Food and habitat supplementation promotes predatory mites and enhances pest control

Lucas Etienne, Cécile Bresch, Louise van Oudenhove, Ludovic Mailleret

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Corresponding author:

Lucas Etienne

INRAE, UR1115, PSH, Plantes et Systèmes de culture Horticoles, F-84000 Avignon, France

Université Côte d'Azur, INRAE, CNRS, ISA, France

lucas.etienne@inrae.fr

Cécile Bresch

Université Côte d'Azur, INRAE, CNRS, ISA, France

cecile.bresch@inrae.fr

Louise Van Oudenhove

Université Côte d'Azur, INRAE, CNRS, ISA, France

louise.vanoudenhove@inrae.fr

Ludovic Mailleret

Université Côte d'Azur, INRAE, CNRS, ISA, France

Université Côte d'Azur, Inria, INRAE, CNRS, Sorbonne Université, Biocore, France

ludovic.mailleret@inrae.fr

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1 Introduction:

Predatory mites are beneficial organisms used to control many different plant pests, including phytophagous mites (Knapp et al., 2018), in vegetable crops (e.g., tomatoes, sweet peppers, or cucumbers); ornamental crops (e.g., roses; Gerson and Weintraub, 2012); and fruit orchards (van Lenteren et al., 2018). Most of the predatory mites used in biological control belong to the family Phytoseiidae, which is composed of more than 2,500 species (Demite et al., 2014). Twelve species from eight genera are commercially available in Europe (Knapp et al., 2018) and commonly employed. Four species account for around 60% of this market: *Neoseiulus cucumeris* (Oudemans), *Amblyseius swirskii* (Athias-Henriot), *Phytoseiulus persimilis* (Athias-Henriot), and *Neoseiulus californicus* (McGregor) (Knapp et al., 2018). *N. cucumeris, N. californicus*, and *A. swirskii* are generalist predators that are mainly used to target thrips, pest mites, and whiteflies (McMurtry and Croft, 1997, 2013). They utilise a wide range of host plants (e.g., vegetable, fruit, and ornamental crops) and are commonly employed in protected cultures. Moreover, their method of rearing is well characterized (Bolckmans et al., 2005), they can be released mechanically (Opit et al., 2005), and their ability to disperse is limited, which helps prevent negative effects on non-target organisms (van Lenteren et al., 2012).

However, biological control utilizing Phytoseiidae tends to be less efficient and more expensive than the use of insecticides or acaricides (Trumble and Morse, 1993; Collier and Van Streewyk, 2004). For instance, to maintain a population under greenhouse conditions, phytoseiid mites must be released on crops several times (Garthwaite et al., 2016). Optimally timing the release is also important to ensure efficacious biological control (Sampson and Kirk, 2016). Moreover, higher release rates do not always provide better levels of biological control (Crowder, 2007). This non-linear relationship between predatory mite population size and the degree of pest control can be explained by different factors: for example, negative effects such as competition, cannibalism, and intraguild interactions among phytoseiid mites may become pronounced as food availability decreases (Schausberger, 2003; Crowder, 2007).

When prey is lacking, different plant resources can be used to provide food to predatory mite populations. These alternative resources include pollen (e.g., Van Rijn et al., 1999, 2002; Duso et al., 2004; Wäckers et al., 2005; Nomikou et al., 2010; Delisle et al., 2015a, b), extrafloral nectar (Van Rijn and Tanigoshi, 1999b), and on occasion, plant tissue (Nomikou et al., 2003). Other food types such as harmless herbivorous mites (Karban et al., 1994) as well as insect eggs or brine shrimp cysts (Nguyen et al., 2014) can also be employed. Furthermore, providing diverse foods can have a stronger positive impact on predatory mites than providing a single food type. For instance, Beltrà et al. (2017) studied the growth dynamics of the phytoseiid *Euseius stipulatus* (Athias-Henriot) on clementine plantlets (*Citrus clementina* Tanaka [Rutaceae]) under supplementation regimes using *Typha* sp. pollen, a sucrose solution, or both. After eight weeks under greenhouse conditions, *E. stipulatus* abundance was 2.5 times in the combined supplementation treatment than in the pollen and sugar treatments combined (Beltrà et al. 2017).

The presence of domatia on plant leaves can also promote the establishment and survival of predatory mites (Norton et al., 2001; Ferreira et al, 2008, 2011). Domatia are small cavities (e.g., pits, pouches, pockets, or, sometimes, hairy tufts; O'Dowd and Willson, 1989; Walter, 1996) that are generally found between the primary and secondary veins on the lower surface of leaves. Domatia can provide shelter for a wide range of predatory mite species (O'Dowd and Willson, 1989, 1991; Walter and O'Dowd, 1992; Grostal and O'Dowd, 1994; Norton et al., 2001) and are known to influence the latter's behaviors (e.g., prospection, feeding, reproduction, and oviposition;

Walter, 1996; Kreiter et al., 2002). Domatia might also help protect younger mites against predators and cannibalism (Ferreira et al., 2008, 2011).

Domatia are found on the leaves of nearly 2,000 species belonging to more than 277 plant families (Brouwer and Clifford, 1990; Agrawal and Karban, 1997); in most cases, these species are woody perennials (Walter and Proctor, 2013). Although crop breeding programs generally aim to develop traits of agricultural value (Cortesero et al., 2000), to our knowledge, characteristics such as domatia presence or size (English-Loeb et al., 2002) have never been a point of focus (Walter and Proctor, 2013). As a consequence, certain crop plants bear fewer and/or smaller domatia than do native species (e.g., grape cultivars; English-Loeb et al., 2002). Past research has explored how predatory mites living in glabrous crop plants are affected by artificially adding materials that mimic the properties of domatia (hereafter, artificial habitats) (Agrawal, 1997; Agrawal and Karban, 1997). The materials that have been tested are cotton, shade netting, polyamide, polyester, and sheep's wool (Roda et al. 2001; Kawashima et al., 2006; Liu et al., 2018; Bresch et al., 2019). Wool may serve as an excellent artificial habitat because it was found to have an equally positive impact on oviposition as natural domatia (Kawashima et al., 2006; Bresch et al., 2019).

