ÍTALO DOS SANTOS FARIA MARCOSSI

TOP-DOWN CONTROL OF A HERBIVORE AND PATHOGEN BY GENERALIST PREDATORY MITES ON TOMATO PLANTS

Thesis submitted to the Entomology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Advisor: Angelo Pallini

Co-advisor: Madelaine Venzon

VIÇOSA - MINAS GERAIS
2023

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T

Marcossi, Ítalo dos Santos Faria, 1994-

M321t
2023

Top-down control of a herbivore and pathogen bygeneralist predatory mites on tomato plants / Ítalo dos Santos Faria Marcossi. – Viçosa, MG, 2023.

1 tese eletrônica (96 f.): il. (algumas color.).

Texto em inglês.
Orientador: Ângelo Pallini Filho.
Tese (doutorado) - Universidade Federal de Viçosa, Departamento de Entomologia, 2023.
Inclui bibliografia.
DOI: https://doi.org/10.47328/ufvbbt.2023.538
Modo de acesso: World Wide Web.

1. Ácaros no controle biológico de pragas. 2. Iolinidae. 3. Phytoseiidae. 4. Tomate - Doenças e pragas - Controle biológico. 5. Oídio. I. Pallini Filho, Ângelo, 1965-. II. Universidade Federal de Viçosa. Departamento de Entomologia. Programa de Pós-Graduação em Entomologia. III. Título.

CDD 22. ed. 595.42

Bibliotecário(a) responsável: Bruna Silva CRB-6/2552

ÍTALO DOS SANTOS FARIA MARCOSSI

TOP-DOWN CONTROL OF A HERBIVORE AND PATHOGEN BY GENERALIST PREDATORY MITES ON TOMATO PLANTS

Thesis submitted to the Entomology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

APPROVED: July 13, 2023

Assent:

Documento assinado digitalmente
ITALO DOS SANTOS FARIA MARCOSSI
Data: 12/09/2023 11:58:46-0300
Verifique em https://validar.iti.gov.br

Ítalo dos Santos Faria Marcossi
Author

Documento assinado digitalmente
ANGELO PALLINI FILHO
Data: 11/09/2023 11:13:35-0300
Verifique em https://validar.iti.gov.br

Angelo Pallini Filho
Advisor

ACKNOWLEDGMENTS

Primeiramente quero agradecer a Deus por tudo que Ele é.

Agradeço a toda minha família.

À Ana Lígia e o Joaquim (sim tive um filho no meio disso tudo) minha nova família.

Aos meus queridos orientadores por todos os ensinamentos no decorrer do trabalho, mesmo eu não sabendo explicar o que pretendia fazer na maioria das vezes.

A todos os amigos e colegas do Laboratório de Acarologia, 6 anos nessa cidade!!!

Aos professores e alunos do programa de Pós-Graduação em Entomologia pelo convívio.

À Universidade Federal de Viçosa e ao Programa de Pós-Graduação em Entomologia pela oportunidade e pela qualidade institucional.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

A Koppert pela experiência e maior contato com o mundo rela do controle biológico, e o auxílio financeiro nesse estudo.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e a Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) pela bolsa de estudo e o financiamento do projeto.

ABSTRACT

MARCOSSI, Ítalo dos Santos Faria, D.Sc., Universidade Federal de Viçosa, July, 2023. **Top-down control of a herbivore and pathogen by generalist predatory mites on tomato plants**. Adviser: Angelo Pallini Filho. Co-adviser: Madelaine Venzon.

The widespread use of pesticides in agriculture poses significant challenges to human health and ecosystems globally. There is therefore a growing need for alternative pest control strategies to reduce pesticide usage. Augmentative biological control, which involves releasing natural enemies into crops to suppress pests, is a promising approach within integrated pest management programs. While there have been successful cases of augmentative biological control, factors such as pesticide use, the selection of natural enemies and plant defence mechanisms can impact its effectiveness. In this thesis, I investigate the use of predatory mites as natural enemies to control two important tomato pests, *Bemisia tabaci* (whitefly) and *Aculops lycopersici* (tomato russet mite), as well as the disease caused by *Oidium neolycopersici* (powdery mildew). The focus is on three species of predatory mites associated with tomato plants: *Amblyseius herbicolus*, *Amblyseius tamatavensis*, and *Homeopronematus anconai*. In Chapter 1, I examine the potential of *A. herbicolus* and *A. tamatavensis* to control *B. tabaci* on tomato plants supplemented with alternative food sources, and evaluate their adaptation to the tomato plant. In Chapter 2, I investigate the population dynamics of *A. lycopersici* in the presence of *A. herbicolus* and *H. anconai*, as well as the ability of *H. anconai* to mitigate the progression of *O. neolycopersici*. Based on the results of Chapter 2, I explore the occurrence of reciprocal intraguild predation between *A. herbicolus* and *H. anconai* in the last chapter, and investigate the potential impact of this interaction on biological control. I discuss the importance of considering factors such as predator adaptation, plant defence mechanisms (e.g., trichomes), and interactions among predators in optimizing biological control strategies. The findings here can contribute to the development of sustainable pest management practices that reduce pesticide use.

Keywords: Biological control. Phytoseiidae. Ionilidae. Whitefly. Tomato russet mite.
Powdery mildew.

RESUMO

MARCOSSI, Ítalo dos Santos Faria, D.Sc., Universidade Federal de Viçosa, julho de 2023. **Controle *top-down* de herbívoros e patógenos por ácaros predadores generalistas em plantas de tomate**. Orientador: Angelo Pallini Filho. Coorientadora: Madelaine Venzon.

O uso generalizado de pesticidas na agricultura apresenta desafios significativos para a saúde humana e os ecossistemas em todo o mundo. Atualmente, há uma necessidade crescente de estratégias alternativas de controle de pragas que reduzam o uso de pesticidas. O controle biológico aumentativo, que envolve a liberação de inimigos naturais nas lavouras para suprimir as pragas, é uma abordagem promissora dentro dos programas de manejo integrado de pragas. Embora existam casos bem-sucedidos de controle biológico aumentativo, fatores como o uso de pesticidas, a seleção de inimigos naturais e os mecanismos de defesa das plantas podem afetar sua eficácia. Nesta tese, investigo o uso de ácaros predadores como inimigos naturais no controle de duas importantes pragas do tomateiro, *Bemisia tabaci* (mosca branca) e *Aculops lycopersici* (ácaro do bronzeamento do tomateiro), bem como a doença causada por *Oidium neolycopersici* (oídio). O foco está em três espécies de ácaros predadores associados ao tomateiro: *Amblyseius herbicolus*, *Amblyseius tamatavensis* e *Homeopronematus anconai*. No Capítulo 1, examino o potencial de *A. herbicolus* e *A. tamatavensis* em controlar *B. tabaci* em plantas de tomate suplementadas com fontes alternativas de alimento, e avalio a adaptação desses predadores à planta de tomate. No Capítulo 2, investigo a dinâmica populacional de *A. lycopersici* na presença de *A. herbicolus* e *H. anconai*, bem como a capacidade de *H. anconai* de mitigar a progressão de *O. neolycopersici*. Com base nos resultados do Capítulo 2, exploro a ocorrência de predação recíproca intraguilda entre *A. herbicolus* e *H. anconai* no último capítulo, investigo o potencial impacto dessa interação no controle biológico. Discuto a importância de considerar fatores como adaptação de predadores, mecanismos de defesa da planta (por exemplo, tricomas) e interações entre predadores na otimização de estratégias de controle biológico. As descobertas aqui podem contribuir para o desenvolvimento de práticas sustentáveis de manejo de pragas que reduzem o emprego de pesticidas.

Palavras-chave: Controle biológico. Phytoseiidae. Ionilidae. Mosca-branca. Ácaro do bronzeamento do tomateiro. Oídio.

SUMMARY

GENERAL INTRODUCTION

The widespread use of pesticides poses a substantial challenge to human health and ecosystems around the world (UN Human Rights Council 2017, Hyland et al. 2019; Jepson 2020). Pesticides are developed and produced with the aim of suppressing pest levels in crops, but the effects of pesticides on target pest densities are typically studied over a single generation (Janssen and van Rijn 2021). However, pests often have multiple generations, which leads to repeated applications of pesticides and to pest resurgence (Janssen and van Rijn 2021). There is a widespread understanding that intensive pesticide application can increase the vulnerability of agricultural systems to pest outbreaks and maintain continued dependence on their use (Guedes et al. 2016; Jepson 2020). There is therefore a growing demand for alternative pest control strategies to reduce pesticide use.

One alternative pest control strategy, known as augmentative biological control, consists of releasing large numbers of natural enemies to suppress the target pest population (van Lenteren 2000, 2012; van Lenteren et al. 2018). Nowadays, augmentative biological control is applied in many agricultural systems, such as fruit and vegetable crops, cereals, maize, cotton, sugarcane, soybean, grapes and many greenhouse crops (van Lenteren et al. 2018). Augmentative biological control is part of Integrated Pest Management (IPM) programs, which provids an environmentally and economically sound alternative to chemical pest control (van Lenteren and Bueno 2003; Cock et al. 2010).

Although there are many cases of success in the use of augmentative biological control in the regulation of pests, most of them concern greenhouse crops (van Lenteren 2012; van Lenteren et al. 2018). Several factors can influence the abundance, effectiveness, and persistence of biocontrol agents. Obviously, the use of pesticides is the main one (Guedes et al. 2016; Jepson 2020), but the types of natural enemies used can also lead to an unsatisfactory result in biological control (Messelink et al. 2014). The selection of natural enemies for augmentative biological control was traditionally focused on specialist natural enemies that were released to obtain rapid control of the pests (van Lenteren and Woets 1988). This implies, however, that natural enemies could only be introduced into the crop after pest invasion, which makes early detection of the pest and timing of release essential. In contrast, the establishment and persistence of generalist predators may provide more sustainable biological control compared to specialist natural enemies, as their broader diet range enables them to persist or even reproduce on alternative prey or plant-provided food sources in the absence of pest organisms (Huffaker and Kennett 1953; Symondson et al. 2002), thus they can be released in the crop before pest invasion. A well-known example is the generalist predatory mite *Amblyseius swirskii*, which is an effective biological control agent of several important pests of various cultures, such as whiteflies, thrips and broad mites (Messelink et al. 2006, 2008; Wimmer et al. 2008; Arthurs et al. 2009; Nomikou et al. 2010; van Maanen et al. 2010; Calvo et al. 2011, 2015; Ghasemzadeh et al. 2017). When provided with alternative food, this predator can be introduced into crops before the occurrence of pests, it has a short generation time and can control several pests simultaneously (Messelink et al. 2008). These characteristics allowed *A. swirskii* to become the most used biocontrol agent in protected cultivations worldwide in just 10 years (Calvo et al. 2015).

Another factor that can limit the action of natural enemies is the interaction between predators and plant defences. Herbivores induce defences in plants that reduce the population growth of pest populations. So-called direct plant defences consist of a collection of physical and chemical barriers that decrease plant accessibility and reduce its quality as food (Kant et al. 2015). Plants also promote the presence of predatory arthropods (indirect plant defences) which also decrease the densities of herbivores (Sabelis et al. 1999; Symondson et al. 2002). Although there are examples of direct defences augmenting indirect defences (Krimmel and Pearse 2013; Thaler 1999), most studies indicate that especially constitutive plant defence traits can negatively affect top-down control. For instance, glandular hairs (trichomes) present on the epidermal surface of plants are one of the main factors correlated with resistance to herbivory (Kennedy 2003; Simmons and Gurr 2005). However, trichomes may interfere with indirect defence by hindering natural enemies of herbivores (Simmons and Gurr 2005; Wei et al. 2012). Evidence of trichomes increasing the mortality of natural enemies has been shown for coccinellids, crisopids, hoverflies (Belcher and Thurston 1982; Elsey 1974; Obrycky and Tauber 1984; Verheggen et al. 2009), parasitoids (Kashyap et al. 1991; Keller 1987; Romeis et al. 1999), and predatory mites (van Haren et al., 1987, Legarrea et al. 2022).

The trichomes of Solanaceae have been studied in detail, especially those of *Solanum* species, because of their role in plant resistance (Glas et al. 2012). For instance, tomato (*Solanum lycopersicum* L.), possesses glandular and non-glandular trichomes that predominantly act as broad-spectrum direct defence against herbivores (Cédola and Sánchez 2003; Kennedy 2003; Glas et al. 2012), but also increase the mortality of natural enemies. Non-glandular trichomes can physically obstruct the movements of prey herbivores, decreasing their density and preventing the establishment and dispersal of predators on the plant (van Lenteren et al. 1995; Simmons and Gurr 2005; Carrilo et al. 2008; Paspati et al. 2021; Legarrea et al.2022). Moreover, glandular trichomes synthesize and store sticky and/or toxic exudates such as terpenoids and acyl sugars that can trap or kill herbivores and predators alike (Simmons and Gurr 2005; Verheggen et al. 2009; van Houten et al. 2013; Paspati et al. 2021). Therefore, understanding the complex interaction between tomato trichomes and their ecological interactions is critical to successful biological pest control in this crop.

A well-studied group of natural enemies with respect to control of various pests in tomatoes is the predatory mites, mainly from the Phytoseiidae family. However, the effectiveness of these tiny predators is reduced by the glandular trichomes (van Haren et al. 1987; van Houten et al., 2013; Schmitd 2014; Paspati et al. 2021; Legarrea et al., 2022), because their survival is reduced on tomato (van Haren et al. 1987; Castagnoli et al. 1999; Paspati et al. 2021). This is because they stick to the glandular trichomes and because the secondary metabolites, such as acyl sugars, present in tomatoes trichomes can be highly toxic to mites and accumulate on their bodies after walking on tomato stems (Paspati et al. 2021).

Adaptation to a crop is one of the main criteria in the search for natural enemies (van Lenteren et al., 2018), and I therefore assumed that predators occurring on wild or feral tomato may be adapted to it. The main objective of this thesis was therefore to investigate the use of three species of predatory mites found on such tomato plants to control two important pests and a severe disease, all attacking tomato. In Chapter 1, I therefore evaluated whether the predatory mites *Amblyseius herbicolus* and *Amblyseius tamatavensis* were adapted to tomato plants, and if they were able to control the whitefly *Bemisia tabaci* on tomato when supplemented with alternative food. In Chapter 2, I evaluated the population dynamics of another important tomato pest, *Aculops lycopersici*, in the presence of the predatory mites *A. herbicolus* and *Homeopronematus anconai*. Furthermore, in this chapter I assessed the ability of *H. anconai* to reduce the progress of the fungus *Oidium neolycopersici* on tomato plants. In Chapter 3, I verified whether reciprocal intraguild predation occurs between *A. herbicolus* and *H. anconai* and explored whether the interaction between these predators affects biological control of *A. lycopersici* and *O. neolycopersici*.

References

Arthurs S, McKenzie CL, Chen J, Dogramaci M, Brennan, M, Houben K, Osborne L (2009) Evaluation of *Neoseiulus cucumeris* and *Amblyseius swirskii* (Acari: Phytoseiidae) as biological control agents of chilli thrips, *Scirtothrips dorsalis* (Thysanoptera: Thripidae) on pepper. Biological control, 49: 91-96.

Belcher DW, Thurston R (1982) Inhibition of movement of larvae of the convergent lady beetle by leaf trichomes of tobacco. Environmental Entomology, 11: 91–94. https://doi.org/10.1093/ ee/11.1.91

Calvo FJ, Bolckmans K, Belda JE (2011) Control of *Bemisia tabaci* and *Frankliniella occidentalis* in cucumber by *Amblyseius swirskii*. Biocontrol, 56:185–192.

Calvo FJ, Knapp M, van Houten YM, Hoogerbrugge H, Belda, J.E (2015) *Amblyseius swirskii*: What made this predatory mite such a successful biocontrol agent? Experimental and Applied Acarology, 65: 419-433.

Carrillo D, Peña JE, Capinera JL (2008) Effect of host plants on successful parasitism by *Haeckeliania sperata* (Hymenoptera: Trichogrammatidae) on *Diaprepes abbreviatus* (Coleoptera: Curculionidae) eggs. Environmental Entomology, 37:1565–1572

Castagnoli M, Liguori M, Simoni S (1999) Effect of two different host plants on biological features of *Neoseiulus californicus* (Mcgregor). International Journal of Acarology, 25:145–150

Cedola CV, Sanchez NE, Liljesthrom GG (2001) Effect of tomato leaf hairiness on functional and numerical response of *Neoseiulus californicus* (Acari: Phytoseiidae). Experimental and Applied Acarology, 25:819–831

Cock MJW, van Lenteren JC, Brodeur J, Barratt BIP, Bigler F, Bolckmans K, Cônsoli FL, Haas F, Mason PG, Parra JRP (2010) Do new access and benefit sharing procedures under the convention on biological diversity threaten the future of biological control? BioControl 55:199–218

Eisner T, Eisner M, Hoebeke ER (1998) When defense backfires: Detrimental effect of a plant's protective trichomes on an insect beneficial to the plant. PNAS, 95:4410–4414. https://doi.org/10.1073/PNAS.95.8.4410

Elsey K.D (1974) Influence of plant host on searching speed of two predators. Entomophaga, 19: 3–6. https://doi.org/10.1007/ BF02371503

Ghasemzadeh S, Leman A, Messelink GJ (2017) Biological control of *Echinothrips americanus* by phytoseiid predatory mites and the effect of pollen as supplemental food. Experimental and Applied Acarology, 73: 209-221.

Glas JJ, Schimmel BC, Alba JM, Escobar-Bravo R, Schuurink RC, Kant MR (2012) Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. International journal of molecular sciences, 13: 17077-17103.

Guedes R, Smagghe G, Stark J, Desneux N (2016) Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. Annual Review of Entomology, 61: 43–62

Huffaker C, Kennett C (1953) Developments toward biological control of cyclamen mite on strawberries in California. Journal of Economic Entomology 46: 802–812.

Hyland C, Bradman A, Gerona R, Patton S, Zakharevich I, Gunier RB Klein K (2019) Organic diet intervention significantly reduces urinary pesticide levels in US children and adults. Environmental Research, 171: 568-575.

Janssen A, van Rijn PCJ (2021) Pesticides do not significantly reduce arthropod pest densities in the presence of natural enemies. Ecology Letters 24:2010–2024. https://doi.org/10.1111/ele.13819

Jepson PC, Murray K, Bach O, Bonilla MA, Neumeister L (2020) Selection of pesticides to reduce human and environmental health risks: a global guideline and minimum pesticides list. The Lancet Planetary Health,4: 56-63.

Kant MR, Jonckheere W, Knegt B, Lemos F, Liu J, Schimmel BCJ, Villarroel CA, Ataide LMS, Dermauw W, Glas JJ, Egas M, Janssen A, van Leeuwen T, Schuurink RC, Sabelis MW, Alba JM (2015) Mechanisms and ecological consequences of plant defence induction and suppression in herbivore communities. Annals of Botany 115:1015–1051. https://doi.org/10.1093/AOB/MCV054

Kashyap RK, Kennedy GG, Farrar RR (1991) Behavioral response of *Trichogramma pretiosum* Riley and *Telenomus sphingis* (Ashmead) to trichome/methyl ketone mediated resistance in tomato. Journal of Chemical Ecology, 17: 543–556. https://doi.org/10.1007/ BF00982125

Keller MA (1987) Influence of leaf surfaces on movements by the hymenopterous parasitoid *Trichogramma exiguum*. Entomologia Experimentalis et Applicata, 43: 55–59. https://doi.org/10.1111/ j.1570-7458.1987.tb02202.x

Kennedy GG (2003) Tomato, pests, parasitoids, and predators: Tritrophic interactions involving the genus *Lycopersicon*. Annual Review of Entomology 48:51–72. https://doi.org/10.1146/ANNUREV.ENTO.48.091801.112733

Krimmel BA, Pearse IS (2014) Generalist and sticky plant specialist predators suppress herbivores on a sticky plant. Arthropod– Plant Interactions, 8: 403–410. https://doi.org/10.1007/s1182 9-014-9318-z

Legarrea S, Janssen A, Dong L, Glas JJ, van Houten YM, Scala A, Kant MR (2022) Enhanced top-down control of herbivore population growth on plants with impaired defences. Functional Ecology 36:2859-2872

Messelink GJ, Bennison J, Alomar O, Ingegno BL, Tavella L, Shipp L, Palevsky E, Wäckers FL (2014) Approaches to conserving natural enemy populations in greenhouse crops: current methods and future prospects. BioControl, 59, 377-393.

Messelink GJ, van Maanen R, van Steenpaal SE, Janssen A (2008) Biological control of thrips and whiteflies by a shared predator: two pests are better than one. Biological Control, 44: 372-379.

Messelink GJ, Van Steenpaal SE, Ramakers PM (2006) Evaluation of phytoseiid predators for control of western flower thrips on greenhouse cucumber. BioControl, 51: 753-768.

Nomikou M, Sabelis MW, Janssen A (2010. Pollen subsidies promote whitefly control through the numerical response of predatory mites. Biocontrol, 55: 253-260.

Obrycky JJ, Tauber MJ (1984) Natural enemy activity on glandular pubescent potato plants in the greenhouse: An unreliable predictor of effects in the field. Environmental Entomology, 13: 679–683. https://doi.org/10.1093/ee/13.3.679

Paspati A, Rambla JL, Gresa MPL, Arbona V, Gómez-Cadenas A, Granell A, González-Cabrera J, Urbaneja A (2021) Tomato trichomes are deadly hurdles limiting the establishment of *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae). Biological Control 157:104572

Riddick EW, Simmons AM (2014) Do plant trichomes cause more harm than good to predatory insects? Pest Management Science 70:1655–1665. https://doi.org/10.1002/PS.3772

Romeis J, Shanower TG, Zebitz CPW (1999) Why *Trichogramma* (Hymenoptera: Trichogrammatidae) egg parasitoids of *Helicoverpa armigera* (Lepidoptera: Noctuidae) fail on chickpea. Bulletin of Entomological Research, 89: 89–95. https://doi.org/10.1017/ S0007485399000115

Rutledge CE, Robinson AP, Eigenbrode SD (2003) Effects of a simple plant morphological mutation on the arthropod community and the impacts of predators on a principal insect herbivore. Oecologia, 135:39–50. https://doi.org/10.1007/s00442-002-1114-2

Sabelis MW, Janssen A, Bruin J, Bakker FM, Drukker B, Scutareanu P, van Rijn PCJ (1999) Interactions between arthropod predators and plants: A conspiracy against herbivorous arthropods?, in: Bruin J, van der Geest LPS, Sabelis MW (Eds.), Ecology and Evolution of the Acari. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 207–229.

Simmons AT, Gurr GM (2005) Trichomes of *Lycopersicon* species and their hybrids: Effects on pests and natural enemies. Agricultural Forest Entomology, 7:265–276. https://doi.org/10.1111/J.14619555.2005.00271.X

Symondson WOC, Sunderland KD, Greenstone MH (2002) Can generalist predators be effective biocontrol agents? Annual review of entomology, 47: 561-594.

Thaler JS (1999) Jasmonate-inducible plant defences cause increased parasitism of herbivores. Nature, 399: 686–688. https://doi. org/10.1038/21420

UN Human Rights Council (2017) Report of the special rapporteur on the right to food. 34th Regular Session. United Nations. Geneva - Switzerland.

van Haren RJF, Steenhuis MM, Sabelis MW, de Ponti OMB (1987) Tomato stem trichomes and dispersal success of *Phytoseiulus persimilis* relative to its prey *Tetranychus urticae*. Experimental and Applied Acarology, 3: 115–121. https://doi.org/10.1007/BF012 70473

van Lenteren JC, Bueno VHP (2003) Augmentative biological control of arthropods in Latin America. BioControl 48:123–139

van Lenteren JC, Woets J (1988) Biological and integrated pest control in greenhouses. Annual Review of Entomology 33:239–269

van Lenteren JC (2000) Success in biological control of arthropods by augmentation of natural enemies. In Biological control: measures of success. Springer, Dordrecht, pp. 77-103.

van Lenteren JC (2012) The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. BioControl, 57: 1-20.

van Lenteren, JC, Bolckmans K, Köhl J, Ravensberg WJ, Urbaneja A (2018) Biological control using invertebrates and microorganisms: plenty of new opportunities. BioControl, 63: 39-59.

van Maanen R, Vila E, Sabelis MW, Janssen A (2010) Biological control of broad mites (*Polyphagotarsonemus latus*) with the generalist predator *Amblyseius swirskii.* Experimental and Applied Acarology, 52: 29-34.

Verheggen FJ, Capella Q, Schwartzberg EG, Voigt D, Haubruge E (2009) Tomato-aphid-hoverfly: A tritrophic interaction incompatible for pest management. Arthropod–Plant Interactions, 3: 141–149. https://doi.org/10.1007/s11829-009-9065-8

Wei J; Yan L; Ren Q; Li C; Ge F; Kang LE (2012) Antagonism between herbivore-induced plant volatiles and trichomes affects tritrophic interactions. Plant Cell Environment, 36: 315-327

Wimmer D, Hoffmann D, Schausberger P (2008) Prey suitability of western flower thrips, *Frankliniella occidentalis*, and onion thrips, *Thrips tabaci*, for the predatory mite *Amblyseius swirskii*. Biocontrol Science and Technology, 18: 533-542.

CHAPTER I

A predatory mite as potential biological control agent of *Bemisia tabaci* on tomato plants

André C. Cardoso[1,5]· Ítalo Marcossi[1,5]· Morgana M. Fonseca[2,5]· Milena O. Kalile[1]·
Leonardo S. Francesco[1]· Angelo Pallini[1]· Thomas V. M. Groot[3]· Arne Janssen[1,4]

[1] Department of Entomology, Section of Acarology, Federal University of Viçosa, Av. PH Rolfs S/N, 36570-900, Viçosa, MG, Brazil

[2] Koppert do Brasil, Piracicaba, SP, Brazil

[3] Koppert Biological Systems, Berkel en Rodenrijs, the Netherlands

[4] IBED, Evolutionary and Population Biology, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, the Netherlands

[5] These authors contributed equally

Part of this chapter was presented as a masters thesis by André Costa Cardoso entitled: Search for predatory mites to control tomato pests

Chapter formatted in the norms of the journal: Journal of Pest Science

Abstract

Several natural enemies are known to be predators of the whitefly *Bemisia tabaci*, which is one of the most invasive pests worldwide and has developed high resistance to pesticides. However, biological control of this pest on tomato is often difficult because the plant's glandular trichomes can release substances that are toxic to arthropods and may hinder the foraging of natural enemies. Therefore, adaptation of natural enemies to this crop is one of the selection criteria for potential biocontrol agents. We collected predatory mites from wild and feral tomato plants and found the species *Amblyseius herbicolus* and *A. tamatavensis*. Whereas the latter is known to feed on whitefly eggs, we investigated the ability of *A. herbicolus* to develop and reproduce when feeding on this prey stage, and assessed whether both species can feed and develop on whitefly crawlers. To verify the adaptation of these predators to tomato, we assessed their ability to disperse on tomato plants and their establishment on clean tomato plants with pollen as an alternative food. Finally, we evaluated whether the predators were effective in controlling *B. tabaci* on tomato plants with different pollen dosages as alternative food. We show that both predators fed and reproduced on immature whiteflies. *Amblyseius herbicolus* established and dispersed better on tomato plants supplemented with cattail pollen than *A. tamatavensis* and only this predator was able to control *B. tabaci* in two population dynamics experiments. Our results suggest that *A. herbicolus* is better adapted to tomato than *A. tamatavensis* and may therefore be a promising biocontrol agent in tomato.

Key words: Biological control · Tomato · Phytoseiidae · Whitefly ·

Introduction

Tomato (*Lycopersicon esculentum* Miller) is one of the most important crops in the world (Naika et al. 2005, Collins et al. 2022). One of the biggest challenges for tomato growers is the susceptibility of tomatoes to attacks by numerous pests and diseases (Picanço et al. 2007; Desneux et al. 2010; Hanssen et al. 2010; Simmons et al. 2018; Wakil et al. 2018; Jones 2021). One of the key pests of tomato is the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (Brown et al. 1995). This pest can seriously damage tomato plants by feeding directly on plant sap and by excreting honeydew, which decreases the rate of photosynthesis and consequently leads to a significant yield reduction (Brown et al. 1995; Oliveira et al. 2001; Calvo et al. 2009). Moreover, *B. tabaci* can transmit a large number of plant-pathogenic viruses (Jones 2003; Navas-Castillo et al. 2011; Andreason et al. 2020), including the devastating tomato yellow leaf curl virus (TYLCV) (Navot et al. 1991; Varma and Malathi 2003) and the tomato chlorosis virus (ToCV) (Wisler et al. 1998). Together, whiteflies and diseases can cause crop losses of up to 100% (Picó et al. 1996; Velasco et al. 2008). Whiteflies are usually controlled on tomato plants with a high number of pesticide applications, from sowing to fruit harvesting (Picanço et al. 1997; Palumbo et al. 2001; Horowitz et al. 2007; Perring et al. 2018). However, besides inflicting damage on human health and ecosystems worldwide, this excessive use of pesticides has led to severe and widespread resistance by whiteflies and other tomato pests (Elver and Tuncak 2017; Hyland et al. 2019; Rani et al. 2021). Moreover, a meta-analysis of field experiments found that chemical pest control is often ineffective in the presence of natural enemies (Janssen and van Rijn 2021). Therefore, there is a growing demand for environmentally safer pest control methods in order to promote the long-term viability of agricultural systems (Pretty and Bharucha 2014).

