our results are consistent with these studies, we showed that biggest instars tested yielded the higher number of offspring, though the last one (fourth) *T. absoluta* instar gave apparently less offspring than the third one. However the values were not significantly different and could be related to the specific behavior exhibited by the fourth instar larvae. It usually goes out of mines to pupate but the parasitoid does not parasitize larvae that are out of their mines; therefore the fourth instar larvae were likely available for parasitism for a shorter time than others instars. However, our experiment do not allow to discriminate if low offspring production are due to female choice i.e. female preference for big host, or to a failure in the parasitoid offspring to development owing low adequacy of the resource or insufficient resource.

Further studies should focus on experiments under more realistic conditions in greenhouse to evaluate efficacy and persistence of the parasitoid during a full cropping

season. Techniques to improve the establishment and, mainly, the persistence of parasitoids should be investigated. The two parasitoids may be difficult to mass-rear owing to their incapacity to parasitize larvae outside tomato mines. Therefore, further studies should investigate possibility for (i) conservative or inoculative biological control methods more than inundative releases, and (ii) means to improve parasitoids persistence during the cropping season thanks to additional food sources or alternative non-pest host sources.

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Chapitre 4 : Les interactions liées à l'introduction de nouveaux auxiliaires au sein de l'agrosystème

I) La lutte biologique basée sur les trichogrammes

Présenté en tant que *Article 6,* sous presse dans *Journal of Pest Science*,
et *Article* 7, soumis à *Journal of Economic Entomology*,
voir annexe 2 pour une photo du dispositif expérimental.

Article 6

Potential for combined use of parasitoids and generalist predators for biological control of the key invasive tomato pest, *Tuta absoluta*

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Potential for combined use of parasitoids and generalist predators for biological control of the key invasive tomato pest, *Tuta absoluta*

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Abstract: The tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) has recently invaded Mediterranean countries and is a major pest in tomato crops. Trichogrammatid oophagous parasitoids have showed promising potential for controlling the pest before yield decreases in greenhouse. In protected tomato crops (greenhouse), mirid predators are commonly used for biological control of whiteflies and they also prey on *T. absoluta*. These predators do not attack *Trichogramma* adults but they may partially decrease overall impact of parasitoids on *T. absoluta* if intraguild predation (IGP) occurs on parasitized eggs. In laboratory conditions we tested if the mirid predator *Macrolophus pygmaeus* shows preference between parasitized and unparasitized *T. absoluta* eggs. We also tested if the predator reduces the number of parasitized eggs on caged tomato plants (microcosms) and assessed the efficacy of natural enemies used alone or together in limiting *T. absoluta* populations. We found that IGP is inflicted on the parasitoid *Trichogramma achaeae* by *M. pygmaeus* and that the risk of IGP depends on the developmental stage of *T. achaeae* inside the egg because the larva is at risk mostly early in its development (when parasitized egg is still yellow). In addition, we observed that non-consumptive events, likely probing of the predator on parasitized eggs, may induce mortality in parasitoid offspring without actual feeding on parasitized eggs. However, both IGP and non-consumptive events decreased when the predator was not confined with parasitized eggs in small arenas. Despite negative effects of the predator to the parasitoid, results demonstrate that adding *Trichogramma* parasitoids may significantly increase the level of control of the pest over what could be attained when only the mirid predator *M. pygmaeus* is present on tomato. Implications of results are discussed regarding potential of these natural enemies for biological control of *T. absoluta* in greenhouse tomato crops.

Keywords: predator, parasitoid, intraguild predation, *Macrolophus pygmaeus*, *Trichogramma*.

Introduction

Invasive species represent a major threat to crops (Suckling and Brockerhoff 2010, Ragsdale et al. 2011) and agricultural pests can reduce yield and increase production costs related to their management (Thomas 1999). The tomato leafminer *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is one of the most devastating pests of tomato in South America (Barrientos et al. 1998). This pest was first detected in 2006 in Spain and it has spread to most of European and Mediterranean basin countries (Desneux et al. 2010a, 2011); in few years it has become a key agricultural threat to tomato production worldwide (Desneux et al. 2011). Plant injury consists of mine-formation within the mesophyll by feeding larvae, thus affecting the plant's photosynthetic capacity resulting in lower fruit yield (Desneux et al. 2010a). Appearance of *T. absoluta* has led to extensive insecticide use by tomato growers, potentially causing a multitude of undesired side effects on non-target organisms (Biondi et al. 2012a, 2012b, and see Desneux et al. 2007 for a thorough review). A comprehensive integrated pest management (IPM) program is needed to keep *T. absoluta* density below the economic injury level on tomato and a key component of such a program is biological control (Desneux et al. 2010a).

Among possibilities for controlling the pest, oophagous parasitoids of the family Trichogrammatidae (Hymenoptera) have showed some potential for reducing pest damages in greenhouse grown tomato (Desneux et al. 2011, Trottin-Caudal et al. 2011, Cabello et al. 2012). Wasps of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are the principal egg parasitoids of lepidopteran agricultural pests (Smith 1996, Andrade et al. 2011, Pizzol et al. 2012, Yuan et al. 2012). Most *Trichogramma* spp. are easy to rear, can be released in fields or greenhouses (Tabone et al. 2010, 2012), and various species have been reported as natural enemies of *T. absoluta* in South America and in Europe (Parra & Zucchi 2004, Pratissoli et al. 2005, Desneux et al. 2010a, Zappala et al. 2012). Inoculative releases of *Trichogramma* spp. have been used against pests in various agricultural systems (Kuhar et al. 2002, Thomson et al. 2003, Hoffmann et al. 2006, Mills 2010). In Europe, studies are in progress to develop *Trichogramma*-based inoculative and augmentative biological control methods against *T. absoluta* (Cabello et al. 2012, Chailleux et al. 2012, Khanh et al. 2012).

Mirid predators, mainly *Macrolophus pygmaeus* Wagner and *Nesidiocoris tenuis* Reuter, are commonly used against whiteflies in greenhouse tomato crops (Bonato et al. 2006, Nannini et al. 2007, Calvo et al. 2009) and they are also biocontrol agents of *T. absoluta* as they prey on eggs (Urbaneja et al. 2009, Calvo et al. 2012, Bompard et al. 2013). However, *N.*

tenuis often prompts insecticide applications at high predator density because of damages it inflicts to both plants and fruits (Calvo et al. 2009, Castane et al. 2011), and *M. pygmaeus* is not fully efficient when used alone against *T. absoluta* (Jaworski C and Desneux N, unpublished data). The predators may feed on *Trichogramma*-parasitized *T. absoluta* eggs on tomato plants. Such intraguild predation (IGP) may decrease the overall efficacy of parasitism of *T. absoluta* if combining *Trichogramma* wasps and mirid predators. IGP occurs when two species that share a prey resource (and therefore may compete) also engage in trophic interaction with each other (e.g. predation) (Polis et al. 1989, Rosenheim et al. 1995, Holt and Huxel 2007). Identification of such trophic links is of primary importance to the success of pest management programs. Therefore, assessment of IGP is required before release(s) of *Trichogramma* parasitoids could be recommended in greenhouse grown tomato crops where mirid predators are used as biocontrol agents for IPM purposes.

In this context, we investigated (i) whether *M. pygmaeus* would attack *Trichogramma*-parasitized eggs in a no-choice test, (ii) whether *M. pygmaeus* would show a preference for parasitized eggs (two-choice test), and (iii) what the combined effect of both natural enemy species would be on *T. absoluta* survival (on single plant microcosm). In addition, we assessed the possible impact of non-consumptive events by *M. pygmaeus* on survival of *Trichogramma* pupae inside *T. absoluta* egg. Such events, notably probing, have been reported to increase drastically the mortality rate in probed prey (e.g. adult insect, host embryo developing in egg, parasitoid developing into host, etc.) in case of attack by Hemipteran predators (Butler & O'Neil 2006), presumably owing to injection of venom (Edwards 1961, Cohen 1990).

Materials and Methods

Biological materials

The plants used in the experiments were four-week old tomato plants, *Solanum lycopersicum* L. cv. Marmande (grown in climatic chambers, 24±1°C, 65±5% RH, 16L:8D). A *T. absoluta* colony was initiated using greenhouse-collected adults (Alenya, France) (initial number of individuals = 190) three months before starting the experiment. The pest was reared on caged tomato plants in climatic chambers (24±1°C, 65±5% RH, 16L:8D). We used the egg parasitoid *Trichogramma achaeae* Nagaraja and Nagarkatti as this species is currently

available for biological control of *T. absoluta* in Europe and showed highest efficacy on *T. absoluta* among various *Trichogramma* species tested (Chailleux et al. 2012). The parasitoid was reared on UV irradiated eggs of a factitious host, *Ephestia kuehniella* Zeller (25±1°C, 70±5% RH, 16L:8D). The parasitoids used for all experiments were 24-h old. *Macrolophus pygmaeus* nymphs were provided by Biotop (Valbonne, France) and kept on potted tomato plants in plastic rearing cages in climatic chambers (23±1°C, 70±5% RH, 16L:8D) until they reached the adult stage. UV irradiated *E. kuehniella* eggs were used as supplemental diet. The predators used during the experiments were 48-h old mated females that had been starved for 24h prior to the start of the tests.

No-choice assay in glass tube

Individual *M. pygmaeus* were caged in glass tubes (length: 7.5cm, diameter: 1cm) with either 12 unparasitized *T. absoluta* eggs, 12 yellow *T. achaeae*-parasitized *T. absoluta* eggs i.e. parasitized for 0-3 days, or 12 black parasitized *T. absoluta* eggs i.e. parasitized by *T. achaeae* for 4-5 days (these conditions and the method for obtained the parasitized eggs were based on results of pilot and preliminary experiments, Desneux et al. 2010b). For all assays, the parasitized eggs were obtained according to the method of Chailleux et al. (2012). The two *T. achaeae*-parasitized *T. absoluta* egg types (yellow or black) were used because predators could exhibit marked tendency to avoid parasitized hosts in favor of unparasitized hosts (Rouechdi and Voegelé 1981, Ruberson and Kring 1991, Roger et al. 2001) and particularly because the age of the parasitoid within the host (i.e. the egg in case of *Trichogramma* spp.) can influence the degree of preference/avoidance (Hoelmer et al. 1994, Brodeur and Boivin 2004). Eggs were deposited onto a paper card and placed in the tube (one paper card per tube). One tomato stem (2 cm) was also inserted into the tube to provide moisture. There were 12-18 replicates per treatment and mated females remained inside the tube in climatic chambers (25±1°C, 70±5% RH, under constant light). After 12h, the numbers of eaten eggs were recorded. Pilot experiments showed that *T. absoluta* eggs (unparasitized, yellow or black), when consumed by *M. pygmaeus*, are devoid of material and are easily seen as having been attacked. It is therefore easy to distinguish between intact (unparasitized or parasitized) eggs and the remains of eggs previously attacked. At the end of the experiment, unconsumed black eggs were collected for further assessment of possible effect of non-consumptive events by *M. pygmaeus* on the survival of *Trichogramma* pupae inside *T. absoluta* egg, i.e. we

assessed adult emergence from black parasitized eggs (see section *Emergence of parasitoids - non-consumptive effects of the predator on parasitized eggs* below).

Data were subjected to ANOVA and pairwise multiple comparisons were performed using a Tukey's post-hoc test. Datasets were first tested for normality and homogeneity of variance using Kolmogorov-Smirnov D test and Cochran's test respectively, and transformed if needed (this test was carried out also for all datasets from other experiments [below], and the SPSS software was used for all analyses).

Choice assay in glass tube

Individual *M. pygmaeus* were caged as above, except that 6 unparasitized *T. absoluta* eggs were paired with 6 yellow or 6 black *T. achaeae*-parasitized *T. absoluta* eggs (for a total of 12 eggs). Thus predators were given a choice of parasitized or unparasitized eggs for 12 hours. In addition, 6 yellow and 6 black *T. achaeae*-parasitized *T. absoluta* eggs were also paired in order to assess predator's preference between early developed vs. late developed parasitized eggs. The control group consisted of exposing *M. pygmaeus* to 12 unparasitized eggs per paper card per tube. Individual *M. pygmaeus* were introduced per glass tube with 12-18 replicates per treatment. All other experimental conditions were identical to the no-choice experiment. In the same way than for the *No-choice assay*, black eggs were collected at the end of the experiment for assessing the effect of non-consumptive events by *M. pygmaeus* on *Trichogramma* pupae (see section

Emergence of parasitoids - non-consumptive effects of the predator on parasitized eggs below).

Numbers of parasitized eggs preyed (yellow and black) were compared to numbers of unparasitized eggs preyed with a paired t-test. Total numbers of eggs eaten per situation were compared among groups using ANOVA and pairwise multiple comparisons were performed using least significant differences (LSD) test.

Behavioral assay

We directly observed predator behavior to assess the propensity of *M. pygmaeus* to attack the different type of *T. absoluta* eggs (unparasitized eggs and yellow and black *T. achaeae*-parasitized *T. absoluta* eggs). We placed one egg of a given type onto the bottom of a Petri

dish using a fine brush and placed the Petri dish under a binocular microscope. Individual *M. pygmaeus* were introduced into a clear plastic dome (d: 1cm, h: 0.65cm) and the dome was placed over an individual egg (Desneux et al. 2009a). Observations began when the predator first encountered the surface of the Petri dish. Predators were observed for five minutes, or until an attack occurred. We recorded predator behaviors as: "contact" (contact with the egg) and "attack" (mouthparts extended and making contact with the prey [egg], Desneux and O'Neil 2008). Fifteen replicates were carried out per egg type, and observations were done in a randomized fashion.

The proportions of unparasitized eggs encountered and attacked by the predator were compared to those of yellow and black parasitized eggs (*T. achaeae*-parasitized *T. absoluta* eggs) using permuted Fisher's exact tests.

Microcosm assay

We studied the effect of *T. achaeae* and *M. pygmaeus* on *T. absoluta* populations on tomato plants in microcosm conditions to determine if any interaction occurred between the two natural enemies. Following the design used in previous studies (Desneux et al. 2009b, Mouttet et al. 2011), microcosms were created by placing a clear acetate cylinder over a potted tomato plant (4-week old plants with four fully expanded leaves were used). Cylinders had a mesh (350 μm) top and windows were cut and covered with mesh (350 μm) for ventilation. Cylinders were 40 cm high x 15 cm in diameter and sand was placed on the soil surface to provide a substrate into which the cylinder could be easily pushed to ensure a complete seal. All experiments were carried out at a temperature of 25±1°C, 70±5% RH and a 16L:8D photoperiod.

To prepare plants for starting the experiment, 10 *T. absoluta* mated females were released per plant (microcosm) and removed after 24 hours. The number of *T. absoluta* eggs was then adjusted to 100 eggs per plant. Then plants were distributed among four different treatments: (i) Control = no natural enemies released, (ii) 50 *T. achaeae* were released per microcosm (number of *T. achaeae* released was based on the rate recommended by Biotop, France), (iii) one *M. pygmaeus* was released per microcosm, and (iv) 50 *T. achaeae* and one *M. pygmaeus* were released per microcosm, (15-27 replicates per treatment). After five days, *T. absoluta* larvae and parasitized eggs (black eggs) in the microcosms were counted. At the end of the experiments, black eggs were collected for subsequent assessment of emergence of *T. achaeae* offspring (see below).

The numbers of *T. absoluta* larvae were analyzed using a generalized linear model (PROC Genmod, SAS 1999) with 'Parasitoid' and 'Predator' as factors (a Tukey's post-hoc test was used for pairwise multiple comparisons). The numbers of parasitized eggs were compared using the same statistical method with one single factor 'Predator'. The proportions of parasitoids that emerged from parasitized eggs (with or without predator) were compared using a chi-square test.

Emergence of parasitoids - non-consumptive effects of the predator on parasitized eggs

We evaluated the possible impact of *M. pygmaeus*, through non-consuming event (e.g. probing, see Butler & O'Neil 2006), on the survival of *Trichogramma* pupae inside *T. absoluta* eggs. We focused our assessment on black parasitized eggs over yellow ones because the latter were shown to be largely preyed on by *M. pygmaeus* (see Results). To compare the success in development of parasitoid offspring in a control situation vs. in presence of the predator (i.e. when black eggs were from *No-choice*, *Choice* and *Microcosm assays*), we prepared batches of *T. absoluta* eggs for parasitism by *T. achaeae* without presence of *M. pygmaeus*, i.e. parasitized control egg batches. All collected black parasitized eggs (from the three assays as well as from the prepared controls) were placed in Petri dishes in a climatic chamber (25±1°C, 70±5% RH and a 16L:8D). After five days, we counted the adults that emerged.

The proportion of black eggs that yielded parasitoid adults among the assays and controls were fitted to a log-linear model (with factors "assay" and "predator" tested). The "assay" factor was included in the statistical model to assess how increasing assay complexity, i.e. one egg or two egg types in glass tubes, or one plant in microcosm, may impact occurrence of non consumptive events on black eggs. In instance, increased complexity in architectural plant characteristics (e.g. plant size, number of nodes and leaves) have been reported to significantly decrease encounter rate between predators and prey (e.g. Coll and Ridgway 1995, Rutledge and O'Neil 2005). In addition, proportions of adults emerged from eggs subjected to predator non-consumptive events (from the various assays) were compared with respective control using chi-square tests.

Results

No-choice and Choice assays in glass tube

In a no-choice situation, *M. pygmaeus* fed on *T. achaeae*-parasitized *T. absoluta* eggs at the same rate as unparasitized eggs when all *T. absoluta* eggs were yellow. The predator however fed significantly less (about 10-fold less) when parasitized eggs were older (i.e. black) (Fig. 1; $F_{2,35} = 40.891$, $P < 0.001$).

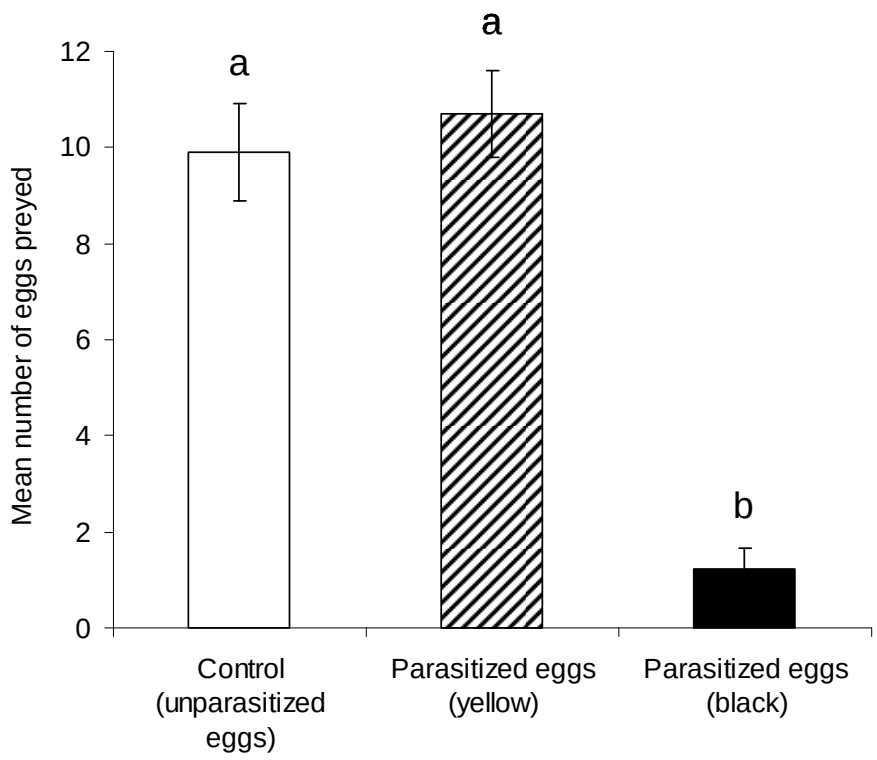


Figure 1: Mean number (± SEM) of *T. absoluta* eggs preyed in the no-choice experiment in glass tubes with one *M. pygmaeus* female exposed to 12 unparasitized *T. absoluta* eggs (control), 12 *T. achaeae*-parasitized *T. absoluta* yellow eggs (i.e. parasitized for 0-3 days), or 12 *T. achaeae*-parasitized *T. absoluta* black eggs (i.e. parasitized for more than 4-5 days). Histograms bearing different letters are significantly different ($P < 0.05$, ANOVA followed by a Tukey's post-hoc test).

When the predators were offered two egg types at the same time (*Choice assay*), the total consumption of eggs per tube was significantly lower in tubes in which black parasitized eggs were provided (Fig. 2; $F_{3,57} = 14.734$, $P < 0.001$). *Macrolophus pygmaeus* did exhibit a preference for unparasitized over black parasitized eggs ($t = 8.884$, $df = 17$, $P < 0.001$) but did not show any preference when both unparasitized eggs and parasitized eggs were still yellow

(t = -1.382, df = 14, P = 0.189). In addition, the predator showed a preference for yellow parasitized eggs over black parasitized eggs (Fig. 2; t = 13.950, df = 12, P < 0.001).

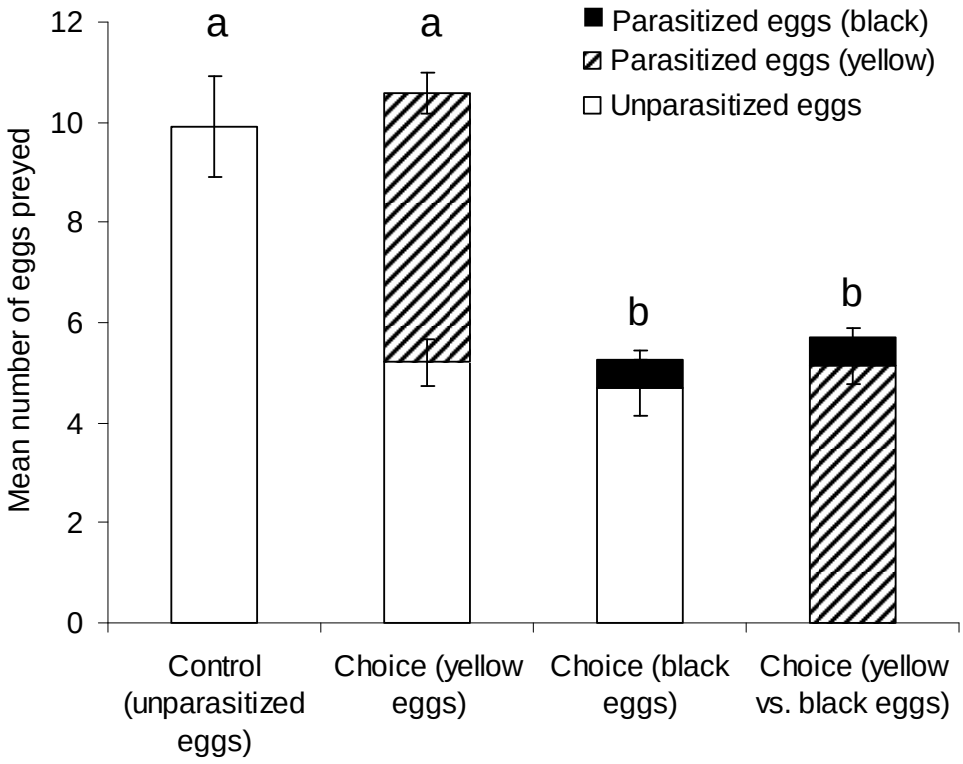


Figure 2: Mean number (± SEM) of *T. absoluta* eggs preyed in the choice experiment in glass tubes with one *M. pygmaeus* female exposed to 12 unparasitized *T. absoluta* eggs (control), or 6 unparasitized *T. absoluta* eggs and 6 *T. achaeae*-parasitized *T. absoluta* eggs (yellow or black) placed together, or 6 *T. achaeae*-parasitized *T. absoluta* yellow eggs and 6 *T. achaeae*-parasitized *T. absoluta* black eggs placed together. Different letters indicate a significant difference in total consumption of *T. absoluta* eggs (P < 0.05, ANOVA followed by a Tukey's post-hoc test).

Behavioral assay

Similar proportions of unparasitized *T. absoluta* eggs, yellow *T. achaeae*-parasitized eggs and black *T. achaeae*-parasitized eggs were encountered by the predator, 0.93, 0.93 and 0.86, respectively. Parasitism had no effect on the rate at which predators encountered eggs in the observation arenas (permuted Fisher's exact tests, all P > 0.05). However, black parasitized eggs were attacked at a significantly lower rate (0.20) than were unparasitized eggs (0.93, P = 0.01), but there was no significant difference between attack rates on yellow parasitized eggs (0.87) and unparasitized eggs (P = 0.494).

Microcosm assay

In microcosms, the number of *T. absoluta* larvae at the end of the experiment varied significantly in function of the presence of the parasitoid *T. achaeae* (Fig. 3; significant 'Parasitoid' factor: Chi-square = 25.99, df = 1, $P < 0.001$) and the presence of the predator *M. pygmaeus* (significant 'Predator' factor: Chi-square = 13.06, df = 1, $P < 0.001$). Both factors interacted significantly (interaction 'Parasitoid' x 'Predator' factors: Chi-square = 4.21, df = 1, $P = 0.040$). Therefore, the effect of *T. achaeae* and *M. pygmaeus* on the survival of *T. absoluta* varied differently as a function of the presence of a second natural enemy (either the parasitoid or the predator) in the microcosms. The lowest number of *T. absoluta* larvae was observed when *T. achaeae* and *M. pygmaeus* were released together into microcosms. When the predator *M. pygmaeus* was released alone it reduced *T. absoluta* populations when compared to the control group but less efficiently than when released together with the parasitoid *T. achaeae* (though we did not observe fully additive effects of the two natural enemies together). The number of *T. achaeae*-parasitized eggs varied as function of the presence of the predator ('Predator' factor: Chi-square = 4.97, df = 1, $P = 0.026$). Indeed, when both natural enemies were released together, the number of parasitized eggs (black eggs) was two-fold lower (18.20±8.61) than in case the parasitoids had been released alone into the microcosm (35.24±4.43).

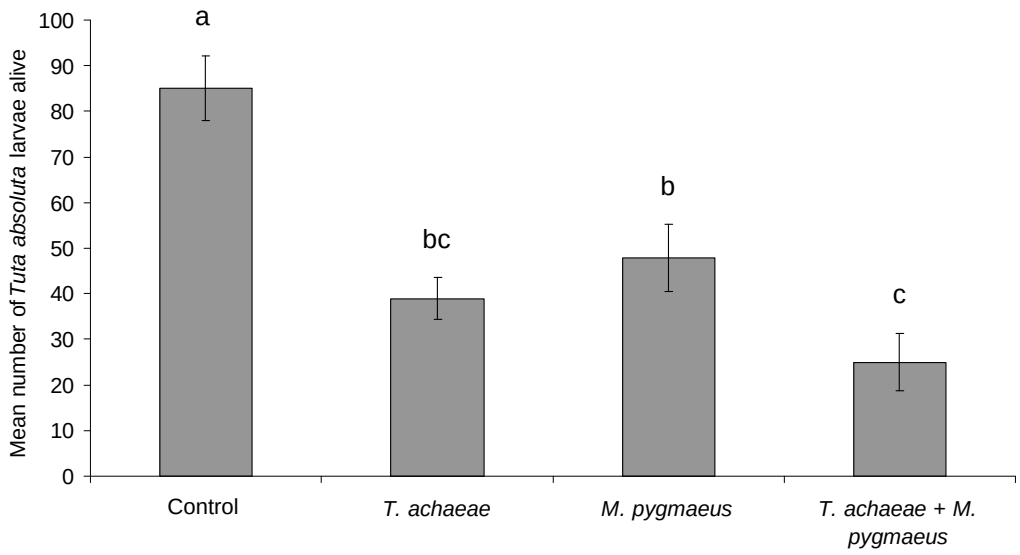


Figure 3: Mean number (± SEM) of *T. absoluta* larvae alive per plant after five days in microcosm as function of the various treatments: (i) Control = no natural enemy, (ii) *T. achaeae* released, (iii) *M. pygmaeus* released, and (iv) *T. achaeae* + *M. pygmaeus* released together. Histograms bearing different letters are significantly different ($P < 0.05$, generalized linear model followed by a Tukey's post-hoc test).

Emergence of parasitoids - non-consumptive effects of the predator on parasitized eggs

The emergence of parasitoids from parasitized eggs varied significantly according to the presence of the predator *M. pygmaeus* (significant 'Predator' factor: Chi-square = 19.91, df = 1, $P < 0.001$) but not according to the assay considered (Fig. 4; 'Assay' factor: Chi-square = 4.12, df = 2, $P = 0.128$). However both factors interacted significantly (interaction 'predator' x 'assay' factors: Chi-square = 6.71, df = 2, $P = 0.035$), hinting that the effect of the predator on parasitoid emergence depended on the assay considered. More specifically, when parasitoid emergence was compared between the various assays with respective controls, we observed significantly fewer parasitoid adults in the assays vs. controls (*No-choice assay*: Chi-square = 25.04, df = 1, $P < 0.001$, *Choice assay*: Chi-square = 10.02, df = 1, $P = 0.002$, *Microcosm assay*: Chi-square = 3.89, df = 1, $P = 0.049$). However, the reduction in parasitoid emergence was more important in *No-choice* and *Choice assays* (58% and 41% reduction respectively) than in the *Microcosm assay* (15% reduction) (Fig. 4).

