Provision of astigmatid mites as supplementary food increases the density of the predatory mite *Amblyseius swirskii* in greenhouse crops, but does not support the omnivorous pest, western flower thrips

Fatemeh Pirayeshfar · **Seyed Ali Safavi** · **Hamid Reza Sarraf Moayeri** · **Gerben J. Messelink**

Abstract Astigmatid mites can be used as prey for mass rearing of phytoseiid predators, but also as a supplemental food source to support predator populations in crops. Here we evaluated the potential of six species of astigmatid mites (living or frozen) as alternative food for the predatory mite *Amblyseius swirskii* Athias-Henriot in greenhouse crops. All prey mites tested were suitable for predator oviposition. In general, oviposition was greater when prey mites were reared on dog food with yeast than when they were reared on wheat bran with yeast. Amongst prey items provided as frozen diet, larvae of *Thyreophagus entomophagus* (Laboulbene), *Acarus siro* L. and *Lepidoglyphus destructor* (Schrank) that had been reared on dog food with yeast, resulted in the highest oviposition rates of *A. swirskii. T. entomophagus*
larvae as frozen diet resulted in the shortest preimaginal developmental time of *A. swirskii*. On chrysanthemum plants, we found that the greatest increase in predator density occurred when living mites of *T. entomophagous* were used as a food source. This increase was greater than when predators were fed cattail pollen, a commonly used supplemental food. Effects on predators of providing living *A. siro* and *L. destructor*, or frozen larvae of *T. entomophagous* as food, were comparable with provision of pollen. Use of supplemental food in crops can be a risk if it is also consumed by omnivorous pests such as western flower thrips, *Frankliniella occidentalis* Pergande. However, we showed that both frozen and living mites of *T. entomophagous* were unsuitable for thrips oviposition. Hence, we believe that provision of prey mite species increases *A. swirskii* density, supporting biological control of thrips and other pests in greenhouse crops.

Keywords Factitious diets · Storage mites · *Thyreophagus entomophagus* · Pollen · *Frankliniella occidentalis*

F. Pirayeshfar (✉) · S. A. Safavi
Department of Plant Protection, Faculty of Agriculture, Urmia University, P.O. Box 57135-165, Urmia, West Azerbaijan, Iran
e-mail: f.pirayeshfar@urmia.ac.ir

F. Pirayeshfar · G. J. Messelink
Business Unit Greenhouse Horticulture, Wageningen University & Research, P.O. Box 20, 2265 ZG Bleiswijk, The Netherlands

H. R. S. Moayeri
Department of Plant Protection, Faculty of Agriculture, University of Zanjan, P.O. Box 45195-313, Zanjan, Iran

Introduction

Predatory mites in the family Phytoseiidae are one of the most important natural enemies used in augmentative biological pest control (Gerson and Weintraub

2007; McMurtry et al. 2013; van Lenteren et al. 2018). A major breakthrough in biological control was the discovery that astigmatid mites could be used as prey/ food for rearing of phytoseiid predatory mites, enabling mass production of predators at low cost (Schliesske 1981; Ramakers and van Lieburg 1982). Many species of predatory mites are now mass produced on astigmatid mites, although the production systems reported in the literature remain limited (Bolckmans and van Houten 2006; Simoni et al. 2006; Midthassel et al. 2013). Generalist phytoseiid predatory mites, classified as Type III, feed on multiple prey species and also pollen (McMurtry et al. 2013), and are the predatory mites best suited to mass rearing on astigmatids (Barbosa and de Moraes 2015). Amongst them, *Amblyseius swirskii* (Athias-Henriot) is widely used because of its efficacy against a wide range of important pests, including thrips, whiteflies and plant-feeding mites (Nomikou et al. 2002; Messelink et al. 2006, 2008, 2010; Arthurs et al. 2009; van Maanen et al. 2010). Currently, *A. swirskii* is used in more than 50 countries for biological pest control in vegetable and ornamental crops, making it one of the most successful biological control agents worldwide (Buitenhuis et al. 2015; Calvo et al. 2015; Janssen and Sabelis 2015). However, releases of predatory mites do not always result in good establishment, mainly due to a lack of suitable food sources in the crops. This is particularly the case in ornamental crops where pest tolerance is low.

One solution to overcoming food scarcity is the provision of alternative food sources (Messelink et al. 2014; Janssen and Sabelis 2015). Pollen has been widely used as an alternative food source and can successfully support predatory mite populations and increase pest control (Nomikou et al. 2010). However, pollen is also an excellent food source for pests such as western flower thrips, *Frankliniella occidentalis* Pergande leading to temporarily high thrips densities under some circumstances (van Rijn et al. 2002; Leman and Messelink 2015; Vangansbeke et al. 2016). Besides pollen, there are many other factitious (nonnatural) hosts that offer opportunities as alternative food sources for predatory mite populations in crops. These include *Artemia* sp. cysts, *Ephestia kuehniella* Zeller eggs and different species of astigmatid mites (Hoogerbrugge et al. 2008; Messelink et al. 2009).

