Entomologia
Experimentalis et Applicata

DOI: 10.1111/eea.12704

# Food supplementation to optimize inoculative release of the predatory bug *Macrolophus pygmaeus* in sweet pepper

Nathalie Brenard[1]*, Vincent Sluydts[1], Luc De Bruyn[1,2], Herwig Leirs[1] &
Rob Moerkens[1,3]

*[1]Evolutionary Ecology Group, University of Antwerp, Antwerp, Belgium, [2]Research Institute of Nature and Forest (INBO), Brussels, Belgium, and [3]Tomato Research, Research Centre Hoogstraten, Hoogstraten, Belgium*

Accepted: 25 May 2018

*Key words*: biological control, integrated pest management, IPM, inoculative release strategy, population dynamics, dispersal, greenhouse vegetables, Miridae, Hemiptera

## Abstract

Biological control is widespread in management of greenhouse sweet pepper crops. Several species of predatory mites, bugs, and parasitoids are used against a wide range of pest species. However, biological control of particular pests like aphids, caterpillars, and the tobacco whitefly, *Bemisia tabaci* Gennadius, remains problematic. *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) is a generalist predatory bug which is used on a large scale in Western European tomato greenhouses. It has already been demonstrated that *M. pygmaeus* is a valuable biocontrol option in sweet pepper crops, but it has yet to find its way into common practice. *Macrolophus pygmaeus* should be introduced at the start of the growing season and determining an optimal release strategy is a key step in this process. In tomato crops, *M. pygmaeus* requires supplemental food releases to reach sufficient population numbers and dispersal levels. In this study, the need for food supplementation in sweet pepper is investigated. Three strategies were tested: (1) no food supplementation, (2) local food supplementation, and (3) full field food supplementation. Both population numbers and dispersal rates of the second generation were higher under the third strategy. *Macrolophus pygmaeus* oviposits near food sources, therefore dispersal rates are higher when food is more spread out. Pest control was achieved in all treatments, but faster and at lower pest levels under the full field strategy.

## Introduction

One of the most illustrative examples of successful biological pest management is found in greenhouse sweet pepper crops in Europe (Ramakers, 2004; van der Blom et al., 2008). Inoculative releases of predatory bugs, predatory mites, and parasitoids are all used to control a variety of sweet pepper related pest species. Well known examples are the use of phytoseiid mites like *Phytoseiulus persimilis* Athias-Henriot against the two-spotted spider mite *Tetranychus urticae* Koch (Gerson & Weintraub, 2007), anthocorid bugs of the genus *Orius* against western flower thrips, *Frankliniella occidentalis* (Pergande) (Dissevelt et al., 1995; Weintraub et al., 2011), and the parasitoids *Encarsia formosa* Gahan and *Eretmocerus eremicus* Rose & Zolnerowich against greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (van Lenteren et al., 1995; Hoddle et al., 1998; Bolckmans et al., 2005).

Despite these successes, the biological control of aphids remains difficult in sweet pepper greenhouses and growers rely mostly on chemicals (R Moerkens, pers. comm.). Releases of the parasitoid wasps *Aphidius* spp. (van Schelt et al., 2011; Prado et al., 2015) and *Aphelinus abdominalis* (Dalman) (Jarosik et al., 1996; Mölck et al., 1999), and the gall midge *Aphidoletes aphidimyza* (Rondani) (Messelink et al., 2011a) are frequently applied against aphids, but control remains ineffective in European sweet pepper greenhouses (Bloemhard & Ramakers, 2008; Messelink et al., 2013). Moreover, these specialists will often disappear after reducing pest levels and are unable to establish a population in the greenhouse. Their use is limited to obtaining rapid control of an occurring pest outbreak by

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*Correspondence: Nathalie Brenard, Evolutionary Ecology Group, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Antwerp, Belgium. E-mail: nathalie.brenard@uantwerpen.be

releasing high quantities at the right moment which requires intensive monitoring. They cannot prevent new outbreaks unless released frequently, but this is not always economically viable (Messelink et al., 2014).