One sustainable pest management practice is augmentative biological control; the release of large numbers of natural enemies to suppress pest densities (van Lenteren 2000, 2012; van Lenteren et al. 2018). Predatory mites, mostly from the family Phytoseiidae, are the dominant agents in commercial augmentative biological control in many countries, currently representing over 60% of the global market for arthropod natural enemies (van Lenteren 2012; Knapp et al. 2018; Vangansbeke et al. 2023). Amongst the factors that explains the success of predatory mites in biological control programs is their generalist feeding habits, which enables then to control multiple pest species (Messelink et al. 2009, 2012; Janssen and Sabelis 2015; Knapp et al. 2018) and their short generation times, resulting in rapid numerical responses to increased pest densities (Helle and Sabelis 1985). Moreover, many of these predators also feed on plant-provided food such as pollen and nectar, which allows them to be introduced before the pest is present or to remain in the crop when target pest densities are low (van Rijn et al. 2002; Messelink et al. 2014; Janssen and Sabelis 2015). Various natural enemies, such as parasitoids, predatory mirids and predatory mites, are commercially employed in augmentative biological control of *B. tabaci*, but their success on tomato lags behind.

Phytoseiid mites are also often ineffective at controlling pests on tomato (van Haren et al. 1987; Gillespie and Quiring 1994; Cedola et al. 2001; Kennedy 2003; Paspati et al. 2021). This is likely due to contact with glandular trichomes and their exudates (van Haren et al. 1987; Sato et al. 2011; van Houten et al. 2013; Paspati et al. 2021). Glandular trichomes are outgrowths of the epidermis in which specialized metabolites are synthesized and stored (Schuurink and Tissier 2020). While trichomes act as a broad-spectrum direct defense against herbivores (Glas et al. 2012), they can also increase the mortality of natural enemies (Belcher and Thurston 1982; van Haren et al. 1987; Verheggen et al. 2009; Paspati et al. 2021). In addition, trichomes can affect the density of herbivorous prey and hinder the establishment and dispersal of predators on the plant (van Lenteren et al. 1995; Simmons and Gurr 2005; Carrilo et al. 2008; Paspati et al. 2021; Legarrea et al. 2022). Therefore, finding phytoseiid mites adapted to tomato is a significant challenge.

In an attempt to find tomato-adapted predatory mites, we sampled tomato plants in the state of Minas Gerais, Brazil. Commercial tomato crops with pesticide applications usually did not contain any predatory mites, therefore, predators were collected from cultivated and feral tomato plants from gardens in urban and rural areas. *Amblyseius herbicolus* Chant (Acari: Phytoseiidae) was the most abundant species of the predatory mites found. This predator reproduces through thelytokous parthenogenesis, hence, populations consist of females only (de Moraes and Mesa 1988). *Amblyseius herbicolus* is a generalist predatory mite, abundant in several crops and it is known that it can prey on several crop pests such as immature whiteflies, broad mites, and spider mites, of which the latter are also tomato pests (Rodríguez-Cruz et al. 2013; Cavalcante et al. 2015; Duarte et al. 2015). It is also able to feed on alternative foods such as pollen (Rodríguez-Cruz et al. 2013; Marcossi et al. 2020). Another predatory mite found on tomato plants was *Amblyseius tamatavensis* Blommers (Acari: Phytoseiidae). This species has also shown potential to prey on whitefly eggs (Cavalcante et al. 2015, 2017; Barbosa et al. 2019). One of these latter studies demonstrated that *A. tamatavensis* reduced the densities of whiteflies in the presence of pollen on bell pepper plants (Cavalcante et al. 2017).

Although is known that these predators can feed on *B. tabaci*, further experiments are still needed to determine their suitability to control this pest on tomato plants. Here, we report the ability of *A. herbicolus* to develop and reproduce when feeding on whitefly eggs. Moreover, we measured oviposition and predation on whitefly crawlers by *A. herbicolus* and *A. tamatavensis.* To verify the adaptation of these predators to tomato, we assessed their ability to disperse on tomato plants and whether they can establish on clean tomato plants with pollen as alternative food. Finally, we evaluated whether thes predator were effective in controlling *B. tabaci* on tomato plants using different pollen dosages.

Materials and Methods

Plant material

Tomato seeds (*Solanum lycopersicum* var. Aguamiel (EX V305), Limagrain®, imported by Vilmorin Seed Generation in Brazil) were sown in polystyrene trays (8 x 16 cells) in a commercial plant substrate enriched with macro and micronutrients (MECPLANT® - Mec Prec, Telêmeco Borba). Twenty days after germination, the seedlings were transplanted to plastic pots (2 - 3.5 L, depending on the experiment) with the same substrate. The plants were fertilized every week with a solution of 50g of N-P-K (20-05-20) and 100g of simple superphosphate in 20L of water and they were tied to a bamboo stick with a string for support. The plants were kept inside cages in a room with natural light at 23 ± 3 °C and 70 ± 10% relative humidity. Cattail pollen used in all experiments was collected from *Typha* sp. plants on the campus of the Federal University of Viçosa. The pollen was dried in an oven at 60º C for 24 h and stored in a freezer at -20 °C according to recommendation (Hagedorn 1968; Pernal and Currie 2000).

Whitefly rearing

A rearing unit of *Bemisia tabaci* (Gennadius) biotype B was started with individuals obtained from the Laboratory of Integrated Pest Management of the Federal University of Viçosa. They were kept on tomato plants inside cages (0.5 x 0.5 x 1.0 m^3). A new tomato plant with at least four completely developed leaves was added to each cage every week. The oldest plants were removed from the cage once a month. The cages were kept in a room with natural light at 23 ± 3 °C and 70 ± 10% relative humidity.

Mite rearings

To collect predatory mites, leaves attacked by pests were removed from selected plants in the field, placed in paper bags, and kept in a cooler with ice packs to reduce predatory mite activity until laboratory evaluation. The leaves were inspected with a stereomicroscope and predatory mites were transferred to arenas made of PVC sheets (15 × 10 cm) on top of foam pads (h = 3 cm), which were kept in plastic trays (29 × 14 × 4 cm) filled with water. The edges of the arenas were wrapped in wet tissue paper, serving as a barrier for mite escapes and as a water source (van Rijn and Tanigoshi 1999). Small pieces of cotton wool were placed on the arenas as oviposition sites and were covered with pieces of tent-shaped PVC sheet (1.5 cm^2) for shelter. Initially, *Typha sp.* pollen and bee pollen (Santa Barbara®, Apiários Macklllani Ltda, Minas Gerais, Brazil) were supplied as food twice per week, until we noticed that *Typha sp.* pollen alone was sufficient to maintain laboratory cultures. After establishing a culture, microscope slides were made and the species was identified as *Amblyseius herbicolus* by Manoel Guedes Correa Gondim Junior from the Universidade Federal Rural de Pernambuco (UFRPE).

A culture of *Amblyseius tamatavensis* was maintained in our laboratory, and had been started with individuals found in our culture of *Tetranychus urticae* Koch (Acari: Tetranychidae) on tomato plants. The arenas of *A. tamatavensis* were made of black plastic dishes (Ø = 13 cm, h = 2 cm) placed on top of foam pads (h= 3 cm), which were kept in plastic trays (29×14×4 cm) filled with water. These predators were fed with cattail pollen and with the mite species *Thyreophagus cracentiseta* Astigmatina (Sarcoptiformes) (Barbosa, OConnor & Moraes) mixed with wheat bran once a week. The culture of *T. cracentiseta* was started with individuals obtained from the Laboratory of Acarology of the Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), in 2019. These astigmatids were reared on sterilized wheat bran in plastic pots (ø = 10; h = 15 cm) covered with mesh (90 µm). Rearing arenas of all mite species were kept in a room at 24±1 °C, 70±10% RH, 12:12 L:D.

Oviposition and development of *A. herbicolus* on whitefly eggs

We first investigated whether *A. herbicolus* was able to reproduce and develop on *B. tabaci* eggs. Because it is already known that *A. tamatavensis* can do so, we did not carry out such experiments with this species, (Cavalcante et al. 2015, 2017; Barbosa et al. 2019). Leaves of a tomato plant from the whitefly rearing (infested for c. 3 days) were removed and leaflets were cut into pieces. Each piece was examined with a stereomicroscope to ensure the presence of at least 20 fresh *B. tabaci* eggs (less than 48-h old, based on their light green coloration) and subsequently added to a Petri dish (Ø = 5 cm, 1.5 cm high). A piece of wet filter paper as source of moisture was placed inside each dish and the dishes were covered with transparent lids. As control, one mg of pollen with a clean piece of leaflet was placed in another set of those dishes. A predator larva was collected from the rearing unit with a fine brush and placed in each dish.

The predators were checked once a day to verify survival and development until adulthood. After becoming adults, individuals were checked once a day to verify oviposition. Every day, the piece of leaf with the food was replaced, and predator eggs were removed to prevent cannibalism. Oviposition of predators was recorded during five more days. Fifteen and sixteen replicates were done for *B. tabaci* eggs and *Typha sp.* pollen respectively. The tests were performed in a room at 23 ± 3 °C, 70 ± 10% relative humidity and 12 hours of light. The effects of diet on survival and development were analysed with a Cox proportional hazards survival analysis of the survival package of R (Therneau 2020). To compare oviposition rates, data were taken from one to five days after the first individuals became adult, which were compared with a linear mixed effects model (LME, package "nlme", Pinheiro et al. 2021) with diet and time as fixed factors and individual as random factor to correct for pseudoreplication. All models here and below were checked with normal error plots and plots of residuals against fitted values. Significance of factors and interactions were determined with likelihood ratio (L.R.) tests. Contrasts among treatments were obtained with the package emmeans with a Tukey correction for multiple comparisons (Lenth 2019). All statistical analyses were done using the software R version 4.2.2 (R Core Team 2022).

Predation and oviposition of *A. herbicolus* on new and old eggs

We evaluated the predation and oviposition rates of *A. herbicolus* on young and old *B. tabaci* eggs (less and more than 48 h old, respectively). The experiment was carried out as above, but we used gravid females of *A. herbicolus*, aged between 4 and 6 days since becoming adults. Predation and oviposition rates were verified every 24 hours during 3 days. Eight replicates were done with exception of the treatment with pollen, which was replicated six times. The first day of oviposition was excluded from the analysis because of the possible effect of the previous diet (Sabelis 1990). Oviposition and predation data were analysed with linear mixed effects models (LME) with replicate as random factor and diet and time as fixed factors.

Predation and oviposition of both predatory mites feeding on whitefly crawlers

We assessed the ability of *A. herbicolus* and *A. tamatavensis* to feed and reproduce on crawlers of *B. tabaci* on tomato leaf discs (Ø = 2 cm) arranged on wet tissue paper (Ø = 3 cm) and placed in Petri dishes as above. Twenty whitefly crawlers of up to 30 hours old were placed on each leaf disc, which is sufficient to avoid prey depletion during the experiment. *Typha* sp. pollen placed on clean leaf discs was offered as food in a control treatment. Gravid females of each predator species were placed in Petri dishes as above. The tests were performed in a room as above. Predation and oviposition rates were verified every 24 hours during 4 days. After each evaluation, each predator was transferred to a Petri dish containing fresh prey. Fifteen replicates were done per treatment. The first day of oviposition was excluded as above. The numbers of crawlers consumed and eggs produced were analysed with a LME with identity of the individual mites as a random factor to correct for repeated measures, predator species as categorical factor and time as continuous factor. The interaction between predator species and time was also included, but was not significant.

Adaptation to tomato plants

Here we investigated the ability of the predatory mites to move and reproduce on tomato plants with cattail pollen during seven days. Tomato plants were three weeks old (about 20 cm high) with three fully developed leaves. Pollen was placed on the release leaflet (the second leaflet from the insertion of the first leaf into the stem) at the beginning of the experiment and on the target leaflet (the leaflet in front of the release leaflet) on the fourth day. Eight gravid females of each predator species, aged between four and six days since becoming adult, were released on the release leaflet of each plant (n = 11 plants). Wet cotton wool was placed around the base of the stem to confine predators to the plants. The position of the plants was randomized in a room at 23 ± 3 °C, 70 ± 10% relative humidity and 12 hours of light. On the seventh day, the predatory mites were scored as dead or alive on the release and target leaflets and on the rest of the plant. Total numbers of dead plus alive predators of the two species on the entire plants were compared with a generalized linear model (GLM) with a Poisson error distribution (log link), numbers of alive individuals on the release plus target leaflet were log(x + 1) transformed and compared with a GLM with Gaussian error distribution (identity link), and the distribution of the alive individuals on the release and target leaflets were compared between the two species with a GLM with a quasi-binomial error distribution (logit link).

We also tested the population growth and movement of the predatory mites on entire tomato plants with cattail pollen during thirty days. Fifteen young females of *A. herbicolus* or *A. tamatavensis* were introduced individually on the lowest (=oldest) leaf from each (four-leaf stage) tomato plant (16 plants per treatment). *Typha* sp. pollen was provided as food two times per week. The plants were kept together outside (mean temperature 24.3°C, range 18.7–27.7° C; mean relative humidity 85%, range 59–96%), hence, could be infested by different organisms. During the experimental period, plants were allowed to develop from four to nine leaves, but the plant apices were subsequently removed, thus maintaining a maximum plant size of nine leaves. Four plants were evaluated per week by cutting them into pieces and storing the pieces in separate boxes according to the stratum of the plant. We assessed the densities of both predatory mites on three different plant strata (bottom, middle and top) under a stereo microscope (25× magnification; Zeiss, Oberkochen, Germany). Moreover, the presence of pests and other organisms that occurred naturally was noted. Densities of the two predatory mite species were compared using a GLM with a quasi-Poisson error distribution, with species, time and their interaction as factors. We also assessed differences in the proportions of the total population consisting of juveniles to detect differences in reproduction on the plants between the two species. This was done with a GLM with a binomial error distribution (logit link). Subsequently, we analysed the distribution of *A. herbicolus* (log(x+1)-transformed numbers) over the three plant strata with a linear mixed effects model with time and strata as factors and individual plant as random factor.

Dynamics on plants

We performed two population dynamics experiments on plants to evaluate the ability of predators to control whitefly populations on tomato plants. In the first experiment, we evaluated whether preventive releases of *A. herbicolus* and *A. tamatavensis* with the addition of pollen could prevent future infestations of whiteflies. We carried out this experiment in 3 large outdoor screen cages (2 x 2 x 2 m – Howitec Netting®), each with 16 tomato plants (mean temperature 21.4°C, range 14.8–25° C; mean relative humidity 58%, range 33–82%). To prevent cross-contamination, the plants were spaced out in the cage, and each plant pot was put in a dish with water, serving as barrier for dispersing mites. Initially, a tomato leaf disc (3 cm^2) with 5 young female predatory mites (4-6 days since becoming adult) was introduced on the bottom, middle and top leaf from each young (4-leaf stage) tomato plant in two cages, hence, in total 15 mites per plant. Plants in one cage received *A. herbicolus*, they received *A. tamatavensis* in a second cage, and no predators were released in the third cage. One milligram of *Typha* sp. pollen was applied above the newly developed leaves of each plant twice per week, including to those in the cage without predators. Most of the pollen fell onto the new leaves, but some also fell onto the older leaves. Plants were not sampled during the first 3 weeks in order not to cause disturbance. Three weeks after the release of predatory mites, we collected whiteflies from the rearing cages using an aspirator. The whiteflies were placed in a refrigerator at 4ºC for 2 minutes to reduce their activity, and subsequently, three pairs of whiteflies were released on each plant in each cage inside a clip cage. Clip cages were placed on the third leaf of all plants and were removed after 2 days, leaving the adult whiteflies and their eggs behind. This procedure was repeated in week 5, so a total of 6 pairs of whiteflies were released on each plant. Note that adult whiteflies were able to migrate among plants of the same treatment after being released, but juveniles were restricted to the plants. The number of predatory mites was evaluated weekly from the 3rd week (day 0) and the number of juvenile whiteflies from the 4th week (day 7) after the introduction of predatory mites. Adult whiteflies were not counted, as they could move from plant to plant, especially when disturbed while counting predators and immature whiteflies. The evaluations were carried out using a handheld magnifying glass and head magnifier with light, sampling all the leaves of each tomato plant. At the last sampling, the plants were cut and stored in separate boxes for evaluation in the laboratory under a stereo microscope. During this last evaluation, it was therefore also possible to assess the densities of whitefly eggs on the plants. Powdery mildew occurred during this experiment, became severe from the 7th week onwards, which forced us to end the experiment after thirty-two days. Numbers of juvenile whiteflies (log(x+1)-transformed) were compared among treatments with an LME with predator treatment and time as factors and plant as random factor. Numbers of whitefly eggs were compared with a Kruskal Wallis ANOVA with a Holm test for pairwise comparisons (package FSA, Ogle et al. 2022).

A second experiment was done to evaluate the capacity of *A. herbicolus* to reduce whitefly populations on tomato plants when supplying a larger amount of pollen distributed throughout the entire plant. It was conducted in six BugDorm-4F insect cages (0.5 x 0.5 x 1.0 m) inside a greenhouse. A tomato plant with four developed leaves and ca. 20 cm high was placed inside each cage. Half of the plants received fifteen adult females of *A. herbicolus*. On the same day, three pairs of adult whiteflies (6 ± 1 days of age) were introduced in each of the six cages. All plants also received 15 mg of *Typha* sp. pollen distributed over all leaves of the plants with the aid of a brush. The experiment was evaluated twice a week by carefully handling all leaves of each plant. Whitefly adults were counted while they were sucked into a pipette tip (1000 µl; Nichiryo, Japan) connected to a transparent hose (ø = 1 cm) closed with a mesh (size 170 µm) to avoid counting the same insect more than once. Subsequently, adult predatory mites were carefully counted on all leaves. Then, more pollen was added to the plants and the adult whiteflies were reintroduced to the cages by inserting the pointed part of the pipette tip closed with Parafilm "M" (Bemis Flexible Packaging, Neenah, WI 54956, USA) into the soil. Plants without predatory mites were always evaluated first to avoid contamination of these control plants with predators. The temperature during the experiment ranged between 13 - 26º C. Differences in numbers of whiteflies per plant between treatments were analysed with an LME with log(x+1)-transformed numbers of whiteflies as dependent variable, time and treatment as fixed factors and replicate as random factor.

Results

Oviposition and development of *A. herbicolus* on whitefly eggs

Nineteen out of 20 juvenile *A. herbicolus* survived on a diet of *B. tabaci* eggs, whereas all 20 juveniles survived on a pollen diet (Fig. 1), but development was significantly faster on a diet of pollen than on whitefly eggs (Fig. 1; Cox proportional hazards: Likelihood ratio (L.R.) = 11.67, d.f. = 1, $p < 0.001$). Per diet, oviposition rates differed significantly through time (Fig. 1, LME, L.R. = 137.9, d.f. = 1, $p < 0.0001$), but there was no significant effect of diet (Fig. 1, LME, L.R. = 2.86, d.f. = 1, $p = 0.091$). Despite the difference in development until adulthood, there was no significant difference in oviposition through time between the two diets (Fig. 1, interaction of diet with time: L.R. = 0.84, d.f. = 1, $p = 0.36$).

Predation and oviposition of *A. herbicolus* on new and old eggs

The predation rate on old eggs was significantly lower than young eggs (Fig. S1a, LME, L.R. = 70.59, d.f. = 1, $p < 0.0001$). The oviposition of *A. herbicolus* also differed significantly among diets (Fig. S1b; LME, L.R. = 18.45, d.f. = 2, $p < 0.0001$). On the third day, oviposition of *A. herbicolus* on a diet of old *B. tabaci* eggs was lower than when feeding on young eggs or pollen (Fig. S1a, b).

Predation and oviposition of both predator mites feeding on whitefly crawlers

The average consumption of *B. tabaci* crawlers did not differ significantly between the two predator species (Fig. 2a; LME: L.R. = 2.04, d.f. = 1, $p = 0.15$) or among days (L.R. = 1.85, d.f. = 1, p = 0.17). There was a significant difference in oviposition rates of both predators on the two diets (Fig. 2b; L.R. = 35.2, d.f. = 3, $p < 0.0001$) and oviposition did not differ among days (L.R. = 1.39, d.f. = 1, $p = 0.24$). Oviposition of both predators was significantly higher on a diet of pollen than on *B. tabaci* crawlers and oviposition of *A. tamatavensis* on crawlers was higher than that of *A. herbicolus* on the same diet (Fig. 2b).

Adaptation to tomato plants

The two species showed no differences in dispersal between leaflets from a leaf (Fig. 3a; GLM: Deviance = 1.48, d.f. = 1, $p = 0.27$). The total number of alive and dead predators after 7 days did not differ significantly between predator species (Fig. 3a; GLM: Deviance = 0.0047, d.f. = 1, $p = 0.95$), showing that reproduction of the two predator species did not differ significantly.

There was also no significant difference in the number of alive predators of the two species on the release plus target leaflets (Fig. 3a; GLM: $F_{1,20} = 0.15$, $p = 0.7$).

In contrast, the numbers of *A. herbicolus* were significantly higher than those of *A. tamatavensis* on tomato plants (Fig. 3b; GLM: $F_{1,30} = 22.4$, $p < 0.0001$) and the numbers of predators of both species varied through time ($F_{1,29} = 14.7$, $p < 0.001$). The proportion of juveniles of *A. herbicolus* was also significantly higher than that of *A. tamatavensis* (Fig. 3b; GLM: $F_{1,27} = 31.2$, $p < 0.001$). Whereas *A. tamatavensis* remained only in the lowest stratum, *A. herbicolus* spread towards the other strata near the end of the experiment, however, the distribution of the numbers over the three strata differed significantly (lme: L.R. = 62.0, d.f. = 2, $p < 0.0001$), with numbers in the top and middle stratum being much lower than in the bottom stratum (Fig. 3c). Besides the predatory mites, we found whiteflies, some thrips, astigmatid mites, iolinid mites, powdery mildew and some spiders on the plants (Suppl. Table 1).

Population dynamics

There was a significant difference in densities of immature whiteflies among the three cages through time (LME: interaction of treatment with time: L.R. = 6.87, d.f. = 2, $p = 0.032$). The predator *A. herbicolus* delayed the increase of whiteflies compared to the treatment with *A. tamatavensis* and without predators, but only significantly so at 7 and 14 days after the beginning of the experiment (Fig. 4a). Tomato plants with *A. herbicolus* had significantly lower whitefly egg densities than treatment without predators, but did not differ from plants with *A. tamatavensis* (Fig. 4a; Kruskal–Wallis ANOVA, $Chi^2 = 7.96$, d.f = 2, $p = 0.019$). Whereas *A. tamatavensis* disappeared from all plants, populations of *A. herbicolus* persisted (Fig. 4b).

In the experiment with a higher quantity of pollen applied, the density of whiteflies in the presence of *A. herbicolus* was significantly lower through time than in their absence (Fig. 4c; LME: interaction of predator presence with time: Likelihood ratio = 18.5, d.f. = 1, $p < 0.001$). The difference in whitefly densities between plants with and without predators became apparent and significant from day 47 onwards, when a new generation of adult whiteflies appeared on the plants (Fig. 4c). At the end of the experiment, there were about 91% more adult whiteflies on plants without predatory mites than on plants with *A. herbicolus.* Predator densities increased until reaching an average of one hundred and twenty predators per plant around the thirty-seventh day, after which when they began to decrease. At the end of the experiment there were still about forty predatory mites visible to the naked eye per plant (Fig. 4d).

Discussion

We show that *A. herbicolus* is able to develop on whiteflies eggs (Fig. 1), and both *A. herbicolus* and *A. tamatavensis* fed and reproduced on a diet of whitefly crawlers (Fig. 2). However, *A. herbicolus* established and dispersed better on tomato plants supplemented with cattail pollen than did *A. tamatavensis* (Fig. 3b, c) and only *A. herbicolus* was able to control *B. tabaci* in the two population dynamics experiments (Fig. 4a, c). These results suggest that *A. herbicolus* is better adapted to tomato plants than *A. tamatavensis* and thus, it may be a more efficient biocontrol agent on tomato.

Predation rates of *A. herbicolus* and *A. tamatavensis* on crawlers were lower than those reported for *A. swirskii*, which is commonly used for whitefly control (Nomikou et al. 2004; Janssen and Sabelis 2015; Calvo et al. 2015), but the host plant used in these papers differed from ours. The oviposition rate of *A. herbicolus* fed with crawlers was lower than that of *A. tamatavensis*, however, the eggs produced by *A. herbicolus* will only result in female offspring, which will increase the intrinsic growth rate of this species compared to sexual species with the same oviposition rate (Cicolani 1979; Janssen and Sabelis 1992). Our findings revealed that both predatory mites can efficiently reproduce when feeding on crawlers and cattail pollen, which is contrary to the results of Cavalcante et al. (2015), who reported low oviposition rates for *A. tamatavensis* when fed with cattail pollen. The ability of predatory mites to feed on alternative resources allows their introduction and persistence in crops with low pest densities or before pest invasion, potentially resulting in an increase in predator densities and enhanced pest control (McMurtry and Johnson 1965; Kennett et al. 1979; van Rijn and Sabelis 1993; Nomikou et al. 2010; Delisle et al. 2015; Duarte et al. 2015; Leman and Messelink 2015).

An important characteristic when selecting natural enemies is their adaptability to the host plant. Negative effects of tomato plants on the survival of phytoseiids have been observed frequently (van Haren et al. 1987; Castagnoli et al. 1999; Paspati et al. 2021; Legarrea et al. 2022) due to contact with glandular trichomes and their exudates (van Haren et al. 1987). Secondary metabolites, such as acyl sugars present in tomato trichomes can be highly toxic to mites and can accumulate on their bodies after walking on tomato stems (Paspati et al. 2021). Whereas our results showed no differences in the capacity of *A. tamatavensis* and *A. herbicolus* to move from leaflet to leaflet of the same leaf (Fig. 3a), they did differ significantly in their capacity to move from leaf to leaf and reproduction on entire tomato plants (Fig. 3b). This was probably caused by the higher density of trichomes on tomato stems than on leaves (van Haren et al. 1987). Although *A. herbicolus* initially showed a high mortality rate in the experiment on entire plants, their numbers increased about 4.2 times during the experiment, with a high number of juveniles (Fig. 3b). Possibly, *A. herbicolus* can adapt to tomato defences within a few generations, as was demonstrated for other predators on tomato (Drukker et al. 1997; Wheeler and Krimmel 2015; Sun et al. 2019).

Whereas *A. tamatavesis* was always found close to their point of release, *A. herbicolus* was found in the different strata of the plant, but 96.8% of *A. herbicolus* were found in the lowest stratum (Fig. 3c). Similar results were found by van Houten et al. (2013) for *Amblydromalus limonicus*. In this last study, predatory mites only successfully established on the plants after degradation of tomato trichomes as a result of *Aculops lycopersici* infestations. A recent study showed that predatory mites do not only perform better on tomato plants lacking defensive hairs, but also that they can suppress herbivore densities better and faster on these hairless plants (Legarrea et al. 2022). Based on the limited mobility of mites on tomato plants, different strategies could be used to improve the density of *A. herbicolus* on plants, for example, increasing the frequency of release of the predator on different parts of the plant.

In the population dynamics experiment with both predators and a low dosage of pollen, *A. herbicolus* was better able to control whiteflies than *A. tamatavensis*. Whiteflies were absent during the first 21 days, thus, the only food available for predators was cattail pollen, which was supplied on the newly developed leaves. Hence, predators had to move to new food patches. This resulted in the extinction of *A. tamatavensis* from the plants and a reduction in the densities of *A. herbicolus* (Fig. 4b). Nevertheless, *A. herbicolus* was able to reduce whitefly immature densities (Fig. 4a). Moreover, *A herbicolus* had reduced whitefly egg densities at the end of this experiment by almost half compared to the treatment without predators, but this difference was not significant. As a considerable number of whiteflies were still present on the plants at the end of the experiment, control by *A. herbicolus* was not considered satisfactory. We therefore decided to test this predator with higher quantities of pollen. With more food available, the predator would not need to move frequently to other parts of the plant, thus preventing contact with the stem trichomes and consequently reducing the initial mortality.