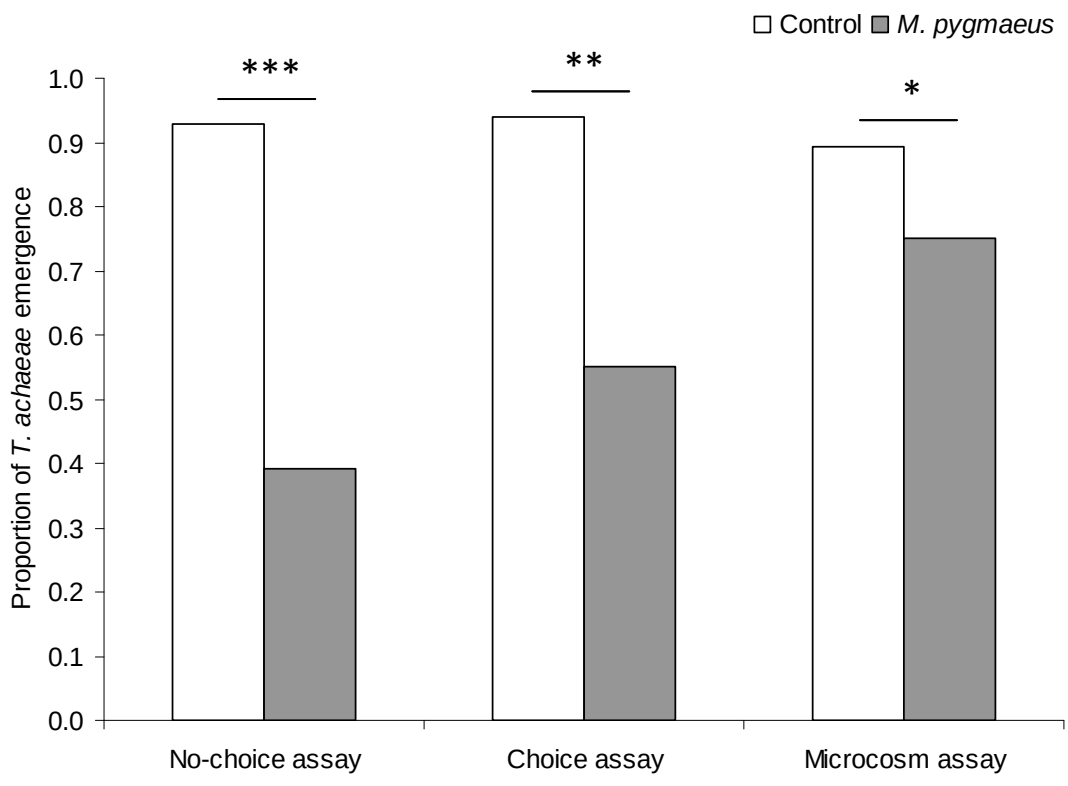


Figure 4: Percentage of parasitoid emerging from black eggs collected during the *No-choice, Choice* and *Microcosm assays* (as well as from black eggs of respective control groups). *** $P < 0.001$, ** $P < 0.01$,* $P < 0.05$ (significant difference with the respective control, chi-square test).

Discussion

Generalist predators are largely acknowledged as providing valuable levels of regulation of pests in crops throughout the world (Symondson et al. 2002, Lu et al. 2012). However, development of biological control programs can be compromised through intraguild predation which is actually a common phenomenon in agro-ecosystems (Rosenheim et al. 1995, Müller and Brodeur 2002, Chacon and Heimpel 2010). We found that IGP, as well as non-consumptive events, did occur on the oophagous parasitoid *T. achaeae* by the generalist predator *M. pygmaeus* under laboratory conditions. The predator can decrease survival of *T. achaeae*'s offspring that are developing in *T. absoluta* eggs because it does feed on parasitized eggs. However, the risk of IGP depends on the developmental stage of *T. achaeae* inside *T. absoluta* parasitized eggs because the larvae are at risk mostly early in their development when parasitized eggs are still yellow (eggs turn black halfway through development of parasitoid). In addition, parasitoid offspring can also suffer mortality when developing in *T. absoluta* eggs through probing behavior exhibited by the predator on parasitized eggs. This behavior is known to increase mortality in prey (Butler and O'Neil 2006) without actual feeding by the predator, probably owing to a toxin injected by hemipteran predators when probing prey (Edwards 1961, Cohen 1990). Despite occurrence of IGP and non-consumptive events, the density of *T. absoluta* larvae at the end of the experiment in microcosms suggested that releasing *T. achaeae* against *T. absoluta* on tomato plants may increase the level of pest suppression achieved over that occurring when only mirid predators are used. Predation of *T. absoluta* eggs by *M. pygmaeus* was consistent with previous studies which reported that *M. pygmaeus* would consume *T. absoluta* eggs under laboratory conditions (Urbaneja et al. 2009, Arno and Gabarra 2011, Bompard et al. 2013). The results of our microcosm assay showed that *M. pygmaeus* does reduce *T. absoluta* population levels on tomato plants.

Predators are known to consume eggs parasitized by oophagous parasitoids (Herrick et al. 2008; Mbata and Shapiro-Ilan 2010) and there are examples of predation on eggs parasitized by *Trichogramma* spp. (Brower and Press 1988, Smith 1996, Kuhar et al. 2002, Philip et al. 2005). By contrast, many studies have reported that egg predators avoid parasitized prey containing parasitoid larvae or pupae (Rouechdi and Voegelé 1981, Ruberson and Kring 1991, Roger et al. 2001). Hemipteran predators, when engaged in IGP with *Trichogramma* spp., are more likely to accept unparasitized host eggs than those that contain pupae of *Trichogramma*, although younger stages of *Trichogramma* appear equally susceptible

(Rouechdi and Voegelé 1981, Brower and Press 1988). In instance, we showed that *M. pygmaeus* preferentially attacked unparasitized eggs and parasitized eggs during the early stages of parasitoid larvae development (yellow eggs) compared to black eggs, i.e. when the parasitoid is in the pupal stage during its final development. In addition, behavioral observations showed that the predator would not primarily feed on black eggs, i.e. only 20% of acceptance at first encounter with black egg vs. 87-93% for yellow eggs. When *Trichogramma* parasitoids reach the prepupa stage, they catalyze the production of melanin which darkens the parasitized eggs (Pintureau et al. 1999, Knutson 2005). Melanin is a pigment involved in the mechanical strengthening of insects' cuticle and other biological materials (Moses et al. 2006). The presence of melanin might make it harder for predators like Hemipterans to pierce parasitized eggs. Melanization alone did not totally prevent feeding by *M. pygmaeus* on these parasitized eggs, but when the parasitoid *T. achaeae* is at late developmental stage (pupae), parasitized eggs (black) are consumed at a lower rate, thus reducing the risk of IGP. Our choice tests confirm that *Trichogramma* parasitoids can be at risk of IGP by *M. pygmaeus* for at least half of their developmental duration (i.e. before pupation) when parasitizing *T. absoluta* on tomato.

However, mortality of *Trichogramma* pupae inside black eggs may still occur through non-consumptive events, i.e. probing which kills parasitoid offspring. The mortality of pupae induced by such non-consumptive behavior was relatively high when the predator was confined in small arenas with black eggs, i.e. *No-choice* and *Choice assays* (Fig. 4). However, behavioral observations suggested that probing on black eggs would occur mostly after several encounters of the predator with black eggs, e.g. probing on black eggs occurred in only 20% of first encounters with black eggs (vs. in 87-93% in the case of encounters with yellow eggs), though learning process in the predator may increase (or actually decrease) acceptance rate of these black eggs by *M. pygmaeus*. Encounter rate between Hemipteran predators and prey are known to decrease when the complexity in architectural plant characteristics increase (Coll and Ridgway 1995, Rutledge and O'Neil 2005). Consistently with this observation, mortality of parasitoid pupae in black eggs was much lower (barely significant when compared to the control) in the *Microcosm assay* than what was recorded for black eggs from *No-choice* and *Choice assays* (glass tubes), likely due to higher architectural complexity in the *Microcosm assay* reducing encounter rate between the predator and black eggs. In addition, in tomato greenhouse, higher architectural complexity as well as lower pest density may further decrease encounter rate between Hemipteran predators and *Trichogramma*-parasitized *T. absoluta* eggs.

We did not observe strong additive effects of the two natural enemies on *T. absoluta* survival in the microcosms (Fig. 3). When the predator was present at the time the parasitoids were parasitizing *T. absoluta* eggs, it is likely that the predator both reduced number of eggs available for the parasitoids, and also preyed on eggs recently parasitized by the parasitoid. In case of *Trichogramma* species adapted to *T. absoluta* as host, it could lead to a parasitoid population decrease if host (*T. absoluta* eggs) density becomes very low. By contrast, the omnivorous predator *M. pygmaeus* could switch to other food sources, e.g. plant or alternative prey, though the presence of *Trichogramma* parasitoids may still induce a decrease in *T. absoluta* eggs available as food.

This work provided insights on what could be expected to occur in tomato greenhouses. Despite occurrence of IGP (predation on early stage of parasitoid development), a likely competition for resources (*T. absoluta* eggs) between the two natural enemies, and an absence of additive effects of the two natural enemies on *T. absoluta* survival in microcosm conditions, our microcosm results demonstrate that adding *Trichogramma* parasitoids would significantly increase the level of control of the pest over what could be attained when using *M. pygmaeus* alone. Our findings suggest that presence of mirid predators in greenhouse tomato crops may not interfere significantly with oophagous parasitoids for *T. absoluta* control though population dynamics studies in greenhouse conditions would have to be performed to confirm this result. For instance, increased habitat complexity is known to decrease IGP rates (Finke and Denno 2002, Langellotto and Denno 2006). In addition, plant material may have provided a supplemental food resource that reduced feeding of mirid predators on prey (e.g. Calvo et al. 2009). In our experiment, tomato plants in the microcosms likely (i) decreased encounter rates between *M. pygmaeus* and parasitized eggs, and (ii) served as food and thus reduced occurrence of IGP and non-consumptive events of the predator over parasitized eggs. It could explain why only half of parasitized eggs were consumed in microcosms whereas most of yellow *T. achaeae* parasitized *T. absoluta* eggs were consumed in glass tubes (no-choice and choice assays). Further, we suggest that combining releases of *Trichogramma* parasitoids and *M. pygmaeus* will not strongly interfere with the parasitoids. Although *M. pygmaeus* would feed on parasitized- but still yellow - *T. absoluta* eggs, there was no evidence this degree of IGP impacted positively *T. absoluta* survival (Fig. 3). In addition, juveniles usually represent an important part of *M. pygmaeus* populations in greenhouse tomato crops, and prey consumption by these juveniles is lower than predation by adults (Fauvel et al. 1987), thus lowering risk of IGP. Efficient integration of both natural

enemies into an IPM program might be achieved via adjustment of the frequency of releases according to predator, *T. absoluta* and alternative prey (e.g. whitefly) densities in the crop.

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Article 7

Suitability of the Host-plant System *Tuta absoluta* (Lepidoptera: Gelechiidae)-Tomato for *Trichogramma* (Hymenoptera : Trichogrammatidae) Parasitoids and Insights for Biological Control

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Suitability of the Host-plant System *Tuta absoluta* (Lepidoptera: Gelechiidae)-Tomato for *Trichogramma* (Hymenoptera : Trichogrammatidae) Parasitoids and Insights for Biological Control

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Abstract: The South American tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), is a major pest that has recently invaded Afro-Eurasia. Biological control, especially by *Trichogramma* parasitoids, is considered to be very promising as a management tool for this. However further development of *Trichogramma*-based biocontrol packages would require assessing the impact of released parasitoid offspring on the pest. Under laboratory conditions, we compared (i) the response of five *Trichogramma* species-strains on the pest-plant system *T. absoluta*-tomato, and (ii) assessed the fitness of parasitoids, previously mass-reared on a factitious host (*Ephestia kuehniella*), when developing on *T. absoluta*. In addition, we evaluated the overall control of two specific *Trichogramma* species when released under greenhouse conditions in combination with a common natural enemy in tomato crop; the predator *Macrolophus pygmaeus* Rambur. Parasitoids emerging from *T. absoluta* on tomato showed lower parasitism rates and reduced fitness, e.g. wing deformations, reduced longevity, when compared to the control on the factitious host under laboratory conditions. Under greenhouse conditions, the parasitoids that developed on *T. absoluta* after initial releases were of low use for the biological control of *T. absoluta*. Parasitism was lower when the predator was present. However, the highest *T. absoluta* control level was achieved by combining the predator and the parasitoids. This study shows that *Trichogramma* parasitoids may not build up populations on the *T. absoluta*-tomato system, but that *Trichogramma* parasitoids can be used in combination with the predator to enhance biological control of the pest in tomato crops.

Keywords: fitness, leafminer, invasive species, inundative release, mass-rearing.

Introduction

The South American tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), is one of the most devastating tomato pests in South America (Gontijo et al. 2013). The pest has recently invaded and spread in the Afro-Eurasian continent and in few years has become a major pest in tomato crops (Desneux et al. 2010, 2011). Appearance of *T. absoluta* has led to extensive insecticide use by tomato growers, potentially causing a multitude of undesired side effects on non-target organisms (Arnó and Gabarra 2011, Biondi et al. 2012, and see Desneux et al. 2007 for a thorough review). Biological control is considered a very promising environmentally- and economically-sound pest management tool for this pest in Europe (Desneux et al. 2010). The role of natural generalist enemies as regulators of pests has been increasingly acknowledged in multiple crops (Van Driesche and Bellows 1996, Lu et al. 2012, Symondson et al. 2002). However, their use as biocontrol agents is often slowed down because their effectiveness in the field is difficult to predict.

Wasps belonging to the *Trichogramma* genus are generalist egg parasitoids, mainly of Lepidoptera. They are largely used in biological control programmes, notably through inundative releases (Smith 1996, Pintureau 2009, Mills 2010). Although the genus *Trichogramma* is not the only group used with this approach, much of our understanding of inundative releases comes from studies done on these oophagous parasitoids (Smith 1996). They have been used in tomato crops against *T. absoluta* through inundative releases both in the native areas, mainly with *Trichogramma pretiosum* Riley (Parra and Zucchi 2004, Pratissoli et al. 2005), and in the newly invaded areas with releases of *T. achaeae* Nagaraja & Nagarkatti (Cabello et al. 2012, Calvo et al. 2012). Considering the effectiveness of these programmes and the natural parasitism of this pest by various *Trichogramma* spp. reported in South America and in Europe (Desneux et al. 2010, Zappalà et al. 2012), screenings for more effective *Trichogramma* species in the invaded area are still ongoing (Chailleux et al. 2012, Khanh et al. 2012).

Various factors can influence the impact of inundative releases of mass-reared parasitoids. For instance, host selection by parasitoids may be influenced by the characteristics of both the host and host plant (Chau and Mackauer 2001, Desneux and Ramirez-Romero 2009). In addition, host preference in parasitoids tends to correlate with the fitness gained from the host (Van Alphen and Vet 1986, Chau and Mackauer 2001, Driessen et al. 1991), this being defined as the *preference-performance* hypothesis (Jaenike 1978). However, very low-quality hosts can also be accepted (Janssen 1989, Heimpel et al. 2003,

Desneux et al. 2009, 2012). Studies on acceptance of mass-reared *Trichogramma* on factitious host species toward natural hosts yielded variable results. El-Wakeil (2007) showed differences in pest parasitism which depended on the rearing host. The size of the natal host affected natural host parasitism rates in the field and female parasitoids usually accepted host eggs of the same size or larger as their natal host (Salt 1940; Nurindah et al. 1999). By contrast, Kolliker-Ott et al. (2003) reported no *preference-performance* relationship for *Trichogramma brassicae* Bezdenko (Hymenoptera, Trichogrammatidae) mass-reared on the factitious host *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) when parasitizing the target host *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae).

The effectiveness of *Trichogramma* releases may depend not only on the biological characteristics of the parasitoid species/strains used, but also on their interactions with a specific pest-plant system (Andrade et al. 2011, Tabone et al. 2010, Yuan et al. 2012). Depending on the affinity of the parasitoids with the pest-plant system, the biocontrol services provided by the released parasitoids most likely vary. *Trichogramma* are mainly used as biocontrol agent through inundative releases, but the impact of *Trichogramma* generations which developed in the crop can play a crucial role in the success of biological control programmes (Mills 2010). For example, use of *T. brassicae* for biological control of the European corn borer in maize takes into account the effect of parasitoids developing in the field for the long-term control of the pest (Pintureau 2009). Extra mass-releases may be reduced and overall crop protection costs can be lowered.

In a first laboratory experiment we evaluated *T. absoluta* eggs parasitism by five *Trichogramma* strains, i.e. different species or strains of the same species, hereafter named "strains", and the subsequent development of their offspring. To document the possible biocontrol impact of these parasitoids when developing on *T. absoluta* in the tomato crops after inundative releases, we compared (i) a *Released generation*: developed in *E. kuehniella* eggs / parasitizing *T. absoluta* eggs, to (ii) a *Field generation*: developed in *T. absoluta* eggs / parasitizing *T. absoluta* eggs. Moreover, a control treatment, i.e. parasitoids developed in *E. kuehniella* eggs / parasitizing *E. kuehniella* eggs, was added. This aimed at assessing possible reduced parasitoid fitness when parasitoids switched from the mass-rearing host to the targeted pest-plant system. In a second experiment, in greenhouse, we tested the overall efficiency of a field release and the subsequent generation (which corresponds to the *Field generation*) of two *Trichogramma* strains against *T. absoluta*. To assess the usefulness of *Trichogramma* parasitoids within the framework of current biocontrol programmes in tomato crops in Europe, we included the mirid predator *Macrolophus pygmaeus* Rambur because it is

widely used for biocontrol, notably to control whiteflies. It is also used as a biocontrol agent of *T. absoluta* as it preys on eggs (Urbaneja et al. 2009, Bompard et al. 2013).

Materials and Methods

Biological materials. The plants used in the experiments were tomato plants, *Solanum lycopersicum* L. cv. Marmande, 5-week old for the laboratory experiments and 7-week old for the cage experiments. Plants were grown in climatic chambers (25 ± 1°C, RH: 65%, L.D. 16:8), pesticide applications were strictly avoided and a nutrient solution was applied daily. A colony of *T. absoluta* was set up using greenhouse-collected individuals in July 2009 (initial number of individuals = 190) at INRA (National Institute for Agricultural Research), Alenya, France. *Tuta absoluta* was reared in growth chambers (25 ± 1°C, RH 70 ± 10%, L.D. 16:8) in cages (55×75×80 cm), containing tomato plants. Adult moths were fed on honey placed on one wall inside the cages. The parasitoids used for the experiments originated from collections (Table 1). Dr. B. Pintureau (French National Institute for Agricultural Research, Lyon, France) identified the species before the experiments. *Trichogramma euproctidis* Girault, and both *T. evanescens* Westwood strains were chosen because they were shown to be promising strains for biocontrol of *T. absoluta* during a previous study (29 strains tested, Chailleux et al. 2012). *Trichogramma pretiosum* was considered because it may have co-evolved with *T. absoluta* and is already commercialized in South America against this pest. *T. achaeae* was tested because it has already been commercialized in Europe and Africa against *T. absoluta*. Colonies of parasitoids were reared on UV-irradiated eggs of a substitute host, *E. kuehniella* (18 ± 1°C, RH 70 ± 10%, L.D. 12:12). Rearing was carried out in glass tubes (l: 4.5 cm; d: 0.7 cm) and the parasitoids were fed on honey droplets. *Ephestia kuehniella* eggs were glued on a piece of cardboard (3×10 mm) with 10% arabic gum (Pizzol et al. 2010). Parasitoids were maintained for at least three generations at the temperature of 25°C on *E. kuehniella* eggs before starting experiments.

In the laboratory trials, the *T. absoluta* eggs used were 0 to 12-h old and the wasps were 0 to 24-h old (as both host and parasitoid ages can play a role in development of the *Trichogramma* offspring, see Pizzol et al. 2012). To obtain parasitoid females that had developed in *E. kuehniella* eggs, females from the colonies were released on *E. kuehniella* eggs in the same conditions as described for the rearing, but female emergence date was checked precisely. To obtain parasitoid females developed on *T. absoluta* eggs, parasitoids from the colonies were released on *T. absoluta* eggs on tomato leaflets for 12 h inside

ventilated plexiglass tubes (d:4 cm, l:14 cm). The parasitoids used in all the experiments were collected daily from these tubes i.e. those containing parasitized eggs of *E. kuehniella* or *T. absoluta*. *Macrolophus pygmaeus* were reared on *E. kuehniella* eggs on tobacco plant in climatic chambers (23±1°C, 70±5% RH, L.D. 16:8). Adult predators used in the experiments were <1 to 3-d old.

Table 1: Country of origin, initial host plant and host and year of collection of the five *Trichogramma* strains tested.

Species	Geographic origin	Host plant	Host moth or butterfly	Year of collection
T. achaeae Nagaraja & Nagarkatti, 1969	Canaries Island	Tomato	*Chrysodeixis chalcites*	2010
T. euproctidis Girault, 1911	Switzerland	-	-	-
T. evanescens Westwood, 1833 (1)	Northern France	Vine	*Lobesia botrana*	1990
T. evanescens Westwood, 1833 (2)	Northern France	Cauliflower	*Argyrotaenia sphaleropa*	2002
T. pretiosum Riley, 1876	Uruguay	Vine	*Argyrotaenia sphaleropa*	1995

Impact of *T. absoluta*-tomato pest-plant system on parasitism and offspring development. The experiment was conducted in growth chambers (25°C, 70±10% RH, L.D. 16:8). Two different combinations were tested by mimicking the development of two generations of *Trichogramma*: (1) the *released generation* (developed in *E. kuehniella* eggs on cardboard and exposed to *T. absoluta* eggs on tomato leaflets) and (2) the *field generation* (developed in *T. absoluta* eggs on tomato and exposed to *T. absoluta* eggs on tomato leaflets). Parasitoids in the rearing situation were used as a control (third treatment). Control individuals developed on *E. kuehniella* egg and exposed to *E. kuehniella* eggs on cardboard.

Control and released generations: Females emerging from *E. kuehniella* eggs (obtained as described in the *Biological materials* section) were used both for the control treatment i.e. releasing the females on *E. kuehniella* eggs, and for the *released generation* treatment i.e. releasing the female on *T. absoluta* eggs. Therefore, young mated females were released individually in glass tube containing 30-40 eggs of *E. kuehniella* (the same technique as previously described for the rearing) and fed honey droplets during 24h to measure the *rearing generation* parameters. On the other hand, for the *released generation*, young mated females were presented individually with 30-40 *T. absoluta* eggs on a tomato leaflet in ventilated plexiglass tubes (d: 4 cm, l: 14 cm) with honey during 24h. The stems of leaflet, sticking out of the tube, were planted into floral foam for watering.

Field generations: To evaluate the *field generation* parameters, young *Trichogramma* females emerging from *T. absoluta*-parasitized eggs (obtained as described in the *Biological*

materials section) were presented individually to 30-40 *T. absoluta* eggs on a tomato leaflet in the ventilated tube (with honey droplets as food source) during 24 hours, i.e. using the same oviposition conditions as the *released generation*, except that females had previously developed in *T. absoluta* eggs vs. *E. kuehniella* eggs.

For the three treatments, tubes containing parasitized eggs were kept in a climatic chamber (25°C, 70±10% RH, L.D. 16:8) and maintained until offspring emergence for 15 days. For each treatment the number of parasitized eggs (black eggs) and of aborted eggs (white eggs with neither hatched nor with the cephalic capsule of the immature larvae visible) were counted after 5 days of parasitisation. The sex-ratio (females/total) and the wings (absent, stump or crumple wings 2-3 days after emergence) of offspring individuals (F1) were recorded after emergence i.e. 15 days after the parasitisation. Ten to15 replicates were carried out per parasitoid strain and treatments were tested in a randomized fashion over time.

Impact of the host system on the longevity. We assessed the longevity of female parasitoids that had developed in *T. absoluta* eggs (= the *field generation*) and in *E. kuehniella* eggs as a control. Females were obtained as described in the *Biological materials* section. Females were placed in glass tubes (d: 0.7 cm, l: 4.5 cm), closed with a cotton ball and provided with honey or left without in climatic chambers (25°C, 70±10% RH, L.D. 16:8), but without water. Twenty-25 replicates were carried out per parasitoid strain and per treatment. The survival was checked daily with a binocular microscope and wasps were considered dead when they did not react after being touched by a fine paint brush.

Cage experiments. A cage experiment was carried out whose aim was to test the efficiency of the *field generation* under realistic conditions. The experiments were carried out in cages (h: 100 cm, l: 70 cm, L: 100 cm, made with insect-proof mesh) that were placed in a greenhouse at the INRA Sophia AgroBiotech Institute (Sophia-Antipolis, France). The greenhouse was similar to the ones used by many tomato growers in France and more broadly in Southern Europe (semi-controlled temperatures, min < mean temperature < max: 15.5°C < 24.2°C < 37.4°C; min < mean RH < max, 18.0% < 63.0% < 90.0%; natural ambient light: mid-July mid-August 2011). The two *Trichogramma* species tested, *T. achaeae* and *T. euproctidis*, which showed the highest parasitism at the *Field generation* in the laboratory (see *Results* section), were chosen for this experiment. Both species were tested alone and in combination with the predator *M. pygmaeus*. The predator was also tested alone and a control with the pest alone was carried out. The following 6 treatments were studied: (1) *T. absoluta*, (2) *T. absoluta* + *M. pygmaeus*, (3) *T. absoluta* + *T. achaeae*, (4) *T. absoluta* + *T. euproctidis*, (5) *T. absoluta* + *T. achaeae* + *M. pygmaeus*, and (6) *T. absoluta* + *T. euproctidis* +

M. pygmaeus. Six replicates (cages) were carried out per treatment. The experiment lasted 16 days to allow first field generation observation.

Eight tomato plants (development stage from 7 to 8 fully expanded leaves) were placed into the cages. First, 20 2-day old *T. absoluta* adults (10 males and 10 females) were released per cage. After 24 hours, predators and/or parasitoids were introduced into the cages. Depending on the treatment, 400 *Trichogramma* adults and/or 8 pairs of *M. pygmaeus* (1/1 male/female) were released. These release rates were chosen according to the recommendations of biocontrol companies. To release *Trichogramma* wasps, open glass tubes containing small cardboard strip that held about 400 parasitized *E. kuehniella* eggs on which adult parasitoids were starting to emerge, were placed in the centre of cages. Honey droplets were provided as food source to the parasitoids. Before releases of the parasitoids, we added four sentinel tomato leaflets bearing 30 *T. absoluta* eggs (15 on adaxial and 15 on abaxial surfaces of each leaflet). These leaflets were placed on the top of a stake so that they always touched a tomato leaf in the upper plant part. The stems of these leaflets were put inside a tube full of water. Five days later, leaflets were collected and parasitized eggs (black eggs) and predated eggs (empty crumpled eggs) were counted under a binocular microscope and percent parasitism and percent predation rates were calculated. Then, the leaflets were put back inside their respective cages.

To evaluate parameters in the same way for the *Field generation*, produced by the released parasitoid, we intended to provide new sentinel leaflet at the field generation emergence time. The *Trichogramma* juveniles (egg to adult) developmental time is 8 days at 30°C (Foerster and Foerster 2009), therefore the new leaflets were placed in each cage 8 days after the *Trichogramma* releases, following the same procedure previously described. The leaflets were collected seven days later to enable an effective assessment of overall parasitism undertaken by the second generation parasitoid females, i.e. they emerged for up to 3 days and adults longevity in greenhouse conditions is $\leq$ 3 days (AC and ND, unpublished data). Parasitized eggs and predated eggs were counted under a binocular microscope as for the *released generation*. At the end of the experiment (after 16 days), a destructive monitoring was carried out. The number of *T. absoluta* larvae and *M. pygmaeus* juveniles were counted in each cage on four plants chosen randomly.

Statistical analyses. All statistical analyses were performed using the R software (R Development Core Team 2009) with the *multcomp* and *agricolae* packages. For the laboratory experiments, the number of parasitized and aborted eggs were analysed using a generalized linear model (GLM) based on Poisson distributed data with a log link function. Sex-ratio

(females/total) and wing malformations were analyzed using a GLM designed for modelling binomial data with a logit link function. For these four parameters the effect of the factor *Emergence host* (host in which females had developed), *Oviposition host* (host parasitized by the females), and *Trichogramma Strain* were tested. Longevity was also analysed using a GLM but it was designed for modelling gamma data with an inverse link function. The effect of the factors *Emergence host*, *Honey supply* and *Strain* were tested. For the cage experiments, the percentages of parasitism, aborted eggs and predated eggs were analyzed using a GLM designed for modelling binomial data. For the parasitism and aborted eggs, the effect of the factors, *M. pygmaeus Presence*, *Parasitoid Generation* and *Strain* were tested. For the predated eggs the effect of the factors *Trichogramma Strain* and *Trichogramma generation* were tested. A GLM designed for Poisson distributed data was used to analyse the number of *T. absoluta* larvae and *M. pygmaeus* juveniles. For *T. absoluta* larvae, the factor *Trichogramma Strain* and *M. pygmaeus Presence* were tested, and for the *M. pygmaeus* juvenile, the *Trichogramma Strain* factor was tested. Finally, multiple comparisons were performed using a Fisher's LSD (Least Significant Difference) post-hoc test for both laboratory and cage experiments.

Results

Impact of *Tuta absoluta*-tomato pest-plant system on parasitism and offspring development. There was a significant effect of the *Oviposition host* (host egg parasitized, $F_{1,182} = 91.780$, $P < 0.001$) and the *Emergence host* (host egg in which parasitoid offspring developed, $F_{1,181} = 80.980$, $P < 0.001$) on the number of parasitized eggs. Parasitism also varied significantly among the *Trichogramma strains* ($F_{(4,177)} = 8.902$, $P<0.0001$). The number of parasitized eggs ranged from 35.54±4.42 for the *Rearing generation* of *T. euproctidis* to 3.00±0.98 for the *Field generation* of *T. evanescens* 2 (Fig. 1). Parasitism was significantly lower when female were exposed to *T. absoluta* (*Released* and *Field generations*) than on *E. kuehniella* (*Control*). Parasitism rate was also lower when females had developed on *T. absoluta* (*Field generation*) than when females had developed on *E. kuehniella* (*Control* and *Released generation*) (Fig.1).