In protected tomato crops, aphids are successfully controlled with inoculative releases of the generalist predatory bug *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) (Perdikis & Lykouressis, 2002; Urbaneja et al., 2009). It is one of the most important natural enemies in biocontrol programs of tomato crops, as it feeds on multiple species affecting the crop such as mites, thrips, and whiteflies (Enkegaard et al., 2001; Blaeser et al., 2004; Castañé et al., 2004; Alomar et al., 2006). In case of prey scarcity, *M. pygmaeus* will feed on plant tissue while lowering its reproduction (Perdikis & Lykouressis, 2000; Lykouressis et al., 2001; Ingegno et al., 2011; Portillo et al., 2012). Therefore, it can maintain its population in the greenhouse, often preventing new pest outbreaks. Several studies have shown that *M. pygmaeus* is able to sustain healthy populations and prevent aphid outbreaks in sweet pepper crops (van Schelt et al., 2011; Messelink & Janssen, 2014; De Backer et al., 2015; Pérez-Hedo & Urbaneja, 2015). *Macrolophus pygmaeus* can also control thrips in sweet pepper, although less efficient compared to *Orius laevigatus* (Fieber) (Heteroptera: Anthocoridae) which is commonly used against thrips (Messelink et al., 2011a; Messelink & Janssen, 2014). Messelink et al. (2011b) identified *M. pygmaeus* as the best choice when sweet pepper crops suffer from both aphid and thrips attacks. The mirid bug can control both pests, whereas *O. laevigatus* fails to control aphids. Despite these results, inoculative releases of *M. pygmaeus* in European greenhouse sweet pepper crops are rare.

To successfully introduce *M. pygmaeus* in sweet pepper crops, an optimal release strategy should be determined. Natural enemies and supplemental food are expensive for growers and releasing them can be labor intensive. Nevertheless, a grower needs a sufficient population, spread throughout the entire greenhouse. Densities should not be too high either, as cannibalism is common (Hamdi et al., 2013) and the bugs can cause fruit damage in tomato (Castañé et al., 2011). Whether *M. pygmaeus* can cause damage in a pepper crop is so far unknown. The right timing, number, and spreading of releases and perhaps supplemental food are very important to gain maximum results at minimum costs.

In greenhouse tomato crops, *M. pygmaeus* is released soon after planting. Distributing the predators over more plants and supplementing food ensures a quick and sufficient population build-up throughout the crop (Moerkens et al., 2017). This supplementary food usually consists of eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), often mixed with cysts of the brine shrimp, *Artemia franciscana* Kellogg (De Clercq et al., 2014). Traditionally, food is supplied directly on the release plants, but Put et al. (2012) demonstrated that uniform application of *E. kuehniella* eggs ensures higher population numbers and higher dispersal rates of *M. pygmaeus*. Quite some variation exists among growers concerning the number of supplementary food applications, ranging from weekly to biweekly for 2–6 weeks or even longer.

No research has been published about the need for supplementary food applications when using *M. pygmaeus* in biocontrol programs of sweet pepper. In contrast to tomato crops, sweet pepper crops produce a lot of pollen which is available as a food source for zoophytophagous bugs. In the case of *O. laevigatus*, food supplementation is unnecessary as the bugs feed on nectar and pollen produced by sweet pepper plants (Cocuzza et al., 1997; Hulshof & Jurchenko, 2000). It has been hypothesized that *M. pygmaeus* also feeds on sweet pepper nectar and pollen (Messelink et al., 2011b, 2015). Vandekerkhove & De Clercq (2010) demonstrated that *M. pygmaeus* could develop and reproduce on a mixed diet of pollen and *E. kuehniella* eggs. *Macrolophus pygmaeus* lays its eggs near food sources (Put et al., 2012; Moerkens et al., 2017), so if food supplementation is advantageous, spreading it throughout the greenhouse might benefit dispersal rate more than supplementing it locally on the release plants.

