



A predatory mite as potential biological control agent of *Diaphorina citri*

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Abstract *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) is a vector of the bacteria that cause Huanglongbing, the fatal disease threatening citriculture worldwide. One of the most important management methods is the control of *D. citri* with pesticides, but their intensive use causes development of resistance and pollution. An alternative method is therefore necessary to manage this vector-pathogen system. The generalist predatory mite *Amblyseius herbicolus* Chant (Acari: Phytoseiidae) can be found on citrus and orange jasmine plants, important hosts of *D. citri* in Brazil. Here we show that this phytoseiid can reproduce and develop on *D. citri* eggs. The predation rate was higher on *D. citri* eggs collected from plants

in the field than on eggs from small plants from a laboratory rearing. Yet the predator preferred eggs from the laboratory. This may indicate that the higher predation rate of eggs from the field served to compensate for these eggs being of lower nutritional quality. This was confirmed with a series of experiments with limited numbers of eggs. We conclude that *Amblyseius herbicolus* is a potential biological control agent of *D. citri*, and its performance and control may be affected by the quality of the host plant.

Keywords Asian citrus psyllid · Nutritional quality · Compensatory predation · Huanglongbing · Citrus

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Introduction

Huanglongbing (HLB) or citrus greening is the most devastating disease of citrus in the world and it is caused by three different phloem-limited gram-negative *Candidatus* bacteria species (Garnier et al. 1984; Grafton-Cardwell et al. 2013). Two insects are vectors of HLB: *Trioza erytreae*, the African psyllid (McClean and Oberholzer 1965), which occurrence was limited to Africa until its arrival on the Iberian Peninsula in 2014 (Pérez-Otero et al. 2015), and *Diaphorina citri*, the Asian citrus psyllid, capable of transmitting the three species of bacteria, including *Ca. Liberibacter americanus*, known only from Brazil (Bové 2006). The disease transmitted by *D. citri* is

characterized as being more severe than that transmitted by *T. erythrae* because of higher heat tolerance (Lopes et al. 2010). This makes *D. citri* currently the major global citrus pest (Bassanezi et al. 2020).

Huanglongbing can affect citrus plants of all cultivars (Zou et al. 2019), is incurable and ultimately fatal (Bové 2006; Grafton-Cardwell et al. 2013). The symptoms, mottling and chlorosis of the leaves (Batool et al. 2008), can easily be mistaken for zinc deficiency. HLB symptoms also include poor development of the root system (Batool et al. 2008), seed abortion, asymmetrical fruits (Bové 2006; Gasparoto et al. 2018), premature fruit drop and bitter juice without economic value (Bové 2006; Gottwald et al. 2007). The visual symptoms can vary greatly (Gottwald et al. 2007) and are commonly preceded by a prolonged asymptomatic period, which may delay detection by months to years (Gottwald et al. 2020). Thus, asymptomatic infected plants act as a source of the disease and contribute to its rapid spread (Lee et al. 2015). Huanglongbing causes quantitative and qualitative yield losses and ultimately plant death (Gottwald et al. 2007; Alvarez et al. 2016), leading to dramatic economic losses worldwide (Alvarez et al. 2016).

Pesticides have been used to control the vector (Grafton-Cardwell et al. 2013; Chen et al. 2020), but their effectiveness is limited because *D. citri* populations have evolved resistance (Chen et al. 2020) due to aggressive insecticidal spray programs. These sprayings concomitantly disrupt the naturally occurring biological control (Monzo et al. 2014) and may cause secondary pest problems (Miranda et al. 2018). Besides not effectively controlling the vector, the frequent and long-term pesticide applications have various undesirable effects on beneficial arthropods, human health and environment, such as citrus orchard pollution (Cunha et al. 2012; Li et al. 2019).

An alternative to chemical control is the use of natural mortality factors of *D. citri*. The main factors that affect the vector are the flushing rhythms of host plants (growing tips are necessary for oviposition and development, Catling 1970), weather extremes and natural enemies (Catling 1970; Kistner et al. 2016). Biological control with natural enemies is an important pest control strategy and does not cause resistance development on pests, in contrast to insecticides (Bale et al. 2008). Thus, one way of controlling *D. citri* would be the use of natural enemies (Grafton-

Cardwell et al. 2013). Natural enemies reported for biological control of *D. citri* include parasitoids such as *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae) (Chen and Stansly 2014), generalist predators such as coccinellid beetles, hoverflies, spiders and lacewing larvae (Michaud 2004; Kistner et al. 2016), entomopathogens (Hall et al. 2012; Arnosti et al. 2019), predatory mites such as *Amblyseius swirskii* and *Neoseiulus cucumeris* (Juan-Blasco et al. 2012; Fang et al. 2013) and *Pyemotes* mites (Prostigmata: Pyemotidae) (Lu et al. 2019). *Tamarixia radiata*, the most important parasitoid of *D. citri* used for biocontrol, attacks nymphs from the third to fifth instar (Chen and Stansly 2014). However, it is laborious to rear and its effectiveness is controversial: *T. radiata* did not seem to reduce the populations of *D. citri* enough to limit the spread of the pathogen in some regions, whereas it is considered an effective biological control agent in other regions (Grafton-Cardwell et al. 2013). Moreover, Michaud (2004) reported that the main natural mortality factor of *D. citri* is predation by generalist predators, which may be more efficient at controlling citrus pests than parasitoids (Symondson et al. 2002; Michaud 2004).

The consumption of *D. citri* eggs is considered crucial (Juan-Blasco et al. 2012; Fang et al. 2013). However, eggs of *D. citri* are oviposited on small developing leaves that are unfolding, where they are protected from the consumption by large predators, but not by small predatory mites (Juan-Blasco et al. 2012). Besides, large predators can feed on the parasitized 4th and 5th instar nymphs, which can result in high mortality of the parasitoid inside these hosts (Michaud 2004). To date, the disease continues to advance and no single approach is effective enough to control *D. citri* and HLB (Juan-Blasco et al. 2012; Lu et al. 2019). Therefore, the combined use of the parasitoid in association with a generalist predator that feeds on *D. citri* eggs and younger nymphs, but not on those parasitized, may be the best alternative.