The host egg species in which the females developed (*Emergence host*) significantly affected the sex-ratio of their offspring (Fig. 2, $F_{(1,127)} = 5.149$, $P < 0.0251$). By contrast, the Female *Oviposition host* (emergence host for the offspring) did not significantly affect the sex-ratio ($F_{(1,126)} = 0.0736$, $P < 0.7866$). The sex-ratio of the offspring also varied significantly

among strains tested (significant *Strain* factor, $F_{(4,122)} = 4.9373$, $P < 0.0010$). The sex-ratio significantly differed among generations only in the case of *T. achaeae* when strains were

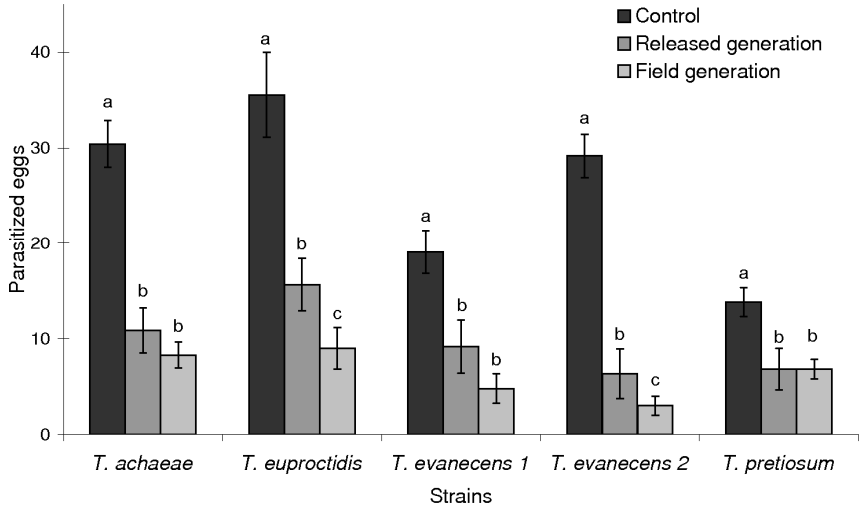


Figure 1: Mean (±SEM) number of parasitized eggs per parasitoid female for the five *Trichogramma* strains tested. *Released generation*: developed in *Ephestia kuehniella* eggs / exposed to *Tuta absoluta* eggs; *Field generation*: developed in *T. absoluta* eggs / exposed to *T. absoluta* eggs; *Control*: developed in *E. kuehniella* eggs / exposed to *E. kuehniella* eggs. Post-hoc tests (Fisher's LSD test) were carried out separately for each strain tested. For each generation tested i.e. *Released* and *Field generations*, and for the *Control* the histograms bearing the same letter are not significantly different at $P < 0.05$.

tested individually (Fig. 2, Fisher's LSD test). The sex-ratio of offspring from the *Field generation* was significantly lower than in the two other generations (*Control* and *Released*

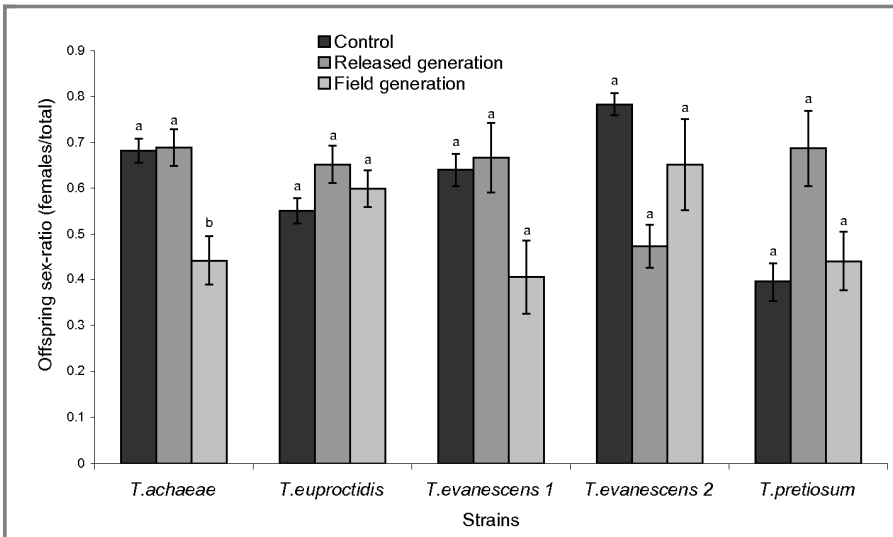


Figure 2: Mean sex-ratio (±SE) (females/total) of offspring for the five *Trichogramma* strains tested. *Released generation*: females developed in *Ephestia kuehniella* eggs / exposed to *Tuta absoluta* eggs; *Field generation*: females developed in *T. absoluta* eggs / exposed to *T. absoluta* eggs; *Control*: females developed in *E. kuehniella* eggs / exposed to *E. kuehniella* eggs. Post-hoc tests (Fisher's LSD test) were carried out separately for each strain tested. For each generation tested i.e. *Released* and *Field generations*, and for the *Control* the histograms bearing the same letter are not significantly different at $P < 0.05$.

generation). We observed similar trends for *T. evanescens* 1 (Fig. 2), but differences among treatments were not significant either for *T. evanscens* 1 or the three other strains (most likely owing to a low number of offspring individuals in the *Field generation*).

Trichogramma Strain, female *Emergence host* and female *Oviposition host* factors all had a significant impact on wing malformations occurring in offspring ($F_{(4,122)} = 39.107$, $P < 0.0001$, $F_{(1,127)} = 24.765$, $P < 0.0001$, $F_{(1,126)} = 7.479$, $P = 0.0072$, respectively). The strain presenting the highest proportion of malformed wings among offspring was *T. euproctidis* (Fig. 3), the highest proportion was observed for the *T. euproctidis Field generation*, with 38±4% of offspring with wing malformations (Fig. 3).

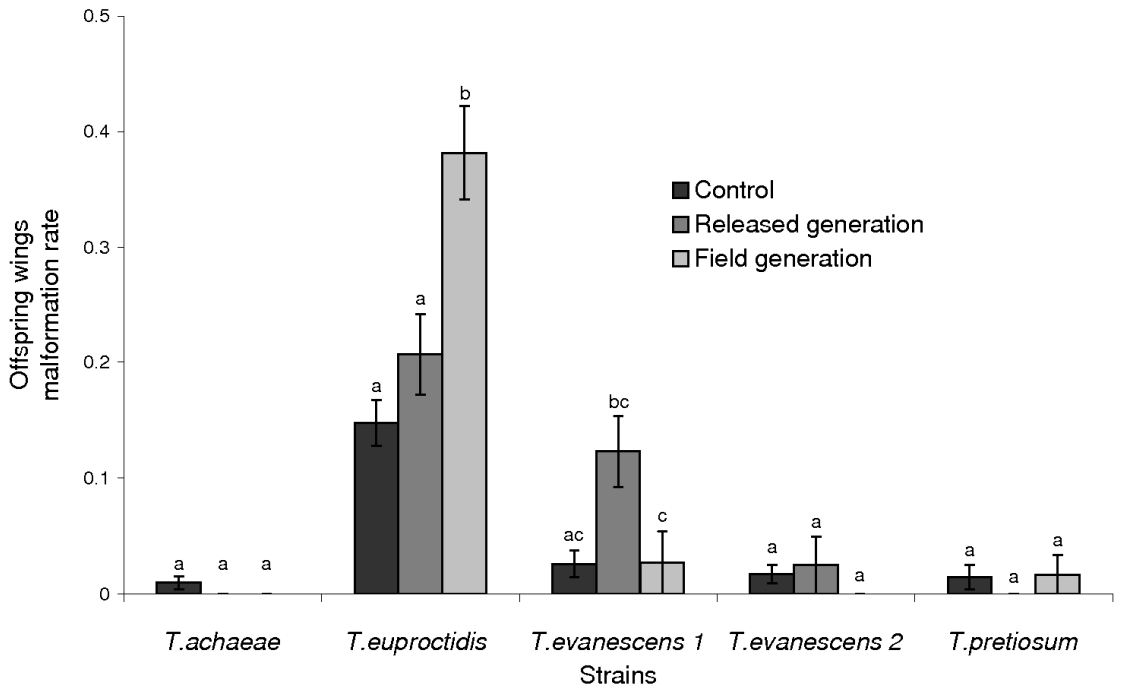


Figure 3: Proportion (±SE) of offspring with wings malformation i.e. absent, stump or crumple wings, for the five *Trichogramma* strains tested. *Released generation*: females developed in *Ephestia kuehniella* eggs / parasitizing *Tuta absoluta* eggs; *Field generation*: females developed in *T. absoluta* eggs / parasitizing *T. absoluta* eggs; *Control*: females developed in *E. kuehniella* eggs / parasitizing *E. kuehniella* eggs. Post-hoc tests (Fisher's LSD test) were carried out separately for each strain tested. For each generation tested i.e. *Released* and *Field generations*, and for the *Control*, the histograms bearing the same letter are not significantly different at $P < 0.05$.

Impact of the host system on the longevity. The effects of the *Emergence host* (χ^2 = 173.319, df = 1, $P < 0.0001$) and the *Honey supply* factors (χ^2 =173.319, df =*1*, $P < 0.0001$) were significant on female parasitoid longevity (Fig. 4). The *Strain* factor also had a significant effect on *Trichogramma* longevity (χ^2 = 28.530, df = 4, $P < 0.0001$) with a

maximum of 18 days on average for *T. evanescens* 1 developed in *E. kuehniella* egg and supplied with honey (Fig. 4a). Without honey, longevity did not exceed four days (for *T. pretiosum* emerged from *E. kuehniella*) (Fig. 4b).

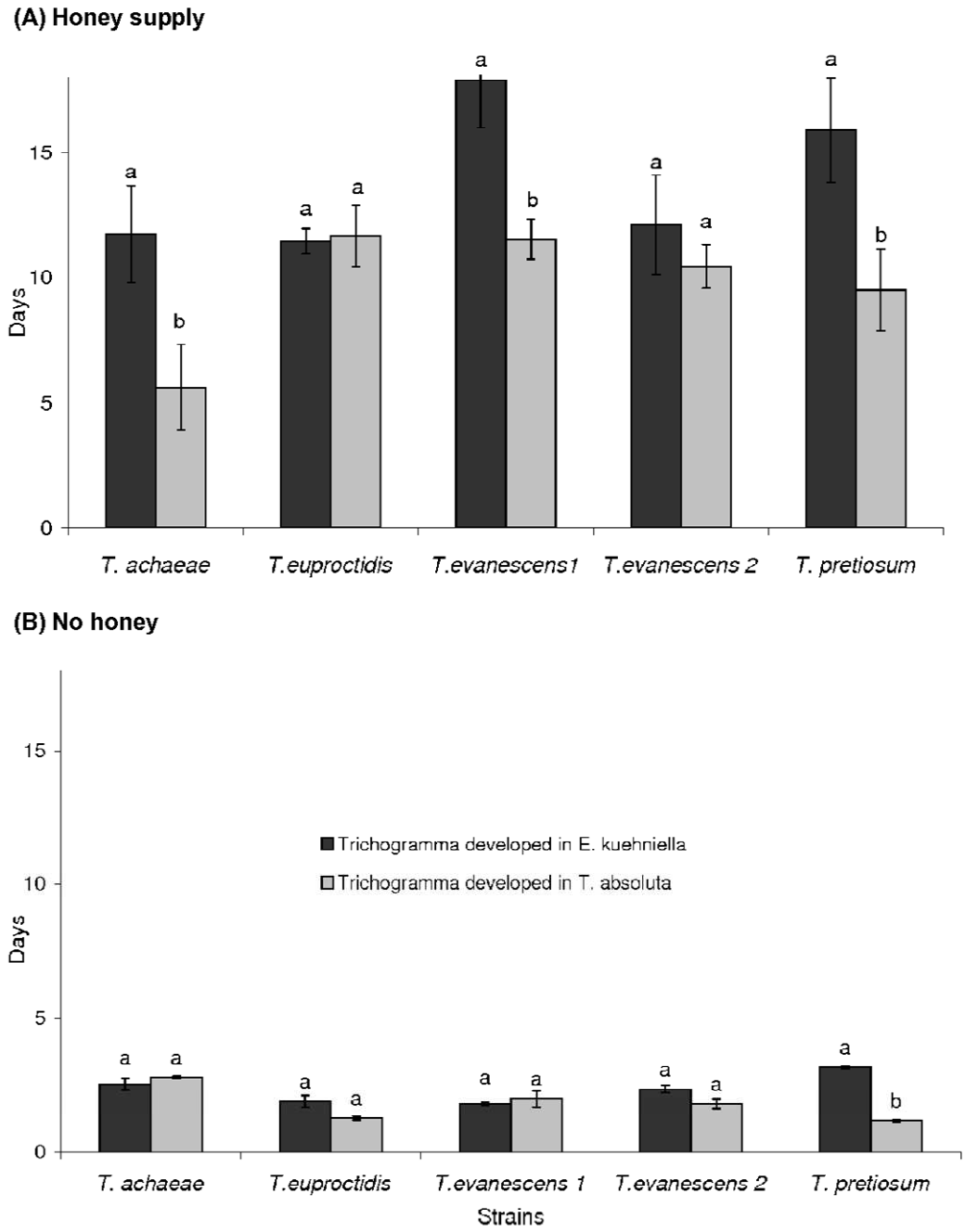


Figure 4: Mean (±SE) longevity of the five *Trichogramma* strains tested. *Ephestia kuehniella*: females developed in *Ephestia kuehniella* eggs (equivalent to the *Control* and the *Released generation* females); *Tuta absoluta*: females developed in *T. absoluta* eggs (equivalent to the *field generation* females); **(a)** in presence of honey as food source and **(b)** without any food sources. Post-hoc tests (Fisher's LSD test) were carried out separately for each strain tested. For each emergence host tested the histograms bearing the same letter are not significantly different at $P < 0.05$.

Cage experiments. The *Trichogramma Strain* and the *M. pygmaeus Presence* factors had a significant effect on the percentage of parasitism observed on the sentinel leaflets (Fig. 5, $F_{(1,29)} = 22.648$, $P < 0.0001$ and $F_{(1,30)} = 8.981$, $P = 0.0066$, respectively). The parasitism of the *Field generation* was significantly lower than the one of the *Released generation* ($F_{(1,28)}=33.542$, $P<0.0001$). The highest level of percent parasitism was obtained with *T. achaeae* alone. On the contrary, no parasitism was observed for the *Field generation* of *T. euproctidis* in the presence of *M. pygmaeus* (Fig. 5).

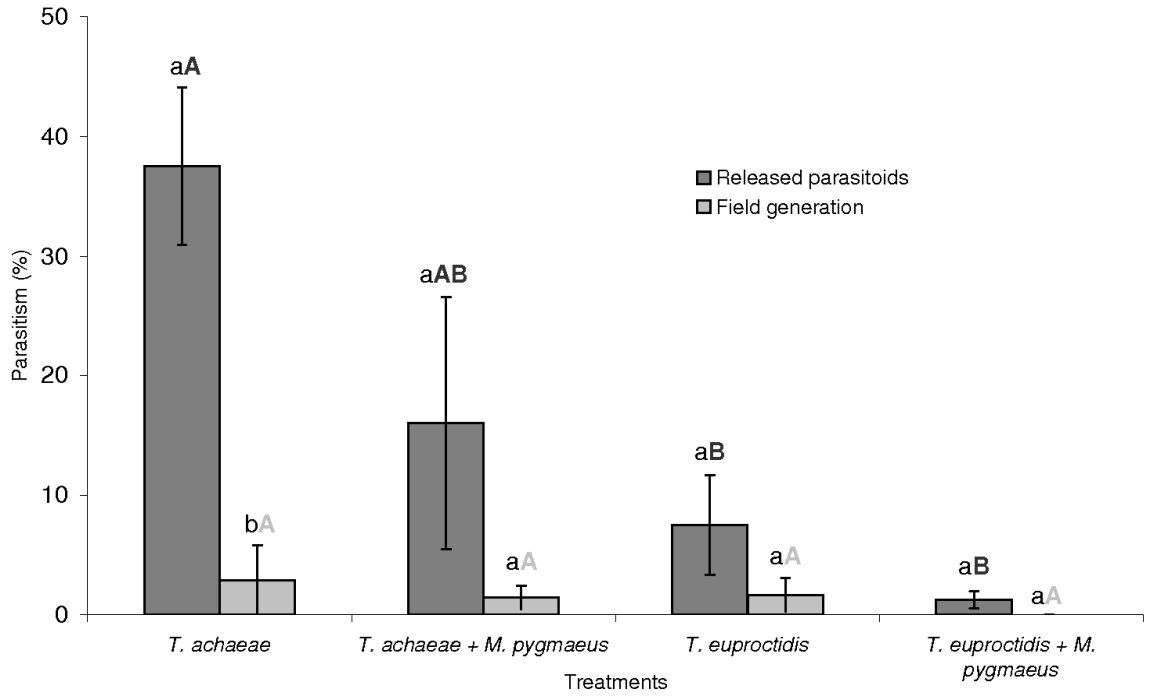


Figure 5: Mean (±SE) percent parasitism of *Tuta absoluta* eggs obtained on sentinel leaflets placed in greenhouse cages for the four treatments with *Trichogramma* releases, for the *Released generation* and the *Field generation.* Two types of post-hoc tests (Fisher's LSD test) have been done separately (i) each strain separately, generations bearing the same small-letters are not significantly different for $P < 0.05$ (ii) each generation separately, strains bearing the same capital letters are not significantly different at $P < 0.05$.

In the three treatments with *M. pygmaeus*, the number of eggs eaten did not vary significantly depending on the *Trichogramma Strain* (*T. achaeae* or *T. euproctidis* or absence) ($F_{(2,21)}=0.080$, $P=0.9228$). No significant effect of the *Trichogramma Generations* factor (*Released* or *Field generations*) was observed ($F_{(1,20)}=0.876$, $P=0.3631$). *Tuta absoluta* eggs were preyed on an average 71.66±3.61%, ranged between 25% and 96.6%. The factors *Trichogramma Strain* (*T. achaeae* or *T. euproctidis* or absence), *M. pygmaeus Presence* and *Trichogramma Generation* had a significant effect on the number of aborted eggs

($F_{(2,44)}$=3.918, P=0.0294, $F_{(1,46)}$=12.429, P=0.0012 and $F_{(1,43)}$=12.429, P=0.0012, respectively). However, the overall percentages of aborted eggs were very low throughout the course of the experiments in cages (≤5%). The maximum values were observed in groups with *Trichogramma* parasitoids without *M. pygmaeus* (*Released generation*).

At the end of the experiment, an overall average of 2.12 ± 0.50 *M. pygmaeus* juveniles per plant was recorded, ranging between from 1 to 3 per plant. No significant differences related to the *Trichogramma Presence* were observed ($F_{2,9}$=0.022, P=0.9780). The number of *T. absoluta* larvae was influenced by *Trichogramma Presence*, though only numerically significant (Fig. 6, $F_{2, 21}$=2.870, P=0.0860) and it was significantly affected by *M. pygmaeus Presence* ($F_{(1,20)}$=9.178, P=0.0080). The highest number of larvae was obtained in the control (*T. absoluta* alone) and the lowest infestation was observed in the treatment combining *T. achaeae* and *M. pygmaeus* (Fig. 6).

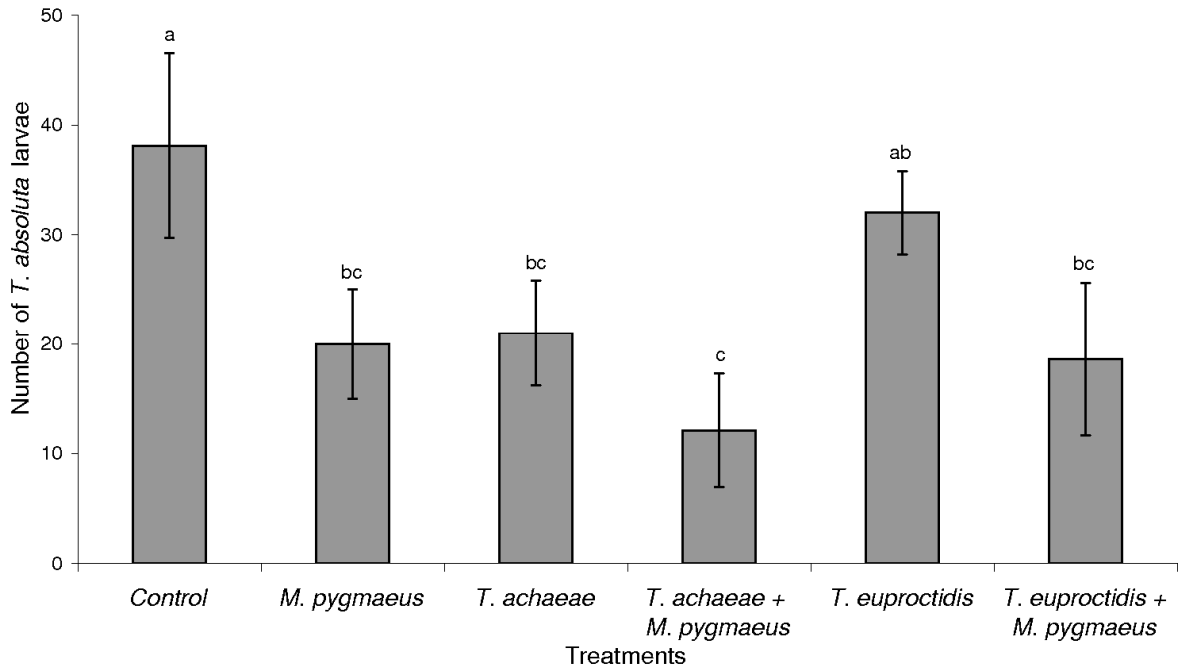


Figure 6: Mean (±SE) number of *T. absoluta* larvae per plant 16 days after insect releases in greenhouse cages for the six treatments tested. Values marked with the same letter do not differ significantly at $P < 0.05$ (Fisher's LSD test).

Discussion

The parasitism of five *Trichogramma* strains, i.e. four species, on *T. absoluta* was evaluated in the laboratory by testing two different parasitoid generations, particularly the ones

potentially occurring in tomato crop after inundative releases of parasitoids. The parasitism of *T. absoluta* eggs on tomato by parasitoids from the *Released* and *Field generations* was related to the parasitism observed in individuals from a *Control* treatment (developed in *E. kuehniella* eggs / parasitizing *E. kuehniella* eggs that is to say: standard rearing conditions). The highest parasitism was recorded for the generations under the standard rearing conditions on the factitious host. Parasitism decreased when oviposition occurred on the new pest-plant system. On this pest-plant system, the lowest parasitism was recorded for the *Field generation* although it was not consistent for *T. euproctidis* and *T. pretiosum*, i.e. these strains showed same parasitism levels in both *Released* and *Field generations*. *Trichogramma euproctidis* showed the highest parasitism rate among the parasitoid strains tested under laboratory conditions. Overall, parasitism efficiency and fitness decreased on the *T. absoluta*-tomato system; this pest-plant system was showed relatively unsuitable for the *Trichogramma* strains tested. Reduced quality of individuals produced on *T. absoluta*-tomato was observed in all traits measured in offspring: decreased sex-ratio, i.e. more males vs. females produced (see Desneux et al. 2009), increased malformations of individuals (wings checked in our study) and reduced longevity (for *T. achaeae*, *T. evanescens* 1 and *T. pretiosum*, with up to a 6-day reduction).

We can assume that differences among parasitoid efficiency recorded for the *Control* and the *Released* and *Field generations* are linked to host physiological suitability for the *Trichogramma* strains tested. Although the host size-parasitoid fitness relationship is not always consistent among parasitoids species (Harvey et al. 2013), they largely depend on the quality and quantity of resources provided by the host for their larval development (Mackauer et al. 1996, Pennacchio and Strand 2006). A close relationship has been generally observed between the size of the host parasitized and the size and fitness of parasitoid offspring, e.g. egg load of emerging females (Harvey et al. 2013). In addition, offspring sex-ratio and longevity can be linked to the size of parasitoid individual (Fidgen et al. 2000) and *Trichogramma* parasitoids commonly show preference for relatively large host eggs (Roriz et al. 2006). *Trichogramma achaeae* is reared on *E. kuehniella* by biocontrol companies and eggs of this host are 3-fold larger than those of *T. absoluta*. Therefore, the decreased quality of parasitoids (parasitism efficiency, sex-ratio, longevity and wing malformations) that developed in *T. absoluta* eggs may result from the small size of the host eggs, e.g. smaller parasitoids emerged from *T. absoluta* eggs (AC, personal observation). Such wing malformations have been scarcely documented for parasitoids belonging to the *Trichogramma* genus. Wings and abdominal malformations were observed in *Trichogramma* wasps reared on

artificial diet (Consoli and Parra 1996), and sexual dimorphism (apterous males and fully winged females) of *T. semblidis* was described by Salt (1937) when the wasps developed in small eggs (*Sialis lutaria* Linn. [Megaloptera:Sialidae]) vs. *E. kuehniella* eggs.

Understanding the suitability of the pest-plant system that underpins the parasitism pattern in the laboratory has important practical implications, as highlighted by our greenhouse cage experiments, i.e. the parasitism activity of the *Field generation* of both *Trichogramma* species was quite low in cages under greenhouse conditions. However, overall *T. absoluta* control achieved by *T. achaeae* was similar to those achieved with *M. pygmaeus* when these natural enemies were used alone (~46% decrease in *T. absoluta* larvae/plant, see Fig. 6), though the experiment was carried out over a short period, i.e. infestation levels were measured 16 days after the pest was introduced into the cages. As opposed to the results under laboratory conditions, *T. euproctidis* both *Released* and *Field generations* (with or without predator) showed the lowest parasitism rates in the greenhouse cage experiments. This species was not able to control *T. absoluta* populations; there was no significant difference in larvae number compared to the control (see Fig. 6). These results indicate that populations of parasitoid released in the tomato crop may disappear soon after the releases. Therefore, the impact of *Trichogramma Field generation*(s) on *T. absoluta* population growth could be considered negligible for biological control, at least with strains tested in our studies.

The drastic decrease of parasitism efficiency of field-born *Trichogramma* parasitoids in the cages points to the fact that tomato host plant impact is more noticeable under the realistic conditions than in a confined laboratory experiment. Reduced efficiency between laboratory and more realistic conditions, such as in a greenhouse cage, has been documented for released *Trichogramma* generations against *T. absoluta* (Chailleux et al. 2012). However, our present study further documents the possible impact of *Trichogramma* generations when parasitoids emerged in the greenhouse from *T. absoluta*-parasitized eggs. Poor effectiveness of the *Field generation* wasp in *T. absoluta* parasitism may be due to various factors. First, continuous tomato plant growth during the course of the cage experiment has led most likely to an increasing plant architecture complexity between the beginning of the experiment, i.e. the time parasitoids were first released, *Released generation*, and the end of the experiment, when parasitoids developing in *T. absoluta*-parasitized eggs emerged. Increased habitat complexity is known to negatively affect efficiency of natural enemies in finding hosts or prey (Rutledge and O'Neil 2005). It may have decreased host searching and/or dispersal ability of the parasitoid females when foraging for hosts (Gingras et al. 2008, Tabone et al. 2012). Second, parasitism effectiveness may have decreased due to (i) the small size of the

parasitoids emerging from *T. absoluta* eggs (such small host eggs make parasitoid females mature fewer eggs, Kazmer and Luck 1995), and (ii) increased sex-ratio in offspring produced (more males in the population). Third, parasitoid mobility may have been reduced on tomato plants because of the smaller individual size and the high pubescence of tomato. Foraging ability was consequently reduced (Carrillo et al. 2008). *Trichogramma* wasps are known to be poor flyers and to forage primarily by walking and jumping on substrates (Keller 1987, Olson and Andow 2006). In addition, previous studies reported that tomato trichomes have negative effects on *Trichogramma* efficiency (Farrar et al. 1994, Kauffman and Kennedy 1989).

This study supports the hypothesis that the pest-plant system *T. absoluta*-tomato shows unfavorable characteristics for the effective establishment of *Trichogramma* parasitoids in tomato crops. More broadly, *Trichogramma* parasitoids should be used through inoculative or inundative strategies according to the biological and physical characteristics of the targeted crop and associated pest eggs (Mills 2010), e.g. suitability for successful offspring development. In contrast to other pest-plant systems in which *Trichogramma* field parasitoid generations provide biocontrol services to some extent after the initial release in the crop, e.g. on the European corn borer in maize crop (Thomson et al. 2003, Hoffmann et al. 2006, Pintureau 2009), the biocontrol service provided by the *Trichogramma* field generations in tomato crop against *T. absoluta* should be considered low. *Trichogramma*-based biocontrol programmes targeting *T. absoluta* on tomato crop therefore need to rely on periodical inundative releases of the parasitoid, at least for *Trichogramma* strains so far identified as potential natural enemies of *T. absoluta* in Europe.

We also evaluated the effect of the generalist predator *M. pygmaeus* presence on the effectiveness of *T. achaeae* and *T. euproctidis* releases against *T. absoluta*. *Macrolophus pygmaeus* decreased the abundance of parasitoids hence their effectiveness on *T. absoluta,* both through direct competition for the resource (moth eggs), and direct predation on *Trichogramma*-parasitized eggs (Chailleux et al. 2013). Parasitism exerted by the *Released* and *Field generations* on both parasitoid strains was lower when the predator was present. The highest *T. absoluta* control level (less than 68% larvae per plant than in the control) was achieved when combining the parasitoid *T. achaeae* with the predator. In addition, the presence of *M. pygmaeus* nymphs attests effective predator establishment after being introduced into cages. Previous studies reported that supplementary releases of *T. achaeae* in tomato crops, in which another mirid predator (*Nesidiocoris tenuis* Reuter) was released during the cropping cycle, improved the biological control of *T. absoluta* (Desneux et al. 2010, Cabello et al. 2012). Our results back up these conclusions. However, additional

releases of *T. achaeae* did not improve control of *T. absoluta* when *N. tenuis* was inoculated by pre-plant releases in nurseries (Calvo et al. 2012), i.e. when the predator populations were already well established in the tomato crop before arrival of *T. absoluta*. Nevertheless, such a strategy based on very early establishment of *N. tenuis* in nursery plants often leads to insecticide applications when the omnivorous predator reaches high densities. Indeed, this predator is known to inflict damage to tomato plants, causing necrotic rings on stems and flowers and punctures in fruits (Calvo et al. 2009, Castañe et al. 2011).

