MILENA OLIVEIRA KALILE

POTENTIAL OF THE PREDATORY MITE *Amblyseius herbicolus* AS BIOCONTROL CONTROL AGENT OF *Diaphorina citri* ON 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*.

Adviser: Arne Janssen

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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*.

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“*Ninguém come PIB (Produto Interno Bruto), come alimentos*”.

(Maria da Conceição Tavares)

ABSTRACT

KALILE, Milena Oliveira, D.Sc., Universidade Federal de Viçosa, March, 2024. **Potential of the predatory mite *Amblyseius herbicolus* as biological control agent of *Diaphorina citri* on plants**. Adviser: Arnoldus Rudolf Maria Janssen. Co-adviser: Angelo Pallini Filho.

Biological control is a sustainable and economically profitable method to control agricultural arthropod pests. However, the biocontrol of phytopathogen vectors can be more complex because it aims not only at the control of pest densities, but also at reducing the incidence of diseases. Here, I investigated the potential of the predatory mite *Amblyseius herbicolus* Chant (Acari: Phytoseiidae) as biological control agent of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). This psyllid pest is the major vector of the bacteria associated with Huanglongbing (HLB). Previously, *A. herbicolus* showed high predation rates, reproduction and development when feeding on *D. citri* eggs. I investigated whether *A. herbicolus* can also feed on nymphs and reduce densities of *D. citri* on host plants in the lab (chapter 1) and outside (chapter 2), which may also potentially reduce the spread of HLB. In population dynamics experiments, there was a reduction of c. 85% of *D. citri* densities in the lab, and 90% on plants outside. In addition, it is crucial to investigate how the presence of predators affects the host plant choice and dispersal of the vector because these have implications for disease transmission. I therefore studied this in the third chapter and observed that *D. citri* did not avoid plants with predatory mites, not even after having received experience with predators feeding on pest offspring. Besides, the pest did not disperse more from plants with predators feeding on eggs and nymphs than from plants without them. In conclusion, *A. herbicolus* is a promising biocontrol agent of *D. citri*. Population dynamics experiments evaluating the effect of this predator on *D. citri* and HLB incidence on citrus plants in the field need to be done.

Keywords: Citrus; Mite; Predator; Psyllid; Vector.

RESUMO

KALILE, Milena Oliveira, D.Sc., Universidade Federal de Viçosa, março de 2024. **Potencial do ácaro predador *Amblyseius herbicolus* como agente de controle biológico de *Diaphorina citri* em plantas**. Orientador: Arnoldus Rudolf Maria Janssen. Coorientador: Angelo Pallini Filho.

Controle biológico é uma alternativa sustentável e economicamente viável para controlar pragas agrícolas. Entretanto, o controle biológico de vetores de fitopatógenos pode ser muito mais complexo porque o objetivo não é apenas controlar densidades da praga, mas também a incidência de doenças. Aqui, foi investigado o potencial do ácaro predador *Amblyseius herbicolus* Chant (Acari: Phytoseiidae) como agente de controle biológico de *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). Essa praga é o principal vetor das bactérias associadas ao Huanglongbing (HLB). Anteriormente, *A. herbicolus* mostrou alta taxa de predação e foi capaz de reproduzir e desenvolver se alimentando em ovos de *D. citri*. Foi investigado se *A. herbicolus* também pode se alimentar de ninfas e reduzir densidades de *D. citri* em plantas no laboratório (Capítulo1) e fora do laboratório (capítulo 2), o que também pode reduzir a disseminação de HLB. Em experimentos de dinâmica populacional, houve redução das densidades de *D. citri* em 85% em plantas no laboratório e em 90% ao ar livre. Além disso, é crucial investigar como os predadores afetam a escolha da planta hospedeira e dispersão de vetores por causa das implicações para transmissão da doença. No terceiro capítulo, foi observado que *D. citri* não evitou plantas com ácaros predadores, mesmo após um período de experiência. Além disso, o vetor não dispersou mais das plantas com predadores. Em conclusão, *A. herbicolus* é um agente de controle biológico do vetor *D. citri* promissor. Experimentos de dinâmica populacional avaliando o efeito desse predador na praga e na incidência de HLB em plantas de citros no campo devem ser realizados.

Palavras-chave: Ácaro; Citrus; Predador; Psilídeo; Vetor.

SUMMARY

CHAPTER 1:

CHAPTER 2:

CHAPTER 3:

GENERAL INTRODUCTION

Humans have always had to deal with strategies to control organisms that directly affect them, such as parasites and pathogens, or that compete with them for resources, such as herbivores of agricultural crops (Hajek and Elienberg, 2018). When an organism has negative impacts from a human perspective, it is called a pest (Rajendran and Singh, 2016). Herbivorous pests compete with humans for food because they eat crop plants (Hajek and Elienberg, 2018). To decrease or eliminate this competition, humans try to kill these herbivores, and chemical control is the most used method to control them (Hajek and Elienberg, 2018; Kole et al., 2019). However, pesticide applications have resulted in the development of resistance in pest populations (Chen and Stelinski, 2017; Kole et al., 2019), necessitating the development of other pesticides. Besides, the use of pesticides generates concerns about their adverse effects on human health, the environment and on beneficial organisms such as pollinators and natural enemies of pests (Hajek and Elienberg, 2018; Monzó and Stansly, 2020). In addition, pesticides are often ineffective in the medium term (i.e. a growing season) because natural enemies are more negatively affected by them than pest populations even when the pesticides are selective and cause lower mortality of the natural enemies than of the pest (Janssen and van Rijn, 2021). Therefore, more sustainable and economic management methods are required. In this context, biological control is one of the most important, sustainable and economically profitable methods of pest control (Stenberg et al., 2021; Monzó and Stansly, 2020). Agriculture is changing and biological control products are increasingly commercialized worldwide (Siegwart et al., 2015 Knapp et al., 2018).

Potential biological control agents comprise pathogens of arthropods, nematodes and mites (Luz et al. 1998), competitors, parasitoids (Gerling et al. 2001; Smyrnioudis et al., 2001) and predators (Moore et al. 2009). In addition to natural biological control, when pests are reduced by natural enemies that already co-occur with the pest (van Lenteren et al. 2018), there are three biological control strategies (Stenberg et al., 2021). First, augmentative biological control consists of the release of natural enemies, either inundative or inoculative (Stenberg et al., 2021). In the inundative approach, large numbers of natural enemies are released to promptly control a pest, whereas inoculative release consists of releasing lower numbers, aiming at more long-term control by several generations of the released natural enemies (Van Lenteren and Bueno, 2003; Stenberg et al., 2021). Second, classical biological control concerns the permanent introduction of non-native natural enemies to control an exotic pest with which the natural enemy co-evolved (Howarth, 1991). Last, conservation biological control aims at preserving and favoring populations of naturally occurring natural enemies through management of the agroecosystem (Barbosa, 1998; Stenberg et al., 2021). To apply conservation biological control, it is necessary understand that natural enemies can have a mutualistic relationship with plants (Price et al., 1980). The plants may offer structure for oviposition and refuge and food such as pollen and nectar (Price et al., 1980; Schmidt, 2014), whereas natural enemies provide protection against herbivores (Price et al., 1980). When plants do not provide good conditions for natural enemies, it is possible to provide it intentionally by supplying alternative food or shelter to the natural enemies. Pollen is often used as alternative food for predators to guarantee their persistence when pest densities are low and also allows for preventive release, before pests have arrived (Murdoch and Oaten, 1975; Hassel and May, 1986; Janssen and Sabelis, 2015). Thus, agroecosystems can be manipulated to improve conditions for natural enemies.

Herbivorous arthropods do not only cause damage by feeding on plants, but they can also transmit plant pathogens (Purcell, 1982; Okamoto and Amarasekare, 2012). Thus, these herbivores pose a dual threat to crops because they can cause economic losses by both herbivory and disease transmission (Okamoto and Amarasekare, 2012). One way of controlling plant pathogens is the control of their arthropod vectors (Purcell, 1982; Okamoto and Amarasekare, 2012). Nevertheless, biocontrol of phytopathogens through control of their vectors can be more complex because it aims not only to control pest densities, but also the incidence of diseases (Jeger et al., 2004; Okamoto and Amarasekare, 2012). This incidence depends not only on the densities of the vector, but also on its behaviour (Jeger et al. 2004; Eigenbrode et al., 2018). Thus, causing changes in the selection of host plants, feeding and dispersal behavior of vectors can increase or reduce the acquisition and transmission of pathogens (Ebert et al., 2019; Eigenbrode et al., 2018).

Natural enemies do not only affect pests by killing them, but also by changing their behaviour. For example, herbivores may stop feeding to escape or defend themselves, and this so-called antipredator behaviour has costs, and can therefore also indirectly affect the growth of herbivore populations (Lind and Cresswell, 2005; Finke, 2012). It is also known that females may avoid ovipositing on plants where the probability of predation of the offspring is high (Mappes and Kaitala, 1995). The whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), for example, learned to avoid plants with the predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) after having experienced predation on offspring (Nomikou et al., 2003). Thus, the perception of predation risk may change the host plant choice and movement, affecting fitness, population dynamics and the spread of the pest (Roitberg and Myers 1978; Lima and Dill, 1990; Lima, 1998; Nomikou et al., 2003; Ferrari et al., 2009). In the case of vectors, these changes may increase or reduce the transmission of disease to plants. For example, vectors that move frequently among host plants may be more efficient at disease transmission than vectors that spend several generations on the same plant. Hence, the use of natural enemies to control vector populations may result in changes in the transmission of the disease (Belliure et al., 2011; Finke, 2012). It is therefore crucial to understand whether a natural enemy is able to reduce vector populations on plants, but also how the presence of a natural enemy affects host plant selection, dispersal and establishment of the vector (Okamoto and

Amarasekare, 2012; Finke, 2012). Therefore, both direct and indirect effect of natural enemies on vector pests need to be investigated.

One famous example of a pest vector is *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), the Asian citrus psyllid, which has become a key citrus pest worldwide because it is involved in the transmission of the phloem-inhabiting bacteria that are associated with Huanglongbing (HLB) or the citrus greening disease (Catling, 1970; Halbert and Manjunatah, 2004). HLB bacteria, *Candidatus* Liberibacter spp., colonize the gut and salivary glands of *D. citri*, and are transmitted when an infected *D. citri* feeds on a host plant (Haapalainen, 2014). Infected trees serve as a source of inoculum for healthy plants, become unproductive and need to be eliminated (Gottwald, 2007). Until now, HLB is the most important threat to the citrus industry, it is incurable, fatal and can cause losses up to 100 % (Gottwald, 2007). Currently, no commercial cultivar is resistant to the disease because the association between bacteria and cultivated citrus is relatively recent (Haapalainen, 2014; Bassanezi et al., 2020).

So far, the most effective measure against HLB is the control of *D. citri*, which is difficult and costly (Gottwald, 2007; Haapalainen, 2014; Monzó and Stansly, 2020). Currently, the major control strategy is the frequent application of pesticides (Monzó and Stansly, 2020). Besides development of resistance in *D. citri* populations (Chen and Stelinski, 2017), pesticides can reduce the natural control and conservation biological control of this pest. According to estimates, conservation biological control of *D. citri* may save about $ 300 million dollars per season in the Florida citrus industry (Monzó and Stansly, 2020). The main potential biological control agents of *D. citri* are the commercially available entomopathogenic fungi *Isaria (Cordyceps) fumosorosea* Wize (Hypocreales: Cordycipitaceae) and *Beauveria (Cordyceps) bassiana* (Bals. -Criv.), the parasitoid *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae) (Chien et al., 1989), predators such as coccinellids, and predatory mites such as *Amblyseius swirskii* Athias-Henriot (Juan-Blasco et al., 2012) and *Amblyseius herbicolus* Chant

(Acari: Phytoseiidae) (Kalile et al., 2021). One advantage of using predatory mites for biological pest control is that many species can be easily mass reared in comparison to other organisms such as parasitoids, especially when they can be reared on stored product mites. Furthermore, they have a limited capacity for active dispersal, which helps to prevent adverse effects on non-target organisms, and many of them can be used to control several pests (Janssen and Sabelis, 2015; Knapp et al., 2018; Van Lenteren, 2012).

Among the predatory mites investigated to control *D. citri*, *A. herbicolus* was shown to reproduce and develop by feeding on *D. citri* eggs, and had a higher predation rate than other predatory mites (Kalile et al., 2021). Subsequently, it was shown that this predator was also able to feed on first and second instar nymphs of *D. citri* (Chapter 1). However, the occurrence of predation is not sufficient to prove that a biological control agent will be effective. I therefore investigated here whether *A. herbicolus* can reduce densities of *D. citri* on host plants, which may also potentially reduce the spread of HLB. Thus, in the first chapter, I released this predatory mite before arrival of the pest on isolated orange jasmine plants, a preferential host plants of *D. citri*. The mites were provided with pollen as alternative food and twines as oviposition sites (Adar et al. 2014, Pekas and Wäckers, 2017). The latter were supplied because plants of the Rutaceae family, such as citrus and orange jasmine, are glabrous (Schmidt, 2014), which means scarcity of structures that serve as shelter and oviposition sites for predatory mites. Subsequently, *D. citri* was released on those plants and their dynamics was followed. I show that *A. herbicolus* successfully reduced densities of adults and nymphs of *D. citri*: plants with the predator had about 85% lower densities than plants without predators.

Whereas in the first chapter the dynamics of *D. citri* and the predatory mites was investigated indoors, in the second I investigated the ability of *A. herbicolus* control *D. citri* outside. The predatory mites were released on orange jasmine plants one month and a half before the pest and were supplied with pollen as alternative food. The predatory mite suppressed

D. citri densities by about 90%, showing that it is also a promising biological control agent outdoors.

However, an effective biological control agent of *D. citri* should not only reduce its densities, but preferably also reduce disease transmission. It is therefore crucial to investigate how the presence of predators on host plants affects the host plant choice and dispersal of the vector and its implications for disease transmission. Host plant choice by *D. citri* is sex dependent and based on experience during adulthood and developmental stages (Stockton et al. 2016). Females that developed on one host plant species may prefer this species when adult, but the experience with other plant species may change their preference. Besides, females of *D. citri* avoided plants exposed to the predator *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae) and its chemical cues, which may consume nymphs and adults of this pest (Seo et al., 2018). It is therefore important to study movement, dispersal and host plant selection of *D. citri* in response to the presence of predators, which I did in the third chapter. It was observed that *D. citri* did not avoid plants with predatory mites and did not disperse more from plants with predators than from plants without them. This means that the predator can feed on *D. citri* offspring without triggering the movement of the vector and the consequent spread of the disease.

In conclusion, *A. herbicolus* is a potential biocontrol agent of *D. citri* and should be further tested on citrus plants in the field, also for its capacity to reduce transmission of HLB.

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CHAPTER 1:

A predatory mite that suppresses *Diaphorina citri* populations on plants with pollen and oviposition sites provided over time

(Published in Entomologia Experimentalis et Applicata)

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Running head: A PREDATORY MITE SUPPRESSES *DIAPHORINA CITRI* POPULATIONS

Key words: alternative food, Asian citrus psyllid, biological control, citrus, citrus greening, huanglongbing, natural enemies, Phytoseiidae, predator, predator-prey dynamics, Hemiptera, Psyllidae

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Abstract

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), is the major citrus pest worldwide because it can transmit the bacteria associated with citrus greening disease (huanglongbing, HLB). The most common management strategy of this pest is chemical control, but this often results in pest resistance and threatens the sustainability of the citrus culture. There is therefore need for sustainable control strategies, such as biological control. Previous experiments showed that the predatory mite *Amblyseius herbicolus* Chant (Acari: Phytoseiidae) is able to develop and reproduce on *D. citri* eggs. Here, we studied the population dynamics of this pest on plants with and without *A. herbicolus* on isolated orange jasmine plants, *Murraya paniculata* (L.) Jack (Rutaceae), an important host plant of the pest. Predatory mites were released 1 week before the release of *D. citri* and were provided with twines with pollen as oviposition sites and food source, respectively, to maintain populations of *A. herbicolus* in the absence of the pest. Densities of adults and nymphs of *D. citri* on plants with predators were about 85% lower than on plants without predators, showing that *A. herbicolus* is a potential biocontrol agent of *D. citri*. However, the stage structure of the *D. citri* populations at the end of the experiment did not differ significantly between control plants and plants with predators. This led us to investigate whether the predator attacked other stages besides eggs. Indeed, predators were able to feed and reproduce on first and second instar nymphs of *D. citri*, but not on instars 3–5. Concluding, the release of *A. herbicolus* with alternative food and oviposition sites can reduce infestations of *D. citri*. We suggest that field experiments should be done in areas where HLB occurs to test whether releases of this predator limit the spread of this devastating disease.

Introduction

Plant traits can affect the natural enemies of herbivores in several ways. For example, nectar and pollen produced by the plant may serve as food for the predators, and morphological plant structures such as hollow thorns and domatia may offer shelter to natural enemies (Price et al., 1980; Dicke & Sabelis, 1988; Marquis & Whelan, 1996; Sabelis et al., 1999). These traits can thus increase the reproduction and survival of natural enemies on the plant, and consequently, reduce pest densities on plants (Nomikou et al., 2002; van Rijn et al., 2002; Schmidt, 2014; Adar et al., 2014). If plants lack such structures or do not produce alternative food for the natural enemies, one way of improving control is to add them to the plants (van Rijn et al., 1999; Agrawal et al., 2000; Nomikou et al., 2002, 2010; Adar et al., 2014; Delisle et al., 2015). We used this method here, providing alternative food and oviposition sites to a host plant without these attributes, to study the possibility of controlling an important citrus pest, the Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), a vector of the plant disease huanglongbing (HLB).

Huanglongbing or citrus greening is an incurable citrus disease. It is caused by bacteria – *Candidatus* Liberibacter africanus, *Candidatus* Liberibacter asiaticus, widespread in several of the largest citrus producing regions, and *Candidatus* Liberibacter americanus that colonize the phloem of host plants (Bové, 2006; Merfa et al., 2019). The last two species are transmitted if infected *D. citri* feed on the phloem of uninfected plants (Bové, 2006). The disease occurs in more than 40 countries, where it is spreading rapidly through orchards (Blaustein et al., 2018). The psyllids may acquire the bacteria during mating and through transovarial transmission (Pelz-Stelinski et al., 2010; Mann et al., 2011), but the most important infection route of the vector is by feeding on infected plants (Pelz-Stelinski et al., 2010; Kelley & Pelz-Stelinski, 2019). The probability of infection with *Candidatus* Liberibacter bacteria of both *D. citri* and plants increases with the time spent feeding by *D. citri* (Pelz-Stelinski et al., 2010; Kelley & Pelz-Stelinski, 2019). Additionally, the feeding of multiple infected individuals increases the transmission of the bacteria to the plant (Lee et al., 2015). Thus, control of *D. citri* may reduce the densities of this pest and consequently the acquisition and transmission of HLB (Juan-Blasco et al., 2012).

Chemical control is one of the main methods to control *D. citri* and limit the spread of the disease (Miranda et al., 2021), but the use of chemical pesticides has known negative side effects on non-target organisms (Desneux et al., 2007; Bryden et al., 2013; Yamamuro et al., 2019), and can interfere with pest control by natural enemies (Janssen & van Rijn, 2021). Therefore, effective, environment-friendly control strategies are urgently required (Blaustein et al., 2018). Biological control of the vector could be a good method to limit the spread of HLB without causing the environmental problems associated with excessive pesticide use (Adami et al., 2019).

Although natural enemies such as ladybugs, syrphids, and lacewings are known to prey on *D. citri* (Michaud & Olsen, 2004; Qureshi & Stansly, 2009; Kistner et al., 2017; Gómez-Marco et al., 2022), only two biocontrol agents are reared and released in the field for this purpose. The parasitoid *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) mainly parasitizes the fourth and fifth nymphal instars of *D. citri* (Sule et al., 2014). In some countries such as the USA and Brazil, it is mainly used in citrus groves where no pesticides are applied and in urban areas (Bassanezi et al., 2020; Milosavljević et al., 2021). The second one is the entomopathogenic fungus *Isaria fumosorosea* Wize that is commercially available for biological control of nymphs and adults of *D. citri* (Bassanezi et al., 2020). However, the efficiency of these biocontrol agents may be limited by the use of chemical pesticides (Janssen & van Rijn, 2021).