Furthermore, combining habitat structural complexity and dietary diversity can promote predatory mite abundance (Pozzebon et al., 2015). For instance, when both pollen and artificial habitats were used as supplements, the establishment of *A. swirskii* and *Euseius scutalis* (Athias-Henriot) on sweet peppers greatly improved (Loughner et al., 2011; Adar et al., 2014). Using a higher degree of food diversity can boost abundance even more. For instance, Pekas and Wäckers (2017) found that the abundance of *E. stipulatus* eggs on *Citrus aurantium* L. (Rutaceae) was up to five times higher for the group where mites had both artificial habitat (fibers) and diverse foods (pollen and sugar) than the combined results for the groups where mites had either the habitat or the foods.

The ecological theory of apparent competition predicts that providing supplemental food resources and/or artificial habitats should trigger indirect negative effects on target pests by boosting predator abundance and density (Holt, 1977, Holt and Bonsall, 2017). Studies to date tend to support this prediction. First, food supplementation has been shown to increase pest suppression in various situations. For instance, providing pollen to predatory mites can improve levels of thrips (van Rijn et al., 1999) and whitefly (Nomikou et al., 2010) control. Second, the presence of domatia on plant leaves can also indirectly enhance biological control by predatory mites (Agrawal, 1997; Agrawal and Karban, 1997). Nevertheless, to ensure that biological control pressures are maintained, supplementation must not divert the attention of predators away from their pest prey (e.g., through satiation or switching), which could end up benefiting the pest (Holt and Lawton, 1994; Abrams and Matsuda, 1996; Nomikou et al., 2010; Li and Zhang, 2020). Moreover, supplementation must not directly benefit pest organisms by providing them with resources and/or refuges (van Rijn et al. 2002). Very few studies have explored the combined effects of providing both alternative food resources and artificial oviposition sites/habitats on the efficacy of biological pest control by generalist predators (Lee and Zhang, 2018). In a rare example of this research, Lee and Zhang (2018) showed that such tandem supplementation could enhance the control of whiteflies by the predatory mite *Amblydromalus limonicus* (Garman & McGregor). However, the effects of supplementation were highly dependent on host plant identity (Lee and Zhang, 2018), revealing the existence of more complex three-way interactions in this plant-pest-predator system.

Our study examined how providing artificial habitat and non-prey food resources to the generalist predatory mite *N. cucumeris* affected levels of biological control. More specifically, we tested whether such supplementation improved (i) the establishment and development of *N. cucumeris* in the absence of prey and (ii) the suppression of the pest mite *Tetranychus urticae* (Koch). First, we explored which resource served as the best alternative food (BAF) for the predatory mite: almond

pollen, moth eggs, or both. Then, we identified which treatment regime served as the best supplementation program (BSP) for the predatory mite: the BAF, wool as an artificial habitat, or both. Our experiments were performed under controlled laboratory conditions and under greenhouse conditions on sweet pepper plants. Finally, the effectiveness of the BSP in the biological control of the pest mite *T. urticae* was tested using sweet pepper plants under greenhouse conditions.

2   Materials and methods:

This study was carried out in the spring of 2019 in laboratory and greenhouse facilities in southern France, at the Sophia Antipolis centre of the French National Research Institute for Agriculture, Food, and Environment (INRAE).

2.1 Biological materials

2.1.1   *Predatory mites:* We used *N. cucumeris* as our experimental predatory mite. This species belongs to the Phytoseiidae family classified as type III: it is a generalist predator that can also feed on pollen or nectar (McMurtry and Croft, 1997, 2013). Its optimal climatic conditions for oviposition are 25°C with 65% relative humidity (RH) (Al-Azzazy et al., 2018). The *N. cucumeris* used in this study came from the Amblyseius-System sold by Biobest (France). The system comes in a package containing vermiculite, the prey *Tyrophagus putrescentiae* (Schrank) Acaridae, and the predator *N. cucumeris*. Before each experiment, the contents of the packages were each sieved (0.315-mm mesh) for 2 minutes and examined with a stereomicroscope (Leica EZ4) to determine the sex ratio and developmental stage distribution of the predatory mite population (seven 1.0-g replicates) and the prey mite population (three 1.0-g replicates) (Table A).

2.1.2   *Pest mites:* We used *T. urticae* as our experimental pest mite. This well-known species belongs to the Tetranychidae family and feeds on chloroplasts, which it accesses inside plant cells with its buccal apparatus (Bounfour et al., 2002). It causes major damage to various crop species (e.g., Walsh et al., 1998; Park and Lee, 2005; Meck et al., 2013) and has often been studied in the context of biological control (Attia et al., 2013). We used mites taken from populations in INRAE greenhouses that had been bred in the laboratory (rearing conditions: 23°C, RH 40%, 16:8 D/N) on dwarf beans for three months before the experiments began.

2.1.3   *Plants:* In the greenhouse experiments, we used the sweet pepper, *Capsicum annuum* (Solanaceae) var. *Doux très long des Landes* as our host plant. We were curious about how the almost glabrous character of its leaves would interact with the addition of artificial habitat. We also chose this variety because of its smaller size and economic importance. Plants were supplied by Saveurs de Provence (Brin-sur-Seille, France) and were grown inside a climate chamber (21°C, 16:8 D/N) for one week. Since an adult thrips was observed on a leaf, treatments using the nematode *Steinernema feltiae* (Sternerneima-System, Biobest) were performed weekly during the entire study. Plants were placed in 3-L pots filled with a mixture of Agrilite® perlite substrate and Tonusol vegetable compost (1:2 ratio). Since pollen can serve as an alternative food for predatory mites (VanRijn and Sabelis, 1990; Weintraub et al., 2007), flowers were removed before blooming and left on the substrate. At the beginning of the greenhouse experiments, foliage on each plant was reduced to 20 leaves to standardize plant size.

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2.1.4 *Alternative foods and artificial habitat:* Two types of alternative foods were used: almond pollen and moth eggs. The pollen came from *Prunus dulcis* Mill. (Rosaceae) because *Prunus* sp. pollen has been shown to markedly improve *N. cucumeris* development (Van Rijn and Tanigoshi, 1999a). It was purchased fresh from Firman Pollen (USA) and frozen upon reception. The eggs came from the flour moth *Ephestia kuehniella* Zeller (Pyralidae), and their use has also been shown to positively affect *N. cucumeris* development (Delisle et al., 2015). They were irradiated to prevent moth development and purchased from Bioline Agrosciences (France). Sheep wool was used as the artificial habitat (Kawashima et al., 2006; Bresch et al., 2019).