Indeed, when a large supply of pollen distributed throughout the plant was offered to *A. herbicolus*, the predator substantially reduced whitefly populations (Fig. 4c). This was certainly due to the high number of predatory mites, with final densities that were on average eight times higher than the release density (Fig. 4d). It is highly likely that the differences in whitefly densities between the two population dynamics experiment was caused by the amount of pollen offered, thus, it is necessary to investigate the ideal pollen dosage to be supplemented to *A. herbicolus*. Moreover, it is important to prevent supplying excess of pollen, because this may result in mold growth on the uneaten pollen and in predators feeding on pollen only. Besides pollen, there are other alternative foods/prey items that can support predator mite populations in crops, such as species of astigmatid mites (Hoogerbrugge et al. 2008; Messelink et al. 2009). These mites have particular potential as an alternative food for predatory mites because of low costs of their mass production (Pirayeshfar et al. 2020). The use of astigmatid mites in greenhouse crops is being increasingly explored in practice (Messelink et al. 2014), and further experiments should evaluate the potential of astigmatid mites as alternative food for *A. herbicolus* on tomato.

To the best of our knowledge, our results are the first to show that a phytoseiid mite can effectively control *B. tabaci* on tomato plants when supplemented with pollen. Moreover, the fact that *A. herbicolus* reproduces through thelytokous parthenogenesis can facilitate their persistence in a crop compared to non-thelytokous species because one individual can give rise to an entire new population. Moreover, thelytoky may be advantageous because adaptive traits of strains will not be diluted due to outcrossing with other strains of this species in the field (Hoy 1985; Hoy and Cave 1986). *Amblyseius herbicolus* is also able to feed on at least three other tomato pests: *Aculops lycopersici*, *Polyphagotarsonemus latus,* and *Tetranychus urticae* (Rodríguez-Cruz et al. 2013; Cavalcante et al. 2015; Duarte et al. 2015; Cardoso 2019). This can reduce the number of natural enemies to be introduced in the crop, thus reducing costs (Messelink et al. 2008). In addition, feeding on more than one pest species can result in better control of those pests through an increase in predator densities (Messelink et al. 2008; Holt and Bonsall 2017).

Taken together, our results demonstrate the potential of *Amblyseius herbicolus* to control whiteflies on tomato plants, both when releasing the predator together with the pest, and when introduced before the pest. Our results do not support the use of *A. tamatavensis* to control whiteflies on tomato plants. Further studies are needed to investigate the possibility of *A. herbicolus* controlling whitefly populations in the field.

References

Andreason SA, Shelby EA, Moss JB et al (2020) Whitefly endosymbionts: biology, evolution, and plant virus interactions. Insects 11: 775

Barbosa, MF, Poletti M, Poletti EC (2019) Functional response of *Amblyseius tamatavensis* Blommers (Mesostigmata: Phytoseiidae) to eggs of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) on five host plants. Biol Control 138: 104030

Belcher DW, Thurston R (1982) Inhibition of movement of larvae of the convergent lady beetle by leaf trichomes of tobacco. Envir Entomol 11:91–94

Brown JK, Frohlich DE, Rosell RC (1995) The sweet potato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? Annu Rev Entomol 40: 511-534

Castagnoli M, Liguori M, Simoni S (1999) Effect of two different host plants on biological features of *Neoseiulus californicus* (Mcgregor). Int J Acarol 25:145–150

Calvo FJ, Bolckmans K, Belda JE (2009) Development of a biological control-based Integrated Pest Management method for *Bemisia tabaci* for protected sweet pepper crops. Entomol Exp Appl 133: 9-18

Calvo FJ, Knapp M, van Houten Y et al (2015) *Amblyseius swirskii*: What made this predatory mite such a successful biocontrol agent? Exp Appl Acarol 65:419–433

Cardoso AC (2019) Search for predatory mites to control tomato pests. MSc thesis, Federal University of Viçosa. Viçosa, Minas Gerais, Brazil

Carrillo D, Peña JE, Capinera JL (2008) Effect of host plants on successful parasitism by *Haeckeliania sperata* (Hymenoptera: Trichogrammatidae) on *Diaprepes abbreviatus* (Coleoptera: Curculionidae) eggs. Environ Entomol 37:1565–1572

Cavalcante ACC, dos Santos VLV, Rossi LC, Moraes GJ (2015) Potential of five Brazilian populations of Phytoseiidae (Acari) for the biological control of *Bemisia tabaci* (Insecta: Hemiptera). J Econ Entomol 108:29–33

Cavalcante ACC, Mandro, ME, Paes ER, de Moraes GJ (2017) *Amblyseius tamatavensis* Blommers (Acari: Phytoseiidae) a candidate for biological control of *Bemisia tabaci* (Gennadius) biotype B (Hemiptera: Aleyrodidae) in Brazil. Int J Acarol 43: 10-15

Cedola CV, Sanchez NE, Liljesthrom GG (2001) Effect of tomato leaf hairiness on functional and numerical response of *Neoseiulus californicus* (Acari: Phytoseiidae). Exp Appl Acarol 25:819–831

Cicolani B (1979) The intrinsic rate of natural increase in dung macrochelid mites, predators of *Musca domestica* eggs. Bolletino Zool 46:171–178

Collins EJ, Bowyer C, Tsouza A, Chopra M (2022) Tomatoes: An extensive review of the associated health impacts of tomatoes and factors that can affect their cultivation. Biology 11:239

de Moraes GJ, Mesa NC (1988) Mites of the family Phytoseiidae (Acari) in Colombia, with descriptions of three new species. Int J Acarol 14:71–88

Delisle JF, Shipp L, Brodeur J (2015) Apple pollen as a supplemental food source for the control of western flower thrips by two predatory mites, *Amblyseius swirskii* and *Neoseiulus cucumeris* (Acari: Phytoseiidae), on potted chrysanthemum. Exp Appl Acarol 65:495–509

Desneux N, Wajnberg E, Wyckhuys KA et al (2010) Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. J Pest Sci 83:197-215

Duarte MV, Venzon M, Bittencourt MC de S et al (2015) Alternative food promotes broad mite control on chilli pepper plants. BioControl 60:817–825

Drukker B, Janssen A, Ravensberg W, Sabelis MW (1997) Improved control capacity of the mite predator *Phytoseiulus persimilis* (Acari: Phytoseiidae) on tomato. Exp Appl Acarol 21:507–518

Elver H, Tuncak B (2017) Report of the Special Rapporteur on the right to food, UN doc. A/HRC/34/48. Human Rights Council, Geneva Switzerland

Gillespie DR, Quiring DJM (1994) Reproduction and longevity of the predatory mite, *Phytoseiulus persimilis* (Acari: Phytoseiidae) and its prey, *Tetranychus urticae* (Acari: Tetranychidae) on different host plants. J Entomol Soc Brit Columbia 91:3–8

Glas JJ, Schimmel BCJ, Alba JM, Escobar-Bravo R, Schuurink RC, Kant MR (2012) Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. Int J Mol Sci 13: 17077–17103

Hagedorn HH (1968) Effect of the age of pollen used in pollen supplements on their nutritive value for the honeybee. I. Effect on thoracic weight, development of hypopharyngeal glands and brood rearing. J Apic Res 7:89–95

Hanssen IM, Lapidot M, Thomma BP (2010) Emerging viral diseases of tomato crops. Mol Plant Microbe Interact 23:539-548

Helle H, Sabelis MW (1985) Spider mites: their biology, natural enemies and control. Elsevier, Amsterdam

Holt RD, Bonsall MB (2017) Apparent competition. Annu Rev Ecol Evol Syst 48:447–471

Hoogerbrugge H, van Houten Y, van Baal E, Bolckmans K (2008) Alternative food sources to enable establishment of *Amblyseius swirskii* (Athias-Henriot) on chrysanthemum without pest presence. IOBC/WPRS Bull 32:79–82

Horowitz R, Denholm I, Morin S (2007) Resistance to insecticides in the TYLCV vector, *Bemisia tabaci*. In: Czosnek H (ed) Tomato yellow leaf curl virus disease. Springer, Dordrecht, pp 305–325

Hoy MA (1985) Recent advances in genetics and genetic improvement of the Phytoseiidae. Annu Rev Entomol 30:345–370

Hoy MA, Cave FE (1986) Screening for thelytoky in the parahaploid phytoseiid, *Metaseiulus occidentalis* (Nesbitt). Exp Appl Acarol 2:273–276

Hyland C, Bradman A, Gerona R, Patton S, Zakharevich I, Gunier RB, Klein K (2019) Organic diet intervention significantly reduces urinary pesticide levels in U.S. children and adults. Environ Res 171:568–575

Janssen A, Sabelis MW (1992) Phytoseiid life-histories, local predator-prey dynamics, and strategies for control of tetranychid mites. Exp Appl Acarol 14:233–250

Janssen A, Sabelis MW (2015) Alternative food and biological control by generalist predatory mites: the case of *Amblyseius swirskii*. Exp Appl Acarol 65:413–418

Janssen A, van Rijn PC (2021) Pesticides do not significantly reduce arthropod pest densities in the presence of natural enemies. Ecol Lett 24: 2010-2024

Jones RA (2021) Global plant virus disease pandemics and epidemics. Plants 10:233

Jones DR (2003) Plant viruses transmitted by whiteflies. Eur J Plant Pathol 109:195–219

Kennedy GG (2003) Tomato, pests, parasitoids, and predators: tritrophic interactions involving the genus *Lycopersicon*. Annu Rev Entomol 48:51–72

Kennett CE, Flaherty DL, Hoffmann RW (1979) Effect of wind-borne pollens on the population dynamics of *Amblyseius hibisci* [Acarina: Phytoseiidae]. Entomophaga 24:83–98

Knapp M, van Houten Y, van Baal E, Groot T (2018) Use of predatory mites in commercial biocontrol: current status and future prospects. Acarologia 58:7282

Leman A, Messelink G (2015) Supplemental food that supports both predator and pest: A risk for biological control? Exp Appl Acarol 65:511–524

Lenth R (2019) emmeans: Estimated Marginal Means, aka Least-Squares Means. https://CRAN.R-project.org/package=emmeans

Legarrea S, Janssen A, Dong L, Glas JJ, van Houten YM, Scala A, Kant MR (2022) Enhanced top-down control of herbivore population growth on plants with impaired defences. Funct Ecol 36:2859-2872

Marcossi Í, Fonseca MM, Carbajal PA et al (2020) High-quality alternative food reduces cannibalism in the predatory mite *Amblyseius herbicolus* (Acari: Phytoseiidae). Exp Appl Acarol 81:189–200

McMurtry JA, Johnson HG (1965) Some factors influencing the abundance of the predaceous mite *Amblyseius hibisci* in southern California (Acarina: Phytoseiidae). Ann Entomol Soc Am 58:49–56

Messelink G, Bennison J, Alomar O et al (2014) Approaches to conserving natural enemy populations in greenhouse crops: current methods and future prospects. BioControl 59: 377–393

Messelink GJ, van Maanen R, van Steenpaal SEF, Janssen A (2008) Biological control of thrips and whiteflies by a shared predator: Two pests are better than one. Biol Control 44:372–379

Messelink GJ, Sabelis MW, Janssen A (2012) Generalist predators, food web complexities and biological pest control in greenhouse crops. In: Larramendy ML and Soloneski S (eds): Integrated pest management and pest control - current and future tactics, pp 191–214

Messelink GJ, Ramakers PMJ, Cortez JA, Janssen A (2009) How to enhance pest control by generalist predatory mites in greenhouse crops. In: Mason PG, Gillespie DR, Vincent C (eds) 3rd international symposium on biological control of arthropods, Christchurch, New Zealand. pp 309–318

Naika S, de Jeude JVL, de Goffau M, Hilmi M, van Dam B (2005) Cultivation of tomato production, processing and marketing (4th ed.). Wageningen: Agromisa Foundation and CTA

Navas-Castillo J, Fiallo-Olivé E, Sánchez-Campos S (2011) Emerging virus diseases transmitted by whiteflies. Annu Rev Phytopathol 49:219–248

Navot N, Pichersky E, Zeidan M, Zamir D, Czosnek H (1991) Tomato Yellow Leaf Curl Virus: a whitefly-transmitted geminivirus with a single genomic component. Virology 161:185–151

Nomikou M, Janssen A, Schraag R, Sabelis MW (2004) Vulnerability of *Bemisia tabaci* immatures to phytoseiid predators: Consequences for oviposition and influence of alternative food. Entomol Exp Appl 110:95–102

Nomikou M, Sabelis MW, Janssen A (2010) Pollen subsidies promote whitefly control through the numerical response of predatory mites. BioControl 55:253–260

Ogle DH, Doll JC, Wheeler AP, Dinno A (2022) *FSA: Simple Fisheries Stock Assessment Methods*. R package version 0.9.4, https://CRAN.R-project.org/package=FSA.

Oliveira MRV, Henneberry TJ, Anderson P (2001) History, current status, and collaborative research projects for *Bemisia tabaci*. Crop Prot 20:709–723

Palumbo JC, Horowitz AR, Prabhaker N (2001) Insecticidal control and resistance management for *Bemisia tabaci*. Crop Prot 20:739-765

Paspati A, Rambla JL, Gresa MPL, Arbona V, Gómez-Cadenas A, Granell A, González-Cabrera J, Urbaneja A (2021) Tomato trichomes are deadly hurdles limiting the establishment of *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae). Biol Control 157:104572

Perring TM, Stansly PA, Liu TX, Smith HA, Andreason SA (2018) Whiteflies: Biology, ecology, and management. In Sustainable management of arthropod pests of tomato. Elsevier, Amsterdam, pp 73-110

Pernal SF, Currie RW (2000) Pollen quality of fresh and 1-year-old single pollen diets for worker honey bees (*Apis mellifera* L.). Apidologie 31:387–409

Picanço MC, Bacci L, Crespo ALB et al (2007) Effect of integrated pest management practices on tomato production and conservation of natural enemies. Agric For Entomol 9: 327-335.

Picanço M, Faleiro FG, Pallini A, Matioli AL (1997) Perdas na produtividade do tomateiro em sistemas alternativos de controle fitossanitário. Hort Bras 15: 88-91

Picó B, Díez MJ, Nuez F (1996) Viral diseases causing the greatest economic losses to the tomato crop. II. The Tomato yellow leaf curl virus — a review. Sci Hortic 67:151–196

Pinheiro J, Bates D, DebRoy S, Sakar D (2021) _nlme: Linear and Nonlinear Mixed Efects Models. R package version 3.1-152, https://CRAN.R-project.org/package=nlme

Pirayeshfar F, Safavi SA, Sarraf Moayeri HR, Messelink GJ (2020) The potential of highly nutritious frozen stages of *Tyrophagus putrescentiae* as a supplemental food source for the predatory mite *Amblyseius swirskii*. Biocontrol Sci Technol 30:403–417

Pretty J, Bharucha ZP (2014) Sustainable intensification in agricultural systems. Ann Bot 114:1571–1596

R Core Team (2022) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria

Rani L, Thapa K, Kanojia N, et al (2021) An extensive review on the consequences of chemical pesticides on human health and environment. J Clean Prod 283:124657

Rodríguez-Cruz FA, Venzon M, Pinto CMF (2013) Performance of *Amblyseius herbicolus* on broad mites and on castor bean and sunnhemp pollen. Exp Appl Acarol 60:497–507

Sabelis MW (1990) How to analyze prey preference when prey density varies? A new method to discriminate between effects of gut fullness and prey type composition. Oecologia 82:289–298

Sato MM, de Moraes GJ, Haddad ML, Wekesa VW (2011) Effect of trichomes on the predation of *Tetranychus urticae* (Acari: Tetranychidae) by *Phytoseiulus macropilis* (Acari: Phytoseiidae) on tomato, and the interference of webbing. Exp Appl Acarol 54:21–32

Schuurink R, Tissier A (2020) Glandular trichomes: micro-organs with model status? New Phytol 225:2251–2266

Simmons AM, Wakil W, Qayyum MA, Ramasamy S, Kuhar TP, Philips CR (2018) Lepidopterous pests: Biology, ecology, and management. In: Wakil W, Brust GE, Perring TM (eds). Sustainable Management of Arthropod Pests of Tomato. Cambridge, Elsevier: pp 131–162

Simmons AT, Gurr GM (2005) Trichomes of *Lycopersicon* species and their hybrids: Effects on pests and natural enemies. Agric For Entomol 7:265–276

Sun R, Jiang X, Reichelt M, Gershenzon J et al (2019) Tritrophic metabolism of plant chemical defenses and its effects on herbivore and predator performance. Elife 8:e51029.

Therneau T (2020) A package for survival analysis in R. R package version 3.2-7. https://CRAN.R-project.org/package=survival

Vangansbeke D, Duarte MV, Pekas A, Wäckers F, Bolckmans K (2023). Mass production of predatory mites: state of the art and future challenges. In: Morales-Ramos JA, Guadalupe Rojas A, Shapiro-Ilan (eds). Mass Production of Beneficial Organisms, Invertebrates and Entomopathogens, Second Edition. Academic Press, Elsevier: pp 195-232.

van Haren RJF, Steenhuis MM, Sabelis MW, De Ponti OMB (1987) Tomato stem trichomes and dispersal success of *Phytoseiulus persimilis* relative to its prey *Tetranychus urticae*. Exp Appl Acarol 3:115–121

van Houten YM, Glas JJ, Hoogerbrugge H, Rothe J, Bolckmans KJF, Simoni S, van Arkel J, Alba JM, Kant MR, Sabelis MW (2013) Herbivory-associated degradation of tomato trichomes and its impact on biological control of *Aculops lycopersici*. Exp Appl Acarol 60:127–138

van Lenteren JC (2000) Measures of success in biological control of arthropods by augmentation of natural enemies. In: Gurr G, Wratten S (eds) Measures of success in biological control. Kluwer, Dordrecht, pp 77–103

van Lenteren JC (2012) The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. BioControl 57:1–20

van Lenteren JC, Bolckmans K, Köhl J et al (2018) Biological control using invertebrates and microorganisms: plenty of new opportunities. BioControl 63:39–59

van Lenteren JC, Hua LZ, Kamerman JW, Rumei X (1995) The parasite-host relationship between *Encarsia formosa* (Hym. Aphelinidae) and *Trialeurodes vaporariorum* (Hom., Aleyrodidae) XXVI. Leaf hairs reduce the capacity of *Encarsia* to control greenhouse whitefly on cucumber. J Appl Entomol 119:553–559

van Rijn PCJ, Sabelis MW (1993) Does alternative food always enhance biological control? The effect of pollen on the interaction between western flower thrips and its predators. IOBC/WPRS Bull 16:123–125

van Rijn PCJ, van Houten YM, Sabelis MW (2002) How plants benefit from providing food to predators even when it is also edible to herbivores. Ecology 88: 2664–2679

Varma A, Malathi VG (2003) Emerging geminivirus problems: A serious threat to crop production. Ann Appl Biol 142:145–164

Velasco L, Simón B, Janssen D, Cenis JL (2008) Incidences and progression of tomato chlorosis virus disease and tomato yellow leaf curl virus disease in tomato under different greenhouse covers in southeast Spain. Ann Appl Biol 153:335–344

Verheggen FJ, Capella Q, Schwartzberg EG, Voigt D, Haubruge E (2009) Tomato-aphid-hoverfly: A tritrophic interaction incompatible for pest management. Arthropod Plant Interact 3:141–149

Wakil W, Brust GE, Perring TM (2018) Tomato and management of associated arthropod pests: past, present, and future. In: W Wakil, EB Brust, TM Perring (eds.) Sustainable management of arthropod pests of tomato. Academic Press, pp 3–12

Wheeler Jr AG, Krimmel BA (2015) Mirid (Hemiptera: Heteroptera) specialists of sticky plants: adaptations, interactions, and ecological implications. Annu Rev Entomol 60:393-414

Wisler GC, Li RH, Liu H-Y, Lowry DS, Duffus JE (1998) Tomato chlorosis virus: a new whitefly-transmitted, phloem-limited, bipartite closterovirus of tomato. Phytopathology 88:402–409

Figure legends

Fig 1. Juvenile development, survival and average oviposition rates of *Amblyseius herbicolus* fed with *Bemisia tabaci* eggs or *Typha* sp. pollen. Juvenile development is shown as the mean (± s.e.) cumulative proportion of adults as a function of time (*B. tabaci*: white circles; Pollen: white squares). Total survival is the final cumulative proportion that reached adulthood on day 6. Average oviposition rates (± s.e.) of *A. herbicolus* on the 4th-9th day are given by black circles (*B. tabaci*) and black squares (Pollen).

Fig 2. Average (± s.e.) predation **(a)** and oviposition rates **(b)** of *A. herbicolus* (circles) and *A. tamatavensis* (squares) on the 2nd – 4th day of feeding on crawlers of *B. tabaci* (white symbols) or *Typha* sp. Pollen (black symbols). Different letters to the right of the data indicate significant differences among treatments (contrasts through model simplification after LME; $p < 0.05$).

Fig. 3 **(a)** Average numbers (± s.e.) of alive and dead predators found on the release and target leaflets or on the rest of the plants after 7 days. Negative (downwards) error bars are of the categories, positive (upward) error bars are of the total. **(b)** Average densities (± s.e.) of juveniles (white symbols) and adults (black symbols) of the predatory mites *A. herbicolus* (circles) and *A. tamatavensis* (squares) on tomato plants when only pollen was offered as food. Note that we tested differences in the total numbers of predators (adults and juveniles), the difference between the two species was significant, and the effect of time was also significant. The proportion of immatures also differed significantly between the two species, with many juveniles for *A. herbicolus* and few for *A. tamatavensis*. **(c)** Average densities (± s.e.) of all motile stage of the predatory mite *A. herbicolus* in three different strata in the same experiment as of **(b)**. Densities in strata with different letters differed significantly (contrasts through model simplification after GLM; $p < 0.05$).

Fig 4. Population dynamics of *Bemisia tabaci* and the predatory mites *A. herbicolus* or *A. tamatavensis* on tomato plants. **(a)** Average densities (± SE) of immature whiteflies and average numbers of whitefly eggs on the 28th day in the presence of *A. herbicolus* (circles), *A. tamatavensis* (triangles) and without predators (squares). For egg density, different letters indicate significant differences among treatments (Kruskal-Wallis rank sum test with Holm method correction; $p < 0.05$). **(b)** Average densities (± s.e.) of all mobile stages of both predatory mites. The *vertical arrows* indicate the weeks that the plants were infested with 3 pairs of whiteflies. **(c)** Average densities (± s.e.) of adult whiteflies in the presence of *A. herbicolus* and without predators. **(d)** Average densities (± s.e.) of all mobile stages of *A. herbicolus*. Asterisks indicate a significant difference among treatments per time step (contrasts through model simplification after LME): $^{*}p < 0.05$; *n.s.*: not significant.

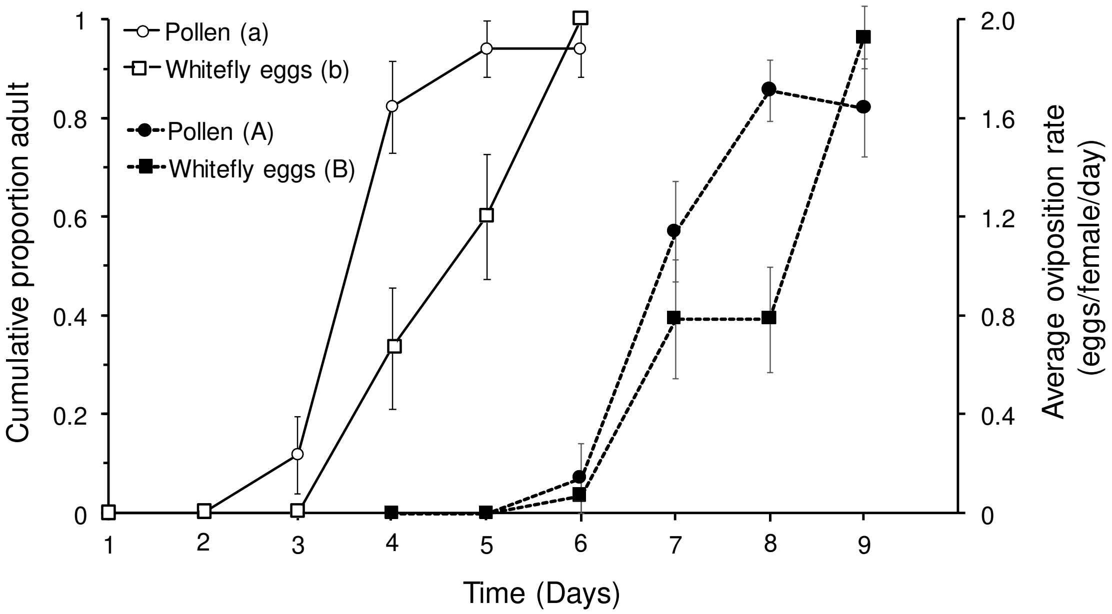
Pollen (a)
Whitefly eggs (b)
Pollen (A)
Whitefly eggs (B)
Cumulative proportion adult
0
0.2
0.4
0.6
0.8
1
Average oviposition rate (eggs/female/day)
0
0.4
0.8
1.2
1.6
2.0
1
2
3
4
5
6
7
8
9
Time (Days)

Figure 1.

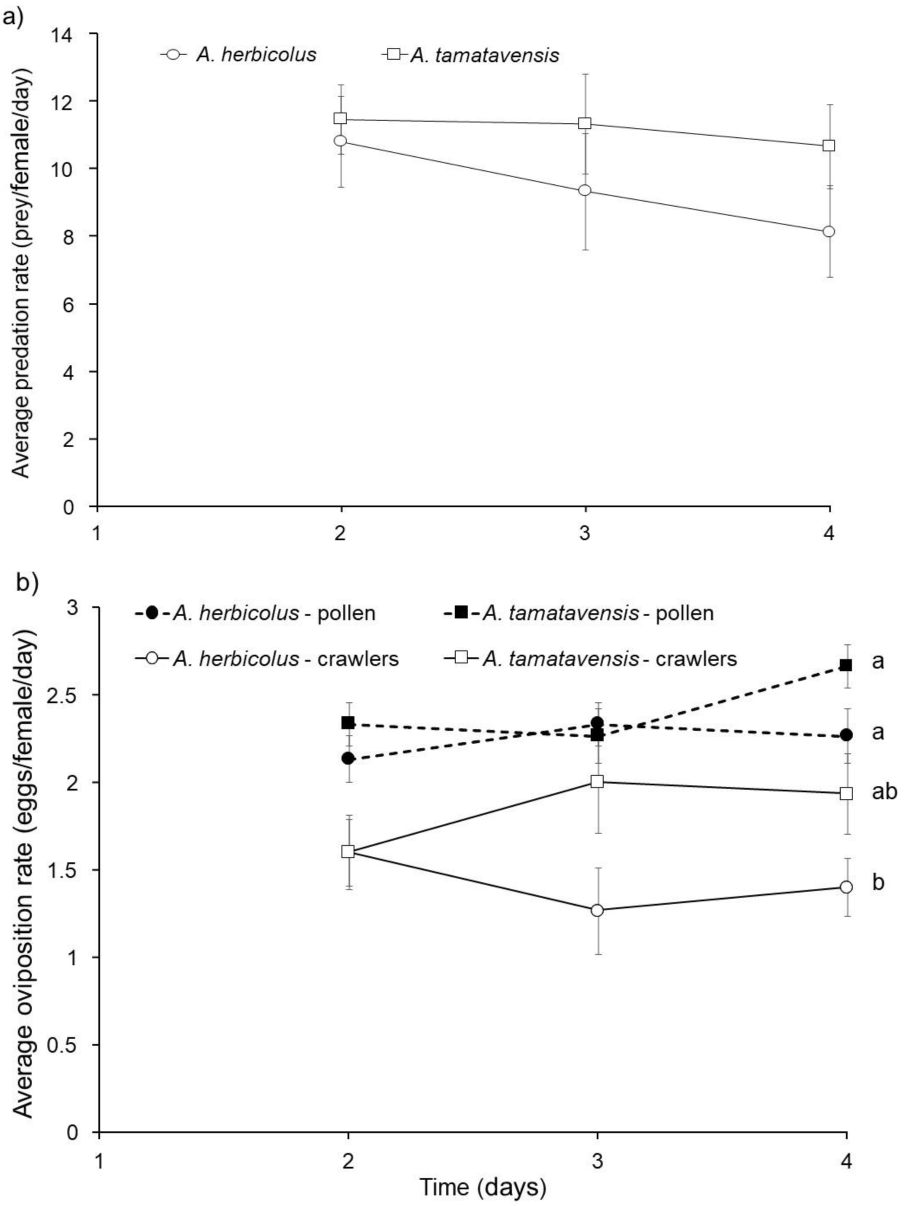
a)
Average predation rate (prey/female/day)
A. herbicolus
A. tamatavensis
0
2
4
6
8
10
12
14
1
2
3
4
b)
Average oviposition rate (eggs/female/day)
A. herbicolus - pollen
A. tamatavensis - pollen
A. herbicolus - crawlers
A. tamatavensis - crawlers
0
0.5
1
1.5
2
2.5
3
a
a
ab
b
1
2
3
4
Time (days)

Figure 2.