This study investigated the need for food supplementation to boost *M. pygmaeus* population build-up and dispersal rate in greenhouse sweet pepper crops at the start of the growing season. Population numbers and dispersal rates were compared between three supplementary food application strategies: (1) no supplementation, (2) local supplementation on release plants, and (3) full field supplementation where food was distributed homogeneously throughout the greenhouse.

## Materials and methods

### Greenhouse location, crop, and climate conditions

The trial was carried out in six compartments (A–F) in a semi-commercial sweet pepper greenhouse at Research Centre Hoogstraten (Hoogstraten, Belgium). Greenhouse compartment surface ranged between 500 and 1 500 $m^2$ (Table 1). Plants were sown on 21 October 2016 and planted in the greenhouse on 7 December 2016. Plant distance was 32 cm, with 2.4 plants $m^{-2}$. Each plant had three stems, which resulted in 7.1 stems $m^{-2}$. This stem density is common practice in Belgian greenhouses. Plants were planted on a rockwool substrate (Cultilene, Tilburg, The Netherlands). The greenhouses were 7 m high and equipped with a gutter growing system (FormFlex/Metazet, Wateringen, The Netherlands). Climate conditions were

**Table 1** Characteristics of the various sweet pepper greenhouse compartments

| Compartment | Surface (m$^2$) | Variety | Mean ± SE temperature (°C) | Mean ± SE relative humidity (%) | Treatment |
|---|---|---|---|---|---|
| A | 500 | Maduro | 17.7 ± 0.1 | 67.0 ± 0.8 | No food |
| B | 500 | Overture | 17.5 ± 0.2 | 68.3 ± 0.9 | Full field |
| C | 1500 | Maduro | 17.7 ± 0.2 | 65.7 ± 0.8 | No food |
| D | 1500 | Allrounder | 17.5 ± 0.2 | 69.5 ± 0.9 | Local |
| E | 500 | Maduro | 17.1 ± 0.2 | 71.3 ± 1.0 | Local |
| F | 500 | Maduro | 17.4 ± 0.2 | 69.2 ± 1.2 | Full field |

automatically logged in each compartment and registered by means of an Electronic Measuring Box (Priva, De Lier, The Netherlands). Main sweet pepper *Capsicum annuum* L. (Solanaceae) varieties were Maduro (Enza Zaden, Enkhuizen, The Netherlands), Allrounder (Rijk Zwaan, De Lier, The Netherlands), and Overture (Syngenta, De Lier, The Netherlands). Variety distribution and climate conditions are given in Table 1. Prior to the experiment, plants were checked and found to be pest free. No plant protection products were used in the greenhouse compartments during the course of the experiment. A single fertilization scheme representable for practical conditions and advised by a crop advisor was used in all compartments.

### Release of *Macrolophus pygmaeus*

*Macrolophus pygmaeus* (product name Mirical; Koppert, Berkel en Rodenrijs, The Netherlands) was released on 16 December 2016. For each 500 m$^2$ of greenhouse compartment, the contents of one Mirical tube were equally distributed across four locations, resulting in four release locations in the small compartments (500 m$^2$), and 12 in the larger ones (1 500 m$^2$). Each location consisted of five consecutive plants. One Mirical tube contains 500 *M. pygmaeus* individuals of which 90% are adults and 10% are N4-N5 nymphal instars, and some wood chips as substrate. Contents were gently mixed while holding the tube horizontally and then divided into four equal parts, containing ca. 125 individuals. Each quarter was then equally distributed over the five plants of a release location. Insects and substrate were placed in a DIBOX (Koppert) for distribution, each release plant having one DIBOX that hung on the petiole of a lower leaf. Insects were counted while placing them in a box so 25 *M. pygmaeus* were released per plant, resulting in an average density of one individual m$^{-2}$ in all greenhouse compartments.