Predatory mites, mainly of the family Phytoseidae (Acari), have been reported to feed and develop on *D. citri* eggs (Juan-Blasco et al., 2012; Fang et al., 2013; Kalile et al., 2021; Jorge et al., 2021) and are well-known effective biocontrol agents of many pests (Huffaker & Spitzer, 1951; Huffaker & Kennett, 1956; Fleschner, 1959; Stenseth, 1979; Ramakers, 1980; de Klerk & Ramakers, 1986; Nomikou et al., 2002; Onzo et al., 2005; Messelink et al., 2006; Fraulo & Liburd, 2007). Several species of predatory mites were studied to control *D. citri*, such as *Amblyseius swirskii* (Athias-Henriot) (Juan-Blasco et al., 2012), *Neoseiulus cucumeris* (Oudemans), *Neoseiulus barkeri* Hughes (Fang et al., 2013), and *Amblyseius herbicolus* (Chant). Previous work showed that a Brazilian strain of this last species reproduced and developed when feeding on *D. citri* eggs, and with a higher predation rate than the other species (Jorge et al., 2021; Kalile et al., 2021). However, the effective application of biological control depends not only on predation, reproduction, and development of natural enemies on the pest, but mainly on the population dynamics of the pest and the natural enemy (Ridgway & Vinson, 1977). We therefore studied the dynamics of *D. citri* in the presence and absence of *A. herbicolus*.

Besides *Citrus*, several plants in the Rutaceae family can serve as hosts for *D. citri,* including orange jasmine, *Murraya paniculata* (L.) Jack, which is one of the preferred host plants of *D. citri* (Halbert & Manjunath, 2004). Plants of the Rutaceae family are usually glabrous, so do not offer many structures for shelter or oviposition for predatory mites (Schmidt, 2014), and nectar and pollen are not permanently available (Krajewski & Rabe, 1995). Previously, it was found that providing pollen on chili pepper plants without flowers enabled the increase of *A. herbicolus* densities, which resulted in the control of broad mites, *Polyphagotarsonemus latus* (Banks) (Duarte et al., 2015). Also, it was observed that providing twines as oviposition sites and pollen as alternative food on plants that lack these traits increased densities of predatory mites (Adar et al., 2014), also on citrus (Pekas & Wäckers, 2017). We therefore supplied the predatory mites on orange jasmine plants with pollen as alternative food and twines as oviposition sites (Adar et al., 2014; Pekas & Wäckers, 2017). In particular, we verified the ability of the predatory mite *A. herbicolus* to suppress populations of *D. citri* on isolated plants. The idea behind this is, that if the predator would not be able to reduce pest populations even on these small plants, control on larger trees in orchards will probably not be feasible. We observed a significant reduction in overall densities of *D. citri*; however, the stage structure of the pest did not differ between populations on plants with vs. without predators. A possible cause of this is that predators did not only feed on eggs, but also on other stages, we therefore verified on which nymphal instars *A. herbicolus* is able to feed and reproduce.

Materials and methods

The experimental system and rearing methods

We used small orange jasmine plants for logistical reasons. They are among the vector's preferred host plant species (Halbert & Manjunath, 2004) and are also host of the HLB-causing bacteria (Damsteegt et al., 2010), although they are poorer hosts for the bacteria than citrus plants (Beloti et al., 2018; Ramsey et al., 2022). Orange jasmine is widely used as an ornamental, which facilitates the dispersal of the vector and of the disease, so *D. citri* needs to be controlled on these plants as well as on citrus, especially because orange jasmine can be used as trap plants to manage *D. citri* (Tomaseto et al., 2019). Orange jasmine plants (4–6 months old, about 25–30 cm high) were used in experiments and for rearing *D. citri.* These plants were obtained from the Viveiro Antuérpia in Viçosa, Minas Gerais, Brazil. The region is free of HLB, which was confirmed with a molecular analysis of plant material (Kalile et al., 2021). Plants were kept in an insect-proof greenhouse (25 ± 2 ºC). They were pruned regularly to stimulate the formation of new growing tips and fertilized every 2 months with NPK 4/14/8 (Nutrisafra Fertilizantes, Barueri, SP, Brazil). The plants used for rearing and experiments were 25–30 cm high and had similar numbers (1–2) and stages of growing tips (developmental phases 2 and 3;

Cifuentes-Arenas et al., 2018), which are oviposition sites for *D. citri*. Adults of *D. citri* were collected from orange jasmine plants on the campus of the Federal University of Viçosa, MG, Brazil (21°76′03″S, 42°87′00″W). The psyllids were incubated in BugDorm-4F insect cages (0.5 × 0.5 × 1.0 m; MegaView Science, Taichung, Taiwan) with orange jasmine plants, which were watered twice a week (Kalile et al., 2021). The cultures were maintained under laboratory conditions (26 ± 2 ºC, 70 ± 10% r.h., natural light). To obtain adults of *D. citri* of similar ages (7–10 days old) for the experiments, 10-cm-long branches with fifth-instar nymphs were collected from the rearing or from orange jasmine plants on the campus. These nymphs were identified through their body size, antennal segmentation, number of antennal setae, and the presence and extension of mesothoracic wing pads (Tsai & Liu, 2000; Hall, 2008). The bases of the orange jasmine branches were wrapped in wet cotton wool and the branches were placed in seeding trays inside an insect cage as described above. Insects that reached adulthood were transferred to a new cage with orange jasmine plants and were separated depending on the day of emergence. Because *D. citri* become sexually mature and mate 2–6 days after emergence, we used 7- to 10-day-old *D. citri* (Shivankar et al., 2000) to ensure that all females were of the same reproductive phase.

Females of the thelytokous *A. herbicolus* (de Moraes & Mesa, 1988) had been collected from tomato plants in Prados, MG (21°03′00″S, 44°04′47″W; Kalile et al., 2021). The predators have been reared in the Laboratory of Acarology of the Federal University of Viçosa since 2014. The rearing units consisted of a plastic tray with water and one wet sponge, on which a plastic sheet (10 × 15 cm) was placed, surrounded by a barrier of moistened cotton wool (McMurtry & Scriven, 1965). The predators were fed twice a week with *Typha* sp. pollen, which is a good food source for this predator (Duarte et al., 2015). The pollen was collected in 2016 from *Typha* sp. plants from marshes around Viçosa, MG, dried in the laboratory (40 ºC for 12 h), and stored in the freezer (–6 ºC). Before being used, small amounts of pollen were placed in an Eppendorf tube (1.5 mL) and dried again (60 ºC for 48 h). After each use, the tube with pollen was stored in the refrigerator (8 ºC) (Kalile et al., 2021). Cotton threads, covered with a piece of black plastic sheet (1 × 1 cm) folded into the shape of a roof, served as oviposition sites and shelter (Kalile et al., 2021). Cohorts of similar-aged females were obtained by allowing adults to oviposit on new arenas for 24 h. The predatory mite cultures were maintained in a climate room at 25 ± 2 ºC, 70 ± 10% r.h., and L12:D12.

Population dynamics experiments

Two experiments were performed to test the capacity of *A. herbicolus* to reduce densities of *D. citri* on orange jasmine plants. Orange jasmine plants were placed inside tubular cages (60 cm diameter, 50 cm high) made of transparent acetate sheet (0.25 mm thick; Promom Industria e Comercio, Andradina, SP, Brazil) and covered with a mesh (90 µm), glued to the top end of the tube to provide air circulation. An opening (10 × 10 cm) at the center of the side of the cage, covered with mesh, also allowed air circulation to avoid excess of humidity. A circular plastic tray (60 cm diameter) was used to close the base of the tube, and cages were placed inside an outer tray (47 × 23 × 9.5 cm) with water and detergent to prevent invasions of undesirable organisms. Because we previously observed some dead adults of *D. citri* in the moist soil after releasing them, we equipped each plant with a circular black plastic sheet at the base of the plant, covering the soil, with an opening in the center for the stem.

Half of the plants received 6 adult females of *A. herbicolus,* collected from a cohort of similar age (10–12 days since the egg stage) and transferred to the plants using a fine brush. The other plants were maintained without predators and served as control. We previously observed that *A. herbicolus* oviposited on twine with pollen on orange jasmine plants and did not establish well on plants without twine, and we therefore provided each plant (including controls) with one 5-cm-long fibrous cotton twine (Indústria Comércio Resíduos Têxteis Boa

Vista, Carmo, RJ, Brazil) with *Typha* sp. pollen (about 0.5 mg) to facilitate oviposition and establishment of *A. herbicolus*. After release of the predatory mites, all plants were irrigated and received about 3 mg of *Typha* sp. pollen twice a week throughout the experiment, which was applied through the mesh at the top of the cage using a brush, so it was concentrated at the top of the plant.

One week after release of the predators, all plants received 7- to 10-day-old adults of *D. citri*. These were individually sucked from the culture plants into a pipette tip (1000 µl; Nichiryo, Saitama, Japan) connected to a transparent hose (1 cm diameter) with a mesh (90 µm) at the interface of the hose and the tip to restrain the *D. citri*. After collection, the pipette tip was detached from the hose and closed on both sides with Parafilm 'M' laboratory film (Bemis Flexible Packaging, Neenah, WI, USA) and the insect was sexed based on the abdominal tip. For release of *D. citri*, the tubular cage was lifted, and the pointed part of the pipette tip was inserted into the soil through the opening of the plastic sheet at the base of the plant close to the stem, but with the wide end extending above the sheet. Thus, the psyllids would end up on the sheet when leaving the tip, and not on the humid soil. Subsequently, the Parafilm was removed so that the insects could move onto the plant and the tubular cage was replaced. The experiments were evaluated twice a week; the presence of *D. citri* eggs on the plants was confirmed, and the adults and fifth instars of *D. citri* were counted through visual inspection. To count *D. citri*, the trays with the cages were placed on a bench and rotated. Parts of the plants that were difficult to inspect with the cage over the plant were examined by quickly raising and putting back the cage. Hands were sanitized with 70% alcohol before evaluating each cage. To prevent invasions of the control plants by predators, plants without predators were always evaluated before plants with predators. The plants were not pruned during the experiment to prevent loss of *D. citri* individuals.

Owing to the small size of the predators (adult female ca. 0.5 mm long) and the complex plant architecture, it was impossible to reliably estimate the densities of the predators without destructive sampling. We therefore confirmed their presence through the presence of eggs on the twines. Twines were removed with tweezers, placed in a Petri dish checked for the presence of eggs under a Stemi 508 binocular stereomicroscope (25× magnification; Zeiss, Oberkochen, Germany). Subsequently, pollen (as above) was applied to the twines with a brush and they were returned to their plants. We also observed adult predators under the black sheet close to the soil, which was therefore also used to confirm their presence.

In the first (pilot) experiment, three males and seven females of *D. citri* were initially released per plant, reflecting the sex ratio observed in the cultures and in the field. More individuals were released on all plants weekly until we observed eggs of *D. citri* on all plants, which amounted to a total of five releases (Figure 1). The numbers released varied with the availability of individuals of the right age (second and third release: 1 male:5 females; fourth release: 2 males). In total, each plant received seven males and 17 females of *D. citri* (24 psyllids). Ten plants were used, half received predators and the other half were controls. Despite precautions, two control cages were invaded by predators after 26 days and were therefore considered as a different treatment. These cages were not included in the statistical analysis, but were evaluated until the end of the experiment and *D. citri* densities were also plotted (Figure 1). The experiment lasted for 100 days and was conducted under laboratory conditions (26 ± 2 ºC, 70 ± 10% r.h., natural light). At the end of experiments, the plants were about 45-50 cm high. The pilot experiment showed that five plants with predators and three plants without predators were sufficient to obtain significant differences in *D. citri* densities between plants with and without predators. We therefore repeated the experiment with five plants with predators and five plants without. This second experiment was done 1 year later, starting in August (as the previous experiment), with some slight modifications. We now provided two twines on each plant, and changed those twines that did not contain predator eggs with new ones every 3 weeks. This time, six releases of *D. citri* were needed until all plants had eggs (males:females = 7:7, 3:2, 2:3, 4:7, 5:5, and 1:3, respectively). In total, each plant received 27 females and 22 males (49 psyllids). The sex ratio and numbers of *D. citri* released differed from the first experiment according to the availability of individuals with standardized age (7–10 days). To ensure that invasions would not occur, water and detergent in the outer trays that isolated the plants was replaced more regularly, and we maintained a high level of the water barrier. Based on the densities observed in the pilot experiment, this second experiment lasted 53 days. At the end, all plants were sampled destructively and all stages of *D. citri* were counted using a Stemi 508 binocular stereomicroscope (20× and 25× magnification). The experiment was conducted under laboratory conditions (26 ± 2 °C, 70 ± 10% r.h., natural light).

Predation and oviposition of *Amblyseius herbicolus* on nymphs of *Diaphorina citri*

To verify which instars of *D. citri* are killed by *A. herbicolus*, we performed a quick test in which we provided orange jasmine growing tips (development phase 3; Cifuentes-Arenas et al., 2018) with three individuals from each instar of *D. citri* separately in an arena consisting of a black plastic dish (5.5 cm diameter, 1.4 cm high), covered with a transparent lid and with a piece of wet cotton wool inside (1 cm diameter) to provide water (Kalile et al., 2021). The nymphs were obtained from the culture of *D. citri* and we checked for predation 24 h later. Controls without predators were also included. We considered dead nymphs that no longer contained visible remains of body fluid as being preyed upon. Dead nymphs encountered in arenas without predators always contained such remains. Additionally, as evidence of predation, we considered the change in color of the predator’s digestive tract, from slightly transparent milky white when feeding on pollen to non-transparent yellow, the predominant color of the eggs and nymphs of this pest. First, we tested the predator's ability to consume each of the five instars of *D. citri*. For this, we offered nymphs of each instar separately to individual predators and evaluated the survival of these nymphs compared to a control without predator. Nymphal stages were differentiated as indicated above (Tsai & Liu, 2000; Hall, 2008). After seeing that *A. herbicolus* fed on instars 1 and 2 but not on instars 3–5, we assessed predation and oviposition rates on instars 1 and 2. To obtain nymphs, we released about 120 adults of *D. citri* in a cage with orange jasmine plants with growing tips (development phases 2 and 3). One week later, first instar *D. citri* were obtained from these plants; second instars were obtained after 2 weeks. Based on the previous tests, we provided 15-16 first instars and 4–5 second instars per individual predator. We placed a growing tip (development phase 3) inside each arena and gently transferred the nymphs one by one with a brush. All predators were tested individually and were of the same age (11–13 days old) and were moved to a new arena with fresh prey nymphs every day. As a control for oviposition, we used the same setup, but instead of adding *D. citri*, we added 1 mg of *Typha* sp. pollen. The numbers of *D. citri* nymphs consumed and oviposition rate of the predator was assessed during 3 days, but the oviposition of the 1st day was not considered to reduce effects from the previous diet (Sabelis, 1990). We tested 15 and 18 predators with first instars and pollen, respectively, and 15 with second instars and its control with pollen. The arenas were maintained in a climate room at 25 ± 2 ºC, 70 ± 10% r.h., and L12:D12.

Statistical analysis

Log(x+1)-transformed numbers of *D. citri* on plants with and without predators were analyzed with a linear mixed effect model (LME) of the package *nlme* (Pinheiro et al., 2017), with ‘replicate’ as a random factor, ‘treatment’ (presence/absence of predators) as categorical factor, and ‘time’ as continuous factor, as well as the interaction between treatment and time. Because of the non-linearities in the densities of *D. citri* through time, we also added a quadratic term of time and its interaction with treatment. Models were checked with normal error plots and plots of residuals against fitted values. Significance of factors and interactions were determined with likelihood-ratio (LR) tests. The interaction of time squared with treatment was not significant and was therefore removed from the models. As expected, the effect of the factor time squared on its own was significant (pilot experiment: LR = 62.7, d.f. = 1, $P < 0.0001$; second experiment: LR = 5.43, d.f. = 1, P = 0.020), and maintained in the models. In the results, we concentrate on the effects of the interaction of treatment with time as the main result of our experiments. Contrasts between treatments per time step were obtained with the package *emmeans* with a Tukey correction for multiple comparisons (Lenth et al., 2020). The total numbers of all *D. citri* stages and the proportions of the various stages at the end of the second experiment (day 53) were analyzed using quasi-Poisson and quasi-binomial generalized linear models (GLMs), respectively. One replicate of the treatment with predators was excluded from the analysis of the proportions of stages because of the absence of pest individuals on the plant. All statistical analyses were done with R v.4.1.2 (R Core Team, 2019).

Results

Population dynamics experiments

In the first (pilot) experiment, there was a significant effect of treatments through time on the densities of *D. citri* (LME, treatment*time: LR = 20.9, d.f. = 1, P = 0.0001; Figure 1). This was because the treatments started with the same number of *D. citri*, but through the course of the experiment, plants with predators had fewer *D. citri* than plants without predators. At the end of the experiment, about 56% fewer *D. citri* were found on plants with *A. herbicolus* than on plants without them, despite repeated releases of *D. citri* (Figure 1). The two control plants that were invaded by predatory mites were not included in the statistical analysis, and showed *D. citri* densities intermediate between uninvaded control plants and plants with predators since the beginning (Figure 1). Predatory mites stopped ovipositing on the twine after some weeks, and in cages with nymphs and new individuals of *D. citri*, the predators started ovipositing on or near exuviae of *D. citri* nymphs (Figure 2).

In the second experiment, plants with predators again had lower densities of *D. citri* through time than plants without predators (LME, treatment*time: LR = 44.1, d.f. = 1, $P < 0.0001$; Figure 3). The difference in densities of *D. citri* between plants with and without *A. herbicolus* was significant from day 24 until the end of the experiment (Figure 3). Final densities were obtained by destructive sampling on day 53, and we found ca. 85% fewer *D. citri* (all stages) on plants with predatory mites than on control plants (GLM: $F_{1,8} = 19.3$, $P = 0.0023$; Figure 3). There was no significant difference in stage structure of the populations on control and treatment plants (GLM, adults: $F_{1,7} = 0.71$, $P = 0.43$; nymphs: $F_{1,7} = 0.27$, $P = 0.62$; eggs: $F_{1,7} = 0.0002$, $P = 0.99$; Figure 4). During the final destructive sampling, we observed a few individuals of *A. herbicolus* on the aerial part of the plants. Later experiments evaluating the performance of *A. herbicolus* on orange jasmine plants showed that most individuals stayed close to the soil, at least during the day (MO Kalile, pers. obs.).

Predation and oviposition of *Amblyseius herbicolus* on nymphs of *Diaphorina citri*

Amblyseius herbicolus fed on first and second instars of *D. citri* but not on the other three instars. It consumed on average 9.55 ± 0.34 (mean ± SE, n = 15) first instar *D. citri* per day during 3 days and deposited on average 1.26 ± 0.11 eggs per day during the last 2 days, compared to 1.81 ± 0.08 eggs (n = 18) per day on pollen. On second instars, the predators consumed on average 2.04 ± 0.14 individuals per day during 3 days and produced on average 1.83 ± 0.06 (n = 15) eggs per day during the last 2 days, compared to 1.96 ± 0.10 (n = 15) on pollen.

Discussion

We previously found *A. herbicolus* on citrus and orange jasmine plants with *D. citri* and showed that this predator can develop and reproduce when feeding on *D. citri* eggs (Kalile et al., 2021). Here we show that *A. herbicolus* can reduce *D. citri* populations on orange jasmine plants given the presence of pollen and oviposition sites, and is also able to feed and reproduce on first and second instars. The first population dynamics experiment lasted 100 days, pest individuals were released repeatedly, and the populations of *D. citri* became 56% lower on plants with predatory mites than on control plants. Unfortunately, several plants without predators were invaded by them, resulting in a loss of replicates, despite our efforts to avoid contamination. On the positive side, this shows that the predators can find plants with *D. citri*, even when they need to cross barriers before ending up on these plants. The densities of *D. citri* on those invaded plants were lower than on control plants but higher than those on plants on which predatory mites were released, and differed from both. This suggests that better control can be reached when the predatory mites are already established on the plants before arrival of the pest. In the second, better controlled population dynamics experiment, the reduction of *D. citri* densities was larger and faster and reached 85%, showing that this predator has potential to suppress *D. citri* on intact plants.