Brazil is the largest producer of oranges in the world (Gasparoto et al. 2018) and the incidence of HLB in the citrus belt of São Paulo, Triângulo and Southeast of Minas Gerais State increased considerably during the last decade (Bassanezi et al. 2020). One of the most important control strategies is to reduce densities of the insect vector (Grafton-Cardwell et al. 2013). The use of biological control in Brazil is growing, but still limited (Parra 2014), and

studies of biological control of *D. citri* with predators are scarce, despite the large diversity of natural enemies (Demite et al. 2014). Thus, the aim of this work was to study a generalist predator as potential biocontrol agent of *D. citri* in Brazil.

Several generalist phytoseiid mites are excellent biological control agents due to their capacity to feed on alternative food or prey, enabling the persistence of populations in the absence of the target pest (van Rijn et al. 2002; Nomikou et al. 2003; Janssen and Sabelis 2015). We therefore investigated the potential of a native Brazilian predatory mite for the control of *D. citri*. This predator can be found on both citrus and orange jasmine plants, which are hosts for *D. citri* and HLB bacteria (Lopes et al. 2010; Alves et al. 2014). At the moment, there are no studies of predators controlling *D. citri* in Brazil. We therefore studied the potential of the phytoseiid mite *Amblyseius herbicolus* Chant (Acari: Phytoseiidae) to feed on *D. citri*. It is a generalist predator which can be easily reared on pollen (Cavalcante et al. 2015; Duarte et al. 2015) and astigmatid mites (Cavalcante et al. 2015). Experiments were carried out in the laboratory to test the capacity of *A. herbicolus* to develop and reproduce when feeding on *D. citri* eggs.

Materials and methods

Predator sampling

One of the preferred host plants of *D. citri* is orange jasmine, *Murraya paniculata* (L.) Jack (Alves et al. 2014). It is also a potential reservoir of *Candidatus Liberibacter* bacteria associated with HLB (Lopes et al. 2010). Orange jasmine is a widespread ornamental plant in Brazil (Lopes et al. 2010), which favors the spread of the pathogen. We therefore first assessed the presence of predatory mites on citrus, including sweet orange (*Citrus sinensis* (L.) Osbeck: varieties Bahia, Lima Sorocaba and Westin; *Citrus reticulata* Blanco, var. Ponkan; *Citrus deliciosa* Tenore var. Mexerica do Rio) and orange jasmine plants on the campus of the Federal University of Viçosa (20°46'9" S, 42°87'02" W) in the state of Minas Gerais, Brazil. Two collection methods were used: the first was beating the leaves above a black tray, which was subsequently covered with plastic film. For the second method, we removed branches

with *D. citri* eggs in the growing tips, which were then placed in paper bags. Tray and paper bags were taken to the laboratory, where slides of predatory mites were made for morphological identification using a phase contrast microscope and a dichotomous key (Chant and McMurtry 1994, 2007). The predatory mite *A. herbicolus* was found on both citrus and orange jasmine plants, in the last case together with *D. citri*.

Acquisition and maintenance of clean plants

Orange jasmine plants were used for rearing *D. citri* and in experiments. Plants (4–6 months old) were obtained from Viveiro Antuérpia in the vicinity of Viçosa, Minas Gerais, Brazil, and were kept in an insect-proof greenhouse (25 ± 2 °C), where they were fertilized every two months with a mixture of NPK (4/14/8). Plants were pruned regularly to induce the development of growing tips, which are oviposition sites of *D. citri*. We assessed the presence of the three *Candidatus Liberibacter* species in plant material as well as in *D. citri* (see Supplementary Material and Table S1).

Diaphorina citri rearing

Adult *D. citri* were collected from orange jasmine trees of about six years old on the campus of the Federal University of Viçosa. They were reared in BugDorm-4F insect cages (0.5 × 0.5 × 1.0 m) to oviposit on pesticide-free orange jasmine plants (see above) and formed the basis of what will be referred to as the laboratory population. These insects were kept under laboratory conditions (26 ± 2 °C, 70 ± 10% RH, natural light conditions). Plants were watered two times a week and adults were regularly transferred to new plants. Besides eggs from this laboratory line, eggs were also obtained directly from trees on the campus. They are referred to as the field line.

Pollen

The pollen used for the predatory mite rearing and experiments was collected from *Typha* sp. plants from rural areas around Viçosa, Minas Gerais. This pollen was chosen because previous studies have shown its suitability (Duarte et al. 2015). It was dried in an oven at 40 °C for 12 h and was stored in a container in the freezer (− 6 °C) and small amounts were removed periodically from the container. Subsequently, the

pollen was placed in 1.5 ml microtubes (Eppendorf), dried at 60 °C for 48 h and then stored in the refrigerator (8 °C).

Amblyseius herbicolus rearing

The predators used for experiments came from a culture of *A. herbicolus* at the Laboratory of Acarology of the Universidade Federal de Viçosa, established since 2014. It was collected from tomato plants in Prados (latitude: 21°03'00" S; longitude: 44°04'47" W), Minas Gerais, Brazil. The predators were reared on arenas consisting of a plastic sheet (10 × 15 cm²) surrounded by a moistened cotton wool barrier, placed on a wet sponge in a plastic tray containing water (McMurtry and Scriven 1965). They were fed with pollen twice a week. Cotton threads were provided to serve as oviposition sites and were covered with a piece of black plastic for shelter (1 × 1 cm²). Threads with eggs were placed on a new arena to obtain cohorts of similar-aged individuals and, after 10–12 days, the young adult females were used for experiments. The rearing arenas were kept under controlled conditions (25 ± 2 °C, 70 ± 10% RH and L:D 12:12).