Astigmatid mites as an alternative food for predatory mites has particular potential because of the low cost of mass production and the recent finding that, for at least one species, they do not support reproduction of thrips (Pirayeshfar et al. 2020). The use of astigmatid mites in greenhouse crops is increasingly being explored in practice (Messelink et al. 2014). Many species of astigmatid mites have been evaluated for use in the mass production of predatory mites, such as *Acarus farris* (Oudemans) (Ramakers and van Lieburg 1982), *Carpoglyphus lactis* (L.) (Bolckmans and van Houten 2006; Nguyen et al. 2013), *Suidasia medanensis* (Oudemans) (Midthassel et al. 2013), *Lepidoglyphus destructor* (Schrank) (Simoni et al. 2006) and *Tyrophagus putrescentiae* (Schrank) (Riahi et al. 2017). However, little is known about the performance of different astigmatid mite species in crops and their potential contribution as alternative food for predatory mite populations.

Prey mites that are suitable for mass rearing are not necessarily suitable for application in the crop, because of the totally different environmental conditions in crops compared with mass rearing systems. Considerations for evaluation include whether astigmatid mites cause any potential feeding damage to young and soft plant tissues (Hiroshi 1991), and whether high numbers of astigmatid mites pose a risk to human health because of their association with allergies (Johansson et al. 1994; Hubert et al. 2018). One method to minimize the risk of allergies is to freeze-kill the mites before application. This was recently evaluated for the astigmatid mite *T. putrescentiae* by Pirayeshfar et al. (2020) who found that frozen stages could support development and oviposition of *A. swirskii* in the laboratory, but not on plants in a greenhouse. Based on this study, we hypothesise that it might be worthwhile testing the same method with other astigmatid mite species. Interestingly, the study of Pirayeshfar et al. (2020) showed that both the prey life stage and the food on which it had been reared affected predatory mite performance. Highest oviposition rates in predatory mites were achieved when they were fed on diets based on frozen prey larvae.

In the current study, the potential of living and frozen stages (eggs and larvae) of six species of astigmatid mites to support oviposition and population increase of *A. swirskii* were compared with two sources of pollen. In addition, we evaluated the suitability of astigmatid mite stages as a food source for *F. occidentalis*. The purpose of this study was to select the most suitable astigmatid mite species based

on nutritional value and how they were presented (living or frozen) as an alternative food source for supporting populations of the predatory mite *A. swirskii* in greenhouse crops.

Materials and methods

Mite and thrips cultures

The stock colony of *A. swirskii* was initially obtained from Koppert Biological Systems (Berkel en Roden-rijs, The Netherlands) and subsequently reared on pollen provided to leaves of sweet pepper, *Capsicum annuum* L. cv. Spider (Enza Zaden, Enkhuizen, The Netherlands). The leaves were placed upside down on water-saturated cotton wool in plastic containers (18 × 12 × 5 cm). Wet tissue papers were attached to the edge of the leaves to provide moisture and prevent predatory mites from escaping (van Rijn and Tanigoshi 1999). The predators were fed every two days with cattail pollen (*Typha angustifolia* L.) as a standard diet for *A. swirskii* rearing (Nguyen et al. 2013). All laboratory oviposition experiments were done with 4–5 day-old *A. swirskii* females collected from the laboratory culture, but for the greenhouse trial, the predatory mites came directly from the commercial product obtained from Koppert (which had been reared on *C. lactis* with bran).

Six species of astigmatid mites from four families were evaluated: (1) Acaridae: *Thyreophagus ento-mophagus* (Laboulbene), *Aleuroglyphus ovatus* (Tro-peau) and *Acarus siro* L.; (2) Suidasiidae: *Suidasia nesbitti* Hughes and (3) Glycyphagidae: *L. destructor* and (4) Carpoglyphidae: *C. lactis*. The astigmatid mites came from various sources and had been maintained for several years by BU Greenhouse Horticulture of Wageningen University and Research in the Netherlands. For each species, two colonies were established and each maintained on a different food source in order to produce prey mites with different nutritional values.

All astigmatid mite species were reared on either wheat bran (Havens, Maashees, The Netherlands) or crushed dry dog food (Royal Canin, Veghel, The Netherlands). Wheat bran represents a low fat, low protein and high carbohydrate diet while crushed dog food represents a high fat, high protein and low carbohydrate diet (Erban et al. 2015). Both diets were supplemented with the same quantity of instant dry bakers' yeast (Mauripan, Hampton, United Kingdom) (50/50 by weight), which is known to be a suitable food source for astigmatid mites (Huang et al. 2013). While yeast also contains proteins, the difference in protein level between the two diets remained constant after addition of the yeast. Although yeast was present in two diets, based on their main constituent, both of diets are referred to as 'dog food' and 'bran' from here.

Each astigmatid mite species was reared for several generations in a plastic box (10 cm in diameter, 6 cm in high) which was embedded in a larger glass container (14 cm diameter, 8 cm high) covered with a lid and filled with a 1 cm layer of a saturated KNO_3 solution to prevent mite escape and provide a high and constant humidity level of 93% (Winston and Bates 1960). Colonies of *A. swirskii* were maintained in a different climate chamber to the astigmatid prey mites but both were under long day illumination (L:D 16:8) at 25 °C and 70% RH.