The densities of *D. citri* on the control plants in the first population dynamics experiment showed considerable variation. This was probably caused by a delay in the establishment of *D. citri* in one of the control cages, despite our efforts to standardize the plants in terms of age, number of growing tips, and fertilization, as well as the numbers and age of *D. citri*. Because considerable numbers of *D. citri* were released on the plants, it is unlikely that this variation can be ascribed to chance events during the establishment of the psyllids. Hence, plants may have varied in some unknown quality independent of this standardization.

Besides affecting the population dynamics of *D. citri*, we previously showed that plant quality may also affect predation rates of *D. citri* eggs by *A. herbicolus* (Kalile et al., 2021). We observed a stronger reduction of pest densities in the second compared to the first population dynamics experiment, whereas each plant in the second experiment received twice as many individuals of *D. citri* than in the first experiment. It has been shown before that phytoseiids failed to establish on citrus plants without providing alternative food and twine as oviposition sites (Pekas & Wäckers, 2017). Therefore, the difference in pest densities observed here may be caused by the synergetic effect of repeated supply of twines as oviposition sites and pollen to the predators.

Fang et al. (2013) released the predatory mite *N. cucumeris* on caged citrus plants at the same time as *D. citri*. This resulted in reductions of *D. citri* densities comparable to those found here, but only with the release of a few thousand predators, much higher numbers than released here. This suggests that control of *D. citri* with *A. herbicolus* can be achieved with considerably lower numbers and possibly with lower costs. An open question still is whether the control of *D. citri* results in reductions of the spread of HLB. Unfortunately, we were unable to test the effects of vector control on disease occurrence because the disease does not occur yet in the region in which the research was carried out.

Experiments lasting more than one pest generation, such as those reported here, are essential when evaluating biological control because they show not only whether pest densities decrease, but also whether this decrease is persistent. The reduction of densities observed during one generation may be ambiguous due to the preponderance of vulnerable stages (Harrison & Cappuccino, 1995). Our experiments were conducted for 100 and 53 days, covering about six and three generations of *D. citri*, respectively (Liu & Tsai, 2000), showing that the reduction of *D. citri* densities was persistent. Nevertheless, we observed prey persistence on some plants with predators and this may have been caused by the presence of stages that are invulnerable to consumption by predators, which may prevent the extinction of the pest (Murdoch et al., 1987; Nomikou et al., 2002), at least in the absence of alternative food.

In our experiments, pollen enabled the persistence of predatory mites on the plants even without *D. citri*. This persistence is important because even a few *D. citri* individuals may transmit the disease, and 'standing armies' of natural enemies are therefore needed to protect plants on which the pest does not occur yet. Our experiments are proof of principle that populations of *A. herbicolus* can reduce the densities of *D. citri*, or even eradicate them, when plants are supplied with pollen and twines. If our experiments would have shown no control of *D. citri*, this would strongly suggest that time-consuming field experiments would be futile. Considering that *A. herbicolus* already occurs on citrus and orange jasmine plants in the field in Brazil, the next question is how to boost populations of this predator and control of the pest on trees in the field. We used the concept of pollen-on-twine here (Adar et al., 2017), and similar methods of providing alternative food and oviposition sites were demonstrated in a greenhouse crop by Lee & Zhang (2013) and by Pekas & Wäckers (2017) on citrus seedlings. Beltrá et al. (2017) showed that biweekly applications of pollen and sugars to citrus trees in two orchards resulted in increased densities of predatory mites, but no effects on pest densities were reported. Furthermore, Tsuchida & Masui (2020) showed that spraying pollen on citrus plants in the field as food for predatory mites resulted in a significant decrease of another citrus pest, the eriophyid mite *Aculops pelekassi* (Keifer), on leaves and a marginal decrease on fruits. Together, these studies show that it is in principle possible to supply predatory mites on citrus with alternative food, and that this may result in increased pest control. Besides using pollen on twine, several other methods have been used to boost densities of natural enemies before the arrival of the pest (van Rijn et al., 2002; Aguilar-Fenollosa et al., 2011; Pijnakker et al., 2020; Irvin et al., 2021), some of which may be more feasible in the field. For example, food and/or oviposition sites may also be provided by intercropped plants (Maoz et al., 2011), which was shown to increase predatory mite densities and to reduce pest densities in citrus orchards (Aguilar-Fenollosa et al., 2011; Aguilar-Fenollosa & Jacas, 2013). Obviously, this needs further research into which companion plants can supply *A. herbicolus* with alternative food and oviposition sites. Plants with flowers and extrafloral nectaries that may favor the parasitoid *T. radiata* and other predators, such as predatory hoverflies (syrphids), are already being investigated (Patt & Rohrig, 2017; Irvin et al., 2021; Irvin & Hoddle, 2021), and it would be interesting to investigate whether these plants affect densities of *A. herbicolus* in citrus orchards and the possible effects of their combined use with other natural enemies.

Much remains to be investigated to achieve successful biological control of *D. citri*, for example, the compatibility of various natural enemies. *Amblyseius herbicolus* does not appear to attack the same stages of *D. citri* as *T. radiata* or larger predators such as lady beetles. However, it is conceivable that larger, generalist natural enemies may also attack the predatory mites (Brødsgaard & Enkegaard, 1995; Rosenheim, 1998; Colfer et al., 2003; Chow et al., 2008). Moreover, ants are known to protect *D. citri* against natural enemies and may reduce the efficiency of biocontrol agents (Milosavljević et al., 2021). Understanding how the presence of *A. herbicolus* may affect both the population densities and behavior of *D. citri* is particularly important for understanding the effects of predator-induced changes in vector behavior on disease transmission and how to reduce the spread of HLB.

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Figure captions

Figure 1 Mean (± SE) number of adults plus fifth-instar nymphs of *Diaphorina citri* on orange jasmine plants with (grey line) and without (black line) *Amblyseius herbicolus* predators, supplied with pollen and oviposition sites, during 100 days (first experiment). Evaluations started 3 days after the first release of *D. citri*. Arrows indicate when adults of *D. citri* were released (see text for numbers released). The hatched line and squares between control and treatment show the mean densities of the two control plants that were invaded by predators (not included in the statistical analysis). The letters on the right indicate significant differences between plants with and without predators (contrast after an LME: $P < 0.0001$).

Figure 2 Eggs of the predatory mite *Amblyseius herbicolus* under an exuvium (about 1.5 mm long) of a *Diaphorina citri* nymph during the first population dynamics experiment.

Figure 3 Mean (± SE) number of adults plus fifth-instar nymphs of *Diaphorina citri* on orange jasmine plants with (grey line) and without (black line) *Amblyseius herbicolus* predators, supplied with pollen and oviposition sites, during 53 days (second experiment). Evaluations started 3 days after the first release of *D. citri*. Arrows indicate when adults of *D. citri* were released. Final densities on plants without (black square) and with predators (white square) were obtained through destructive sampling on day 53, when adults, all nymphal stages, and eggs were counted. Asterisks (*) indicate significant differences between treatments per day of evaluation (contrasts after an LME: $*0.001 < P < 0.05$, $**P < 0.001$).

Figure 4 Proportion of eggs (black), nymphs (gray), and adults (white) of *Diaphorina citri* on orange jasmine plants with and without *Amblyseius herbicolus* predators. Numbers above the bars indicate the total numbers of *D. citri*.

Fig. 1

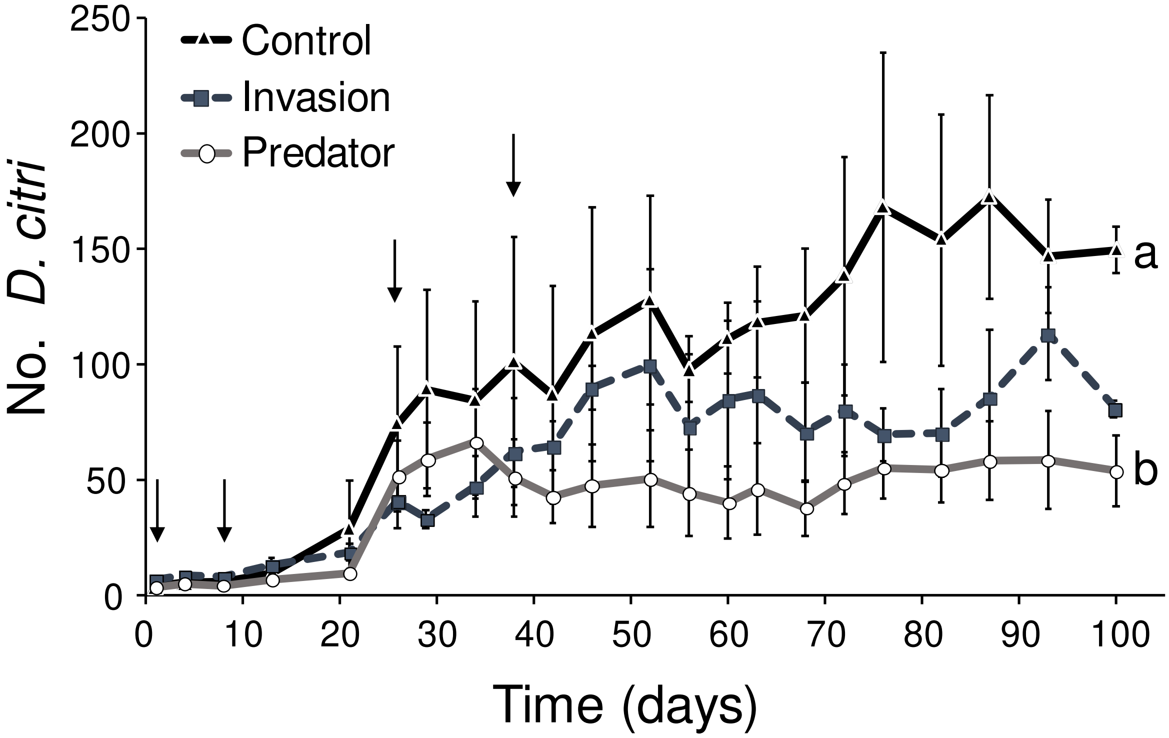

Control
Invasion
Predator
No. D. citri
250
200
150
100
50
0
0
10
20
30
40
50
60
70
80
90
100
Time (days)
a
b

Fig. 2

Fig. 3

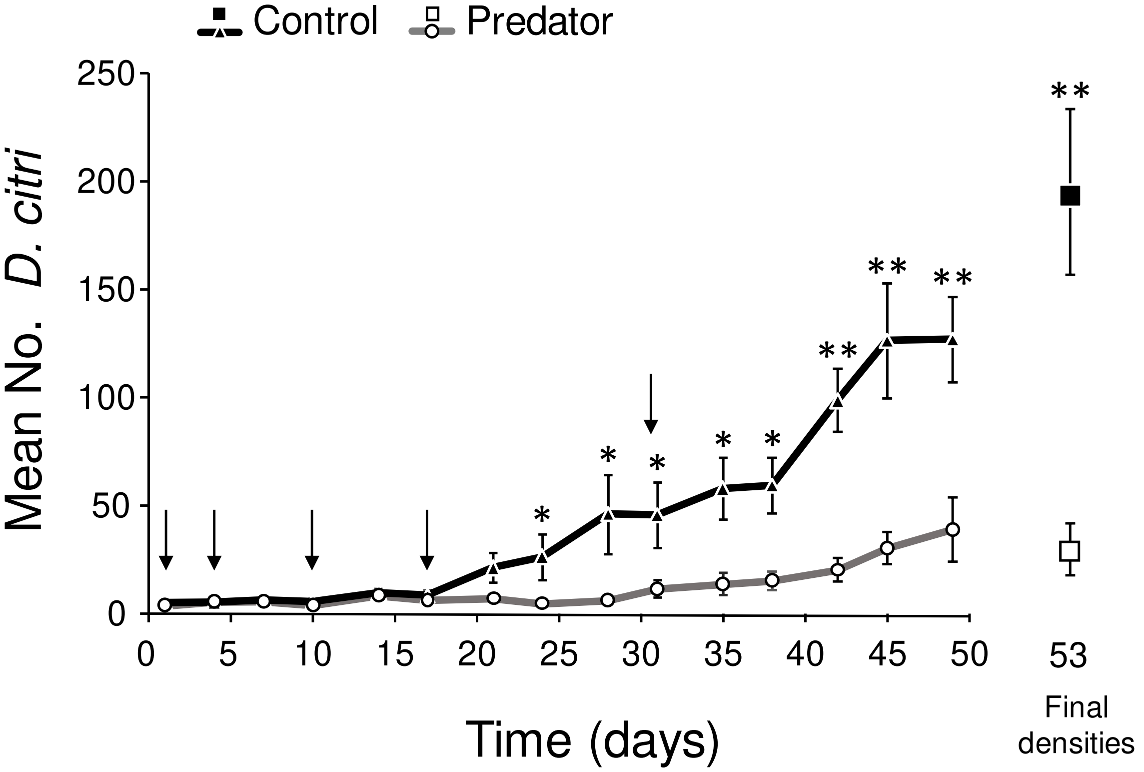
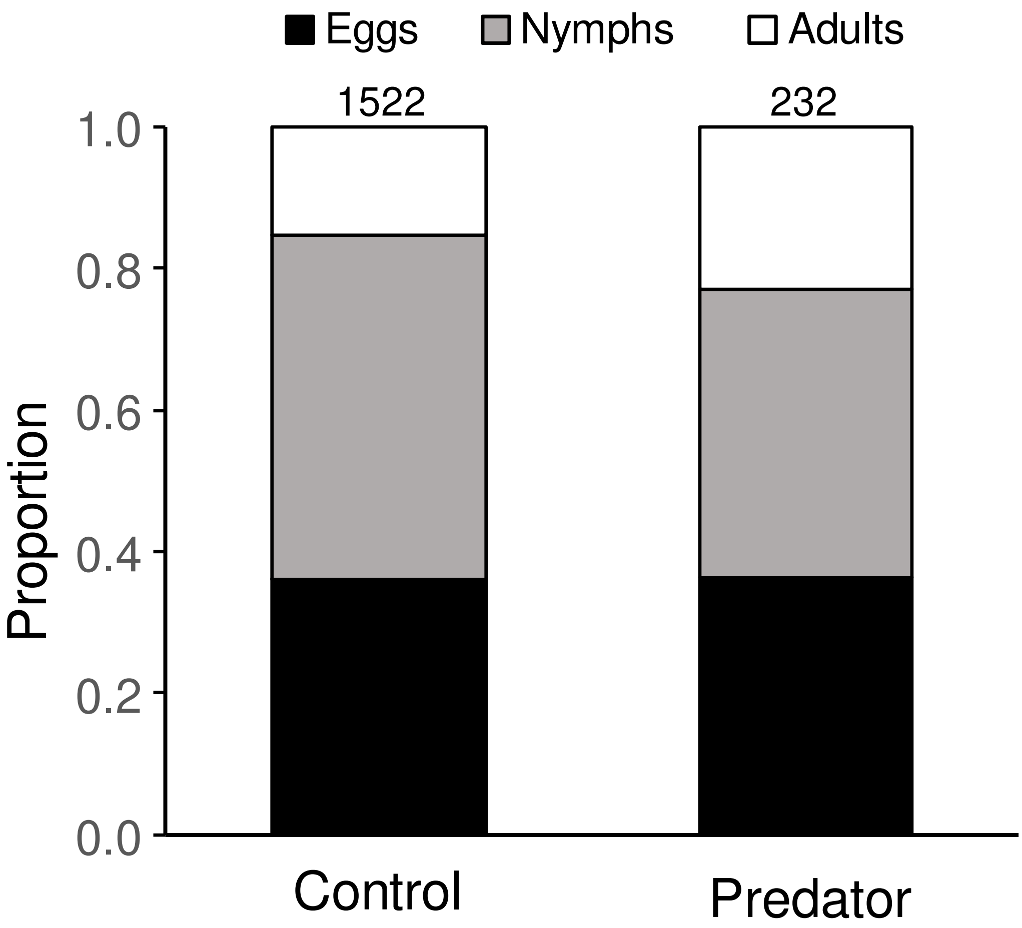

Control
Predator
Mean No. D. citri
0
50
100
150
200
250
0
5
10
15
20
25
30
35
40
45
50
53
Time (days)
Final densities
*
**

Fig. 4


■ Eggs
▨ Nymphs
☐ Adults
1522
232
1.0
0.8
0.6
0.4
0.2
0.0
Proportion
Control
Predator

CHAPTER 2:

A predatory mite reduces outdoor *Diaphorina citri* populations

(Edited according to the journal Biological Control)

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ABSTRACT

Maintaining predator populations in the absence of pests is one of the objectives of preventive biological control. To achieve this, predator populations can be provided with alternative food so that they persist in the crop and serve as a standing army against pest invasions. Here we provided pollen as alternative food and pieces of twine as oviposition sites to the predatory mite *Amblyseius herbicolus* Chant (Acari: Phytoseiidae) on orange jasmine plants outside. This resulted in the establishment of populations of the predators, and we subsequently released *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) on these plants and on a similar group of plants without predators. *Diaphorina citri* is an important pest of citriculture industry worldwide because it transmits the bacteria that cause Huanglongbing (HLB) or greening, a fatal and incurable disease. However, the region in which the experiments were carried out was still free of this disease, so we could only observe the effect of the predator on pest densities and not on HLB incidence. *Amblyseius herbicolus* was released 45 days before the pest and predator presence was monitored weekly. The numbers of *D. citri* were assessed during 63 days. *Diaphorina citri* densities on plants with predators were 90% lower than on plants without predators. Being outdoors, other organisms were able to settle on the plants, such as mealybugs, aphids, whiteflies, thrips, phytophagous and fungivores mites, three species of predatory mites, spiders, lacewings and *T. radiata*, a parasitoid of *D. citri*. We therefore also present the distribution of these organisms on plants with and without predatory mites. We conclude that *A. herbicolus* is a potential biocontrol agent of *D. citri* in the field. Further tests should evaluate the suppression of this pest in citrus orchards and the possible impacts on HLB spread.

Keywords: Asian citrus psyllid; biological control; Greening; HLB; population dynamics; pollen; predator

INTRODUCTION

The side effects of pesticides on the environment, wildlife, beneficial organisms and human health have raised increasing concerns over the years (Bryden et al., 2013; Desneux et al., 2007; Köhler and Triebskorn, 2013). Although pesticides can have immediate negative effects on pest densities, in the long term they can lead to pest resurgence through their direct and indirect impact on natural enemies (Janssen and Rijn, 2021). Besides, problems such as the presence of pesticide residues, pest resistance and secondary pest outbreaks (Dutcher, 2007; Guedes et al., 2016; Hardin et al., 1995) have led producers to look for more sustainable and effective alternatives. In addition, environmental regulations are increasingly limiting long-term use of pesticides, while finding new molecules is expensive and difficult (Sparks et al., 2019; Topping et al., 2020). One sustainable alternative for chemical pesticides is the use of biological control, which is the intentional use of natural enemies to control target pests (Waage and Greathead, 1988). In the last years, biological control of arthropods increased about 15% per year (Knapp et al., 2018; Van Lenteren et al., 2020), whereas pesticide use has stabilized in several countries (Bourguet and Guillemaud, 2016). Usually, economic evaluations of pesticide use take only their purchase costs into account, but not their side effects (Bourguet and Guillemaud, 2016; Naranjo et al., 2019). Therefore, the benefits of biological control to producers, society and environment are immense and economically underestimated (Naranjo et al., 2019).

Plant pathogens are a major threat to many crops worldwide (Collinge et al., 2022), and most plant pathogens depend on motile vectors for transmission from infected to heathy plants (Purcell and Almeida, 2005). In particular, whiteflies and aphids are major vectors of plant viruses (Purcell and Almeida, 2005; Perilla-Henao and Casteel, 2016), and leafhoppers and psyllids are important vectors of plant pathogenic bacteria (Purcell and Almeida, 2005; Perilla-Henao and Casteel, 2016). Vectors of plant-pathogenic bacteria transmit diseases that cause devastating economic losses to crops (Perilla-Henao and Casteel, 2016). Vectors such as whiteflies, aphids and thrips can already be controlled effectively with natural enemies (Arnó et al., 2009; Boivin et al., 2012; Calvo et al., 2015; Mouden et al., 2017), resulting in protection of plants against diseases (Tellez et al., 2017). However, pesticides are still often used to control of these vectors, but often fail to control the spread of diseases (Purcell and Almeida, 2005; Okamoto and Amarasekare, 2012). The biocontrol of pathogen vectors with arthropod natural enemies can be complex because besides the direct effect of consumption, indirect effects such as antipredator behaviour of the vector may affect pathogen transmission (Finke, 2012). Nevertheless, biocontrol is receiving increasing attention as a potential alternative to control disease vectors, especially those which developed pesticide resistance, or when suitable pesticides are not available (Collinge et al., 2022).

