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Ringed micro-habitats as supplementations for predatory mites

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Abstract: In augmentative biological control, the reproduction, survival and efficiency of predatory mites can be improved by providing supplementations such as artificial micro-habitats and alternative food sources. Building on previous results on food and micro-habitat supplementations for *Neoseiulus cucumeris*, the present study aimed to i) design a prototype of Ringed Micro-habitats (RM) easily usable in agricultural cropping systems and optimise its position on the plant; ii) test the effect of this RM on the reproduction of three predatory mite species: two generalists (*Neoseiulus cucumeris* and *Amblyseius swirskii)*, and one specialist (*Neoseiulus californicus*); iii) test the effect of this RM on the reproduction of two phytophagous mite species: *Polyphagotarsonemus latus* and *Tetranychus urticae*. We evidenced that *A. swirskii* preferred to lay eggs in the RM prototype when it was installed in direct contact with the underside of a rose leaf instead of being suspended on the plant. We also demonstrated that leaves bearing RM prototypes significantly increased the reproductive output of both generalist predatory mite species, but they had no impact on the reproductive output of the specialist species. In addition, the presence of RM on plants had no effect on the reproductive output of the studied pest species. Results indicate that the provision of supplementations such as the RM can favour generalist predatory mite reproduction with no risk on pest promotion, opening new avenues for more efficient biological control with predatory mites.

Key words: biocontrol, predatory mites, pest mites, microhabitat, alternative food

Introduction

Augmentative biological control is based on inoculative or repeated introductions of natural enemies of plant pests (Bout et al. 2022). This control method has become one of the pillars of biological and integrated protection programs in the majority of greenhouse crops worldwide. Predatory mites represent 60% of this market (by value) as their use steadily increased since 2000 (Knapp et al. 2018). A part of this growth concerns practices of preventive predator introductions combined with techniques of alternative food supplementations (Leman and Messelink, 2015).

Combining food supplementations with artificial micro-habitats proved to have a much higher positive impact on populations of predatory mites than bare food supplementations (eg. Adar et al. 2014; Pekas and Wackers 2017; Etienne et al., 2021). Recent studies in greenhouse conditions demonstrated that this positive effect translates into more efficient pest population suppression (Lee and Zhang 2018; Etienne et al. 2021). However, according to our knowledge, no micro-habitat technology is currently on the market. This might be explained by the complexity of the functional role of micro-habitats whose different dimensions may depend on an intimate association with the crop.

In a previous study, we explored the influence of artificial micro-habitats, combined with alternative food sources, on the generalist predatory mite *N. cucumeris* (Etienne et al., 2021). Related designs were also used by Pekas and Wäckers (2017) and Lee and Zhang (2018). In our study, the micro-habitat was made of raw frayed wool patches that were glued under the leaves of plants (Figure 1a) mimicking a domatium (Grostal and O'Dowld, 1994). Alternative foods were both plant-based (pollen of *Prunus dulcis* (Mill.) (D.A.Webb, 1967) and animal-based (*Ephestia kuehniella* Zeller eggs). Experiments under both laboratory and greenhouse conditions showed that the additional provision of artificial micro-habitat drastically increased the reproductive output of the predatory mite, as compared to bare food supplementation. Furthermore, the efficiency of this combined supplementation program was also evaluated on the suppression of *Tetranychus urticae* pest on pepper plants in greenhouse conditions. Combining the presence of *N. cucumeris* with the supplementations of food and microhabitat was the only effective treatment to control *T. urticae* in the long term (Etienne et al., 2021).