Western flower thrips, *F. occidentalis* were maintained for many generations on flowering chrysanthemum plants (*Dendranthema grandiflora* Tzvelev cv. Tapas Time), in a separate greenhouse compartment, and provided with artificial light and heating during winter. The plants were kept in cages to avoid contamination by other herbivores and replaced frequently.

Factitious prey diets

For each astigmatid mite species, six different factitious diets were prepared (36 treatments in total) as follows: (1) by life stage (mixed living, frozen eggs or frozen larvae) and (2) by prey mite diet (dog food or bran) (Table 1). The eggs of all astigmatid mite species were individually separated from other life stages by sieving colonies through a 100 µm mesh screen and stored at − 20 °C for at least 4 h before use in the experiments. A proportion of the sieved eggs (instead of being stored at − 20 °C) were incubated for 48–72 h under the conditions described previously, until more than 80% developed into larvae, which was confirmed under a dissecting microscope. Newly hatched larvae were also stored at − 20 °C for at least 24 h before use in experiments (Pirayeshfar et al. 2020). Before experimental use, frozen diets were thawed at room temperature (20 ± 2 °C) for ca. 30 min. Cattail pollen (Nutrimite TM) was supplied by Biobest N.V.,

Table 1 Different diets based on six species of astigmatid prey mites, *Thyreophagus entomophagus*, *Aleuroglyphus ovatus*, *Acarus siro*, *Suidasia nesbitti*, *Lepidoglyphus destructor* and *Carpoglyphus lactis*

Mite composition	Food substrate
Mixed life stages	Wheat bran + dry yeast
Mixed life stages	Dog food + dry yeast
Frozen eggs	Wheat bran + dry yeast
Frozen larvae	Wheat bran + dry yeast
Frozen eggs	Dog food + dry yeast
Frozen larvae	Dog food + dry yeast

(Westerlo, Belgium) and used as a reference for all laboratory and greenhouse trials. Olive (*Olea europaea* L.) pollen was used as a second reference, because it is easily collected in large quantities and is known to support reproduction of *A. swirskii* (Kumar et al. 2014; Nemati et al. 2019). The olive pollen in our study was provided by the Olive Research Station of Tarom, Zanjan, Iran. For long-term storage, both pollens were stored at − 20 °C.

Oviposition of *A. swirskii*

Oviposition rates of the predatory mite, *A. swirskii* feeding on the 36 factitious diets and two pollen sources (cattail or olive) were measured over three consecutive days. Peak oviposition rates of phytoseiid mites (which occur during the first days after the pre-oviposition period) are known to be strongly correlated with population growth rates (Janssen and Sabelis 1992) and so we limited the time period for measurement of oviposition to only three days. Data from the first day were omitted to limit effects of the pre-experimental diet (Sabelis 1990). Gravid female *A. swirskii* (4–5 days since adult emergence) were placed individually on 2.5 cm sweet pepper (*C. annuum*) leaf discs in 5 cm diameter Petri dishes. Each leaf disc was floating on water with the abaxial side uppermost and fixed with a modified paper clip stand that had been glued to the base of the Petri dish before filling with water (Pirayeshfar et al. 2020). Dishes were closed with a fine mesh lid to allow ventilation. Each disc had a leaf axil as a domatium for *A. swirskii* oviposition (Faraji et al. 2002). There were 20 replicate experimental units for each food treatment. According to food treatment, the diet was introduced into each unit in the following quantities:

0.01 g of pollen, 0.01 g frozen diet, or 40–50 mixed living life stages (predominantly eggs and larvae). Predatory mite eggs were counted and removed daily to prevent cannibalism. Based on this oviposition experiment, we selected the astigmatid mite species with greatest potential for subsequent trials. The experiment was done in a climate chamber under long day illumination (L:D 16:8) at 25 °C and 70% RH.

Survival and juvenile development

Based on the observed oviposition rates of *A. swirskii* fed on different food diets (pollens and frozen diets), we selected five food treatments to evaluate immature developmental time and survival of *A. swirskii*. To obtain synchronized *A. swirskii* eggs, ca. 100 mated females were transferred from the cultures to a new rearing arena (larger than the oviposition experimental unit) with cattail pollen. After 24 h, their eggs were transferred individually to the experimental units, as described for the oviposition experiment. Diets were added to each unit (0.01 g) after larval emergence. Old diet was removed every 48 h and replaced with fresh diet. The duration of each life stage was determined based on the presence of exuvia as evidence of moulting. Survival and development of individuals were recorded daily until mites reached adulthood. There were 20 replicates for each diet. The experiment was done in a climate chamber under long day illumination (L:D 16:8) at 25 °C and 70% RH.