Predation and oviposition of *A. herbicolus* on *D. citri*

This experiment was designed to assess the predator performance on *D. citri* eggs from field and laboratory plants. Young adult female predators were individually tested in black plastic dishes (ø = 5.5 cm; 1.4 cm high) with a piece of wet cotton wool inside, covered with a transparent lid. Growing tips (development phases 2 and 3, Cifuentes-Arenas et al. 2018) with prey eggs were obtained from orange jasmine plants from the laboratory and the field. Other species of herbivores were removed from the tips, the number of prey eggs was assessed using a binocular stereo microscope (20 × magnification; Zeiss Stemi 508, Germany) and growing tips were placed in a closed arena with one adult female *A. herbicolus*. Moistened cotton wool (ø = 1 cm) was placed in each arena to prevent dehydration of *D. citri* eggs and as water source for the predator. Previous experience showed that the moistened cotton is sufficient to prevent dehydration of *D. citri* eggs for more than 24 h. All replicates were done with 60–62 prey eggs from the laboratory or from the field. This number of eggs

allowed for *ad libitum* feeding. Individuals were moved to a new arena with fresh prey eggs every day. The same methodology was used for oviposition experiments, but using an extra treatment with 1 mg of pollen as control. The numbers of *D. citri* eggs consumed and the predator oviposition rate were scored for three days, but the score of the first day of oviposition was excluded to reduce effects from the previous diet (Sabelis 1990). During the experiment, a few nymphs hatched (maximum five), but some were also preyed upon by the predatory mites. The numbers of females tested were 12 and 13 for prey eggs from the field and the laboratory, respectively, and 13 for the pollen treatment. All experiments were performed at 25 ± 2 °C, 70 ± 10% RH and L:D 12:12, similar to the conditions for rearing *D. citri* and the predators. Predation and oviposition data were analyzed with linear mixed effects models (LME) with individual as a random factor to correct for repeated measures. Contrasts between treatments were obtained with the Tukey method (function `clm` of the `multcomp` package of R, Hothorn et al. 2008) and models were checked for normality of errors. Contrasts among treatments were assessed with the package `emmeans` with a Tukey correction for multiple comparisons (Lenth 2019). All statistical analyses were done using R version 3.5.3 (R Core Team 2019).

Choice test

Because the previous experiment showed that the predators consumed many more prey eggs from the field than from the laboratory, we further investigated possible differences in prey eggs of these different origins by offering the predators a choice between them. Similar quantities of prey eggs were offered on two growing tips (as above), one from the field and one from the laboratory, each on one side of a circular arena made of a black plastic sheet (ø = 5.5 cm) on wet cotton wool and surrounded with water. Care was taken to select pairs of growing tips that were of approximately the same size. The side of the arena on which the growing tips of each origin were offered was alternated to avoid possible bias of the predators for one side. An adult female was placed in the center of each arena, and its position was checked every hour. All females tested were of the same age (10–12 days old). After 6 h, the consumption of prey eggs from both origins was evaluated. The predators showed no

significant preference for either side of the arena (binomial test, all $p > 0.05$). The number of females tested was 13. The data were analyzed first with a binomial test per female, with the expectation that they would choose eggs of either origin with a probability of 0.5 (Siegel and Castellan 1988). Subsequently, we compared the number of preyed *D. citri* eggs from each origin with a generalized mixed effects model with a Poisson error distribution and a log link function, including individual as random factor and origin of eggs as fixed factor (GLMER, Bates et al. 2015). Sizes of the eggs from the field (nine eggs) and from the culture (ten) were measured by taking multifocal photographs using a Stereomicroscope (LEICA M205A) with a coupled camera (LEICA MC170 HD). Lengths and widths of the eggs were measured using the Leica application suite LAS EZ software (V3.1.1) and the volume of the eggs was calculated assuming that the shape corresponded to a combination of two opposing ellipsoids, which is a good approximation of the shape of the eggs. The volumes were compared with a t-test. Because we could not control the age of eggs from plants of both origins, we also investigated whether *A. herbicolus* had a preference for eggs of different ages. We offered predators a choice between new eggs (one day old) and the oldest eggs (3.5–4 days old) from the laboratory lines ($25 \pm 2^\circ\text{C}$, $70 \pm 10\%$ RH, natural light condition). This experiment was conducted as above and the number of predators tested was ten. The predators showed no significant preference for either side of the arena (binomial test, all $p > 0.05$). We compared the number of preyed *D. citri* eggs from each age with a generalized mixed effects model with a Poisson error distribution and a log link function, including individual as random factor and age of eggs as fixed factor (GLMER, Bates et al. 2015).

Predation and oviposition of *A. herbicolus* on limited numbers of *D. citri* eggs

Because the previous experiments indicated a possible difference in quality of prey eggs from the field and from the laboratory, we further measured oviposition and predation on eggs from the field and the laboratory. We investigated whether offering limited numbers of eggs from the field would result in a larger reduction of oviposition than eggs from the laboratory because feeding on more eggs from the field to compensate for

their lower quality would no longer be possible. Two experiments were performed, one providing 30 and the other ten eggs of both origins per day. The number of females tested was 11 for eggs from the field and 12 for eggs from the laboratory with 30 eggs provided per day and 14 and ten in the experiment with ten eggs provided per day. Differences in numbers of females tested were caused by differences in the availability of eggs from the field and the laboratory. The experiments were performed as described above, but without a control with pollen. The data were analyzed as predation and oviposition data above.

Juvenile development

This experiment was designed to verify whether *A. herbicolus* could develop by feeding on *D. citri* eggs only. Adult *A. herbicolus* were removed from the rearing and placed in a new arena, similar to those used in the predation and oviposition experiments, for 24 h to oviposit. Forty-eight h after removing the adults (eggs hatch in 24 h), larvae were taken from this cohort and each were placed in a separate black dish (as above). There were three treatments: one group of larvae received 1 mg of pollen, another group was supplied with a surplus of *D. citri* eggs (at least five eggs) from the field and a third group with a surplus of prey eggs from the laboratory (also at least five eggs). Every day, individuals were transferred to new dishes with fresh food and were checked for survival and development until adulthood. Upon observing the first adult, evaluations were done every 12 h. The number of replicates was 27 for eggs from the field, 23 for eggs from the laboratory and 26 for pollen. Effects of diet on survival and developmental were analyzed with a Cox's proportional hazards model (survival package in R, Therneau 2020).