2.2 Experimental setup

2.2.1 *Laboratory experiments:* Two experiments were conducted in thermoregulated incubators (24.7 ± 0.2°C [SD], 16:8 D/N). The experimental unit was a plastic box (16 x 10 x 5.5 cm) with a 2.6-cm diameter hole covered by Parafilm® on top. A small piece of towel strip (1 cm x 1 cm) was placed inside the box and soaked with 3 mL of demineralized water. A Petri dish of 9 cm in diameter was also placed inside the box; it contained the food resources and/or artificial habitat, depending on the treatment. During each experiment, there were 4 treatment groups with 20 replicates per treatment (i.e., total of 80 boxes). The experiments were launched over two days. Forty boxes were started on the day the Amblyseius-System packages arrived, and 40 boxes were started the day after. The same number of boxes within each treatment group were started each day.

- **BAF**: In this experiment, we identified the best alternative food under laboratory conditions. There were four treatment groups: predatory mites without supplemental food (Pe), predatory mites given 0.001 g of pollen (P) or 0.001 g of *E. kuehniella* eggs (E), and predatory mites given both foods (0.001 g of each; [P+E]). On day 1 of the experiment, 2 g of the bulk package contents (i.e., 10 ± 7.4 [SD] female *N. cucumeris*, Table A) were introduced into a 5.5-cm-diameter Petri dish containing a rose bush leaflet (1.81 ± 0.23 cm [SD]) with its stipule inside a 0.3 mL Eppendorf tube filled with wet cotton. The Petri dish was placed in an experimental unit, which was then subject to one of the four treatments. On day 7, we counted *N. cucumeris* eggs, larvae, nymphs, and adults using a stereomicroscope.

- **BSP1**: In this experiment, we identified the best supplementation program under laboratory conditions. There were four treatment groups: predatory mites without supplemental food (Pe), predatory mites given the BAF identified in the previous experiment (i.e., 0.001 g of pollen and 0.001 g of *E. kuehniella* eggs (food = F)), predatory mites given 0.005 g of frayed wool (wool = W), and predatory mites given both foods and the wool (F+W). On day 1 of the experiment, 0.95 g of the bulk package contents (i.e., 20 ± 6.6 [SD] female *N. cucumeris*, Table A) were introduced into a 5.5-cm-diameter Petri dish that was placed in an experimental unit, which was then subject to one of the four treatments. On day 7, *N. cucumeris* eggs, larvae, nymphs, and adults were counted using a stereomicroscope.

2.2.2 *Greenhouse experiments:* We performed two experiments in two separate compartments (surface area of each: 40 m^2) of a glass greenhouse. In each experiment, there were 4 treatment groups with 24 replicates each; consequently, 96 sweet pepper plants were grown in each compartment. Each pepper plant served as an independent experimental replicate because mites could not move between plants: the plants did not touch and were placed on 4-cm high stands in a tray filled with 2 cm of water. In addition, the exteriors of the plant pots and the irrigation tubes were covered with Vaseline® to prevent mites from escaping. Climatic conditions and irrigation in the greenhouse were

controlled using ARIA automation software (Table B). In the treatment groups receiving food supplementation, 6 mg of pollen and 6 mg of *E. kuehniella* eggs were sprinkled on top of the plants weekly (Delisle et al., 2015). In the treatment groups given artificial habitat, the 0.005 g of frayed wool was divided in three and flattened into patches. Each patch was affixed to the underside of a leaf, between the primary and secondary veins, using a 1:2 mixture of water and water-based glue (Rubafix®). The three leaves used were randomly chosen at the beginning of the experiment and were located at the top, middle, and bottom of the plant. The experiments each lasted six weeks. Once a week, the number of leaves on each plant were counted. Mite populations were counted using a handheld magnifying glass (x4); for the counts, depending on the treatment group, we either chose eight evenly distributed leaves or five evenly distributed leaves plus the three leaves with the artificial habitat. The mite population on a given plant was estimated by multiplying the mean number of mites on the observed leaves by the number of leaves on the plant. In the treatment group with artificial habitat supplementation, separate estimates were calculated for the leaves with and without the wool and then combined to obtain an estimate for the whole plant. In the experiments, individual plants were randomly assigned to the treatment groups.

- **BSP2**: In this experiment, we identified the best supplementation program under greenhouse conditions. There were four treatment groups: predatory mites without supplemental food (Pe), predatory mites given the BAF from the first lab experiment (food = F), predatory mites given frayed wool (wool W), and predatory mites given both food and wool (F+W). On day 1 of the experiment, 1.19 g of the bulk package contents (i.e., 30 ± 11.5 [SD] of mobile *N. cucumeris*, including larvae, nymphs, and adults) was added to the substrate of each plant (Table A). The number of *N. cucumeris* eggs was counted weekly for eight leaves as previously described.

- **Biocontrol**: In this experiment, we assessed the efficiency of the best supplementation program in controlling a pest mite under greenhouse conditions. All the experimental plants were inoculated with *T. urticae*. There were four treatment groups: the group with neither supplementation nor predatory mites (control = C), the group experiencing the BSP from the previous experiment (F+W), the group with predatory mites but no supplemental food or habitat (Pe), and the group with predatory mites that experienced BSP (Pe+F+W). To ensure that the pest mite cycled through one generation (Sabelis, 1982) and became established (Parolin et al., 2013), pest mites were introduced twice—two weeks and one week before the beginning of the experiment. Fourteen days before the experiment began, plants were inoculated with pest mites: a piece of bean leaf infested by *T. urticae* (10 adults and 14 ± 9.6 [SD] eggs) was placed at the top of each plant. Seven days before the experiment began, 10 *T. urticae* adults and 29 ± 20.5 (SD) *T. urticae* eggs were added to each plant. For the Pe and Pe+F+W treatments, on day 1 of the experiment, 1.84 g of the bulk package contents (i.e., 30 ± 16.9 [SD] mobile *N. cucumeris*, including larvae, nymphs, and adults) was added to the substrate of each plant (Table A). The number of mobile pest mites (larvae, nymphs, and adults) was estimated weekly from counts of eight leaves, as described above.

