Potential of two omnivorous iolinid mites as predators of the tomato russet mite, *Aculops lycopersici*

Lore Vervaet (✉ lore.vervaet@ugent.be)

  Ghent University: Universiteit Gent   https://orcid.org/0000-0003-4522-4190

Gokul Parapurath

  Ghent University: Universiteit Gent

Raf De Vis

  Research Station for Vegetable Production

Thomas Van Leeuwen

  Ghent University: Universiteit Gent   https://orcid.org/0000-0003-4651-830X

Patrick De Clercq

  Ghent University: Universiteit Gent   https://orcid.org/0000-0003-0664-1602

Abstract

Two iolinid predatory mites were studied in the laboratory as potential biological control agents of the tomato russet mite, *Aculops lycopersici* (Acari: Eriophyidae). The development, reproduction and predation capacity of *Pronematus ubiquitus* and *Homeopronematus anconai* (Acari: Iolinidae) on *A. lycopersici* were investigated. Developmental time from egg to adult at 25°C averaged 9.59, 9.31 and 9.52 days for *P. ubiquitus* on *A. lycopersici*, *A. lycopersici* and *Typha angustifolia* pollen, and pollen only, respectively. *H. anconai* required 11.18, 10.39 and 11.90 days to complete development on the respective diets. Survival of the immature stages exceeded 83% on all diets for both iolinids. In experimental arenas, both predators caused a substantial reduction of the immature population development of *A. lycopersici*. The number of tomato russet mite offspring was reduced by 78% and 57% by *P. ubiquitus* and *H. anconai*, respectively. The addition of pollen to the arena lowered this population reduction to 35% and 27% for the respective predators. However, supplementing a diet of tomato russet mites with pollen significantly increased the fecundity of both predatory mites from 14–15 to 24–25 eggs over a five-day period. The results suggest that both *P. ubiquitus* and *H. anconai* have good potential to suppress *A. lycopersici* populations and that *T. angustifolia* pollen can support population establishment of the predators.

Key Message

- *P. ubiquitus* and *H. anconai* are potential biological control agents of *A. lycopersici*
- *T. angustifolia* pollen can support population establishment of both predators
- Although pollen supplementation lowered predation rates, it substantially increased the fecundity of both iolinids
- Overall, *P. ubiquitus* developed faster and suppressed *A. lycopersici* stronger as compared to *H. anconai*
- Survival and fecundity was similar for both iolinids on all diets tested
- Further field studies are required

Introduction

Tomato, *Solanum lycopersicum* L., is globally the second most important solanaceous vegetable crop after potato (*Solanum tuberosum* L.) (Quinet et al. 2019). The annual worldwide production of tomatoes steadily increases every year and in 2020, approximately 187 million tonnes of tomatoes were produced on a ca. 5 million ha surface area (FAOSTAT 2022). The tomato russet mite, *Aculops lycopersici* (Tryon, 1917), is an economically important pest of tomatoes in many tomato growing areas for which no commercial biological control solution exists (Vervaet et al. 2021). Feeding of this mite causes massive destruction of the epidermal cell layer, resulting in the characteristic bronzing of the damaged tissue and substantial yield losses (Al-Azzazy et al. 2018; Bailey et al. 1943). In Europe, management practices are generally limited to pesticide application (e.g. abamectin and sulphur) which is often perceived as ineffective and it moreover interferes with biological control of other greenhouse tomato pests (e.g., whiteflies, spider mites and aphids) (Bailey et al. 1943; Brodeur et al. 1997; Duso et al. 2010). Additionally, due to concerns regarding the adverse effects of chemical pesticides on human health and the environment, there is an increasing demand for pesticide-free vegetables (Gerson et al. 2007; Van Lenteren et al. 2020). Consequently, it is imperative to find effective biological control agents against *A. lycopersici*.

Although *A. lycopersici* is a suitable food source for various natural enemies in the laboratory, especially for predatory mites of the Phytoseiidae, they are unable to control the tomato russet mite on tomato crops (van Houten et al. 2013; Vervaet et al. 2021). Tomato plants are covered with trichomes. These protect the plants from a range of herbivores but also hamper or kill natural enemies, including phytoseiid predators (Kant et al. 2015; van Houten et al. 2013). The minute size of the tomato russet mite (150–200μm) allows it to move between the trichomes that create a shelter against most of its competitors and predators (van Houten et al. 2013).