Greenhouse trial

Based on the two laboratory experiments, eight food treatments, including the two reference pollens (cattail and olive) and six factitious prey diets (frozen and living stages of three species of the prey mites) were selected to assess their influence on establishment and population growth of *A. swirskii* on chrysanthemum plants in a greenhouse trial. The selected prey mites were *A. siro*, *L. destructor* and *T. entomophagus*, which were all reared on dog food. The trial was done in two adjacent greenhouse compartments at Wageningen University & Research, BU Greenhouse Horticulture in Bleiswijk, The Netherlands. Each compartment had an area of 24 m^2 and three tables, each of 7 m^2. The trial was set up using a randomized complete block design with five replicates using each table as a block with eight treatments (two tables in

one compartment and three in the other). Young chrysanthemum plants (*Dendranthema × grandiflorum* cv. Baltica) were supplied by Deliflor (Maasdijk, The Netherlands) and were planted in 12 cm diameter pots filled with peat. Each experimental unit (serving as a replicate) contained nine pots, each with one plant bearing 6–8 leaves at the start of the trial. All plants were placed on water-saturated irrigation mats to prevent predatory mite migration and minimise contamination amongst treatments. Plants were irrigated with a standard nutrient solution using an ebb-and-flow irrigation system for 10 min per day. Predatory mites were introduced by adding three sweet pepper leaf discs (4 cm diameter) each containing 15 gravid female *A. swirskii* on top of the nine plants in each treatment (45 in total, thus approximately five per plant). The predatory mites were collected using a fine brush directly from the commercial product and placed on the pepper leaves for transfer. Food treatments (0.06 g plant^{-1}) were added shortly after the predator releases by dusting with a fine brush to achieve an even distribution. For treatments containing living stages of the prey mites, approximately 500 different mobile stages were released per plant (counted based on weight). Population density of *A. swirskii* was monitored weekly for five consecutive weeks, before the flowering stage of chrysanthemum began, starting one week after releasing the predatory mites. All stages of *A. swirskii* on 18 randomly picked leaves per replicate (two leaves per plant) were counted. Temperature and RH during the experiment were recorded every 5 min with a climate recorder (Hoogendoorn Growth Management, Vlaardingen, the Netherlands). The climatic conditions in the two adjacent compartments were similar: the average temperature was 19.3 °C (range 12.2–29.6 °C) and 19.3 °C (range 12.8–28.4 °C) and the average RH 72% (range 46–88%) and 72% (range 47–87%).

Oviposition of *F. occidentalis* on astigmatid mite diets

Based on the results from the laboratory experiments and greenhouse trial, we selected the most promising astigmatid food treatments and evaluated their suitability as food sources for western flower thrips. The mite diets were compared with the two reference pollen diets. Food suitability was assessed by measuring the oviposition rates of *F. occidentalis* females when fed on these diets for four consecutive days, using a double parafilm method modified from that of Teulon and Penman ([1991](#)). Each experimental unit was made of a perspex cylinder (30 mm height and 25 mm diameter) that was closed with a mesh (size 80 μm) at one end to allow ventilation and by two layers of stretched parafilm at the other end. Four food diets were evaluated: (1) cattail pollen (0.03 g unit^{-1}), (2) olive pollen (0.03 g unit^{-1}), (3) mixed living stages of *T. entomophagus* (about 100 mites, predominantly eggs and larvae per unit) and (4) frozen larvae of *T. entomophagus* (0.03 g unit^{-1}). After adding the diets, three *F. occidentalis* females of unknown age were introduced into each unit and then immediately covered with a first layer of parafilm to retain the thrips inside the cylinder. After placing small droplets of water on the surface of the first layer of parafilm, the second layer was also covered. The number of eggs laid in the double parafilm membrane was counted daily. Each food treatment had 17 replicates (units).

Statistical analysis

To analyse the effects of diet on oviposition rates of *A. swirskii* (mean of days 2 and 3) and oviposition rates of thrips (mean of days 2, 3 and 4), we fitted generalised linear models (GLM) with a Poisson error distribution and log link function. Dispersion parameters were estimated to correct for overdispersion. Diet effects on juvenile developmental time of *A. swirskii* were also analysed with GLM, but with a normal distribution and identity link function for protonymphs and deutonymphs and a gamma distribution with a reciprocal link function for the total juvenile developmental time. Pairwise t-test were performed assuming that parameter estimates are approximately normally distributed (McCullagh and Nelder, [1983](#)). We used Fisher's exact test to analyse differences in juvenile mortality among treatments. Population dynamics of predatory mites over time (total numbers of all stages) in the greenhouse trial were analysed using a generalized linear mixed model (GLMM) with a Poisson error distribution and log link function and an estimated dispersion parameter. We evaluated the statistical significance using an approximate F-test (Kenward and Roger [1997](#)). Predatory mite density was the response variate, and food treatment was the fixed factor. Both block and time were included as random factors (time nested in block). Differences

amongst treatments were compared using pairwise *t* tests. All statistical analyses were done using the statistical package GenStat (Release 19.1).