Results

Predation and oviposition of *A. herbicolus* on *D. citri*

Amblyseius herbicolus consumed *D. citri* eggs and the predators obtained a yellow colour after consumption. There was a significant effect of the interaction between day and origin of eggs of *D. citri* on predation ($\chi^2 = 4.53$, d.f. = 1, $p = 0.033$; Fig. 1a). The

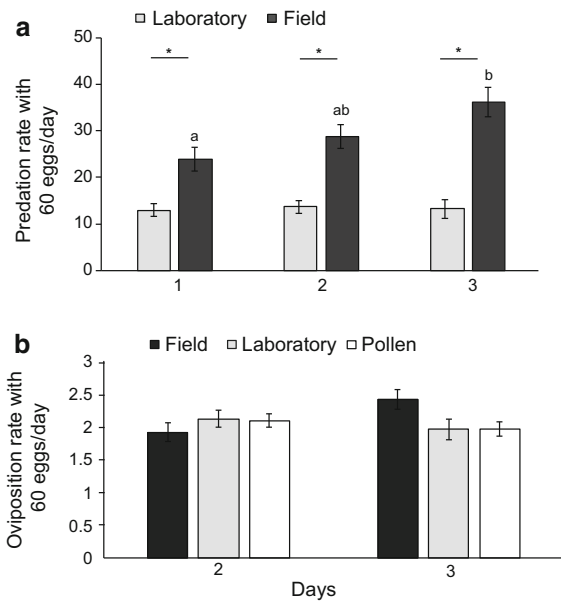


Fig. 1 **a** Average (\pm SE) predation rate of *Amblyseius herbicolus* fed on 60 eggs of *Diaphorina citri* from the laboratory (light grey) or the field (dark grey) during three days and **b** oviposition rate (\pm SE) also on pollen (medium grey) during two days. Different lower-case letters indicate significant differences in egg consumption among days in the treatment with eggs from the field ($p < 0.05$). The consumption of eggs from the laboratory did not differ significantly among days. Asterisks indicate significant differences in consumption of eggs from the field and the laboratory per day (all differences were assessed with contrasts after lme, $p < 0.05$). Oviposition rates did not differ significantly among treatments or between days ($p > 0.05$)

predators consumed more prey eggs from the field than from the laboratory on all three days. In addition, the numbers of eggs consumed from the laboratory did not vary significantly during the three days, but consumption of eggs from the field increased with time (Fig. 1a). The oviposition rate did not differ significantly between days ($\chi^2 = 2.02$, d.f. = 1, $p = 0.155$; Fig. 1b) and did not differ significantly with predator diet ($\chi^2 = 1.82$, d.f. = 2, $p = 0.40$; Fig. 1b). The mean total consumption of eggs from the field over the three days was about 3.5 times higher than that of eggs from the laboratory ($\chi^2 = 530.5$, d.f. = 1, $p < 0.0001$; Fig. 2a), but the oviposition during the last two days of the experiment did not differ significantly between these two diets or pollen ($\chi^2 = 0.11$, d.f. = 1, $p = 0.74$; Fig. 2b). This suggests that there were differences in quality between prey from the field and from the laboratory.

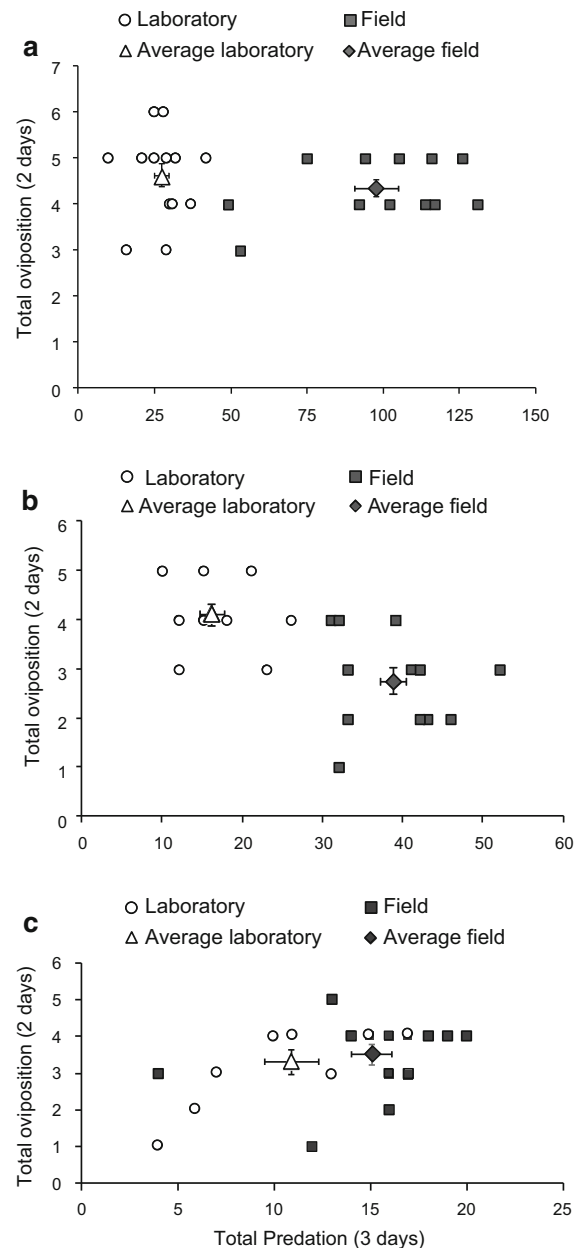


Fig. 2 Total oviposition of *Amblyseius herbicolus* during three days (vertical axis) as a function of total predation (horizontal axis) of *Diaphorina citri* eggs during the same period. Eggs were from the laboratory colony (light grey circles) or from the field (dark grey squares), and 60 (**a**), 30 (**b**) or ten (**c**) eggs were provided per day. The larger symbols show the averages (\pm SE), smaller symbols are individual measurements

Choice test

There was a significant preference for eggs from the laboratory relative to eggs from the field ($\chi^2 = 85.0$,

d.f. = 1, $p < 0.0001$; Fig. 3). In general, five times more eggs from the laboratory were consumed than eggs from the field. In addition, we observed that more than half of the eggs from the laboratory were partially eaten, which was about six times more than observed for eggs from the field ($\chi^2 = 79.2$, d.f. = 1, $p < 0.0001$; Fig. 3). Hence, predators consumed more eggs from the field than from the laboratory if they had no choice (Figs. 1a and 2a), but had a clear preference for eggs from the laboratory when given a choice. The sizes of eggs from both origins did not differ significantly (eggs from the field: $0.0022 \pm 0.00006 \text{ mm}^3$, eggs from the laboratory: $0.0024 \pm 0.0004 \text{ mm}^3$, t-test: $t = 0.52$, $df = 17$, $p = 0.61$). Although the trend is towards eggs from the laboratory being bigger, the difference was about 10%, which is not sufficient to explain the difference in predation rate of about 258% (Fig. 3a). Additionally, predators did not show a significant preference for eggs of different ages ($\chi^2 = 0.0001$, d.f. = 1, $p = 0.99$). Together, this suggests that the eggs from the laboratory were of better quality than the eggs from the field, and that predators perhaps consumed more eggs from the field to compensate for low egg quality when they had no choice. To further test this, we limited the numbers of prey eggs.