The iolinid predatory mites *Homeopronematus anconai* (Baker, 1943) and *Pronematus ubiquitus* (McGregor, 1932), however, are considerably smaller (225–280μm) than phytoseiid mites (Baker 1943; Knop et al. 1983; McGregor 1932). This allows them to navigate under and between the tomato trichomes without being hindered and may thus enable them to contribute to the biological control of the tomato russet mite. Both iolinids are distributed throughout the world and have been observed on citrus (Ueckermann et al. 2007), apple (Bayan 1986), grape (Knop et al. 1983), fig (Abou-Awad et al. 1999) and different solanaceous crops, including tomato (Abou-Awad 1979; Brodeur et al. 1997; Hessein et al. 1986; Kawai et al. 2004). Moreover, they are predators of different eriophyids, including *A. lycopersici* (Abou-Awad 1979; Hessein et al. 1986; Kawai et al. 2004; Pijnakker et al. 2021; Van Houten et al. 2020). Besides predation on other arthropods, these iolinids display various feeding habits and have been reported to feed on pollen and plant tissue (without causing obvious damage), fungi and dead arthropods (Flaherty et al. 1971; Hessein et al. 1986; Jeppson et al. 1975; Knop et al. 1983; Pijnakker et al. 2021; Walter et al. 2009). The pollinivory of both iolinids offers potential to pre-establish populations in tomato crops with supplementation of pollen and enables them to sustain their populations in a tomato crop when prey is scarce or absent (Pijnakker et al. 2021). In a recent greenhouse study, *P. ubiquitus* was shown to be capable of simultaneously controlling a tomato russet mite infestation and powdery mildew infection (*Oidium neolycopersici* L.) on potted tomato plants (Pijnakker et al. 2021). The simultaneous protection of this mite against pest and pathogen was thought to show potential to solve the interference of fungicides and sulphur applications with biological control programs.

The objective of the present laboratory study was to compare the potential of *P. ubiquitus* and *H. anconai* as biological control agents of *A. lycopersici*. In particular, the developmental and reproductive performance of the two predatory mites was assessed, using *A. lycopersici*, *Typha angustifolia* pollen or a combination of both as food.

Materials And Methods

Cultures and plant materials

All cultures and experimental arenas were maintained in climatically controlled chambers (PHCBI MLR-352H-PE, Japan). Plants of tomato (*Solanum lycopersicum.*, cv. 'Moneymaker') and common bean (*Phaseolus vulgaris* L. cv. 'Prelude') were grown in an experimental greenhouse at the Department of Plants and Crops, Ghent University. Fresh cattail pollen (*Typha angustifolia* L., Nutrimite™) was supplied by Biobest N.V. (Westerlo, Belgium) and stored at -18°C. Before use in the experiments, a small amount of pollen was thawed and kept at 4°C for maximum one week.

The laboratory colony of *P. ubiquitus* was initiated with mites collected on angel's trumpet (*Brugmansia* sp.) in Ternat, Belgium (50.866798 N, 4.159654 E). The colony of *H. anconai* was established from individuals collected on *Viburnum burkwoodii* Burkwood & Skipwith and *Stephanandra incisa* L. at the Research Station for Vegetable Production in Sint-Katelijne-Waver, Belgium (51.078727 N, 4.529423 E). The colonies were reared on bean leaves which were placed with the lower side down on a 2 cm layer of water-saturated cotton in a plastic tray. The plastic trays (17.4 x 12.4 x 4.2 cm) were covered with a plastic lid (17.8 x 12.4 x 4.2 cm) having a central hole of 5 cm diameter. Tissue paper strips were placed half on the leaf margins and half on the cotton to provide water to the mites and prevent them from escaping. Once a week, the mites were supplied ad libitum with cattail pollen. The colonies of both iolinids were maintained at 25 ± 0.5°C, 65 ± 5% relative humidity (RH) and a photoperiod of 16:8 h (L:D).

Tomato russet mites, *A. lycopersici*, were collected from a natural infestation on tomatoes (*S. lycopersicum* cv. 'Merlice') grown in a greenhouse at the Research Centre Hoogstraten in Belgium (51.471169 N, 4.798792 E) and were maintained on young tomato plants (*Solanum lycopersicum*, cv. 'Moneymaker') in an environmental chamber at 27 ± 0.5°C, 50 ± 5% RH and a photoperiod of 16:8 h (L:D).