Results

Oviposition of *A. swirskii*

There was a significant effect of diet treatment on oviposition rates of *A. swirskii* ($F_{37,759}$ = 9.68, p < 0.001). In general, oviposition rates of *A. swirskii* increased when prey mites had been fed with dog food compared with bran, but this increase was only significant for *A. siro*, *A. ovatus* and *L. destructor* (Fig. 1). Oviposition rates of *A. swirskii* on prey mites were highest in the *A. siro* treatment (when fed with dog food), reaching similar rates as that achieved by *A. swirskii* in the pollen treatment. Oviposition rates of *A. swirskii* in all other diets of living prey mite species (when fed dog food) were similar to each other (Fig. 1). In general, highest *A. swirskii* oviposition rates were achieved in frozen prey mite treatments (when fed with dog food) (Fig. 1). Highest *A. swirskii* oviposition rates were achieved on the prey mite *A. siro*, followed by *L. destructor*, *T. entomophagus*, *S. nesbitti* and *C. lactis*. A lower oviposition rate was observed in the *A. ovatus* prey mite treatment (Fig. 1). The mean two-day oviposition rate of *A. swirskii* on olive pollen (not shown in Fig. 1) was not significantly different from cattail pollen (3.45 ± 0.34 versus 4.25 ± 0.32, respectively).

Survival and juvenile development

The predatory mite *A. swirskii* survived and successfully developed on all diets evaluated. Significant differences amongst treatments were observed for the duration of the protonymph stage ($F_{4,99}$ = 19.02, p < 0.001), deutonymph stage ($F_{4,99}$ = 5.60, p < 0.001) and the total juvenile development time from larva to adult ($F_{4,99}$ = 18.98, p < 0.001) (Table 2). Developmental time was shortest in the olive pollen treatment, but mortality was also higher on olive pollen than the other diets (p = 0.045; Fisher's exact test, Table 2). When fed the frozen prey diets, developmental time of *A. swirskii*, from larva to adult, was significantly longer for *L. destructor* than for *T. entomophagous* and *A. siro*.

Consumption of all frozen diets resulted in a longer developmental time for *A. swirskii* than consumption of the pollen treatments (Table 2).

Greenhouse trial

There was a significant effect of treatment on the population densities of predatory mites on chrysanthemum plants ($F_{7, 168}$ = 46.17, p < 0.001, Fig. 2). Supplementation with mixed living stages of *T. entomophagus* (Te) resulted in the highest overall densities of *A. swirskii* (Fig. 2). Frozen diets based on *A. siro* (frozen As) and *L. destructor* (frozen Ld) did not support the *A. swirskii* population, but the diet based on larvae of *T. entomophagous* (frozen Te) was as effective in supporting *A. swirskii* as the diet based on living mites of *A. siro* (As) and slightly less effective than the diet based on cattail, pollen (Fig. 2). Moreover, when supplied with olive pollen, predator density was significantly lower than when supplied with cattail pollen (Fig. 2).

Oviposition of *F. occidentalis* on astigmatid mite diets

The oviposition rates of western flower thrips, *F. occidentalis*, were significantly affected by food treatment ($F_{4,84}$ = 98.06, p < 0.001, Fig. 3). Oviposition rates were higher in the cattail and olive pollen treatments than in the treatments providing living and frozen stages of *T. entomophagus*, which were not significantly different from the treatment without food (Fig. 3).

Discussion

This study shows that providing prey mites in crops has substantial potential for increasing predatory mite densities. All six tested prey mite species were suitable food sources for reproduction of *A. swirskii* in small arenas. Three selected species, *T. entomophagous*, *A. siro* and *L. destructor*, were also successful in increasing densities of predatory mites in the greenhouse. The most successful prey mite in the greenhouse was *T. entomophagous*. When feeding on *T. entomophagous* diets, predatory mites reached even higher densities than when fed on cattail pollen, which is a supplemental food source that is commonly used