An example of a vector of an incurable plant disease is the psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). It vectors the bacteria associated with Huanglongbing or Greening, the major disease of citrus plants during the last 20 years (Guo et al., 2021). This disease is devastating, spreads quickly and is fatal to plants (Bové, 2006; Ghosh et al., 2022). Nowadays, it occurs in about 65 countries, including the major citrus producers (Ghosh et al., 2022). Control of the vector is a key HLB management strategy (Guo et al., 2021) and tolerance for its presence in orchards is close to zero (Bové, 2006; Singerman and Rogers, 2020), hence, the economic threshold is the first observation of the vector (Singerman and Rogers, 2020). Increased pesticide applications to eliminate *D. citri* raised the costs of citrus production, induced vector resistance and reduced populations of natural enemies (Guo et al., 2021; Kanga et al., 2016). This reduction of enemies of *D. citri* and their regulation services costs millions of dollars annually only in Florida (Monzó and Stansly, 2020). Biological control of *D. citri* is potentially a sustainable alternative for pesticide use (Grafton-Cardwell et al., 2013; Monzó and Stansly, 2020), and can reduce the dependency on pesticides (Grafton-Cardwell et al., 2013). The feasibility of biological control is supported by the observation that lower densities of *D.*

citri were found in organic orchards than in those that were sprayed (Shrestha et al., 2021).

Natural enemies of *D. citri* encompass parasitoids, predators and microorganisms (Michaud and Olsen, 2004). *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), the main parasitoid of *D. citri* (Chen and Stansly, 2014; Flores and Ciomperlik, 2017), was introduced in several countries to control this vector and had variable success rates (Chen and Stansly, 2014; Diniz et al., 2020). In general, results were better without pesticide applications (Chen and Stansly, 2014; Chow and Sétamou, 2022; Michaud and Olsen, 2004). Recently, it was reported that *T. radiata* is able to acquire and transmit the bacteria horizontally during the probing of *D. citri* nymphs (Guo et al., 2021), thus, this is perhaps not the best natural enemy to reduce the spread of HLB. To date, the entomopathogenic fungus *Cordyceps fumosorosea* (Wize) Kepler, Shrestha & Spatafora (Hypocreales: Cordycipitaceae) (basionym: *Isaria fumosorosea*) and *Beauveria (Cordyceps) bassiana* (Bals. -Criv.) are available biological agents to control *D. citri* (Maluta et al., 2022). These microbial biocontrol agents showed a reduction of about 80% of *D. citri* adults and nymphs on citrus when plants were highly infested, but were not effective against light infestations (Chow et al., 2018; Saldarriaga et al., 2017;). Most field studies report coccinellids, lacewings, syrphids, spiders and formicids as the most common natural enemies of *D. citri* (Kistner et al., 2017; Michaud and Olsen, 2004; Shrestha et al., 2021). Perhaps because they are microscopic, studies usually disregard the role of predatory mites. However, they are one of the most common natural enemies in citrus orchards and they are crucial in biological control of several citrus pests (Aguilar-Fenollosa et al., 2011; Beltrà et al., 2017; McMurtry and Croft, 1997). The use and commercialization of predatory mites increased during the last decades and now more than 60% of the arthropod biocontrol market consists of predatory mites (Knapp et al., 2018). They are used to control pests such as thrips, whiteflies and phytophagous mites (Knapp et al., 2018). The family Phytoseiidae is the most important group of predatory mites commercialized as biocontrol agents and there are actually about 20 species available worldwide (Knapp et al., 2018).

Amblyseius herbicolus (Chant) (Acari: Phytoseiidae) is a generalist predatory mite that occurs on several host plants of *D. citri* (Demite et al., 2024; Kalile et al., 2021; Reis et al., 2007). It can survive and multiply by feeding on non-prey food sources such as pollen (Cavalcante et al., 2015; Duarte et al., 2015), which enables preventive releases and maintenance of predatory mite populations on plants when pests are scarce or absent (Calvo et al., 2015; de Klerk and Ramakers, 1986; Janssen and Sabelis, 2015; van Rijn et al., 2002). Thus, in addition to the ability to control pests, the possibility of establishing *A. herbicolus* populations on plants without the pest makes it a promising biocontrol agent, because it can be used as a standing army of plant bodyguards. Besides, *A. herbicolus* is a good candidate to control multiple pests such as whiteflies, *Bemisia tabaci* (Gennadius), the broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) and thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Duarte et al., 2015; Rodríguez-Cruz et al., 2013). Another characteristic of this predator is that it reproduces by thelytokous parthenogenesis, so populations consist exclusively of females (Reis et al., 2007) and this can facilitate establishment in the field. In addition, it can be reared on astigmatid mites, which facilitates its multiplication for mass rearing and future commercialization (Zhang and Zhang, 2021). Considering these favourable characteristics, we performed experiments to evaluate the potential of this predatory mite to control *D. citri* outside.

The selection of suitable natural enemies to control pests in the field requires a series of tests, starting with small-scale laboratory experiments (Lesna et al., 1995). After we verified in the literature that *A. herbicolus* occurs on citrus plants, we sampled several plant species from the Rutaceae family (Kalile et al., 2021), confirming that this predator also occurs on orange jasmine plants, one of the preferred hosts of *D. citri*. However, co-occurrence does not imply a predator-prey association (Lesna et al., 1995), thus we showed that *A. herbicolus* consumes *D.*

citri eggs and can develop and reproduce when feeding on it (Kalile et al., 2021). Subsequently, we showed that the predatory mite reduced *D. citri* densities by about 85% on orange jasmine plants in the laboratory, and that it feeds on first – and second-instar nymphs of the psyllid (Kalile et al., 2023). Here we report on an outdoor experiment to control *D. citri* on small orange jasmine plants.

MATERIAL AND METHODS

Plants

We obtained orange jasmine plants from Viveiro Antuerpia in the vicinity of Viçosa, Minas Gerais, Brazil (Kalile et al., 2023). The plants used for the rearings of *D. citri* were about 25-30 cm high; plants used in the experiment were about 1 m high. After the plants were sprayed with a mixture of water and detergent (10%), washed and inspected to ensure they were free of insects and mites, they were kept in a greenhouse (25 ± 2 °C). The plants were fertilized bimonthly with a mixture of NPK (4/14/8, Nutrisafra Fertilizantes Ltda, Barueri – SP) and they were pruned regularly to stimulate the formation of new growing tips because these are the oviposition sites for *D. citri*.

Pollen

We used *Typha* sp. pollen collected from plants in rural areas around Viçosa, Minas Gerais (21°76′03″S, 42°87′00″W) (Kalile et al., 2021). After collection, the pollen was dried at 40 °C for 12 h in an oven and stored in a container in the freezer (− 6 °C). Periodically, we removed small amounts from the container and placed them in 1.5 ml microtubes (Eppendorf). This pollen was dried at 60 °C for 48 h and then stored in the refrigerator (8 °C) for daily use (Kalile et al., 2021). *Typha* sp. pollen was used to rear *A. herbicolus* and as alternative food for this predator on plants during the experiment.

Diaphorina citri rearing

The rearing of *D. citri* was established in the Laboratory of Acarology of the Federal University of Viçosa since 2018. When necessary, adults were collected from orange jasmine plants from the campus or from the rural area around Viçosa to increase or establish new rearings (Kalile et al., 2023). Viçosa and its surroundings are considered free of HLB, which was confirmed with molecular analysis of plant material and psyllids (Kalile et al., 2021). The psyllids were collected using a manual aspirator and released inside BugDorm-4F insect cages, MegaView Science Co., Ltda, Taichung City – Taiwan (0.5 × 138 0.5 × 1.0 m) with orange jasmine plants (4-6 months old) with growing tips, which were watered twice a week (Kalile et al., 2021). The adults were transferred regularly to new cages with uninfested plants (Kalile et al., 2021). The rearings were maintained under laboratory conditions (26 ± 2 ºC, 70 ± 10% RH, natural light).

Predatory mite rearing

Initially, we used a predator strain collected from tomato plants in lab experiments (Kalile et al., 2023), but after discovering a strain feeding on eggs of *D. citri* on orange jasmine plants, *Murraya paniculata*, (L.) Jack, Rutaceae in the rural area of Viçosa – Minas Gerais, Brazil (20º.79’56’’S, 42º.89’61’’W), we decided to use this strain in the experiments. This strain also established better on orange jasmine plants than the “tomato” strain in experiments in the lab (MOK, pers. obs.). The predators were reared on arenas that consisted of a black plastic sheet (10 × 15 cm²) surrounded by a wet cotton wool barrier. Underneath the plastic, there was a wet sponge in contact with water inside a plastic tray to ensure that the cotton wool remained wet and to prevent escapes of the predators (McMurtry and Scriven, 1965). This plastic tray was placed inside a larger tray with water to further ensure isolation (Kalile et al., 2021). We offered cotton threads covered with a piece of black plastic sheet folded into a roof shape (1 × 1 cm²) as a substrate for oviposition and a hiding place, respectively. The predators were fed with

Typha sp. pollen twice a week under controlled conditions (25 ± 2 °C, 70 ± 10% RH and L:D 12:12) (Kalile et al., 2021). This strain from orange jasmine was reared for about one year before being used in the experiment outside.

Population dynamics

The objective of this experiment was to verify the ability of *A. herbicolus* to decrease *D. citri* populations on plants outdoors. We used twelve orange jasmine plants which were pruned about 15 days before the start of the experiment to produce growing tips, which are the main oviposition sites of *D. citri*. Afterwards, we separated the plants into pairs of similar size and the same number of growing tips. All plants were about 0.7-1m tall at the beginning of the experiment, with at least three growing tips. They were transplanted into 10 L pots and placed outdoors. The plant pairs were arranged side by side, about one meter from each other and from the next pair, forming two rows of six plants. The positions of the control and treatment plants was alternated between rows to avoid bias due to variation in environmentalfactors. The experiment was conduct outside the insectary of the Department of Entomology at the campus of the Federal University of Viçosa, Minas Gerais, Brazil (20º 75’85’’S, 42º86’86’’W). Besides orange jasmine plants, various other plants occurred in this area, such as *Ligustrum japonicum* Thunb., *Tecoma stans* L., *Acalypha wilkesiana* Müll.Arg., and *Rosa* sp. The position of control and treatment plants were alternated in the area to reduce any bias caused by variation in environmental factors. Behind the experimental area, there was an area with remnants and regrowth of Atlantic Coastal Rain Forest. We used a physical barrier (Formifita, Divinut, Rio Grande do Sul – Brazil) surrounding each potted plant (5 cm x 1 m) to avoid attacks of the plants by leaf-cutting ants. It consists of a smooth plastic strip on one side with a sponge adhered to the opposite side and serves to prevent ants from walking up and reaching the plant (Estay, 2020). Besides, each plant was placed on top of a circular support (ø = 15cm; 3 cm high) inside a larger rectangular tray (30 cm x 21 cm x 6.5 cm) filled with water and detergent. The water was replaced when necessary and the plants were irrigated twice a week. The rectangular tray was surrounded with an adhesive tape on which entomological glue was applied to the non-sticky side, to further prevent invasions by ants, which indeed did not occur. We released 15 predatory mites on half of the plants in June, 2022, when it was winter (average temperature 18 ± 9 °C, Boletim Meteorológico – UFV, 2022) and the natural densities of *D. citri* were still low due to low temperatures and humidity. The predatory mites were released on the same day that the plants were taken to the experimental area. Citrus and orange jasmine plants belong to the Rutaceae family, which is known to have glabrous leaves without domatia, hence, there may be a lack of hiding structures for predatory mites (Krajewski and Rabe, 1995; Schmidt, 2014). Although the plants flower several times during the year in the tropics, there are periods without flowers, nectar and pollen (Krajewski and Rabe, 1995), so a temporary lack of alternative food for predatory mites, especially on younger plants. To provide oviposition sites for the predators, we added three fibrous cotton twines (Indústria Comércio Resíduos Têxteis Boa Vista Ltda., RJ-BR) (5 cm long) as oviposition sites with *Typha* sp. pollen as food (about 0.5 mg) to the aerial part of each plant, one at the top, one in the middle and one closer to the plant base (Kalile et al., 2023). Pollen (3 mg) was supplied as alternative food for the predators twice a week by sprinkling it with a brush on the top of the plants and on twines, including on control plants. It was applied at the top where the highest number of growing tips are found and where *D. citri* oviposits. It was not possible to assess the numbers of *A. herbicolus* without destroying the plants. Instead, we assessed the presence of eggs, larvae, nymphs and adults on the twines once per week. After 3 weeks, no predators were observed and the twines were replaced with new ones with pollen. Before the release of *D. citri*, we observed predators or their exuviae on the plants and we released 15 more *A. herbicolus* one month and a half after the first release. One week later, we confirmed the presence of predators on all plants on which they were released and then released 15 *D. citri* on all plants as follows. Psyllids were collected from the rearing units using a manual aspirator consisting of a transparent hose (ø = 1 cm) fitted with a pipette tip (1000 µl; Nichiryo, Japan) and a mesh (90 µm) at the interface of the hose and the tip to restrain *D. citri*. We did not select psyllids according to age or mating status; instead, we ensured that all plants received a mix of males and females, so that the experiment corresponded as closely as possible to what happens in the field when *D. citri* populations begin arriving on an orchard. Subsequently, the pipette tip was detached from the hose and closed on both sides with Parafilm "M" laboratory film (Bemis Flexible Packaging, Wisconsin, USA). The pipette tip was tied with a twine near the top of each plant close to a growing tip. At the time of release, all plants had at least three growing tips at the top of the plant. We placed a mesh bag (90 µm; 10 cm x 5 cm) with an elastic band at the end to isolate the growing tip with the pipette tip with *D. citri.* The parafilm was taken from the larger end of the pipette tip, which was facing upwards, to allow the psyllids to move onto the plant. All mesh bags were removed two days later. Four days and two weeks later, we released 15 and 10 more adult *D. citri* on all plants, respectively, following the same procedure. To further guarantee the co-occurrence of adult *A. herbicolus* with *D. citri* offspring, we also released 10 *A. herbicolus* on the treatment plants during the last release of the pest. In total, 40 adults *D. citri* were released on each plant and 40 *A. herbicolus* were released on each plant with predators. We did not prune the plants during the experiment to avoid discarding predator and pest individuals.

After the release of *D. citri*, the numbers of 4th and 5th nymphal instars and adults of this pest on the plants were assessed during 63 days. At the same time, we stopped monitoring the presence of predatory mites on the plants so as not to disturb and perhaps induce dispersal of adult *D. citri*. Instead, we confirmed the presence of the predators on the plants at the last, destructive sampling on September 27th, when we counted all organisms, including all stages of *D. citri*. We also registered the size of the plants, the numbers of leaves and the numbers of growing tips. The predatory mites and other arthropods of similar size were counted using a binocular stereomicroscope (20 x and 25 x magnification; Zeiss Stemi Germany). We classified the organisms to species groups and identified individuals of *T. radiata*, the parasitoid of *D. citri*, mealybugs, predatory mites and a predatory thrips to species level.

Statistical analysis

We used a linear mixed effect model (LME of the package nlme, (Pinheiro et al., 2017) to compare the (log (x+1)-transformed) sum of adults and 4th and 5th nymphal instars of *D. citri* per plant in the presence or absence of predators through time. To correct for repeated measures, individual plant was entered as a random factor. We used likelihood-ratio tests (L.R.) to determine the significance of factors and interactions and we checked the models using plots of residuals and normal error distributions. Significance of contrasts between treatments per time step was assessed using the package emmeans with a Tukey correction for multiple comparisons (Lenth et al., 2020). The data of the destructive sampling at the end of the experiment were analysed with a quasi-Poisson test (GLM, log link). The proportions of the various *D. citri* stages (eggs and 1 – 3rd nymphal instars, 4th – 5th instars and adults) on control and treatment plants were compared using a quasi-binomial test, (GLM, logit link). We compared the numbers of other organisms found on plants with or without predators using a GLM with a Poisson error distribution (log link). All statistical analyses were done with R v.4.1.2 (R Core Team, 2021).

RESULTS

After the first release of *A. herbicolus* on plants, we found different stages of the predators on the twines of all treatment plants. Subsequently, predators were regularly encountered on the twines of plants that received them, but never on the other plants. During the destructive sampling at the end of experiment, predators were found on all plants on which they were released (on average12.5 ± 8.8 individuals per plant) and one predator was encountered on one of the control plants. There was a significant effect of the interaction between the presence/absence of predators and time on *D. citri* densities (LME, L.R. = 28.8; d.f. = 1; $p < 0.0001$), because numbers of *D. citri* on plants without predators started increasing after 20 days, whereas the numbers on plants with predators remained significantly lower (Fig. 1). After day 40, *D. citri* densities started to decrease on all plants. On average, plants with predators had about 4 times fewer adults plus 4th and 5th instar nymphs of *D. citri* than control plants (Fig. 1). At the final destructive sampling, we counted all stages of *D. citri* and there were about 90% fewer *D. citri* on the plants with predators than on control plants (GLM, $F_{1,10} = 10.3$; d.f. = 1; $p = 0.009$; Fig. 1). The final stage structure of the populations of psyllids differed significantly between plants with and without predators (Fig. 2). There were no *D. citri* eggs on any of the plants with predators at the end of the experiment, and only one plant contained two young instar nymphs, in agreement with *A. herbicolus* feeding on these stages (Kalile et al., 2021, 2023). This resulted in a significantly smaller proportion of eggs and young nymphs (1st – 3rd instars) on plants with predatory mites than on plants without predators ($F_{1,10} = 8.25$; $p = 0.017$), but not in differences in the proportion of old nymphs (4th – 5th instars) ($F_{1,10} = 3.05$; $p = 0.11$), which are not consumed by the predator (Kalile et al., 2023). The low densities of prey stages that can be attacked by the predators also shows the importance of the presence of alternative food to maintain predator populations on plants when densities of the target pest stages are low.

The plants also harboured herbivores such as mealybugs, aphids, whiteflies, thrips, phytophagous and fungivores mites, and natural enemies, including three species of predatory mites, spiders, lacewings and the parasitoid *T. radiata*. We found a total of 13 individuals of other predatory mites on plants, of which 12 were on control plants and 1 on a treatment plant, the mean number was 2 ± 3.1. These predatory mites on plants were of three species *Phytoseius* *litoralis* (Silva et al., 2013), *Typhlodromalus transvaalensis* (Nesbit) and *Typhlodromalus* afins *aripo* sp. (DeLeon) (Acari: Phytoseiidae). The thrips species was identified as *Franklinothrips vespiformis* (Crawford) (Thysanoptera: Insecta), which was found feeding on nymphs of *D. citri* at the end of experiment. There were significant effects of treatment on numbers of other organisms found on plants with or without predators (GLM, Deviance = 942.7, d.f. = 1, $p < 0.001$, Supplementary material Fig. S3). We found more mealybugs (*Coccus viridis* (Green) (Hemiptera Coccidae)) (Deviance = 1639.6, d.f. = 1, $p < 0.001$), aphids (Deviance = 116.1, d.f. = 1, $p = 0.005$) and lacewings (Deviance = 4.33, d.f. = 1, $p = 0.037$) on plants with *A. herbicolus*, and more thrips (Deviance = 12.1, d.f. = 1, $p < 0.001$), phytophagous mites (Deviance = 9.58, d.f. = 1, $p = 0.002$) and phytoseiid mites (Deviance = 11.0, d.f. = 1, $p < 0.001$) on plants without *A. herbicolus* (Fig. S3). We found *T. radiata* and whiteflies in very low numbers (two and three individuals respectively), so we did not observe differences in distribution of these organisms.