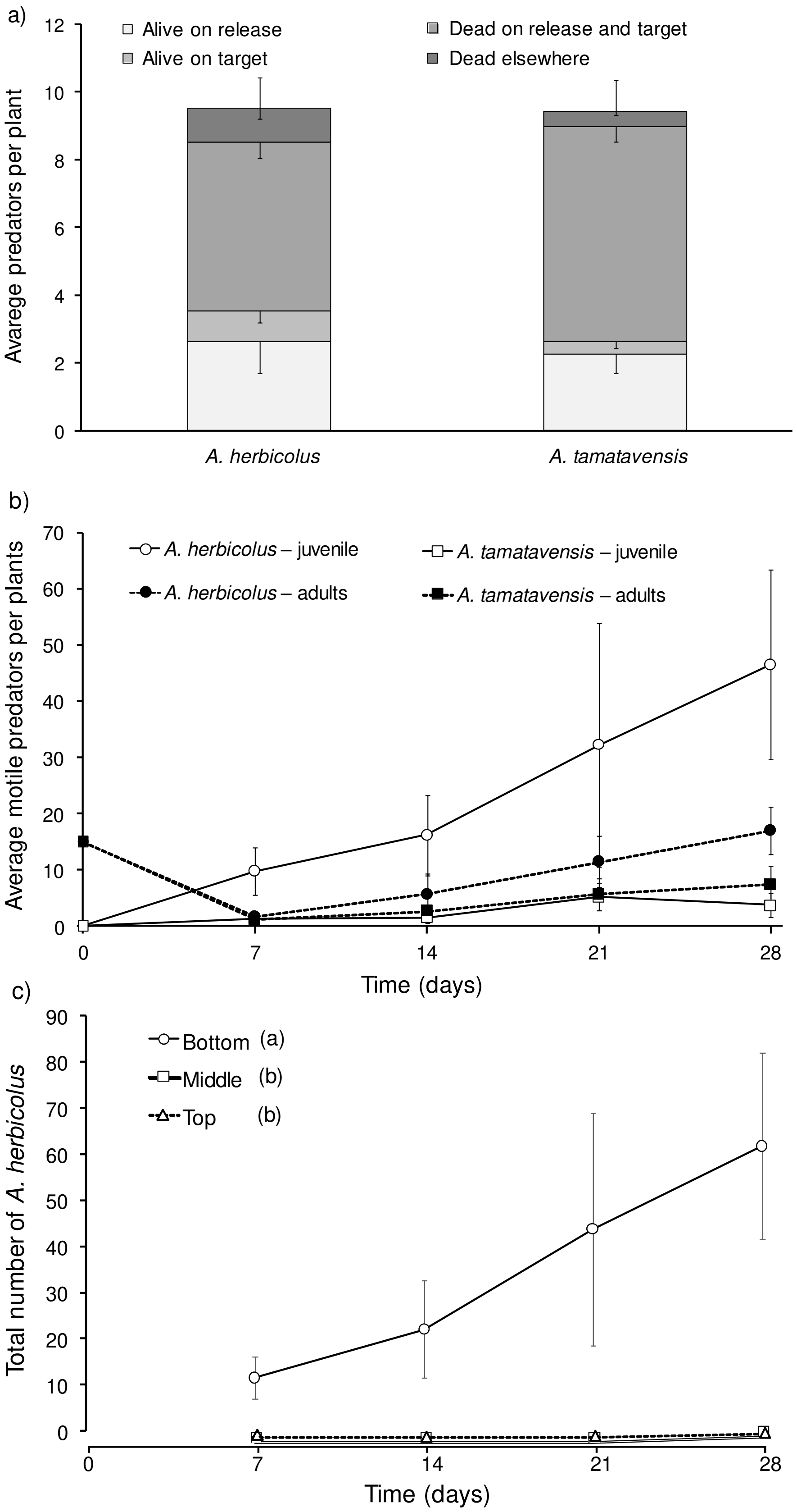
a)
Alive on release
Alive on target
Dead on release and target
Dead elsewhere
Avarege predators per plant
0
2
4
6
8
10
12
A. herbicolus
A. tamatavensis
b)
A. herbicolus – juvenile
A. herbicolus – adults
A. tamatavensis – juvenile
A. tamatavensis – adults
Average motile predators per plants
0
10
20
30
40
50
60
70
0
7
14
21
28
Time (days)
c)
Bottom (a)
Middle (b)
Top (b)
Total number of *A. herbicolus*
0
10
20
30
40
50
60
70
80
90
0
7
14
21
28
Time (days)

Figure 3.

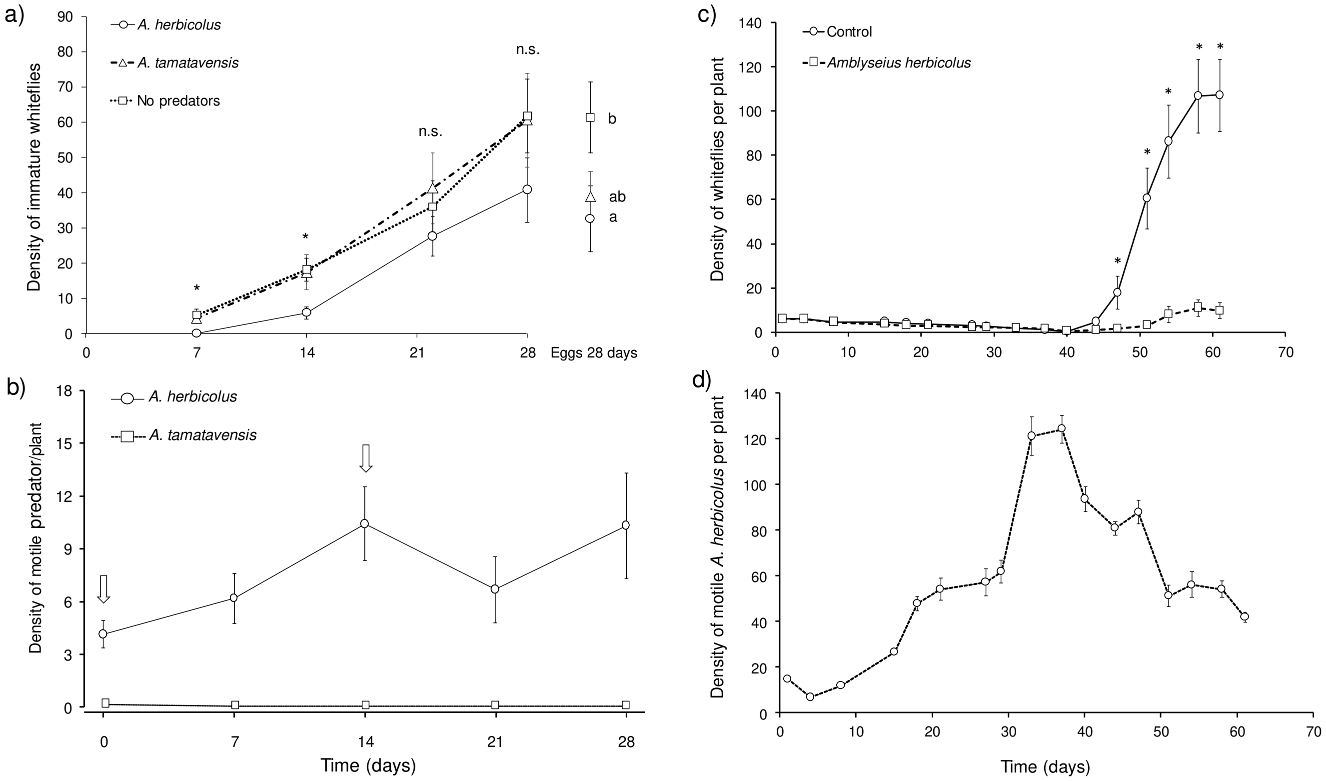
a)
A. herbicolus
A. tamatavensis
No predators
Density of immature whiteflies
90
80
70
60
50
40
30
20
10
0
0
7
14
21
28
Eggs 28 days
*
*
n.s.
n.s.
b
ab
a
b)
A. herbicolus
A. tamatavensis
Density of motile predator/plant
18
15
12
9
6
3
0
0
7
14
21
28
Time (days)
c)
Control
Amblyseius herbicolus
Density of whiteflies per plant
140
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
*
*
*
*
*
*
d)
Density of motile A. herbicolus per plant
140
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
Time (days)

Figure 4.

Supplementary Figure and Table

Table 1. Infestation of tomato plants with the predatory mite *A. herbicolus* or *A. tamatavensis* and pollen by different organisms.

Treatment	Species	Guild	Presence on plants[a]
Amblyseius herbicolus	*Bemisia tabaci* (whitefly)	Herbivore	62.5%
	Oidium sp. (powdery mildew)	Plant-pathogenic fungus	62.5%
	Tyrophagus putrescentiae	Generalist	31.25%
	Thrips (unidentified)	Herbivore	6.25%
	Spider (unidentified)	Carnivore	6.25%
Amblyseius tamatavensis	*Bemisia tabaci* (whitefly)	Herbivore	50.0%
	Oidium sp. (powdery mildew)	Plant-pathogenic fungus	62.5%
	Tyrophagus putrescentiae	Generalist	18.25%
	Iolinid mite	Generalist	18.75%
	Spider (unidentified)	Carnivore	6.25%

[a] Proportion of plants infested with different organisms in the adaptation experiment on tomato plants. (n=16)

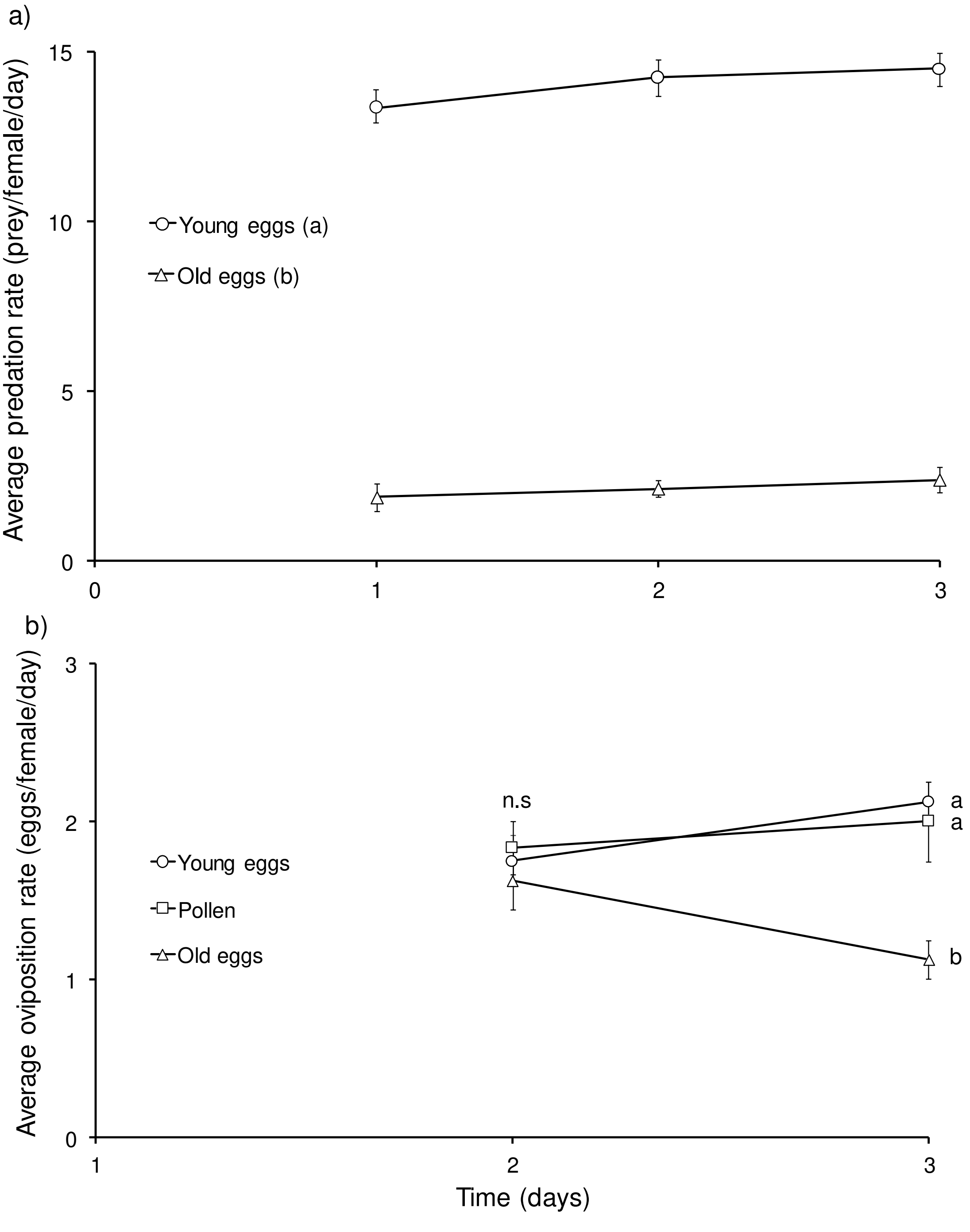

Figure S1: Average (± s.e.) predation **(a)** and oviposition rates **(b)** of *A. herbicolus* of feeding on eggs of *B. tabaci* or *Typha* sp. pollen. Young eggs were < 48 h-old; old eggs were > 48 h-old. Oviposition rates in treatments with different letters in the legend and following the key entries differed significantly (contrasts through model simplification after LME; $p < 0.05$): *n.s* not significant.

CHAPTER II

Predatory mites as potential biological control agents for tomato russet mite and powdery mildew on tomato crops

Ítalo Marcossi[1] · Leonardo S. Francesco[1] · Morgana M. Fonseca[2] ·

Angelo Pallini[1] · Thomas Groot[3] · Raf De Vis[4] · Arne Janssen[1,5]

[1]Department of Entomology, Section Acarology, Federal University of Viçosa, Viçosa, MG, Brazil.

[2]Koppert do Brasil, Piracicaba, SP, Brazil

[3]Koppert BV, Berkel en Rodenrijs, The Netherlands

[4]Research Station for Vegetable Production, Sint-Katelijne-Waver, Belgium

[5]IBED, Section Population Biology University of Amsterdam, The Netherlands

Chapter formatted in the norms of the journal: Journal of Pest Science

Abstract

Tomato crops are normally attacked by numerous pests and diseases. Among the main threats to this crop are the tomato russet mite *Aculops lycopersici* and powdery mildew, *Oidium neolycopersici*. Natural enemies of tomato pests are often hindered by the tomato trichomes, whereas the russet mite lives under and among these leaf hairs. It is expected that natural enemies that occur on tomato plants are more adapted to these direct defences. We found the omnivorous predatory mites *Amblyseius herbicolus* and *Homeopronematus anconai* on tomato plants and investigated their potential as biological control agents for pests in this crop. First, we show that both predators were able to feed and reproduce on russet mites but had higher oviposition rates when they were feeding on pollen. Subsequently, we show that both predators established on tomato plants with pollen as alternative food, but only *H. anconai* increased in densities and effectively controlled tomato russet mites. Moreover, it was also effective in controlling powdery mildew. Altogether, our results show that *H. anconai* is an efficient biocontrol agent of two key pests on tomato plants. The use of a single predator that is effective in controlling both a pest and a pathogen can be a new strategy for biological crop protection.

Keywords Biological control · Tomato · Iolinidae · Phytoseiidae · Tomato russet mite · Powdery mildew

Introduction

Herbivores are suppressed by both bottom-up (host plants) and top-down (natural enemies) forces (Price et al. 1980; Stiling and Rossi 1997; Denno et al. 2002). Bottom-up forces are related to direct plant defences, such as chemical and physical defences that have negative effects on herbivore performance (Price et al. 2011; Kant et al. 2015). Top-down forces are mediated by the natural enemies of the herbivores, often in interaction with the plants, which possess traits that enhance attraction or arrestment of the natural enemies (Sabelis et al. 1999; Schmitz et al. 2000; Sabelis et al. 2001; Pearse et al. 2020). These two forces are not necessarily compatible; direct plant defences can negatively impact natural enemies (Rutner et al. 1998; Rutledge et al 2003; Simmons and Gurr 2005; Riddick and Simmons 2014; Legarrea et al. 2022). For example, glandular trichomes, which are outgrowths of the epidermis in which specialized metabolites are synthesized and stored (Schuurink and Tissier, 2020), predominantly act as broad-spectrum direct defence against herbivores (Glas et al. 2012), but can also increase the mortality of natural enemies (Belcher and Thurston 1982; van Haren et al. 1987; Verheggen et al. 2009; Paspati et al. 2021; Legarrea et al. 2022). Therefore, the interaction between direct plant defences and natural enemies can directly affect the control of herbivores.

A classic example of a plant species with trichomes is tomato (*Solanum lycopersicum* L.), which possess glandular and non-glandular trichomes (Cédola and Sánchez 2003; Kennedy 2003). These trichomes are multicellular and consist of basal, stalk, and apical cells. The apical cells contain metabolites that can entrap small insects and predatory mites and can also be highly toxic to mites (van Haren et al. 1987; Sato et al. 2011; van Houten et al. 2013; Paspati et al. 2021; Legarrea et al. 2022). Nevertheless, tomato plants are attacked by herbivores and pathogens adapted to their direct defences (Schneider and Grogan 1977; Sarmento et al. 2011; Glas et al. 2014). For example, the tomato russet mite *Aculops lycopersici* Tryon (Acari: Eriophyidae) is one of the most destructive pests of tomato crops worldwide (Abou-Awad, 1979; de Moraes and Lima 1983; Greenhalgh et al. 2020; Vervaet et al. 2021). It is so small (adults are 150–200 µm in length, Abou-Awad, 1979; Vervaet et al. 2021) that it moves easily among the stalks of the trichomes without touching their apical cells, which thus create a shelter against most of its competitors and predators (van Houten et al. 2013). Adults and nymphs feed on the epidermis of foliage, stems, inflorescences and (young) fruits, causing browning, shrivelling and necrosis of stems and leaves, flower abortion and russeting of fruits (Royalty and Perring 1988; Duso et al. 2010; Vervaet et al. 2021). At high infestations, tomato plants shrivel and production can be lost completely (Al-Azzazy and Alhewairini 2018). The russet mites are actually only controlled with pesticide applications (e.g., abamectin and sulphur) (Duso et al. 2010; Vervaet et al. 2021), but this is often ineffective (Lindquist et al.1996; van Houten et al. 2013). Furthermore, a meta-analysis of published data from field experiments demonstrated that chemical pest control often does not work when natural enemies are present (Janssen and van Rijn 2021), and it is therefore better to search for efficient natural enemies, thus preventing the use of pesticides.

Predatory mites of the Phytoseiidae family are one of the main groups of natural enemies studied for the control of the russet mite (Brodeur et al. 1997; Park et al. 2010; Al-Azzazy et al. 2018). However, the effectiveness of these mites on tomato plants is hindered because of the glandular trichomes. Although some phytoseiid mites may be or become adapted to tomato plants and their trichomes (Drukker et al. 1997; Silva et al. 2010; Sato et al. 2011), so far, none of them can control the russet mite on tomato with trichomes. Possibly, predatory mites found in natural association with tomato plants and their pests are more adapted to these trichomes and may therefore control pests on this crop. Recently, it was demonstrated that the predatory mite *Amblyseius herbicolus* (Acari: Phytoseiidae), originally collected from feral tomato plants (see Materials and Methods), was able to establish and reduce whitefly densities on pollen-supplemented tomato plants (Cardoso 2019). This last study also demonstrated that this predator develops and reproduces when feeding on *A. lycopersici*, but its effectiveness in controlling russet mite on tomato plants has not been evaluated yet.

Besides this phytoseiid mite, the iolinid predatory mites *Homeopronematus anconai* (Baker) and *Pronematus ubiquitus* (McGregor) (Acari: Iolinidae) were reported to feed and reproduce on *A. lycopersici* (Hessein and Perring 1986; Kawai and Haque 2004; van Houten et al. 2020; Pijnakker et al. 2022a, b; Vervaet et al. 2022). Both species are also so small that they move in between the stalks of the trichomes without touching their apical cells. They can successfully establish on tomato plants (Kawai and Haque 2004; Pijnakker et al. 2022b) and find and consume *A. lycopersici*. They are also herbivorous and iolinid mites in general need to feed on plant sources (Moraes and Flechtmann 2008; Pijnakker et al. 2022b; Vervaet et al. 2022) which is proven by the fact that they can only be reared on or in the presence of plant tissue (except for *Pronematus* sp., De Vis et al. 2006). However, they cannot complete their cycle on tomato leaves alone and addition of prey, pollen or fungi boosts their reproduction (Hessein and Perring 1986; Kawai and Haque 2004; Pijnakker et al. 2022a, b; Vervaet et al. 2022). Therefore, the application of pollen allows preventive establishment of the predators in the crop in the absence of prey. In a recent study, Pijnakker et al. (2022b) showed that establishment of *P. ubiquitus* with pollen on tomato plants resulted in preventing *A. lycopersici* infestations in a greenhouse. They also demonstrated that *P. ubiquitus* effectively controlled powdery mildew infections (*Oidium neolycopersici* L.), a devastating tomato disease (Jones et al. 2001; Lebeda et al. 2014).

Current management practices of powdery mildew include fungicide applications, the use of sulphur, the development of resistant crop varieties and technology-based climate control in greenhouses crops (Jarvis 1992; Castañe et al. 2020; Vielba-Fernandez et al. 2020). Successful biological control of powdery mildew in vegetables is achieved almost exclusively by antagonistic microorganisms (Kiss 2003), such as the fungal antagonist *Ampelomyces quisqualis* (Kiss et al. 2004; Caffi et al. 2013; Legler et al. 2016). A poorly evaluated possibility in the biological control of diseases is the use of fungivores. There are studies demonstrating the ability of fungivorous arthropods (mainly mites) to reduce powdery mildew infestations (Hessein and Perring 1986; English-Loeb et al. 1999; Norton et al. 2000; Kawai and Haque 2004; Sutherland and Parrela 2009; Pijnakker et al. 2022b). Here, we evaluated the reproductive performance of the omnivorous predators *A. herbicolus* and *H. anconai* feeding on *A. lycopersici* and pollen. Subsequently, we assessed the control of russet mites by both predators on tomato plants when pollen was offered as an alternative food, as well as their effect on fruit production. Furthermore, we evaluated the effectiveness of the iolinid mite *H. anconai* in suppressing natural powdery mildew infections on tomato plants.

Materials and methods

Plant materials

Tomato seeds (*Solanum lycopersicum* var. Aguamiel (EX V305), Limagrain®, imported by Vilmorin Seed Generation, Campinas - Brazil) were sown in a commercial plant substrate enriched with macro - and micronutrients (MECPLANT® - Mec Prec, Telêmeco Borba), in polystyrene trays (8 x 16 cells). This variety is susceptible to powdery mildew, but is resistant to various plant-pathogenic viruses. Fifteen days after germination, the seedlings were transplanted to plastic 3 L pots with the same substrate. The plants were fertilized every week with a solution of 50g of N-P-K (20-05-20) and 100g of simple superphosphate in 20L water and were supported with bamboo sticks, to which they were tied with a string. The plants were kept inside cages in a room with natural light at 23 ± 3 °C and 70 ± 10% relative humidity. Cattail pollen used in all experiments was collected from *Typha* sp. plants from the campus of the Federal University of Viçosa. The pollen was dried in an oven at 60º C for 24 h and stored in a freezer at -20 °C according to recommendation (Hagedorn 1968; Pernal and Currie 2000).

Mite rearings

Tomato russet mites were collected from tomatoes grown in a rural area of Coimbra in 2021 (MG, Brazil, 20° 51' 42.4" S, 42° 50' 14.4" W) and were maintained on tomato plants inside cages (0.5 x 0.5 x 1.0 m). A new tomato plant with at least four completely developed leaves was added to each cage every month and the oldest plants removed. The cages were kept in a room at 22 ± 2 °C, 70 ± 10% RH, 12:12 L:D.

The laboratory rearing of *H. anconai* was initiated with mites collected on russet-mite-infested tomato plants in the house of the first author in Viçosa in 2021. The species identification was conducted by Tairis Da-Costa and Noeli Juarez Ferla from UNIVATES (RS, Brazil). They were reared on tomato plants, to which cattail pollen was added twice a week as extra food. The tomato plants were kept inside cages (0.5 x 0.5 x 1.0 m), which were kept in climate rooms as above. The rearing of *A. herbicolus* was started with individuals collected from tomato plants in gardens in the urban and rural areas of Prados (MG, Brazil) (Cardoso 2019). The species identification was carried out by Manoel Guedes Correa Gondim Junior from UFRPE (PE, Brazil). This predator reproduces through thelytokous parthenogenesis, hence, populations consist of females only (de Moraes and Mesa 1988). They were reared on arenas made of black PVC sheets (15 × 10 cm) on top of foam pads (h = 3 cm), which were kept in plastic trays (29 × 14 × 4 cm) filled with water (cf. van Rijn and Tanigoshi 1999). The edges of the arenas were wrapped in wet tissue paper to prevent the mites from escaping, but the tissue also served as a water source. Small pieces of cotton wool were placed on the arenas as oviposition sites and were covered with pieces of black, tent-shaped PVC sheet (1.5 cm²) for shelter. Cattail pollen was offered as food two times per week. The rearing was maintained in climate rooms as above.

Predation and oviposition

First, we investigated the ability of both predatory mites to feed on adult russet mites. This experiment was performed on tomato leaf discs (Ø = 2 cm) arranged on wet tissue paper (Ø = 3 cm) and placed in Petri dishes (Ø = 5 cm, 1.5 cm high), which were closed with lids to prevent mites from escaping. Preliminary experiments were done to check how many individuals to offer to avoid prey depletion. We placed ten adult russet mites on each leaf disc, together with a single adult female of each predator. These adult females were placed singly in the experimental units and were starved for 24 h prior to the experiments to prevent effects from the previous diet. Predation was scored 24 hours later. Ten replicates were carried out per predator species. The predation rates of *A. herbicolus* and *H. anconai* on russet mite adults were compared using a generalized linear model (GLM) with a Poisson error distribution.

To assess the ability of both predatory mites to reproduce on russet mites, we introduced a young adult female (12-15 days after egg eclosion) of one of the two predatory mite species on a tomato leaf disc with c. 100 russet mites (mixed stages, including eggs) in experimental units as above. *Typha* sp. pollen, placed on clean leaf discs, was used as food in a control treatment. Oviposition rates were assessed every 24 hours for 4 days. After each evaluation, predators were transferred to new experimental units containing fresh prey or pollen. Ten replicates were carried out per predator species. To prevent effects from the previous diet, oviposition of the first day was excluded from the analysis (Sabelis 1990). The tests were performed in a room at 22 ± 2 °C, 70 ± 10% RH, 12:12 L:D. Because of considerable non-normality of the errors, also after transformation, oviposition rates were summed per individual over the last three days of the experiment and compared with a Wilcoxon-Mann-Whitney test (function wilcox.test of the R package coin, Hothorn et al. 2008). Nevertheless, we present oviposition data per day below to show trends through time.

Tomato russet mite control on tomato plants, powdery mildew levels and fruit production

To test for the capacity of *A. herbicolus* and *H. anconai* to control russet mites, we carried out experiments in 3 screen cages (2 x 2 x 2 m – Howitec Netting®, Joure, the Netherlands), under natural conditions (mean temperature 21.5°C, range 19–26.1° C; mean relative humidity 62%, range 41–77%), but protected from occasional rain. Each cage contained 12 tomato plants; each plant was a replicate. Initially, a tomato leaf disc (3 cm^2) with 5 *A. herbicolus* adult females was put on all leaves of the tomato plant (4-leaf stage), with a total of 20 predators per plant. *Homeopronematus anconai* was introduced in a similar way, but with 10 mobile stages per leaf disc at a total of 40 individuals per plant. About 1 mg of *Typha* sp. pollen was applied twice a week, including in the control treatment (without predators). To prevent cross-contamination, the plants were spaced out in the cage, and each plant pot was put in a dish with water, serving as barrier for dispersing mites. Plants were not sampled for mites during the first 4 weeks to avoid disturbances. However, a few days after setting up the trial, natural infestations of powdery mildew occurred in all treatments. The presence of powdery mildew was quantified during this period (4-weeks) by non-destructive counting of the leaflets containing visual mildew lesions. This was done until the release of the russet mite in the fourth week.