### Supplementary food applications

Three supplementary food application strategies were tested in six greenhouse compartments, two for each strategy. In compartments A and C no supplementary food was provided after release of *M. pygmaeus*. In compartments D and E, supplementary food was distributed on the release plants of *M. pygmaeus* only. To this end, the correct amount of food was placed on a small Petri dish and carefully blown onto the plant. In compartments B and F supplementary food was applied as a full field application using a mini-airbug (Koppert). These three strategies will be called ‘no food’, ‘local’, and ‘full field’ throughout the text.

Supplementary food was provided, according to the feeding strategy, at the time of *M. pygmaeus* release and weekly during the next 6 weeks. As we are testing a full-field supplementary food application, the costs for the growers should not be ignored. Therefore, we selected cheaper *A. franciscana* (product name Artefeed; Koppert) over *E. keuhniella* for this study. Food was provided ad libitum on the release plants under the local food strategy. The producer’s advice of 0.40 g per release plant was followed, resulting in 0.017 g m$^{-2}$. More food was more homogeneously distributed in the compartments with the full field strategy (0.04 g per plant; 0.08 g m$^{-2}$). In this case, the dosage per plant is determined by the speed at which the user of the mini-airbug walks by the plants. This resulted in a total of 0.119 g m$^{-2}$ at a density of 2.8 g per release plant added in each compartment of the local strategy and 0.56 g m$^{-2}$ at a density of 0.28 g per plant in each compartment of the full field strategy by the end of the experiment.

### Population dynamics and dispersal

The population growth of *M. pygmaeus* was recorded weekly from 23 December 2016 until 15 March 2017. Four count plots were created in each greenhouse compartment, each consisting of 11 consecutive plants. Two plots per compartment included release plants of *M. pygmaeus* and are hereafter referred to as ‘release plots’. The other two plots were selected two plant rows further and insect counts at these locations give an indication of the dispersal rate of *M. pygmaeus* in the greenhouse. These plots are cited as ‘dispersal plots’ throughout the text. The diagonal plot distance between a release plot and its corresponding dispersal plot was 8.4 m and no other release plants were located within a closer range than 8.4 m. To keep sufficient distance between a dispersal and a non-corresponding release plot, no more than four count plots fitted a compartment of 500 $m^2$. The same number of plots was created in the two large compartments. For each of the three supplementary food application strategies, there are four release and four dispersal plots divided over two compartments. A release plot and its corresponding dispersal plot are considered to be one replicate.

The *M. pygmaeus* individuals were counted on two flowers and three random leaves per plant, divided over three plant heights (upper, middle, lower). The five nymphal instars and adults were categorized in three groups: N1–3, N4–5, and adults.

### Statistical analysis

Of all observed *M. pygmaeus* individuals, 92% were found in the flowers of the pepper plants. Therefore, we restricted our analysis to the count data from the flowers only. To assess the effect of supplementary food applications on the population build-up, counts of *M. pygmaeus* at the times of peak abundance of the first and second generation were compared. The first two generations of an introduced *M. pygmaeus* population do not overlap. Prior to the analysis, counts of the various life stages on a flower were summed to obtain a total number of *M. pygmaeus*. Next, *M. pygmaeus* totals of the 22 flowers in a plot were summed. To obtain the overall abundance per replicate, counts of the release plot and the corresponding dispersal plot were grouped. *Macrolophus pygmaeus* counts were analyzed by constructing a generalized linear mixed model (GLMM), adding 'compartment' as a random factor. Treatment, generation, their interaction, and sweet pepper variety were treated as fixed effects. Residuals were assumed to follow a Poisson distribution.

Dispersal rate was determined at the time of peak abundance of the second generation as the ratio of the individuals counted in dispersal plots vs. the total number of individuals in the population. Counts of *M. pygmaeus* individuals on the 22 flowers in each plot were summed prior to analysis. Again a GLMM was constructed, treating compartment as a random factor and treatment, life stage, their interaction, and variety as fixed effects. The number of adults counted in the crop was too low (16 over all compartments) so this life stage was omitted from the analysis. In this case, a binomial distribution was used.