DISCUSSION

We show that the predatory mite *A. herbicolus* prevented the increase of *D. citri* populations on plants under natural conditions by consumption of eggs and early nymphal instars. The most important question in the control of plant pathogenic vectors is how much the vector needs to be reduced to prevent economic losses. Lee et al. (2015) suggested that a 75% reduction of *D. citri* densities would lead to a reduction in the incidence of the disease. The densities of *D. citri* on plants with predator were always lower than on control plants and we observed 90% less *D. citri* on plants with predators than on plants without them at the end of the experiment, which would then be sufficient to reduce disease incidence (Lee et. all, 2015).

It is known that *A. herbicolus* can kill a high number of *D. citri* eggs daily (about 32 eggs per day) (Kalile et al., 2021). Previous experiments showed that *A. herbicolus* consumed four times more 1st instar nymphs than 2nd instars (Kalile et al., 2023). The predation rate of *A.*

herbicolus may depend on the nutritional quality of the prey, and we previously showed that the quality of eggs of *D. citri* was affected by quality of the plants on which the adult females fed (Kalile et al., 2021). Here we used non-infected psyllids and plants, and the predation rate of infected eggs and nymphs of *D. citri* by *A. herbicolus* is not known. A high proportion of *D. citri* eggs and 1st and 2nd instar nymphs of infected mothers can also be infected (Nian et al., 2023), hence, the consumption of these stages will prevent that these individuals become adults and transmit HLB pathogens. Because we carried out experiments in an HLB-free area, it was not possible to evaluate the effect of the predator on the incidence of HLB, or how nutritional changes caused by the disease affect the nutritional quality of *D. citri* offspring for the predator.

We released and maintained *A. herbicolus* populations on plants for about a month and a half with alternative food and oviposition sites before the release of *D. citri*. We used orange jasmine plants, which are one of the preferred host plant species of *D. citri* (Halbert and Manjunath, 2004). They are often used as ornamentals in urban areas, which favours the dispersal and survival of the pest (Laranjeira et al., 2020). Although orange jasmine is also a host of HLB bacteria, it is more resistant than citrus plants and it can be used in push-pull strategies (Tomaseto et al., 2019). This plant is attractive and can be used as trap plant for *D. citri* at the border of orchards (Tomaseto et al., 2019). The continuous provision of pollen and twines for the predators resulted in their persistence until the final evaluation, where densities of the pest stages that can be attacked by the predatory mites were low (Fig. 2). This reinforces the importance of continuously providing alternative food. Besides offering alternative food, the provision of twines for oviposition helped in verifying the presence of predators on the plants. In previous laboratory experiments, we used small plants (25-30 cm) and most of the eggs were found on the twines, indicating that these were essential for oviposition and establishment of predators (Kalile et al., 2023). Here, we found the majority of eggs and immature stages of the predator on the stems and leaves (Fig. S2), not on the twines. This suggests that the addition of twines is perhaps not essential for maintaining populations of the predator on plants with more structure.

Populations of *A. herbicolus* survived large temperature variations, especially during winter in the first month, when temperatures dropped to 12.3 ºC (±6), (Boletim Meteorológico – UFV, 2022) at night. This indicates that this predator was adapted to the conditions outside, whereas it was reared under controlled conditions in the laboratory. We observed the highest densities of *A. herbicolus* on two plants that received more shade and *D. citri* was controlled better on these two plants throughout the experiment. The latter was not caused by differences in the numbers of growing tips on these plants (MOK, personal observation). The higher densities of predators was possibly caused by these plants receiving lower amounts of UV radiation, which is known to be avoided by predatory mites (Onzo et al., 2003, 2010; Vangansbeke et al., 2015). It is known that higher densities of *D. citri* are observed on trees at the perimeter of orchards (Sétamou and Bartels, 2015). This distribution is often attributed to innate behaviour of this pest (Sétamou and Bartels, 2015), however, it may also be a consequence of higher densities of natural enemies such as *A. herbicolus* on shaded plants at the centre of orchards. Therefore, more studies are needed to investigate if plants that are shielded from direct sunlight or plants with more foliar structure harbour more predators and whether this affects pest suppression in citrus orchards.

Whereas we showed before that *A. herbicolus* reduced *D, citri* populations in the lab (Kalile et al., 2023), here we show that it also can control *D. citri* under natural conditions and in the presence of other invading herbivores and natural enemies. By maintaining populations of this predatory mite by supplying pollen, we show the potential use of this predatory mite in preventive biological control of *D.* citri. We suggest that experiments should be carried out in citrus orchards where HLB occurs to assess the potential of this predatory mite to limit the incidence of the disease through reduction of *D. citri* densities.

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Figure legends

Fig. 1 Mean (± SE) number of adults, fifth and fourth-instar nymphs of *Diaphorina citri* on orange jasmine plants outside the lab. The black line represents plants without the predatory mite *Amblyseius herbicolus* (control) and the grey line the plants with predators, during 60 days. The black arrows indicate when adults of *D. citri* were released. The final evaluation was a destructive sampling on day 63 and all stages of *D. citri* were counted. The black square indicates final densities on control plants and white triangle on treatment plants. The asterisks (*) indicate significant differences between plants of control and treatment per evaluation (contrasts after LME: *$p<0.05$; **$p<0.001$).

Fig. 2 Proportion of eggs, young nymphs (black) and old nymphs (white) of *Diaphorina citri* on plants without *A. herbicolus* (control) and with this predator (treatment). The total numbers are indicated above the bars. Different letters indicate significant differences between treatment and control plants (GLM, $p<0.05$).

Fig. 1

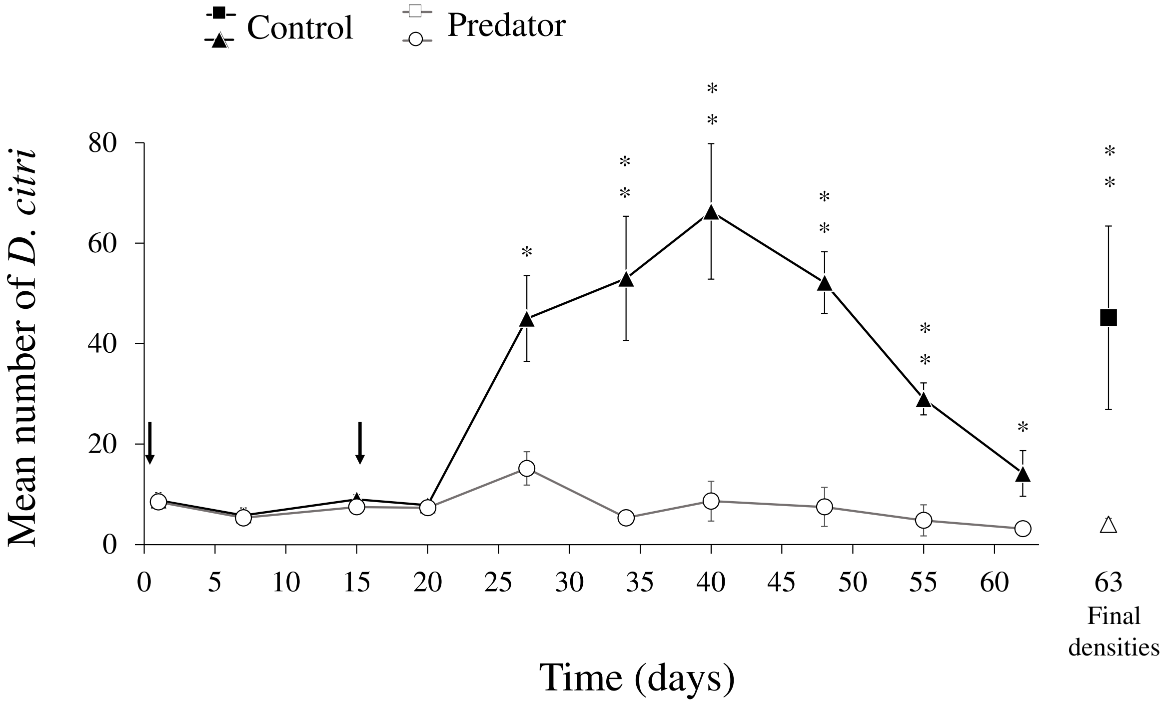
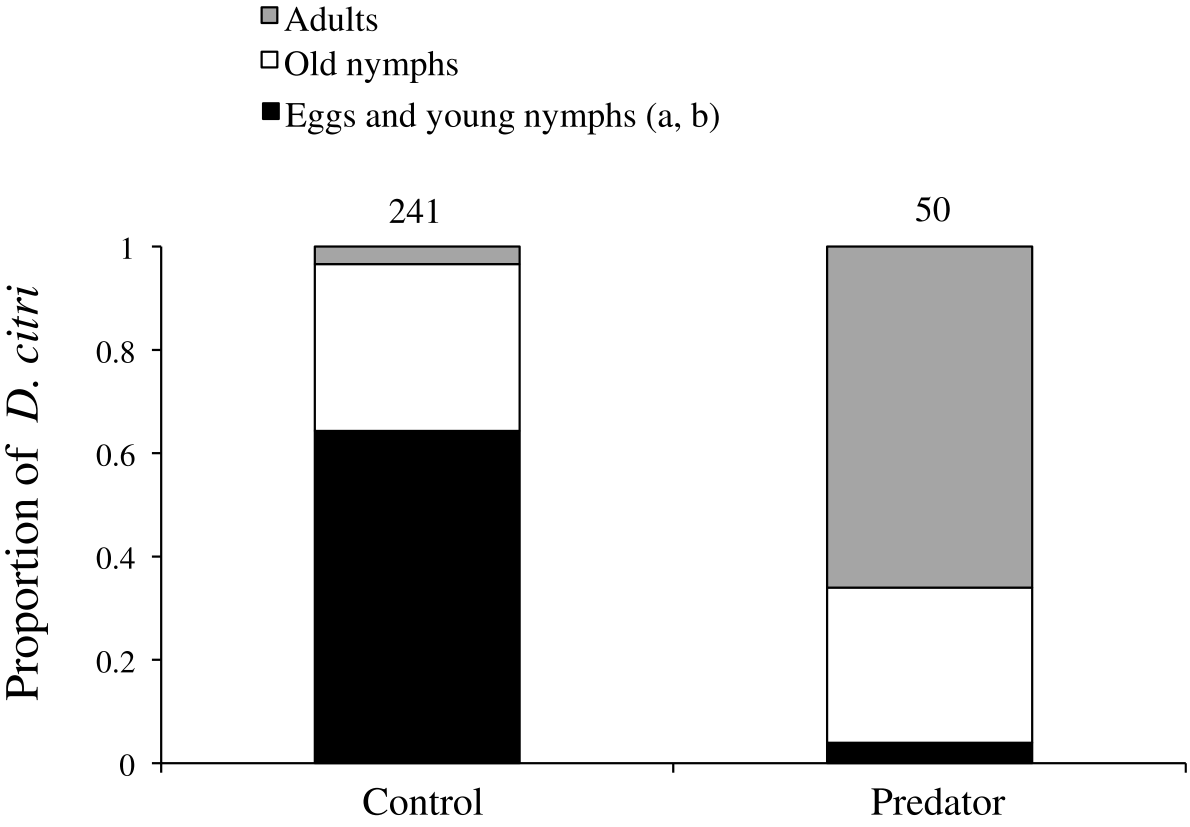

Control
Predator
Mean number of D. citri
0
20
40
60
80
0
5
10
15
20
25
30
35
40
45
50
55
60
63
Final densities
Time (days)
*
* *
* *
* *
* *
*
* *

Fig. 2


▨ Adults
☐ Old nymphs
■ Eggs and young nymphs (a, b)
241
50
1
0.8
0.6
0.4
0.2
0
Proportion of *D. citri*
Control
Predator

SUPPLEMENTARY MATERIAL

Throughout the experiment, other natural enemies and herbivores could colonize the plants, but they were only quantified and identified during the destructive sampling at the end of the experiment. The numbers of these organisms differed between plants with or without *A. herbicolus*. The most abundant herbivores we found on plants with *A. herbicolus* were aphids and the mealybug green scale, *C. viridis*, which is a polyphagous parthenogenic pest (George et al., 2022). Both established better on the plants with predators and with reduced *D. citri* populations. Their increased densities may be the result of weaker competition with *D. citri*, which were reduced by *A. herbicolus*. It is known that aphids and psyllids avoid each other, probably because they compete for the growing tips (Tena et al., 2013). Furthermore, *D. citri* may induce plant defences that have negative effects on mealybugs and aphids (Karban and Baldwin 1997). *Coccus viridis* is common mealybug on citrus, coffee and some ornamental plants (Fredrick, 1943). The main natural enemies of *C. viridis* are coccinelids, parasitoids and entomopathogenic fungi (Rosado et al., 2014; Souza et al., 2023), whereas aphids are mainly controlled by coccinelids and parasitoids (Boivin et al. 2012). The predatory mite *A. herbicolus* is able to control whiteflies, psyllids, broad mites and thrips (Cavalcante et al., 2015; Duarte et al., 2015; Kalile et al., 2023 (chapter 1); Lam et al., 2021; Rodríguez-Cruz et al., 2013), but it is still unknown if it, or any other phytoseiid, is able to feed on aphids or mealybugs. We also found more lacewings on plants with predatory mites, and these are used to control aphids in greenhouses (Hassanpour et al., 2021) and can feed on psyllids (Gómez et al., 2022; Hassanpour et al., 2021; Messelink et al., 2016). Considering the low numbers of psyllids on treatment plants, they were probably attracted by plants to feed on aphids. We observed no natural enemies of mealybugs on the plants, which may explain the high densities of this pest.

Ants are known to impair parasitoids, syrphids and coccinellids, all natural enemies of *D. citri* (Kistner et al., 2017; Shrestha et al., 2022). It is not yet known whether ants disrupt the control exerted by *A. herbicolus*, and we therefore excluded ants from our plants. Future experiments should assess whether ants interfere with control of *D. citri* by *A. herbicolus*.

We found more phytophagous and astigmatid mites on the plants without predators than on plants with them, possibly because the generalist *A. herbicolus* could feed on them, but this needs further research. Besides phytophagous and astigmatid mites, we found more predatory mites on the plants without *A. herbicolus*. We found 12 predatory mites on control plants: 3 *Phytoseius litoralis,* 6 *Typhlodromalus transvaalensis* and 3 *Typhlodromalus aripo*, and one *T. transvaalensis* on one plant with *A. herbicolus*. As far as we know, this is the first time that *P. litoralis* was reported on orange jasmine plants and in Minas Gerais, Brazil. *Typhlodromalus aripo* is a neotropical predatory mite and potential biocontrol agent of phytophagous mites from the Tetranychidae and Tarsonemidae families (Cañarte et al., 2017). The third predatory mite we found was *T. transvaalensis*, which is a generalist predatory mite, capable of feeding on pollen, insects and phytophagous mites (McMurtry et al., 2013; Cañarte et al., 2017). We also observed the larvae of the generalist predatory thrips *F. vespiformis* feeding on *D. citri* nymphs. The larvae of this predator were found twice as often on the control plants. This is the first record of this predator feeding on psyllids, but it was already reported feeding on spider mites, other thrips, whiteflies and pollen (Hussain et al., 2022). The thrips may have been attracted to the control plants because of the availability of food: besides higher densities of *D. citri* (Fig. 1), there was probably more pollen available on the control plants because of the absence of *A. herbicolus* consuming it. In addition, these predators may have preferred plants without *A. herbicolus* to avoid competition or intraguild predation (Janssen et al., 1997; Marques et al., 2018; Rosenheim et al., 1995).

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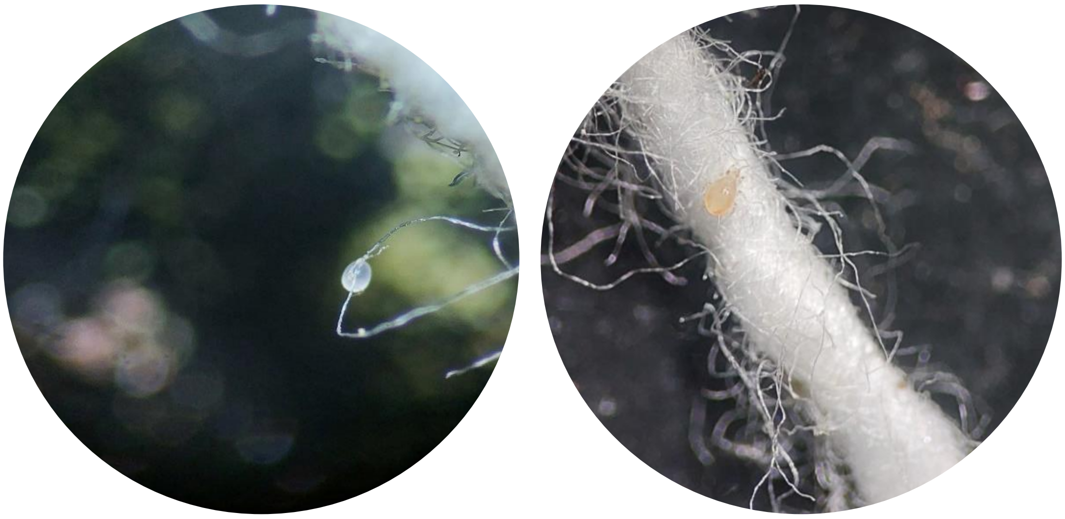

Fig. S1 Egg and adult of *A. herbicolus* found on twines that were placed on orange jasmine plants of the treatment to favour the establishment of this predatory mite.

Fig. S2 Egg of *A. herbicolus* found in an incision in the stem of an orange jasmine plant at the destructive evaluation.

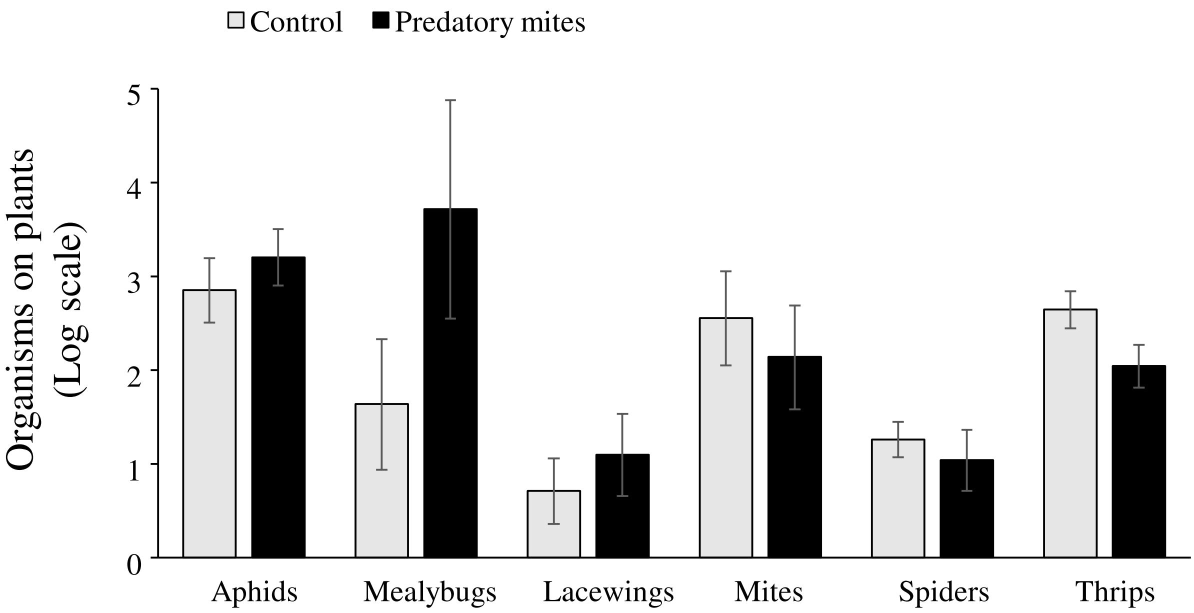

Fig. S3 Mean numbers (± SE) of organisms (log scale) found on plants with or without *A. herbicolus*. Mites consisted of herbivorous and astigmatid mites. We observed significant differences among the organisms found on plants with (black colums) or without (gray columns) *A. herbicolus*.