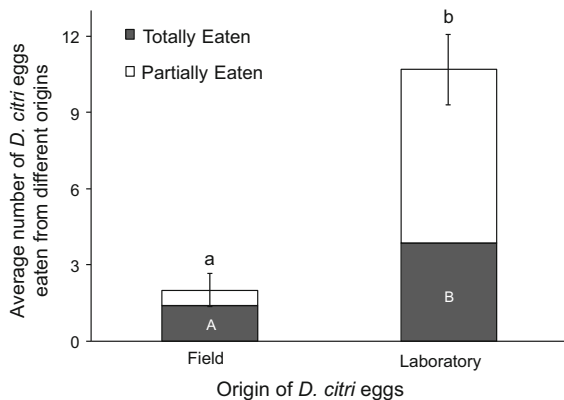


Fig. 3 Mean numbers (\pm SE) of eggs of *Diaphorina citri* from the field or from the laboratory consumed by *Amblyseius herbicolus* when offered a choice. Light gray bars refer to partially consumed eggs, dark gray to completely consumed eggs. Different upper-case letters indicate significant differences in partial consumption of eggs and lower-case letters indicate significant differences in total consumption (totally eaten plus partially eaten) of eggs from both origins (all differences were assessed with contrasts after GLMER, $p < 0.05$)

Predation and oviposition of *A. herbicolus* on limited number of *D. citri* eggs

When 30 *D. citri* eggs were provided per day, the average total predation on eggs from the laboratory was lower than on eggs from the field ($\chi^2 = 41.9$, d.f. = 1, $p < 0.0001$; Fig. 2b), but oviposition of predators feeding on eggs from the laboratory was higher ($\chi^2 = 9.47$, d.f. = 1, $p = 0.0021$; Fig. 2b). This confirms that eggs from the field were of lower quality than eggs from the laboratory. When ten *D. citri* eggs were provided per day, the predation of eggs from the field was again higher than predation of eggs from the laboratory ($\chi^2 = 7.75$, d.f. = 1, $p = 0.0054$; Fig. 2c). However, the mean total oviposition of predators fed on prey eggs from both origins did not differ statistically ($\chi^2 = 0.24$, d.f. = 1, $p < 0.627$; Fig. 2c).

Juvenile development

There was a significant effect of diet on the developmental time of *A. herbicolus* ($\chi^2 = 30.01$, d.f. = 2, $p < 0.001$; Fig. 4). Development took longer when predators fed on *D. citri* eggs from the field or from the laboratory than when they fed on pollen. Survival was high on all diets, and there was no significant effect of diet on survival ($\chi^2 = 4.61$, d.f. = 2, $p = 0.1$),

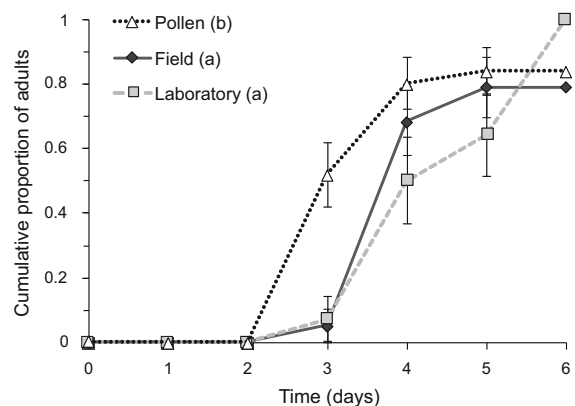


Fig. 4 Juvenile development and survival of *Amblyseius herbicolus* fed *Diaphorina citri* eggs from the field or from the laboratory or *Typha sp.* pollen. Shown is the cumulative proportion (\pm SE) of adults as a function of time. Total survival is the final cumulative proportion of individuals that reached adulthood (i.e. the points at day 6). Survival was high on all diets, but developmental rate was significantly higher on pollen than on eggs of *D. citri* ($p < 0.05$, indicated by the different letters in the legend)

although there was a trend of the survival being somewhat higher on a diet of eggs from the laboratory.

Discussion

We show that the predatory mite *A. herbicolus* can feed, develop and reproduce on eggs of *D. citri*. We also observed that the predation rates were affected by the origin of prey eggs, whereas predator oviposition rates differed only when the supply of prey eggs from the field was limited. In a preference experiment, about five times more eggs from the laboratory were consumed than eggs from the field (Fig. 3), and this could not have been caused by differences in age or size of the eggs. Predators are generally known to prefer high-quality prey (Dicke et al. 1990), and prey preference of phytoseiid mites is often positively related to fitness and reproductive success (Dicke et al. 1990; Rovenská et al. 2005). We also observed that some eggs were partially consumed. After some hours, the partially consumed eggs dehydrated and shrunk, and it was no longer possible to see the difference between partially consumed and totally consumed prey eggs. Hence, partial consumption could only be assessed by frequent observation. When satiation of predators decreases, the tendency to capture prey increases, and partial prey consumption occurs when less than the content of an entire prey is needed for full satiation (Sabelis 1990). Most of the eggs from the laboratory were partially consumed, whereas most eggs from the field were completely consumed. This suggests that the predators were satiated sooner when they fed on eggs from the laboratory than when they fed on eggs from the field.