2.3 Statistical analyses:

All the statistical analyses were performed using R (version 1.3.1056; R Core Team, 2019).

2.3.1 *Laboratory experiments*: The statistical procedure was similar for both the BAF and BSP1 experiments. There were two dependent variables: (i) survival, represented by the number of nymphs and adults and (ii) reproduction, represented by the number of eggs and larvae. For each variable, the differences between treatments

were tested using a Wilcoxon multiple pairwise comparison non-parametric test with a false discovery rate (FDR) post-hoc correction. Next, the presence of synergies between the two supplement types (the two foods in the BAF experiment and the foods and wool in the BSP experiment) was quantified using statistical models in which survival or reproduction was the response variable, and the two supplement types and their interaction were the explanatory variables. In addition, to account for any potential bias due to the day of introduction, the day the experiment started (a two-level factor) was also included as an explanatory variable. The data were overdispersed. For survival, a generalized linear model with a negative binomial distribution was used (glm.nb function, MASS package, Venables and Ripley, 2002). For reproduction, because of the high number of zero values, we used a Hurdle model with a negative binomial distribution for the truncated data (hurdle function, pscl package, Jackman et al., 2007). This model separates out the effect of the explanatory variables on (i) the probability of reproduction (binary response = zeros vs. non-zeros) and (ii) the number of eggs and larvae given the occurrence of reproduction. In both cases, stepwise backward model selection was performed. The best-fit model was the one with the lowest Akaike's information criterion (AIC) when the difference in AIC between two models was > 2. When AIC was < 2, the most parsimonious model was selected. Using the best-fit model, the significance of the explanatory variables was assessed using a Wald test applied to the estimated coefficients.

2.3.2 *Greenhouse experiments*: In both experiments, the first step was to check whether the number of leaves per plant was similar among treatment groups. For each experimental timepoint (i.e., weekly counts), a Kruskal-Wallis test was performed to assess differences among treatment groups in the number of leaves per plant. When the difference was not significant (alpha level = 0.05), the count data were left unchanged. If a difference was present, the data were standardized: for each plant, the number of leaves was multiplied by the mean number of leaves for all treatments, divided by the mean number of leaves in the plant's treatment group, and rounded to the nearest whole unit.

- **BSP2:** The best supplementation program for the predatory mites was identified using generalized linear mixed models (GLMM). The number of eggs was modeled as a function of time, treatment group, and their interaction; plant identity was included as a random effect to account for temporal pseudoreplication. Due to the large number of zero values, we used a Hurdle model with mixed effects—GLMM hurdle (glmmTMB function, glmmTMB package, Brooks et al., 2017) and a Poisson error distribution. With this model structure, the effect of the supplementation treatments could be interpreted separately for (i) the probability of oviposition (binary response: zeros vs. non-zeros) and (ii) the number of eggs given the occurrence of oviposition. Stepwise backward model selection was performed, and the best-fit model was the model with the lowest AIC, as previously described.
- **Biocontrol:** The efficiency of the best supplementation program in controlling the pest mite was assessed using GLMM. The number of mobile pest mites (larvae + nymphs + adults) was modeled as a function of time, treatment group, and their interaction, and plant identity was included as a random effect to account for temporal pseudoreplication; a Poisson error distribution was employed (glmer function, lme4 package, Bates et al., 2015). The best-fit model was selected as explained above.

3   Results:

3.1 BAF

The best alternative food was the combination of almond pollen and *E. kuehniella* eggs (Pe+P+E) because it was the only treatment to significantly increase both predatory mite survival and reproduction relative to the control (see the Wilcoxon multiple pairwise comparison results in Fig. 1). Therefore, this food regime was selected as the BAF in the subsequent experiments. The survival data showed that predatory mites were significantly more abundant when fed pollen (Z[79] = 3.4, p < 0.001; Fig. 1a) or *E. kuehniella* eggs (Z[79] = 2.9, p = 0.004). Indeed, the best-fit model contained the two food treatments and their interaction, as well as the day the experiment started. The effects of the foods were not additive (negative interaction between food types: Z[79] = -2.1, p = 0.04; Fig. 1a), and predatory mite abundance was higher in the boxes started on the second day (Z[79] = 5.8, p < 0.001). The reproduction data showed that supplementation with both foods positively affected the likelihood of egg and larvae production (Z[77] = 2.7, p = 0.007 for pollen; Z[77] = 2.3, p = 0.02 for *E. kuehniella* eggs; Fig. 1b). The treatments had no effect on the number of eggs and larvae when reproduction occurred.

3.2 BSP1

The best supplementation program was the combination of the two foods and the artificial habitat (Fig. 2). It was therefore used as the BSP in the subsequent experiments. The survival data showed that the two supplementation types had a positive additive effect on the number of nymphs and adults (Z[79] = 6.3, p < 0.001 for both foods; Z[79] = 2.6, p = 0.009 for the wool; Fig. 2a). Indeed, the best-fit model contained the combined foods and the artificial habitat, as well as the day the experiment started. As in the BAF experiment, abundance was higher in the boxes started on the second day (Z[79] = 7.3, p < 0.001). The best-fit model for the reproduction data included the combined foods, the artificial habitat, and their interaction. The presence of the wool increased the likelihood of egg and larvae production (Z[77] = 2.6, p = 0.009; Fig. 2b). Moreover, the two supplementation types synergistically interacted to increase egg and larvae number when reproduction occurred (interaction: Z[77] = 2.7, p = 0.007). Indeed, the number of eggs and larvae was about 2.5 times higher in the combined supplementation treatment than in the treatment with artificial habitat alone (Fig. 2b).

3.3 BSP2

The first greenhouse experiment yielded similar results to the BSP1 experiment, underscoring the pronounced effects of both supplementation types on the long-term dynamics of predatory mite populations (Fig. 3). As in the laboratory experiments, the presence of the two food types and the artificial habitat synergistically increased the likelihood of egg production (Table 1). Moreover, when the supplemental foods were absent, the positive effect of the wool's presence on oviposition probability decreased over time (Table 1). Egg number increased when the predatory mites were given the two supplemental foods (Table 1). By itself, the wool's presence had a negative effect on egg number. However, its interaction with time was positive, meaning that these negative effects diminished over the course of the experiment (Table 1).