The installation of wool patches under plant leaves was proved efficient but this method requires a lot of handling time, limiting its interest for growers. To solve this problem, we designed and optimised a prototype of microhabitat like a sticky ring of frayed wool, a "ringed micro-habitat" (RM, Figure 1b-c). It can be easily mass-produced and efficiently arranged on crop plant leaves in real life conditions, making the RM more adapted and transferable to growers. In this study, we evaluated to which extent an intimate relationship between plant leaves and RM was necessary or if the mere presence of the RM in the plant was sufficient to boost predatory mites reproductive output. Then, we quantified the effects of RM on the reproduction of two generalist (type III) and one more specialist (type II) species of predatory mite (the Phytoseiidae *Neoseiulus cucumeris* Oudemans, *Amblyseius swirskii* Athias-Henriot and *Neoseiulus californicus* MCGregor, respectively). *Neoseiulus cucumeris* and *A. swirskii* feed on a wide range of resources of plant (pollen, nectar) or animal origin (arthropods) (Mcmurtry et al., 2013). *Neoseiulus californicus* is mainly used to control *T. urticae* (Easterbrook, Fitzgerald, & Solomon, 2001) and can also feed on alternative food (Khanamani et al. 2017a) although to a lesser extent than the other two generalist species.

Finally, we tested a potential effect of the supplementations on the reproduction of plant pests. We assessed the effects of RM on the reproduction of two economically important phytophagous mites: *Polyphagotarsonemus latus* Banks (Tarsonemidae) and *Tetranychus urticae* Koch (Tetranychidae) (Malais and Ravensberg, 2006).

Material and methods

Supplementations

Ringed-Micro-habitat (RM) prototype

The RM prototype was made of green wool yarns. Wool proved to be effective egg shelters for several species of predatory mites (Etienne et al., 2021; Bresch et al., 2019; Loughner et al., 2010a,b; Kawashima et al., 2006). The RM was made of 0.05g of frayed wool that was glued to the non-sticky side of a reinforcement ring (Figure 1b). The RM is a self-adhesive ring that might subsequently be stuck on the underside of plant leaves (Figure 1b).

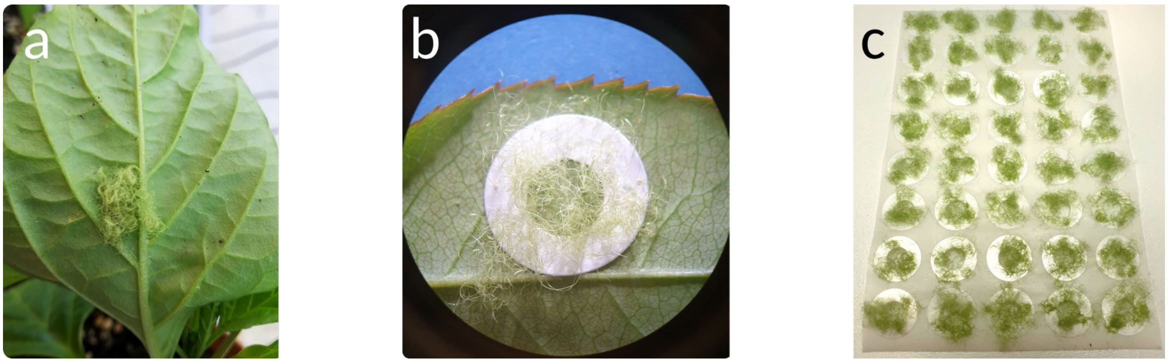

Figure 1. (a) Patch of frayed wool stuck under pepper plant leaf. (b) Ringed Micro-habitat prototype stuck on the lower face of a leaf of *Rosa sp.* (c) Plate of ringed micro-habitats in serial production.

Food supplementation
RM was combined with alternative foods composed of 50% *P. dulcis* pollen (Firman pollen, USA) and 50% *E. kuehniella* eggs (Bioline Agrosciences, France). Almond pollen is an important source of nutrients such as lipids, sugars and proteins that support growth, immunocompetence and mite reproduction (Khanamani et al., 2017b). *Ephestia kuehniella* eggs are a complementary efficient food source for Phytoseiidae, especially for the generalist species *A. swirskii* and *N. cucumeris* (Delisle et al., 2015).