Experimental set-up

All experiments were conducted in a controlled environmental chamber at 25 ± 0.5°C, 65 ± 5% RH and a photoperiod of 16:8 h (L:D). Freshly excised tomato leaf disks (*Solanum lycopersicum*, cv. 'Moneymaker', 2.1 by 2.1cm) were used as arenas. The leaves were placed with the upper surface facing up on water-saturated cotton in plastic Petri dishes (ø 5.1cm) with a mesh-covered hole (ø 1.4 cm) in the lid. The edges were covered with moist pieces of tissue paper to provide free water and prevent the mites from escaping and drowning.

Effects of diet on development and survival of *P. ubiquitus* and *H. anconai*

For each predator and diet, 20 to 30 fresh eggs (less than 8 hours old) from the stock colony were transferred individually to a tomato leaf arena. The predators were supplied ad libitum with one of the following food sources: (1) cattail pollen, (2) mixed stages of *A. lycopersici*, and (3) mixed stages of *A. lycopersici* + cattail pollen. The mixed stages of *A. lycopersici* were provided by placing a 1-cm section of infected stem from the stock colony on the tomato leaf disk for 24h. Pollen was placed on the arenas using a fine brush, twice a week. Development and survival of the predators were monitored twice a day. Once the adult stage was reached, the sex of the predators was determined. Whenever the quality of a leaf substrate began to deteriorate, it was replaced with a fresh leaf. To avoid injury to a predatory mite during transfer, a small piece of the leaf disk containing the mite was cut out and transferred to the new leaf disk. As soon as the predator had moved to the new disk, the old leaf section was removed.

Fecundity and impact of P. ubiquitus **and** H. anconai **on the population development of** A. lycopersici, **in the presence or absence of pollen**

In this experiment, the predation and reproduction capacity of *P. ubiquitus* and *H. anconai* on *A. lycopersici* was assessed. In addition, the effect of pollen on the performance of the predatory mites was evaluated. Four treatments were set up: *P. ubiquitus* with or without pollen and *H. anconai* with or without pollen. Two-day-old female predatory mites were starved for 24 h, i.e. they were kept on a tomato leaf without any additional food, and then confined individually on tomato leaf disks inoculated with fifty tomato russet mite adults. In the treatments with pollen, fresh cattail pollen were provided at the start of the experiment and on the third day. Controls consisted of tomato leaf disks infested with fifty tomato russet mites, with or without pollen and without a predatory mite. For each treatment, 14 to 21 replicates were set-up. Over the next five days, the number of tomato russet mite eggs, nymphs and adults was recorded daily, as well as the number of predatory mite eggs.

Statistical analysis

All data were analyzed using RStudio 4.0.2. Data from mites that drowned were not included in the analysis. Normality was visually assessed using normal QQ-plots, as well as using Shapiro-Wilk normality tests. The data for developmental time were analyzed in hours using a generalized linear model (GLM). A quasi-Poisson distribution was used instead of a Poisson distribution to correct for overdispersion. The significance of interactions and factors was assessed by removing them from the model using the ANOVA function of R until a minimum adequate model was reached (Crawley 2007). In addition, contrasts among treatments were determined with general linear hypothesis testing (glht function from the multcomp package) to perform Tukey HSD tests for post-hoc pairwise comparisons (Hothorn et al. 2008). Survival rates and sex ratios had a binomial distribution and were analyzed using a GLM with a logit link and a binomial error function. Tukey's post hoc analysis was used to identify significant differences between treatments. The non-normally distributed predation data were analyzed using generalized linear mixed effect models (GLMMs). A negative binomial distribution was used instead of a Poisson distribution to correct for overdispersion. The number of predatory mite eggs and tomato russet mite adults that were killed after five days were compared using a GLM with a quasi-Poisson distribution due to the overdispersion of the data. In addition, contrasts among treatments were determined with general linear hypothesis testing. For all tests, the level of statistical significance was set at p = 0.05.