3.4 Biocontrol

The combined supplementation treatment did not promote pest mite development. Indeed, neither supplementation type had an effect on *T. urticae* populations in the absence of the predatory mites (Table 2, Fig. 4). Furthermore, the combined supplementation treatment significantly enhanced pest suppression. When the predatory mites were absent, *T. urticae* abundance dramatically increased over time (Fig. 5). Introducing *N. cucumeris* significantly slowed this growth (Table 2, Fig. 4). Providing the two foods and the artificial habitat in tandem significantly boosted the level of biological control (Table 2). Indeed, based on the model's estimates, the two supplementation

types helped reduce the *T. urticae* population so much that its rate of reproduction became negative, which suggests pest control would be highly effective in the long term (Fig. 5).

4   Discussion:

This study found that providing alternative food resources and artificial habitat boosted biological control by the predatory mite *N. cucumeris* because the two supplementation types interacted synergistically to increase *N. cucumeris* survival and oviposition. These findings were supported by data obtained under both laboratory and greenhouse conditions. The best alternative food was almond pollen and *E. kuehniella* eggs used together. In the best supplementation program, the two foods were coupled with artificial habitat, a regime that increased egg and larvae production by *N. cucumeris*. Furthermore, we demonstrated that this supplementation program resulted in more effective control of the pest mite *T. urticae* under greenhouse conditions*.*

Our results add to a growing body of research that highlights the positive influence of alternative food supplementation on predatory arthropods such as ladybird beetles or mirids (Berkvens et al., 2007; Vandekerkhove and De Clercq., 2010) and mites (Van Rijn et al., 1999, 2002; Nomikou et al., 2010; Duarte et al., 2015). In particular, we observed that almond pollen could serve as a useful alternative food for *N. cucumeris*, which, to our knowledge, is a new discovery. Furthermore, we found that the combination of the two foods—almond pollen and *E. kuehniella* eggs—had a positive effect on oviposition by *N. cucumeris*. Similar results were reported by Beltrà et al. (2017) for *E. stipulatus*, in a study that also employed two alternative foods (i.e., *Typha* sp. pollen and a sugar solution). One potential explanation for these results is that *N. cucumeris* directly benefited from increased food availability. Another potential explanation is that increased food availability and quality decreased levels of cannibalism (Zannou et al., 2005; Calabuig et al., 2018). Mite population development could also have been enhanced because of the dietary complementarity of the food resources (Nguyen et al., 2013). For instance, De Clercq et al. (2005) showed that diversified diets can be important: pollen-based supplementation compensated for suboptimal prey in a polyphagous ladybird beetle species. In our study, we observed a similar effect when combining plant- and animal-based foods.

We also observed that the artificial habitat stimulated *N. cucumeris* oviposition. The availability of nesting sites and refuges has previously been shown to benefit various predatory mite species, like *N. californicus* or *A. limonicus* (Roda et al., 2001; Kawashima et al., 2006; Liu et al., 2018; Bresch et al., 2019). In both our laboratory and greenhouse experiments, *N. cucumeris* larvae and eggs were mostly found in the wool, indicating that this type of artificial habitat provides a favorable environment and a certain degree of protection to earlier mite stages. This pattern is likely mechanistically driven by thigmotaxis, negative phototropism, or the wool's optimal microclimatic conditions. The availability of artificial habitat might also limit intraspecific competition and cannibalism in the same way that plant acarodomatia do (Ferreira et al., 2011). The aggregation of early stages of *N. cucumeris* in artificial habitats could also trigger social interactions that are beneficial to young predatory mites (Schausberger et al., 2017).

The results of the laboratory and greenhouse experiments demonstrated that the development of the predator population was enhanced when food and habitat supplementation were combined. A similar result was seen for *A. swirskii* and *E. scutalis* on sweet pepper plants (Loughner et al., 2011; Adar et al., 2014). We found that the two supplementation types acted synergistically; their influence extended beyond the simple sum of their individual effects. Comparable results were obtained in an experiment with *E. stipulatus* on *Citrus aurantium* plants, in which the effects of supplementation with Typha's pollen, sucrose solution, and cotton fibers were tested (Pekas and

Wäckers, 2017). Moreover, the Pekas and Wäckers (2017) study and our study highlight that such tandem supplementation programs can maintain predatory mite populations on crops for several weeks, even in the absence of prey (up to 6 weeks in our experiments). This time period is much longer than the introduction delay that biological control companies recommend for their biological control programs.

More importantly, this study has increased our understanding of how combined supplementation programs can enhance pest control efforts and boost biological control methods based on predatory mites. Indeed, in the biocontrol experiment, combining the presence of *N. cucumeris* with the combined supplementation regime was the only effective strategy for controlling *T. urticae* in the long run. Although *N. cucumeris* can easily consume inert food resources such as pollen or eggs, it did not permanently switch food or become satiated in this study. Indeed, pest suppression was more effective with than without the supplementation regime. Short-term experiments have observed a decrease in predatory activity by generalist arthropods given supplemental alternative foods (Skirvin et al. 2007, Leman and Messelink, 2015), a pattern seen in a recent study that also used *N. cucumeris* and *T. urticae* (Li and Zhang, 2020). These dynamics might explain why the supplementation program did not have an effect during the two first weeks of the biocontrol experiment. However, from week 3 onward, there were significant impacts on the pest mite population, probably because the predatory mite population was growing faster, as was seen in the experiments to identify the best supplementation program under laboratory conditions (BSP1) and greenhouse conditions (BSP2). The supplementation program thus appeared to trigger a form of long-term apparent competition between the supplemented resources and the focal pest, as has been observed in other predator-prey systems (Van Rijn et al., 2002; Nomikou et al., 2010; Leman and Messelink, 2015).