Four weeks after the release of predatory mites, all treatments were infested with russet mite. Discs were cut from leaflets picked from a well-infested tomato plant, and each disc harboured on average c. 50 mobile stages. Each tomato plant received a leaf disc with russet mites on the 2nd to the 6th leaf from the bottom leaf, hence, about 250 mobile stages in total. The discs were positioned on the middle of each leaf. The number of predatory mites was evaluated weekly from the 4th week and the number of russet mites from the 5th week after the introduction of predatory mites. This was done by collecting six leaflets per plant (two leaflets from the bottom, middle and top section of each plant, respectively). The leaflets were stored in separate boxes for evaluation in the laboratory under a stereo microscope. At the last sampling, tomato fruits were collected, counted and weighed using a semi-micro analytical balance (Balança-AD200, Marte Científíca, São Paulo).

The densities of *A. lycopersici* were log (x+1) transformed before the analysis and compared with a linear mixed effects model (LME) with time and treatment and their interaction as fixed factors (lme of the package nlme, Pinheiro et al. 2021). The replicate (plants) was used as a random factor to correct for repeated measures. Models were checked with normal error plots and plots of residuals against fitted values. Significance of factors and interactions were determined with likelihood ratio (L.R.) tests. The numbers of tomato fruits and weights of fruits per plant were compared among treatments using a GLM with Gaussian error distribution (identity link). The number of leaflets with powdery mildew lesions in the first four weeks of the population dynamics experiment was compared using a generalized linear mixed effects model (GLMER, glmer of the package lme4, Bates et al. 2015) with a Poisson error distribution, treatment and time as fixed factors and replicates (plants) as random factor to correct for repeated measures.

Powdery mildew control by *H. anconai*

Based on the results with powdery mildew obtained in the above experiment, we conducted an experiment to evaluate whether *H. anconai* could affect mildew progress on different strata (bottom, middle and top) of tomato plants. As powdery mildew initially starts on lower leaves of plants, fifty *H. anconai* motiles were introduced on the bottom (= oldest) leaf from each (6-leaf stage) tomato plant (5 plants per treatment). *Typha* sp. pollen (1 mg) was applied two times per week in all leaves, also on plants of a control treatment without predators. The plants were kept in two separate cages (1 x 1 x 1 m). Plant apices were subsequently removed to keep plants at a constant size and prevent the leaves from touching the top of the cages. For four weeks, we counted the leaflets with visible powdery mildew lesions on the entire plants. Powdery mildew occurrence on tomato plants was compared in two steps using a GLMER with a binomial error distribution: first the occurrence of mildew per leaf in the three strata, and then the number of leaflets with mildew for those leaves that had symptoms of mildew. Contrasts among treatments in the different trials were assessed with the Tukey method using the package emmeans (Lenth 2016). All statistical analyses were performed with software R, version 4.2.1 (R Core Team 2022).

Results

Predation and oviposition

The average total consumption of russet mite adults by the predatory mites *H. anconai* (0.8 ± 0.2) and *A. herbicolus* (5.6 ± 0.8) (mean ± SE) differed significantly (GLM; Deviance = 42.7; *d.f.* = 1; $p < 0.001$). The phytoseiid mite consumed about 7 times more russet mite adults than *H. anconai.*

The two predators were able to reproduce by feeding on *A. lycopersici*. Oviposition rates were significantly affected by diet in both *H. anconai* (Fig. 1A; Wilcoxon test: $Z = 3.52$, $p = 0.0004$) and *A. herbicolus* (Fig. 1B; $Z = 3.75$, $p = 0.0002$). Feeding on cattail pollen resulted in the highest oviposition rates for both predators.

Tomato russet mite control on tomato plants, powdery mildew levels and fruit production

There was a significant effect of the presence of predators through time on densities of *A. lycopersici* (Fig. 2A; LME: interaction of treatment with time: L.R. = 14.4, d.f. = 2, $p = 0.0007$). The predator *H. anconai* successfully suppressed the population growth of *A. lycopersici*, but *A. herbicolus* did not (Fig. 2A). During the last evaluations, we observed a slight increase in the densities of russet mites in the treatment with *H. anconai*. However, the densities were low, whereas they increased in the treatments with *A. herbicolus* and without predators (Fig. 2A). Whereas *H. anconai* population increased twelve-fold during the experiment, *A. herbicolus* disappeared from all plants from week 7 onwards (Fig. 2B). In the seventh week of evaluation, the plants showed a high incidence of powdery mildew, except in the treatment with *H. anconai*. The severity of the disease resulted in the drying and consequent senescence of some leaflets, directly affecting the predators still present on the plants.

The different treatments significantly affected the number of leaflets with powdery mildew lesions through time during the first four weeks (Fig. 3; interaction of treatment with time, GLMER; $Chi^2 = 26.6$, d.f. = 2, $p < 0.0001$). *Homeopronematus anconai* reduced the spread of the disease with 86.5% and 87.6% compared to the treatments with *A. herbicolus* and without predators, respectively. There was no significant effect of treatment on the numbers of fruits produced (Fig. 4A; GLM; *Deviance* = 5.79, d.f. = 2, $p = 0.055$). Tomato plants with *H. anconai* produced fewer fruits than plants in the other treatments. However, fruits from plants with *H. anconai* were somewhat heavier than those of plants with *A. herbicolus* and without predators, but this effect was also not significant (Fig. 4B; GLM; $F_{2,33}$= 2.0; $p = 0.15$), and the total fruit weight per plant (Fig. 4C) also did not differ significantly among treatments (GLM; $F_{2,33} = 0.55$, $p = 0.58$).

Powdery mildew control by *H. anconai*

The occurrence of powdery mildew on leaves in the three strata of tomato plants was significantly affected by the presence of *H. anconai* (Fig. 5A-C; interaction of treatment with strata, GLMER; $Chi^2 = 7.3$; $p = 0.026$). However, there was no significant difference in the bottom stratum. The proportion of leaflets of the leaves that had mildew was also affected by the presence of *H. anconai* (interaction of treatment with strata and time, GLMER; $Chi^2 = 31.5$; $p < 0.0001$). Although plants with *H. anconai* showed lower rates of infection with powdery mildew in the bottom part, this difference was not significant (Fig. 5C). In the upper parts of the plant, almost no leaflets with powdery mildew lesions were found in the presence of *H. anconai*. Only 0.6 ± 0.4 (mean ± SE) infected leaflets were observed in the middle stratum (Fig. 5B), and 0.05 ± 0.36 with powdery mildew lesions were found at the top of the plants (Fig. 5A). Therefore, the control of powdery mildew by *H. anconai* was evident, limiting the spread of the disease to higher parts of the tomato plant.

Discussion

We show that both the phytoseiid *A. herbicolus* and the iolinid *H. anconai* were able to feed and reproduce on tomato russet mites, but *A. herbicolus* consumed about 7 times more adult russet mites than *H. anconai.* These findings agree with other studies that reported low consumption rates of adult russet mites by *H. anconai* (Brodeur et al. 1997; Vervaet et al. 2022). Here, both predatory mites reached higher oviposition rates when the mites were feeding on cattail pollen (Fig. 1A-B) and providing pollen before pest introduction allowed the establishment of both mites on tomato plants. However, only *H. anconai* was able to increase in density and effectively control russet mites (Fig. 2A). Moreover, our results show that *H. anconai* was also effective in controlling the spread of powdery mildew (Fig. 3 and 5). Thus, early releases of *H. anconai* with the addition of *Typha* pollen can possibly prevent both infestations by russet mites and powdery mildew on tomato plants. This is the second report of an arthropod biological control agent acting against both this pest and disease (Pijnakker et al. 2022b).

Several studies have analysed the effects of direct plant defences on pest-natural enemy interactions and observed adverse effects of plant defences such as trichomes on natural enemies (Riddick and Simmons 2014; Peterson et al. 2016; Legarrea et al. 2022). On tomato plants, few natural enemies are known to be able to cope with glandular trichomes (Legarrea et al. 2022). Hence, it is expected that predators that naturally occur on tomatoes are more adapted to the plant direct defences. Our results demonstrate that *H. anconai* was indeed not hampered by the high densities of glandular trichomes to such an extent that they could not control russet mites. The glandular trichomes provide a refuge for russet mites, protecting them against many predatory mites (van Houten et al. 2013), but iolinid mites are also small and navigate unhindered under the glands of the trichomes, thus, they can find and consume russet mites. Similar results were reported by Pijnakker et al. (2022b) for the related iolinid *P. ubiquitus*. Both iolinid mites seem adapted to the direct defences (i.e., toxic glandular trichomes) of tomato plants and can thus contribute to the biological control of the russet mite.

Previous work has demonstrated how supplementing natural enemies with pollen as alternative food can be used to improve biocontrol (van Rijn et al. 2002; Duso et al. 2004; González-Fernández et al. 2009; Nomikou et al. 2010; Delisle et al. 2015; Leman and Messelink 2015), including by *A. herbicolus* (Duarte et al. 2015). The establishment of *H. anconai* with pollen was important to achieve the rapid population growth of this predator. For example, four weeks after their introduction, the densities of *H. anconai* had already increased by 45%. Although the addition of pollen may result in lower predation rates in the short term, it results in better control after some time (Nomikou et al. 2004; Vangansbeke et al. 2016; Samaras et al. 2021). The presence of alternative food such as pollen on a crop can enhance the efficacy of pest control because it increases the densities of natural enemies, which subsequently causes a reduction in pest populations, a phenomenon similar to apparent competition (Holt 1977; van Rijn et al. 1999; Nomikou et al. 2002; Nomikou et al. 2010; Duarte et al. 2015). We show here that the establishment of *H. anconai* with pollen on tomato plants before infestation by a pest indeed resulted in effective control: the density of russet mites per leaflet sampled in the presence of *H. anconai* was close to zero (Fig. 2A). Predator satiation and perhaps a preference of *H. anconai* for pollen over prey were eventually offset by an increase in predation at the population level. Similarly, *P. ubiquitus*, another iolinid mite, has also been reported to effectively control russet mites on tomato plants supplemented with pollen (Pijnakker et al.

2022b). Furthermore, both *P. ubiquitus* and *H. anconai* prevent progression of powdery mildew.

Feeding on fungal spores has previously been demonstrated for tydeoid mites (Flaherty and Hoy 1971; Hessein and Perring 1988; English-Loeb 1999, 2007; Norton et al. 2000; Duso et al. 2005; Pijnakker et al. 2022b). Hessein and Perring (1988) reported that adults of *H. anconai* survived when they fed on *Cladosporium cladosporioides* spores, but this diet did not allow reproduction. We did not evaluate whether *H. anconai* can develop on a diet of powdery mildew only.

It is also known that plant feeding by various omnivorous natural enemies, including predatory mites, can trigger the induction of plant defences (Pérez-Hedo et al. 2015; Zhang et al. 2018; Cruz-Miralles et al. 2019; Pérez-Hedo et al. 2022; Silva et al. 2022). Therefore, the suppression of powdery mildew by *H. anconai* may also be caused by the induction of plant defences by plant feeding of *H. anconai*, and this might also be the case for tomato russet mite. Although *A. herbicolus* had a higher predation rate and similar oviposition rates when compared to *H. anconai*, this was not reflected in a better control on tomato plants. To the contrary, the russet mite control by *A. herbicolus* was also limited at the start of the experiment. The better control by *H.* anconai is therefore difficult to explain by direct control only. We therefore hypothesise that the russet mite control might also be indirect and that herbivory by *H. anconai* might trigger not only resistance to powdery mildew but also to tomato russet mite. Further research is needed to elucidate these hypotheses.

Many phytoseiid mites are negatively affected by glandular trichomes of tomato plants, and this hampers biocontrol (van Haren et al. 1987; Sato et al. 2011; Paspati et al. 2021; Legarrea et al. 2022; Pijnakker et al. 2022a). However, our results show that *A. herbicolus* populations were initially established on the plants (Fig. 2B). Moreover, the reduction of final densities of russet mites on the plants with *A. herbicolus* by 26.1% compared to the treatment without predators indicates some effect of phytoseiid mite in the weeks after pest introduction, although this difference was not significant. Cardoso (2019) showed that *A. herbicolus* supplemented with a high pollen dosage can significantly reduce whitefly densities in tomato plants and the predator densities increased until reaching an average eight times higher than the release density. We observed that *A. herbicolus* disappeared from the plants after the seventh week, most likely due to the increasing incidence of powdery mildew and subsequent deterioration of the plants. We expected to continue the experiment for a longer time, but the high powdery mildew infestation in the treatments with *A. herbicolus* and control (without predators) forced us to interrupt the experiment of population dynamics because leaves of the plants started to dry out. Severe powdery mildew infestations can lead to leaf chlorosis which results in reduced plant quality (Jones et al. 2001). Possibly, the limitation of physical resources such as oviposition sites, directly affect the predators present in the leaves attacked by powdery mildew. Perhaps better control of russet mites by *A. herbicolus* would have been achieved if the plants had been less affected by powdery mildew. The combined release of *A. herbicolus* and *H. anconai* could indicate a new biological control strategy for tomato crops. While the iolinid mite would reduce the density of russet mites and powdery mildew, *A. herbicolus*, besides to contributing to the reduction of *A. lycopersici*, would also control the whitefly *B. tabaci* (Cardoso 2019). However, the interaction between these two predators needs to be investigated.

Tomato plants with *H. anconai* produced fewer, but heavier fruits than plants of the other treatments (Fig. 4A), although this difference was not significant. As explained above, it is possible that plant feeding by the iolinid induces plant defences, and some of the plant hormones involved in defences (i.e. jasmonic acid) are also involved in flowering (Wasternack et al. 2013). Hence the induction of plant defences may result in the differential allocation of resources to vegetative growth and reproduction (Herms and Mattson 1992; Zhang et al. 2019). For example, a previous study showed that sweet pepper plants exposed to the omnivorous plant-feeding predator *Macrolophus pygmaeus* have fewer leaves and flowers than unexposed plants (Zhang et al. 2019). It should be noted though, that the tomato plants with *H. anconai* were still of good quality at the end of our experiment, in contrast to the plants of the other treatments, which were of such low quality due to high infestations with powdery mildew that the experiment had to be aborted. It is therefore highly likely that the plants with *H. anconai* would continue to produce fruits for a much longer time than the plants of the other treatments and further studies are required to assess life-time fruit production of plants with and without the predator.

In conclusion, we show that the early establishment of the omnivorous iolinid mite *H. anconai* simultaneously controls russet mites and powdery mildew on tomato plants. Our results are in agreement with the study by Pijnakker et al. (2022b), who reported that the establishment of the related iolinid *P. ubiquitus* before infection with herbivores provides dual protection against a key pest and pathogen in tomatoes. Further studies are required to determine whether augmentative releases of *H. anconai* can maintain *A. lycopersici* and/or powdery mildew under economic threshold densities in the field. In addition, powdery mildew is a disease of a wide range plants, and the economic importance of eriophyid mite pests is increasing worldwide (e.g., on vines: Knop & Hoy 1983; on figs: Abou-Awad et al. 1999). Therefore, the potential of the iolinid predatory mite *H. anconai* as a biological control agent in other crop systems warrants further investigation.

References

Abou-Awad BA (1979) Über die rotgelbe Tomatenmilbe, *Aculops lycopersici* (Massee) (Acari, Eriophyidae) in Ägypten. Anz Schadlingskde Pfl 52:153–156. https://doi.org/10.1007/BF01905641

Abou-Awad BA, El-Sawaf BM, Abdel Kader AA (1999) Life history and life table of *Pronematus ubiquitus* (Mc Gregor) as a predator of eriophyoid mites in Egypt (Acari: Tydeidae), Acarologia 40:29–32

Al-Azzazy MM, Al-Rehiayani SM, Abdel-Baky NF (2018) Life tables of the predatory mite *Neoseiulus cucumeris* (Acari: Phytoseiidae) on two pest mites as prey, *Aculops lycopersici* and *Tetranychus urticae*. Arch Phytopathol Plant Prot 51:637–648. https://doi.org/10.1080/03235408.2018.1507013

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48. https://doi.org/10.18637/jss.v067.i01

Belcher DW, Thurston R (1982) Inhibition of movement of larvae of the convergent lady beetle by leaf trichomes of tobacco. Envir Entomol 11:91–94. https://doi.org/10.1093/ee/11.1.91

Brodeur J, Bouchard A, Turcotte G (1997) Potential of four species of predatory mites as biological control agents of the tomato russet mite, *Aculops lycopersici* (Masse) (Eriophyidae). Can Entomol 129:1–6. https://doi.org/10.4039/Ent1291-1

Caffi T, Legler SE, Bugiani R, Rossi V (2013) Combining sanitation and disease modelling for control of grapevine powdery mildew. Eur J Plant Pathol 135:817–829. https://doi.org/10.1007/s10658-012-0124-0

Cardoso AC (2019) Search for predatory mites to control tomato pests. MSc Dissertation, Federal University of Viçosa

Castañé C, van der Blom J, Nicot PC (2020) Tomatoes. In: Gullino, M, Albajes R, Nicot P. (eds) In: Integrated Pest and Disease Management in Greenhouse Crops. pp 487-511, Springer, Cham. https://doi.org/10.1007/978-3-030-22304-5_17

Cédola CV, Sánchez NE (2003) Effect of tomato pubescence on development, survival and fecundity of *Tetranychus urticae* Koch and *Neoseiulus californicus* (McGregor) [Acari: Tetranychidae: Phytoseiidae]. Acarologia 43:255-260

Cruz-Miralles J, Cabedo-López M, Pérez-Hedo M, Flors V, Jaques JA (2019) Zoophytophagous mites can trigger plant-genotype specific defensive responses affecting potential prey beyond predation: the case of *Euseius stipulatus* and *Tetranychus urticae* in citrus. Pest Manag Sci 75:1962-1970

de Moraes GJ, Lima HC (1983) Biology of *Euseius concordis* (Chant) (Acarina: Phytoseiidae) a predator of the tomato russet mite. Acarologia 24:251–255

de Moraes GJ, Mesa NC (1988) Mites of the family Phytoseiidae (Acari) in Colombia, with descriptions of three new species. Int J Acarol 14:71–88

De Vis RMJ, de Moraes GJ, Bellini MR (2006) Initial screening of little-known predatory mites in Brazil as potential pest control agents. Exp Appl Acarol 39:115–125. https://doi.org/10.1007/s10493-006-9004-7

Delisle JF, Shipp L, Brodeur J (2015) Apple pollen as a supplemental food source for the control of western flower thrips by two predatory mites, *Amblyseius swirskii* and *Neoseiulus cucumeris* (Acari: Phytoseiidae), on potted chrysanthemum. Exp Appl Acarol 65:495–509

Denno RF, Gratton C, Peterson MA, Langellotto GA, Finke DL, Huberty AF (2002) Bottom-up forces mediate natural-enemy impact in a phytophagous insect community. Ecology 83:1443–1458

Drukker B, Janssen A, Ravensberg W, Sabelis MW (1997) Improved control capacity of the mite predator *Phytoseiulus persimilis* (Acari: Phytoseiidae) on tomato. Exp Appl Acarol 21:507–518. https://doi.org/10.1023/B:APPA.00000 18885.35044.C6

Duarte MV, Venzon M, Bittencourt MCDS, Rodríguez-Cruz FA, Pallini A, Janssen A (2015) Alternative food promotes broad mite control on chilli pepper plants. BioControl 60: 817-825

Duso C, Castagnoli M, Simoni S, Angeli G (2010) The impact of eriophyoids on crops: recent issues on *Aculus schlechtendali*, *Calepitrimerus vitis* and *Aculops lycopersici*. Exp Appl Acarol 51:151–168. https://doi.org/10.1007/s10493-009-9300-0

Duso C, Malagnini V, Paganelli A, Aldegheri L, Bottini M, Otto S (2004) Pollen availability and abundance of predatory phytoseiid mites on natural and secondary hedgerows. BioControl 49:397–415

Duso C, Pozzebon A, Capuzzo C, Malagnini V, Otto S, Borgo M (2005) Grape downy mildew spread and mite seasonal abundance in vineyards: effects on *Tydeus caudatus* and its predators. Biol Control 32: 143-154.

Eisner T, Eisner M, Hoebeke ER (1998) When defense backfires: Detrimental effect of a plant's protective trichomes on an insect beneficial to the plant. Proc Natl Acad Sci U.S.A 95:4410–4414. https://doi.org/10.1073/PNAS.95.8.4410

English-Loeb G, Norton AP, Gadoury DM, Seem RC and Wilcox WF (1999) Control of powdery mildew in wild and cultivated grapes by a Tydeid mite. Biol Control 14:97–103

English-Loeb G, Norton AP, Gadoury DM, Seem RC and Wilcox WF (2007) Biological control of grape powdery mildew using mycophagous mites. Plant Dis 91: 421-429

Flaherty DL, Hoy MA (1971) Biological control of Pacific mites and Willamette mites in San Joaquin Valley vineyards: part III. Role of tydeid mites. Res Popul Ecol 13:80-96

Glas JJ, Alba JM, Simoni S, Villarroel C A, Stoops M, Schimmel BCJ, Schuurink, RC, Sabelis MW, Kant MR (2014) Defense suppression benefits herbivores that have a monopoly on their feeding site but can backfire within natural communities. BMC Biology 12:1–14. https://doi.org/10.1186/S1291 5-014- 0098-9

Glas JJ, Schimmel BCJ, Alba JM, Escobar-Bravo R, Schuurink RC, Kant MR (2012) Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. Int J Mol Sci 13: 17077–17103. https://doi.org/10.3390/ijms1 31217077

González-Fernández JJ, de la Peña F, Hormaza JI, Boyero JR, Vela JM, Wong E, Trigo MM, Montserrat M (2009) Alternative food improves the combined effect of an omnivore and a predator on biological pest control. A case study in avocado orchards. Bull Entomol Res 99:433–444

Greenhalgh R, Dermauw W, Glas JJ, Rombauts S, Wybouw N, Thomas J, Alba JM, Pritham EJ, Legarrea S, Feyereisen R, van de Peer Y, van Leeuwen T, Clark RM, Kant MR (2020) Genome streamlining in a minute herbivore that manipulates its host plant. Elife 9:e56689

Hagedorn HH (1968) Effect of the age of pollen used in pollen supplements on their nutritive value for the honeybee. I. Effect on thoracic weight, development of hypopharyngeal glands and brood rearing. J Apic Res 7:89–95

Herms DA, Mattson WJ (1992) The dilemma of plants: to grow or defend. Q Rev Biol 67:283–335. https://doi.org/10.1086/417659.

Hessein NA, Perring TM (1986) Feeding habits of the Tydeidae with evidence of *Homeopronematus anconai* (Acari: Tydeidae) predation on *Aculops lycopersici* (*Acari: Eriophyidae*). Int J Acarol 12:215–221. https:// doi. org/ 10. 1080/ 01647 95860 86834 67

Hessein NA, Perring TM (1988) The importance of alternate foods for the mite *Homeopronematus anconai* (Acari: Tydeidae). *Ann Entomol Soc Am* 81:488–492. https://doi.org/10.1093/aesa/81.3.488

Holt RD (1977) Predation, apparent competition, and the structure of prey communities. Theor Popul Biol 12:197-229.

Hothorn T, Hornik K, Wiel MA van de, Zeileis A (2008) Implementing a class of permutation tests: The coin package. J Stat Soft 28:1-23. https://doi.org/10.18637/jss.v028.i08

Janssen A, van Rijn PCJ (2021) Pesticides do not significantly reduce arthropod pest densities in the presence of natural enemies. Ecol Lett 24:2010–2024. https://doi.org/10.1111/ele.13819

Jarvis WR (1992) Managing diseases in greenhouse crops. APS Press, St. Paul

Jones H, Whipps JM, Gurr SJ (2001) The tomato powdery mildew fungus *Oidium neolycopersici*. Mol Plant Pathol 2:303–309

Kant MR, Jonckheere W, Knegt B, Lemos F, Liu J, Schimmel BCJ, Villarroel CA, Ataide LMS, Dermauw W, Glas JJ, Egas M, Janssen A, van Leeuwen T, Schuurink RC, Sabelis MW, Alba JM (2015) Mechanisms and ecological consequences of plant defence induction and suppression in herbivore communities. Ann Bot 115:1015–1051. https://doi.org/10.1093/AOB/MCV054

Kawai A, Haque MM (2004) Population dynamics of tomato russet mite, *Aculops lycopersici* (Massee) and its natural enemy, *Homeopronematus anconai* (Baker). Jpn Agric Res Q 38:161–166. https:// doi. org/ 10. 6090/ jarq. 38. 161

Kennedy GG (2003) Tomato, pests, parasitoids, and predators: Tritrophic interactions involving the genus *Lycopersicon*. Annu Rev Entomol 48:51–72. https://doi.org/10.1146/ANNUREV.ENTO.48.091801.112733

Kiss L (2003) A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. Pest Man Sci 59:475-483

Kiss L, Russell JC, Szentivanyi O, Xu X, Jeffries P (2004) Biology and biocontrol potential of *Ampelomyces mycoparasites*, natural antagonist of powdery mildew fungi. Biocontrol Sci Technol 14:635–651.

Knop N, Hoy M (1983) Biology of a tydeid mite, *Homeopronematus anconai* (n. comb.) (Acari: Tydeidae), important in San Joaquin Valley vineyards. Hilgardia 51(5):1–30. https://doi.org/10.3733/hilg.v51n05p030

Lebeda A, Mieslerová B, Petřivalský M, Luhová L, Špundová M, Sedlářová M, Nožková-Hlaváčková V, Pink DAC (2014) Resistance mechanisms of wild tomato germplasm to infection of *Oidium neolycopersici*. Eur J Plant Pathol 138:569–596

Legarrea S, Janssen A, Dong L, Glas JJ, van Houten YM, Scala A, Kant MR (2022) Enhanced top-down control of herbivore population growth on plants with impaired defences. Funct Ecol 36:2859-2872

Legler SE, Pintye A, Caffi T, Gulyás S, Bohár G, Rossi V, Kiss L (2016) Sporulation rate in culture and mycoparasitic activity, but not mycohost specificity, are the key factors for selecting *Ampelomyces* strains for biocontrol of grapevine powdery mildew (*Erysiphe necator*). Eur J Plant Pathol 144:723–736. https://doi.org/10.1007/s10658-015-0834-1

Leman A, Messelink G (2015) Supplemental food that supports both predator and pest: a risk for biological control? Exp Appl Acarol 65:511–524

Lenth R (2016) Least-squares means: the R package lsmeans. J Stat Softw 69:1–33

Lindquist EE, Sabelis MW, Bruin J (eds) (1996) Eriophyoid mites—their biology, natural enemies and control, vol World Crop Pest Series vol 6. Elsevier, Amsterdam, p 790

Moraes GJ, Flechtmann CHW (2008) Manual de acarologia: acarologia básica e ácaros de plantas cultivadas no Brasil. Holos, Ribeirão Preto

Nomikou M, Janssen A, Schraag R, Sabelis MW (2004) Vulnerability of *Bemisia tabaci* immatures to phytoseiid predators: Consequences for oviposition and influence of alternative food. Entomol Exp Appl 110:95–102

Nomikou M, Sabelis MW, Janssen A (2010) Pollen subsidies promote whitefly control through the numerical response of predatory mites. BioControl 55:253–260

Norton AP, English-Loeb G, Gadoury D, Seem RC (2000) Mycophagous mites and foliar pathogens: Leaf domatia mediate tritrophic interactions in grapes. Ecology 81:490–499

Park HH, Shipp L, Buitenhuis R (2010) Predation, development, and oviposition by the predatory mite *Amblyseius swirkii* (Acari: Phytoseiidae) on tomato russet mite (Acari: Eriophyidae). J Econ Entomol 103:563–569. https://doi.org/10.1603/ec09161

Paspati A, Rambla JL, Gresa MPL, Arbona V, Gómez-Cadenas A, Granell A, González-Cabrera J, Urbaneja A (2021) Tomato trichomes are deadly hurdles limiting the establishment of *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae). Biol Control 157:104572. https://doi.org/10.1016/j.biocontrol.2021.104572

Pearse IS, LoPresti E, Schaeffer RN, Wetzel WC, Mooney KA, Ali JG, Ode PJ, Eubanks MD, Bronstein JL, Weber MG (2020) Generalising indirect defence and resistance of plants. Ecol Lett 23:1137–1152. https://doi.org/10.1111/ ELE.13512

Pérez-Hedo M, Bouagga S, Zhang NX, Moerkens R, Messelink G, Jaques JA, Flors V, Broufas G, Urbaneja A & Pappas ML (2022) Induction of plant defenses: the added value of zoophytophagous predators. J Pest Sci 95:1501-1517. https://doi.org/10.1007/s10340-022-01506-3

Pérez-Hedo M, Urbaneja-Bernat P, Jaques JA, Flors V, Urbaneja A (2015) Defensive plant responses induced by *Nesidiocoris tenuis* (Hemiptera: Miridae) on tomato plants. J Pest Sci 88:543-554

Pernal SF, Currie RW (2000) Pollen quality of fresh and 1-year-old single pollen diets for worker honey bees (*Apis mellifera* L.). Apidologie 31:387–409

Peterson JA, Ode PJ, Oliveira-Hofman C, Harwood JD (2016) Integration of plant defense traits with biological control of arthropod pests: Challenges and opportunities. Front Plant Sci 7:1794

Pijnakker J, Hürriyet, A, Petit C, Vangansbeke D, Duarte M, Arijs Y, Moerkens R, Sutter L, Maret D, Wäckers F (2022a) Evaluation of Phytoseiid and Iolinid mites for biological control of the tomato russet mite *Aculops lycopersici* (Acari: Eriophyidae). Insects 13: 1146

Pijnakker J, Moerkens R, Vangansbeke D, Duarte M, Bellinkx S, Benavente A, Merckx J, Stevens I, Wäckers F (2022b) Dual protection: A tydeoid mite effectively controls both a problem pest and a key pathogen in tomato. Pest Manag Sci 78:355–361. https://doi. org/ 10. 1002/ ps. 6647

Pinheiro J, Bates D, DebRoy S, Sakar D (2021) _nlme: Linear and Nonlinear Mixed Efects Models. R package version 3.1-152, https://CRAN.R-project.org/package=nlme

Price PW, Bouton CE, Gross P, McPheron BA, Thompson JN, Weis AE (1980) Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. Annu Rev Ecol Syst 11:41–65

Price PW, Denno RF, Eubanks MD, Finke DL, Kaplan I (2011) Insect ecology: behavior, populations and communities. Cambridge University Press.