Results

Effects of diet on development and survival of *P. ubiquitus* and *H. anconai*

Both *P. ubiquitus* and *H. anconai* have six life stages: egg, larva, three nymphal stages (protonymph, deutonymph, tritonymph) and adult; in between the active stages, a distinct quiescent phase (chrysalis) occurs. Feeding was observed in all active stages. The durations of the different immature stages and the total developmental times on the three tested diets are shown in Table 1; the results of the GLMs conducted are reported in Supplementary Table 1. Total developmental time (egg to adult) was affected by predator species (GLM: $F_{1,139}$ = 117.16; $p < 0.001$), diet (GLM: $F_{2,137}$ = 8.12; $p < 0.001$) and by their interaction (GLM: $F_{2,135}$ = 4.90; $p = 0.009$). Diet had a clear effect on the total developmental time of *H. anconai* but not on that of *P. ubiquitus*. The latter completed its development faster as compared to *H. anconai* on all three diets. *P. ubiquitus* showed similar egg to adult developmental times of 9.31−9.59 days on the different diets while *H. anconai* required 10.39−11.90 days. Diet had no significant effect on the durations of the different developmental stages of *P. ubiquitus*. *H. anconai*, on the contrary, developed significantly faster on a mixed diet as compared to pollen alone ($p < 0.001$) but not as compared to *A. lycopersici* alone ($p = 0.120$). However, there was no significant difference between the two single diets ($p = 0.205$). In *H. anconai*, the developmental times of the deutonymph and the four chrysalis stages were not affected by diet. In contrast, in the larval, protonymph and tritonymph stage, diet did have a significant effect on the developmental time of these stages. The larval stage developed faster on *A. lycopersici* alone as compared to pollen alone ($p = 0.016$) but not as compared to a mixed diet ($p = 0.969$). Developmental time of the protonymph stage on a mixed diet was shorter than that on pollen alone ($p = 0.001$) but was similar to that on *A. lycopersici* alone ($p = 0.413$). The tritonymph stage was significantly shorter on the mixed diet compared to either pollen ($p = 0.045$) or tomato russet mites alone ($p = 0.001$), whereas there was no difference between the single diets ($p = 0.892$).

Table 1

Effect of three diets on the developmental time (days) of *P. ubiquitus* and *H. anconai* on tomato leaf disks at 25 ± 0.5°C and 65 ± 5% RH.

Predator/Diet	Developmental time									
	n	Egg	Larva	Chrysalis 1	Protonymph	Chrysalis 2	Deutonymph	Chrysalis 3	Tritonymph	Chrysalis 4	Total
P. ubiquitus											
Pollen[a]	29	4.17 ± 0.06a	1.16 ± 0.04abc	0.52 ± 0.02a	0.60 ± 0.04a	0.49 ± 0.01a	0.64 ± 0.04ab	0.49 ± 0.02a	0.90 ± 0.05ab	0.55 ± 0.03a	9.52 ± 0.08a
A. lycopersici	29	4.19 ± 0.05a	1.10 ± 0.07ab	0.53 ± 0.02a	0.66 ± 0.07ab	0.48 ± 0.01a	0.59 ± 0.03a	0.49 ± 0.02a	0.90 ± 0.08ab	0.64 ± 0.05ab	9.59 ± 0.19a
A. lycopersici + pollen[a]	30	4.28 ± 0.05ab	0.98 ± 0.04a	0.53 ± 0.03a	0.56 ± 0.03a	0.52 ± 0.03a	0.59 ± 0.04a	0.49 ± 0.02a	0.73 ± 0.05a	0.65 ± 0.04ab	9.31 ± 0.10a
H. anconai											
Pollen[a]	20	4.23 ± 0.05ab	1.95 ± 0.16d	0.83 ± 0.05b	1.10 ± 0.10c	0.53 ± 0.03a	0.83 ± 0.07ab	0.48 ± 0.03a	1.13 ± 0.09bc	0.85 ± 0.05c	11.90 ± 0.28b
A. lycopersici	20	4.06 ± 0.13a	1.40 ± 0.11bc	0.78 ± 0.06b	0.87 ± 0.09bc	0.55 ± 0.04a	0.87 ± 0.14b	0.53 ± 0.03a	1.26 ± 0.12c	0.79 ± 0.05bc	11.18 ± 0.33bc
A. lycopersici + pollen[a]	23	4.59 ± 0.09b	1.52 ± 0.18cd	0.70 ± 0.05b	0.68 ± 0.05ab	0.55 ± 0.03a	0.68 ± 0.07ab	0.55 ± 0.03a	0.79 ± 0.07a	0.76 ± 0.06bc	10.39 ± 0.20c

[a]pollen from *T. angustifolia*. Means ± SE within a column followed by different letters are significantly different (Multiple Comparisons of Means: Tukey HSD tests; $p < 0.05$).