At first, fully parameterised models were constructed. Non-significant interactions and factor effects were sequentially dropped until significance level reached 0.05 or less using a log-likelihood ratio test ($\chi^2$). Post-hoc comparisons were performed by least square means with Tukey adjustments of P-values. Statistical analyses were carried out in R v.3.4.1 (R Core Team, 2017), using packages lme4 (Bates et al., 2015) and lsmeans (Lenth, 2016).

## Results

The experiment lasted for ca. 13 weeks in which two generations of *M. pygmaeus* developed (Figure 1). Total numbers rose steeply during the early life stages (N1–3)

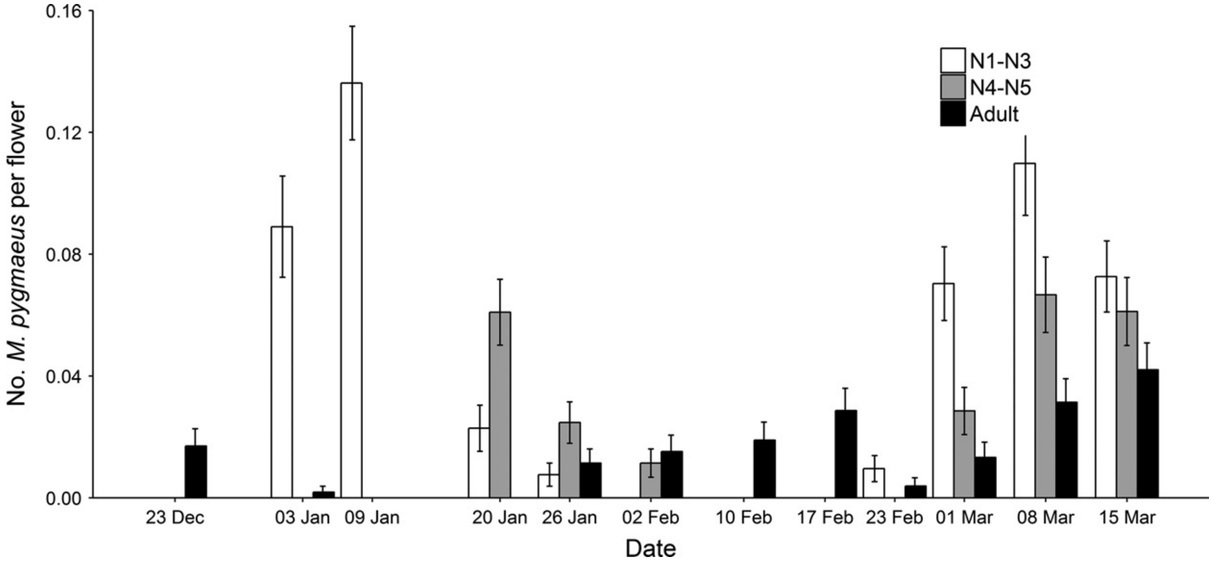

**Figure 1** Mean (± SE) number of individuals of the various *Macrolophus pygmaeus* life stages [nymphal stages 1–3 (N1–3), 4–5 (N4–5), and adults] per flower in sweet pepper greenhouse compartments in 2016/2017.

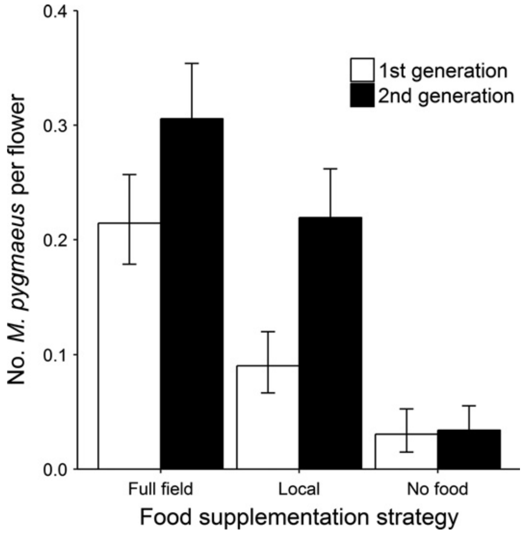

**Figure 2** Mean (± SE) number of *Macrolophus pygmaeus* individuals per sweet pepper flower for each food supplementation strategy at two generation peaks.