The potential differences in the quality of eggs from different origins can have several causes. The plants in the field and from the laboratory were of the same population: the nursery used seeds from the field to produce seedlings. Hence, there was not much difference in genetic background of the plants. Moreover, no pesticides were used in the field or the nursery. Differences in plant quality may not only affect herbivores but also the third trophic level (Price et al. 1980; Teder and Tammaru 2002), and it is possible that herbivores feeding on plants with lower nutritional value produce eggs that are also lower in nutritional value. This would imply that, here, plants in the field were of lower quality for the herbivores

than the plants from the laboratory. This may have several reasons. First, it may have been caused by differences in environmental conditions (e.g. humidity and temperature), which may affect the eggs directly or indirectly via the host plant. Second, plants can also be of reduced quality for herbivores by containing fewer nutrients, and plant quality is also reduced by the presence of phytotoxins and secondary metabolites (Guidolin and Cónsoli 2020). The development of constitutive defences is known to be age-dependent (Boege and Marquis 2005; Quintero and Bowers 2011), and plants used in the laboratory (six months) were younger than the plants in the field (ca. four years). We rarely found *D. citri* on orange jasmine plants older than ten years in the field, which may have been caused by well-developed constitutive defences of these older plants. Hence, the plants in the field may have had better-developed defences against herbivores than the plants in the laboratory, and this may have indirectly affected the prey quality of *D. citri* eggs. In addition, the plants from the laboratory were fertilized bimonthly, but the plants in the field not.

Third, plants in the field were attacked by other species of herbivores besides *D. citri* and this may have induced defence responses in the plants (Walling 2000), which could have affected *D. citri* and its quality as prey. Fourth, differences in nutritional quality of the eggs might in theory have been caused by differences in the HLB infection of the plants (Maris et al. 2004; Mauck et al. 2010; Cen et al. 2012), but a molecular analysis showed no evidence for the infection of plants and insects from the field or from the laboratory (Supplementary Material).

In conclusion, the differences in predation rate were probably caused by nutritional differences or by the presence of hazardous compounds that needed to be detoxified (Sabelis 1990). Other possible explanations can be the different environmental conditions. It is known that animals may compensate nutritional imbalances in their diet by consuming more food that is deficient in some aspect (Mayntz et al. 2005), and the increased consumption of eggs from the field is perhaps an example of such compensatory feeding (Price et al. 1980; Rueda et al. 1991; Cruz-Rivera and Hay 2000). In contrast, when toxic compounds need to be detoxified, we would expect increased excretion but not consumption rates (Dowd et al. 1983). This compensation, in turn, then ensured that the

oviposition rate of the predators was not negatively affected by the deficient diet if the latter was present in sufficient quantity. Based on this theory, we expected an effect of diet on oviposition rates of *A. herbicolus* when it could only feed on limited numbers of eggs. A comparison of predation and oviposition with different densities of prey eggs (Fig. 2a–c) shows that predation on eggs from the field was always higher than on eggs from the laboratory. When high numbers of eggs were provided, the increased predation of eggs from the field apparently compensated for their lower quality, resulting in similar oviposition rates (Fig. 2a). With intermediate numbers of eggs (Fig. 2b), total predation of eggs from the field was still higher than that of eggs from the laboratory, but this apparently did not compensate for the low quality, resulting in lower oviposition rates. When the numbers of eggs were further limited, eggs from the rearing also did not offer sufficient resources to maintain a high oviposition rate. Hence, these results indeed further confirm the idea that eggs from the field were of lower quality than eggs from the laboratory, and that the predators can maintain high oviposition rates by compensatory feeding.

The predation rate of *A. herbicolus* on *D. citri* eggs was high, and the oviposition rate on this prey was comparable to that on pollen, a good food source for this predator (Duarte et al. 2015; Fig. 3b). Although a direct comparison of our results with the other two studies of predatory mites feeding on *D. citri* is difficult because of the different methodologies used, *A. herbicolus* seems to be a more promising biocontrol agent than *A. swirskii*, *N. cucumeris* and *N. barkeri*. The predation rates found here were much higher than those of the other three species (Juan-Blasco et al. 2012; Fang et al. 2013), and *A. herbicolus* was capable of ovipositing when feeding on *D. citri*, whereas such information is not available for *A. swirskii* (Juan-Blasco et al. 2012), and oviposition of *N. cucumeris* was low (Fang et al. 2013). Furthermore, it is not known whether *A. swirskii* and *N. cucumeris* can develop into adults when feeding on *D. citri* eggs, whereas *A. herbicolus* can (Fig. 4). Hence, our results suggest that the *A. herbicolus* is a potential good biological agent of *D. citri*.

The compatibility of different natural enemies needs to be evaluated, because using several natural enemies may have synergistic or antagonistic effects on biological control (Ferguson and Stiling 1996).

Besides the parasitoid *T. radiata*, lacewings and ladybirds are important mortality factors of *D. citri*, but coccinellids may feed on parasitized *D. citri* (Michaud 2004), which may limit the efficiency of the parasitoid. Although we have not performed predation experiments with *D. citri* nymphs as prey, we observed that *A. herbicolus* also consumed 1st instar nymphs of *D. citri*, but that they did not consume 5th instar nymphs. Further experiments are needed to confirm if other instars can be eaten by the predator, especially because the 4th and 5th instar are the preferred host stage of *T. radiata* (Chen and Stansly 2014).

In conclusion, *A. herbicolus*, a predator encountered on citrus and orange jasmine, proved to be promising as a potential biological agent for *D. citri*, consuming large amounts of eggs and being able to develop and survive when feeding on this pest. Thus, it deserves further research as biocontrol agent of *D. citri*, and indirectly of HLB, on entire plants and in the field.

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Data availability The authors provided a supplementary material for publication.