A major drawback of supplementation programs is that target pests could benefit from the supplemented resources. Such is the case for the thrips species *Frankliniella occidentalis* (Pergande), whose growth is increased by Typha pollen, a biocontrol product marketed as food supplementation for generalist predatory mites (e.g., Vangansbeke et al., 2016). The combined supplementation program did not have such an effect on *T. urticae* in this study. Although *T. urticae* produces fine webs that enhance its survival and fitness (Oku et al., 2009; Le Goff et al., 2010), it did not appear to be affected by the presence of the wool microhabitats. Likewise, food supplementation did not boost the development of *T. urticae* populations. However, if the supplementation program described here were to be used in a different system, such as one where *N. cucumeris* is used to control *F. occidentalis*, for example, further research would be needed to confirm that the new pest would not be positively affected. Additionally, the program's broader applicability would need to be tested using plants other than sweet pepper. Indeed, supplementation effects could be plant dependent, as seen with *A. limonicus* (Lee and Zhang, 2018).

To mount effective biological control efforts, it is essential to study the practical aspects involved in implementing combined supplementation programs in agricultural systems. In this regard, our supplementation program holds promise because of the low cost and minimal quantities of the alternative foods and wool. However, the technique used to create the microhabitat on the plants is not at all feasible for crop farmers. Further applied research is needed to develop a technological solution that is more easily deployed. Adar et al. (2014) developed a promising solution: pollen-coated twine rings hung over host plants. However, this solution did not achieve better results than simply adding pollen to the leaves. That said, predatory mite populations drastically increased in size when plain twine rings were used in combination with the addition of pollen to the leaves, suggesting that it may be important to separate nesting sites from food resources (see also Faraji et al., 2002). Moreover, the spatial distribution of microhabitats could also play a role—past

research has shown that the increase in predatory mite populations was more pronounced with dispersed as opposed to aggregated habitats (Liu et al. 2018). Therefore, it is crucial to consider the spatial and temporal aspects of combined supplementation programs to ensure that they translate into effective biological pest control.

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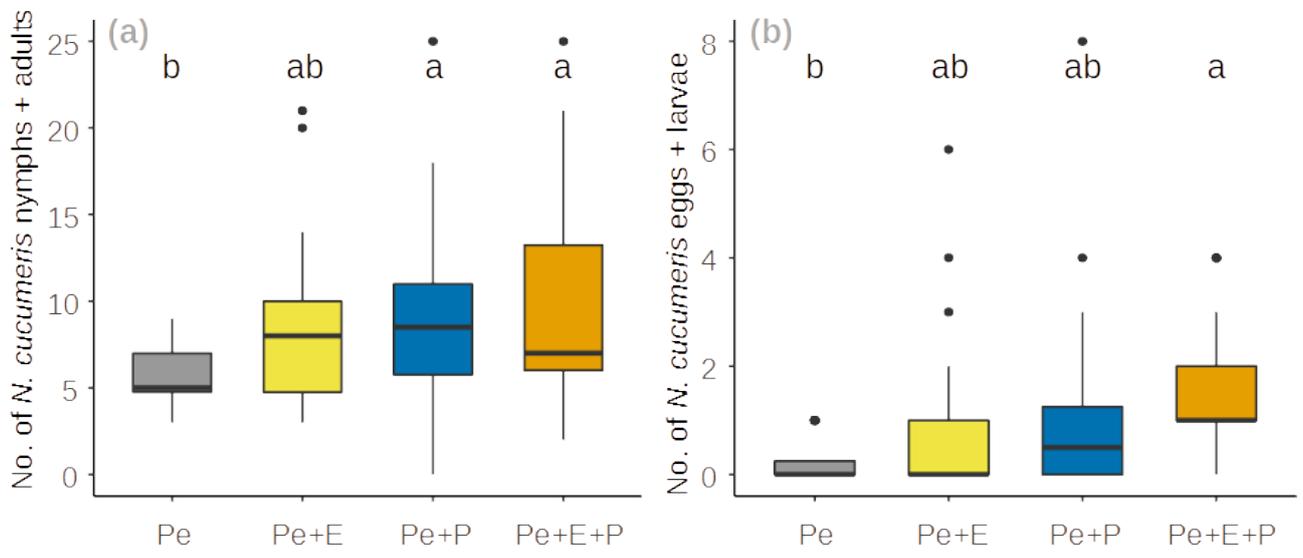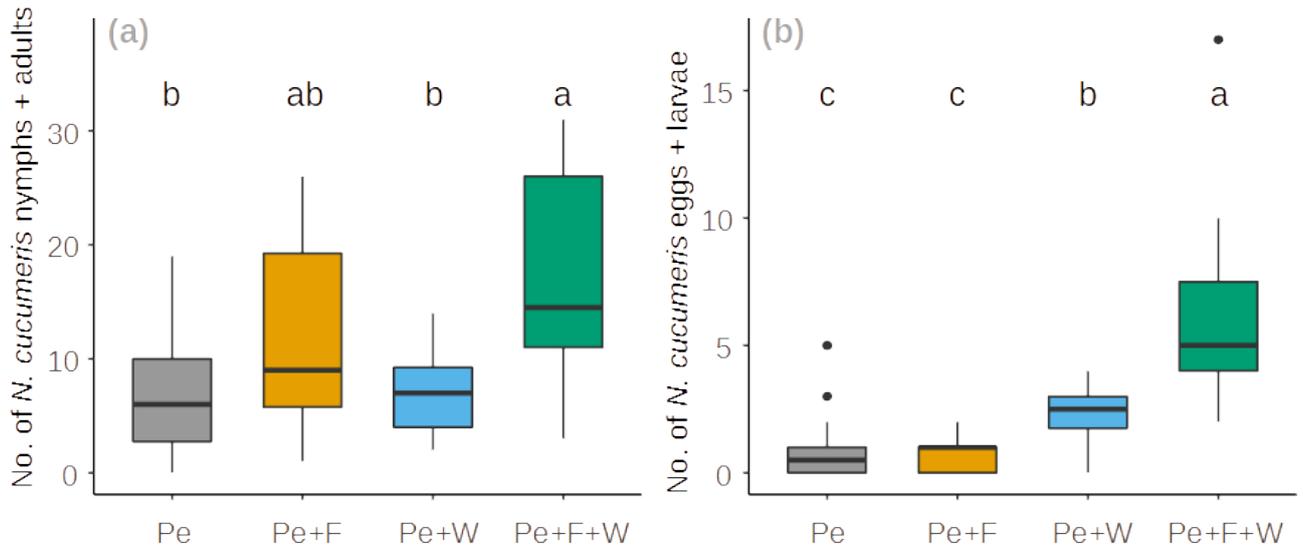
Figure 1