Mites
Predatory mites
Two generalists' predatory mite (type III) species (*A. swirskii* and *N. cucumeris*) and one more specialist species (type II) (*N. californicus*) were considered in this study.

All three species were purchased in bulk boxes from Koppert B.V. (The Netherlands). Predatory mites were then reared in the laboratory (Nguyen and al., 2014) to produce egg cohorts of the same age. Eggs of the same cohort were reared together until maturity, fed with food supplementation every two days and carefully transferred to experimental devices. All predatory mite species were reared at 25+/-0.6°C with 72+/-8% RH and 16L:8D photoperiod. In such conditions, *A. swirskii* reached adult stage in 7 days, *N. cucumeris* in 11 days and *N. californicus* in 9 days.

Pest mites
Polyphagotarsonemus latus were collected on star jasmine plants (*Trachelospermum jasminoides* (Lindl.) Lem.) by the Regional Horticultural Development Committee of the Centre-Val-de-Loire region (CDHR, France). *Tetranychus urticae* populations were collected on *Calystegia sepium* (L.) R. Br. (Convolvulaceae) from the site of the Mediterranean Agricultural Research and Experimentation Center (CREAM, France). Both pest mite species were reared on bean plants in growth chambers (23°C, RH<50% for *T. urticae*, RH >80% for *P. latus*, 16L:8D photoperiod).

Experiments
Ringed Micro-habitat position
To evaluate if the RM are more effective for predatory mite oviposition when stuck on a leaf or hanging on plants, a choice experiment was performed. 20 individuals of *A. swirskii* were released in a plastic-coated cardboard arena where two RM were disposed: one was directly

stuck under a leaf of rosebush associated with an empty plastic label and the second was stuck to a plastic label associated with an empty leaf (Figure 2). A small plate containing food supplementations was placed in the centre of the arena and renewed every 2 days in order to prevent pollen rotting. In order to guarantee the hydration of the rose leaves during the experiment, their petiole was placed between two sheets of damp paper towel bordering the arena. 15 replicates were randomly deposited in thermoregulatory climatic cabinets (16L:8D photoperiod, 25.9 +/- 2.8°C, 68.1 +/- 12.8% RH). After 7 days, eggs on each RM were counted and both the rose leaves and the prototypes were renewed. Living mites collected with food, leaves or RM were returned to the arena. Two days later, eggs on each RM were counted.

Effect of RM on predatory mites

For each predatory mite species, 10 adults (3 males and 7 females) were deposited on a plastic-coated cardboard arena either with or without RM. The cardboard stood on the top of a wet sponge in a plastic box. In both cases (with or without RM), predatory mites were fed with food (mix of pollen and moth eggs). The boxes (n=31 for *A. swirskii*, n=20 for *N. cucumeris* and n=16 for *N. californicus*) were deposited in thermoregulatory cabinets (16L:8D photoperiod, 25,3°C +/- 0,78, 72,5% +/- 7 RH). Three days after the beginning of the experiment, the number of eggs and larvae laid in the food, on the plastic floor and in the RM were counted.

Effect of RM on pest mites

For each pest mite species, a bean plant composed of ten leaves was prepared with or without a RM fixed on the lower side of the biggest leaf. Ten adults of either *T. urticae* or *P. latus* (2-3 males and 7-8 females) were then introduced. For each species, the 28 plants (14 with and 14 without RM) were reared in climatic chambers (26°C, RH<50% for *T. urticae*, RH>80% for *P. latus*, 16L:8D photoperiod). Three days after pest inoculation, the plants were cut individually by separating the leaves and stems of each plant and stored in a zipped plastic bag placed in a cold room (4°C). Eggs and larvae present on each plant were then counted separately.

Statistical analysis

Statistical analyses were performed with R software (version 4.2.2, R Core Team, 2022). Package tidyverse and ggplot2 (Wickham et al. 2019) were used for data manipulation and graphics.