Our study suggests that the integration of *Trichogramma* parasitoids for biological control strategies against *T. absoluta* in tomato crops should rely on the use of several inundative releases and should also take into account the population levels of generalist predators either artificially released and/or naturally occurring in the tomato crop.

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II) La lutte biologique basée sur les parasitoïdes larvaires

Présenté en temps que *Article 8*, en préparation,

voir annexe 3 pour une photo du dispositif expérimental,

Article 9, en préparation,

voir annexe 4 pour une photo du dispositif expérimental,

et *Article 10*, en préparation,

voir annexe 1 pour une photo du dispositif expérimental.

Article 8

No avoidance behavior of an omnivorous predator by a specialist parasitoid under exploitative competition and kleptoparasitism risk

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No avoidance behavior of an omnivorous predator by a specialist parasitoid under exploitative competition and kleptoparasitism

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Abstract

Optimal habitat choices are essential in species survival in ecosystems. Among all ecological mechanisms that are known to be important in this, competition is commonly considered to be the primary explanation for observed patterns of species association. Species are expected to avoid habitat were other species that can affect their fitness are present. Specialists are thus expected to avoid competitor and predator. For specialists, and especially parasitoids, omnivorous predatory species are both competitor and predator, because many of these predators behave as intraguild predator. We present a study highlighting the short-term interactions between a larval parasitoid and a predator when sharing an invasive species, *Tuta absoluta*. We observed a strong effect of kleptoparasitism instead of classical intraguild predation. However, we do not provide evidence that the parasitoid avoid the omnivorous predator. Reasons of such a result are discussed.

Keywords: interspecific interaction, coexistence, intraguild interference, *Tuta absoluta*, *Stenomesius japonicus*, *Macrolophus pygmaeus*.

Introduction

Optimal foraging theory explores decisions that animals make while foraging for resources and that contribute to maximise their reproductive success (Stephens and Krebs 1986). Among the different behavioral strategies studied, optimal habitat choices and investment decisions are complicated by spatial and temporal dynamic variation in factors affecting the quality of habitats, such as the amount and quality of food resources, intensity of predation and competition, and microclimate conditions (e.g. Gustafsson 1987, Robinson et al. 1995, Sinervo and DeNardo 1996, Martin 2001, Shima and Osenberg 2003, Marshall and Cooper 2004).

Competition is commonly considered to be one of the primary explanation for observed patterns in ecology and evolutionary theory (for reviews: Connell 1980, 1983, Schoener 1983, Gurevitch et al. 1992, Schluter 2001, Eccard and Ylönen 2003). However, the role of inter-specific competition may depend on the type of competitive interaction (Morris 1999). Exploitative competition, for example, involves indirect negative interactions arising from the use of a common resource (e.g. Case and Gilpin 1974). In contrast, interference competition involves direct negative interactions arising from territoriality, overgrowth, predation or chemical competition (Schoener 1983), where consumers might alter other's ability to exploit the resource at any level of abundance (e.g. Vance 1984). These two types of competition for resources may lead to the competitive exclusion, or at least the decline, of one or more native species that are utilizing the same resources (e.g. Wheeler and Hoebeke 1995, Elliott et al. 1996, Human and Gordon 1996, Westman et al. 2002). Therefore, it may be strategic for the inferior competitor to avoid patches of resources occupied by the superior competitor. In animal communities, species often avoid detrimental effects of competition by segregating their niches in time or in space (Rosenzweig 1995, Morris 1999). Among arthropods, avoidance of hetero-specific competitors has been demonstrated between bark beetles (Byers 1993), parasitoids (Janssen et al. 1995, Tamo et al. 2006, Cancino et al. 2012), phytophagous mites (Pallini et al. 1997), and between predators (Janssen et al. 1997, Gnanvossou et al. 2003). However, avoidance is not always systematic (Janssen et al. 1999) and only a few data accurately demonstrated such interaction in the natural enemy trophic guild. Actually, most of the study had focus on avoidance of conspecific competitors which has been reported among several species (Danchin et al. 2004, Dall et al. 2005).

The theory of habitat selection usually assumes that individuals are fully aware about the relative differences in quality among potential habitat patches and choose the best possible

option (Morris 2003). Parasitic wasps searching for suitable hosts to deposit their offspring have been ideal models for such studies (Godfray et al. 1994, Quicke 1997). Besides choosing the best patch of resources, parasitoids frequently have to decide whether to lay their eggs or not in already parasitized hosts i.e. superparasitism, which may represent inferior resources compared to unparasitized hosts (van Alphen and Visser 1990, Godfray 1994). Actually, since they usually share their habitat, they often have to compete for resources with omnivorous predators. Predators are considered omnivorous when they feed on more than one trophic level. In term of population dynamics, this type of predators may be considered as superior competitors compared to specialist as they are able to outstand low level of prey by feeding on other trophic level (Grover 1990).

Omnivorous predators are not only exploitative competitor, but can also compete through interference competition. Besides feeding on their usual prey, omnivorous predator species are sometimes attacking and feeding their intraguild competitors i.e. a so-called intraguild predatory behavior, hence reinforcing their superior competitor status. Such behavior can increase the risk of the specialist exclusion (Montserrat et al. 2008). Kleptoparasitism, also called food robbing or cleptobiosis, is also a widespread form of interference competition by which many animals reduce the costs of searching for, handling, and obtaining food by stealing competitor of already procured food (Curio 1976). This can lead to victim fitness decrease, and in some case can have the same consequences as intraguild predation in term of fitness when the theft of the resource leads to the death of the owner (Reader et al. 2006). In such a case, specialists would benefit by developing avoidance responses to cues from intraguild predatory species living in the same habitats to reduce predation risks. Actually, most of the hetero-specific competitor avoidance studies had focused on competitors involved in intraguild predation. For example, this has been demonstrated for the coccinelids-aphids biological control model (Nakashima et al. 2006, 2004) as well as in other models like predatory mites (Gnanvossou et al. 2003).

Direct (interference competition) and indirect (exploitative competition) negative interactions between competitors are known to adversely affect species coexistence (Holt and Polis 1997). On the contrary, adaptive behaviors like avoidance may favour competitor species to coexist. Therefore, such phenomenon has to be accurately studied in order to understand species associations in ecosystems. When species do not have co-evolved on a shared resource, however, intraguild interactions may be different from systems in which they have co-evolved. This situation occurs when an exotic species invades a new area, natural enemies able to attack or parasitize this new invasive species may compete for the new shared

resource. Behaviors in such situation are unknown, especially regarding to avoidance behavior, since this is usually gain during co-evolution (Magurran 1993). Therefore, we present in this work a study highlighting the short-term interactions between a larval parasitoids and an insect predator sharing an invasive species, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae). *T. absoluta* is a tomato leafminer originated from South America that has invaded the European Mediterranean basin since 2006 (Desneux et al. 2010). This pest has become a major risk for tomato crop in a few years and is now suspected to invade other continents (Desneux et al. 2011). Indigenous entomophagous species have been reported to be efficient biological control agents against this pest (Zappala et al. in prep., Bompard et al. 2013) and some have been shown to be able to reproduce efficiently on it (Urbaneja et al. 2009, Chailleux et al. submitted). Thus, these species are likely to compete between each other on *T. absoluta* infested tomato plants, leading to new species associations in agro-ecosystems. Nevertheless, natural enemy species interactions are not accurately known in this case and the viability of the natural enemies associations is remaining uncertain. Fitness costs of coexistence in mixed species communities, which may have historically led to segregation, are difficult to study, prevented by the "ghost of competition past": competition had led to the species reparation and are no more observable in nowadays ecosystems (Connell 1980). Our study shed some light on new natural enemy species interactions when sharing an invasive pest through laboratory experiments. The work presented here aims at assessing the potential short-term negative impact of the predator on the parasitoid and the avoidance behavior of the parasitoid. Implications in terms of biological control against a new invasive pest are discussed.

Materials and methods

Biological materials

Five-week old tomato plants, *Solanum lycopersicum* L. cv. Betalux, were used. They were grown in climatic chambers (24 ± 1°C, HR: 65%, photoperiod 16L:8D) and a nutrient solution was applied daily. Insect were reared in growth chambers (25 ± 1°C, RH 70 ± 10%, 16L:8D). A colony of *Tuta absoluta* was set up using greenhouse-collected individuals in July 2009 at INRA, Alenya, France (initial number of individuals = 190). Adults were kept in cages (55×75×80 cm) containing tomato plants and fed with honey placed on one wall inside

the cages. The parasitoid species is *Stenomesius japonicus* Ashamed (Hymenoptera: Eulophidae), a larval parasitoid species specialist of leafminer that behaves as an ectoparasitoid (James and Stevens 1992, Chailleux et al. submitted). It was reared in the same cages like those used for *T. absoluta* rearing. A constant supply of tomato plants infested with *T. absoluta* larvae and honey drop were put on the plants. Rearing was initiated using individuals from Spain and France (n = 10, n = 5, respectively) collected in greenhouses. The predator, *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae), is an omnivorous predator widely used in European greenhouses against whiteflies, and is also able to fed on plants (Fauvel 1987, Bonato 2006). Individuals came from the Biotop company in France, and were reared for one generation at the laboratory on tobacco plants and fed on *Ephestia kuehniella* UV-sterilized eggs before experiment.

All parasitoid females used in experiments came from the rearing (mixed ages) and had previously been isolated from host in a transparent plastic box (d: 9 cm; h: 12cm) covered with a mesh and containing a tomato trifoliate (see Biondi et al. 2012, for a thorough description of the design) during five days, which corresponds to an optimal duration to obtain the best oviposition level according to preliminary experiments. Honey droplets were provided on boxes side. This type of designs i.e. arenas, was used for all experiments except for the behavioral study. In the same way, all *M. pygmaeus* females used in the experiment had previously been isolated from insect food sources for 24 h using the same arenas than those used for parasitoids isolation.

Short-term negative interactions

A laboratory experiment was carried out in arenas to evaluate the effect of the predator on the parasitoid immature stages. *S. japonicus* females were individually transferred to an arena containing a trifoliate infested with five third instars *T. absoluta* larvae. Larvae were collected from the rearing and were deposited on the tomato trifoliate 1 h before the parasitoid release to enable host larvae mine digging. Parasitoid females were allowed to parasitize the hosts during 48 h in a climatic chamber. Parasitoids were then removed and a pair of one male and one female of *M. pygmaeus* was introduced in the box. Three days after their introduction, predators were removed and the mines were open under a binocular microscope to record the number of parasitoid offspring. The number of living larvae was also noted. The same design was carried out but without *M. pygmaeus* to allow for comparison. Twenty repetitions were done for the two treatments i.e. with and without *M. pygmaeus*.

A second experiment consisted in direct behavioral observations. It was carried out since a negative effect of the predator on the parasitoid offspring was observed in the previous experiment (see Results section). One *T. absoluta* larva of the third instar was placed on leaflet in Petri dishes 1 h before the parasitoids release to ensure their establishment in a mine. Leaflet stems were planted in flower foam for watering. Then, parasitoid females were allowed to oviposit individually under a binocular to observe oviposition and thus to ensure eggs presence in the mines without opening the mines. Petri dishes were kept two days in climatic chamber corresponding to the time required to obtain parasitoid larvae. Then, one *M. pygmaeus* female was released in the Petri dishes during 5 h. Attacks upon the *T. absoluta* larvae or the parasitoid immature stages were then recorded by opening the mines under a binocular microscope. The percentage of the *T. absoluta* attacked by *M. pygmaeus* (hemolymph sucking) was visually evaluated. As a control, *M. pygmaeus* females were also released on healthy *T. absoluta* larvae in a leaflet, in Petri dishes, using the same experimental design and at the same time as the treatment with parasitized larvae. Eleven repetitions were carried out for the two treatments.

Avoidance behavior

The capacity of the parasitoid to avoid the predator was tested using both a choice and a non-choice experiment. In the non-choice essay, four treatment were carried out (1) *T. absoluta* + *S. japonicus*; (2) *T. absoluta* + *S. japonicus* + *M. pygmaeus*; (3) *T. absoluta* + *S. japonicus* + *M. pygmaeus* semiochemicals (4) *T. absoluta* + *M. pygmaeus*; and (5) a control with *T. absoluta* alone was also done. Five third instars *T. absoluta* larvae were placed per arena 1 h before the natural enemies release, as described for the first experiment. Then, isolated *S. japonicus* females and/or, when needed, a pair of one male and one female *M. pygmaeus* were added during 48 h. For treatment 4, a pair of one male and one female *M. pygmaeus* was added in the arena previously to the experiment during 24 h and removed just before the host larvae established i.e. 1 h before the parasitoid introduction. After the 48 h of experiment, mines were opened under a binocular to record the number of parasitoid offspring and the number of living larvae. Twenty repetitions were carried out per treatment.

A Y-tube olfactometer was used to study the response of adult female *S. japonicus* in choice situation. The Y-tube consisted of a plastic tube (L: 8 cm; d: 1.6 cm) connected at the two sides to the middle of arenas. This tube was perforated in the centre and connected to a removable tube (L: 4 cm; d: 1 cm). This removable tube was used to place the parasitoids 15

min before the experiment to avoid decision due to stress. Then on side of this tube was fixed on a perforation at the centre of the olfactometer tube and the other side to a suction pump that produced an air flow from the two side of the tube to the centre. The air flow chosen was 0.25 $m.s^{-1}$, which did not inhibit parasitoid movement. The odour sources in the two arenas consisted in a tomato trifoliate infested with two third instar larvae with, for one side, and without, for the other side, a pair of one male and one female *M. pygmaeus*. Larvae were placed on the trifoliate 2 h before the experiment and the predator was released in the arena 1 h before the experiment, so larvae were in the mines when predators were released. Each arena was used three times and then replace by a new one. Parasitoid that did not reach one of the two ends of the tube after 30 min were excluded from the experiment. Twenty repetitions were carried out.

Statistical analyses

All statistical analyses were performed using the R software (R Development Core Team 2009) with the *multcomp* and *agricolae* packages. For the predator effect on parasitoid immature stages experiment, the number of *S. japonicus* offspring and the number of living *T. absoluta* larvae were analysed using a Generalized Linear Model (GLM) with a Poisson distribution with a log link function, the factor *M. pygmaeus* presence was tested. For the avoidance non-choice essay, a GLM with a Poisson distribution was also used to analyse the number of *S. japonicus* offspring (*M. pygmaeus* presence as a factor) and the number of living *T. absoluta* larvae (*M. pygmaeus* presence and *S. japonicus* presence as factors). When necessary, means were separated using a Least Significant Difference post-hoc test (LSD test) for multiple comparisons. For the behavioral observations experiments, a χ^2 test was performed to compare the proportion of larvae eaten by the predator. The parasitoid choice in the avoidance choice essay experiment was analysed using an exact binomial test.

Results

Short-term negative interactions

The laboratory experiment evaluating the predator interaction with parasitoid immature stages showed that the number of *S. japonicus* offspring recorded after three day was reduced by the

M. pygmaeus presence. We found 0.25 ± 0.10 (Average ± SE) offspring with the predator and 0.85 ± 0.20 without, the difference was significant ($F_{1, 38} = 8.18$, $P = 0.007$). However, the number of living *T. absoluta* was not affected by the *M. pygmaeus* presence ($F_{1, 38} = 1.24$, $P = 0.273$): on average, half of the *T. absoluta* larvae were living after three days in both treatments (2.20 ± 0.29 with the predator and 2.65 ± 0.29 without the predator).

In the behavioral observations, none of the parasitoid juveniles were directly attacked by the predator. On the contrary, *M. pygmaeus* feed on the paralysed and parasitized host larvae, 81.8 ± 11.8 % of these were subjected to a more or less important hemolymph sucking by predator. When *T. absoluta* larvae were healthy, none of them were attacked by the predator, this was significantly different from the proportion of parasitized larvae attacked ($\chi^2 = 12.03$, d.f. = 1, $P < 0.001$). The hemolymph sucking of parasitized *T. absoluta* larvae by the predator was 48.3 ± 26.3 %. However, even if *M. pygmaeus* do attack directly the parasitoid juveniles, all of them died two days after the experiment, probably because of a lack of food resource i.e. resource robbed by the predator.

Avoidance behavior

In the non-choice essay, the number of *S. japonicus* offspring produced (Fig. 1) was not

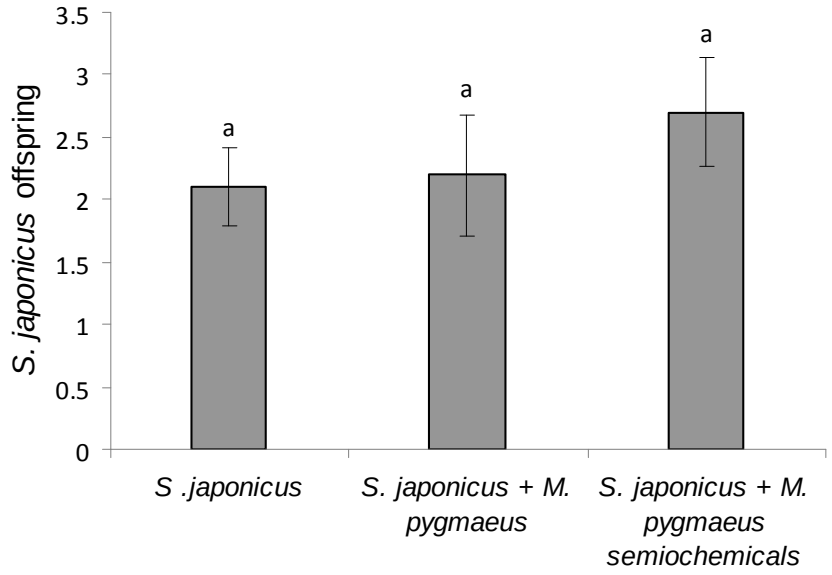


Figure 1: Mean (± SE) number of *S. japonicus* offspring produced per parasitoid female when alone with *T. absoluta* larvae, when *M. pygmaeus* was present, and when only *M. pygmaeus* semiochemicals were present under laboratory conditions. Values with the same letters are not significantly different at $P < 0.05$.

significantly affected by the factor *M. pygmaeus* presence ($F_{1, 58}$ = 0.47, *P* = 0.497). An average of 2.33 ± 0.41 offspring were produced in 48 h. The number of living *T. absoluta* larvae (Fig. 2) was significantly affected by the factor *S. japonicus* presence ($F_{1, 98}$ = 112.21, P < 0.001). On the contrary, the *M. pygmaeus* presence had not a significant effect on this parameters ($F_{2, 97}$ = 1.65, *P* = 0.197). Consistently, in the choice essay, parasitoids did not significantly avoid the predator (binomial exact test: *P* = 0.824), only 55 ± 0.11 % of the parasitoid chose the box without *M. pygmaeus*.

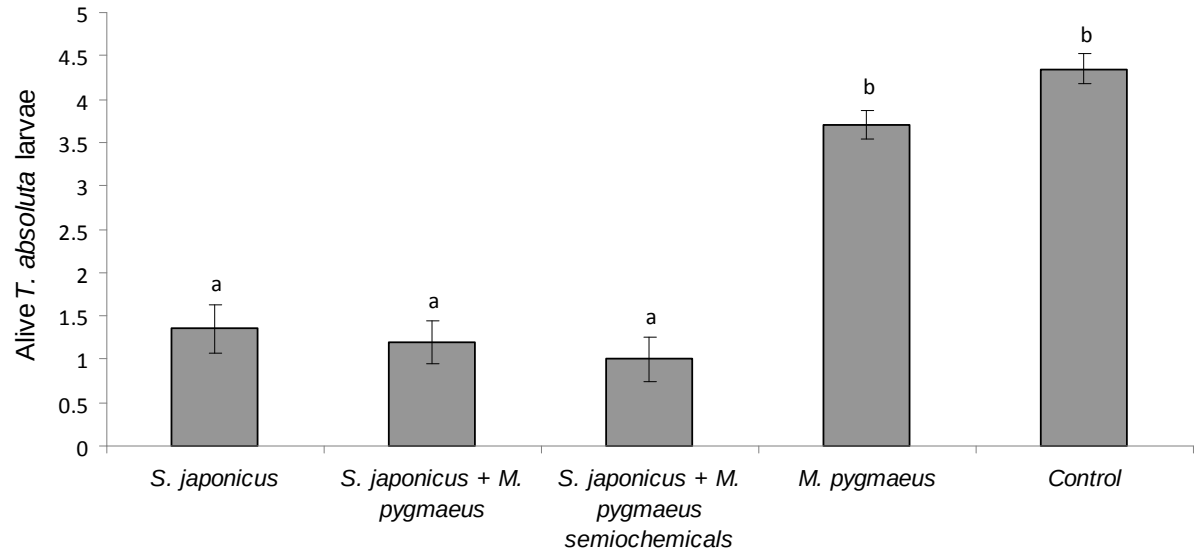


Figure 2: Mean (± SE) number of living *T. absoluta* larvae per after 48 h in five treatments under laboratory conditions: (i) *S. japonicus* female alone, (ii) *S. japonicus* female + *M. pygmaeus*, (iii) *S. japonicus* female + *M. pygmaeus* semiochemicals (iv) *M. pygmaeus* alone (v) control, i.e. *T. absoluta* larvae alone. Values with the same letters are not significantly different at *P* < 0.05.

Discussion

Results provide evidence of a negative effect of the omnivorous predator on the parasitoid immature stages and observations highlight that it was not accurately intraguild predation but rather kleptoparasitism. The predator fed on *T. absoluta* larvae attacked by parasitoids females, thus robbing the exploitative resources of the parasitoid juvenile leading to its death. This is really closed to intraguild predation because each attack leads to the death of a competitor. However, in our experiments, the parasitoid was not keen to limit oviposition when the predator was present in non-choice tests, neither to avoid the predator in choice tests. Regarding the negative effect of the predator in the offspring, this result is surprising

and will lead to a decrease in the parasitoid fitness when sharing its ecosystem with the omnivorous predator.

Cases of intra- and inter-specific cleptobiosis have been reported in vertebrates, including fishes (Dominey and Snyder 1988), birds (Garrido et al. 2002), mammals (Di Bitetti and Janson 2001, Honer et al. 2002), marine invertebrates (Morissette and Himmelman 2000), spiders (Whitehouse et al. 2002), and insects such as thrips (Crespi and Abbot 1999), wasps (Kronauer 2004, LaPierre et al. 2007), bees (Michener 1974), ants (Richard et al. 2004), and phytophagous flies (e.g. Sivinski et al. 1999, Reader 2003), but never in a biological control context. Inter-specific kleptoparasitism has never been reported in predatory bugs, but scavenger behavior, which consists in feeding on dead and decaying organic matter, has already been observed in bugs (Sugiura and Yamazaki 2006). In our study, kleptoparasitism is closed to scavenging, because the larvae that are robbed are dead and sometime decaying. It seems that *M. pygmaeus* adopts an opportunistic behavior when it encounters a parasitized larva that represents a big amount of food compared to *T. absoluta* eggs. Moreover, healthy *T. absoluta* larvae adopt a defensive behavior: they squirm (personal observation) and escape from *M. pygmaeus* attack. Defensive behavior has been observed for the moth *Barathra brassicae* (Tautz and Markl 1978) and has been shown to be efficient in protecting larvae from wasp attacks: squirming caterpillars usually fall off the leaf into the undergrowth and can escape. On the contrary, when *T. absoluta* larvae have previously been attacked by a parasitoid and are paralyzed, attack becomes easier for *M. pygmaeus* since larvae do not exhibit defensive behavior anymore, hence limiting predation cost.

Kleptoparasitism of *M. pygmaeus* on *S. japonicus*, as it leads to the death of the parasitoids, likely have similar consequences in term of population dynamics compared to intra-guild predation and thus undoubtedly increases risk of parasitoid exclusion from the agrosystems, hence reducing probability of long-term coexistence between interacting species. However, occurrence of this behavior in the field is unknown and it would be hard to discriminate it from exploitative resource competition. Parasitisation of larvae provides access of *M. pygmaeus* to a prey stage that it cannot feed on when alone in ecosystems. Accordingly, parasitoids presence might favour predator since prey used by the parasitoid become easily available to the predator. The parasitized larvae constitute a big amount of food with reduced energetic predation cost. It has been demonstrated that on stage-structured prey population i.e. when tow competitors do not attack the same stage of the shared prey/host, two parasitoids attacking different stages can coexist if the first stages attacked by one of the competitor is still available for the competitor attacking the later stages (Briggs 1993). If first-attacked

instars are no more available for the predators attacking the later stages, coexistence becomes not possible (Briggs 1993). On the contrary, in our biological model, the late parasitized stages remain available for the competitor attacking the first instar. So, this means that *M. pygmaeus*, which decreases the resources for the parasitoid by preying on eggs, is also favoured because it can feed on paralysed larvae in the presence of the parasitoid. This increases the asymmetry of the competition and consequences in terms of population dynamics would adversely affect long-term coexistence.

We demonstrated that the parasitoid suffered a decrease in fitness in the predator presence because of kleptoparasitism. However, the parasitoid was not keen to avoid patches with the predator. Kleptoparasitism and exploitative resource competition should imply a decrease in fitness for the parasitoids, so avoidance behavior should be expected. Four main hypotheses might explain these results: (i) the absence of previous co-evolution between the two protagonists, (ii) biased interpretation about the effect of hetero-specific presence, (iii) avoidance does not occur between species that never meet at the adult stage, and (iv) the use of inappropriate experimental design. Absence of odour-mediated interactions have been already demonstrated for species that did not previously co-evolve (Janssen et al. 1999) and may be due to the fact that the parasitoids have not been selected to recognise cues from the predator as being potential competitor and kleptoparasite. However, the two species studied here share the same geographical area and, even if this had never been reported so far, may have co-evolved on other leafminer species. Second, contrary to our interpretation, the parasitoid may use the predator semiochemicals as an indication of available habitat, or habitat quality. Recent theoretical studies predict that hetero-specific presence may be used by some species to detect a potential habitat, with optimal conditions relative to competitor density occurring at some intermediate density (Mönkkönen et al. 1999, Fletcher 2006). However, we did not find a parasitoid preference for predator presence, which reduces the probability of this hypothesis to be correct. Third, it is essential to note that both negative interactions i.e. exploitative competition and kleptoparasitism via the shared resources, reported here are indirect. Moreover, since the two competitors attack different stages, they never meet trying to use the same individual resource. If the parasitoid would have encountered the predator when attacking a host or have suffered real intraguild predation, avoidance would have been more likely to occur. Finally, *S. japonicus* might be able to discriminate between odours of plants either with or without hetero-specific predators, although this cannot be analysed using the experimental set-up used here. Experiments in other conditions, e.g. the predator alone without *T. absoluta* larvae in tomato trifoliate, or a

higher density of predators, may have produced different results. Nevertheless, the experimental designs in the present work are typically used to test avoidance behavior, and the predator alone or higher predator densities would not have been representative of the reality and would not have provided behavior susceptible to occur in agro-ecosystem.

Our results suggest that resource competition on different stages of the prey and kleptoparasitism on the juvenile stages are two interactions that do not allow parasitoids imago to meet their competitors and thus do not lead to direct hetero-specific competitor avoidance. This hypothesis remains to be tested on other biological models. The apparent absence of adaptive behaviors that may limit the strength of negative interactions may have a drastic effect on parasitoid populations. Thus, further studies should evaluate such natural enemies association during long-term dynamics and under real crop conditions.

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Article 9

Survival of a specialist natural enemy when experiencing resource competition with an omnivorous predator

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Survival of a specialist natural enemy when experiencing resource competition with an omnivorous predator

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Abstract

Do specialist parasitoids can persist in the agrosystem when competing with an omnivorous for their shared prey/hosts? Consequences of omnivory have been studied theoretically, but empirical studies are lacking. However, omnivory is frequent in nature and omnivorous predator coexists with specialist in many ecosystems, even when they behave as intraguild predator. This type of association is also frequent in agro-ecosystem when biological control strategies are used. Our study provides an example of the outcome of an association in the context of biological control of the invasive pest *Tuta absoluta* in the tomato agro-ecosystem. The two natural enemies tested, the specialist parasitoid *Stenomesius japonicus* and the omnivorous predator *Macrolophus pygmaeus*, were able to coexist during three months. In this biological model omnivory does not lead to the parasitoid population local extinction. Mechanisms that may have promoted coexistence as well as consequences for inoculative biological control programme are discussed.

Keywords: coexistence, intraguild predation, interspecific interaction, *Tuta absoluta*, *Stenomesius japonicus*, *Macrolophus pygmaeus*.

Introduction

Multiple species of natural enemies commonly attack single prey/host species (Price 1971, Hawkins 1990, Polis 1991, Hawkins and Mills 1996, Polis and Strong 1996). How do they coexist in ecosystem using a common resource is a key question to understand ecosystem functioning (Chase and Leibold 2003, Finke and Snyder 2008). It has been established that relatively strong intra-specific competition coupled with relatively weak inter-specific competition fosters species coexistence and promotes biodiversity (e.g. Hutchinson 1959, MacArthur 1970, McKane et al. 2002, Chase 2003). Several mechanisms can lead to a decrease of intra-specific competition, whereas four mechanisms are recognize to be effective at decreasing inter-specific competition (i) aggregation behavior, (ii) diet breath, (iii) resource segregation and (iv) trade-off in life history trait. The effect of the diet breadth on coexistence had been theoretically studied but empirical experiments are lacking, especially those studying the effect of true omnivory, i.e. feeding on different trophic level, as plant and herbivores, and not only on different prey species (Coll and Guershon 2002). However, association between species sharing the same resources, but yet having different diet breadth is frequent in nature.

Under equilibrium conditions old theories have shown that the presence of omnivory destabilizes food webs (Pimm and Lawton 1978, Coll and Izraylevich 1997), correspondingly, omnivory ought to be rare in real food webs. This theory have then been re-evaluated by showing that omnivory can stabilize ecosystem (Mc Cann and Hastings 1997, Lalonde et al. 1999). Indeed, this diet breadth is considered to stabilise herbivore populations because, for example, switching between plant and prey feeding provides a refuge for the prey when it is at low densities. Another factor that may stabilize the system is the relatively poor searching efficiency of omnivores (Lalonde et al. 1999, Peers et al. 2012). Thus, it is likely that omnivory, by decreasing the intensity of the prey used, may also limit the strength of the competition between the omnivorous predator and other natural enemies. However, there are two main limits in these theories. First, omnivorous diet breadth enable the predator to persist at low level of prey by feeding on plants (Crawley 1975, Pimm and Lawton 1977, 1978, Wald 1994, Eubank and Deno 1999), thus potentially reducing prey density at levels that cannot allow other competitors to persist. Second, many omnivorous predators are often engaged in intraguild predation, i.e. when one of the competitor attack and feed directly on the other one.