CHAPTER 3:

Diaphorina citri does not avoid plants with predatory mites

(Edited according to Journal of Pest Science)

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KEY MESSAGE

- Predation risk can be perceived by pests and impact their host plant choice and distribution;
- We investigated the impact of predation risk on behaviour of the pest and phytopathogenic vector *Diaphorina citri*;
- *D. citri* did not avoid host plants with predatory mites;
- The presence of predators on plants did not increase dispersal of the pest from the plants;
- The implications for biocontrol of *D. citri* and spread of greening disease are discussed.

ABSTRACT

Natural enemies are usually the major mortality factor of herbivorous pests and are considered as allies in plant defense. Besides directly killing pest individuals, the presence of natural enemies can also affect the behaviour of the pest, which may indirectly also affect pest densities. For example, the perception of predator cues may change host plant choice and movement of the pest, affecting fitness, population dynamics and the distribution of the pest. In the case of herbivorous vectors of phytopathogens, these changes induced by the perception of predators and associated cues may increase or reduce the transmission of diseases to plants. Here we show that *Diaphorina citri*, the most important vector of the citrus disease Huanglongbing (HLB) or Greening, did not avoid plants harbouring the predatory mite *Amblyseius herbicolus*. The pest was more attracted to plants with conspecifics and to plants with conspecifics and predatory mites than to clean plants. Plants with predators feeding on *D. citri* eggs were also not avoided. Experience of adult *D. citri* on plants with predators attacking eggs and juveniles of the pest did also not trigger avoidance of the pest. In addition, the presence of predators on plants did not trigger increased dispersal of the pest from these plants. These results suggest that *A. herbicolus* can reduce *D. citri* densities by feeding on its eggs and nymphs without triggering the movement of adults to other plants, and thus possibly reducing the transmission of the disease. Clearly, the control of *D. citri* by *A. herbicolus* and its effect on the spread of HLB deserve further study.

Keywords: Asian Citrus Psyllid; Biological Control; Greening; HLB; Predatory mite; Predation risk

INTRODUCTION

The host range of herbivores is not only determined by the nutritional quality and the defence of potential host plants, but also by the presence of competitors and natural enemies such as predators and parasitoids (Sih et al. 1985; Ohsaki and Sato 1994; Pallini et al. 1999; Scheirs et al. 2000; Ingerslew and Finke 2016; Jandricic et al. 2016). Natural enemies are often a major mortality factor of herbivores and they can regulate herbivore populations (Cappuccino and Price 1995; Rosenheim 1998), and the interactions between plants and natural enemies are therefore often considered as an indirect plant defence (Price et al. 1980).

Natural enemies are known to directly affect prey densities by killing them, but they can also have indirect effects that also result in reduction of prey densities (Jeffries and Lawton 1984; Schmitz 1998; Preisser et al. 2005). For example, prey can avoid patches that contain (cues of) predators (Mappes and Kaitala, 1995; Ohsaki and Sato, 1994; Nomikou et al., 2003) to avoid being eaten, but this also imposes costs (Jeffries and Lawton 1984; Crawley 1992; Pallini et al. 1998; Oku et al. 2004). The detection of predation risk can trigger changes in feeding and reproductive behaviours (Bernays 1997; Gotthard 2000; Smyrnioudis et al. 2001; Lavoie and Oberhauser 2004; Kaplan 2012), in addition to increasing metabolic rates (Hawlena and Schmitz 2010), oxidative stress (Janssens and Stoks 2013) and movement (Roitberg and Myers 1978). Besides, prey often have multiple natural enemies, each with different foraging behaviours, and they need to navigate and forage to obtain sufficient high-quality food while avoiding being eaten (Losey and Denno 1998; Kaplan et al. 2014; Doherty and Ruehle 2020). This can affect individual fitness and mortality, but also population and community dynamics (Schmitz et al. 1998; Preisser et al. 2005; Fill et al. 2012; Doherty and Ruehle 2020; Culshaw-Maurer et al. 2020). Thus, in addition to consumptive effects of natural enemies on their prey, it is important to investigate the non-consumptive effects, such as the avoidance behaviour mediated by the perception of predation risk. This holds in particular for prey that are crop pests and therefore need to be controlled (Preisser et al. 2005; Doherty and Ruehle 2020).

One way of controlling crop pests is through biological control (Stenberg et al. 2021). Research on biological control typically focuses on the lethal effects of the natural enemies (Culshaw-Maurer et al. 2020), but even when these kill few individuals, they can have a significant negative effect on prey densities because of the risk they represent (Jeffries and Lawton 1984; Lima and Dill 1990; Schmitz 1998; Preisser et al. 2005). Biological control programs also use predators to control pests that are vectors of plant pathogens and in these cases, predation risk may affect the transmission of diseases through changes in the behaviour of the vector (Lee et al. 2022). For example, predation risk may affect host plant selection and dispersal and, therefore accelerate or reduce pathogen transmission to host plants (Smyrnioudis et al. 2001; Hodge et al. 2011; Belliure et al. 2011; Lee et al. 2022). For instance, vectors may avoid plants with predators (Lee et al. 2011), hence, these plants will be protected against pathogens. The arrival of predators on a host plant can induce dispersal of the vector (Roitberg and Myers 1978), which will reduce the vector's feeding time, thus reducing disease spread when prolonged feeding is necessary to acquire and transmit pathogens (Long and Finke 2015). In contrast, the increased movement of vectors can increase the transmission of rapidly acquired plant pathogens (Roitberg and Myers 1978). Besides, predators can also change the vector's feeding behaviour and disease transmission without increasing movement of vectors but by reducing ingestion or by inducing ingestion of plant tissue that is unsuitable for pathogens (Tholt et al. 2018). Because of these implications in acquisition and transmission of pathogens, the effects of predation risk on host plant selection and dispersal of vectors of plant pathogens need to be investigated (Dáder et al. 2012).

It is known that pests can recognize and avoid plants with their enemies (Nomikou et al. 2003). For example, the phytophagous mite *Tetranychus urticae* Koch (Tetranychidae) can detect and avoid cues associated with predators (Grostal and Dicke 1999; Pallini et al. 1999). In addition, a strain of *T. urticae* collected from greenhouses was able to avoid a predatory mite used for its control for years, but not a predatory mite that was recently introduced (Pallini et al. 1999). The risk may be perceived through predator cues, prey alarm signals (Kats and Dill 1998; Culshaw-Maurer et al. 2020), habitat characteristics (Verdolin 2006) and may depend on cognitive abilities of the pest (Kats and Dill 1998; Fill et al. 2012). Besides, prey without a coevolutionary history or experience with an enemy may exhibit weak avoidance responses (Culshaw-Maurer et al. 2020), as in the case of adults of the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), which did not avoid plants with predators when it did not have experience with predators (Nomikou et al. 2003).

An important vector of a plant disease is the Asian citrus psyllid *Diaphorina citri*. It transmits Huanglongbing (HLB) or Greening, an untreatable and fatal disease of citrus trees and it is of high economic importance (Alquézar et al. 2022). There is little information on the effects of natural enemies and of predation risk on host plant selection by this vector. It disperses over long distances and reproduces quickly, which favours the spread of HLB (Lewis-Rosenblum et al. 2015). The excessive application of pesticides to control this vector raises concerns about sustainability and is problematic because several *D. citri* populations have developed resistance (Tiwari et al. 2011; Chen et al. 2018; Shrestha et al. 2021). Moreover, the pesticides may also induce psyllids dispersal (Johnston et al. 2019) and consequently, the spread of the disease (Bassanezi et al. 2020; Alquézar et al. 2022). Nevertheless, the use of biological control agents is still incipient compared to the chemical approach, whereas there are indications that natural enemies can reduce densities of *D. citri* in orchards that were not sprayed with pesticides to levels below those found in orchards sprayed with pesticides (Shrestha et al. 2021). This is in line with a recent meta-analysis of the effects of pesticides in general, which showed that the application of pesticides often does not reduce pest densities when natural enemies are present (Janssen and van Rijn 2021). Hence, there is scope for biological control of *D. citri*.

Diaphorina citri is attacked by several predators, such as ladybeetles, lacewings, syrphids and predatory mites (Michaud and Olsen 2004; Kalile et al. 2021; Shrestha et al. 2021), as well as by parasitoids (especially *Tamarixia radiata*) and by fungal pathogens (Chen and Stansly 2014; Patt and Rohrig 2017; Arnosti et al. 2019). To date, only the entomopathogenic fungi *Isaria (Cordyceps) fumosorosea* Wize (Hypocreales: Cordycipitaceae) and *Beauveria (Cordyceps) bassiana* (Bals. -Criv.) are commercially available to control this pest (Arnosti et al. 2019; Liu, et al. 2023). Nowadays, c. 60% of commercialized arthropod natural enemies are predatory mites (Knapp et al. 2018) for several reasons. Many of them are generalists and able to control multiple pests (Janssen and Sabelis, 2015; Knapp et al. 2018), they also can be released preventively in crops with alternative food such as pollen (van Rijn et al., 2002; Janssen and Sabelis, 2015). Furthermore, they multiply quickly and can be cheaply produced on a large scale with astigmatid mites as food (Knapp et al., 2018).

Amblyseius herbicolus is a generalist predatory mite and it is known to potentially control populations of whiteflies, broad mites and thrips (Duarte et al., 2015; Rodríguez-Cruz et al., 2013; Cavalcante et al., 2015; Lam et al., 2021). This predator occurs on several host plants (Demite et al. 2014), including host plants of *D. citri* in citrus orchards (Kalile et al. 2021). It is a promising biological control agent of *D. citri* (Kalile et al. 2021, Chapter 1). Previously, we showed that *A. herbicolus* can feed, develop and reproduce on eggs, first and second nymphal instars of *D. citri*, and that it reduces *D. citri* densities on plants (Kalile et al. 2021, 2023). However, it is not yet known whether *D. citri* changes its behaviour because of the risk of predation associated with the presence of *A. herbicolus*. The effects of this predatory mite on *D. citri* densities, behaviour and distribution are unknown. Thus, we investigated if *D. citri* changes its host plant preference and dispersal behaviour in the presence of this predator. In line with earlier research (Nomikou et al. 2003), we also investigated if previous experience of adult *D. citri* with the predator would impact host plant selection and dispersal behaviour of this pest.

MATERIALS AND METHODS

Plants

Orange jasmine plants were used to rear *D. citri,* as described in Kalile et al. (2021). In short, plants (4–6 months old and free of pesticides) were obtained from a commercial grower near Viçosa, Minas Gerais, Brazil, and were kept in a greenhouse (25 ± 2 °C). The plants were fertilized bimonthly with a mixture of NPK (4/14/8) and pruned regularly to induce the development of growing tips, which are used by *D. citri* for oviposition (Cifuentes-Arenas et al. 2018).

Pollen

The pollen used for rearing the predatory mites and for experiments was collected from *Typha* sp. plants from rural areas around Viçosa, Minas Gerais (21°76′03″S, 42°87′00″W). It was dried in an oven at 40 °C for 12 h and stored in a container in the freezer (− 6 °C). Small amounts of pollen were placed in 1.5 ml microtubes (Eppendorf), dried at 60 °C for 48 h and then stored in the refrigerator (8 °C) for daily use (Kalile et al. 2021).

Diaphorina citri

Diaphorina citri was collected in 2018 from orange jasmine plants of about six years old on the campus of the Federal University of Viçosa and in rural areas of Viçosa (20º 80’47’’S; 42º88′59″ W). The region of Viçosa is considered HLB-free and this was further confirmed through molecular analyses of the insects and the plants (Kalile et al. 2021). Insects were reared in BugDorm-4F insect cages (0.5 × 0.5 × 1.0 m), MegaView Science Co., Ltda, Taichung City – Taiwan, with pesticide-free orange jasmine plants that were watered twice a week. New plants with growing tips were regularly placed inside the cages and adult *D. citri* were transferred to new cages when necessary. The cages were kept under laboratory conditions (26 ± 2 °C, 70 ± 10% RH, natural light conditions).

Predators

We used two strains of *A. herbicolus*. The first strain was collected on tomato plants in Prados, Minas Gerais (21°03′00″ S; 44°04′47″ W) in 2014 (Kalile et al. 2021). The second strain was collected in 2021 on orange jasmine plants, *Murraya paniculata*, (L.) Jack, Rutaceae, infested with *D. citri* in the rural area of Viçosa. Both predator strains were reared on arenas consisting of a plastic sheet (10 × 15 cm²) surrounded by a moistened cotton wool barrier, which also served as a water source, placed on a wet sponge in a plastic tray containing water (McMurtry and Scriven 1965). This plastic tray was placed inside a larger tray with water and detergent to further ensure isolation. Cotton threads were offered as oviposition substrate, covered with a piece of black plastic sheet (1 × 1 cm²), folded into a roof shape as shelter. The predators were fed with *Typha* sp. pollen twice a week (Kalile et al. 2021). The two strains were reared in different rooms under controlled conditions (25 ± 2 °C, 70 ± 10% RH and L:D 12:12) to prevent cross-contamination. The strain from orange jasmine was reared for three months before being used in the experiments.

Infestation of plants with *Diaphorina citri* and predatory mites

For experiments that required plants infested with *D. citri*, we selected plants with growing tips (development stage 2) and covered the aerial part of plus part of the pot of each plant with a separate mesh bag (90 µm; 64 cm high, ø = 28cm). The mesh bag had an adjustable elastic band at both ends and we fixed bamboo sticks (60 cm) into the soil so that the mesh did not touch the leaves. Mixtures of male and female *D. citri* were collected from the culture plants into pipette tips (1000 µl; Nichiryo, Japan) using a manual aspirator (Kalile et al. 2023). The openings of the tips were subsequently closed with Parafilm "M" (Bemis Flexible Packaging, Neenah, WI 54956, USA). We then lifted the bottom part of the mesh bag and inserted the pointed part of the pipette tip into the soil (Kalile et al. 2023). The parafilm was removed from the wide end, allowing *D. citri* to colonize the plant. After establishment (2 days), the sleeve was removed and the plants and/or psyllids were used for experiments. Plants with predatory mites were obtained by collecting adult predatory mites from the rearing units with a fine brush and releasing them on a plant. One twine (5 cm long), serving as oviposition site (Chapter 1, Adar et al. 2014, Pekas and Wäckers, 2017) was added to all plants. Pollen (about 0.5 mg) was provided twice a week as alternative food for the predators. To restrain predators to the aerial part of the plant, we applied entomological glue (Colly, Mombuca -SP, Brazil) at the base of all plants stem, close to the soil. At the end of experiment, predators were present on all plants on which we released them.

Choice experiments with *Diaphorina citri*

To offer *D. citri* a choice between plants with different treatments, we used cages consisting of a plastic tray (51.5 x 30 x 9.5 cm) with vertical bamboo sticks glued to each of the four corners, extending 60 cm above the tray. This bamboo frame was covered with a mesh (90 µm) that was attached to the base of the tray with an elastic band. Inside each cage, two orange jasmine plants, each with a different treatment, were placed on opposite sides. The plants were about 25 cm high and had one or two growing tips (developmental stage 2, Cifuentes-Arenas et al. 2018). The different treatments are shown in Table 1. Plants were standardized within each cage with respect to size and number and stage of growing tips (developmental phases 2 and 3, Cifuentes-Arenas et al., 2018). The position of the clean and treated plants relative to the environment was alternated among replicates to correct for asymmetries in the behaviour of *D. citri* with respect to light intensity and other environmental factors. *Diaphorina citri* were collected in pipette tips as described above, and the tips were positioned vertically, extending from a tube in the middle of the cage between the two plants. Subsequently, the parafilm from the top of each pipette tip was removed, allowing *D. citri* to leave and choose between the plants. After 48 h, which is long enough to allow *D. citri* making a choice (M. Kalile, pers. obs.), alive and dead *D. citri* were counted, their position (on the plants, on the cage walls or the floor) registered, and their gender verified under a binocular stereomicroscope (20 x magnification; Zeiss Stemi 508, Germany). The experiments were conducted in the laboratory and in a greenhouse to confirm that the effects observed occur under different environmental conditions, especially differences in light intensity because of the strong phototactic behaviour of *D. citri* (Sétamou et al. 2011; Patt et al. 2015; Supp. Material). In the greenhouse, we used BugDorm-4F insect cages (0.5 × 0.5 × 1.0 m), such as used for rearing *D. citri*, and two plants were again placed on opposite sides inside each cage, again alternating the positions of the clean and treated plants. The experiments in the greenhouse were conducted as described above (26 ± 12.5 ℃ and 70 ± 10% RH, natural light conditions – Supplementary Material). The proportions of *D. citri* that made a choice in the greenhouse and the lab were similar, hence, the data of these two environments were analysed together, with lab or greenhouse as factor. We also analysed the proportions of dead individuals and psyllids that did not choose a plant in each experiment

Preference for plants with conspecifics

To evaluate if the plant choice of *D. citri* was influenced by the presence of conspecifics, we first infested orange jasmine plants with marked *D. citri* as follows. We collected 12 groups of 12 *D. citri* from the culture plants, using an aspirator as above. Subsequently, we marked them using about 0.2 mg of biodegradable silver glitter (Reflex Ind. e Com. de Materiais Escolares LTDA, Araçatuba- SP, Brasil). The glitter was added to the pipette tip with the collected *D. citri* with a brush, taking care that no animals escaped. The tip was then tapped and rotated lightly so that the glitter adhered to the insects. This mark remained visible until the end of the experiment (M. Kalile, pers. obs.). Immediately after marking them, the *D. citri* were released on plants inside a mesh bag (as above), and the psyllids were allowed to establish on the plants for two days. After that, plants harboured on average 5.1 ± 1.5 females plus 3.3 ± 1.7 males, and all plants had a total of 8-10 marked *D. citri*. The plants were then each placed in a cage together with a control plant of similar age, height and developmental stage, but without *D. citri*. Subsequently, 10 unmarked *D. citri* were collected, released and recaptured as described above (**Choice experiments with *Diaphorina citri***). This experiment was repeated 12 times simultaneously in the laboratory (12 cages with two plants) and six times in the greenhouse (six cages with two plants), so a total of 18 replicates. Marked *D. citri* did not move between plants during the experiment. Because unequal numbers of males and females were released, we used the proportion of the total numbers of males and of females released that chose for either of the two plants, hence, separate proportions for males and females. These proportions were analysed with a Generalized Linear Mixed-Effect Model (GLMER) using a binomial error distribution (Crawley 2012) with the presence/absence of conspecifics, the place of the experiment (lab or greenhouse), the sex of the recaptured *D. citri* and their interactions as factors. Models were checked by plotting residuals against fitted values and checking normality of the error distribution. As explained above, the position of the plants relative to the environment was alternated among cages, and this position was entered as random factor. Significance of factors and interactions were determined with deletion tests. Contrasts among treatments were assessed with the package emmeans with a Tukey correction for multiple comparisons (Lenth et al. 2020). To test whether the sex ratio of the marked *D. citri* on the plants affected the numbers of male or female unmarked *D. citri* that were found on the plants we used a similar analysis, but with the presence/absence of conspecifics, the sex of the recaptured *D. citri* and the sex ratio of the marked *D. citri* plus their interactions as factors. All statistical analyses were done using R version 4.1.2 (R Core Team 2021).

Preference for clean plants or plants with conspecifics plus predatory mites

We offered *D. citri* the choice between clean plants or plants with conspecifics and predators. Methods were as above, but with 12 plants with marked *D. citri* plus predatory mites and 12 clean plants. Plants with marked *D. citri* were prepared as above, and 20 predatory mites were released on each of these plants 6 hours before the experiment. Twelve unmarked *D. citri* were released in each cage and allowed to choose a host plant. Experimental procedures and analyses were as described above, but experiments were only done in the laboratory.

Preference for plants with conspecifics or plants with conspecifics plus predatory mites

To further confirm if *D. citri* did not avoid predators and that predators did not reduce the attractiveness of plants to this pest, the psyllids were offered a choice between plants with conspecifics and plants with predatory mites plus conspecifics. Twenty-four plants with marked *D. citri* were prepared as above and half of them received 20 predatory mites 6 hours before the experiment. Unmarked *D. citri* were released as above. Experimental procedures and analyses were as described above.