Compliance with ethical standards

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References

- Alvarez S, Rohrig E, Solís D, Thomas MH (2016) Citrus greening disease (Huanglongbing) in Florida: economic impact, management and the potential for biological control. *Agric Res* 5:109–118
- Alves GR, Diniz AJF, Parra JRP (2014) Biology of the Huanglongbing vector *Diaphorina citri* (Hemiptera: Liviidae) on different host plants. *J Econ Entomol* 107:691–696
- Arnosti A, Delalibera Junior I, Conceschi MR, D'Alessandro CP, Travaglini RV, Camargo-Mathias MI (2019) Interactions of adjuvants on adhesion and germination of *Isaria fumosorosea* on adults of *Diaphorina citri*. *Sci Agric* 76:487–493
- Bale JS, van Lenteren JC, Bigler F (2008) Biological control and sustainable food production. *Philos Trans R Soc Lond B Biol Sci* 363:761–776
- Bassanezi RB, Lopes SA, Miranda MP, Wulff NA, Volpe HXL, Ayres AJ (2020) Overview of citrus huanglongbing spread and management strategies in Brazil. *Trop Plant Pathol* 45:251–264
- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48
- Batool A, Iftikhar Y, Mughal SM, Khan MM, Jaskani MJ, Abbas M, Khan IA (2008) Citrus greening disease—a major cause of citrus decline in the world—a review. *Hort Sci (Prague)* 34:159–166
- Boege K, Marquis RJ (2005) Facing herbivory as you grow up: the ontogeny of resistance in plants. *Trends Ecol Evol* 20:441–448
- Bové JM (2006) Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. *J Plant Pathol* 88:7–37
- Catling HD (1970) Distribution of the psyllid vectors of citrus greening disease, with notes on the biology and bionomics of *Diaphorina citri*. *FAO Plant Prot Bull* 18:8–15
- Cavalcante ACC, dos Santos VLV, Rossi LC, Moraes GJD (2015) Potential of five Brazilian populations of Phytoseiidae (Acari) for the biological control of *Bemisia tabaci* (Insecta: Hemiptera). *J Econ Entomol* 108:29–33
- Cen Y, Yang C, Holford P, Beattie GAC, Spooner-Hart RN, Liang G, Deng X (2012) Feeding behaviour of the Asiatic citrus psyllid, *Diaphorina citri*, on healthy and huanglongbing-infected citrus. *Entomol Exp Appl* 143:13–22
- Chant DA, McMurtry JA (2007) Illustrated keys and diagnoses for the genera and subgenera of the Phytoseiidae of the world (Acari: Mesostigmata). Indira Publishing House, West Bloomfield
- Chant DA, McMurtry JA (1994) A review of the subfamilies Phytoseiinae and Typhlodrominae (Acari: Phytoseiidae). *Int J Acarol* 20:223–310
- Chen X, Stansly PA (2014) Biology of *Tamarixia radiata* (Hymenoptera: Eulophidae), parasitoid of the citrus greening disease vector *Diaphorina citri* (Hemiptera: Psylloidea): a mini review. *Fla Entomol* 97:1404–1413
- Chen XD, Ebert TA, Pelz-Stelinski KS, Stelinski LL (2020) Fitness costs associated with thiamethoxam and imidacloprid resistance in three field populations of *Diaphorina citri* (Hemiptera: Liviidae) from Florida. *Bull Entomol Res* 110:512–520
- Cifuentes-Arenas JC, Goes A, Miranda MP, Beattie GAC, Lopes SA (2018) Citrus flush shoot ontogeny modulates biotic potential of *Diaphorina citri*. *PLoS ONE* 13(1):e0190563
- Cruz-Rivera E, Hay ME (2000) Can quantity replace quality? Food choice, compensatory feeding, and fitness of marine mesograzers. *Ecology* 81:201–219
- Cunha JP, Chueca P, Garcerá C, Moltó E (2012) Risk assessment of pesticide spray drift from citrus applications with air-blast sprayers in Spain. *Crop Prot* 42:116–123
- Dowd PF, Smith CM, Sparks TC (1983) Detoxification of plant toxins by insects. *Insect Biochem* 13:453–468
- Demite PR, McMurtry JA, de Moraes GJ (2014) Phytoseiidae database: a website for taxonomic and distributional information on phytoseiid mites (Acari). *Zootaxa* 3795:571–577
- Dicke M, Sabelis MW, De Jong M, Alers MPT (1990) Do phytoseiid mites select the best prey species in terms of reproductive success? *Exp Appl Acarol* 8:161–173
- Duarte MVA, Venzon M, de Bittencourt MC, S, Rodríguez-Cruz FA, Pallini A, Janssen A, (2015) Alternative food promotes broad mite control on chilli pepper plants. *BioControl* 60:817–825
- Fang X, Lu H, Ouyang G, Xia Y, Guo M, Wu W (2013) Effectiveness of two predatory mite species (Acari: Phytoseiidae) in controlling *Diaphorina citri* (Hemiptera: Liviidae). *Fla Entomol* 96:1325–1333
- Ferguson KI, Stiling P (1996) Non-additive effects of multiple natural enemies on aphid populations. *Oecologia* 108:375–379
- Garnier M, Danel N, Bové JM (1984) The greening organism is a gram negative bacterium. In: Garnsey SM, Timmer LW, Dodds JA (eds) International organization of citrus virologists conference proceedings. University of California, Riverside, pp 115–124
- Gasparoto MCG, Hau B, Bassanezi RB, Rodrigues JC, Amorim L (2018) Spatiotemporal dynamics of citrus huanglongbing spread: a case study. *Plant Pathol* 67:1621–1628
- Gottwald TR, da Graça JV, Bassanezi RB (2007) Citrus huanglongbing: the pathogen and its impact. *Plant Health Prog* 8:31–67
- Gottwald T, Poole G, McCollum T, Hall D, Hartun J, Bai J, Luo W, Posny D, Duan YP, Taylor E, da Graça J, Polek M, Louws F, Schneiderf W (2020) Canine olfactory detection of a vectored phyto-bacterial pathogen, *Liberibacter asiaticus*, and integration with disease control. *PNAS* 117:3492–3501
- Grafton-Cardwell EE, Stelinski LL, Stansly PA (2013) Biology and management of Asian citrus psyllid, vector of the huanglongbing pathogens. *Annu Rev Entomol* 58:413–432
- Guidolin AS, Cônsoli FL (2020) Influence of host plant on oligophagous and polyphagous aphids, and on their obligate symbiont titers. *Biologia* 75:71–81
- Hall DG, Hentz MG, Meyer JM, Kriss AB, Gottwald TR, Boucias DG (2012) Observations on the entomopathogenic fungus *Hirsutiella citrififormis* attacking adult *Diaphorina citri* (Hemiptera: Psyllidae) in a managed citrus grove. *BioControl* 57:663–675
- Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biom J* 50:346–363