Riddick EW, Simmons AM (2014) Do plant trichomes cause more harm than good to predatory insects? Pest Manag Sci 70:1655–1665. https://doi.org/10.1002/PS.3772

Royalty RN, Perring TM (1988) Morphological analysis of damage to tomato leaflets by tomato russet mite (Acari: Eriophyidae). J Econ Entomol 81:816–820. https://doi.org/10.1093/jee/81.3.816

Rutledge CE, Robinson AP, Eigenbrode SD (2003) Effects of a simple plant morphological mutation on the arthropod community and the impacts of predators on a principal insect herbivore. Oecologia 135:39–50. https://doi.org/10.1007/s00442-002-1114-2

Sabelis MW (1990) How to analyze prey preference when prey density varies? A new method to discriminate between effects of gut fullness and prey type composition. Oecologia 82:289–298

Sabelis MW, Janssen A, Kant MR (2001) The enemy of my enemy is my ally. Science 291: 2104–2105

Sabelis MW, van Baalen M, Bakker FM, Bruin J, Drukker B, Egas CJM, Janssen A, Lesna IKA, Pels SH, van Rijn PCJ, Scutareanu P (1999) The evolution of direct and indirect plant defence against herbivorous arthropods. In Olff H, Brown VK, Drent RH (Eds.). Herbivores: Between plants and predators (pp. 109–166). Blackwell.

Samaras K, Pappas ML, Pekas A, Wäckers F, Broufas GD (2021) Benefits of a balanced diet? Mixing prey with pollen is advantageous for the phytoseiid predator *Amblydromalus limonicus*. Biol Control 155:104531.

Sarmento RA, Lemos F, Bleeker PM, Schuurink RC, Pallini A, Oliveira MGA, Lima ER, Kant M, Sabelis MW, Janssen A (2011) A herbivore that manipulates plant defence. Ecol Lett 14:229–236. https://doi.org/10.1111/J.14610248.2010.01575.X

Sato MM, de Moraes GJ, Haddad ML, Wekesa VW (2011) Effect of trichomes on the predation of *Tetranychus urticae* (Acari: Tetranychidae) by *Phytoseiulus macropilis* (Acari: Phytoseiidae) on tomato, and the interference of webbing. Exp Appl Acarol 54:21–32. https://doi.org/10.1007/s10493-011-9426-8

Schmitz OJ, Hambäck PA, Beckerman AP (2000) Trophic cascades in terrestrial systems: A review of the effects of carnivore removals on plants. Am Nat 155: 141–153

Schneider RW, Grogan RG (1977) Tomato leaf trichomes, a habitat for resident populations of *Pseudomonas tomato*. Phytopathology 67: 898–902

Schuurink R, Tissier A (2020) Glandular trichomes: micro-organs with model status? New Phytol 225:2251–2266. https://doi.org/10.1111/nph.16283

Silva DB, Hanel A, Franco FP, de Castro Silva-Filho M, Bento JMS (2022) Two in one: The neotropical mirid predator *Macrolophus basicornis* increases pest control by feeding on plants. Pest Manag Sci 78:3314-3321

Silva FR, de Moraes GJ, Gondim Jr MG, Knapp M, Rouam SL, Paes JL, de Oliveira GM (2010) Efficiency of *Phytoseiulus longipes* Evans as a control agent of *Tetranychus evansi* Baker & Pritchard (Acari: Phytoseiidae: Tetranychidae) on screenhouse tomatoes. Neotrop Entomol 39:991–995.

Simmons AT, Gurr GM (2005) Trichomes of Lycopersicon species and their hybrids: Effects on pests and natural enemies. Agric For Entomol 7:265–276. https://doi.org/10.1111/J.14619555.2005.00271.X

Stiling P, Rossi AM (1997) Experimental manipulations of top-down and bottom-up factors in a tri-trophic system. Ecology 78:1602–1606

Sutherland AM, Parrella MP (2009) Biology and co-occurrence of *Psyllobora vigintimaculata* (Coleoptera: Coccinellidae) and powdery mildews in an urban landscape of California. Ann Entomol Soc Am 102:484–491

van Haren R, Steenhuis M, Sabelis M, De Ponti O (1987) Tomato stem trichomes and dispersal success of *Phytoseiulus persimilis* relative to its prey *Tetranychus urticae*. Exp Appl Acarol 3:115–121

van Houten YM, Glas JJ, Hoogerbrugge H, Rothe J, Bolckmans KJF, Simoni S, van Arkel J, Alba JM, Kant MR, Sabelis MW (2013) Herbivory-associated degradation of tomato trichomes and its impact on biological control of *Aculops lycopersici*. Exp Appl Acarol 60:127–138. https://doi.org/10.1007/s10493-012-9638-6

van Houten YM, Hoogerbrugge H, Knapp M (2020) Potential of *Pronematus ubiquitus* to control tomato russet mite, *Aculops lycopersici*. IOBC/WPRS Bull 149:87–92

van Rijn PCJ, Tanigoshi LK (1999) Pollen as food for the predatory mites *Iphiseius degenerans* and *Neoseiulus cucumeris* (Acari: Phytoseiidae): dietary range and life history. Exp Appl Acarol 23:785–802

van Rijn PCJ, van Houten YM, Sabelis MW (2002) How plants benefit from providing food to predators even when it is also edible to herbivores. Ecology 83:2664–2679

Vangansbeke D, Nguyen DT, Audenaert J, Verhoeven R, Gobin B, Tirry L, De Clercq P (2016) Supplemental food for *Amblyseius swirskii* in the control of thrips: feeding friend or foe? Pest Manag Sci 72:466-473

Verheggen FJ, Capella Q, Schwartzberg EG, Voigt D, Haubruge E (2009) Tomato-aphid-hoverfly: A tritrophic interaction incompatible for pest management. Arthropod Plant Interact 3:141–149. https://doi.org/10.1007/s1182 9009-9065-8

Vervaet L, De Vis R, De Clercq P, Van Leeuwen T (2021) Is the emerging mite pest *Aculops lycopersici* controllable? Global and genome-based insights in its biology and management. Pest Manag Sci 77:2635–2644. https://doi.org/10.1002/ps.6265

Vervaet L, Parapurath G, De Vis R, Van Leeuwen T, De Clercq P (2022) Potential of two omnivorous iolinid mites as predators of the tomato russet mite, *Aculops lycopersici*. J Pest Sci 95:1671-1680 https://doi.org/10.1007/s10340-022-01544-x

Vielba-Fernández A, Polonio Á, Ruiz-Jiménez L, de Vicente A, Pérez-García A, Fernández-Ortuño D (2020) Fungicide resistance in powdery mildew fungi. Microorganisms 8:1431

Wasternack C, Forner S, Strnad M, Hause B (2013) Jasmonates in flower and seed development. Biochimie 95:79–85. https://doi.org/10.1016/j.biochi.2012.06.005

Zhang NX, Messelink GJ, Alba JM, Schuurink R, Kant MR, Janssen A (2018) Phytophagy of omnivorous predator *Macrolophus pygmaeus* affects performance of herbivores through induced plant defences. Oecologia 186:101-113

Zhang NX, Messelink GJ, Verdonkschot S, Janssen A (2019) Plant feeding by an omnivorous predator affects plant phenology and omnivore performance. Biol Control 135:66-72

Figure legends

Fig. 1 Average (±SE) oviposition rates of *H. anconai* (**A**) and *A. herbicolus* (**B**) on the 2nd – 4th day of feeding on all stages of the tomato russet mite *A. lycopersici* or cattail pollen. Asterisks indicate a significant difference in oviposition rates between diets (***$p < 0.001$).

Fig. 2 Population dynamics of *Aculops lycopersici* and the predatory mites *H. anconai* (squares) and *A. herbicolus* on tomato plants. (**A**) Shown are average densities (± SE) of tomato russet mites in the presence of the predatory mites *H. anconai* (triangles), *A. herbicolus* (squares) and no predators (circles). (**B**) Shown are log densities (± SE) of all stages of both predatory mites, *H. anconai* (squares) and *A. herbicolus* (circles). The vertical arrows indicate the week that the plants were infested with 250 mobile individuals of *A. lycopersici*. Predators were released in week 0 and were fed *Typha* sp. pollen. Different letters behind the legends indicate significant differences between treatments and asterisks after the week number significant differences within each week, respectively (contrasts through model simplification after LME; $p < 0.05$).

Fig. 3 Average (± SE) number of leaflets with powdery mildew lesions in the first four weeks of the population dynamics experiment in presence of the predatory mites *H. anconai* (triangles), *A. herbicolus* (squares) and no predators (circles). Different letters in the legend indicate significant differences among treatments through time and asterisks after the week number show significant differences within each week between the treatment with *H. anconai* and the two other treatments respectively (contrasts through model simplification after GLMER; $p < 0.05$).

Fig. 4 (**A**) Average numbers of fruits per tomato plant (± SE) (**B**) average weight of individual tomato fruits per plant and (**C**) total fruit weight per plant infested with *A. lycopersici* in the presence of the predatory mites *H. anconai*, *A. herbicolus* and no predators, after an 8-week experiment.

Fig. 5 Average (± SE) number of leaflets with powdery mildew lesions in three different plant strata: top (**A**), middle (**B**) and bottom (**C**) of tomato plants in the presence and absence (no predator) of *H. anconai*. Different letters behind the legend indicate significant differences between treatments (contrasts through model simplification after GLMER; $p < 0.05$). n.s = not significant

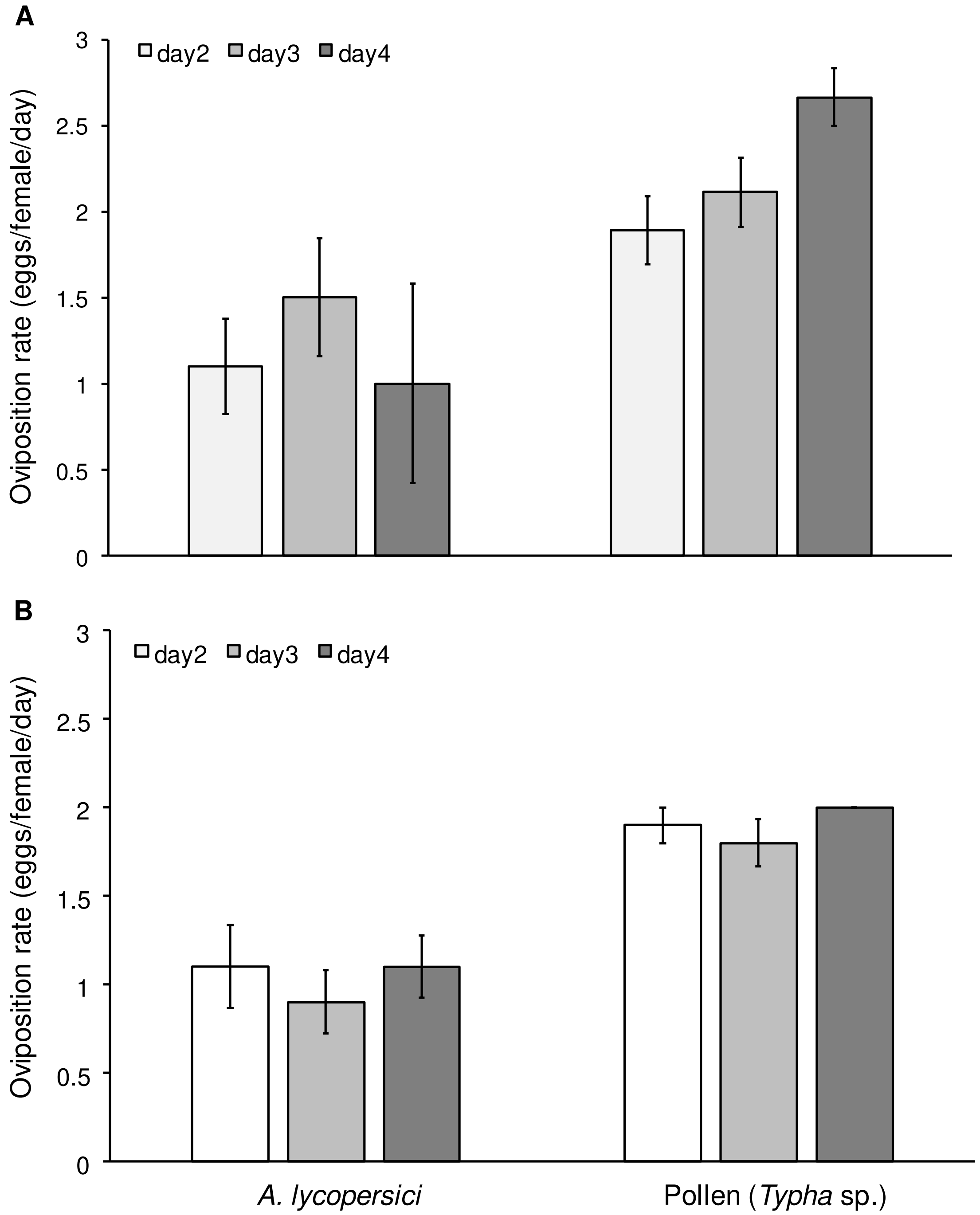
A
B
Oviposition rate (eggs/female/day)
0
0.5
1
1.5
2
2.5
3
day2
day3
day4
A. lycopersici
Pollen (Typha sp.)

Figure 1.

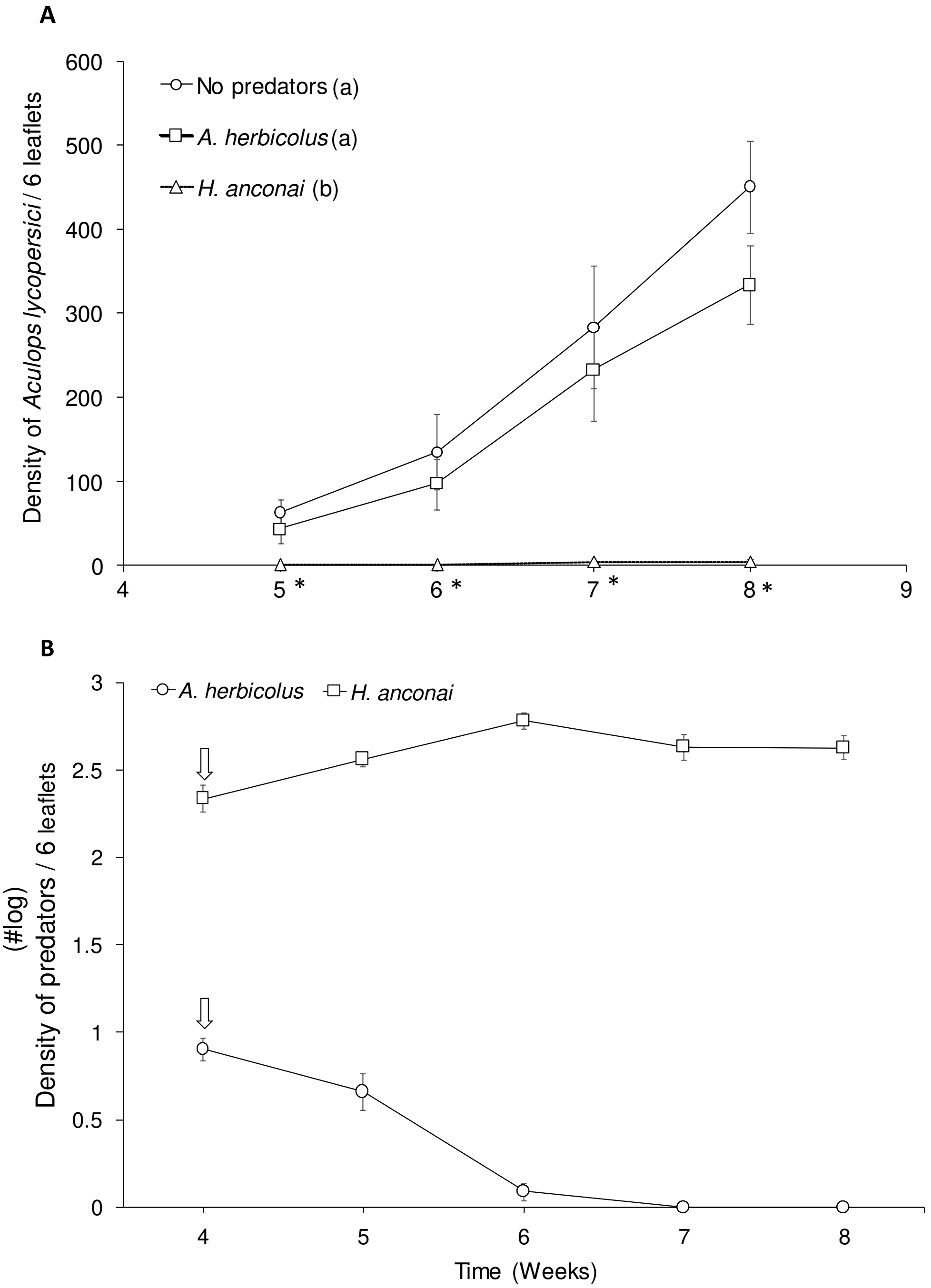
A
600
500
400
300
200
100
0
Density of *Aculops lycopersici* / 6 leaflets
No predators (a)
A. herbicolus (a)
H. anconai (b)
4
5 *
6 *
7 *
8 *
9
B
3
2.5
2
1.5
1
0.5
0
(#log)
Density of predators / 6 leaflets
A. herbicolus
H. anconai
4
5
6
7
8
Time (Weeks)

Figure 2.

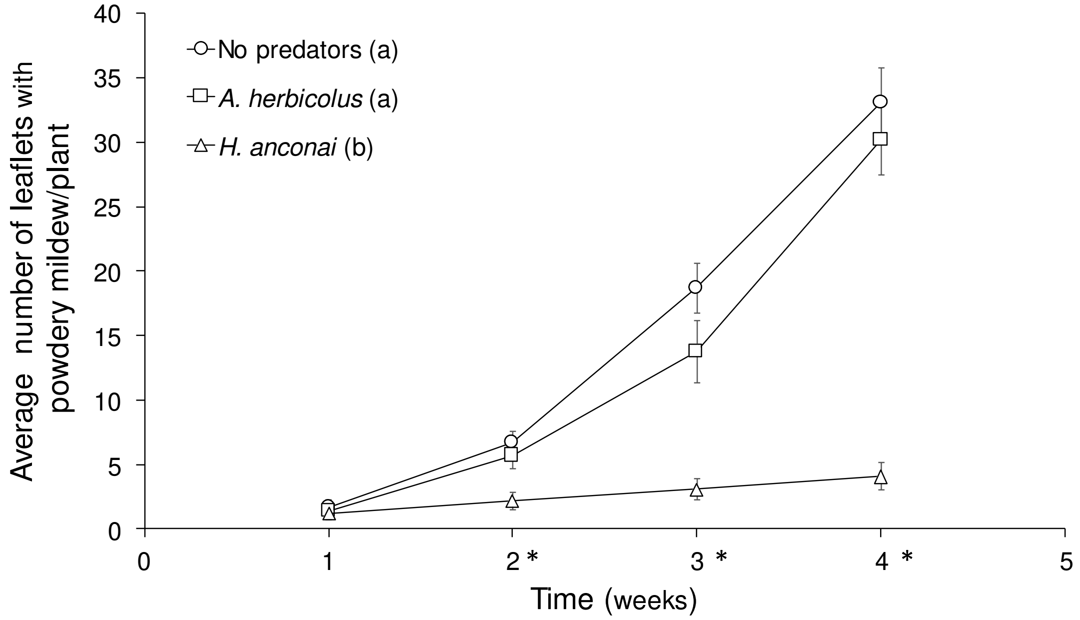
Average number of leaflets with powdery mildew/plant
0
5
10
15
20
25
30
35
40
No predators (a)
A. herbicolus (a)
H. anconai (b)
0
1
2 *
3 *
4 *
5
Time (weeks)

Figure 3.

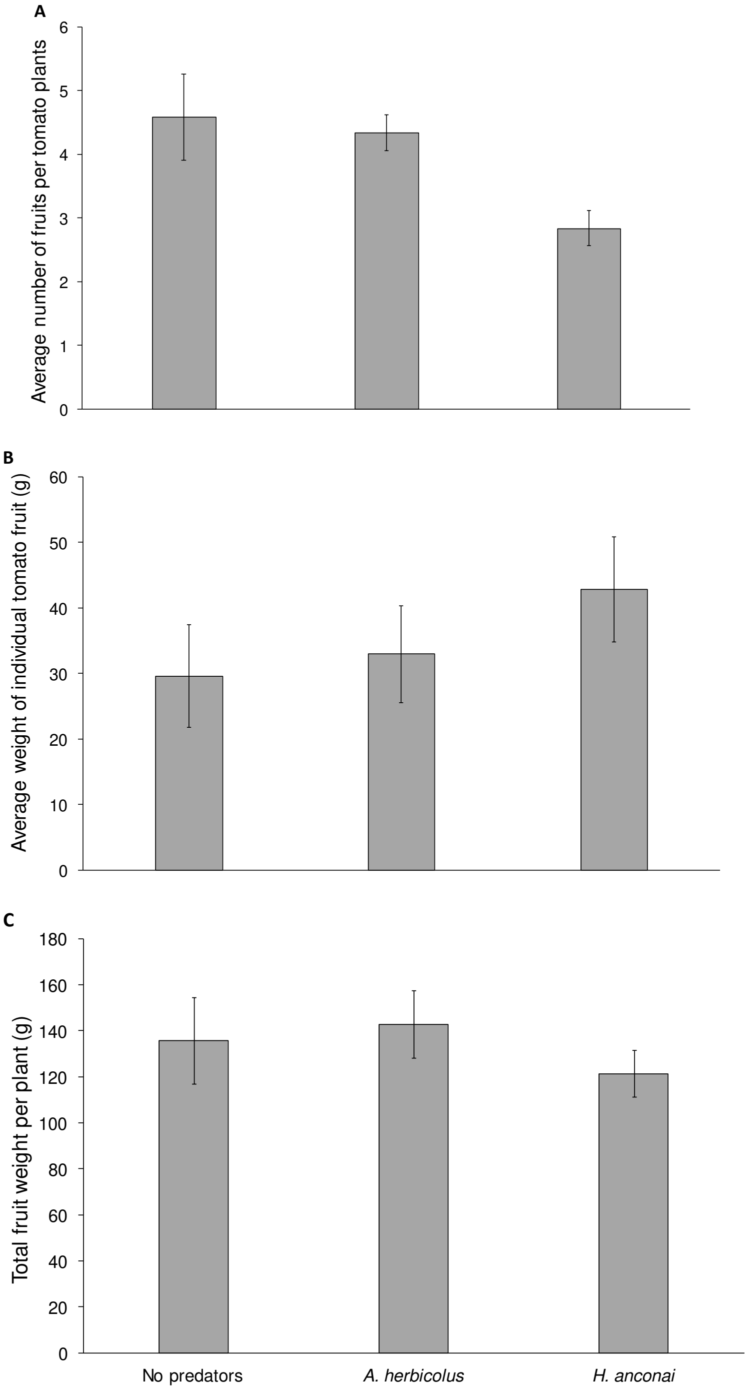
A
Average number of fruits per tomato plants
0
1
2
3
4
5
6
B
Average weight of individual tomato fruit (g)
0
10
20
30
40
50
60
C
Total fruit weight per plant (g)
0
20
40
60
80
100
120
140
160
180
No predators
A. herbicolus
H. anconai

Figure 4.

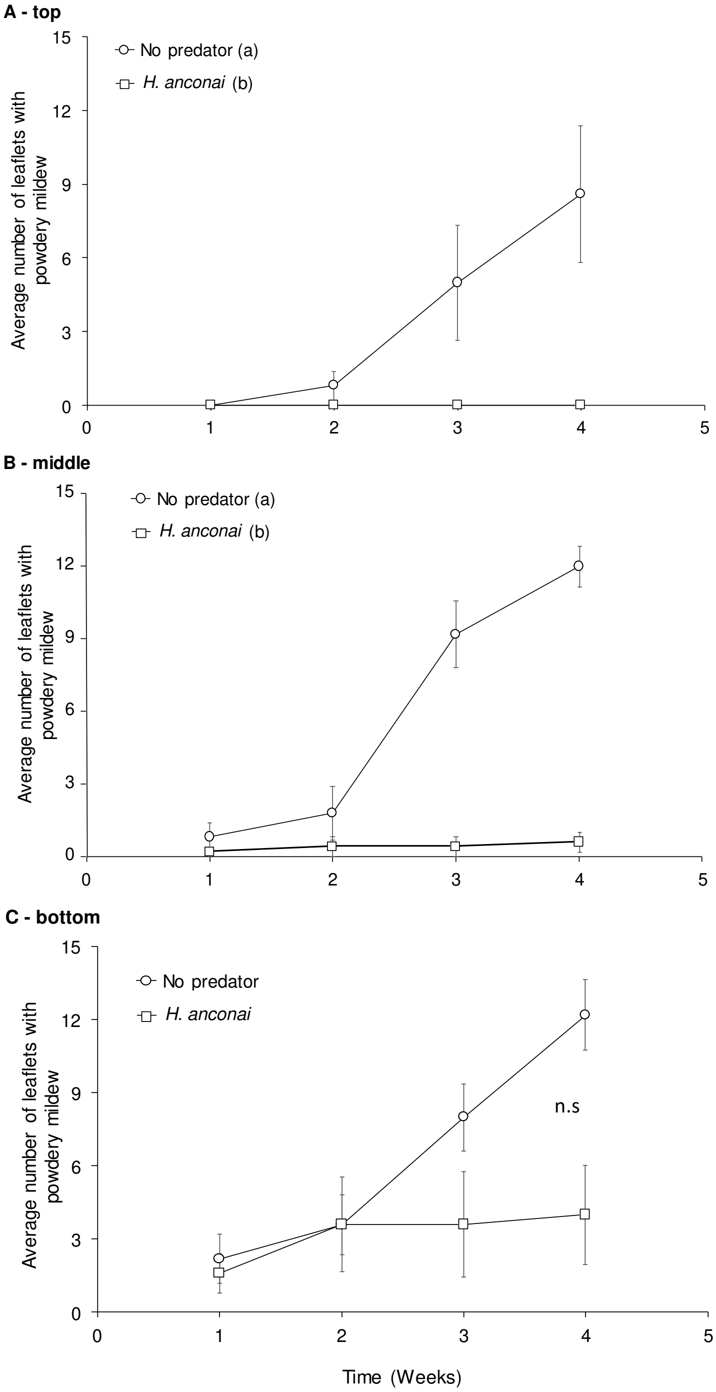
A - top
No predator (a)
H. anconai (b)
Average number of leaflets with powdery mildew
0
3
6
9
12
15
0
1
2
3
4
5
B - middle
No predator (a)
H. anconai (b)
Average number of leaflets with powdery mildew
0
3
6
9
12
15
0
1
2
3
4
5
C - bottom
No predator
H. anconai
n.s
Average number of leaflets with powdery mildew
0
3
6
9
12
15
0
1
2
3
4
5
Time (Weeks)

Figure 5.