Preference for plants with or without predatory mites

We offered to *D. citri* individuals a choice between orange jasmine plants with and without *A.*

herbicolus, both without conspecific psyllids. Plants with predators received 20 adult female *A. herbicolus* as described above and were placed in the experimental cages together with a plant without predators. All plants received a piece of twine and pollen, so that the only difference between the two plants was the presence of predators. *D. citri* were released as described above. This experiment was repeated 16 times in two blocks of 8 replicates, done at different times in the laboratory, and was repeated eight times in the greenhouse in cages as explained above, amounting to a total of 24 replicates. Twenty *D. citri* were released to choose a host plant as above. Data were collected and analysed as above.

Preference for plants with or without predatory mites attacking *D. citri* eggs

Here, we evaluated if *D. citri* was able to recognize and avoid plants with predators feeding on *D. citri* eggs. Fifteen adult *D. citri* were collected from the rearing units and released on plants in sleeves (as above), where they were allowed to oviposit for 4 days, after which the cage and adult *D. citri* were removed. The presence of eggs was confirmed, and 20 predatory mites were released on the plants as described above. Subsequently, the plants were placed in a cage together with a clean plant. Pollen and twine were added to each plant, including controls, as above. *Diaphorina citri* were released as described above. This experiment was repeated 16 times in two blocks of 8 replicates in time in the laboratory, and six times in one block in the greenhouse, amounting to a total of 22 replicates. Twenty *D. citri* were released per cage as above. Data were collected and analysed as above. We also confirmed the presence of predatory mites and consumed *D. citri* eggs on the plants using a stereomicroscope.

Preference experienced *D. citri*

In this and the following experiment, we used a strain of *A. herbicolus* collected from orange jasmine because it established better on these plants than the tomato strain used in the previous experiments (M. Kalile, pers. obs.). We collected and released about fifteen *D. citri* individuals on each of 24 plants and allowed them to oviposit for 4 days, after which *D. citri* eggs were observed on all plants. After these 4 days, 20 predatory mites from the orange jasmine strain were released on 12 plants. The *D. citri* adults were left on the plants with predators for seven days to gain experience with predators feeding on their eggs and first nymphal instars. Subsequently, they were collected using a manual aspirator and were separated in six groups of 20 in pipette tips as above. The groups of experienced (from plants with predators) or inexperienced (from plants without predators) *D. citri* were released as above, where they could choose between a clean plant and the plant from which they were collected, thus, on which predators were feeding on pest eggs since the experience period. In the case of inexperienced *D. citri*, the predators were released on the treatment plants with *D. citri* eggs 6h before the experiment. This experiment was performed in the lab in two blocks through time, with a total of 12 replicates for both experienced and inexperienced *D. citri*, amounting to a total release of 240 experienced psyllids and 240 inexperienced psyllids. Data were collected and analysed as above.

Dispersal of *Diaphorina citri*

To evaluate whether the presence of *A. herbicolus* triggered the dispersal of *D. citri* from plants, we compared the dispersal of established populations of *D. citri* from plants with or without predatory mites. We released 20 *D. citri*, collected from the culture on 12 plants inside sleeves as above and we allowed them to establish and oviposit for 4 days. We then removed the sleeves, counted the *D. citri* on each plant and placed the plants with the eggs and adult *D. citri* in separate cages. A clean plant to which *D. citri* could disperse was added on the opposite side of each cage. The position of the plants was alternated as described above. In half of the cages, we released 20 *A. herbicolus* from the rearing unit on the plants on which *D. citri* had established. We provided pollen, one twine and entomological glue as above. Because *D. citri* is known to disperse more frequently during the first part of the afternoon (Paris et al. 2015), we evaluated the position of each *D. citri* once a day after 4:00 PM during four days. We could not determine the sex of the individuals without disturbing them, and we therefore collected all individuals and recorded their sex on the last day only. We also confirmed the presence of predators on the plants on which they were released on this last day. The numbers of dispersed *D. citri* were compared with GLMER with time and presence/absence of predators on the release plant as factors and side and replicate as random effects. We also analysed the proportions of females and males that had dispersed using the data of the last day with a GLMER with the presence/absence of predators and sex as factors and side as random effect. Models were checked as above.

RESULTS

Preference for plants with conspecifics

Of the 180 marked *D. citri* released on the plants, we recaptured 85%, and 60% of those were female. We recovered 90% of the 180 unmarked *D. citri* that had been released in the middle of the cages, most on plants with conspecifics (Fig. 1, GLMER, $\chi^2 = 14.8$, d.f. = 1, p = 0.0001). Together, these results showed that the experimental set-up served to study host plant selection of this pest. Both males and females were more attracted to plants with conspecifics than to clean plants and we did not observe significant differences between males and females (Fig. 1, GLMER: $\chi^2 = 2.9$, d.f. = 1, p = 0.09). We conducted this experiment in the laboratory and greenhouse, but there was no significant difference between the two places (GLMER: $\chi^2 = 0.13$, d.f. = 1, p = 0.72). The sex ratio of the marked *D. citri* on the plants varied from 55.6% to 100% females and had no significant effect on the choice of *D. citri* (GLMER, $\chi^2 = 0.59$, d.f. = 1, p = 0.44).

Preference for clean plants or plants with conspecifics plus predatory mites

More adult *D. citri* were recovered from plants with conspecifics and predators than from clean plants (Fig. 2), but this difference was not significant (Fig. 2; GLMER: $\chi^2 = 3.29$, d.f. = 1, p = 0.069). We also did not observe differences between males and females in the choice of plants (Fig. 2; GLMER, $\chi^2 = 0.94$, d.f. = 1, p = 0.33).

Preference for plants with conspecifics or plants with conspecifics plus predatory mites

More *D. citri* were recovered from plants with conspecifics plus predatory mites than from plants with only conspecifics (Fig. 3, GLMER: $\chi^2 = 4.24$, d.f. = 1, p = 0.040). Males and females did not differ significantly in the choice of plants (Fig. 3, GLMER: $\chi^2 = 2.64$, d.f. = 1, p = 0.10).

Preference for plants with or without predatory mites

Diaphorina citri did not show a significant preference for clean plants or plants with predatory mites (Fig. 4, GLMER, $\chi^2 = 2.07$, d.f. = 1, p = 0.15). In addition, females and males did not differ in their preference for plants with or without predators (Fig. 4, GLMER: $\chi^2 = 0.0013$, d.f. = 1, p = 0.97). Slightly, but significantly more individuals made a choice in greenhouse (67%) than in the lab (50%) (GLMER: $\chi^2 = 4.16$, d.f. = 1, p = 0.041).

Preference for plants with or without predatory mites attacking *D. citri* eggs

We found preyed eggs and predators on all plants on which predators were released, and a pilot experiment showed a decrease of the numbers of eggs on plants with predators with a factor 5.9. *Diaphorina citri* did not show a significant preference for plants with predators feeding on *D. citri* eggs or clean plants (Fig. 5, GLMER: $\chi^2 = 0.21$, d.f. = 1, p = 0.65), and there was no difference in the choice of females or males (Fig. 5, GLMER: $\chi^2 = 0.48$, d.f. = 1, p = 0.49). We did not observe difference between lab and greenhouse in the choice of *D. citri* (GLMER: χ^2 =

2.23, d.f. = 1, p = 0.136)

Preference of experienced *D. citri*

If experience of *D. citri* with predatory mites would result in a change in preference, this would result in a significant effect of the interaction of experience and the presence/absence of predators on the plants on plant choice, but this interaction was far from significant (GLMER, $\chi^2 = 0.35$, d.f. = 1, p = 0.56). Experienced and inexperienced *D. citri* did not differ significantly in their choice (GLMER, $\chi^2 = 0.84$, d.f. = 1, p = 0.36), but there was a significant interaction between the presence of predators (treatment) and sex (Fig. 6, GLMER, $\chi^2 = 7.7$, d.f. = 1, p = 0. 006). This was because females had a preference for plants with predators while males preferred clean plants (Fig. 6).

Dispersal of *Diaphorina citri*

The presence of *A. herbicolus* did not trigger dispersal of *D. citri* from the plants (Fig. 7a, GLMER, $\chi^2= 0.013$, d.f. = 1, p = 0.90). We observed a significant effect of time because the proportion of individuals that dispersed during the first 24h differed from 48h, 72h and 106h (Fig. 7a, GLMER, $\chi^2= 14.2$, d.f. = 1, p = 0.0001). We also observed a higher proportion of females than of males dispersing from both control and treatment plants (Fig. 7b, GLMER, $\chi^2= 10.04$, d.f. = 1, p = 0.001).

DISCUSSION

We showed that adult *D. citri* did not avoid host plants that harboured the predatory mite *A. herbicolus*, which preys on eggs and young nymphs of this pest. The experiment in which *D. citri* were offered a choice between plants with or without conspecifics showed a clear preference for plants with conspecifics (Fig. 1), in line with earlier results with *D. citri* and citrus plants (Martini et al. 2014). This also shows that the method used here was suitable for testing host plant selection by this pest. In subsequent experiments, *D. citri* showed no sign of avoiding plants with predators, even not when having previous experience on plants on which *A. herbicolus* was consuming *D. citri* offspring. Moreover, the presence of predatory mites feeding on eggs and nymphs of the psyllid on plants did not trigger dispersal of *D. citri*. These results are crucial for understanding the effects of predators on the spread of HLB: apparently the predator will not increase the spread of HLB disease while controlling *D. citri* densities.

The attraction of *D. citri* by plants with conspecifics was observed before (Mann et al. 2013; Martini et al. 2014), but the reason is not completely understood. It is known that males of *D. citri* use cues of females and damaged plants to find mates (Wenninger et al., 2008; Martini et al. 2014). Females are also attracted to damaged plants, but plants with many females are repellent, probably to avoid competition (Martini et al. 2014). *Diaphorina citri* mates, feeds and oviposits on the growing tips (Hall and Abrigo, 2007; Sétamou et al. 2016), which may therefore be a limited resource. The perception of cues from damaged host plants and conspecifics may therefore increase mating probabilities, facilitate plant colonization and finding plants with growing tips, unless densities of conspecifics are high (Wenninger et al., 2008; Wenninger et al. 2009; Martini et al. 2014). In our experiments, the plants did not have a high density of conspecifics, which could help explain the attraction to plants with conspecifics.

One possible explanation for the lack of avoidance is that *D. citri* is not able to detect *A. herbicolus* cues or at least not accurately. Predators and prey are involved in an arms race where predators are selected to avoid detection and prey are selected to become better in avoiding predation (Abrams 1986). *Amblyseius herbicolus* may have been selected for being undetectable by this prey. Perhaps more time together with predators on plants is needed for *D. citri* to start avoiding plants harbouring predatory mites.

The detection of predation risk may be dependent on predator densities (Bowler et al. 2013). Low predator densities may not pose a high predation risk, thus elicit no antipredator response in the prey. However, the numbers of predators on the plants during the experiments were high compared to the numbers of predators that were found elsewhere to cause a significant reduction of *D. citri* densities (Chapter 1 and 2). Furthermore, we observed preyed *D. citri* eggs on plants with predators at the end of experiments, hence, there was substantial predation risk for *D. citri* on plants with predators.

Furthermore, it could be argued that *D. citri* perceives some cues of the predator, but instead of avoiding the plant with the predators, it avoids the entire area in which the predators occur. In our experiments, this would be manifested by lower numbers of *D. citri* choosing for any plant when predators were present somewhere in the set-up. We would therefore expect fewer *D. citri* making a choice for any plant when predators were present. We registered psyllids which died or which did not choose either of the plants in all experiments. Although the results of the different experiments are not directly comparable because they were done at different moments, the overall numbers of these psyllids were not correlated with the presence of predators in the experimental set-up.

Several herbivores have been observed to respond to the presence of predators by moving to other parts of the same plant (Lee et al. 2022), or to other plants (Tamaki et al. 1970; Lima and Dill 1990; McCauley and Rowe 2010). Especially winged insects can easily escape from wingless, slowly dispersing predatory mites (Meng et al., 2012). It is known that *D. citri* are good dispersers (Stelinski 2019), so the lack of dispersal from plants with predators that was observed here was not caused by limited dispersal capacity. There are other examples of flying herbivores, such as whiteflies, which were reluctant to leave plants with predatory mites and they dispersed less with increasing distance to plants without predators (Meng et al., 2012). Here, the plants without predatory mites were close to the plants with predators (about 35 cm), thus, the winged adult *D. citri* could easily have escaped to a nearby plant. Nevertheless, we did not observe increased dispersal when predators were present.

Diaphorina citri is phototactic and aggregates close to intense light sources (Sétamou et al. 2012; Patt et al. 2015). It is attracted to certain wavelengths such as UV (Paris et al. 2015; Kalile et al. 2022), which are used in orientation towards host plants (Sétamou et al. 2012, 2015; Volpe et al. 2020). Besides, *D. citri* is also attracted to yellow and green wavelengths reflected by growing tips, but not by old leaves (Paris et al. 2017). Field observations have shown that *D. citri* aggregates on plants at the borders of citrus orchards where the plants receive more light (Sétamou et al. 2012; Sétamou and Bartels 2015). The results of both our lab and greenhouse experiments confirmed this attraction to light (Supplementary material). However, we compensated for differences in light conditions and attraction to wavelengths in the experimental set-up by systematically changing positions of treatment and control plants.

The reaction of vectors to predators may have implications for the spread of a disease. For example, when attacked by the syrphid *Sphaerophoria rueppellii* (Wiedemann) (Diptera: Syrphidae), the aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), vector of potato virus Y (PVY), showed less dispersal and this did not enhance virus spread (Belliure et al. 2011). However, the coccinellid *Adalia bipunctata* (Linnaeus) (Coleoptera: Coccinellidae) induced strong antipredator behaviour and increased virus transmission (Belliure et al. 2011). *Amblyseius herbicolus* is capable of significantly reducing *D. citri* densities (Chapter 1 and 2) and the current results suggest that the presence of the predators does not increase the risk of transmission because of changes in choice and dispersal behaviour of *D. citri* in response to the presence of the predator. We were unable to investigate the effect of *A. herbicolus* on infective *D. citri* and spread of HLB because the area where the research was carried out (Viçosa, MG) is HLB-free. Therefore, future experiments should test whether the presence of predatory mites affects the host plant choice and dispersal of infected *D. citri* and the effects on acquisition and transmission of HLB.

Field studies have focused on the direct impact of natural enemies on population dynamics and little is known about the effects of predation risk on the behaviour and dynamics of *D. citri*. We have shown elsewhere that *A. herbicolus* is a promising natural enemy for controlling *D. citri* (Chapter 1 and 2), and here we show that there is no evidence that the presence of the predators changes the host plant choice and dispersal behaviour of *D. citri*. This indicates that *A. herbicolus* can potentially control *D. citri* without increased spread of HLB. Future experiments should investigate the impact of *A. herbicolus* on *D. citri* and HLB incidence in the long term in the field.

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Author contributions

AJ and MOK conceived and designed research. MOK, TAFJ and ACC conducted the experiments. AJ analysed the data. MOK and AJ wrote the manuscript with contributions of ACC and AP. All authors read and approved the manuscript.

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Data availability statement

Upon acceptance, data will be available on UvA/AUAS figshare.

Competing Interest

The authors declare that there is no conflict of interest.

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Table 1 The host plant choices offered to *Diaphorina citri*

Control plants	**Treatment plants**
Clean	Marked conspecifics
Clean	Marked conspecifics and predatory mites
Marked conspecifics	Marked conspecifics and predatory mites
Clean	Predatory mites
Clean	Predatory mites feeding on pest offspring
Clean with experienced or inexperienced *D. citri* choosing	Predatory mites feeding on pest offspring with experienced or inexperienced *D. citri* choosing

To avoid repellence caused by high densities of conspecifics, 10-12 *D. citri* were released per cage to make a choice in experiments with marked conspecifics on plants. In the other experiments, 20 *D. citri* were released per cage.

Figure legends

Fig. 1 Proportion of female (white) and male (black) *D. citri* that were recaptured on a clean plant or on a plant with adult conspecifics and their eggs. *Diaphorina citri* were released between the two plants 48h earlier to make a choice. Different lower-case letters indicate significant differences in the choice between the two plants (GLMER, ***p<0.0001). The bars represent confidence intervals.

Fig. 2 Proportion of female (white) and male (black) *D. citri* recaptured on clean plants or on plants with conspecifics, *D. citri* eggs and predatory mites. See legend to Figure 1 for further explanation.

Fig. 3 Proportion of female (white) and male (black) *D. citri* recaptured on plants with conspecifics and *D. citri* eggs or on plants with predatory mites, conspecifics and *D. citri* eggs. Different lower-case letters indicate significant differences in the choice between the two plants (GLMER, *p<0.05). See legend to Figure 1 for further explanation.

Fig. 4 Proportion of female (white) and male (black) *D. citri* recaptured on clean plants or on plants with predatory mites. See legend to Figure 1 for further explanation.

Fig. 5 Proportion of females (white) and males (black) *D. citri* recaptured on clean plants or on plants with predatory mites preying on *D. citri* eggs (Predator with *D. citri* eggs). See legend to Figure 1 for further explanation.

Fig. 6 Proportion of experienced and inexperienced females (white) and males (black) of *D. citri* recaptured on clean plants or on plants with predatory mites. Asterisk indicate significant difference in choice for clean plants or plants with predatory mites. Different letters indicate significant differences between males and females (GLMER, $*p < 0.05$). The bars represent confidence intervals.

Fig. 7 **(a)** Proportion of *D. citri* that dispersed over time from plants with predators (black squares) or without predators (white squares) to clean plants and to the walls of the experimental cage (circle). **(b)** Proportion of females (white) and males (black) of *D. citri* that had dispersed at the end of experiment from clean plants or from plants with predators. The bars show the confidence intervals. Different lowercase letters indicate significant differences (GLMER $***p < 0.001$)

Figure 1

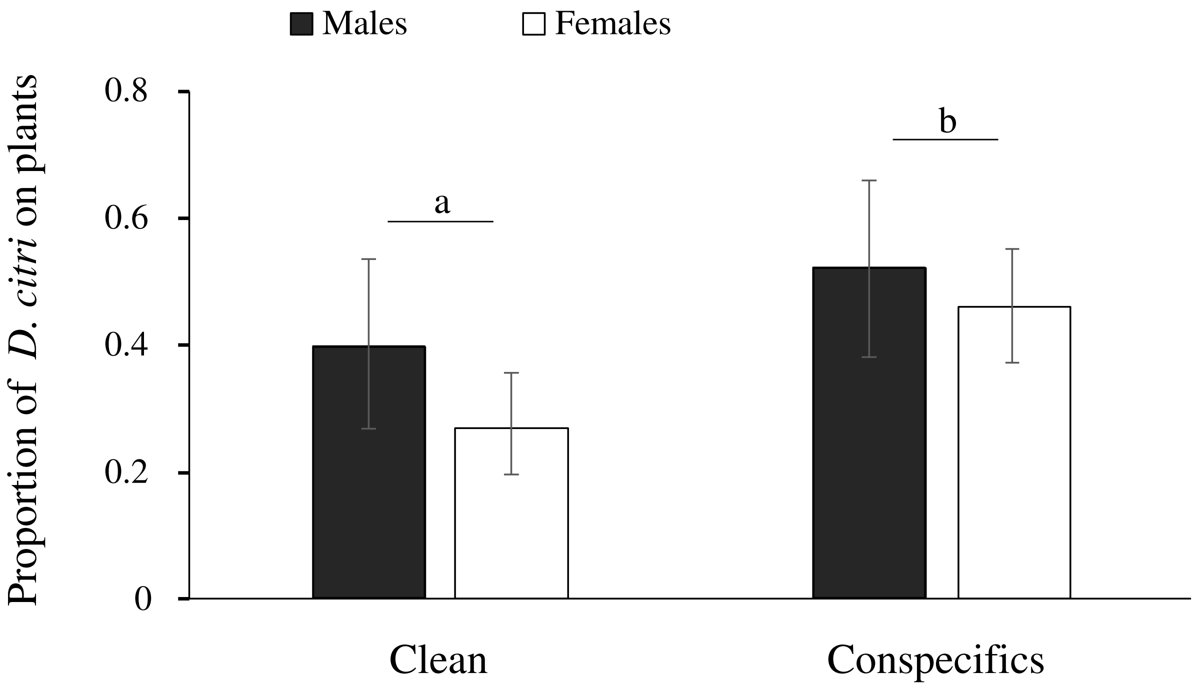

■ Males
□ Females
Proportion of *D. citri* on plants
0.8
0.6
0.4
0.2
0
a
b
Clean
Conspecifics