On all diets, survival of the immature stages exceeded 90% for *P. ubiquitus* and 85% for *H. anconai* (Fig. 1). The interaction between predator species and diet was not significant (Logit: χ^2 = 2.8375, df = 2, $p = 0.2420$) and there was no significant main effect of predator species (Logit: χ^2 = 3.4404, df = 1, $p = 0.0636$) on survival from egg to adult. Diet did have a significant effect on survival from egg to adult (Logit: χ^2 = 9.6712, df = 2, $p = 0.0082$). A Tukey post hoc analysis indicated a significant difference in survival between the pollen and *A. lycopersici* + pollen diet treatments ($p = 0.0210$) but there were no differences for the other contrasts ($p > 0.05$). Sex ratios (mean percentage of females ± SE) on pollen, *A. lycopersici* and *A. lycopersici* + pollen were 48 ± 9%, 41 ± 9% and 44 ± 10% for *P. ubiquitus* and 70 ± 10%, 65 ± 12% and 53 ± 11% for *H. anconai*, respectively. *P. ubiquitus* had a significantly lower proportion of females on each diet compared to *H. anconai* (Logit: χ^2 = 7.2995, df = 1, $p = 0.0069$). On the other hand, diet did not have an effect on sex ratio in either predator species (Logit: χ^2 = 0.3941, df = 2, $p = 0.8214$). Interaction between diet and predator species was not significant (Logit: χ^2 = 0.0083, df = 2, $p = 0.9958$).

Fecundity and impact of P. ubiquitus **and** H. anconai **on the population development of** A. lycopersici, **in the presence or absence of pollen.**

From the first day onwards, the population of tomato russet mites increased quickly in the controls, reaching up to 290 offspring individuals per tomato leaf arena. Both *P. ubiquitus* and *H. anconai* successfully suppressed the population growth of *A. lycopersici* on the small scale laboratory arenas, with or without pollen as a supplementary food source (Fig. 2). In the absence of pollen (Fig. 2a), the number of nymphs and eggs of *A. lycopersici* on the fifth day averaged 290 ± 11 (mean ± SE) in the control compared to 64 ± 10 when *P. ubiquitus* was present. The population of immature tomato russet mites was thus reduced by 78% in the presence of *P. ubiquitus*. When pollen was available as a supplementary food source (Fig. 2b) the reduction of the immature pest population on day five averaged 35% (236 ± 21 offspring in the control versus 155 ± 21 in the treatment). A similar trend was found for *H. anconai*. With *A. lycopersici* as a sole food source (Fig. 2c), *H. anconai* reduced the prey population from 234 ± 10 in the control to 110 ± 17 in the treatment (-57%) on day five. When pollen was supplemented (Fig. 2d), offspring numbers of *A. lycopersici* after 5 days averaged 219 ± 19 in the control versus 159 ± 10 in the treatment (-27%). The addition of pollen significantly lowered the predation of adult tomato russet mites by both iolinids (Fig. 3; GLM, $F_{1,68}$ = 70.059; $p < 0.001$). There was a significant difference in predation of adult *A. lycopersici* between the two mite species (GLM, $F_{1,69}$ = 12.618; $p < 0.001$). At the end of the experiment, *P. ubiquitus* showed the highest predation rates on tomato russet mite adults in the absence of pollen, with an average of 13 ± 2.2 killed adults, versus 8 ± 1.1 adults for *H. anconai* ($p = 0.038$). When pollen was added the difference between the two predators disappeared ($p = 0.578$) and only 2.0 ± 0.3 and 1.0 ± 0.3 tomato russet mite adults were killed after 5 days by *P. ubiquitus* and *H. anconai*, respectively.

P. ubiquitus and *H. anconai* showed similar fecundity on either diets (GLM, $F_{1,69}$ = 0.0717; $p = 0.7897$). However, in both predatory mites fecundity was significantly affected by diet (Fig. 4; GLM, $F_{1,69}$ = 29.968; $p < 0.001$). On a tomato russet mite diet, total oviposition over the course of 5 days averaged 14.5 ± 1.7 and 14.4 ± 1.4 eggs for *P. ubiquitus* and *H. anconai*, respectively ($p > 0.999$). A combination of pollen and *A. lycopersici* resulted in an average oviposition of 24.5 ± 2.3 and 24.4 ± 2.0 eggs over the course of 5 days for the respective predators ($p > 0.999$). The addition of *T. angustifolia* pollen to the diet of *A. lycopersici* and plant sap (obtained from the leaf disk) substantially increased the reproductive performance of both *P. ubiquitus* ($p = 0.003$) and *H. anconai* ($p < 0.001$).