Fig. 1 Daily oviposition rates of the predatory mite *Amblyseius swirskii* on different factitious foods based on six species of astigmatid mites [*Thyreophagus entomophagus* (**a**), *Acarus siro* (**b**), *Lepidoglyphus destructor* (**c**), *Aleuroglyphus ovatus* (**d**), *Suidasia nesbitti* (**e**) *and Carpoglyphus lactis* (**f**)], in comparison with cattail pollen (*Typha angustifolia*). All prey mite species were reared on dog food (high protein) or bran (high carbohydrate) as food substrates. Data presented are mean numbers of eggs (± SE) per female per day based on oviposition on day 2 and 3, since the predators were allowed to feed on the food treatments. Different letters above bars represent significant differences amongst treatments (pairwise *t* tests after a Poisson regression with overdispersion: p < 0.05)
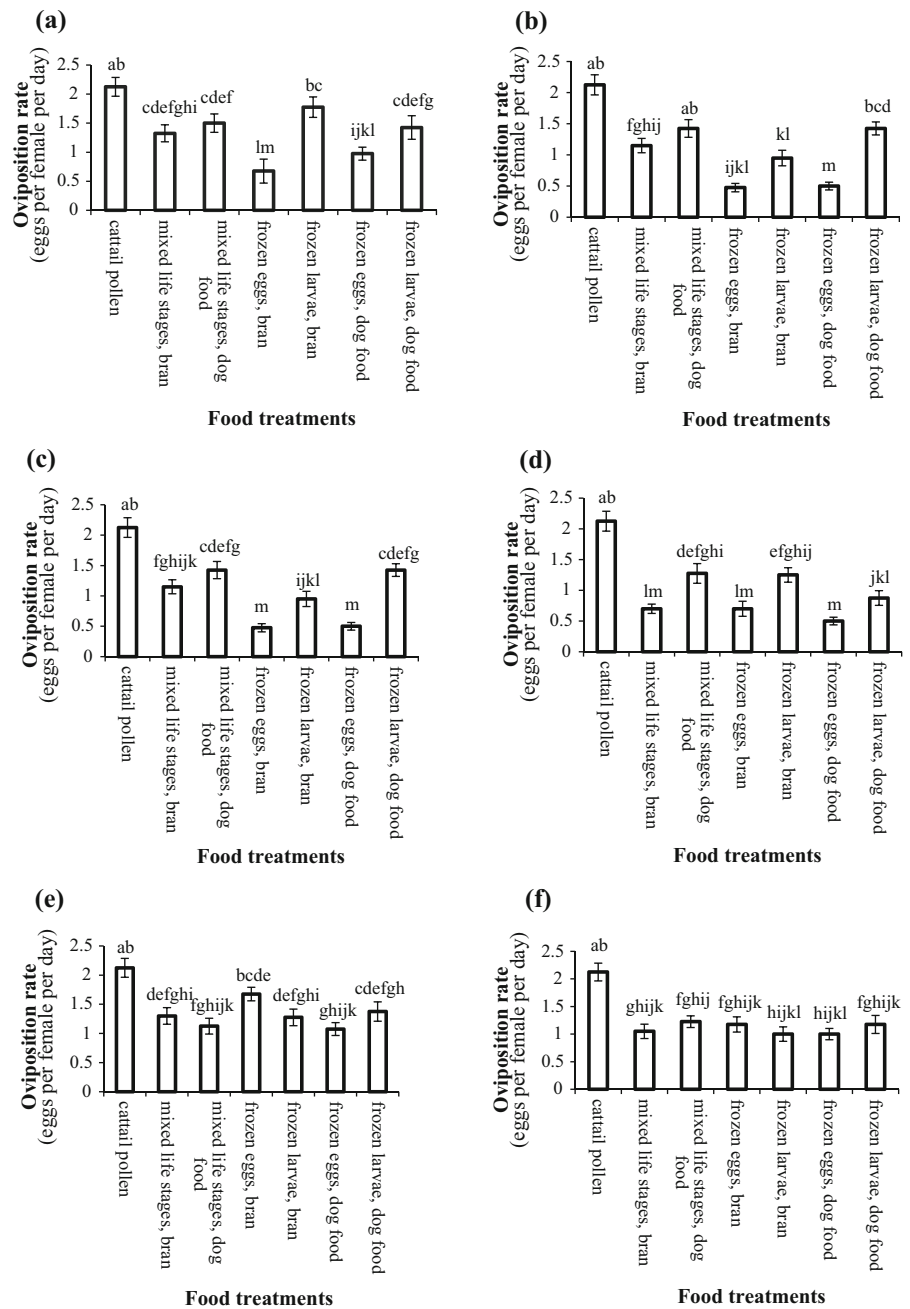
in greenhouse crops (Pijnakker et al. 2016). *Lepidoglyphus destructor* was the next best supplementary diet, increasing predatory mite densities to similar levels as the treatment with cattail pollen. Predatory mites performed less well on a diet of *A. siro* in the greenhouse trial compared with the other two prey mite species. Based on the laboratory oviposition experiment, we would have expected a different outcome. In this experiment we found the highest *A. swirskii* oviposition rates in the *A. siro* treatments. Maybe the longer developmental time of juvenile predatory mites on *L. destructor* and *A. siro* compared with *T. entomophagous* (tested on frozen stages) could explain this difference between laboratory oviposition and greenhouse results. However, other aspects of the greenhouse trial might have contributed, such as the

Table 2 Survival and mean developmental time (days ± SE) of immature stages of the predatory mite *Amblyseius swirskii* fed on different food treatments

Food treatments	Larva	Protonymph	Deutonymph	Total	Survival (%)
Cattail pollen	1.0 ± 0.0a	1.9 ± 0.1c	1.4 ± 0.1b	4.3 ± 0.2c	100.0 ± 0.0a
Olive pollen	1.0 ± 0.0a	1.1 ± 0.1d	1.6 ± 0.2b	3.8 ± 0.2d	75.0 ± 0.2b
Frozen larvae of *Acarus siro*	1.0 ± 0.0a	2.6 ± 0.2ab	1.7 ± 0.1b	5.4 ± 0.2b	100.0 ± 0.0a
Frozen larvae of *Lepidoglyphus destructor*	1.0 ± 0.0a	2.7 ± 0.2a	2.3 ± 0.1a	6.0 ± 0.2a	100.0 ± 0.0a
Frozen larvae of *Thyreophagus entomophagus*	1.0 ± 0.0a	2.2 ± 0.2bc	1.7 ± 0.2b	5.0 ± 0.2b	100 ± 0.0a