To protect crop from insect pest in agro-ecosystem, omnivorous predators are frequently used in combination with specialist natural enemies (Snyder and Ives 2003). Many

species of biological control agents are also known to be intraguild predators and to feed on plant, i.e. true omnivory. For example, spiders are known to feed on pollen and to attack other predators, some predatory bugs, such as Heteroptera, are known to feed on sap or pollen and do feed on various predators (Schmidt et al. 1998). Thus, these predators, having access to multiple food resources, could be considered as the superior competitors that may exclude specialist feeders at the population level (Grover 1997). Here we provide an empirical study on the coexistence of an omnivorous predator and a parasitoid, i.e. the specialist, to highlight the outcome of such association.

Does omnivory will or will not allow the parasitoids survival in a closed system, where mechanism of meta-community i.e. emigration and re-colonisation, at the landscape level, cannot occur? Most experimental studies on predation/parasitism have only examined effects of one natural enemy species at a time (Sih et al. 1998) or multiple natural enemies with the same diet breadth, e.g. between specialist or between omnivorous (Batchelor et al. 2005, Sanders et al. 2011, Moreno-Ripoll et al. 2012). Few data deriving from field experiments address questions crucial for inter-specific competition between specialist and omnivorous species and its impact on natural enemies-host dynamics (Godfray et al. 1994).

In this work, we assessed the competitive interactions between two natural enemy species, a specialist parasitoid and an omnivorous predator. Both of them attack the major tomato invasive pest in Europe, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) (Desneux et al. 2010, 2011). The omnivorous is the predatory bug *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae), it is mainly used to control whiteflies and able to feed on plant food sources, such as sap or pollen (Calvo et al. 2009). This predator was recently included in *T. absoluta* control programs and it preferentially attacks eggs and rarely young larvae instar of this pest (Urbaneja et al. 2009). At the same time, several ecto- or endo-parasitoids have also been reported (mainly Eulophidae, Braconidae and Ichneumonidae) attacking *T. absoluta* in the Mediterranean basin (Zappala et al. 2012, and in prep.). Among them, the idiobiont ectoparasitoid *Stenomesius japonicus* Ashamed (Hymenoptera: Eulophidae), naturally occurred in newly invaded areas (Urbaneja et al. 2012, Biondi et al. submitted) attacking preferentially old larvae (3^{rd} instar larvae; Chailleux et al. submitted). It should be possible to use this species through inoculative releases to control *T. absoluta* although its capacity to persist in tomato crops in the presence of *M. pygmaeus* remains to be assessed. Moreover the generalist predator exerts kleptoparasitism on *S. japonicus* by feeding on paralyzed and parasitized larvae (Chailleux et al. in prep.). In this biological model the consequences of kleptoparasitism on the parasitoid population can not be distinguished from intraguild

predation because each kleptoparasitism attack leads to the death of the parasitoid larvae that have no more enough resource to reach the pupae instar (Chailleux et al. in prep.).

The objectives of this study was thus: (i) to quantify the outcome of a frequent species association in agro-ecosystem, i.e. a specialist parasitoid and an omnivorous predatory bug both feeding on the same pest, and (ii) to identify resource utilization patterns that may promote coexistence of the two natural enemies.

Materials and methods

Biological materials

Five-week and six-week old pesticide-free tomato plants, *Solanum lycopersicum* L. (cv. Betalux) were used in the laboratory and greenhouse experiments, respectively. They were grown in climatic chambers (24 ± 1°C, HR: 65%, photoperiod 16L:8D) and a nutrient solution was applied daily. Insect were reared in growth chambers (25 ± 1°C, RH 70 ± 10%, 16L:8D). A colony of *T. absoluta* was set up using greenhouse-collected individuals in July 2009 at INRA, Alenya, France (initial number of individuals = 190). The colony was kept in cages (55 × 75 × 80 cm), containing tomato plants and honey was provided on one wall inside the cages to feed adults. *Stenomesius japonicus* colony was reared in cages (same as for *T. absoluta* rearing) with a constant supply of tomato infested with *T. absoluta* larvae and honey droplets were provided on the plants as food supply for imagos. The laboratory rearing was initiated using individuals from Spain and France (n = 10, n = 5 respectively) collected in commercial tomato crops. The predator specimens originally came from the commercial insectary of the company Biotop (France). They were then reared for at least one generation on tobacco and fed on *Ephestia kuehniella* UV-sterilized eggs in the cages and environmental conditions described above.

Population dynamics experiment

Experimental setup

The experiments were carried out in cages (100 × l: 70 × L: 100 cm) made with insect-proof mesh and placed in a glasshouse in the facilities of the INRA AgroBiotech Institute (Sophia-Antipolis, France). Inside each cage, six tomato plants in pot were weaved vertically on stake and side stems were removed every week and left underneath the plant to allow insect eggs to hatch. Plants were watered automatically with a nutrient solution. Temperature and humidity were regulated with fog, shade and airing and kept as possible close to 25°C [semi-controlled temperatures, mean temperature: 23.6°C (range 9.8°C – 39.5°C); mean RH: 67.5% (range: 19.0% – 95%); natural ambient light: May-July 2012].

Three combinations of natural enemies were carried out: (i) *T. absoluta* + *S. japonicus,* (ii) *T. absoluta* + *M. pygmaeus* and (ii) *T. absoluta* + *S. japonicus* + *M. pygmaeus.* Furthermore, since prey density could potentially impacts population dynamics, these three combinations were tested on two *T. absoluta* densities. The low density corresponded to four pairs (one male and one female, hereafter called pair) and the high density to 16 pairs of young imagos (< 1 week-old) release per each cage at the beginning of the experiment. The two natural enemies were released at a ratio of two pairs per plant, 12 pairs per cage in total. Treatments with only one natural enemy were repeated three times, while the one with both natural enemies was repeated four times. Placement of the treatments within the greenhouse was randomized.

To avoid cycling populations because of the release of only one insect stage, each species was released twice, i.e. half-quantities each time. A first release and then a second one after a time interval corresponding to the half of their life-cycle duration, namely one week for mirid bug and *S. japonicus*, and two weeks interval for *T. absoluta*. Young *M. pygmaeus* adults (< 3 days) were released first, i.e. two weeks before, the first *T. absoluta* release. Commercially available UV-sterilized eggs of *E. kuehniella* (Biotop, France) were put on the plant as an initial food source for *M. pygmaeus* to ensure its establishment on the crop. This is recommended by company selling the predator and thus mimicked a preventive inoculative release as done by tomato producers. *Stenomesius japonicus* imagos (mixed ages) were released once larvae of *T. absoluta* had reached the ideal stage (3rd instar larvae) for parasitoid offspring production (Chailleux et al. submitted). Insect releases began on the 30th of April 2012 and the last release of *S. japonicus* was done on the 11th of June. Monitoring started in

week 1, on the 13th of June, i.e. one month and a half after the first release and the same week as the last parasitoid release.

Monitoring and Sampling

Plants were monitored weekly during eight weeks after the last insect releases. In each cage, two plants were randomly selected every week for the monitoring. *M. pygmaeus* adults and nymphs were counted on the entire selected plants. Eggs and larvae of *T. absoluta* were monitored on six leaves, two leaves were selected at random from the upper, the middle and the bottom third of each selected plant. *T. absoluta* larvae were observed by shining a torch lamp under each leaf and eggs were observed with the help of a hand-lens. All *S. japonicus* adults observed in the cages (on all the plants and in the cage walls) were counted. Parasitism was evaluated on six leaflets (containing *T. absoluta* mines) per selected plant. Leaflets were collected and for each leaflet one mine containing a *T. absoluta* larva was dissected subsequently in the laboratory with a binocular microscope to count the number of larvae, eggs and pupae of *S. japonicus*. Thus in total 12 mines containing a *T. absoluta* larva were examined per cage each week.

Resource partitioning

The impact of the resource partitioning along the tomato plant axis was tested under laboratory conditions. Potted tomato plants were covered with a plastic cylinder (d: 15 cm, h: 30 cm) closed on the top with a mesh. Seventeen eggs and ten larvae of *T. absoluta* were deposited on the plants with a paint brush. The three following treatments were set up and tested: (i) eggs on the upper third of the plant larvae on the bottom third of the plant i.e. natural pest instar distribution within the tomato plant (Torres et al. 2011); (ii) the reversed distribution, eggs on the bottom third and larvae on upper third and (iii) mixed eggs and larvae all over the plant. *Tuta absoluta* eggs used were 0 to 12-h old and the larvae were late second and early third instars.

Five *S. japonicus* and one *M. pygmaeus* females were introduced in the cylinder one hour after the larvae to allow the larvae to dig mines. Three days after, natural enemies were removed; mines were collected to assess under a binocular the egg predation, the parasitism and predation of larvae. Fifteen replications were carried out for each treatment.

Statistical analyses

All the statistical analyses were performed using R software (R Development Core Team 2009) with the package *geepack*. In the population dynamics experiment, differences in population dynamics of pests and natural enemies among the treatments were analysed using Generalized Estimating Equations (GEE) adapted to repeated measures over time. A GEE based on Poisson distributed data with a log link function was applied for the numbers of *T. absoluta, M. pygmaeus* and *S. japonicus* imago. For parasitism rate, a binomial distribution was used. For the *T. absoluta* larvae and eggs dynamics, factors tested were the natural enemy combination, the initial release quantity of *T. absoluta* i.e. pest density factor, and the date i.e. time factor. For the *S. japonicus* imago and the parasitism dynamics, factors tested were the predator presence, the initial release quantity of *T. absoluta* and the date. Finally for the predator population dynamics, factors tested were the parasitoid presence and the initial release quantity of *T. absoluta* and the date. In all the equation, the interactions between factors were tested, but they are provided in the results section only when significance and meaning is essential.

Results of the resource partitioning experiment were analysed using a GLM for Poisson data with the treatments as a factor. When necessary, means were separated using a Least Significant Difference post-hoc test (LSD test) for multiple comparisons.

Results

Population dynamics experiment

Each species was found at the end of the experiment in the cages where they were initially released. Neither the natural enemies nor the host/prey went to local extinction, hinting that natural enemy might coexist, at least during the length of the experiment. The number of *S. japonicus* adults in the cages (Fig. 1) was significantly affected by both the predator presence and the date ($\chi^2 = 13.03$, d.f. = 1, $P < 0.001$; $\chi^2 = 82.93$, d.f. = 1, $P < 0.001$, respectively). A huge increase in parasitoid number was observed on week 8, especially in the treatment without predator (see Fig. 1). Whereas, there was no effect of the *T. absoluta* release density on the parasitoid population levels ($\chi^2 = 0.36$, d.f. = 1, $P = 0.548$).

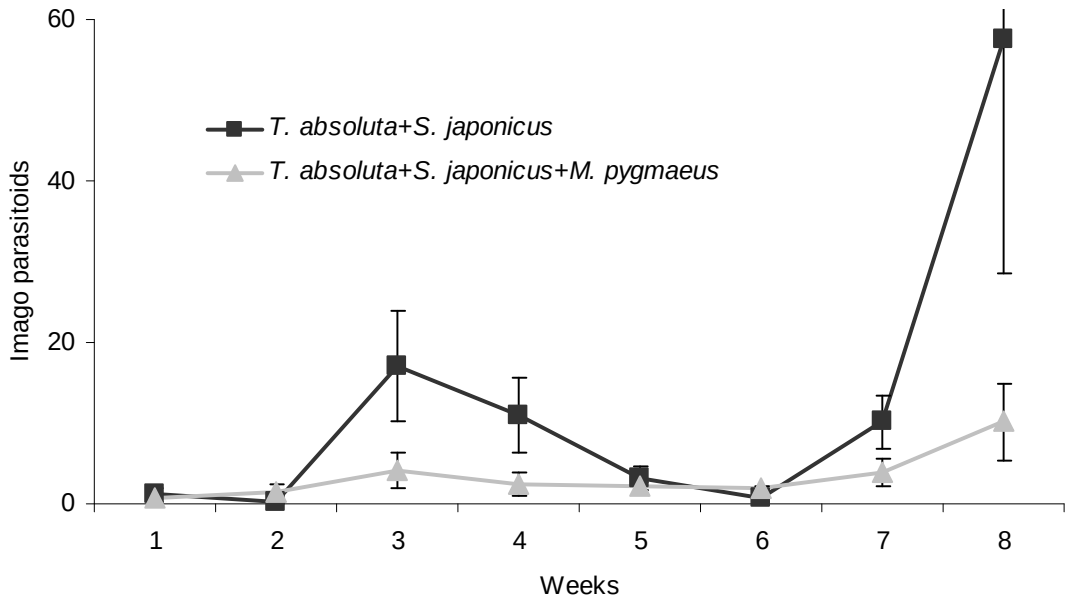


Figure 1: Mean (±SE) number of *S. japonicus* adults during eight weeks when alone with *T. absoluta* or with the concomitant presence of *M. pygmaeus*. The two initial densities of *T. absoluta* are pooled.

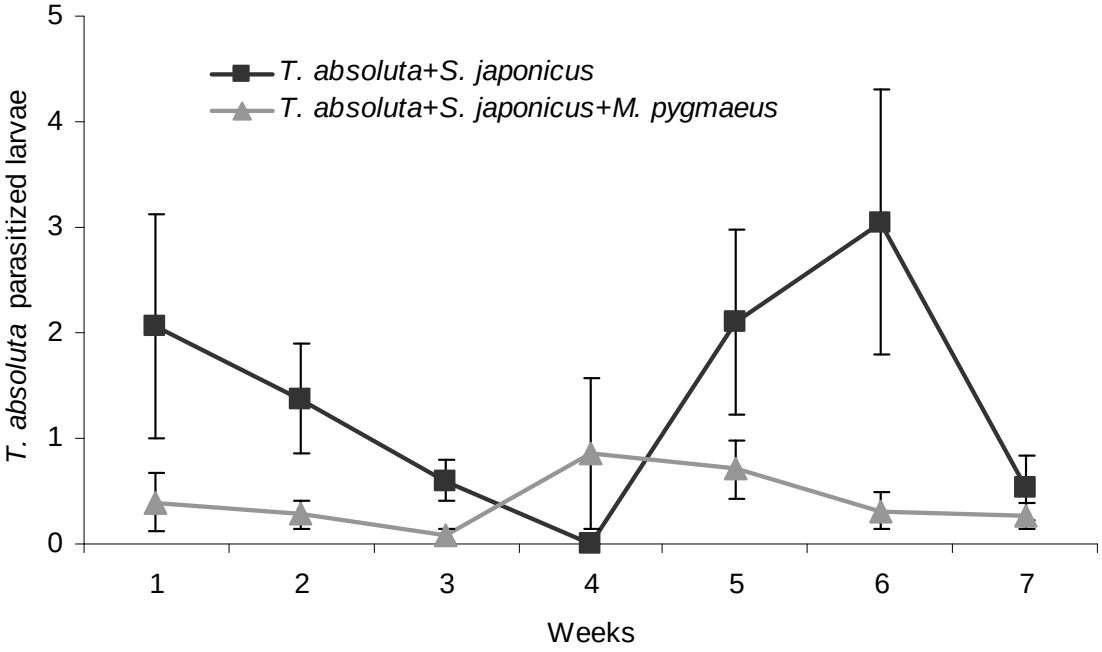


Figure 2: Mean (±SE) number of *T. absoluta* parasitized larvae per leaf (percent parasitism × number of larvae per leaf) during eight weeks in the presence of the larval parasitoid alone or in the concomitant presence of *M. pygmaeus* too. The two initial densities of *T. absoluta* are pooled.

No significant effect of the predator on the parasitism rate (Fig. 2) was observed when the eight weeks were taken into account ($\chi^2 = 0.85$, d.f. = 1, $P = 0.360$). However, when only the first seven weeks were used in the test, the effect of the predator was significant in interaction with the week ($\chi^2 = 2744.37$, d.f. = 5, $P < 0.001$). Whereas, the effect of the week alone was marginally significant ($\chi^2 = 9.89$, d.f. = 5, $P = 0.078$) (Fig. 2). Finally, *T. absoluta* release density had a marginally significant effect on the parasitism ($\chi^2 = 3.64$, d.f. = 4, $P = 0.057$).

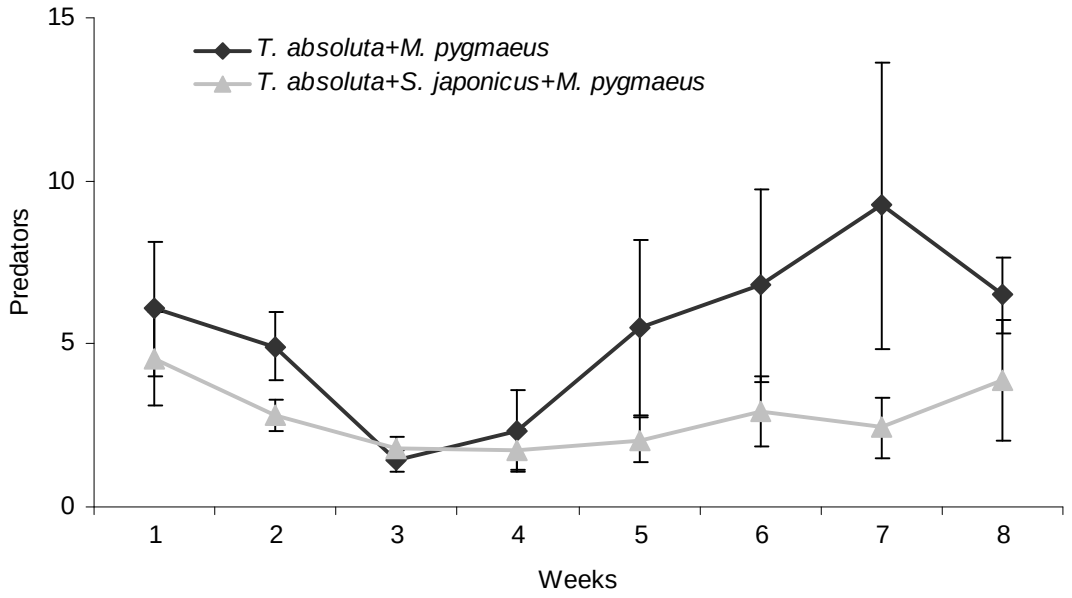


Figure 3: Mean (±SE) number of *M. pygmaeus* during eight weeks under greenhouse conditions when alone with *T. absoluta* or in the presence of *S. japonicus*. The two initial densities of *T. absoluta* are pooled.

Predator numbers were significantly affected by the presence of the larval parasitoid ($\chi^2 = 4.33$, d.f. = 1, $P = 0.037$) and by the date ($\chi^2 = 53.92$, d.f. = 7, $P < 0.001$) (Fig. 3), the mirid population was higher at the end of the experiment and in absence of the parasitoid. *T. absoluta* release density had no effect on their population ($\chi^2 = 0.003$, d.f. = 1, $P = 0.955$).
T. absoluta eggs and larvae (Fig. 4) were significantly affected by the natural enemy combination ($\chi^2 = 14.72$, d.f. = 2, $P < 0.001$; $\chi^2 = 10.19$ d.f. = 2, $P = 0.006$, respectively) and by the date ($\chi^2 = 494.92$, d.f. = 7, $P < 0.001$; $\chi^2 = 94.31$, d.f. = 7, $P < 0.001$, respectively). *T. absoluta* release density had no effect on the egg number ($\chi^2 = 0.24.$, d.f. = 1, $P = 0.621$) and had a marginally significant effect on larva number ($\chi^2 = 2.92$, d.f. = 1, $P = 0.088$).

Overall, less *T. absoluta* eggs and larvae were recorded in the treatment were both natural enemies were released (Fig. 4).

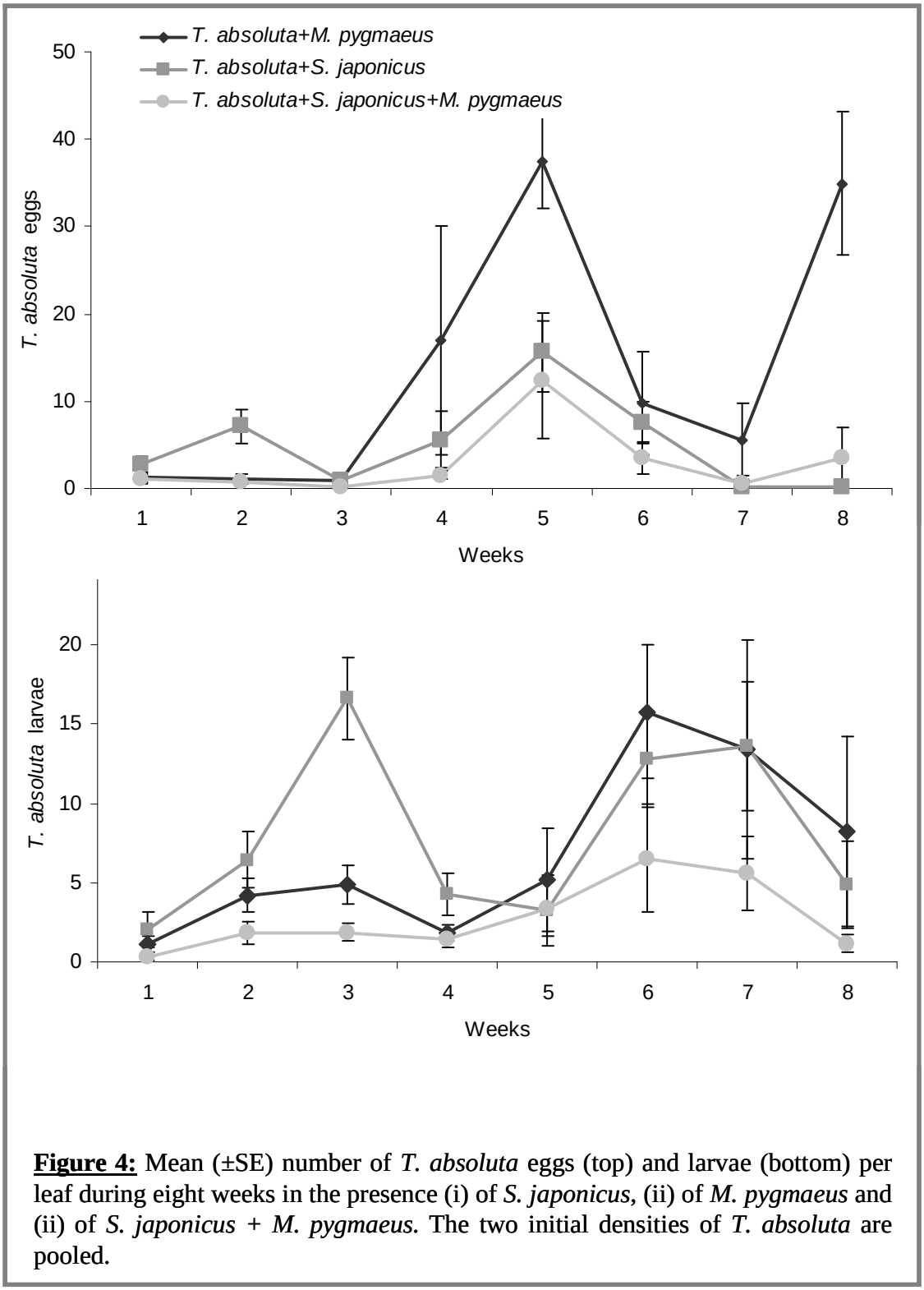


Figure 4: Mean (±SE) number of *T. absoluta* eggs (top) and larvae (bottom) per leaf during eight weeks in the presence (i) of *S. japonicus*, (ii) of *M. pygmaeus* and (ii) of *S. japonicus* + *M. pygmaeus*. The two initial densities of *T. absoluta* are pooled.

Resource partitioning

Although the number of parasitized larvae was marginally significantly affected by the resource distribution ($F_{2, 42} = 2.82$, $P = 0.071$), the highest level of parasitism was obtained for the *mixed* treatment (Fisher's LSD post hoc test: mixed-reversed: Z = 1.89, $P = 0.059$; and natural-mixed Z = -2.09, $P = 0.036$) (Fig. 5). The treatments *natural* and *reversed* showed no difference (Fisher's LSD post hoc test: Z = -0.22 and $P = 0.827$). The numbers of eggs and larvae consumed were not affected by resource distribution ($F_{2, 34} = 2.43$, $P = 0.103$; $F_{2, 42} = 0.38$, $P = 0.684$, respectively) (Fig. 6).

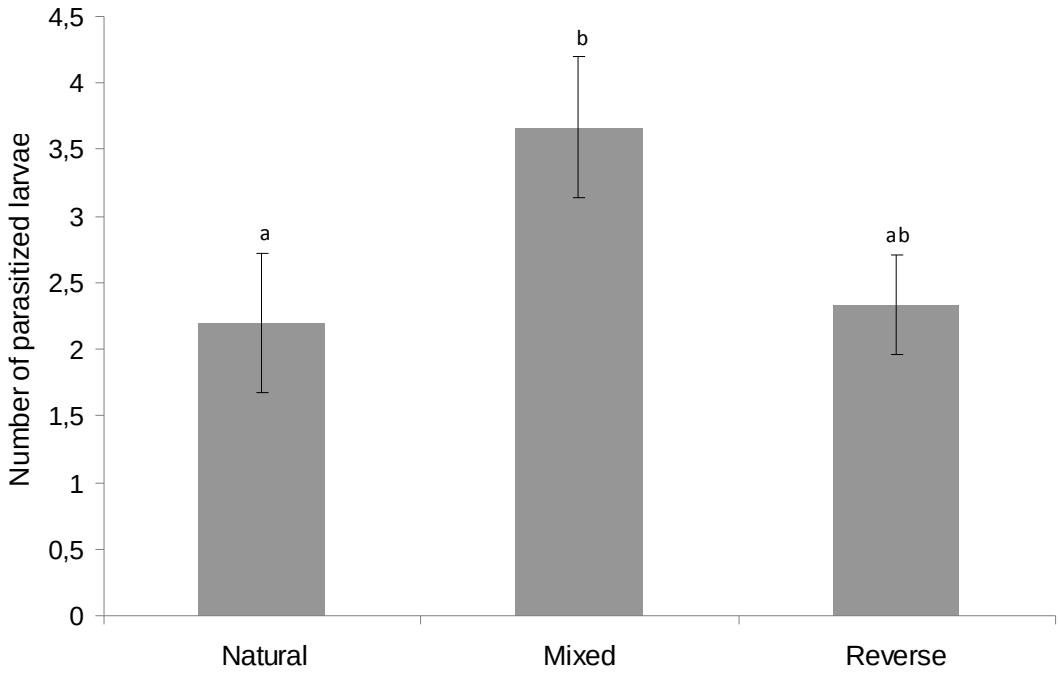


Figure 5: Mean (±SE) number of *T. absoluta* parasitized larvae after three days under laboratory conditions in the three *T. absoluta* stage distributions along the plant axis: (i) when *T. absoluta* eggs were on the upper part of the plant and larvae on bottom part of the plant (i.e. *natural*), (ii) when *T. absoluta* eggs and larvae were mixed all along the plant axis (i.e. *mixed*), or (ii) when *T. absoluta* eggs are on the bottom part of the plant and larvae on upper part of the plant (i.e. *reversed*). Columns bearing the same letter are not significantly different at $P < 0.05$.

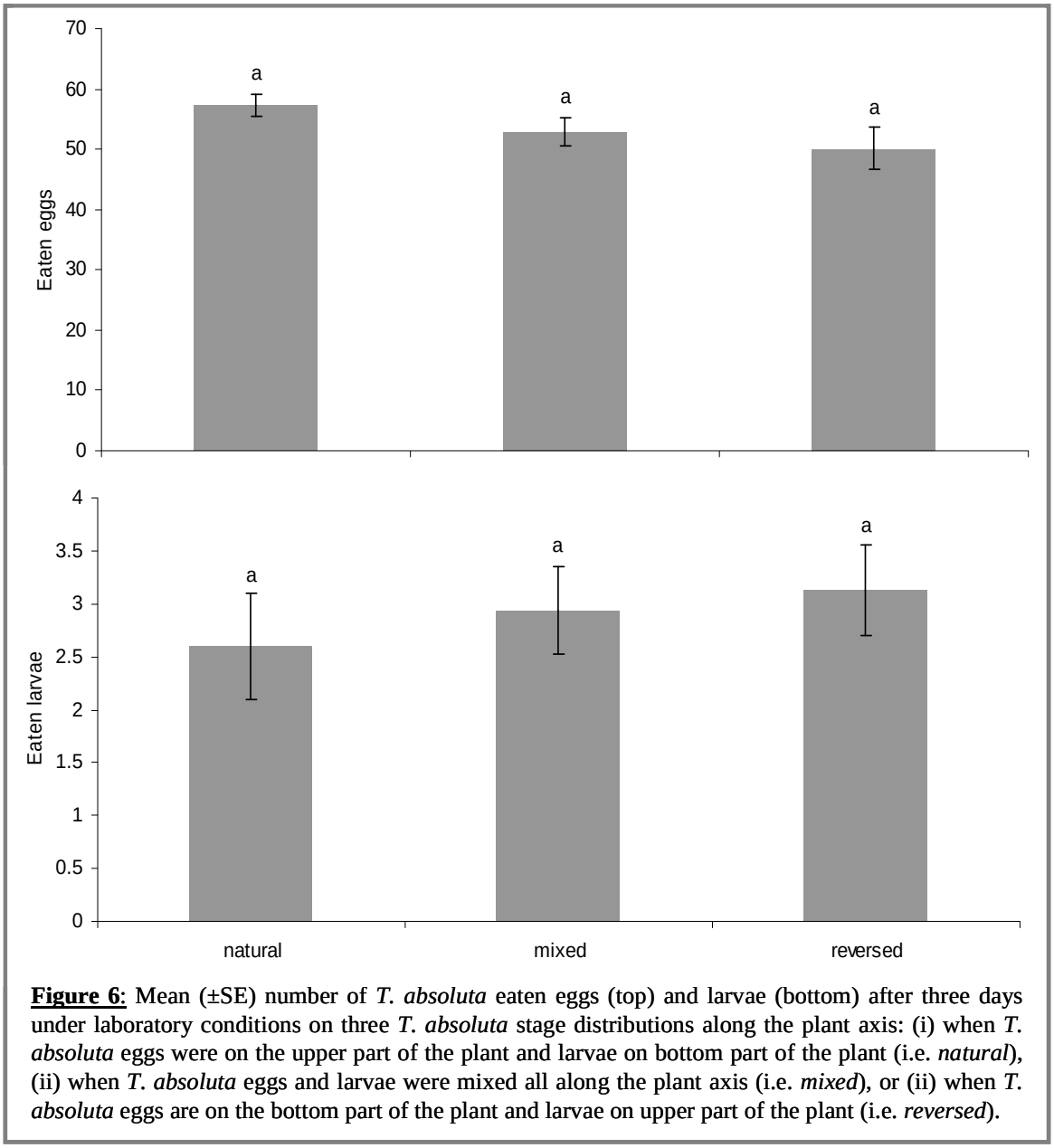


Figure 6: Mean (±SE) number of *T. absoluta* eaten eggs (top) and larvae (bottom) after three days under laboratory conditions on three *T. absoluta* stage distributions along the plant axis: (i) when *T. absoluta* eggs were on the upper part of the plant and larvae on bottom part of the plant (i.e. *natural*), (ii) when *T. absoluta* eggs and larvae were mixed all along the plant axis (i.e. *mixed*), or (ii) when *T. absoluta* eggs are on the bottom part of the plant and larvae on upper part of the plant (i.e. *reversed*).