Discussion

This laboratory study shows that *A. lycopersici* is an acceptable prey for both *P. ubiquitus* and *H. anconai*. The iolinids were able to attack and kill the tomato russet mites and effectively exploit them as food, allowing complete development. Both predatory mites were observed piercing and sucking the contents of all tomato russet mite stages. They were also able to successfully develop from egg to adult on fresh cattail pollen or on a mixed diet of pollen and tomato russet mites.

The durations of the different life stages of *P. ubiquitus* on the diets tested in the present study are considerably shorter than those reported by Abou-Awad et al. (1999) for this predator when feeding on the fig leaf mite *Rhyncaphytoptus ficifoliae* (Keifer) or the fig bud mite *Eriophyes ficus* (Cotte) at 29°C and 70−80% RH. These authors reported a mean total developmental time of *P. ubiquitus* females of 19 and 19.5 days on the respective prey species. Under similar climatic conditions (24−25°C), the total developmental duration of *H. anconai* in this study was shorter than that reported by Knop et al. (1983) who offered the iolinid cattail pollen on blackberry leaves (13.6 days). In the present study, the developmental time of *H. anconai* was shorter on a mixed prey-pollen diet compared to pollen alone. Hessein et al. (1988) noted a positive influence of a mixed diet of *A. lycopersici* and *Typha latifolia* L. pollen on the survival of *H. anconai* compared to either food alone. A beneficial effect of a mixed diet on life history parameters has been reported for other predatory mites as well (Messelink et al. 2008; Muñoz-Cárdenas et al. 2014; van Rijn et al. 2002). Whereas this beneficial effect of a mixed diet was not observed on immature survival in our experiment, survival of the immature stages exceeded 83% for both mites on all diets.

Both *P. ubiquitus* and *H. anconai* effectively suppressed the population increase of the tomato russet mite on the small scale laboratory arenas, with or without pollen as a supplementary food source (Fig. 2). These results confirm earlier reports of effective control of *A. lycopersici* by *H. anconai* (Kawai et al. 2004) and *P. ubiquitus* (Pijnakker et al. 2021). However, the suppressive effect of *P. ubiquitus* on the pest's population growth was stronger compared to that of *H. anconai*, with or without pollen supplemented. Additionally, our results indicate that the presence of pollen decreases the overall predation of immature and adult tomato russet mites by both predatory mites. Without pollen, a significant reduction of the tomato russet mite population was found from the second day onwards for *P. ubiquitus* compared to the control treatment, while this effect was only significant from the third day for *H. anconai*. With pollen available, a significant reduction of the pest population was found only from the fourth day onwards for *P. ubiquitus*, whereas this effect was further delayed to the fifth day for *H. anconai*. In the absence of pollen, *P. ubiquitus* kills more adult tomato russet mites than *H. anconai*. When pollen is present, this difference between the iolinid species disappears but only few adults are killed. These findings agree with those of Hessein et al. (1988), who reported that *H. anconai* consumed less tomato russet mite adults in the presence of *Typha latifolia* L. pollen.

Although the addition of pollen to the arena lowered the tomato russet mite predation by both iolinids, it substantially increased the fecundity of the predatory mites. The average number of 3−5 eggs/female/day in *P. ubiquitus* observed over a five-day period in the present study is higher compared to the 1.6−1.9 eggs/female/day reported by Abou-Awad et al. (1999) on the fig leaf mite and the fig bud mite, both on fig leaf arenas, but similar to the 2.5 eggs/female/day found by Duarte et al. (2021) on *T. angustifolia* pollen supplied on tomato leaf arenas. Similarly, studies done by Hessein et al. (1988) showed that *H. anconai* laid a significantly higher number of eggs on *A. lycopersici* plus cattail pollen and on cattail pollen alone than on a sole diet of *A. lycopersici*. The numbers of eggs laid by *H. anconai* reported in the latter study (1−2 eggs/female on day 5) are lower than those in our study (3−5 eggs/female). This difference could be related to the plastic substrate used in the study by Hessein et al. (1988). The availability of leaf tissue is key to the fitness of *H. anconai* (Flaherty et al. 1971; Hessein et al. 1988; Knop et al. 1983) and *P. ubiquitus* (Vervaet et al., unpublished). According to Knop et al. (1983), *H. anconai* fails to reproduce on an artificial substrate (plastic or cork) with or without cattail pollen and survival is low. Hessein et al. (1988)