Figure 2

Table 1

Likelihood of oviposition	Estimate	Standard error	Z value	P value
(Intercept)	1.26	0.33	3.86	**0.0001**
Foods	-1.37	0.25	-5.38	**< 0.0001**
Wool	-2.34	0.49	-4.77	**< 0.0001**
Week	-0.07	0.07	-0.89	0.37
Foods*Wool	-1.07	0.45	-2.36	**0.02**
Wool*Week	0.31	0.12	2.63	**0.008**
Egg number				
(Intercept)	1.23	0.15	8.30	**< 0.0001**
Foods	0.46	0.15	3.13	**0.002**
Wool	-0.70	0.19	-3.66	**0.0003**
Week	0.04	0.02	1.68	0.09
Foods*Wool	0.36	0.20	1.84	0.07
Wool*Week	0.11	0.03	3.82	**0.0001**

Figure 3

Table 2

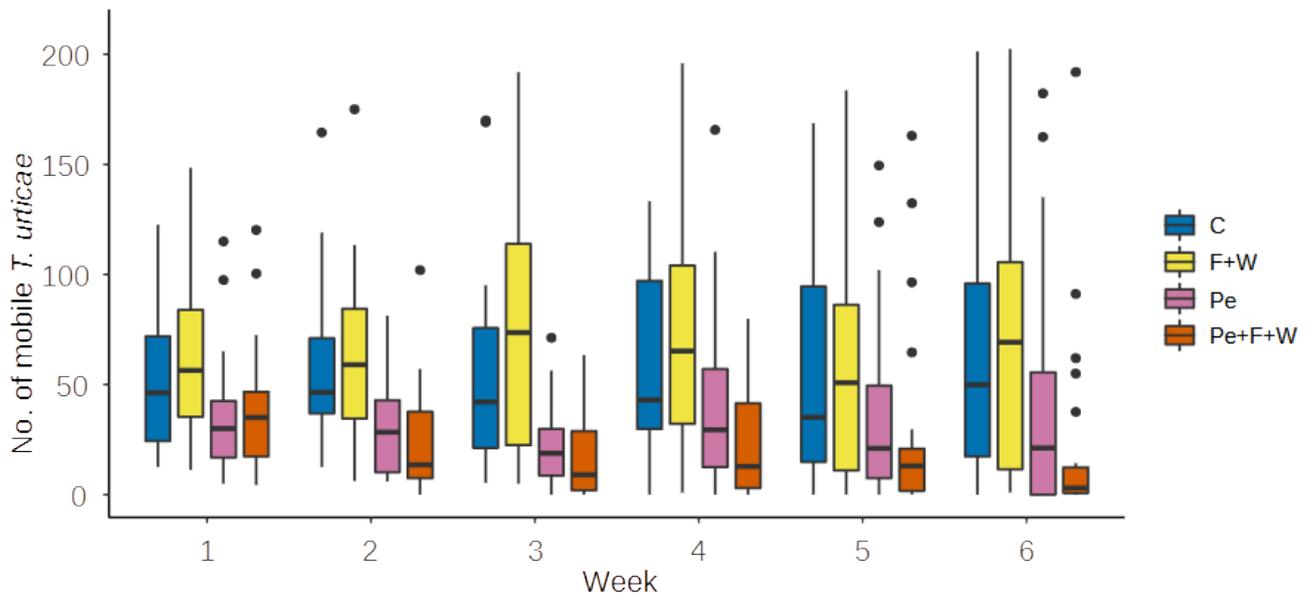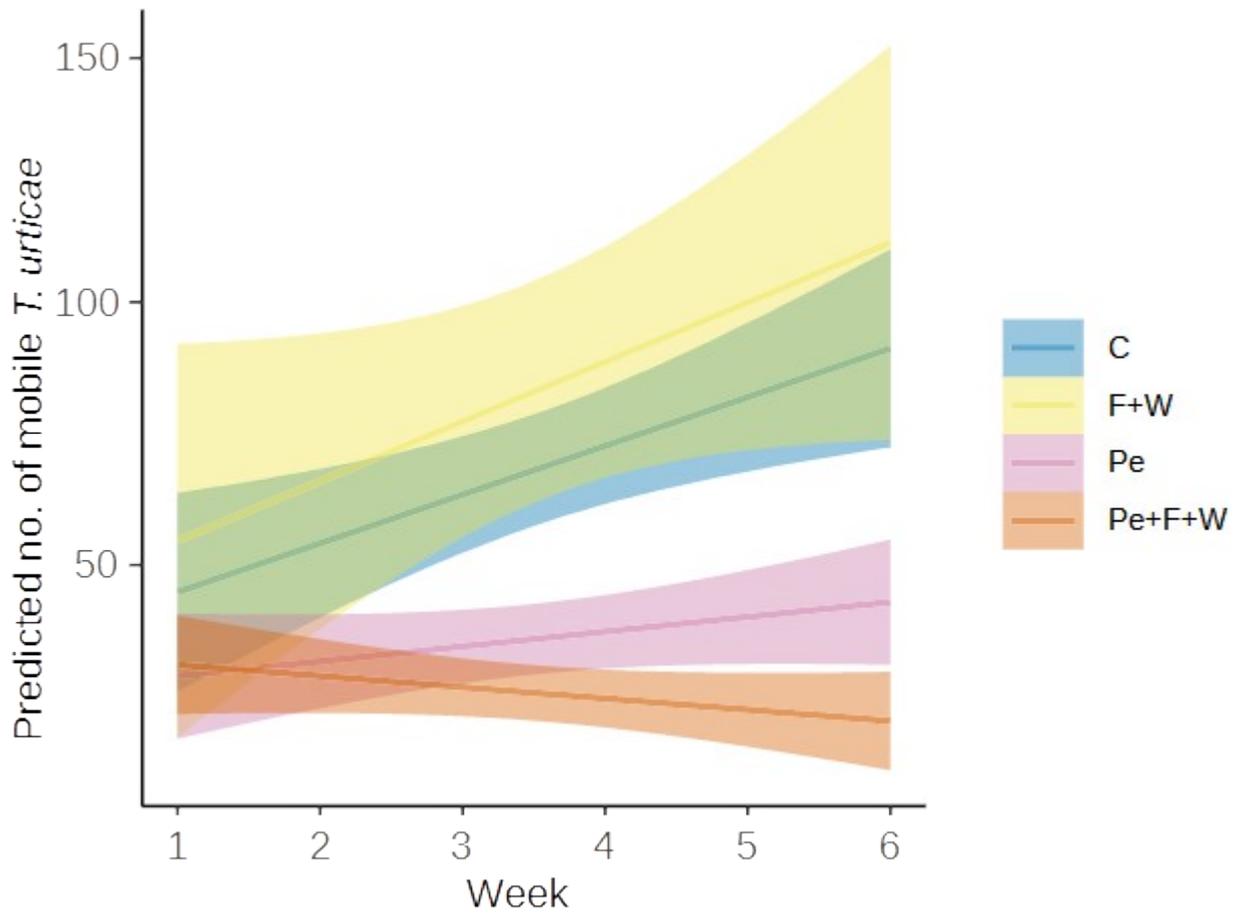|	Estimate	Standard error	Z value	P value
(Intercept)	3.40	0.19	17.63	**< 0.0001**
Supp.	0.07	0.27	0.24	0.81
Pred.	-0.48	0.27	-1.77	0.08
Week	0.14	0.006	22.88	**< 0.0001**
Supp.*Pred.	0.11	0.39	0.29	0.77
Supp.*Week	0.0005	0.008	0.06	0.95
Pred.*Week	-0.06	0.01	-5.73	**< 0.0001**
Supp.*Pred.*Week	-0.16	0.02	-10.83	**< 0.0001**