Discussion

Results showed that coexistence was possible between the two species, despite the asymmetry in their diet breadth and kleptoparasitism: *S. japonicus* and *M. pygmaeus* established successfully in all the treatments in which they were released and were present throughout the whole experiment. On the other hand, for both biocontrol agent species, their population sizes were significantly lower when they were in competition to each other vs. when they were alone. Moreover, the strongest impact on the population of the shared host/prey was observed when the two natural enemies were present together.

Contrary to the results of Bogran et al. (2002), we found no or marginal effects of the *T. absoluta* initial release quantity on the population dynamics. In our experiment, the generalist predator was released before the pest which makes sense from an ecological point of view as the predator is able to feed on the plant. This also mimics preventive releases of generalist predators as usually done by producers (Calvo et al. 2012). Moreover, *M. pygmaeus* has a functional response on *T. absoluta* density (Jaworski et al. in prep.) attacking more prey when more prey are available, this behavior may have lead to an initial regulation of the first pest oviposition that has levelled the two initial densities.

Under the experimental conditions of our study, coexistence was possible and the parasitoid was not excluded, however both natural enemy species suffer a negative interaction in presence of its competitor. Thus, it is likely that some mechanisms probably decreased the strength of the competition (either exploitative competition of the shared resources or kleptoparasitism). Lalonde et al. (1999) suggested that a poor efficiency in foraging for prey of omnivorous may reduce their impact on herbivore populations. This mechanism may have occurred in our experiment and favoured the coexistence of the two natural enemies. Effectively it is known that *M. pygmaeus* spend only few time at foraging for prey, mainly because female spend most of their time on the stem to find suitable oviposition sites (Montserrat et al. 2004). But herbivore prey are scarce on the stem and are usually grouped on the leaves. So, encounter rate between prey and *M. pygmaeus* female may be decreased because of this behavior. Moreover, even if it has been observed that *M. pygmaeus* stay in patches with low level of prey, it exhibited really low level of predation in these patches (Montserrat et al. 2004). This low efficiency in resource use may be a mechanism that promotes coexistence by reducing both exploitative competition and kleptoparasitism. Differences in resource utilisation have been shown to promote coexistence theoretically (Wilson et al. 1999). In practice, Brown et al. (1997) demonstrate the ability of a forager to profitably harvest food at low abundances and to utilize the foraging opportunities left behind by the less efficient forager as a mechanism promoting coexistence.

Then, a second mechanism may reduce the impact of kleptoparasitism. We initially hypothesized that one of the mechanism susceptible to reduce the strength of kleptoparasitism can be resource segregation along the plant axis. If the predator feed preferentially on eggs as demonstrated by Urbaneja et al. (2009), it may stay most of the time where eggs are the most abundant i.e. on upper part of the plant, thus reducing the probability to encounter paralyzed and parasitized larvae. Effectively in real field conditions, owing to the plant growth larvae are generally on the middle part of the plant (Torres et al. 2011). However, our laboratory

experiments did not provide any evidence of such a mechanism and do not allow considering the resource segregation along the plant axis to be effective at reducing kleptoparasitism. We can not exclude that an experiment using taller plant may have shown different results. Nevertheless, segregation was artificially amplified in the aim to detect possible effects of the segregation, which should have counterbalanced the relatively small plant size.

On the other hand, our results outline that the predator also strongly suffered the parasitoid presence. This may arise because of a strong effect of the parasitoid in decreasing the density of the shared resource. Specialists are generally considered to have a higher effect on herbivore populations and to respond better to herbivore population fluctuations than generalists (Snyder and Ives 2003). Moreover, in our study the parasitoid species may have a higher effect on the pest population than the predator because it attacks later pest stages that are more likely to reach the reproductive stages successfully. While a portion of eggs killed by the predator may have died because of natural mortality or climate (Miranda et al. 1998). Similar hypotheses have also been formulated to explain the superiority of a parasitoid preferring the bigger host thus removing the individuals with the higher reproductive value (Lin and Ives 2003).

In terms of pest biological control, the pest population dynamics resulted from each of the two natural enemy presences differed. As a specialist, *S. japonicus* exerted a less efficient suppression of *T. absoluta* growth early, with stronger suppression becoming visible thereafter. In contrast, the omnivorous predator caused an immediate decrease in the pest population growth rate but provide a bad control later (Snyder and Ives 2003). Parasitoid characteristics i.e. short life cycle and specificity, can allow them to mount a strong numerical response when prey outbreaks occur, perhaps leading to outbreak suppression (Hassell 1980, Hassell and May 1986, Berryman 1992, Murdoch 1994, Turchin et al. 1999). On the contrary, omnivorous predators have a longer generation time than herbivores, thus, even if there is a numerical response to changes in the density of a single herbivore species (e.g. Symondson et al. 2002), the response is unlikely to occur quickly enough to lead to outbreak suppression (Hassell and May 1986, DeBach and Rosen 1991). However, their ability to appear in the crop before the pest ensures an immediate control (Snyder and Ives 2003, Calvo et al. 2012). When both the parasitoid and the omnivorous predator were present, *T. absoluta* dynamics reflected the impacts of both types of natural enemy: initial pest population increase was similar to the treatment with the predator alone; whereas, pest densities peaked at levels close to the one of the treatment with the parasitoid alone (Snyder and Ives 2003). This study corroborates other experimental studies that support the idea that intraguild predation, even

when reducing the intraguild prey population, do not erase the pest control efficiency (e.g. Heinz and Nelson 1996, Snyder and Ives 2003, Bilu and Coll 2007, Messelink et al. 2013). Therefore, from a practical point of view, this study suggests that efficient biological control programmes can be based on joint inoculative releases of an omnivorous and a specialist natural enemy that are complementary. We also provided promising results for the development of biological control programmes against *T. absoluta* that rely on endemic biocontrol agent.

The present work provides an example of the outcome when various parameters regulating population dynamics occur simultaneously. The parasitoid, suffering both exploitative competition and kleptoparasitism, was able to survive in the presence of the omnivorous predator, and also reduce the population dynamics of the omnivorous predator. Thus, in our biological model and experimental conditions, omnivory do not lead to the specialist exclusion and parameters favoring coexistence seem to outweigh the omnivory negative effects.

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Article 10

Effects of an alternative prey on the survival of a specialist parasitoid when competing with a generalist predator

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Effects of an alternative prey on the survival of a specialist parasitoid when competing with a generalist predator

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Abstract

The impact of diet breadth on competitor coexistence is puzzling because different diet breadths occur in shared ecosystems. Moreover, generalist predators are frequently engaged in intraguild predation with their competitors. Some species may be able to coexist when sharing a resource despite competition, but adding an alternative prey for one of the two competitors in a given system may actually disrupt coexistence. By manipulating species association on cropped plants (tomato), we aimed at providing an empirical study of the effect of an alternative prey on the coexistence of a generalist predator with a more specialized natural enemy, a parasitoid. Our results showed that the specialist was not excluded from the system when an alternative prey was added, and that the coexistence of the four species (the pest, the alternative prey and the two natural enemies) was possible. In addition to resource competition between the two natural enemies, we observed commensalism and apparent amensalism in the agro-ecosystem. The possible mechanisms that may have favored coexistence are discussed.

Keywords: coexistence, diet breadth, interspecific interaction, Kleptoparasitism, *Tuta absoluta*, *Stenomesius japonicus*, *Macrolophus pygmaeus.*

Introduction

Population dynamics of various species co-occurring in ecosystems are regulated through many direct and indirect interactions (Paine 1980, Wootton 1994). How species coexist when competing for a shared resource is a key-question in ecology (Chase and Leibold 2003, Finke and Snyder 2008). Among the mechanisms that may reduce interspecific competition and thus favoring coexistence of two competitors sharing a same resource, a difference in diet breadth have been shown to promote coexistence (Miller 1967, Schoener 1974, Belovsky 1986, Hassell and May 1986). However, species sharing resources frequently show asymmetric diet breadths, e.g. one has a restricted diet breadth (specialist) and the other species a large one (generalist or even omnivore). What are the conditions that enable such species to coexist is unclear in most systems, notably because in most cases, only one competitor has access to an exclusive resource, and secondly because generalists can behave as intraguild predators by feeding directly on competitors (Schmidt et al. 1998). Theoretical studies by Daugherty et al. (2007) and Holt and Huxel (2007) have suggested that diet breadth enrichment of the intraguild predator could increase the risk of exclusion of a specialist natural enemy (the intraguild prey). However, empirical studies testing this hypothesis are lacking yet.

Theoretical and empirical studies have described possible effects of alternative prey in predator-prey system. In such systems, predator-mediated interactions occur between the two prey (Harmon and Andow 2004). Short term effect could be a reduction of predation pressure on the two prey sharing the predator (dilution effect) (Abrams and Matsuda 1996) i.e. apparent mutualism (++) (Wootton 1994). Such short term interactions have been reported in manipulative greenhouse experiments by Van Maanen et al. (2012) and Bompard et al. (2013). At a long term scale i.e. higher than predator generation time, the predation on the two pests may increase owing to a higher predator numerical response to increased prey availability in comparison with unique prey systems (Holt and Lawton 1994, Messelink et al. 2008, Jaworski et al. in prep.). This leads to a negative indirect interaction between the prey i.e. apparent competition. Such interactions may help to predict the consequences of adding an alternative prey in a given system when involving generalist and specialist natural enemies that share a common prey. At a short time scale, the competition between natural enemies might be reduced by the addition of the alternative prey. In contrast, there might be risk of exclusion of the specialized natural enemy after the predator numerical response (at a long term scale) if the increase of the generalist predator density leads to an increase of the competition strength exerts by this one on the specialist.

Mechanisms may reduce risk of exclusion of specialist natural enemies: (i) a preference of the generalist predator for the alternative prey, or (ii) if specialist natural enemies also benefit from alternative prey. Generalist predators often exhibit preference for some prey (Eubanks and Denno 2000, Meyling et al. 2003, Reitz et al. 2006) and such preference may be dependent of prey density i.e. switching behavior (Murdoch and Oaten 1975). Predator preference may decrease both resource competition and intraguild predation. Switching behavior is not a strict preference, it happens when the predator attack the most abundant species more than hazard encounter rate and this may also promote coexistence of natural enemies i.e. generalist predator may switch to the alternative prey when the density of the shared prey is low (thus leaving this later for the specialist natural enemy). Second, it is also possible that the specialist obtains a direct benefit from the alternative prey presence. Specifically, in agro-ecosystem, many prey are sap feeders and produce honeydew, and parasitoid imago activity is enhanced by sugar sources as nectar and honeydew (e.g. Wackers 2001). Thus if the alternative prey is a species that produce honeydew, the parasitoid would gain a benefit from the alternative prey presence, even without feeding directly on it.

Under field conditions (greenhouse), we studied the impact of an alternative prey on coexistence of a specialist parasitoid with a generalist natural enemy when sharing a common prey. The invasive pest *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is attacked by various indigenous natural enemies (Urbaneja et al. 2012), notably the omnivorous predator *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) and the larval parasitoid *Stenomesius japonicus* Ashamed (Hymenoptera: Eulophidae). *Macrolophus pygmaeus* is omnivorous and generalist i.e. it is able to feed on the plant (sap, pollen and nectar) as well on various herbivores pest species. It is used in biological control in Europe mainly against whiteflies but it is also able to eat *T. absoluta* eggs and young larvae (Urbaneja et al. 2009). This predator has been shown to be engaged in a direct interaction (kleptoparasitism) with the larval parasitoid *S. japonicus* (Chailleux et al. in prep.a), which preferentially attacked *T. absoluta* third larvae instar (Chailleux et al. submitted). *Macrolophus pygmaeus* feed on parasitized *T. absoluta* larvae, and food-probing events ultimately lead to the death of the parasitoid juvenile through starvation before reaching the pupae stage. So, this is indistinguishable from intraguild predation in its impact on the parasitoid population dynamics. However these two species were able to coexist, in cages experiment when no alternative prey was present in the system (Chailleux et al. in prep.b).

In this study we assessed the impact of adding an alternative prey in a *generalist predator-specialist parasitoid-shared prey* system on possible coexistence of the two natural

enemies in the long term (one cropping season). We also evaluated potential benefits of the alternative prey presence on the two natural enemies in laboratory, on longevity and fecundity, to assess possible mechanisms promoting coexistence.

Materials and Methods

Biological materials

The plants used in the laboratory experiments were five-week old tomato plants, the one used for the greenhouse experiment were four-week old tomato plants, *Solanum lycopersicum* L. cv. Marmande. They were grown in climatic chambers (24 ± 1°C, HR: 65%, photoperiod 16L:8D) with a nutrient solution applied daily.

A colony of *T. absoluta* was initiated using greenhouse-collected individuals (n = 190) in July 2009 (INRA, Alenya, France). The colony was reared in cages containing tomato plants and adult moths were fed on honey placed on one wall inside the rearing cages. *T. vaporariorum* colony was initiated with greenhouse-collected individuals (n = 100) that were subsequently reared on tobacco plants. The predator *M. pygmaeus* was provided by Biotop (Valbonne, France) and it was reared for at least one generation on tobacco with *Ephestia kuehniella* UV-sterilized eggs as food. Finally, the parasitoid *S. japonicus* was reared on tomato plants infested by *T. absoluta* larvae, and adults were fed with honey droplets placed on the plants inside the cages. Rearing was initiated using individuals collected in Spain and France (n = 10 and n = 5, respectively) in greenhouses. All insects were reared in climatic chamber (25 ± 1°C, RH 70 ± 10%, 16L:8D) in cages (55×75×80 cm).

Greenhouse experiment

Experimental setup

The experiment was conducted in four greenhouse compartments located at INRA Sophia-Antipolis (Southern France). Each compartment contained four double lines of 16 tomato plants grown on mineral-wool under hydroponic conditions. Mesh nets were placed on each double line to create four isolated groups of 16 plants i.e. tunnels (L: 5m, H: 2m, W: 1m), per greenhouse compartment. Within tunnels, plants were attached vertically on wires. Temperature and humidity were regulated with fog, cooling fan, shade and openings with

25°C as target (min < mean temperature < max: 14.2°C < 22.8°C < 34.1°C; min < mean RH < max, 32% < 79.7% < 99%; with natural ambient light: May-July 2012).

We evaluated possible coexistence of the two natural enemies and possible impact of the alternative prey *T. vaporariorum* through a 2 x 2 factorial design, in which the parasitoid *S. japonicus* was present in all treatments. The first two-level factor consisted of the presence or absence of the generalist predator *M. pygmaeus*. The second two-level factor consisted of the presence or absence of the alternative prey *T. vaporariorum*. These two factors were fully crossed which resulted in four possible combinations (all with the parasitoid and *T. absoluta*): *T. absoluta + S. japonicus, T. absoluta + S. japonicus + T. vaporariorum, T. absoluta + S. japonicus + M. pygmaeus* and *T. absoluta + S. japonicus + T. vaporariorum + M. pygmaeus*. Every treatment had four replicates, one per compartment. The replicates were distributed randomly inside each greenhouse compartment.

Insects were released homogeneously along the tunnel. Each species were released twice: a first initial release and a second release at the half of the generation time (two weeks interval for mirid bug, whiteflies and *T. absoluta* and one week interval for *S. japonicus*) to avoid cycling populations due to the release methods. *Macrolophus pygmaeus* were released first (two pairs of one male and one female per plant in total), two weeks before the prey, and UV-sterilized eggs of *E. kuehniella* were put on the plant in the same time to promote establishment of the predator in the tomato crop (as advised by biocontrol agent producers). The densities of prey chosen for releases were representative of heavy infestations by the two prey (two pairs of one male and one female per plant in total for *T. absoluta*, and 20 individuals per plant for *T. vaporariorum*). This situation was selected to ensure getting rapidly high prey populations so we could detect coexistence or extinction of the insects studied. The parasitoid *S. japonicus* was released once *T. absoluta* larvae had reached L2/L3 stage i.e. the ideal stage for parasitoid offspring production (two pairs of one male and one female per plant). Releases started on April 10th and the last one (*S. japonicus*) was done on May 22nd.

Monitoring and Sampling

Monitoring started on May 24th (week 1) and plants were checked weekly for 11 weeks. For each tunnel four plants were randomly selected every week. The generalist predator, *M. pygmaeus,* adults and nymphs were recorded on the entire plant. The larvae of *T. absoluta* were monitored on six leaves per plant (24 leaves per tunnel); two leaves selected randomly from the upper part of each plant, two in the middle and two in the bottom. Larvae of *T.*

absoluta were observed by shining a torch lamp under the leaves to see through the mines. *Tuta absoluta* eggs were observed with a hand-lens on the top four leaves (young leaves are preferred ovipositing site for *T. absoluta*). Larvae and eggs of *T. vaporariorum* were surveyed on six leaflets taken randomly from the upper, middle and bottom parts of plants and they were counted in the laboratory using a binocular microscope. All *S. japonicus* adults observed in the tunnel during surveys were counted, and six leaflets per plant containing *T. absoluta* mines were collected. Mines were then dissected and observed using a binocular microscope and larvae, eggs and pupae of *S. japonicus* were counted.

Laboratory experiments: effect of the alternative prey on fitness of the natural enemies

We evaluated the effect of whitefly honeydew on longevity and fecundity of *S. japonicus* females, of *M. pygmaeus* females and on the longevity of *M. pygmaeus* males. For *S. japonicus* females, treatments tested consisted of *S. japonicus* + *T. absoluta*, provided or not with the alternative prey *T. vaporariorum*. Tests were carried out in plastic boxes containing leaves of tomato plants, with the stems kept in water (see Biondi et al. 2012 for a thorough description of the design) i.e. arenas. Two-day old *S. japonicus* females were placed individually with a male in the arena (males were replaced when found dead during the time span of the experiment). Arenas contained eight *T. absoluta* L2/L3 larvae placed on a tomato leaf. After three days, the tomato leaf and *T. absoluta* larvae were removed and a new tomato leaf + *T. absoluta* larvae were provided. Each leaf was dissected under a binocular microscope and parasitized larvae and parasitoid offspring were counted. For the treatment containing *T. vaporariorum*, a tomato leaf previously infested with whitefly larvae was added in the boxes.

A similar design was used for trials with *M. pygmaeus*. Two-day old predator females were placed individually for three days together with a male before the beginning of the experiment (no male were present with females during the experiment because they also feed on eggs, thus they were tested separately). Females were transferred individually into plastic boxes containing either 20 *T. absoluta* eggs or 20 whitefly larvae (preferred instars for each prey species) depending on the treatment, per day. As males are also predators of these species (in contrast to parasitoid males), the effect of the prey species on their longevity was also studied thanks to the same treatments. For each treatment, 6-10 repetitions were carried out.

Data analyses

All statistical analyses were performed using R software (R Development Core Team 2009) with the packages *geepack*. For the greenhouse experiment, differences in population dynamics of pests and natural enemies among the treatments were analysed using Generalized Estimating Equations (GEE) adapted to repeated measure over time. The effect of the compartment was tested and was never significant. A GEE based on Poisson distributed data with a log link function were applied for the numbers of *S. japonicus* imagos, *T. absoluta* eggs and larvae, *T. vaporariorum* juveniles (eggs and larvae pooled) and *M. pygmaeus* (all stages grouped). For the parasitism rate, a binomial distribution was used. For *S. japonicus* adult population dynamic, parasitism rate, *T. absoluta* eggs and larvae, the factors tested were whitefly presence, predator presence, date and greenhouse compartment. For *M. pygmaeus* population dynamic, factors tested were whitefly presence, date and greenhouse compartment. For whitefly population dynamic, we tested the factors *M. pygmaeus* presence, date and compartment. Main interactions among factors were also tested.

Longevity was analysed using a log rank test with the package *survival* and the fecundity (total number of offspring per female) was analysed using a GLM (Generalized Linear Model) designed for Poisson distributed data.

Greenhouse experiment

Effect of the alternative prey T. vaporariorum and the predator M. pygmaeus on S. japonicus

Stenomesius japonicus established in all treatments and was present throughout the duration of the experiment (Fig. 1). Parasitoid densities increased in all treatments by week 6; however populations increased more rapidly in treatments with *T. vaporariorum* and without predator (140 individuals on week 7). In the other treatments, where only *T. absoluta* was present as herbivore, parasitoid population peaked with a 3-week lag. Population dynamic of *S. japonicus* adults was affected by both *M. pygmaeus* and *T. vaporariorum*, though only marginally significant ($\chi^2 = 3.4$, d.f. = 1, $P = 0.066$; $\chi^2 = 3.2$, d.f. = 1, $P = 0.072$, respectively), and by the date ($\chi^2 = 59.3$, d.f. = 1, $P < 0.001$). Moreover the interactions of *M. pygmaeus* presence and *T. vaporariorum* presence with the date factor were significant (*M. pygmaeus** date: $\chi^2 = 10.2$, d.f. = 1, $P = 0.043$; and *T. vaporariorum**date: $\chi^2 = 3.2$, d.f. = 1, $P = 0.001$). *S. japonicus* populations were largely lower when the generalist predator was present, with an average of 18 ± 4 and 35 ± 6 *S. japonicus* adults per tunnel with and without *M. pygmaeus*,

respectively. In contrast, *T. vaporariorum* had a slight positive effect on the number of *S. japonicus*, though this effect was clear mostly for the treatments with no predator, and not significant overall (Fig. 1). However, there was no significant interaction between *M. pygmaeus* and *T. vaporariorum* factors on *S. japonicus* population (χ^2 = 1.8, d.f. = 1, P = 0.175).

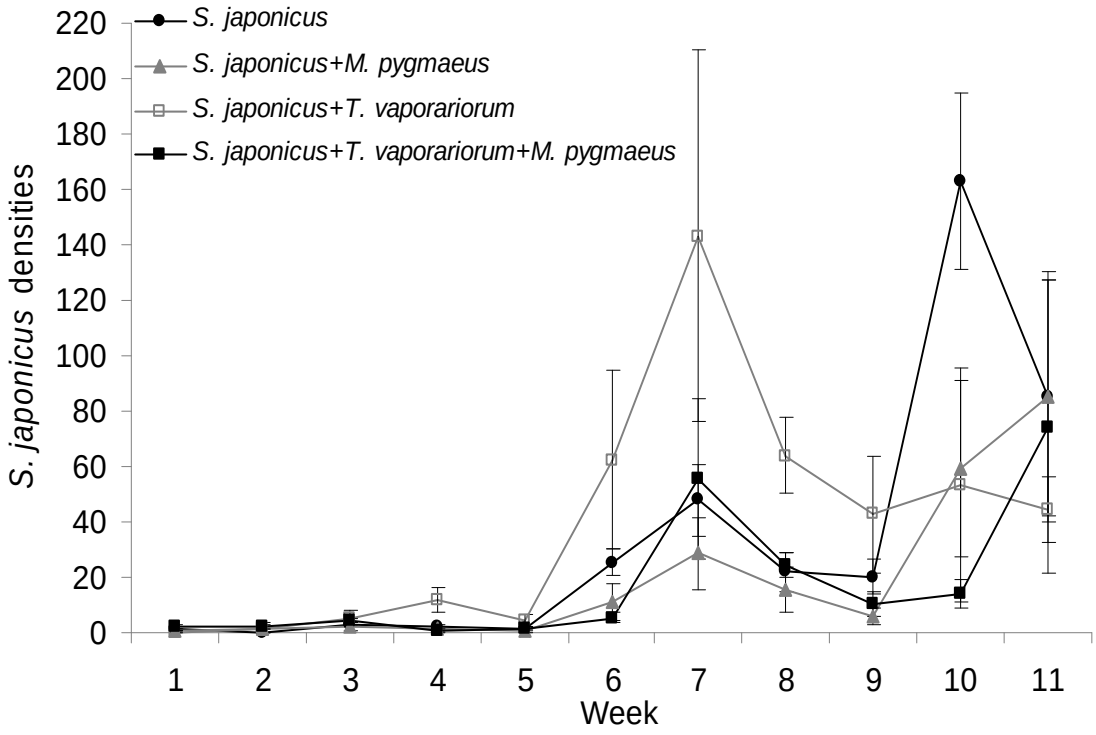


Figure 1: Mean (± SE) of the parasitoid *Stenomesius japonicus* imagos per tunnel during an 11-week greenhouse experiment. The four treatments tested involved *S. japonicus*, and *T. absoluta* was also present in all treatments.

Larvae of *T. absoluta* were parasitized by *S. japonicus* in all treatments, with the highest rates of parasitism recorded in treatments with *T. vaporariorum* alone (double peak to 40% parasitism in week 5 and 9, Fig. 2). The effect of *T. vaporariorum* on parasitism by *S. japonicus* was significant (χ^2 = 30.39, d.f. = 1, P < 0.001) and the lowest parasitism rates were observed in treatments with the predator alone (5% parasitism). The *M. pygmaeus* presence factor had a marginally significant effect on parasitism rate of *T. absoluta* by *S. japonicus* (χ^2 = 2.81, d.f. = 1, P = 0.094). Treatments with a combination of both natural enemies and both prey varied for rates of parasitism recorded through time with a peak to 20% in week five and in week ten (Fig. 2). The date factor had a marginally significant effect on the parasitism (χ^2 = 2.87, d.f. = 1, P = 0.090).

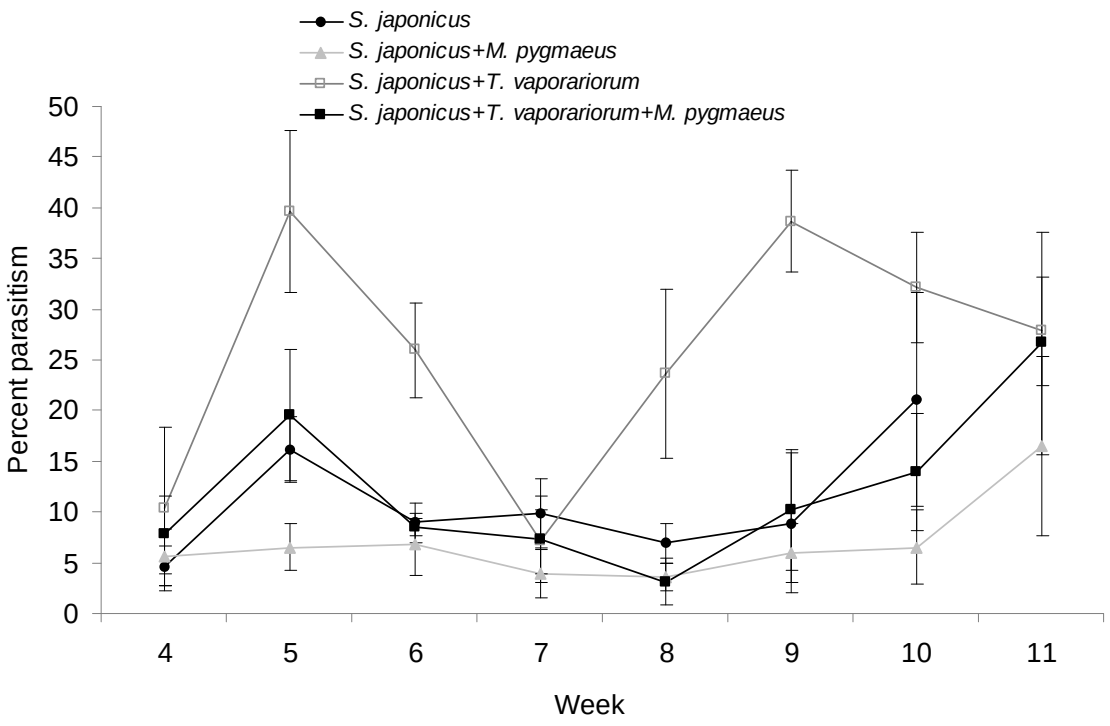


Figure 2: Mean (± SE) percentage of parasitized *T. absoluta* larvae during an 11-week greenhouse experiment. The four treatments tested involved *S. japonicus*, and *T. absoluta* was also present in all treatments.