reported some reproduction of *H. anconai* on a plastic substrate when offered *A. lycopersici* alone or *A. lycopersici* with cattail pollen, but the availability of leaf tissue clearly enhanced population development. Duarte et al. (2021) reported that *P. ubiquitus* females taken from the mass-rearing units and allowed to lay eggs for four days on a tomato leaf-only diet produced less than 0.5 eggs/female/day. However, this reproductive output may be the result of their food uptake during the rearing. On the other hand, attempts to rear *H. anconai* on grape leaf alone (Flaherty et al. 1971; Hessein et al. 1988; Knop et al. 1983) or *P. ubiquitus* on tomato leaf alone (Vervaet et al., unpublished) failed. Thus, whereas feeding on leaf tissue alone provides sufficient nutrients to support survival of both iolinids to some extent, it is insufficient to sustain reproduction. Whether leaf tissue provides one or more essential nutrients, moisture, specific microhabitat requirements, ovipositional cues, or a combination of these, remains unclear.

An effective biological agent against *A. lycopersici* should meet several requirements (Vervaet et al. 2021). First, tomato is an unsuitable host plant for many predators because of the abundant (glandular) trichomes. The results obtained in our study confirm earlier reports that both *H. anconai* and *P. ubiquitus* are not hindered by these trichomes and perform well on tomato leaf surfaces (Kawai et al. 2004; Pijnakker et al. 2021). Furthermore, a tomato russet mite infestation is difficult to detect and early symptoms are easily missed or misdiagnosed as a nutritional deficiency, plant disease or water stress (Vervaet et al. 2021). The preventive establishment of a predator to create a 'standing army' against *A. lycopersici* offers a solution to this problem (Pijnakker et al. 2021). The suitability of pollen as a diet may increase the potential of biological control agents by enabling them to pre-establish well in the crop before the pest is present and to sustain their population when pest densities are low, as well as by making mass-production easier and cheaper (Duarte et al. 2021; McGregor et al. 2020). Our results corroborate earlier reports that pollen is an excellent food source for *H. anconai* and *P. ubiquitus* (Duarte et al. 2021; Flaherty et al. 1971; Hessein et al. 1988; Knop et al. 1983). Duarte et al. (2021) reported that pollen needs to be supplemented at least every other week to allow a sufficient population build-up of *P. ubiquitus* in tomato. Whether pollen supplementation needs to be continued even when *A. lycopersici* is present in the crop remains to be investigated in greenhouse conditions. Based on the findings of the present study, the positive effect of provisioning pollen on the fecundity of both iolinids is expected to compensate the associated decrease in predation on *A. lycopersici* in the longer run, but this also remains to be investigated under practical field conditions. Finally, compatibility with other management strategies in the greenhouse is key to the success of a biological control agent. Pesticides can negatively interfere with the performance of natural enemies in the crop. Five compounds (dicofol, abamectin, sulphur, cyhexatin and thuringiensin) tested on *A. lycopersici* proved toxic to *H. anconai*, but selective doses of abamectin showed the best potential to control *A. lycopersici* without reducing predator numbers (Royalty et al. 1987). Sulphur, widely used in greenhouse production as a fungicide, was also noted to be highly detrimental to *P. ubiquitus* (Van Houten et al. 2020). Interestingly, *P. ubiquitus* was recently found to simultaneously control a tomato russet mite infestation and a powdery mildew infection (*Oidium neolycopersici*) on potted tomato plants, suggesting that augmentative releases of this iolinid might reduce the dependence on sulphur in tomato greenhouses (Pijnakker et al. 2021). In addition, intraguild predation with other beneficials in the greenhouse ecosystem should be investigated. Given the small size of both iolinids, the direct effects of most of the currently used beneficials in tomato greenhouses are expected to be limited. However, as both iolinids feed on plant sap as well, their performance might be influenced by indirect effects associated with induced plant defenses triggered by other omnivorous natural enemies (e.g. Miridae predatory bugs (Pérez-Hedo et al. 2015)).

In conclusion, the present laboratory study indicates that the omnivorous predatory mites *P. ubiquitus* and *H. anconai* can play a crucial role in the biological control of the tomato russet mite in protected tomato cultivation. *A. lycopersici, T. angustifolia* pollen or a combination of both as a diet are adequate food sources to sustain development and reproduction of both iolinid mites. Overall, *P. ubiquitus* developed faster and suppressed the population development of *A. lycopersici* stronger as compared to *H. anconai*. Survival and fecundity on the tested diets was similar for both iolinids. Further field studies are required to determine whether augmentative releases of *P. ubiquitus* or *H. anconai* can maintain *A. lycopersici* under economic threshold levels as well as to optimize pollen supplementation to help establish and sustain populations of the iolinids.