Ringed Micro-habitat position

A Generalised Linear Model (GLM) from the binomial family was built where the dependent variable was both the number of eggs laid on the leaf's RM and the number of eggs laid on the label's RM. The estimated intercept of this simple model (with no explanatory variables) was compared to 0 with a Z test.

Effect of RM on predatory and pest mites

The number of eggs laid with or without RM was compared using a quasi Poisson GLM with the whole number of eggs laid as the dependent variable and the presence of RM as the explanatory variable. Its effect was tested by removing this variable and comparing the resulting scaled deviances with an F test.

Results and discussion

Ringed Micro-habitat position

The predatory mite *A. swirskii* showed a strong preference for laying eggs on RM attached to a leaf rather than to a plastic label placed on a leaf (Figure 2, Z=5.3, p<0.001). RM effects shall therefore be maximised when positioned on leaves, as real acarodomatia, and not on hanging plastic labels. This indicates that the holed design of the RM is important to ensure intimate exchanges between predatory mites and plants. Owing to this direct contact, the predatory mite appeared to benefit from physico-chemical exchanges with the plant, including evapotranspiration (Walzer et al., 2007).

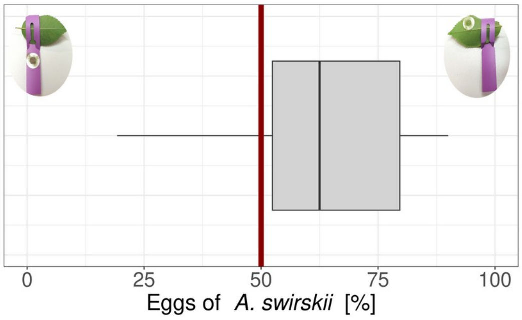

Figure 2. Percentage of *A. swirskii* eggs laid in leaf's RM compared to plastic labels' RM.

Effect of RM on predatory mites

The presence of the AM prototype had a different effect depending on the species of predatory mites considered, with significant effects on the two generalist type III species (Figure 3). The presence of RM boosted by 3.8 times the reproductive output of *N. cucumeris* ($F(1)=24.2$, $p<0.001$) and by 1.4 times that of *A. swirskii* ($F(1)=36.4$, $p<0.001$). No significant difference was observed for *N. californicus* ($F(1)=0.4$, $p=0.5$).

On the one hand, these results support the potential of RM combined with food supplementation to support generalist predatory mite species (Etienne et al., 2021; Lee and Zhang, 2018; Pekas and Wäckers, 2017). In particular, the suitability of wool as artificial micro-habitat for predatory mites is confirmed (Bresch et al., 2019; Loughner et al., 2010a,b; Kawashima et al., 2006). Indeed, when the RM was present, mites laid preferentially in the RM's wool rather than anywhere else in the arena. This attraction might be explained by different factors such as the high moisture retention potential of wool (Tuczu, 2007) or for supporting thigmotactic behaviours (Putman, 1962).

On the other hand, such supplementation programs seemed not adapted to less generalist species such as *N. californicus* and most probably completely useless for true specialist species (e.g. *Phytoseiulus persimilis* Athias Henriot). This lack of interest of *N. californicus* for the RM was not expected since this species lay preferentially eggs on plants with domatia (Bresch et al., 2015), or other artificial micro-habitats (Kawashima et al., 2006). In this study, *N. californicus* displayed the lowest survival rate per cohort. Even if *N. californicus* seems able to develop when exclusively fed by *P. dulcis* pollen (Khanamani et al., 2017b) or *E. kuehniella* eggs (Song et al., 2019), a most suitable diet might have been necessary to ensure *N. californicus* population growth (Song et al., 2019).