Effect of T. vaporariorum and M. pygmaeus on T. absoluta

The generalist predator had a significant effect on *T. absoluta* eggs ($\chi^2 = 3.97$, d.f. = 1, $P = 0.046$) but not *T. absoluta* larvae ($\chi^2 = 2.39$, d.f. = 1, $P = 0.122$), and *T. vaporariorum* affected significantly both eggs and larvae ($\chi^2 = 27.99$, d.f. = 1, $P < 0.001$; $\chi^2 = 27.83$, d.f. = 1, $P < 0.001$, respectively). Lowest densities were observed when the whitefly was present (Fig. 3). The factors *M. pygmaeus* and *T. vaporariorum* did not interact significantly (*M. pygmaeus***T. vaporariorum* $\chi^2 = 0.32$, d.f. = 1, $P = 0.573$; $\chi^2 = 0.24$, d.f. = 1, $P = 0.625$, for eggs and larvae, respectively). *T. absoluta* larvae densities peaked in week 8, as high as 60 ± 19 larvae per leaf in the treatments where only *S. japonicus* was present. *T. absoluta* densities dropped by week 9 in all treatments, whereas densities of generalist predator and parasitoid increased. Variations among the weeks were significant ($\chi^2 = 28.49$, d.f. = 1, $P < 0.001$, $\chi^2 = 22.03$, d.f. = 1, $P < 0.001$ for eggs and larvae, respectively).

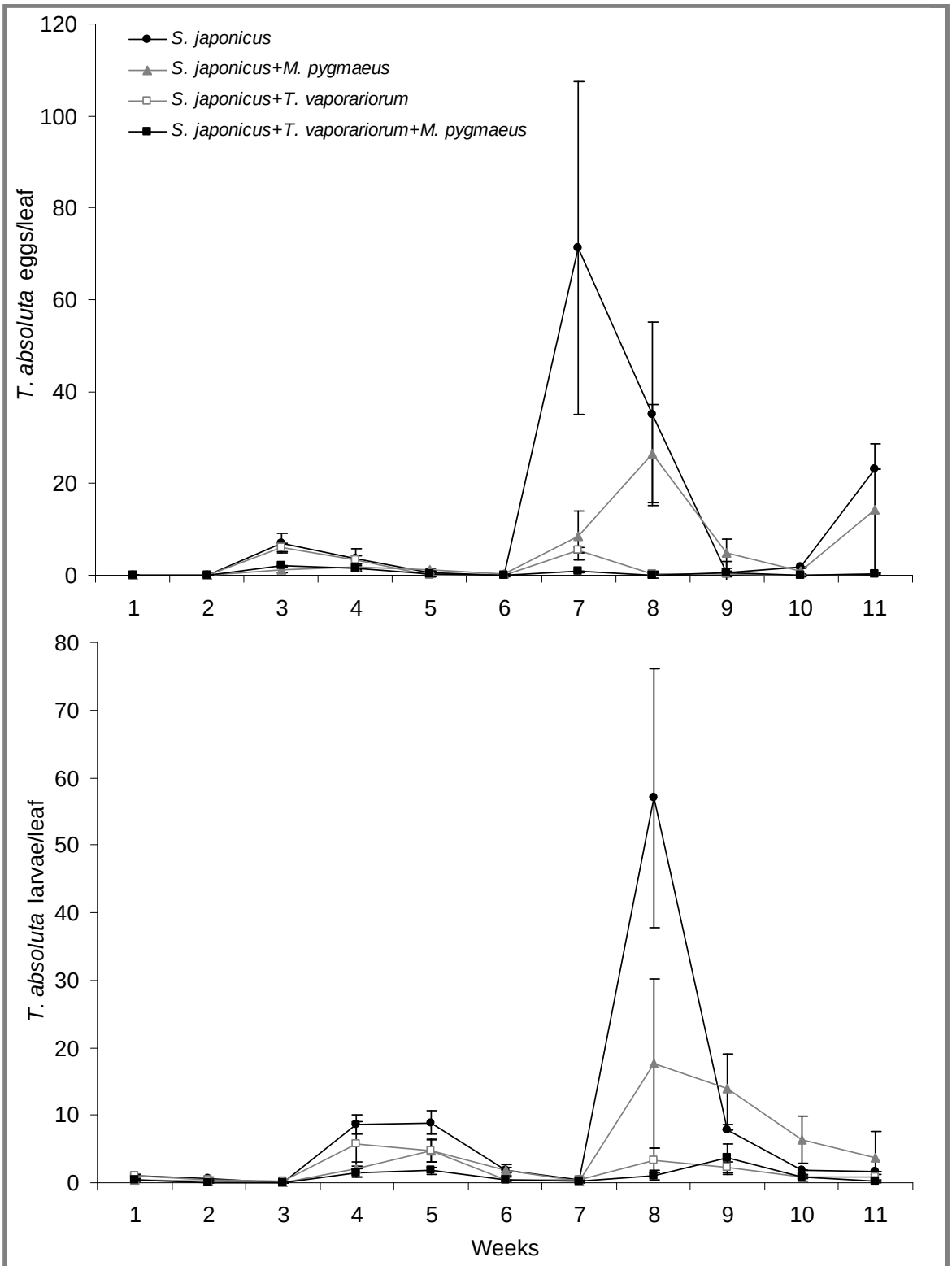


Figure 3: Dynamics (mean ± SE) of the shared prey, *T. absoluta*, eggs (top) and larvae (bottom) per leaf during an 11-week greenhouse experiment. The four treatments tested involved *T. absoluta*.

Effects between M. pygmaeus on T. vaporariorum

Macrolophus pygmaeus had a significantly negative effect on whiteflies (χ^2= 141.9, d.f. = 1, $P < 0.001$) and effect varied as function of dates (significant interaction *M. pygmaeus**date: χ^2 = 5.7; d.f. = 1, $P = 0.017$). The predator reduced whitefly densities (46-fold difference when compare to groups without the predator) during the course of the experiment (Fig. 4) and *T. vaporariorum* populations significantly varied among dates (χ^2 = 128.6, d.f. = 1, $P < 0.001$).

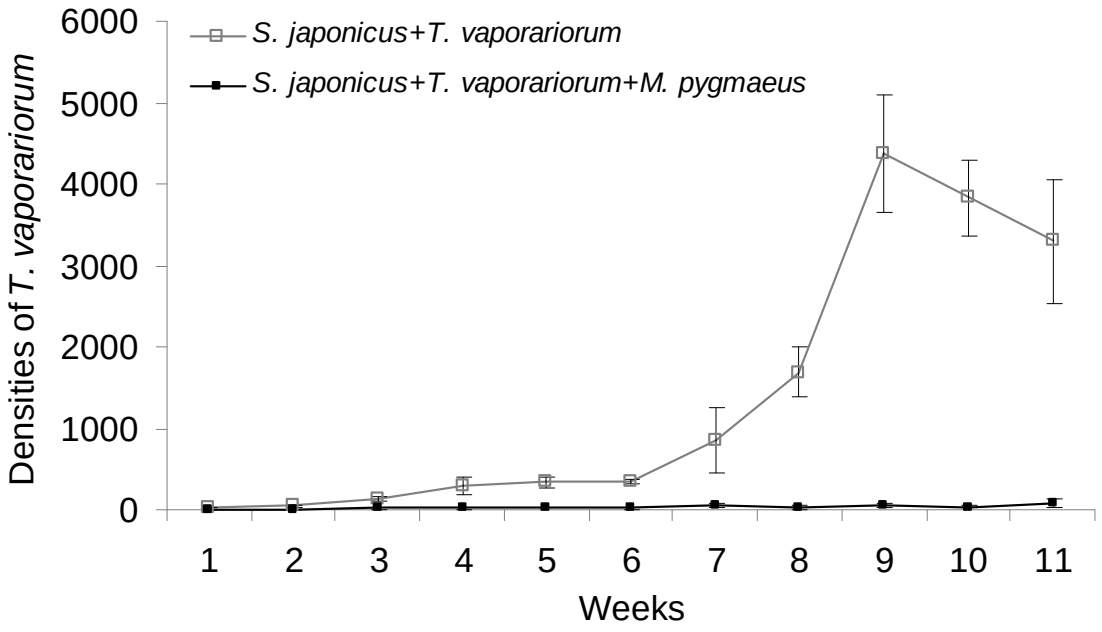


Figure 4: Mean (± SE) of the alternative prey, the whitefly *T. vaporariorum*, per leaflet during an 11-week greenhouse experiment. Two of the four treatments tested involved *T. vaporariorum*, and *T. absoluta* was present in all treatments.

The presence of *T. vaporariorum* had a significant positive effect on the densities of *M. pygmaeus* (χ^2 = 12.69, d.f. = 1, $P < 0.001$) and the interaction between whitefly and date factors were not significant ($\chi^2 < 0.001$, d.f. = 1, $P = 0.993$). Densities of *M. pygmaeus* were higher in the treatments with *T. vaporariorum* (9.8 ± 0.8 vs. 6.3 ± 0.6 *M. pygmaeus* per plant per tunnel) and predator populations showed steadily increase in numbers in week 7 (week factor: χ^2 = 5.79, d.f. = 1, $P = 0.016$) whereas predator densities remained relatively constant in absence of *T. vaporariorum* (Fig. 5).

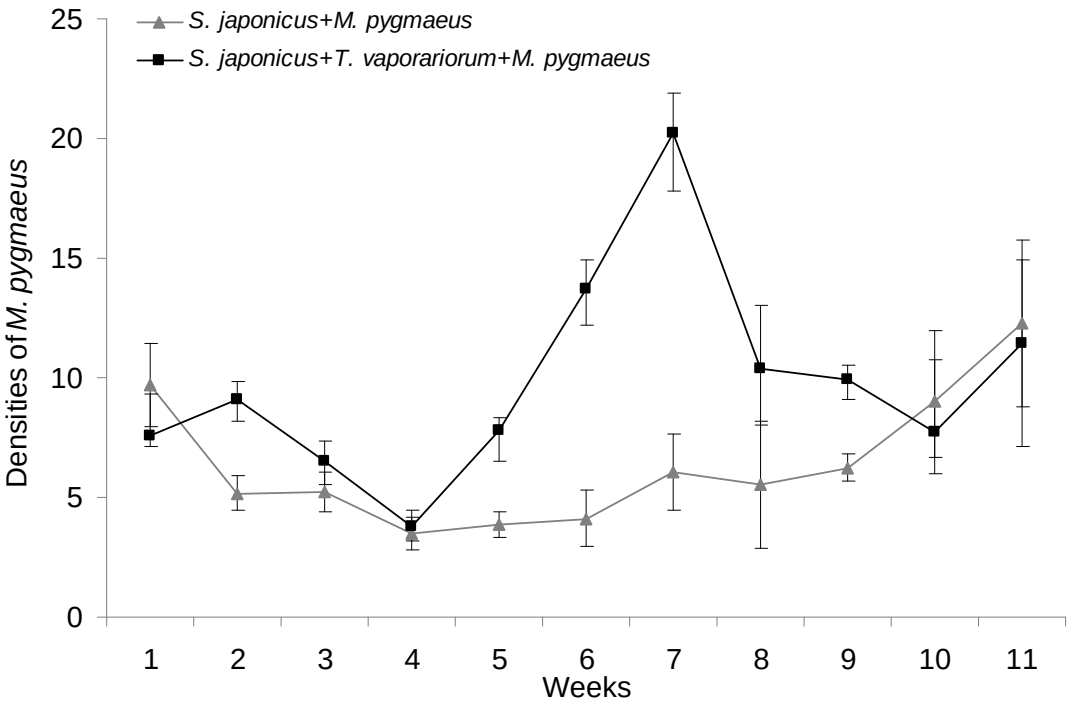


Figure 5: Mean (± SE) of the generalist predator, *M. pygmaeus*, per plant during an 11-week greenhouse experiment. Two of the four treatments tested involved *M. pygmaeus*, and *T. absoluta* was present in all treatments.

Laboratory experiments: effect of the alternative prey on fitness of the natural enemies

Stenomesius japonicus

The presence of whiteflies had no significant effect on the parasitoid longevity ($\chi^2 = 0.06$; d.f. = 1, $P = 0.805$). Females lived 17.4 days ± 1.55 with whiteflies and 18.7 days ± 2.82 without. In contrast, the presence of whiteflies slightly increase the parasitoid fecundity (total ovipostion per female: 5.65 eggs ± 2.30 with whiteflies, 3.2 eggs ± 0.63 without), though it was only marginally significant ($F_{1,14} = 3.47$; $P = 0.083$).

Macrolophus pygmaeus

The prey species i.e. witheflies larvea or *T. absoluta* eggs, had a significant effect on predator longevity ($\chi^2 = 5.6132$; d.f. = 1, $P = 0.0178$) and sex factor was significant i.e. longevity differed between males and females ($\chi^2 = 7.2551$; d.f. = 1, $P = 0.0071$). In addition the interaction between the two factors (prey species**M. pygmaeus* sex) was significant, hinting that the effect of whitefly on predator longevity varied between the sexes (prey species**M. pygmaeus* sex : $\chi^2 = 4.3429$; d.f. = 1, $P = 0.0372$). In fact, only males longevity increased

owing to availability of whiteflies. Effectively, females and males lived 22.0 days ± 4.30 and 28.4 ± 5.52 respectivelly with *T. absoluta* eggs, and 21.7 ± 3.08 and 61.7 ± 9.29 respectivelly with whiteflies. Moreover the effect of prey species on the fecundity was also significant ($F_{1,14}$ = 6.1236; P = 0.0267). Total ovipostion per female was 15.75 eggs ± 4.55 with *T. absoluta* and 48.12 eggs ± 14.38 with whitefly larvae.

Discussion

The four species were able to coexist for the time span of our experiment i.e. 3 months, and the alternative prey did not promote exclusion of the specialist natural enemy (the parasitoid *S. japonicus*). The generalist predator populations were enhanced owing to the presence of the alternative prey (whitefly). However, the specialist parasitoid seemed benefiting from alternative prey presence in the absence of the generalist predator; the highest parasitoid density was obtained 3 weeks earlier in the treatment with the alternative prey vs. in the one without the whitefly. This beneficial effect of whitefly on the specialist parasitoid was actually more marked on parasitism rates recorded.

Our laboratory experiment hinted the occurrence of a commensalism interaction (+0) between the alternative prey and the parasitoid. Despite that the alternative prey was consumed by the generalist predator, it also benefited the specialist because the honeydew produced by whiteflies served as food for the parasitoid. Honeydew is a source of sugar and amino-acids (Douglas 2006) and it provides energetic intake and helps proteins production in synovigenic parasitoids e.g. in *S. japonicus*. Effectively, honeydew from host or non-host species is commonly exploited by parasitoids and has been shown to have a positive effect on various fitness-related traits e.g. survival, egg production, and fat reserves (England and Evans 1997, Eijs et al. 1998, Hirose et al. 2009). We did not observe an increase in parasitoid longevity owing the honeydew availability, however there is usually a trade off between longevity and fecundity (Barnes and Partridge 2003, Mukhopadhyay and Tissenbaum 2007). Therefore, the increase in fecundity that we recorded in our study may have lowered the possible beneficial effect of honeydew on parasitoid longevity. Parasitoids of honeydew-producing hosts do not waste much time for foraging for food as it can be obtained directly when parasiting the hosts (Jervis et al. 1996). On the contrary, some studies have shown that time needed for searching for food sources that are not directly related to the host (or even the host-plant system), e.g. nectar or others, can substantially reduce available host-searching

time (Takasu and Lewis 1995). This is an important cost for parasitoids, notably those that are time-limited (Papaj 2000). In our study, the presence of whiteflies in the greenhouse experiment benefited to the parasitoid, and this even if the parasitoid had to feed on honeydew from a non-host species.

Macrolophus pygmaeus is known to show a numerical response to whitefly species (Hamdan 2006). In concordance, we observed higher *M. pygmaeus* densities in treatments which had the whitefly *T. vaporariorum* as alternative prey. However, *M. pygmaeus* is known to exhibit a switching behavior when feeding on whitefly and *T. absoluta*, so it feeds preferentially on the most abundant prey (Jaworski et al. submitted). Therefore, even if the coexistence of the two natural enemies (parasitoid and predator) might have been disfavored due to increased predator density in response to the alternative prey, the switching behavior exhibited by the predator, may have promoted coexistence. Switching behavior in predators has been shown to promote coexistence under certain conditions in a two prey-one predator, though this is based only on modelling studies (Hutson 1984, Krivan 2003). The impact of this behavior on predator coexistence with heterospecific species had been poorly studied. However, Holt and Polis (1997) suggested that adaptive behaviors may facilitate coexistence when documenting intraguild predation in an intraguild predator-intermediate predator-shared prey system. More specifically, these authors proposed that, if adaptive foraging by the intraguild predator leads to switching between the primary resource and the intermediate predator, the system should be stable. Hence, two main mechanisms, both related to predator switching behavior, may help coexistence in our biological systems. First, the low predation pressure on a shared resource when this one reaches low density levels may favor natural enemy coexistence. The generalist predators switch to other resources leaving remaining shared resource available to the specialist natural enemy. Second, when the specialist is a parasitoid, the switching of the generalist predator between the shared and the alternative prey is similar to the switching between an intermediate consumer (parasitized prey items) and an alternative prey, as described by Holt and Polis (1997). Such process may have favored coexistence by reducing kleptoparasitism events on parasitized *T. absoluta* larvae. In our study, kleptoparasitism and intraguild predation could not be distinguished from each other when studying effects on parasitoid population dynamics because thefts ultimately led to the death of parasitoid juveniles (Chailleux et al. in prep.a).

In terms of biological control, the fact that the generalist predator and the specialist parasitoid were able to coexist during the cropping season, and this despite that a common alternative prey for the predator is present in the crop, shows promising avenues for possible

inoculative biological control of *T. absoluta* through release of the larval parasitoid *S. japonicus*. A parasitoid-mediated indirect interaction, apparent amensalism (-0), occurred between *T. absoluta* and whitefly. The presence of whiteflies improved the parasitism levels by providing extra food sources (honeydew) to the parasitoid. In the presence of whiteflies, the addition of the generalist predator did not strongly improve the pest control and a similar efficient control of *T. absoluta* was observed in the two treatments containing whiteflies. Nevertheless, *M. pygmaeus* was essential in the cropping system because the whitefly population showed important outbreaks when it was absent. Substantial damages to plants and fruits were recorded owing to the production of honeydew by whiteflies in this case. The best results in terms of crop protection were recorded when the four species were present in the system, similar results were recently reported by Messelink et al. (2013) in an other biological system, where authors observed a positive effect of the association of a generalist predator, *Orius majusculus* Reuter, with a specialist parasitoid, *Aphidius colemani* Viereck, on the control of two major pest species in sweet pepper: the green peach aphid *Myzus persicae* Sulzer and the western flower thrips *Frankliniella occidentalis* Pergande.

Overall, our study demonstrated the capacity of specialist parasitoids and generalist natural enemies to coexist when they share a common host/prey. This occurred despite the presence of an alternative prey that promoted the generalist predator population. We highlighted possible mechanisms that may promote such coexistence in the presence of the alternative prey. Two main mechanisms may counter-balance the detrimental effect of the alternative prey i.e. the increased predator density in response to the richer ecosystem. First whitefly honeydew increased parasitoid fecundity, second the predator may have weaken the competition thanks to a switching behavior in the presence of the two prey. However these hypotheses should be verified in further studies on other biological systems.

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Discussion générale

Discussion générale

Le travail de thèse se divisait en trois parties, l'objectif général étant de contribuer à la mise en place de la protection biologique contre une espèce invasive, *T. absoluta*, en mettant notamment en évidence l'importance des interactions multi-trophiques dans les agrosystèmes et leurs rôles dans le succès de la mise en place d'un programme de lutte biologique. Dans un premier temps, nous avons recherché qu'elles étaient les interactions modifiées/ajoutées par l'invasion du ravageur exotique, puis nous avons cherché quels nouveaux auxiliaires pouvaient permettre d'améliorer le contrôle biologique de cette espèce en nous concentrant sur des auxiliaires autochtones, dont l'utilisation est moins risquée vis à vis des écosystèmes locaux. Finalement, nous avons testé l'impact potentiel de l'introduction de ces nouveaux auxiliaires dans le programme de lutte biologique préexistant dans les systèmes de culture sous serre de tomates.

I) Les interactions indirectes liées à l'invasion et la perturbation potentielle de la lutte biologique préexistante

En arrivant dans un agrosystème, une espèce invasive peut perturber, positivement ou négativement, le contrôle biologique des autres ravageurs si l'on est en présence d'un prédateur généraliste capable de s'attaquer à la nouvelle proie. L'impact de l'invasion peut être négatif si le prédateur a une préférence pour la proie invasive. Cependant, il semble que *M. pygmaeus* ait plutôt un comportement de « switching » entre ces deux proies (Jarowski *et al.* in prep.), or ce comportement, qui consiste à préférer la proie la plus abondante (Murdoch et Oaten 1975), aurait plutôt un effet régulateur sur la dynamique des populations de proies (Huston 1984). L'effet peut aussi être négatif à court terme, i.e durée inférieure à la durée d'un cycle du prédateur, si l'on observe un effet de dilution de la prédation, et donc un mutualisme apparent, cet effet n'a pas pu être observé dans notre expérimentation. Sous serre, il a cependant était mis en évidence par Messelink *et al.* (2013) ; il est possible que dans notre expérimentation les densités de proies aient été trop faibles pour que les méthodes de comptage aient pu permettre de le détecter, cependant cela pourrait être mis en évidence au laboratoire à une échelle d'expérimentation plus restreinte. Au contraire, on peut observer des effets positifs à long terme sur le contrôle biologique si l'on observe de la compétition apparente. C'est ce qui a été observé dans nos conditions expérimentales. Le prédateur étant

capable d'avoir une réponse numérique sur les deux types de proie, l'arrivée de l'espèce invasive a plutôt un effet bénéfique sur le contrôle biologique, ce type de résultat a rarement été mis en évidence clairement, mais des résultats assez similaires ont été observés sous serre sur poivron (Messelink *et al*. 2008). Pourtant quand l'espèce invasive, *T. absoluta*, est seule, la réponse numérique n'est pas visible, cela peut être du a deux choses différentes, tout d'abord du fait que les populations étaient cycliques (Abrams 1998). Mais cela peut aussi être du au fait que le nombre de proies qui permet une réelle réponse numérique du prédateur ne lui soit pas accessible, soit à cause d'une densité de proies trop faible, soit par une mauvaise exploitation de cette ressource i.e mauvaise capacité de recherche de l'hôte. Dans ce cas, ce que l'on observait en présence des deux proies pourrait éventuellement être du au bénéfice d'une nourriture mixte. Evans *et al.* (1999) ont comparé la production d'œufs par deux coccinelles aphidiphages *Coccinella septempunctata* et *C. transversoguttata*, nourrit de pucerons et de charançons. Les femelles produisaient un plus grand nombre d'œufs lorsqu'un régime alimentaire de pucerons en nombre limité était complété par des larves de charançon, même si ce dernier, fourni seul, ne permettait pas la reproduction de ces coccinelles.

Perspectives : Il serait intéressant de tester l'existence de la compétition apparente en présence d'un parasitoïde pour chaque espèce, comme c'est le cas dans la majorité des serres, afin d'établir si cette interaction joue un rôle, même dans un système plus complexe. Cela aurait aussi un intérêt du point du vue de la lutte biologique, puisque le contrôle par le prédateur généraliste, même en présence des deux espèces, n'était pas suffisant pour être acceptable en serre de production et nécessiterait donc l'ajout d'auxiliaires, tels que des parasitoïdes.

II) La recherche de nouveaux auxiliaires autochtones pour le contrôle biologique de *T. absoluta* en Europe

La recherche de nouveaux auxiliaires a du se focaliser sur l'utilisation d'espèces autochtones pour éviter les risques sur les écosystèmes naturels liés à une nouvelle introduction (Article 3). Si la majorité des programmes de lutte biologique contre des espèces invasives porte sur l'introduction d'auxiliaires exotiques (Allendorf et Lundquist 2003, Hoddle 2004) c'est parce que, justement, l'espèce a pu devenir invasive car elle a été capable de surmonter les différentes barrières de l'écosystème envahi et notamment la barrière constituée par les ennemis naturels autochtones (voir la section *Les Invasions biologiques*

pour plus de détails). Les espèces invasives n'ont pas d'ennemis naturels suffisamment efficaces pour assurer un contrôle biologique naturel des cultures. Cependant, nous disposons de méthodes, telles que la lutte biologique par augmentation, qui permettent de manipuler les densités de populations d'ennemis naturels dans un agrosystème, ce qui est d'autant plus vrai sous serre.

Parmi les prédateurs généralistes et les spécialistes, l'intérêt a été porté sur les parasitoïdes, puisqu'un prédateur généraliste, *M. pygmaeus*, déjà présent dans les serres s'était montré efficace contre *T. absoluta* dès les première détections de l'espèce invasive (Urbaneja *et al*. 2009, Bompard *et al.* 2013). Les parasitoïdes pourraient ainsi venir compléter efficacement son action (Snyder and Ives 2003). Il a fallu s'intéresser à des parasitoïdes suffisamment généralistes pour s'attaquer à une proie, avec laquelle ils n'ont pas coévolué. C'est le cas des trichogrammes qui sont connus pour parasiter une gamme de lépidoptères très large (Smith 1996). De plus, ces derniers sont élevés facilement à grande échelle, ce qui rendait possible la lutte biologique par augmentation, notamment celle par inondation. Cependant les résultats obtenus ont montré, malgré une bonne efficacité de certaines souches en laboratoire, notamment *Trichogramma euproctidis*, que les espèces/souches testées ne permettaient pas un meilleur contrôle que la souche déjà commercialisée, *T. achaeae* (Article 4) en conditions réalistes (cages et compartiments de serre). Nos résultats ne vont pas dans le sens d'une amélioration de la lutte via l'utilisation d'une nouvelle souche de trichogrammes, cependant une approche différente aurait peut-être donné d'autres résultats. Les résultats obtenus ont rappelé l'importance de faire correspondre les besoins et les caractéristiques des ennemis naturels avec ceux de l'agrosystème cible. Si les souches testées ne se sont pas montrées prometteuses, cela peut être du à deux possibilités: (i) les trichogrammes ne sont globalement pas adaptés à ce système ; (2) les souches testées n'étaient pas adaptées au système, mais elles ne sont pas représentatives des populations disponibles en Europe. En effet, des organisations comme l'IOBC (International Organization for Biological and integrated Control of noxious animals and plants) avaient déjà soulignées l'importance d'évaluer l'efficacité des auxiliaires commercialisés afin de s'assurer que les conditions d'élevages ne conduisaient pas à une chute de leur efficacité, or certaines des souches testées dans notre étude étaient en élevage depuis plusieurs années. Un effet négatif des conditions d'élevage sur les auxiliaires n'est pas systématique (Hoffmann *et al.* 2001, Kölliker-Ott *et al.* 2003), mais des cas de réduction de fitness et d'acceptation des hôtes naturels ont été observés (Ashley 1973 *et al.* Van Bergeijk *et al.* 1989, Salmonova *et al.* 1992), c'est pourquoi un test d'efficacité comme celui du « test de vol » mis en place par l'IOBC, et qui semble

efficacement discriminant (Prezotti *et al*. 2002), aurait peut-être pu permettre d'écarter certaines souches. Il apparait en effet que l'efficacité des trichogrammes est associée à la fois au parasitisme et à la mobilité. La variation de la vitesse de déplacement a été utilisée pour estimer la capacité de localisation et l'efficacité des souches de *T. maidis* pour des lâchers inondatifs (Bigler *et al.* 1988). Il y a également eu des tentatives pour combiner les paramètres de qualité dans l'élaboration d'un indice de qualité (Liu et Smith, 2000). En particulier, Dutton *et al.* (1996) ont mesuré quatre paramètres de qualité : la vitesse de marche, la durée de vie et la fécondité sur l'hôte naturel, ainsi que l'hôte d'élevage. Si notre étude a bien pris en compte la fécondité sur l'insecte hôte et la plante hôte, la capacité de déplacement a été négligée dans ces tests. En effet, le déplacement des micro-hyménoptères par la marche sur la tomate est connu pour être particulièrement difficile, le vol est donc un élément clé de l'efficacité des ennemis naturels sur tomates (e.g. Kauffman et Kennedy 1989, Kennedy 2003). Finalement, les expérimentations suivantes à échelle réaliste (article 3) ont permis d'éviter de se lancer dans un programme de lutte biologique à grande échelle avec des agents inefficaces. Effectivement, ce type de test en grandes cages sous serre est recommandé avant tout choix définitif d'ennemis naturels (Hoelmer et Kirk 2005).

Par conséquent, il est ensuite apparu intéressant de se tourner vers d'autres familles de parasitoïdes (Article 5), ainsi nous avons évalué l'efficacité et la biologie de deux parasitoïdes larvaires de la famille des Eulophidae, fréquemment retrouvés sur *T. absoluta* sous serre de tomates (Urbaneja *et al.* 2012, Zappala *et al.* 2012 et article 2). Bien plus gros que les trichogrammes et très bon voiliers (observations personnelles), ils étaient par conséquent moins sensibles aux trichomes de la tomate. Au contraire des résultats obtenus pour les trichogrammes, ces deux espèces se sont avérées prometteuses. Elles ont montré de très bons niveaux de parasitisme sur *T. absoluta* sur tomates (y compris sur plante entière), elles étaient aussi toutes deux capables de se reproduire sur plusieurs cycles sur cet hôte (élevage de laboratoire effectué sur le système *T. absoluta*-tomate). Ces meilleurs résultats peuvent s'expliquer par le fait que les parasitoïdes larvaires provenaient de collectes sous serre de tomates sur le terrain, au contraire des trichogrammes qui provenaient de collections de laboratoire. Les collectes de terrain, bien que longues et fastidieuses, permettent une présélection des espèces à la fois capables de parasiter et attirées par la nouvelle espèce dans l'écosystème cible. Aux vues de nos résultats, il semblerait que cette technique soit à privilégier lors de la recherche de nouveaux ennemis naturels pour tout les parasitoïdes en général, mais aussi pour les trichogrammes comme recommandé par les spécialistes (Hassan 1994, Smith 1996).