- Janssen A, Sabelis MW (2015) Alternative food and biological control by generalist predatory mites: the case of *Amblyseius swirskii*. *Exp Appl Acarol* 65:413–418
- Juan-Blasco M, Qureshi JA, Urbaneja A, Stansly PA (2012) Predatory mite, *Amblyseius swirskii* (Acari: Phytoseiidae), for biological control of Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae). *Fla Entomol* 95:543–551
- Kistner EJ, Melhem N, Carpenter E, Castillo M, Hoddle MS (2016) Abiotic and biotic mortality factors affecting Asian citrus psyllid (Hemiptera: Liviidae) demographics in southern California. *Ann Entomol Soc Am* 109:860–871
- Lee JA, Halbert SE, Dawson WO, Robertson CJ, Keesling JE, Singer BH (2015) Asymptomatic spread of huanglongbing and implications for disease control. *PNAS* 112:7605–7610
- Lenth R (2019) emmeans: estimated marginal means, aka least-squares means. <https://cran.r-project.org/package=emmeans>
- Li J, Li L, Pang Z, Kolbasov VG, Ehsani R, Carter EW, Wang N (2019) Developing citrus huanglongbing (HLB) management strategies based on the severity of symptoms in HLB-endemic citrus-producing regions. *Phytopathology* 109:582–592
- Lopes SA, Frare GF, Camargo LEA, Wulff NA, Teixeira DC, Bassanezi RB, Beattie GAC, Ayres AJ (2010) Liberibacters associated with orange jasmine in Brazil: incidence in urban areas and relatedness to citrus liberibacters. *Plant Pathol* 59:1044–1053
- Lu H, Li L, Yu LC, He LM, Ouyang GC, Liang GW, Lu YY (2019) Ectoparasitic mite, *Pyemotes zhonghuaqia* (Prostigmata: Pyemotidae), for biological control of Asian Citrus Psyllid, *Diaphorina citri* (Hemiptera: Liviidae). *Syst Appl Acarol* 24:520–524
- Maris PC, Joosten NN, Goldbach RW, Peters P (2004) Tomato spotted wilt virus improves host suitability for its vector *Frankliniella occidentalis*. *Phytopathology* 94:706–711
- Mauck KE, De Moraes CM, Mescher MC (2010) Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts. *PNAS* 107:3600–3605
- Mayntz D, Raubenheimer D, Salomon M, Toft S, Simpson SJ (2005) Nutrient-specific foraging in invertebrate predators. *Science* 307:111–113
- McClellan APD, Oberholzer PCJ (1965) *Citrus psylla*, a vector of the greening disease of sweet orange. *S Afr J Agric Sci* 8:297–298
- McMurtry JA, Scriven GT (1965) Insectary production of phytoseiid mites. *J Econ Entomol* 58:282–284
- Michaud JP (2004) Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida. *Biol Control* 29:260–269
- Miranda MP, Dos Santos FL, Bassanezi RB, Montesino LH, Barbosa JC, Sétamou M (2018) Monitoring methods for *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) on citrus groves with different insecticide application programmes. *J Appl Entomol* 142:89–96
- Monzo C, Qureshi JA, Stansly PA (2014) Insecticide sprays, natural enemy assemblages and predation on Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae). *Bull Entomol Res* 104:576–585
- Nomikou M, Janssen A, Sabelis MW (2003) Phytoseiid predators of whiteflies feed and reproduce on non-prey food sources. *Exp Appl Acarol* 31:15–26
- Parra JRP (2014) Biological control in Brazil: an overview. *Sci Agric* 71:420–429
- Pérez-Otero R, Mansilla JP, del Estal P (2015) Detección de la psila africana de los cítricos, *Triozia erytreae* (Del Guercio, 1918) (Hemiptera: Psylloidea: Trioziidae), en la Península Ibérica. *Arq Entomol* 13:119–122
- Price PW, Bouton CE, Gross P, McPherson BA, Thompson JN, Weis AE (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annu Rev Ecol Syst* 1:41–65
- Quintero C, Bowers MD (2011) Plant induced defenses depend more on plant age than previous history of damage: implications for plant-herbivore interactions. *J Chem Ecol* 37:992–1001
- R Core Team (2019). R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. <https://www.r-project.org/>
- Rovenská GZ, Zemek R, Schmidt JEU, Hilbeck A (2005) Altered host plant preference of *Tetranychus urticae* and prey preference of its predator *Phytoseiulus persimilis* (Acari: Tetranychidae, Phytoseiidae) on transgenic Cry3Bb-eggplants. *Biol Control* 33:293–300
- Rueda AA, Slansky F, Wheeler GS (1991) Compensatory feeding response of the slug *Sarasinula plebeia* to dietary dilution. *Oecologia* 88:181–188
- Sabelis MW (1990) How to analyse prey preference when prey density varies? A new method to discriminate between effects of gut fullness and prey type composition. *Oecologia* 82:289–298
- Siegel S, Castellan NJ (1988) Nonparametric statistics for the behavioral sciences. McGraw-Hill, New York, NY
- Symondson WOC, Sunderland KD, Greenstone MH (2002) Can generalist predators be effective biocontrol agents? *Annu Rev Entomol* 47:561–594
- Teder T, Tammaru T (2002) Cascading effects of variation in plant vigour on the relative performance of insect herbivores and their parasitoids. *Ecol Entomol* 27:94–104
- Therneau T (2020) A package for survival analysis in R. R package version 3.1-12. <https://cran.r-project.org/package=survival>
- van Rijn PCJ, van Houten YM, Sabelis MW (2002) How plants benefit from providing food to predators even when it is also edible to herbivores. *Ecology* 83:2664–2679
- Walling LL (2000) The myriad plant responses to herbivores. *J Plant Growth Regul* 19:195–216
- Zou X, Bai X, Wen Q, Xie Z, Wu L, Peng A, He Y, Xu L, Chen S (2019) Comparative analysis of tolerant and susceptible citrus reveals the role of methyl salicylate signaling in the response to huanglongbing. *J Plant Growth Regul* 38:1516–1528

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