All species of the astigmatid prey mites were reared on dog food. Different letters within the same column show significant differences amongst treatments (LSD test: $p < 0.05$)
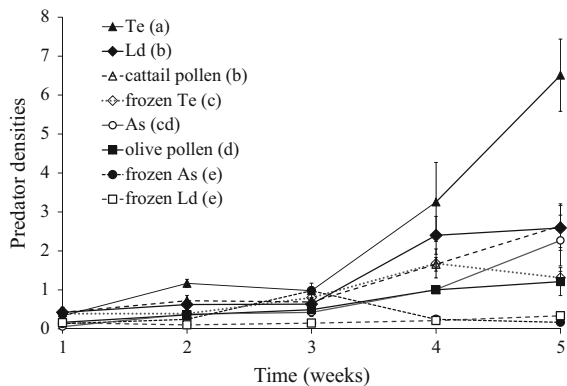
Fig. 2 Population dynamics of the predatory mite *Amblyseius swirskii* on chrysanthemum plants when fed on one of eight food treatments: cattail pollen (*Typha angustifolia*); olive pollen (*Olea europaea*); mixed living stages of *Acarus siro* (As), *Lepidoglyphus destructor* (Ld) and *Thyreophagus entomophagus* (Te); frozen larvae of *A. siro* (frozen As), *L. destructor* (frozen Ld) and *T. entomophagus* (frozen Te). All prey mite species were reared on dog food. Data presented are the mean (± SE) densities of all stages of predatory mites per 18 leaves. Different letters represent significant differences amongst treatments over time (pairwise *t* tests after a Poisson regression with overdispersion: $p < 0.05$)

Fig. 3 Mean (± SE) number of eggs per female western flower thrips, *Frankliniella occidentalis* per day, when feeding on one of four food treatments over three consecutive days. The food treatments were: cattail pollen (*Typha angustifolia*), olive pollen (*Olea europaea*), mixed living stages of *Thyreophagus entomophagus* reared on dog food (living T. e), or frozen larvae of *T. entomophagus* reared on dog food (frozen T. e). Different letters above bars represent significant differences amongst treatments (pairwise *t* tests after a Poisson regression with overdispersion: $p < 0.05$), based on the average number of eggs per female produced in three days

behaviour and survival of the living prey mites on plants. For example, *L. destructor* is known to run much faster than the other prey mite species evaluated, which might have affected the encounter rates with predatory mites on plants (based on personal observations). Based on the oviposition rates observed in the laboratory, we would also have expected a stronger population growth of *A. swirskii* on plants provided with cattail pollen than on plants with prey mites. This difference between the laboratory and greenhouse trial might be explained by the different background 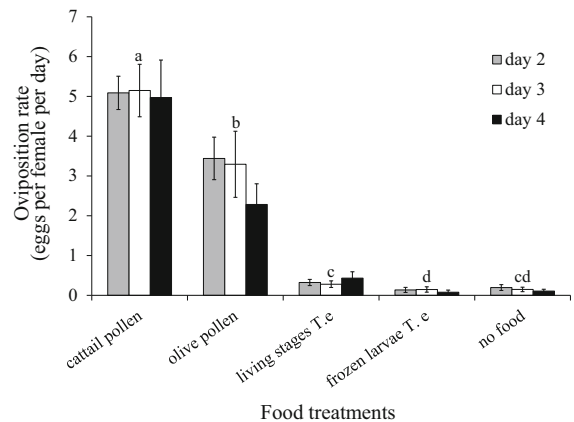of the predatory mites we used. The predatory mites used in the laboratory experiment came from cultures reared on pollen, but the predatory mites used in the greenhouse trial came from a commercial product reared on bran and prey mites. A recent study by Nemati and Riahi (2020) showed that the performance of *A. swirskii* increased after multiple generations on pollen. Thus, the predatory mites used in the laboratory experiment might have been better adapted to pollen feeding than the predatory mites used in the greenhouse trial. Another possible reason might be that pollen quality decreased faster in the greenhouse than in the laboratory.

Although prey mites have been used for decades in mass rearing systems (Ramakers and van Lieburg 1982), little was known about their potential when applied on crops as supplemental food for predators. They have been introduced in crops, but always incidentally alongside predatory mite release, either in the carrier material applied directly to the crop or in slow-release rearing sachets (Calvo et al. 2015). Moreover, these combined releases of predatory and prey mites were mainly done with prey mite species that were optimal for mass rearing, although less suitable prey mite species have been included in slow-release sachets to extend the release time (Bolckmans et al. 2013). Our results indicate that a wider range of prey mite species are suitable as a supplementary food source in crops than are suitable for mass rearing (predominantly the prey mite *C. lactis; Calvo* et al. 2015). Also the prey mite *T. putrescentiae* is suitable for supporting populations of *A. swirskii*, as shown in our previous study (Pirayeshfar et al. 2020), but can also cause feeding damage in young plants (Hiroshi 1991). So this limitation makes it less suitable for releasing on crops. The differences between the suitability of prey mites for mass rearing systems compared with crop application may relate to the strong effects of accumulating defensive oils that occur at high prey mite densities (Midthassel et al. 2016) in closed mass rearing systems, but are not apparent in small arenas in the laboratory or on plants where these volatiles are more diffused.