Declarations

Author contributions

All authors contributed to the study conception and design. Experiments were conducted by Lore Vervaet and Gokul Parapurath. Data analysis was performed by Lore Vervaet and Patrick De Clercq. The first draft of the manuscript was written by Lore Vervaet and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

The datasets generated and/or analyzed in the current study are available from the corresponding author on request.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals (vertebrates) performed by any of the authors.

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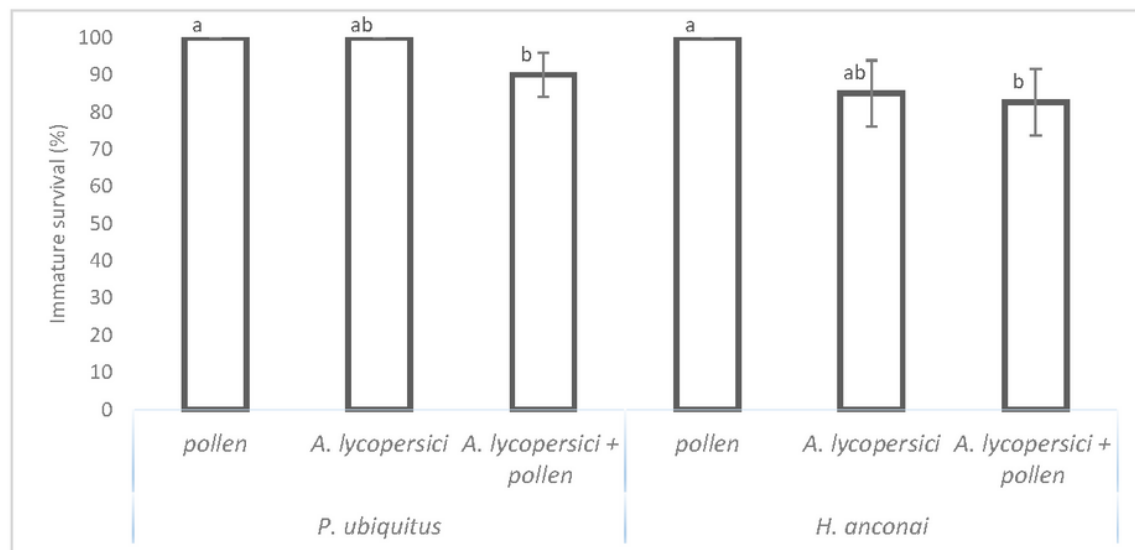
Figures

Figure 1

Immature survival (mean ± SE) of P. ubiquitus and H. anconai on three diets (T. angustifolia pollen, A. lycopersici and A. lycopersici + pollen) on tomato leaf disks at 25 ± 0.5°C and 65 ± 5% RH. Different letters above bars indicate significant differences among treatments (contrasts after GLM, p < 0.05)

Figure 2

Effect of *P. ubiquitus* without (a) or with (b) pollen and *H. anconai* without (c) or with (d) pollen on the population dynamics of *A. lycopersici* (mean number of tomato russet mite (TRM) eggs and immatures ± SE) over a five-day period at 25 ± 0.5°C and 65 ± 5% RH on tomato leaf disks. Different lowercase and uppercase letters denote significant differences among treatments of TRM eggs and TRM immatures, respectively (contrasts after GLM, p < 0.05).

Figure 3

Total number of *A. lycopersici* adults killed (mean ± SE) over a five-day period by *P. ubiquitus* and *H. anconai* on tomato leaf disk with tomato russet mites alone (*A. lycopersici*) or in combination with *T. angustifolia* pollen (*A. lycopersici + pollen)* at 25 ± 0.5°C and 65 ± 5% RH. Different letters denote significant differences among treatments (contrasts after GLM, p < 0.05)

Figure 4

Total number of eggs (mean ± SE) laid over a five-day period by *P. ubiquitus* and *H. anconai* on tomato disks when fed with tomato russet mite alone (*A. lycopersici*) or in combination with *T. angustifolia* pollen (*A. lycopersici* + pollen) at 25 ± 0.5°C and 65 ± 5% RH. Different letters denote significant differences among treatments (contrasts after GLM, p < 0.05)

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