Figure 2

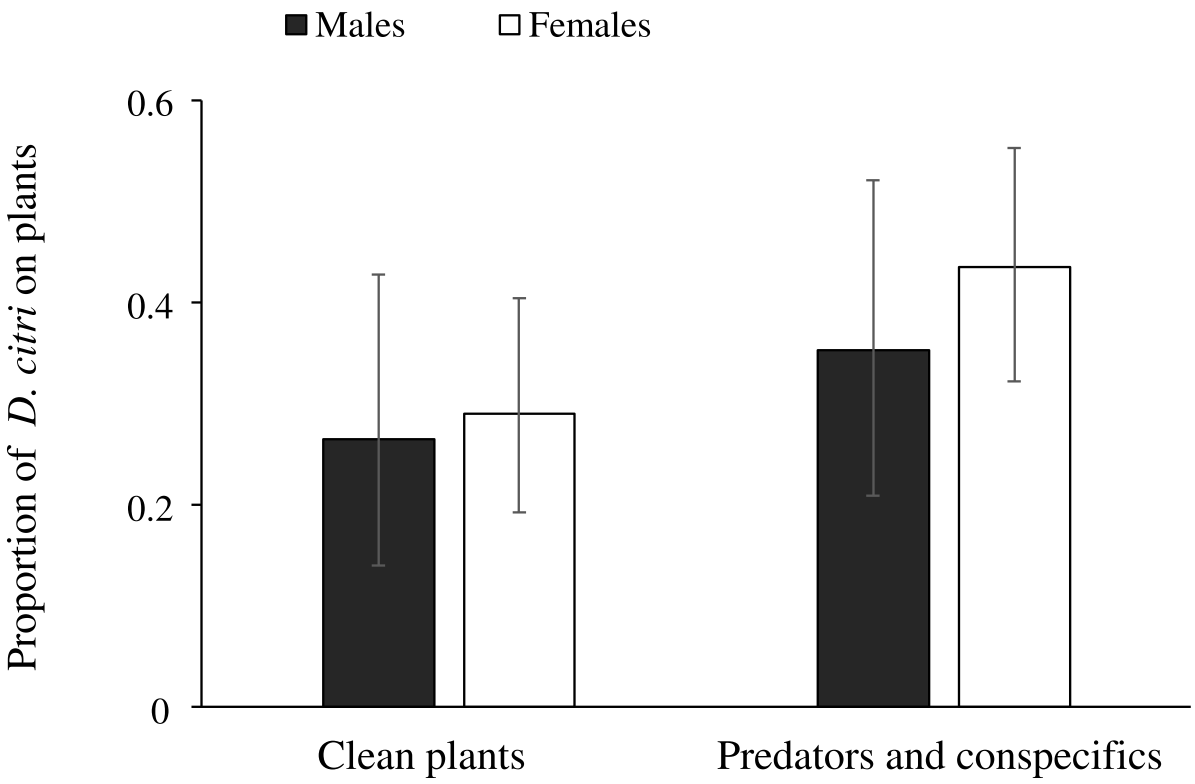

■ Males
□ Females
Proportion of D. citri on plants
0.6
0.4
0.2
0
Clean plants
Predators and conspecifics

Figure 3

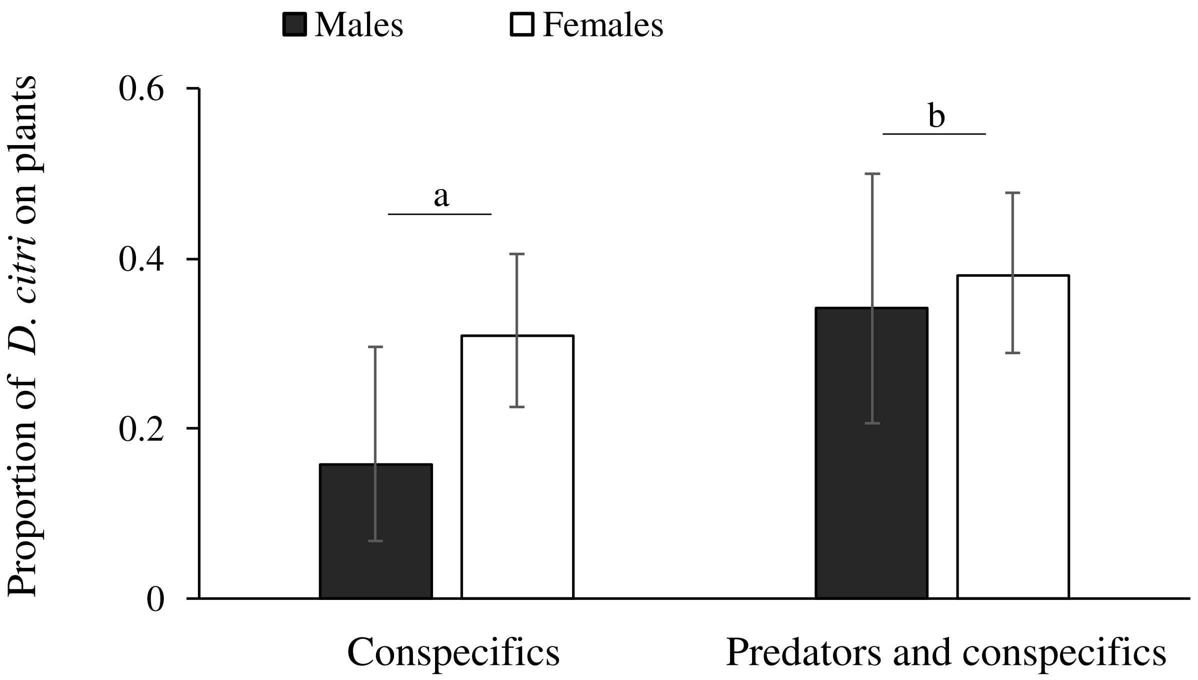

■ Males
□ Females
Proportion of D. citri on plants
0.6
0.4
0.2
0
a
b
Conspecifics
Predators and conspecifics

Figure 4

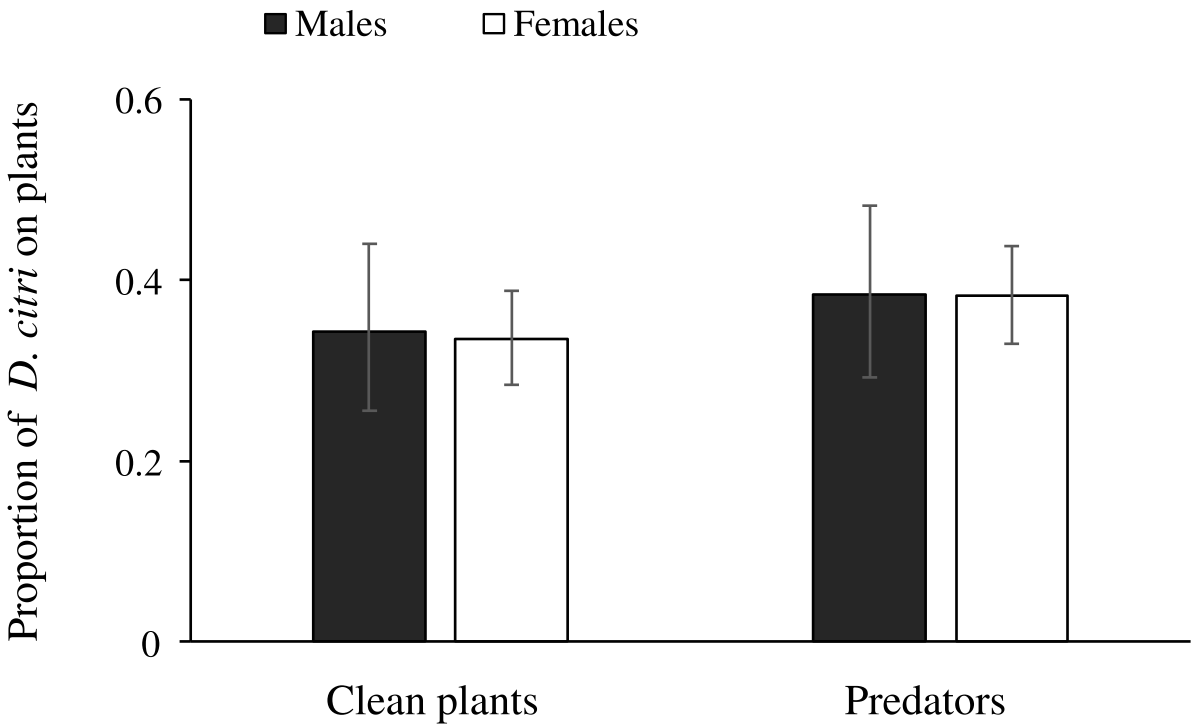

■ Males
□ Females
Proportion of *D. citri* on plants
0.6
0.4
0.2
0
Clean plants
Predators

Figure 5

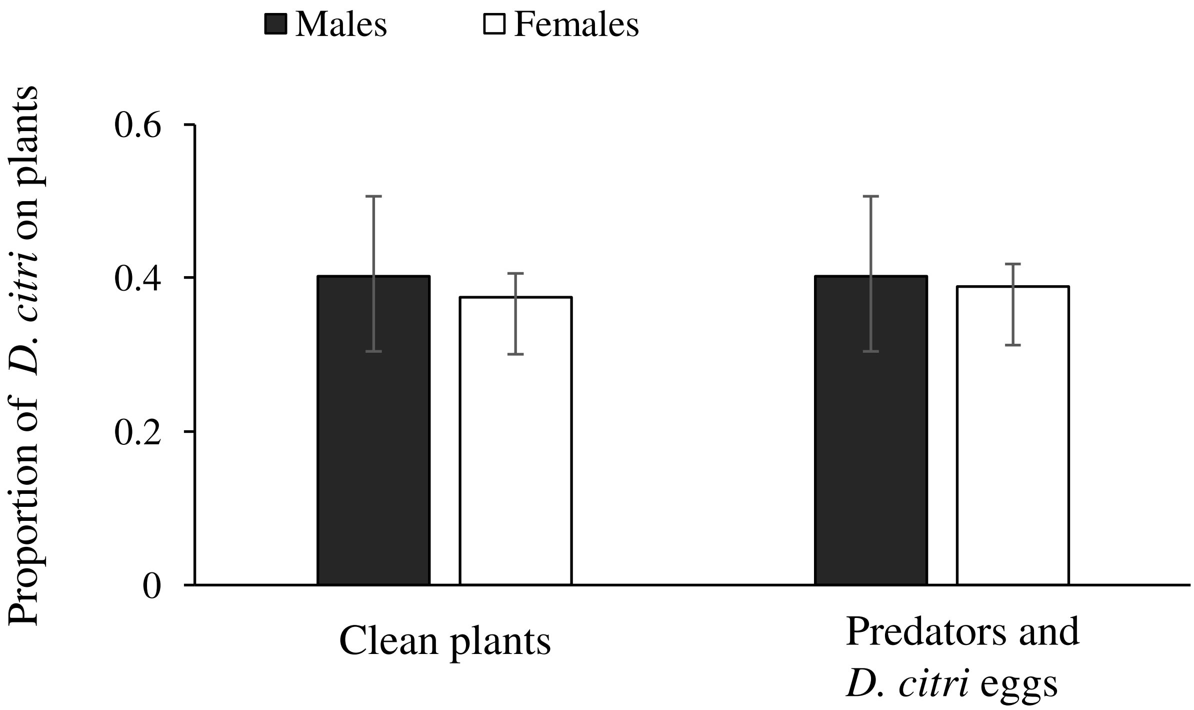

■ Males
□ Females
Proportion of *D. citri* on plants
0.6
0.4
0.2
0
Clean plants
Predators and *D. citri* eggs

Figure 6

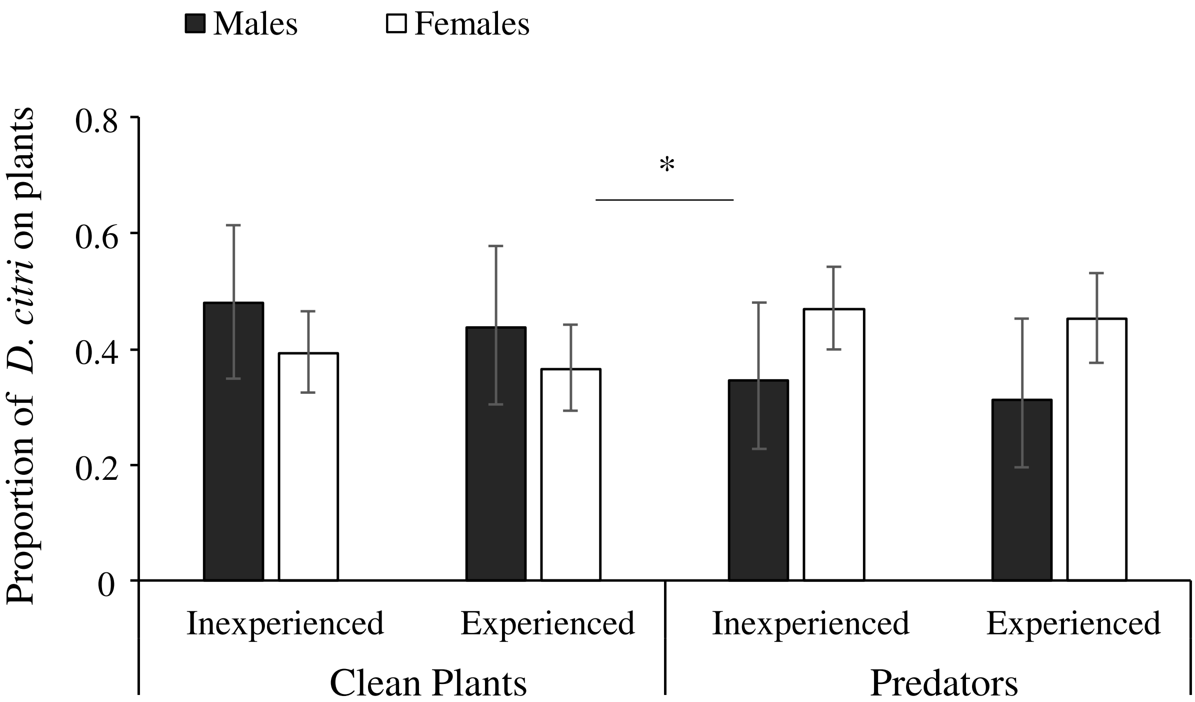
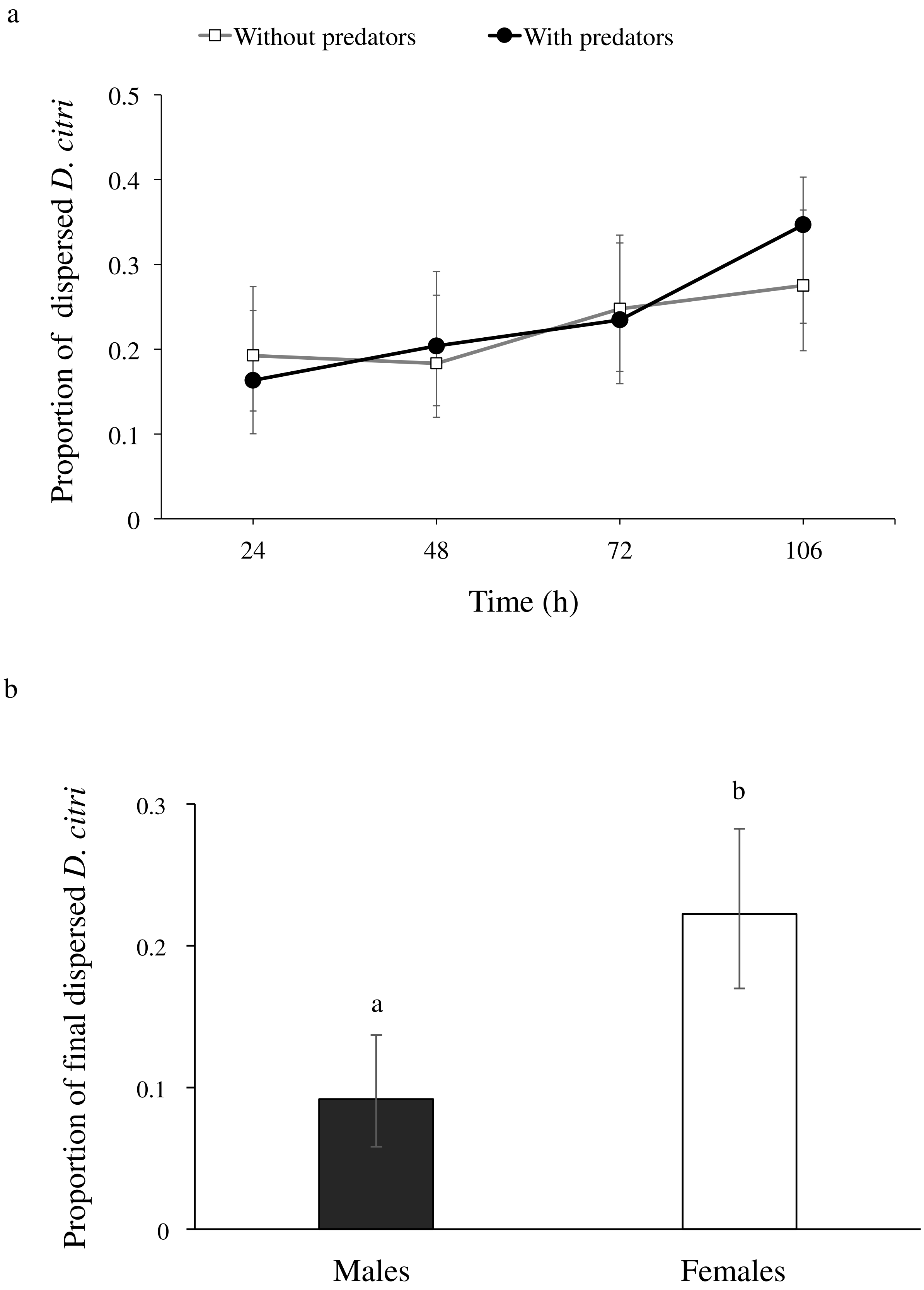

■ Males
□ Females
Proportion of D. citri on plants
0.8
0.6
0.4
0.2
0
*
Inexperienced
Experienced
Inexperienced
Experienced
Clean Plants
Predators

Figure 7


a
Without predators
With predators
Proportion of dispersed *D. citri*
0.5
0.4
0.3
0.2
0.1
0
24
48
72
106
Time (h)
b
Proportion of final dispersed *D. citri*
0.3
0.2
0.1
0
a
b
Males
Females

SUPPLEMENTARY MATERIAL

Materials and Methods

Light conditions

Most replicates of the choice experiments were conducted in the laboratory (26 ± 2 ºC, 70 ± 10% RH, natural light conditions), where the cages were positioned on a bench in front of windows providing natural daylight. *Diaphorina citri* shows positive phototaxis (Yasuda et al. 2005), and although the distance between the plants inside the cage was only about 25 cm, we alternated the positions of the clean plants and treated plants to randomize effects of possible directionality in *D. citri* behaviour due to differences in light conditions. We observed a general preference of *D. citri* to the lighter side (closest to the window), and we therefore also performed some replicates of the choice experiments in a greenhouse with more diffuse light. The light intensity at the position of the plants and on the top of the cages was measured using a digital lux meter (MLM-1001 Minipa, SP- Brazil), both in the lab and greenhouse experiments. We observed that *D. citri* moved to the top of the cage at the lighter side and then chose a plant. We therefore measured the light at the top of the cage as well as on the plants.

Results

Light conditions

The luminosity differed on plants inside the cages in the lab ($F_{1,\,162} = 14.1$, $p = 0.0002$). There was a general preference for the lighter side in the experiments in the lab, but this did not interact with the overall preference for plants with different treatments because we changed position of the treatments among replicates. In the lab, the light intensity at the lighter side averaged 2601 ± 240 lx while it averaged 1426 ± 150 lx away from the window. In the greenhouse, where the light was more diffuse, the luminosity on top of the cages at the lighter side was 5828 ± 549 lx and 4707 ± 460 lx on the darker side. These data help explaining why *D. citri* preferred plants close to the window in the lab (lighter side), but did not show a significant directionality in the greenhouse.

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