CHAPTER III

An iolinid mite controls tomato russet mite, powdery mildew and promotes phytoseiid mite density on tomato plants

Ítalo Marcossi[1] · Sarah F. J. Souza[1] · Morgana M. Fonseca[2] · Angelo Pallini[1] · Thomas Groot[3] · Arne Janssen[1,4]

[1]Department of Entomology, Section Acarology, Federal University of Viçosa, Viçosa, MG, Brazil.

[2]Koppert do Brasil, Piracicaba, SP, Brazil

[3]Koppert BV, Berkel en Rodenrijs, The Netherlands

[4]IBED, Section Population Biology University of Amsterdam, The Netherlands

Chapter formatted in the norms of the journal: Biological Control

HIGHLIGHTS

- We evaluated the co-occurrence of two generalist predators' mites on tomato plants
- The iolinid mite was better at reducing tomato russet mites densities, even in the presence of the intraguild predator
- The predators coexisted on tomato plants supplemented with alternative food

ABSTRACT

The combined release of species of generalist predators can enhance biological control, but, in theory, predators may be excluded through predation on each other. We evaluated the co-occurrence of the generalist predator's mite *Amblyseius herbicolus* and *Homeopronematus anconai* and their control of the tomato russet mite (*Aculops lycopersici*). Besides the prey, both predators consume pollen, and *A. herbicolus* is an intraguild predator of *H. anconai*. Moreover, both predators are able to cope with the direct defence of tomato. A population dynamic experiment showed that *H. anconai* was better at reducing tomato russet mites densities on plants supplemented with pollen, even in the presence of the intraguild predator. The iolinid mite also increased the density of *A. herbicolus* on plants, in two ways, directly as an alternative prey and indirectly by controlling powdery mildew on tomato leaves. Although *A. herbicolus* reduces the densities of *H. anconai* by about half, the predators are able to persist in one crop for a sufficiently long period. The increased joint use of generalist predators for biological control highlights the importance of studies that evaluate the interaction of these natural enemies.

Key words: *Amblyseius herbicolus* - *Homepronematus anconai* - Intraguild predation – Coexixtence- *Aculops lycopersici*

1. Introduction

Augmentative biological control has been widely and successfully applied to several crops, especially in greenhouses. (Sanchez et al., 2000; van Lenteren et al., 2018, 2020). This approach consists of the repeated release of natural enemies to suppress outbreaks of the target pest species (Hajek, 2004; van Lenteren et al., 2018). As most crops are attacked by more than one pest species, augmentative biological control programs often use generalist predators as biological control agents (Collyer, 1964; Ramakers, 1990; van Rijn et al., 1999, 2002; Nomikou et al., 2002, 2010; Gerson and Weintraub, 2007; Messelink et al., 2008; Urbaneja et al., 2009; van Lenteren, 2012; Messelink and Janssen, 2014; Leman et al., 2020; Mendoza et al., 2022). This group of natural enemies feeds on more than one prey species and is capable to survive and reproduce on plant-derived resources such as nectar, pollen, or plant sap (Ramakers and Rabasse, 1995; van Rijn et al., 2002; Bouagga et al., 2018). Moreover, providing alternative food sources to generalist predators allows their introduction before the pest is present in crops and enables their persistence when the target pest is scarce (Altieri and Letourneau, 1982; Eubanks and Denno, 2000; Landis et al., 2000; Janssen and Sabelis, 2015; Gurr et al., 2017).

Although generalist predators have a wide range of prey, a single generalist predator is often not capable of suppressing all pest species present in the crop (Symondson et al., 2002). In such situations, a commonly employed strategy involves releasing multiple natural enemies, that may have complementary effects in controlling the pest species (Sih et al., 1998; Cardinale et al., 2003; Chow et al., 2008; Straub and Snyder, 2008; Messelink and Janssen, 2014). The coexistence of various natural enemies can give rise to diverse types of interactions, further increasing food web complexity (Janssen et al., 1998; Sih et al., 1998; Messelink et al., 2012). For example, when generalist predators share the same prey, they can interfere with each other through competition and intraguild predation (IGP) (Polis et al., 1989; Rosenheim et al., 1995; Janssen et al., 2006; Messelink et al., 2012; van Lenteren et al., 2018). If such interactions lead to the exclusion of one of these predators, the combined release of these natural enemies should be reconsidered. The identification of such trophic links is of primary importance to the success of pest management programs (Janssen et al., 2006; Chailleux et al., 2013; Fonseca et al., 2020). However, alternative food sources, either present in crops or provided, can decrease the negative effects among predators and facilitate coexistence for long enough to complement each other in pest control (Chailleux et al., 2013; Messelink et al., 2013; Messelink and Janssen, 2014).

Another factor that can affect the effectiveness of control by natural enemies is the interaction between the direct defences of plants and the population dynamics of generalist predators on those plants. For example, few natural enemies are known to be able to cope with direct defences of tomato plants (Legarrea et al., 2022). Tomato plants possess glandular trichomes, which are outgrowths of the epidermis in which specialized metabolites are synthesized and stored (Schuurink and Tissier, 2020). They predominantly act as broad-spectrum direct defence against herbivores (Glas et al. 2012), but they can also induce mortality of predators (van Haren et al., 1987; Coll et al., 1997; Lambert, 2007; Verheggen et al., 2009; van Houten et al., 2013; Paspati et al., 2021; Legarrea et al., 2022). Among the generalist predators that are able to establish on tomato plants, mirid bugs, such as *Macrolophus pygmaeus* and *Nesidiocoris tenuis* stand out, commonly introduced to control whiteflies (Bonato et al., 2006; Calvo et al. al., 2009; Bueno et al., 2019). Furthermore, a few species of predatory mites mainly from the Phytoseiidae family (Drukker et al., 1997; Silva et al., 2010; Sato et al., 2011), are used for pest control on tomato, however, their effectiveness is reduced by the glandular trichomes (van Houten et al., 2013; Legarrea et al., 2022).

We have recently shown that two species of generalist predatory mites are adapted to direct defences of tomato. These predatory mites, *Amblyseius herbicolus* Chant (Acari: Phytoseiidae) and *Homeopronematus anconai* (Baker) (Acari: Iolinidae), were originally collected from tomato plants. They are true omnivores, feeding on several trophic levels such as plant materials (pollen, nectar, plant sap), herbivores and fungi (Hessein and Perring, 1986; Kawai and Haque, 2004; Reis et al., 2007; Moraes and Flechtmann, 2008; Rodríguez-Cruz et al., 2013; Duarte et al., 2015, Marcossi et al., 2020; Kalile et al., 2021; Vervaet et al., 2022). Both predators established on tomato plants with pollen as alternative food and share the same prey, the tomato russet mite *Aculops lycopersici* (Acari: Eryophidae) (Hessein and Perring, 1986; Kawai and Haque, 2004; Rodríguez-Cruz et al., 2013; Duarte et al., 2015, Cardoso, 2019; Marcossi et al., 2020; Vervaet et al., 2022). While the iolinid mite can reduce the densities of russet mites and powdery mildew, *A. herbicolus* can contribute to the control of *A. lycopersici*, but can also control other tomato pests, such as the whitefly *Bemisia tabaci* (Chapter 1). However, only *H. anconai* was able to increase in density and effectively controlled tomato russet mites on tomato plants (Chapter 2). Moreover, the iolinid mite was also effective in controlling powdery mildew (*Oidium neolycopersici*) (Chapter 2). Severe powdery mildew infestations can result in reduced plant quality (Jones et al. 2001), and can directly affect generalist predators that do not feed on fungi, such as *A. herbicolus* (Chapter 2), for example by limiting the physical resources of predators, such as oviposition sites and prey patches. This means that the combined release of

A. herbicolus and *H. anconai* should be studied in more detail before they are used in combination to control several pests in tomato.

As a first step, we evaluated whether the combined release of *A. herbicolus* and *H. anconai*, with pollen as alternative food, on tomato plants supplemented with pollen can contribute to the biological control of *A. lycopersic*i. Laboratory observations showed that *A. herbicolus* adults prey on all stages of *H. anconai*, but not vice versa. As both predators share the same prey and the same alternative food, they can engage in intraguild predation., with *A. herbicolus* as the intraguild predator and *H. anconai* as intraguild prey. We therefore expected that the presence of *H. anconai* would increase the densities of *A. herbicolus* both because it serves as food and because it controls powdery mildew, hence, exploring how these potentially interacting predators respond to the presence of each other is an important step toward the successful release of both natural enemies. To investigate this, we evaluated the performance of the omnivorous predator *A. herbicolus* feeding on all stages of *A. lycopersici*, *H. anconai* and pollen. Subsequently, we evaluated the control of tomato russet mites with releases of *H. anconai* alone, *A. herbicolus* alone, or in a combined release, in all cases with pollen as alternative food for both species. Furthermore, we evaluated the effectiveness of the iolinid mite in the different treatments in suppressing natural powdery mildew infections of tomato plants.

2. Materials and methods

2.1. Plant materials

Tomato seeds (*Solanum lycopersicum* var. Aguamiel (EX V305), Limagrain®, imported by Vilmorin Seed Generation in Brazil) were sown in a commercial plant substrate enriched with macro - and micronutrients (Tropstrato HT vegetables®), in polystyrene trays (8 x 16 cells). Fifteen days after germination, the seedlings were transplanted to plastic 3 L pots with the same substrate. The plants were fertilized every week with a solution of 50g of N-P-K (20-05-20) and 100g of simple superphosphate in 20L water and were supported with bamboo sticks, to which they were tied with a string. The plants were kept inside cages in a room with natural light at 23±3 °C and 70±10% relative humidity. Cattail pollen used in all experiments was collected from *Typha* sp. plants from the campus of the Federal University of Viçosa. The pollen was dried in an oven at 60° C for 24 h and stored in a freezer at -20 °C according to recommendation (Hagedorn 1968; Pernal and Currie 2000).

2.2. Mite rearings

Tomato russet mites were collected from tomatoes grown in a rural area of Coimbra (MG, Brazil, 20° 51' 42.4" S, 42° 50' 14.4" W) and were maintained on tomato plants inside cages (0.5 x 0.5 x 1.0 m). A new tomato plant with at least four completely developed leaves was added to each cage every month and the oldest plants removed. The cages were kept in a room at 22±2 °C, 70±10% RH, 12:12 L:D.

The laboratory rearing of *H. anconai* was initiated with mites collected on *A. lycopersici*-infested tomato plants in the house of the first author in Viçosa. The species identification was carried out by Tairis Da-Costa and Noeli Juarez Ferla from UNIVATES (RS, Brazil). The iolinid mites were reared on tomato plants with cattail pollen as food, offered twice a week. The tomato plants were kept inside cages (0.5 x 0.5 x 1.0 m), which were kept in climate rooms as above. The rearing of *A. herbicolus* was started with individuals collected from tomato plants in gardens in the urban and rural areas of Prados (MG, Brazil) (Cardoso 2019). They were reared on arenas made of PVC sheets (15 × 10 cm) on top of foam pads (h = 3 cm), which were kept in plastic trays (29 × 14 × 4 cm) filled with water (cf. van Rijn and Tanigoshi 1999). The edges of the arenas were wrapped in wet tissue paper to prevent the mites from escaping, but the tissue also served as a water source. Small pieces of cotton wool were placed on the arenas as oviposition sites and were covered with pieces of tent-shaped PVC sheet (1.5 cm²) for shelter. Cattail pollen was offered as food two times per week. The rearing was maintained in climate rooms as above**.**

2.3. Juvenile development and oviposition

Whereas *A. herbicolus* can prey on all stages of *H. anconai*, the iolinid mite can not to prey on any stage of *A. herbicolus*, probably due to the large size difference between the predators. Adults females of *A. herbicolus* (313–352 µm in length) are bigger than adult of *H. anconai* (150-200 µm in length) (Chant, 1959; Abou-Awad, 1979; Akyazi et al., 2016; Vervaet et al. 2021) and this might render adults of H. anconai vulnerable to predation by adults of the phytoseiid mite. Therefore, we compared the performance of *A. herbicolus* feeding on different diets including *H. anconai*. For this, we measured peak oviposition rate, development rate and juvenile survival (Janssen and Sabelis, 1992; Nomikou et al., 2001).

First, we measured the development and survival of juvenile *A. herbicolus* feeding on mixed stages of *A. lycopersici*, *H. anconai* and *H. anconai* plus cattail pollen. Juveniles feeding on pollen were used as control. This experiment was performed on tomato leaf discs (Ø = 2 cm) arranged on wet tissue paper (Ø = 3 cm) and placed in Petri dishes (Ø = 5 cm, 1.5 cm high), which were closed with lids to prevent predatory mites from escaping. Newly hatched larvae of *A. herbicolus* were removed from the stock colony and each individual was placed in an experimental unit on a tomato leaf disc according to the treatment. To avoid depletion and loss of quality of the food, new discs were offered daily and those from the previous day were removed. Juvenile development and survival were monitored daily until the individuals reached adulthood or had died. To assess the oviposition rate of *A. herbicolus* on the same diets, we introduced a young adult female (12-15 days after egg eclosion) in similar experimental units. Oviposition rates were assessed every 24 hours during 4 days. New discs were offered after each evaluation. Ten replicates were carried out per treatment in both experiments.

Development data were analysed with a Cox proportional hazards model (package coxph, Therneau, 2020). Because the treatment with *A. lycopersici* was the only that resulted in death over time, survival data were analysed with the Kaplan–Meier estimates (package survdiff Therneau, 2020). To prevent possible effects from the previous diet, oviposition of the first day was excluded from the analysis (Sabelis, 1990). The effect of different diets on the oviposition rate was assessed with a linear mixed-effects model (LME) with treatment and time as fixed factors and individuals as a random factor to correct for repeated measures. Contrasts among treatments here and below were done with the function emmeans from the package with the same name (Lenth, 2019). The tests were performed in a room at 22 ± 2 °C, 70 ± 10% RH, 12:12 L:D.

2.4. Dynamics on plants

We carried out this experiment in twenty-eight BugDorm-4F® insect cages (0.5 x 0.5 x 1.0 m), under natural conditions (mean temperature 23.6°C, range 17.9–27.7° C; mean relative humidity 68%, range 42–86%), but protected from occasional rain. Each cage contained a single tomato plant, distributed over three treatments (seven replicates per treatment). The treatments consisted of releases of either *H. anconai* alone, *A. herbicolus* alone, a combined release of both predators, and a treatment without predators. Initially, a tomato leaf disk (3 cm^2) with 5 adult females of *H. anconai* was placed on all leaves of the tomato plants (4-leaf stage) receiving the two treatments with *H. anconai*, with a total of 20 predators per plant. A week later, we released 10 adult females of *A. herbicolus* per tomato plant in a similar way, on the plants receiving the two treatments with this predator. About 1 mg of *Typha* sp. pollen was applied to all leaves of the plants twice per week, including to those of the treatment without predators, starting from the release of *H. anconai*.

Two weeks after the release of *H. anconai*, thus one week after the introduction of *A. herbicolus*, all treatments were infested with russet mites. Discs were cut from leaflets picked from a well-infested tomato plant; each disc harboured on average c. 25 mobile stages. Each tomato plant received a leaf disc with russet mites on the 2nd to the 6th leaf from the bottom leaf, hence, about 100 mobile stages in total. The discs were positioned on the middle of each leaf. The numbers of *A. herbicolus* were assessed weekly from the 2nd week onwards, using a handheld magnifying glass and head magnifier with light, sampling all the leaves of each tomato plant that received this predator. The numbers of *H. anconai* and russet mites were measured from the 4th week after the introduction of these mites. This was done by collecting two leaflets from the bottom, middle and top section of each plant, respectively. The leaflets were stored in a separate box per plant for evaluation in the laboratory under a stereo microscope. Each leaflet was photographed to measure the leaflet area using ImageJ® software. Subsequently, we took a disk (Ø = 2 cm) from each leaflet and counted all *H. anconai* and *A. lycopersici* on each disk. Based on these numbers and the surfaces of the discs and the leaflets, we calculated pest and predator densities for each leaflet.

From the sixth week after the introduction of *H. anconai*, natural infestations of powdery mildew occurred, mainly in treatments without this mite. The presence of powdery mildew was quantified from that week onwards by non-destructive counting of leaflets containing visual powdery mildew lesions. This was done until the end of the experiment.

The densities of *A. lycopersici* were log (x+1) transformed before the analysis and compared with a linear mixed effects model (LME) with time and treatment and their interaction as fixed factors (lme of the package nlme, Pinheiro et al. 2021). The replicate (plants) was used as a random factor to correct for repeated measures. Models were checked with normal error plots and plots of residuals against fitted values. Significance of factors and interactions were determined with likelihood ratio (L.R.) tests. The density of both predators were analysed similarly. To compare the number of leaflets with powdery mildew lesions, we counted all the leaflets (healthy and infected by powdery mildew) of the tomato plants in the last week of the population dynamics experiment. The proportion of leaflets with damage was compared using a generalized linear mixed (GLM) with a binomial error distribution. All statistical analyses were done using the software R version 4.2.2 (R Core Team 2023).

3. Results

3.1. Juvenile development and oviposition

There was significant effect of predator diet on juvenile development (Fig. 1A, Cox proportional hazards: $Chi^2 = 23.8$, d.f. = 3, $p < 0.001$). Feeding on tomato russet mites resulted in significantly longer juvenile development than on the other diets. Juvenile survival was also significantly affected by diet (Fig. 1a, Mantel–Heanszel test of Kaplan–Meier survival estimates: $Chi^2 = 13.8$, d.f. = 3, $p = 0.003$). While all individuals reached adulthood in the treatments with *H. anconai*, pollen and both diets combined, in the treatment with *A. lycopersici*, 40% of the individuals did not survive.

There was a significant difference in oviposition rate of *A. herbicolus* on the four diets (Fig. 1B; LME: L.R. = 12.9, d.f. = 3, $p = 0.005$) and oviposition did not differ among days (L.R. = 0.27, d.f. = 3, $p = 0.603$). Feeding on cattail pollen and *H. anconai* with pollen resulted in the highest oviposition rates, but oviposition on *H. anconai* with pollen did not differ significantly from the other treatments.

3.2. Dynamics on plants

Aculops lycopersici densities differed significantly among treatments through time (Fig. 2A; LME: interaction of treatment with time: L.R. = 34.6, d.f. = 3, $p < 0.0001$). Tomato plants of treatments with only the predatory mite *H. anconai* and with both predators harboured significantly lower densities of russet mites than plants of the treatment with only *A. herbicolus*, hence, without *H. anconai* (Fig. 2A). Moreover, from the first week of evaluation, plants with *H. anconai* alone already showed a significantly lower density of russet mite density than the other treatments (Fig. 2A). There was a reduction in the density of tomato russet mites on plants without predators and with *A. herbicolus* alone from the 7th and 9th week onwards, respectively, likely due to the invasion of *H. anconai* in these treatments. However, *A. lycopersici* had already reached high densities, resulting in higher plant damage (Fig. 3). This indicates that early release of *H. anconai* is essential for the effective control of russet mites.

Densities of *H. anconai* were significantly affected by the presence of *A. herbicolus* (intraguild predator) (Fig. 2B; LME: L.R. = 11.7, d.f. = 1, $p < 0.0001$), and the densities differed significantly through time (LME: L.R. = 29.80, d.f. = 3, $p < 0.0001$). Throughout the experiment, the numbers of iolinid mites in the presence of *A. herbicolus* were lower, and this difference was significant in weeks five, eight and nine (Fig. 2B. The presence of *H. anconai* (intraguild prey) also affected the densities of *A. herbicolus* (Fig. 2C; LME: interaction of treatment with time: L.R. = 5.89, d.f. = 1, $p = 0.0152$). The density of *A. herbicolus* was always higher in all weeks evaluated in the presence of *H. anconai*, but significant differences were only found in the last week of evaluation (Fig. 2C).

The different treatments significantly affected the number of leaflets with powdery mildew lesions (Fig. 2D; GLM; $F_{3,24} = 41.2$, d.f. = 3, $p < 0.0001$). Plants with only *H. anconai* did not show any leaflets with powdery mildew, and the presence of this predator even combined with *A. herbicolus* reduced disease spread by 90.6% and 94.3% compared to treatments with *H. anconai* and without predators respectively (Fig. 2D).

4. Discussion

We previously showed that *H. anconai* was able to increase in densities and effectively controlled russet mites and the spread of powdery mildew on tomato plants (Chapter 2). Here, we found similar results for *H. anconai*: its controlled *A. lycopersici* and *O. neolycopersici*, even with an intraguild predator present on the plants (Fig. 2A), although predation by *A. herbicolus* reduced the density of the iolinid mites (Fig. 2B). Moreover, the presence of *H. anconai* increased the phytoseiid mite density on tomato plants (Fig. 2C). Probably, in addition to serving as food, the control of powdery mildew by *H. anconai* positively affected the number of *A. herbicolus* present in the leaves.

Both predators feed on *A. lycopersici*, but *H. anconai* was better at reducing tomato russet mite densities. When released alone, *H. anconai* supplemented with pollen reached an average density of over 1200 individuals per plant, which is about 60 times the density initially released, likely caused by the presence of pollen. Several studies have already demonstrated how the presence of alternative food such as pollen on a crop can enhance the efficacy of pest control because it increases the densities of natural enemies, which subsequently causes a reduction in pest populations, a phenomenon similar to apparent competition (Holt, 1977; van Rijn et al., 1999; Nomikou et al., 2002; Nomikou et al., 2010; Duarte et al., 2015). However, in the presence of the intraguild predator *A. herbicolus*, the densities of the iolonids were reduced by about 50% compared to populations without *A. herbicolus*. Nevertheless, *H. anconai* and *A. herbicolus* together controlled the densities of *A. lycopersici* (Fig. 2A). The iolinid mites is not harmed by the direct defences (e.g., toxic glandular trichomes) of tomato plants (Pijnakker et al., 2022; Chapter 2). While the glandular trichomes serve as a refuge for russet mites, offering protection against many predatory mites (van Houten et al., 2013), iolinid mites, being small in size, can also freely navigate under the trichomes. Hence, they can locate and prey on russet mites while they may experience some protection against predation by *A. herbicolus* from the trichomes.

Our data show that the two species coexisted for a rather long period. The basic theory of IGP predicts restricted conditions for stable coexistence: this occurs only when the intraguild prey (*H. anconai* in our case) is the superior competitor for the shared prey and only at intermediate levels of productivity (Polis et al., 1989; Holt and Polis, 1997). Here, it was evident that *H. anconai* was the better competitor for the shared prey. Another factor that contributes to the coexistence of two predators is spatial structure (Finke and Denno, 2006; Janssen et al., 2007). For example, Marques et al. (2018) showed that the predatory mites *Iphiseiodes zuluagai* and *Euseius concordis* involved in reciprocal intraguild predation coexist for much longer on *Jatropha* plants, which offer spatial structure, than on experimental arenas without structure. In our case, the ability of *H. anconai* to move under the trichomes is likely to reduce predation by the intraguild predator.

Recently, we demonstrated that the initial mortality of *A. herbicolus* was high, but that it can adapt to direct tomato defences within a few generations. To reduce the impact of the trichomes, which are especially dense on the tomato stems, a high dosage of alternative food can be supplemented (Chapter 1), because the predator do not need to move frequently to other plant parts in search of food, thus avoiding contact with the stem trichomes.

When feeding on *H. anconai*, *A. herbicolus* showed the same oviposition rate as when feeding on russet mites, but a shorter developmental time (Fig. 1A-B). Indeed, the presence of *H. anconai* increased the final densities of the phytoseiid mite by almost 8 times compared to the treatment in which *A. herbicolus* was released alone (Fig …). The use of alternative prey to maintain populations of predatory mites in crops has received ample attention (Hoogerbrugge et al., 2008; Messelink et al., 2009; Messelink et al., 2014; Pirayeshfar et al., 2020). Perhaps the iolinid mite can be used as an alternative prey for the maintenance of *A. herbicolus* in crops, although this requires research into mass production of *H. anconai*.

It is known that modifications of leaf structure can reduce the effectiveness of predators (Schmitd, 2014). Severe powdery mildew infestations can cause leaf chlorosis and desiccation of leaves and can thus limit predator physical resources such as oviposition sites and prey patches. Therefore, *H. anconai* can also have indirectly increased the densities of *A. herbicolus* on tomato plants through reducing the progress of powdery mildew (Fig. 2D).

It is not entirely clear how *H. anconai* controls powdery mildew. Hessein and Perring (1988) showed that *H. anconai* feeds on fungal spores has previously been demonstrated for: *Cladosporium cladosporioides*. In addition to feeding of spores, plant defence mechanisms induced by herbivory of *H. anconai* (Moraes and Flechtmann, 2008; Vervaet et al., 2022) may also be involved in powdery mildew control. Moreover, *H. anconai* might induce not only defences that decrease the growth of powdery mildew but also that of tomato russet mites. The induction of plant defences through herbivory by *H. anconai* therefore needs to be investigated.

Combined inoculative releases of *A. herbicolus* and *H. anconai* did not result in better control of russet mites. The iolinid mite alone was capable of reducing the densities of *A. lycopersici* and simultaneously preventing the progress of powdery mildew. Despite being the intraguild prey of *A. herbicolus*, the predators persisted on plants for a considerable period, probably caused by the presence of plant structures in the form of trichomes. Although these trichomes were found to impede biocontrol of other tomato pests because of their negative effects on natural enemies (Legarrea et al. 2022), here, the trichomes were possibly the cause of persistence of the two natural enemy species. The effects of such plant structures on trophic interactions therefore deserve further study. Future studies should also assess whether the impact of *H. anconai* on *A. herbicolus* densities will result in the more efficient control of another key tomato pest, the whitefly *B. tabaci*.

References

Akyazi, R., Ueckermann, E.A., Soysal, M., 2016. The new distribution of *Amblyseius herbicolus* in Turkey (Parasitiformes, Phytoseiidae) with a key of *Amblyseius* species found in Turkey. Acarologia 56, 237-244

Altieri, M.A., Letourneau, D.K., 1982. Vegetation management and biological control in agroecosystems. Crop. Prot. 1, 405–430

Bonato, O., Couton, L, Fargues, J., 2006. Feeding preference of *Macrolophus caliginosus* (Heteroptera: Miridae) on *Bemisia tabaci* and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). J Econ. Entomol. 99, 1143–1151

Bouagga, S., Urbaneja, A., Rambla, J.L., Flors, V., Granell, A., Jaques, J.A., Pérez-Hedo, M., 2018. Zoophytophagous mirids provide pest control by inducing direct defences, antixenosis and attraction to parasitoids in sweet pepper plants. Pest Manag. Sci. 74, 1286-1296.

Bueno, V.H., Lins Jr, J.C., Silva, D.B., van Lenteren, J.C., 2019. Is predation of *Tuta absoluta* by three Neotropical mirid predators affected by tomato lines with different densities in glandular trichomes? Arthropod-Plant Inte 13, 41-48.

Calvo, J., Blockmans, K., Stansly, P.A., Urbaneja, A., 2009. Predation by *Nesidiocoris tenuis* on *Bemisia tabaci* and injury to tomato. Biocontrol 54, 237–246

Cardinale, B.J., Harvey, C.T., Gross, K., Ives, A.R., 2003. Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem. Ecol Lett. 6, 857–865

Cardoso, A.C., 2019. Search for predatory mites to control tomato pests. MSc Dissertation, Federal University of Viçosa.

Chailleux, A., Bearez, P., Pizzol, J., Amiens-Desneux, E., Ramirez-Romero, R., Desneux, N., 2013. Potential for combined use of parasitoids and generalist predators for biological control of the key invasive tomato pest *Tuta absoluta*. J. Pest. Sci. 86, 533-541.