Figure 4

Figure 5

Figure 1: Abundance of predatory mites (*N. cucumeris*) in the BAF experiment: (a) numbers of nymphs and adults as a proxy for survival and (b) numbers of eggs and larvae as a proxy for reproduction. Different letters show the presence of significant differences ($p < 0.05$; Wilcoxon multiple comparison tests). Pe = predatory mites only, E = moth egg supplementation, P = almond pollen supplementation.

Figure 2: Abundance of predatory mites (*N. cucumeris*) in the BSP1 experiment: (a) numbers of nymphs and adults as a proxy for survival and (b) numbers of eggs and larvae as a proxy for reproduction. Different letters show the presence of significant differences ($p < 0.05$; Wilcoxon multiple comparison tests). Pe = predatory mites only, F = food supplementation (almond pollen and moth eggs), and W = wool supplementation.

Figure 3: Abundance of predatory mite (*N. cucumeris*) eggs in the BSP2 experiment over time and among treatment groups. Pe = predatory mites only, F = food supplementation (almond pollen and moth eggs), and W = wool supplementation. One point is not shown in the graph: the number of eggs in the Pe+F treatment group in week 1, which equaled 30.

Figure 4: Abundance of (larvae + nymphs + adults) *T. urticae* in the biocontrol experiment over time and among treatment groups. C = control, Pe = predator presence, F = food supplementation (almond pollen and moth eggs), and W = wool supplementation. Ten points are not shown for the F+W treatment group: weeks 4, 5, and 6—514, 725, and 1276, respectively; for the control treatment group: week 4—264, week 5—336 and 285, and week 6—369, 297, and 292; and for the Pe treatment group: 282 in the week 5.

Figure 5: Predicted abundance of (larvae + nymphs + adults) *T. urticae* in the biocontrol experiment over time and among treatment groups. The lines represent the abundances estimated from the GLMM model (Table 2). The envelopes were constructed using glm smoothing. C = control, Pe = predator presence, F = food supplementation (almond pollen and moth eggs), and W = wool supplementation.

Table 1: Statistical results of the BSP2 experiment (GLMM hurdle). The best-fit model was Eggs ~ Foods * Wool + Week * Wool; plant identity was also included as a random variable. Below are the effects of the variables on the likelihood of oviposition (binomial data) and egg number (count data). Degrees of freedom: 563. Significant results are in bold (p-value < 0.05).

Table 2: Statistical results of the biocontrol experiment (GLMM). The best-fit model was Abundance of mobile pest mites (larvae + nymphs + adults *T.urticae*) ~ Supplementation * Predator presence * Week; plant identity was also included as a random variable. Supp. = supplementations (F+W) and Pred. = predator presence (Pe). Degrees of freedom: 567. Significant results are in bold (p-value < 0.05).

Appendices :

Table A: Abundances and sex ratios of the predatory mite (*N. cucumeris*) and the prey mite (*T. putrescentiae*) at the start of the experiments.

		BAF		BSP1		BSP2		Biocontrol	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Predatory mite	larvae	2.3	1	6.7	3.3	1.6	1.6	1	1.5
	males	4	1.5	9.9	4.2	7	2.2	4.6	3.3
	g. females	1.3	1.1	6.6	3.3	3.9	1.8	4	2.1
	females	4	2.6	14.6	3.3	12.7	3.8	6.7	4.3
	eggs	22.9	7.1	54.3	19.4	5.7	2.1	21.3	16.3
Prey mite	adults + nymphs + larvae	48	7	171.3	19.7	55.7	9.3	27.3	6
	eggs	96.7	17.7	1212	175.9	100	38.2	67.3	26

g. females: gravid females and SD: standard deviation

Table B: Climatic conditions in the greenhouse experiments (BSP2 and Biocontrol). The temperature and relative humidity (RH) were set to 25°C and 75%, respectively. An RH level of 65% is optimal for *N. cucumeris*. The plants were watered twice a day using a drip irrigation system (30 L per watering session per compartment). Screens helped reduce light intensity (600-700 W/m^2).

	BSP2		Biocontrol	
	Temperature (°C)	RH (%)	Temperature (°C)	RH (%)
Minimum	17.1	51	17.5	42
Maximum	30.6	82	31.7	98
Mean	20.9	75	21.6	82
Standard deviation	3.4	5	3.9	8.6