Reproduction of predatory mites was slightly better on the mites fed with dog food (protein-rich and fat-rich diet) than on mites fed with bran (carbohydrate-rich diet) confirming our previous finding with *T. putrescentiae* (Pirayeshfar et al. 2020). This indicates that a highly nutritious prey mite diet increases the nutritional value for predatory mites. Since recent studies proved that generalist predatory mites actively balance their diet by feeding on different prey sources (Marques et al. 2015), providing high quality supplemental prey will be particularly important when other pests/prey are scarce but less important when other pests or prey are more abundant.

Frozen prey mite diets were evaluated as a possible alternative to the application of living prey mites into greenhouse crops. Frequent releases of high densities of living prey mites into crops may cause some risks for human health, since some studies indicate that astigmatid mites are able to survive in human body parts and cause acariasis (Li et al. 2003). This risk would be absent when applying dead mites into crops. Our previous study with *T. putrescentiae* showed that frozen larvae were a very suitable food source for *A. swirskii* in the laboratory, but it did not increase predator populations when added to plants (Pirayeshfar et al. 2020). In the present study we found similar effects for the prey mite species *A. siro* and *L. destructor*. Frozen diets based on larvae of these species showed good results in the laboratory but not on plants in the greenhouse. However, the results with frozen larvae of *T. entomophagous* were much better in the greenhouse achieving similar effects to cattail pollen and performing better than olive pollen. The laboratory experiment also showed faster juvenile development of *A. swirskii* on the frozen *T. entomophagous* diet compared with the frozen diets of *L. destructor* and *A. siro*. For this reason, it might be interesting to develop a supplemental food product based on frozen larvae of *T. entomophagous*, although provided living *T. entomophagous* clearly resulted in higher predatory mite densities.

A huge benefit of using prey mites as a food source for predatory mites compared with pollen or other supplemental food sources is the unsuitability of prey mites as a food source for thrips. Our laboratory experiment showed that neither living nor dead (frozen) *T. entomophagous* mites increased thrips reproduction compared with the absence of food entirely. As a result of the strong numerical response of predatory mites, pollen is not a risk for thrips, in most cases. However, when thrips densities are high and predator densities are low, supplemental pollen can lead to an increase in thrips numbers (van Rijn et al. 2002; Leman and Messelink 2015; Vangansbeke et al. 2016). While olive pollen was less suitable for thrips reproduction than cattail pollen, it was also less suitable for predatory mite development in the greenhouse trial, reducing its potential as a supplemental food.

A second benefit of using prey mites as supplementary food sources is the low cost of mass production. More biocontrol companies are selling prey mite products to support predatory mite populations in greenhouse crops, as a part of a 'standing army' strategy (Messelink et al. 2014). Applying prey mites may also have other unexpected positive effects for biological control by supporting a number of other natural enemy species. Many other predators are able

to prey and reproduce on astigmatid mites including: soil-dwelling predatory mites (Grosman et al. 2011; Munoz-Cardenas et al. 2017), anthocorid predatory bugs (Bonte et al. 2017; Bernardo et al. 2017), spiders and rove beetles (our personal observations). Overall, we believe that providing prey mites in crops is a very interesting approach for increasing predator numbers and enhancing biological control. Specifically, for *A. swirskii* we showed that the prey mite *T. entomophagous* is the most promising candidate when applied either as living mites or as frozen larvae.

Declaration

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The authors would state that they only worked with small arthropods (insects and mites) and the international guiding principles are not applicable for these organisms.

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Fatemeh Pirayeshfar is PhD of Agricultural Entomology (ecology and biological control) from Urmia University, Iran. Her research interests focuses on the development of new sources of alternative foods for biological control agents, especially predators.

Seyed Ali Safavi was born in Tabriz, Iran. He received his PhD degree in Agricultural Entomology from University of Tehran, Karaj, Iran in 2007. He is currently the full professor of Urmia University, Urmia, Iran. His research work has been mainly concerned with the biological control of insect pests including predators, parasitoids and entomopathogens with special emphasis on fungal biocontrol agents.

Hamid Reza Sarraf Moayeri recieved his PhD degree from University of Tehran, Karaj, Iran in 2007. His research interests focus on insect biocontrol and ecology. He currently serves as an associate professor at Department of plant protection, University of Zanja, Iran.

Gerben Messelink is an applied entomologist at Wageningen University & Research, BU Greenhouse Horticulture in The Netherlands. His work focuses on the development of biological control systems for pests in greenhouse crops.