Chant, D.A., 1959. Phytoseiid mites (Acarina: Phytoseiidae). Part I. Bionomics of seven species in southeastern England. Part II. A taxonomic review of the family Phytoseiidae, with descriptions of thirty-eight new species — The Canadian Entomologist, Canada, Supplement 12: 166

Chow, A., Chau, A., Heinz, K.M., 2008. Compatibility of *Orius insidiosus* (Hemiptera: Anthocoridae) with *Amblyseius* (*Iphiseius*) *degenerans* (Acari: Phytoseiidae) for control of Frankliniella occidentalis (Thysanoptera: Thripidae) on greenhouse roses. Biol. Control. 44, 259-270.

Coll, M., Smith, L.A., Ridgway, R.L., 1997. Effect of plants on the searching efficiency of a generalist predator: the importance of predator-prey spatial association. Entomol Exp. Appl. 83; 1–1

Collyer, E., 1964. The effect of an alternative food supply on the relationship between two *Typhlodromus* species and *Panonychus ulmi* (Koch) (Acarina). Entomol. Exp. Appl. 7, 120–124.

Daugherty, M.P., Harmon, J.P., Briggs, C.J., 2007. Trophic supplements to intraguild predation. Oikos 116, 662–677.

Drukker, B., Janssen, A., Ravensberg, W., Sabelis, M.W., 1997. Improved control capacity of the mite predator *Phytoseiulus persimilis* (Acari: Phytoseiidae) on tomato. Exp Appl. Acarol. 21, 507–518. https://doi.org/10.1023/B:APPA.00000 18885.35044.C6

Duarte, M.V., Venzon, M., Bittencourt, M.C.D.S., Rodríguez-Cruz, F.A., Pallini, A., Janssen, A., 2015. Alternative food promotes broad mite control on chilli pepper plants. BioControl 60, 817-825

Eubanks, M. D., Denno, R. F., 2000. Health food versus fast food: the effects of prey quality and mobility on prey selection by a generalist predator and indirect interactions among prey species. Ecol. Entomol. 25, 140-146.

Finke, D.L., Denno, R.F., 2006. Spatial refuge from intraguild predation: implications for prey suppression and trophic cascades. Oecologia 149, 265–275.

Fonseca, M. M., Pallini, A., Marques, P. H., Lima, E., Janssen, A., 2020. Compatibility of two predator species for biological control of the two-spotted spider mite. Exp Appl. Acarol. 80, 409-422.

Gerson, U., Weintraub, P.G., 2007. Mites for the control of pests in protected cultivation. Pest Manag. Sci. 63, 658-676.

Glas, J.J., Schimmel, B.C.J., Alba, J.M., Escobar-Bravo, R., Schuurink, R.C., Kant, M.R., 2012. Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. Int. J. Mol. Sci. 13, 17077–17103. https://doi.org/10.3390/ijms1 31217077

Gurr, G.M., Wratten, S.D., Landis, D.A., You, M., 2017. Habitat management to suppress pest populations:progress and prospects. Annu Rev. Entomol. 62, 91–109

Hajek, A.E., 2004. Natural enemies: an introduction to biological control. Cambridge University Press, Cambridge

Hessein, N.A, Perring, T.M., 1988. The importance of alternate foods for the mite *Homeopronematus anconai* (Acari: Tydeidae). Ann Entomol. Soc. Am. 81:488–492. https://doi.org/10.1093/aesa/81.3.488

Hessein, N.A., Perring, T.M., 1986. Feeding habits of the Tydeidae with evidence of *Homeopronematus anconai* (Acari: Tydeidae) predation on *Aculops lycopersici* (Acari: Eriophyidae). Int. J. Acarol. 12, 215–221. https:// doi. org/ 10. 1080/ 01647 95860 86834

Holt, R.D., 1977. Predation, apparent competition, and the structure of prey communities. Theor Popul. Biol. 12:197-229.

Holt, R.D., Huxel, G.R., 2007. Alternative prey and the dynamics of intraguild predation: theoretical perspectives. Ecology 88, 2706–2712.

Holt, R.D., Polis, G.A., 1997. A theoretical framework for intraguild predation. Am. Nat. 149, 745–764.

Hoogerbrugge, H., van Houten, Y., van Baal, E., Bolckmans, K. 2008. Alternative food sources to enable establishment of *Amblyseius swirskii* (Athias-Henriot) on chrysanthemum without pest presence. IOBC/WPRS Bull 32:79–82

Janssen A., Montserrat M., Hille Ris Lambers R et al (2006) Intraguild predation usually does not disrupt biological control. In: Brodeur J, Boivin G (eds) Trophic and guild in biological interactions control. Springer, Dordrecht, pp 21–44

Janssen, A., Montserrat, M., HilleRisLambers, R., de Roos, A.M., Pallini, A., Sabelis, M.W., 2006. Intraguild predation usually does not disrupt biological control. In: Brodeur, J., Boivin, G. (Eds.), Trophic and Guild Interactions in Biological Control. Springer, Netherlands, Dordrecht, pp. 21–44.

Janssen, A., Pallini, A., Venzon, M., Sabelis, M.W., 1998. Behaviour and indirect interactions in food webs of plant inhabiting arthropods. Exp. Appl. Acarol. 22, 497–521

Janssen, A., Sabelis, M.W., 1992. Phytoseiid life-histories, local predator-prey dynamics, and strategies for control of tetranychid mites. Exp. Appl. Acarol 14, 233–250

Janssen, A., Sabelis, M.W., 2015. Alternative food and biological control by generalist predatory mites: the case of *Amblyseius swirskii*. Exp. Appl. Acarol. 65, 413-418.

Jones, H., Whipps, J.M., Gurr, S.J., 2001. The tomato powdery mildew fungus *Oidium neolycopersici*. Mol. Plant. Pathol. 2, 303–309

Kalile, M.O., Cardoso, A.C., Pallini, A., Fonseca, M.M., Elliot, S.L., Fialho, V.S., Janssen, A., 2021. A predatory mite as potential biological control agent of *Diaphorina citri*. BioControl. 66, 237-248.

Kawai, A., Haque, M.M., 2004. Population dynamics of tomato russet mite, *Aculops lycopersici* (Massee) and its natural enemy, *Homeopronematus anconai* (Baker). Jpn. Agric. Res. Q. 38, 161–166. https:// doi. org/ 10. 6090/ jarq. 38. 161

Lambert, A.M., 2007. Effect of prey availability, facultative plant feeding, and plant defenses on a generalist insect predator. Arthropod-Plant Inte. 1, 167–173

Landis, D.A., Wratten, S.D., Gurr, G.M., 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annu. Rev. Entomol. 45, 175–201

Legarrea, S., Janssen, A., Dong, L., Glas, J.J., van Houten, Y.M., Scala, A., Kant, M.R., 2022. Enhanced top-down control of herbivore population growth on plants with impaired defences. Funct Ecol. 36, 2859-2872

Leman, A., Ingegno, B.L., Tavella, L., Janssen, A., Messelink, G.J., 2020. The omnivorous predator *Macrolophus pygmaeus*, a good candidate for the control of both greenhouse whitefly and poinsettia thrips on gerbera plants. Insect Sci.27, 510-518.

Marcossi, Í., Fonseca, M.M., Carbajal, P.A., Cardoso, A. Pallini, A. Janssen, A., 2020. High-quality alternative food reduces cannibalism in the predatory mite *Amblyseius herbicolus* (Acari: Phytoseiidae). Exp. Appl. Acarol. 81, 189–200

Marques, R.V., Sarmento, R.A., Oliveira, A.G., Rodrigues, D.D.M., Venzon, M., Pedro-Neto, M., Pallini, A., Janssen, A., 2018. Reciprocal intraguild predation and predator coexistence. Ecol. Evol. 8, 6952-6964.

Mendoza, J.E., Balanza, V., Rodríguez-Gómez, A., Cifuentes, D., Bielza, P., 2022. Enhanced biocontrol services in artificially selected strains of *Orius laevigatus*. J Pest. Sci. 95, 1597-1608.

Messelink, G. J., Bloemhard, C.M.J., Sabelis, M.W., Janssen, A., 2013. Biological control of aphids in the presence of thrips and their enemies. Biocontrol 58, 45–55. https ://doi.org/10.1007/s1052 6-012-9462-2

Messelink, G.J, Sabelis, M.W., Janssen, A., 2012. Generalist predators, food web complexities and biological pest control in greenhouse crops. In: Larramendy ML, Soloneski S (eds) Integrated pest management and pest control—current and future tactics. InTech, Rijeka, pp 191–214

Messelink, G.J., Bennison, J., Alomar, O., Ingegno B.L., Tavella, L., Schipp, L., Palevsky, E., Wackers F.L., 2014. Approaches to conserving natural enemy populations in greenhouse crops: current methods and future prospects. BioControl 59: 377–393

Messelink, G.J., Janssen, A., 2014. Increased control of thrips and aphids in greenhouses with two species of generalist predatory bugs involved in intraguild predation. Biol. Control. 79, 1-7.

Messelink, G.J., Ramakers PMJ, Cortez JA, Janssen A (2009) How to enhance pest control by generalist predatory mites in greenhouse crops. In: Mason PG, Gillespie DR, Vincent C (eds) 3rd international symposium on biological control of arthropods, Christchurch, New Zealand. pp 309–318

Messelink, G.J., van Maanen, R., van Steenpaal, S.E.F., Janssen, A., 2008. Biological control of thrips and whiteflies by a shared predator: Two pests are better than one. Biol. Control. 44, 372-379.

Moraes, G.J., Flechtmann, C.H.W., 2008. Manual de acarologia: acarologia básica e ácaros de plantas cultivadas no Brasil. Holos, Ribeirão Preto

Nomikou, M., Janssen, A., Schraag, R., Sabelis, M.W., 2001. Phytoseiid predators as potential biological control agents for *Bemisia tabaci*. Exp. Appl. Acarol. 25, 271–291

Nomikou, M., Janssen, A., Schraag, R., Sabelis, M.W., 2002. Phytoseiid predators suppress populations of *Bemisia tabaci* on cucumber plants with alternative food. Exp. Appl. Acarol. 27, 57–68

Nomikou, M., Sabelis, M.W., Janssen, A., 2010. Pollen subsidies promote whitefly control through the numerical response of predatory mites. BioControl 55, 253-260.

Paspati, A., Rambla, J.L., Gresa, M.P.L., Arbona, V., Gómez-Cadenas, A., Granell, A., González-Cabrera, J., Urbaneja, A., 2021. Tomato trichomes are deadly hurdles limiting the establishment of *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae). Biol. Control. 157, 104572. https://doi.org/10.1016/j.biocontrol.2021.104572

Pijnakker J, Moerkens R, Vangansbeke D, Duarte M, Bellinkx S, Benavente A, Merckx J, Stevens I, Wäckers F (2022b) Dual protection: A tydeoid mite effectively controls both a problem pest and a key pathogen in tomato. Pest. Manag. Sci. 78:355–361. https://doi. org/ 10. 1002/ ps. 6647

Pirayeshfar F, Safavi SA, Sarraf Moayeri HR, Messelink GJ (2020) The potential of highly nutritious frozen stages of Tyrophagus putrescentiae as a supplemental food source for the predatory mite Amblyseius swirskii. Biocontrol Sci. Technol. 30:403–417

Polis, G. A., Myers, C. A., Holt, R. D., 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annu. Rev. Ecol. Evol. Syst. 20, 297-330.

R Core Team., 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria

Ramakers, P.M.J., 1990. Manipulation of phytoseiid thrips predators in absence of thrips. IOBC/WPRS Bull. 13, 169–172

Ramakers, P.M.J., Rabasse, J.-M., 1995. Integrated pest management in protected cultivation. In: Reuveni, R. (Ed.), Novel Approaches to Integrated Pest Management. CRC Press, Florida, pp. 199-229.

Reis, P.R., Teodoro, A.V., Pedro Neto, M., da Silva, E.A., 2007. Life history of *Amblyseius herbicolus* (Chant) (Acari: Phytoseiidae) on coffee plants. Neotrop. Entomol. 36, 282–287

Rodríguez-Cruz, F.A., Venzon, M., Pinto, C.M.F., 2013. Performance of *Amblyseius herbicolus* on broad mites and on castor bean and sunnhemp pollen. Exp. Appl. Acarol. 60, 497–507

Rosenheim, J.A., Kaya, H.K., Ehler, L.E., et al 1995. Intraguild predation among biological-control agents: theory and evidence. Biol. Control. 5, 303–335. https ://doi.org/10.1006/bcon.1995.1038

Sanchez, J.A., Alcazar, A., Lacasa, A., Llamas, A., Bielza, P. 2000. Integrated pest management strategies in sweet pepper plastic houses in the Southeast of Spain. Integrated pest management strategies in sweet pepper plastic houses in the Southeast of Spain. 23, 21-30.

Sato, M.M., de Moraes, G.J., Haddad, M.L., Wekesa, V.W., 2011. Effect of trichomes on the predation of *Tetranychus urticae* (Acari: Tetranychidae) by *Phytoseiulus macropilis* (Acari: Phytoseiidae) on tomato, and the interference of webbing. Exp. Appl. Acarol. 54, 21–32. https://doi.org/10.1007/s10493-011-9426-8

Schmidt, R.A., 2014. Leaf structures affect predatory mites (Acari: Phytoseiidae) and biological control: a review. Exp. Appl. Acarol. 62, 1-17.

Schuurink, R., Tissier, A., 2020. Glandular trichomes: micro-organs with model status? New Phytol. 225, 2251–2266. https://doi.org/10.1111/nph.16283

Sih, A., Englund, G., Wooster, D., 1998. Emergent impacts of multiple predators on prey. Trends. Ecol. Evol. 13, 350–355

Silva, F.R., de Moraes, G.J., Gondim Jr, M.G., Knapp, M., Rouam, S.L., Paes, J.L., de Oliveira, G.M., 2010. Efficiency of *Phytoseiulus longipes* Evans as a control agent of *Tetranychus evansi* Baker & Pritchard (Acari: Phytoseiidae: Tetranychidae) on screenhouse tomatoes. Neotrop. Entomol. 39, 991–995.

Straub, C.S., Snyder, W.E., 2008. Increasing enemy biodiversity strengthens herbivore suppression on two plant species. Ecology 89, 1605–1615

Symondson, W.O.C., Sunderland, K.D., Greenstone, M. H., 2002. Can generalist predators be effective biocontrol agents? Annu. Rev. Entomol. 47, 561-594.

Urbaneja, A., Monton, H., Molla, O., 2009. Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. J. Appl. Entomol. 133, 292–296.

van Haren, R., Steenhuis, M., Sabelis, M., De Ponti, O., 1987. Tomato stem trichomes and dispersal success of *Phytoseiulus persimilis* relative to its prey *Tetranychus urticae*. Exp. Appl. Acarol. 3, 115–121 van Houten, Y.M., Glas, J.J., Hoogerbrugge, H., Rothe, J., Bolckmans, K.J.F., Simoni, S., van Arkel, J., Alba, J.M., Kant, M.R., Sabelis, M.W., 2013. Herbivory-associated degradation of tomato trichomes and its impact on biological control of *Aculops lycopersici.* Exp. Appl. Acarol. 60, 127–138. https://doi.org/10.1007/s10493-012-9638-6

van Lenteren, J.C., 2012. The state of commercial augmentative biological control: Plenty of natural enemies, but a frustrating lack of uptake. BioControl 57, 1-20.

van Lenteren, J.C., Alomar, O., Ravensberg, W.J., Urbaneja, A., 2020. Integrated pest and disease management in greenhouse crops. Springer International Publishing

van Lenteren, J.C., Bolckmans, K., Köhl, J., Ravensberg, W.J., Urbaneja, A., 2018. Biological control using invertebrates and microorganisms: plenty of new opportunities. Biocontrol 63, 39–59. https://doi.org/ 10.1007/s10526-017-9801-4

van Rijn, P.C.J., Tanigoshi, L.K., 1999. Pollen as food for the predatory mites *Iphiseius degenerans* and *Neoseiulus cucumeris* (Acari: Phytoseiidae): dietary range and life history. Exp. Appl. Acarol 23,785–802

van Rijn, P.C.J., van Houten, Y.M., Sabelis, M.W., 2002. How plants benefit from providing food to predators even when it is also edible to herbivores. Ecology 83, 2664–2679

Verheggen, F.J., Capella, Q., Schwartzberg, E.G., Voigt, D., Haubruge, E., 2009. Tomato-aphid- hoverfly: A tritrophic interaction incompatible for pest management. Arthropod-Plant Inte. 3, 141–149. https://doi.org/10.1007/s1182 9009-9065-8

Vervaet, L., Parapurath, G., De Vis, R., Van Leeuwen, T., De Clercq, P., 2022. Potential of two omnivorous iolinid mites as predators of the tomato russet mite, *Aculops lycopersici*. J. Pest. Sci. 95, 1671-1680. https://doi.org/10.1007/s10340-022-01544-x

Figure legends

Fig. 1 Juvenile development, survival and average oviposition rates of *Amblyseius herbicolus* fed with all stages of *A. lycopersici*, *H. anconai* and cattail pollen. (**A**) Shown are the mean (± SE) cumulative proportions of individuals that reached adulthood as a function of time. Survival is given by the final proportion of adults. (**B**) Show are the oviposition rates of *H. anconai* on the 2nd – 4th day.
Different letters following the key entries indicate significant differences among treatments (contrasts after survival analysis and through model simplification after LME $P < 0.05$).

Fig. 2 Population dynamics of *Aculops lycopersici* and the predatory mites *H. anconai* (alone), *A. herbicolus* (alone) and the both predators released combined on tomato plants. (**A**) Shown are average densities (± SE) of tomato russet mites in the presence of the predatory mites *H. anconai* – alone (squares), *A. herbicolus* – alone (diamond + dotted line), the both predators (circles) and no predators (triangle + dotted line). (**B**) Shown are log densities (± SE) of all stages of *H. anconai*, released alone (squares) and combined with *A. herbicolus* (circles). (**C**) Shown are log densities (± SE) of all stages of *A. herbicolus*, released alone (triangle) and combined with *H. anconai* (circles). (**D**) Average (± SE) number of leaflets with powdery mildew lesions from the sixth week of the population dynamics experiment in presence of the predatory mites *H. anconai* – alone (squares), *A. herbicolus* – alone (diamond + dotted line), the both predators (circles) and no predators (triangle + dotted line). Predators were released in week 0 (*H.anconai*) and week 1 (*A. herbicolus*) and were fed *Typha* sp. pollen. Different letters indicate significant differences between treatments and asterisks indicate significant differences within each week, respectively (contrasts through model simplification after LME and GLM; $p < 0.05$).

Fig. 3 Tomato plants in the presence of predatory mites at the end of the population dynamics experiment.

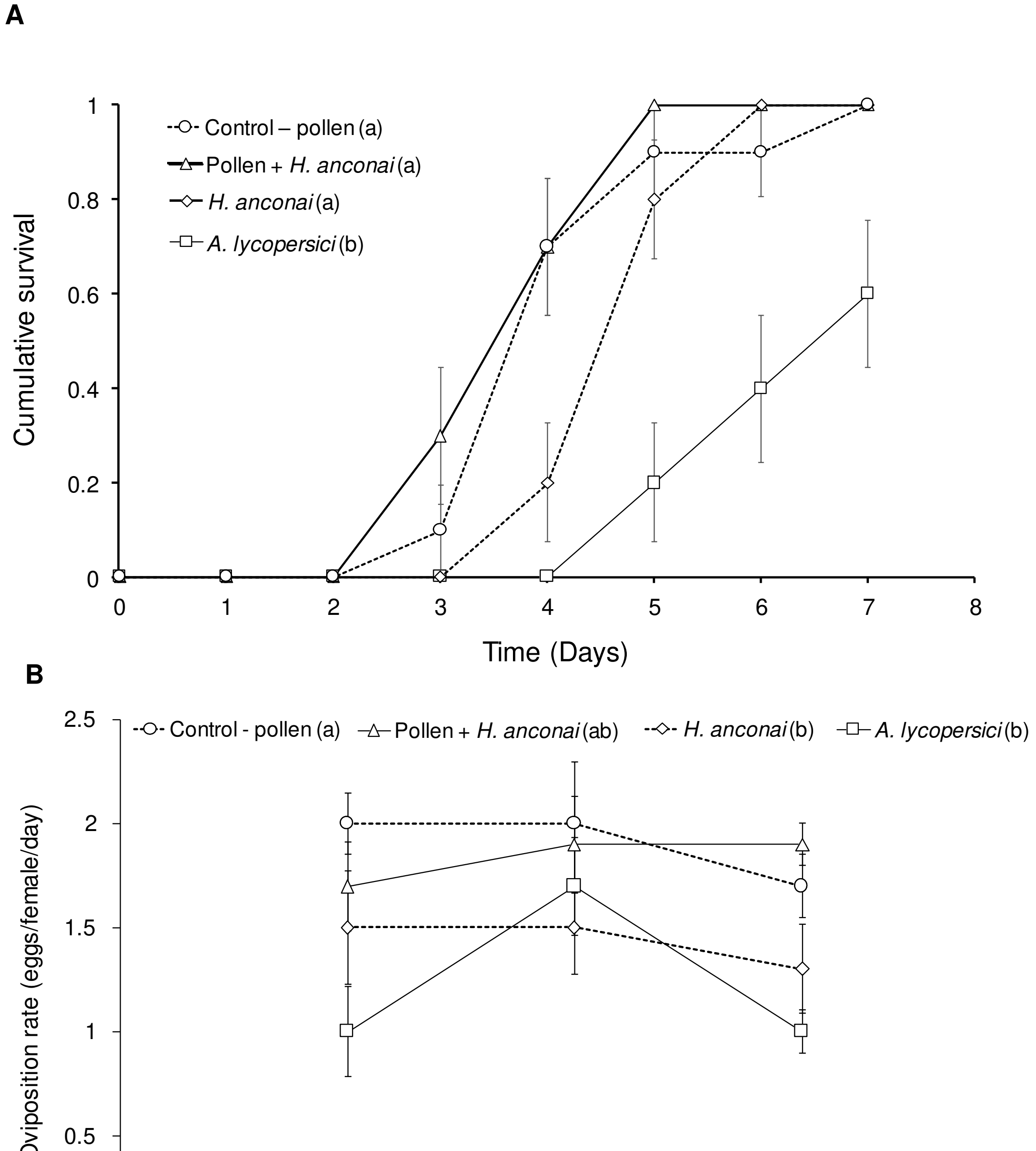
A
Control – pollen (a)
Pollen + *H. anconai* (a)
H. anconai (a)
A. lycopersici (b)
Cumulative survival
0
0.2
0.4
0.6
0.8
1
0 1 2 3 4 5 6 7 8
Time (Days)
B
Control - pollen (a)
Pollen + *H. anconai* (ab)
H. anconai (b)
A. lycopersici (b)
Oviposition rate (eggs/female/day)
0
0.5
1
1.5
2
2.5
1 2 3 4 5
Time (Days)

Fig. 1

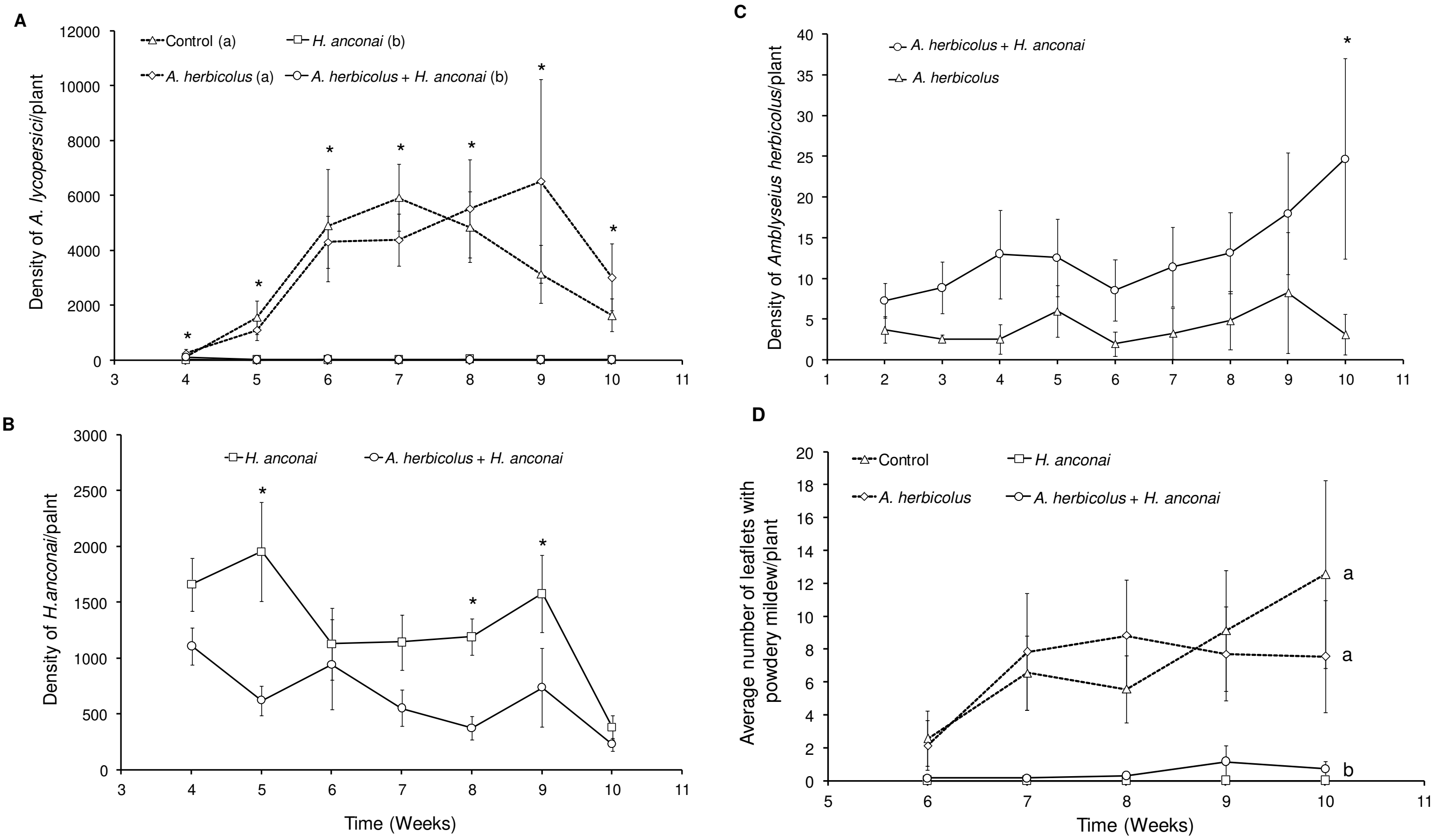
A
Density of A. lycopersici/plant
Control (a)
H. anconai (b)
A. herbicolus (a)
A. herbicolus + H. anconai (b)
0
2000
4000
6000
8000
10000
12000
3
4
5
6
7
8
9
10
11
*
B
Density of H.anconai/palnt
H. anconai
A. herbicolus + H. anconai
0
500
1000
1500
2000
2500
3000
Time (Weeks)
C
Density of Amblyseius herbicolus/plant
A. herbicolus + H. anconai
A. herbicolus
0
5
10
15
20
25
30
35
40
1
2
D
Average number of leaflets with powdery mildew/plant
Control
H. anconai
A. herbicolus
A. herbicolus + H. anconai
0
2
4
6
8
10
12
14
16
18
20
a
a
b
Time (Weeks)

Fig. 2.


H. anconai
H. anconai + A. herbicolus
A. herbicolus
Control (no predators)

Figure 3.

CONCLUSION GENERAL

In this thesis, I investigated the use of three different predatory mites to control important pests and a pathogen in tomato crops. The results showed that the phytoseiid mite *A. herbicolus* is effective in controlling the whitefly (*B. tabaci*), in both situations, whether introduced before or after the pest. However, the amount of alternative food offered, in this case pollen, directly affects the biological control performed by *A. herbicolus* (Chapter I).

In the second chapter, I showed that the early establishment of the omnivorous iolinid mite *H. anconai* simultaneously controls russet mites and powdery mildew on tomato plants. This is the second report of an arthropod biological control agent acting against both a pest and disease.

Based on the results of the second and first chapters, I assumed that the combined use of *A. herbicolus* and *H. anconai* could result in increased biological control. However, the combined inoculative releases of both predators did not result in better control of russet mites. The iolinid mite alone was capable of reducing the densities of *A. lycopersici* and simultaneously preventing the progress of powdery mildew.

In conclusion, this study demonstrates promising results from two generalist predatory mites that effectively control pests in tomatoes, a plant known to be a hostile host for natural enemies. Furthermore, the use of a predatory arthropod to control a disease could be a new biological control strategy for powdery mildew not only in tomato crops.