Il est difficile de comparer ces études à d'autres travaux visant à évaluer l'efficacité d'espèces autochtones pour lutter contre une espèce invasive car, bien que conseillée, cette méthode a rarement été mise en place (Michaud 2002). L'efficacité de prédateurs autochtones a cependant été remarquée pour le contrôle biologique du puceron du soja en Amérique du Nord, invasif sur ce continent (Desneux *et al*. 2006). Une autre étude a aussi démontré l'efficacité d'un Eulophidae autochtone, *Cameraria ohridella*, sur une mineuse exotique (Grabenweger *et al*. 2009). L'étude de l'efficacité des parasitoïdes ainsi que sur la mise en place d'une technique d'élevage sont souvent complexes pour les parasitoïdes spécialistes de mineuses. C'est pourquoi les auteurs de cette étude proposaient l'utilisation du parasitoïde en lutte biologique inoculative, car elle nécessite moins d'individus. C'est aussi une proposition que l'on peut faire pour *Stenomesius japonicus*, dont on pourrait envisager des lâchers inoculatifs sous serre dès l'apparition de larves de *T. absoluta* dans la culture.

Perspectives : Il pourrait être intéressant de faire des prospections sur le terrain en prélevant des œufs de *T. absoluta* sous serre de tomates, soit par collecte, soit en utilisant des œufs sentinelles, afin de trouver des souches de parasitoïdes oophages sur le terrain. Cependant, suite aux grosses quantités de lâchers inondatifs de *T. achaeae*, il est probable que cette espèce soit retrouvée systématiquement. Les trichogrammes (ou autres parasitoïdes oophages, type *Trichogrammatoidae*, par exemple) ainsi récupérés pourraient être testés à l'aide du « test de vol » puis évalués directement en cage sous serre. En ce qui concerne les parasitoïdes larvaires, la priorité doit être mise sur la recherche de moyens d'élevage de masse. Les parasitoïdes spécialistes de mineuses ne parasitent que les larves dans leurs mines, ce qui complique l'élevage. Cela a cependant déjà été mis en place au niveau industriel pour un autre parasitoïde larvaire de mineuse, *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae), ectoparasitoïde de la mouche mineuse de la tomate, mais l'élevage reste coûteux (Chow et Heinz 2006).

III) Les interactions liées à l'introduction de nouveaux auxiliaires au sein de l'agrosystème

On sait, grâce aux théories d'écologie que, pour que l'association d'ennemis naturels pour le contrôle d'un seul ravageur augmente le contrôle biologique, les ennemis naturels ne doivent pas partager totalement la même niche écologique (Gause 1934, Tilman 1982). C'est d'ailleurs ce qui est à l'origine de l'émergence de l'idée de gérer le paysage dans le but de

promouvoir le contrôle biologique (Wratten *et al.* 1998). Sous serre, l'agrosystème est totalement contrôlé par l'homme, mais dès qu'il s'agit de contrôle biologique, les interactions naturelles ont un rôle essentiel, aussi dans ces systèmes protégés. De plus, la serre constitue un environnement particulièrement favorable à l'étude des interactions de par le contrôle accru de tous les paramètres, par rapport au champ, et la possibilité de manipuler les espèces présentes (Van Lenteren 2000, Enkegaard et Brødsgaard 2006). Les prédateurs omnivores sont très utilisés en lutte biologique pour leur capacité à contrôler plusieurs ravageurs à la fois et pour leur capacité à être présents sur la culture avant les ravageurs. Ils sont aussi très communs sous serre, cependant leur large gamme de proies et leur capacité à se nourrir sur différents niveaux trophiques en font la source de nombreuses interactions directes et indirectes (Symondson *et al.* 2002).

Les résultats des études présentées dans les articles 6 et 7 ont porté sur l'adéquation entre les trichogrammes et l'agrosystème tomates sous serre, en prenant en compte la présence du prédateur généraliste *M. pygmaeus*. L'intérêt ici, était d'envisager une potentielle utilisation des trichogrammes en lutte biologique inoculative, ou tout au moins de prendre en compte une éventuelle efficacité de la première génération née sur le terrain, afin de réduire le nombre d'individus dans les lâchers suivants, dans le but de diminuer la contrainte économique limitant l'utilisation des trichogrammes par les producteurs. En effet, puisqu'aucune souche plus efficace que *T. achaeae*, ou ayant une capacité à être stockée grâce à des mécanismes de diapause ou de quiescence, n'a été mise en évidence par les études précédentes (article 4), une autre possibilité était de modifier l'utilisation des trichogrammes via une réduction des doses de lâchers. Cependant, nos études à ce sujet tendent à montrer que l'écosystème cible ne présente pas des caractéristiques très favorables aux trichogrammes pour deux raisons principales, (1) les trichogrammes subissent de la prédation intra-guilde de la part du prédateur omnivore (Fig. 6), et (2) le système insecte hôte-plante hôte leur est peu favorable.

La prédation intra-guilde est fréquemment rencontrée dans les agrosystèmes (Rosenheim *et al.* 1995), celle-ci n'a pas toujours un effet négatif sur la protection des cultures, il semblerait en fait que les cas de perturbation de lutte biologique surviennent surtout quand le prédateur intermédiaire n'est pas un parasitoïde, et que par conséquent, la consommation du prédateur, en plus de réduire la population d'ennemis naturels, détourne le prédateur généraliste de la consommation du ravageur (Messelink *et al.* 2011). Mais dans notre étude, la prédation intra-guilde vient s'ajouter à une mauvaise adéquation du parasitoïde avec le système hôte i.e. *T. absoluta*+tomate, et conduit à une efficacité négligeable de la

première génération fille, née sur le terrain. Comme discuté dans l'article 7, il semble que la mauvaise adéquation des trichogrammes avec le système hôte soit en partie due à la taille des œufs de *T. absoluta*, étant donné qu'aucun mécanisme de défense immune ni d'encapsulation n'a été mis en évidence chez des œufs de lépidoptère. La taille, et donc la performance des imagos, dépend en effet de la taille de l'œuf dans lequel ils se sont développés (Smith 1996). Ce qui s'ajoute aux caractéristiques de la tomate qui sont peu appropriées pour les micro-hyménoptères, comme évoqué précédemment. Ainsi, les trichogrammes contre *T. absoluta* doivent continuer d'être utilisés via des lâchers inondatifs réguliers, comme ils le sont actuellement sur ce ravageur, bien que la lutte inoculative ait pu se révéler efficace sur d'autres systèmes (Kuhar *et al.* 2002, Wright *et al.* 2002, Hoffmann *et al.* 2006). Dans ces conditions, la prédation intra-guilde, même si elle se produit sur le terrain, a pour seule conséquence que l'efficacité des deux auxiliaires ne s'additionne pas exactement, leur efficacité respective se recoupe. De plus, ce recoupement est faible puisque le prédateur évite les œufs mélanisés (noirs) (article 6). Sans installation des trichogrammes dans le système, la prédation intra-guilde n'a pas d'impact sur la dynamique des populations, le prédateur omnivore et les parasitoïdes oophages peuvent donc continuer d'être utilisés ensemble.

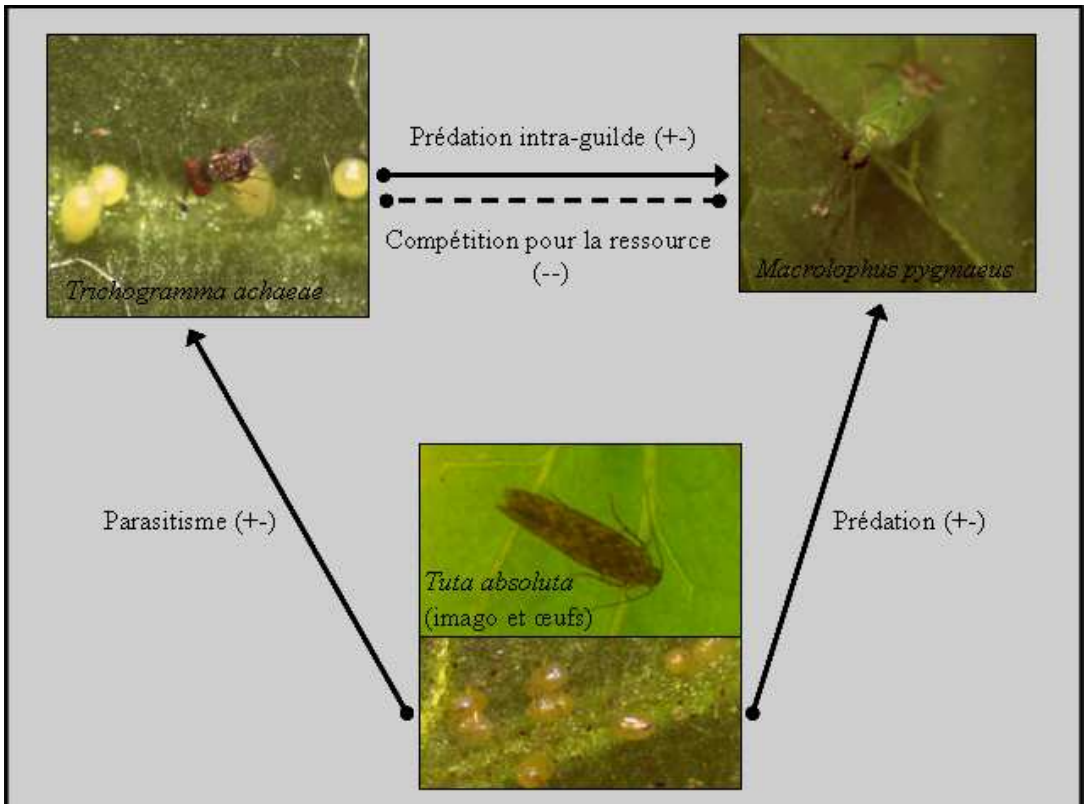


Figure 6 : Réseau trophique autour de *T. absoluta* en situation de contrôle biologique avec des trichogrammes et *M. pygmaeus*. Les ronds schématisent les effets négatifs, les pointes les effets positifs, les traits continus les interactions directes et les pointillés les interactions indirectes.

Les articles 8, 9, 10 ont porté sur la possibilité d'utiliser le parasitoïde larvaire en lutte biologique inoculative même en prenant en compte la présence du prédateur généraliste dans la culture, comme pour les trichogrammes. Mais, à l'inverse de ces derniers, les études des articles 8 et 9 montrent plutôt une bonne adéquation entre le système tomates sous serre et le parasitoïde larvaire, malgré une interaction de type cléptoparasitisme (article 6) avec le prédateur, qui vole la ressource des parasitoïdes juvéniles. Tout d'abord, seul, le parasitoïde a montré une efficacité supérieure à celle du prédateur seul. Ensuite, la coexistence s'est avérée possible avec et sans proie alternative pour le prédateur omnivore, mais elle s'est aussi montrée bénéfique pour le contrôle biologique.

Du point de vue des mécanismes de coexistence, il est assez surprenant que le parasitoïde ait pu survivre en présence du prédateur généraliste. On s'attendait à ce que, dès que la densité de population de proies chute sous un certain seuil, le parasitoïde soit exclu du système, ne tolérant pas des niveaux de proies très faible. En présence d'une proie alternative, il était plus difficile de faire des pronostics, puisque le parasitoïde devait être à la fois favorisé par la présence du miellat produit par les aleurodes (Douglas 2006), et défavorisé à cause d'une réponse numérique accrue du prédateur généraliste en présence d'aleurodes (Hamdan 2006). Si de nombreux auteurs ont étudié la coexistence entre espèces par une approche de modélisation, le nombre d'études expérimentales de long terme dans ce domaine reste encore limité (Amarasekare 2003). En théorie, l'addition d'une proie exclusivement disponible pour le prédateur omnivore, quand le parasitoïde a seulement accès à la ressource commune, augmenterait les risques d'exclusion du parasitoïde (Holt et Huxel 2007). Cependant, les parasitoïdes, ici, bénéficient aussi de la proie alternative via le miellat. Nos études ont donc montré que (1) un parasitoïde peut se maintenir dans un écosystème fermé quand il partage son unique ressource avec un prédateur omnivore pendant plusieurs mois, cependant nous n'avons pas pu mettre en évidence le mécanisme favorisant cette coexistence (article 9), et que (2) la coexistence était toujours possible en ajoutant une proie alternative pour le prédateur omnivore (article 10).

En l'absence d'aleurodes, plusieurs niches écologiques peuvent expliquer la coexistence. D'après nos résultats de laboratoire, le partitionnement de la ressource selon l'axe de la plante n'a pas pu favoriser la coexistence entre *M. pygmaeus* et *S. japonicus*. Nous n'avons donc pas pu conclure quant à l'existence d'une niche liée à la répartition des stades attaqués sur la plante. Il est probable que ces espèces étant toutes deux très mobiles, une répartition spatiale de la ressource ne permette pas de limiter les interactions intraspécifiques. Dans notre modèle biologique, la spécialisation sur des stades larvaires différents ne constitue

pas une niche, comme discuté dans l'article 9. Il semble donc que l'on ait plutôt affaire soit (1) à des comportements d'agrégation qui vont favoriser la coexistence, soit (2) à des compromis entre les traits d'histoire de vie. En ce qui concerne les comportements d'agrégation, *M. pygmaeus* a, en effet, une prédation dépendante de la densité d'hôtes et a tendance à rester plus longtemps dans les patchs à forte densité (Enkegaard *et al.* 2001), tandis que chez de nombreux parasitoïdes d'insectes mineurs, le comportement d'exploitation de la ressource en hôtes est plutôt inversement proportionnel à leur densité (Connor et Cargain 1994). Cependant, à l'échelle d'une cage, il y a peu de chance que cela ait permis de limiter la compétition pour la ressource, par contre, cela a pu avoir un impact sur le cléptoparasitisme, et réduire, par ce biais, la pression du prédateur omnivore sur le parasitoïde larvaire. On peut aussi expliquer la coexistence par des compromis entre les traits d'histoire de vie, la question étant de savoir comment le parasitoïde, qui n'a d'autres possibilités que de consommer son unique proie, a pu supporter les périodes à faible densité de proies. Il y a deux explications envisageables à cela (1) *M. pygmaeus* a une faible capacité à rechercher l'hôte, il n'exploite donc qu'une très faible partie de la ressource qui reste donc disponible pour le parasitoïde ; (2) la longévité du parasitoïde lui permet d'attendre que les populations d'hôtes ré-augmentent après une chute. Il est vrai que *M. pygmaeus* reste inactif pendant une grande partie de son temps par rapport au temps alloué à la recherche de proies (Montserrat *et al.* 2004). On peut donc supposer qu'il existe un compromis entre le régime alimentaire et la capacité de recherche de la ressource entre les deux espèces, qui permettrait au parasitoïde d'exploiter la ressource que le prédateur n'a pas trouvé. Ce phénomène a favorisé la coexistence dans de nombreuses études (Brown *et al.* 1997, Amarasekare 2003, Bonsall *et al.* 2004). En ce qui concerne la deuxième explication, la longévité du parasitoïde, ce paramètre est connu comme favorisant la coexistence mais cela n'est vrai que si les deux espèces en compétition ont des durées de vie différentes (Bonsall *et al*. 2002). Or ici, même si les durées de vie varient pour la même espèce selon les auteurs, elles sont toutes deux connues pour avoir une durée de vie longue (~2 mois), ce mécanisme n'a donc probablement pas permis de favoriser, à lui seul, la coexistence. Il est cependant possible que les deux mécanismes associés aient été en mesure de favoriser la coexistence, à la fois par une utilisation partielle de la ressource par le prédateur, qui a permis d'éviter une extinction de la population de la proie commune et de laisser suffisamment de ressource pour soutenir la population du parasitoïde, mais aussi la longévité du parasitoïde qui lui a permis de survivre aux périodes à faible densité de proies. En effet, en termes de lutte biologique, le problème rencontré si le ravageur disparaît, est que le parasitoïde va lui aussi disparaître. En cas de nouvelle

infestation, venue de l'extérieur de la serre, il ne sera plus présent dans l'agrosystème pour contrôler les populations de ravageurs, or on a vu dans nos résultats que le prédateur n'offrait pas un contrôle suffisant, même lorsqu'il est installé avant la proie. C'est pourquoi il paraît plus intéressant de maintenir un faible niveau de proies sur la culture, qui provoquera des niveaux de dégâts négligeables, mais maintiendra les populations d'auxiliaires qui assureront ainsi un contrôle permanent, même en cas de nouvelles invasions.

L'impact des aleurodes dans le système était essentiel à tester, ceux-ci sont présents dans la majorité des systèmes sous serre, et ils constituent une proie appréciée des punaises prédatrices (Heinz *et al.* 2004). Le parasitoïde consommant du miellat, source d'énergie et d'acides aminés (Douglas 2006), il était logique que la présence d'aleurode le favorise, et c'est bien ce qui a été observé. Il est probable que ce bénéfice, associé au comportement de « switching » du prédateur, est favorisé la coexistence et évité l'exclusion du parasitoïde. En effet, comme évoqué précédemment, *M. pygmaeus* a une préférence pour la proie la plus abondante, la délaissant ensuite quand elle atteint une faible densité. Si le niveau à partir duquel le prédateur omnivore abandonne *T. absoluta* est supérieur à celui nécessaire pour la survie du parasitoïde (Tilman 1990), le « switching » peut être un mécanisme ayant favorisé la coexistence. Dans notre étude, en présence de la proie alternative, les mécanismes favorisant la coexistence ont contrebalancé les effets négatifs et permis le maintient du parasitoïde dans l'agrosystème. Il semblerait donc que la coexistence entre un spécialiste et un omnivore soit possible, même en présence d'une proie alternative.

D'un point de vue appliqué, ces résultats montrent que les deux ennemis naturels peuvent coexister ensemble même en présence de proies alternatives pour le prédateur. C'est d'ailleurs dans la modalité avec les quatre espèces que le meilleur contrôle a été obtenu, corroborant ainsi les études qui montrent qu'une augmentation de la biodiversité améliore le contrôle biologique (Waage et Hawksworth 1991, Bianchi *et al*. 2006), y compris en serre (Messelink *et al.* 2010, 2013).

Perspectives : D'un point de vue théorique, il faudrait expérimenter la coexistence d'associations similaires sur d'autres modèles biologiques où le parasitoïde tire un bénéfice de la présence de la proie alternative, comme un système avec pour proie commune une espèce de pucerons, puisqu'ils produisent eux aussi du miellat, afin de vérifier si la coexistence en milieu fermé est aussi possible. D'autre part, il serait intéressant d'évaluer mathématiquement l'impact du miellat et du « switching » sur la coexistence, en isolant les mécanismes. L'impact du miellat pourrait aussi être comparé a celui d'une vrai proie alternative pour le parasitoïde. Contrairement à une consommation de proies, il s'agit d'une source de nourriture

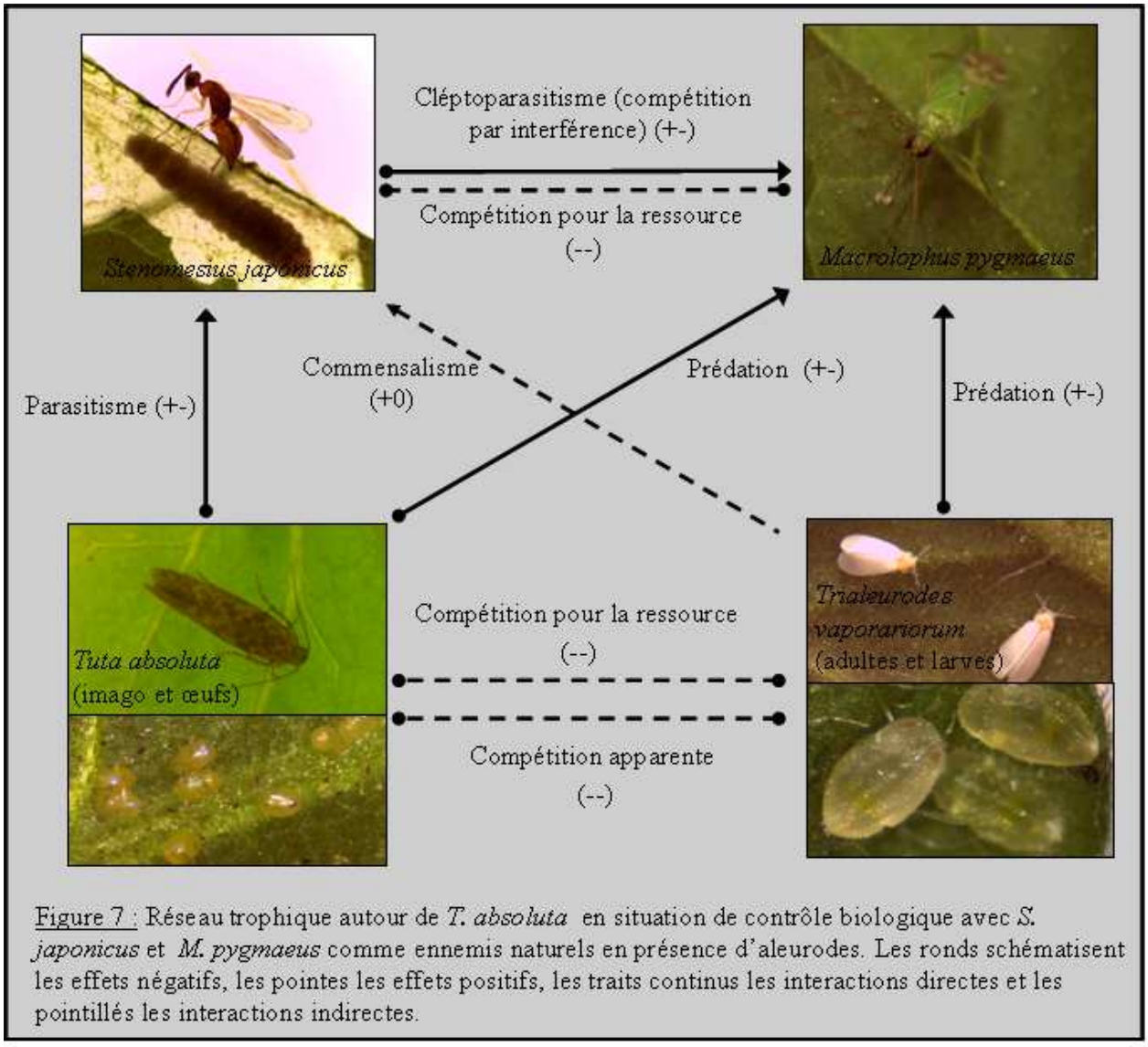


Figure 7 : Réseau trophique autour de *T. absoluta* en situation de contrôle biologique avec *S. japonicus* et *M. pygmaeus* comme ennemis naturels en présence d'aleurodes. Les ronds schématisent les effets négatifs, les pointes les effets positifs, les traits continus les interactions directes et les pointillés les interactions indirectes.

qui n'est pas aussi riche que celle obtenue par nourrissage sur l'hôte, or la ressource en acides aminés est nécessaire aux parasitoïdes synovigéniques pour assurer une production continue d'œufs. Il ne fournit pas non plus d'hôte supplémentaire pour l'oviposition. Les expérimentations empiriques, où l'on manipule les espèces présentes et leur densité, comme celles que nous avons menées en cage et sous tunnel, sont essentielles afin d'étudier les mécanismes de dynamique des populations dans les écosystèmes. Ce type d'expérimentations a d'ailleurs été recommandé par de nombreux écologistes (e.g. Rosenheim *et al.* 1995, Cardinale *et al.* 2003, Letourneau *et al.* 2009). D'un point de vue pratique, l'association prédateur généraliste et parasitoïde larvaire a montré son efficacité dans des conditions réalistes variées, mais il reste à évaluer différentes doses de lâchers pour envisager des lâchers plus faibles, mais aussi à tester l'efficacité de l'association en serre de production. D'une manière plus globale, les associations durables d'ennemis naturels, spécialistes et généralistes,

sont en générale complémentaires en termes de contrôle du ravageur (Snyder et Ives 2003), et la lutte biologique par inoculation étant économiquement plus soutenable que les lâchers inondatifs, ces associations sont à développer. Cependant, le milieu sous serre est un milieu clos et possède une diversité en plantes et en proies limitée, il ne permet que peu ou pas l'action du mécanisme de dispersion-colonisation au niveau méta-populationnel. Par conséquent, il n'est pas propice à la coexistence sur le long terme (ou au moins sur la durée de la culture) des ennemis naturels qui risquent de subir une très forte compétition. C'est pourquoi, les études sur le sujet doivent se poursuivre afin de pouvoir favoriser les mécanismes qui vont permettre cette coexistence. Il est probable que, par exemple, l'efficacité du parasitoïde puisse être amélioré via l'insertion de plantes nectarifères dans la serre, mais les adultes de *T. absoluta* bénéficient aussi du nectar, ainsi toutes les différentes approches d'amélioration du contrôle biologique doivent être testées en conditions réalistes afin d'en vérifier les bénéfices.

Finalement, cette thèse a contribué à mettre en évidence les perturbations liées à l'arrivée d'une espèce invasive dans un agrosystème, puis celles liées à l'ajout de nouveaux auxiliaires contre ce ravageur dans le système. Ce travail a aussi mis en évidence l'importance de la prospection, comme meilleur moyen de rechercher des espèces, plutôt que l'utilisation d'ennemis naturels « en stock ». Il a aussi contribué à mettre en évidence le rôle des auxiliaires autochtones dans le contrôle des espèces invasives, au moins en serre. D'après les expérimentations menées en conditions réalistes, il semble qu'il soit tout à fait possible d'obtenir un contrôle efficace de l'espèce invasive ainsi que des espèces ravageuses autochtones par des moyens de lutte biologique inoculative, grâce à de nombreux mécanismes qui vont favoriser la coexistence des espèces au sein de l'agrosystème. Les espèces omnivores ne conduisent pas nécessairement à l'exclusion des spécialistes, au contraire, leurs caractéristiques biologiques et comportementales semblent favoriser la coexistence dans nos études, même quand les conditions semblaient être réunies pour l'exclusion du parasitoïde. De plus, ces espèces se sont montrées complémentaires en termes de contrôle biologique.

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Annexes

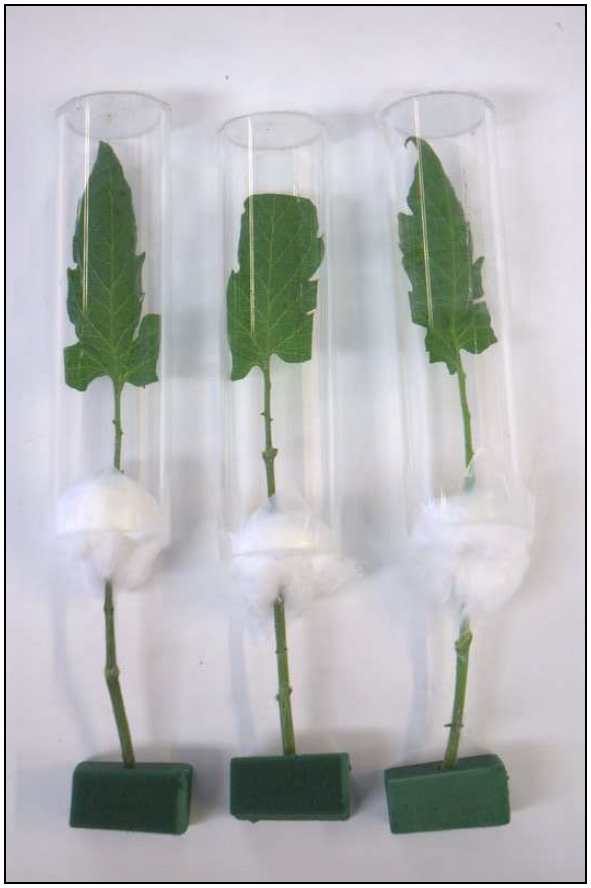
Annexe 1





Dispositif expérimental utilisé pour les expérimentations en tunnels sous serre.

Annexe 2



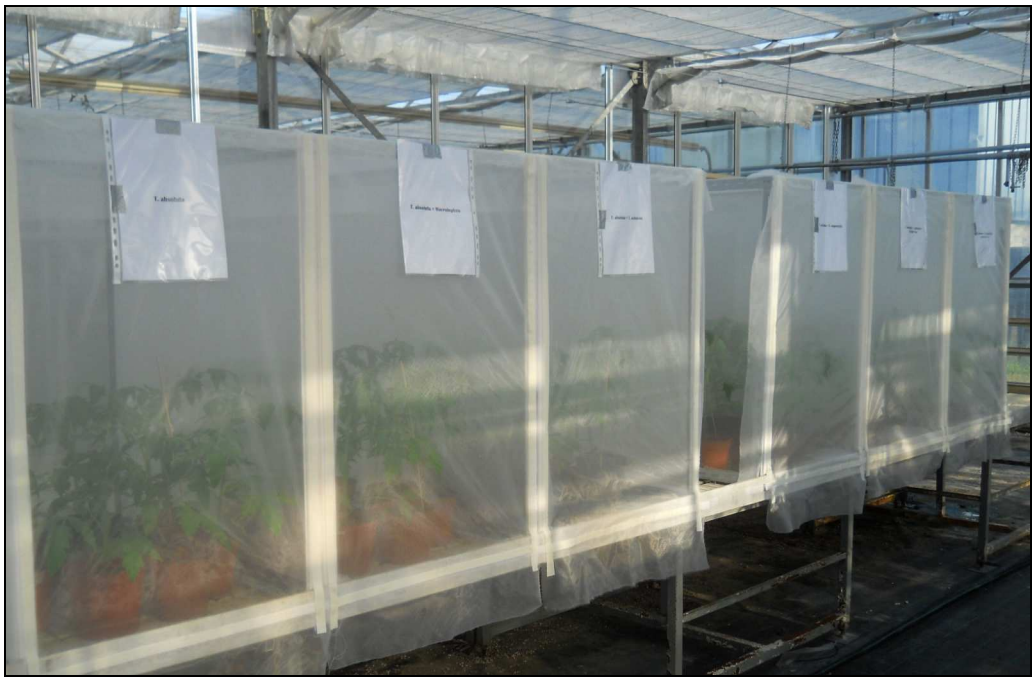
Dispositif expérimental utilisé pour l'évaluation des trichogrammes au laboratoire.

Annexe 3



Dispositif expérimental utilisé pour les tests au laboratoire (parasitisme, longévité, etc.).

Annexe 4



Dispositif expérimental utilisé pour les expérimentations en cage sous serre.