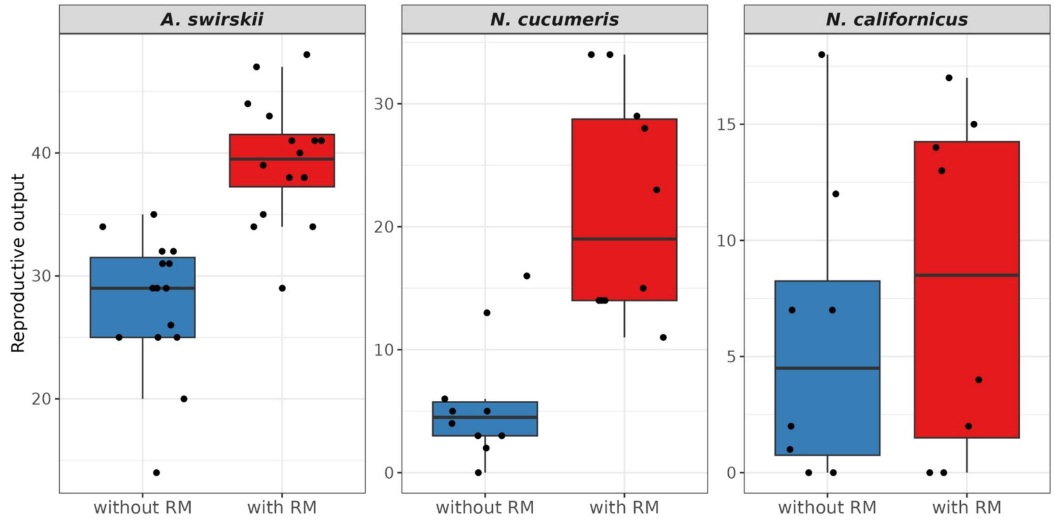

Figure 3. Reproductive output (number of eggs and larvae) of the predatory mites *A. swirskii* (left), *N. cucumeris* (centre) and *N. californicus* (right) according to the presence of RM after three days. Ten adults (3 males and 7 females) for each species of predatory mite and each experimental unit.

Effect of RM on pest mites

The presence of the RM did not favour the development of the pest mites *P. latus* ($F(1)=0.001$, $p=0.97$) and *T. urticae* ($F(1)=0.003$, $p=0.96$) on plants (Figure 4). Furthermore, not a single pest egg was laid on RM during the three days of experimentation. This lack of interest of pest mites for RM might be explained by the fact that pest mites seem not particularly favoured by natural acarodomatia (Grostal and O'Dowd, 1994). As an example, *T. urticae* adults produce their own webs to shelter their eggs (Clotuche et al., 2010).

These results confirm that artificial micro-habitats do not benefit pest mites (Lee and Zhang 2018; Etienne et al. 2021). Providing shelter and food to natural enemies without favouring pest mites, might thus be an efficient tool for augmentative biocontrol (Leman and Messelink, 2015).

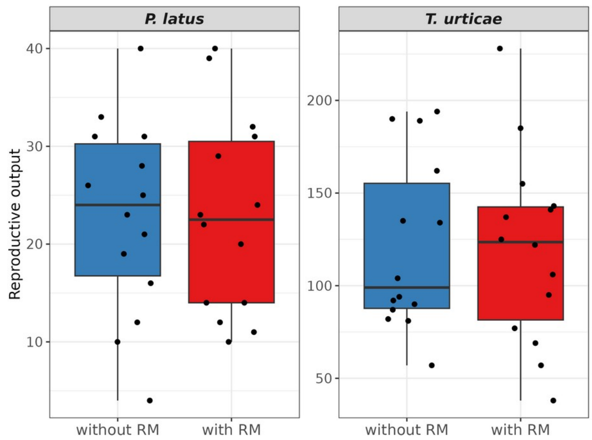

Figure 4. Reproductive output (number of eggs and larvae) of the pest mites *P. latus* (left) and *T. urticae* (right) depending on the presence or absence of RM.

Conclusion

The RM is a promising tool to enhance generalist predatory mites survival and development. It stimulates predatory mites egg laying without affecting pest mite populations. With little technological effort to automate the manufacturing process, RM could be an efficient solution to boost efficiency and low financial cost of augmentative biological control programs.

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