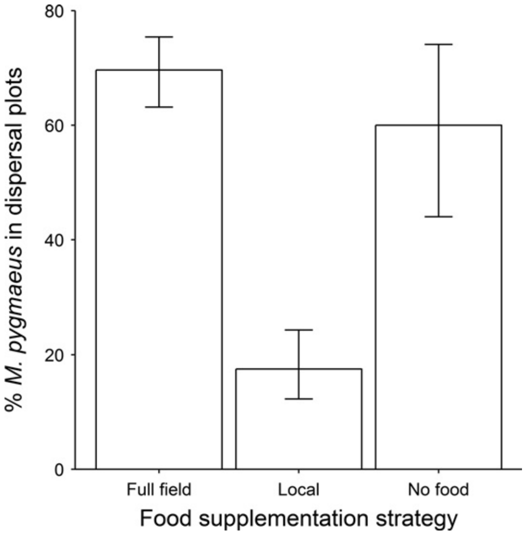

**Figure 3** Mean (± SE) percentage of the second generation *Macrolophus pygmaeus* population found in dispersal plots vs. the total population for each food supplementation strategy.

and gradually lowered when the later life stages appeared. The first generation reached its maximum numbers 24 days after release on 9 January 2017, the second 81 days after release on 8 March 2017.

### Population build-up

Population increase between first and second generation was highest when food was supplemented with the full field strategy and lowest when no food was supplemented (Figure 2, Table 2). This effect occurred in both generations (Table 2). Providing food supplements following a full field strategy increased population levels significantly as compared to both local (P = 0.015) and no food supplementation (P<0.0001). The difference between local and no food supplementation was also strongly significant (P = 0.0001). The second generation reached significantly higher population levels compared to the first in all treatments (P = 0.003). Differences in sweet pepper variety had no effect on the population build-up of *M. pygmaeus* (Table 2).

### Dispersal

Both the full field and the no-food strategy caused higher dispersal rates than the local supplementation strategy (P<0.0001 and P = 0.03, respectively) (Figure 3, Table 2); 69.6 and 60%, respectively, of the second generation population was found in dispersal plots. In case of the local supplementation strategy, only 17.5% of the population was present in dispersal plots. No significant difference in dispersal rate was found between the full field and no-food strategies (P = 0.82). Sweet pepper variety and *M. pygmaeus* life stage had no significant effect on dispersal rate (Table 2).

## Discussion

To prevent pest outbreaks in greenhouse crops with inoculative releases of generalist predators, predator populations must reach sufficient numbers, well spread throughout the greenhouse, before pests emerge. If not, a first pest

**Table 2** Results of the GLMM's testing effects of treatment, generation, and plant variety on population build-up and dispersion

| | Population build-up | | | Dispersion | | |
|---|---|---|---|---|---|---|
| | $\chi^2$ | d.f. | P | $\chi^2$ | d.f. | P |
| Treatment*generation | 1.88 | 2 | 0.39 | – | – | – |
| Treatment*life stage | – | – | – | 0.35 | 2 | 0.84 |
| Treatment | 15.05 | 2 | 0.001 | 11.03 | 2 | 0.004 |
| Generation | 8.87 | 1 | 0.003 | – | – | – |
| Life stage | – | – | – | 0.32 | 1 | 0.57 |
| Variety | 4.43 | 2 | 0.11 | 0.27 | 2 | 0.87 |

outbreak cannot be prevented and the use of chemicals will often be necessary. These chemicals may then harm the predator populations as well. Releasing generalist predators early in the season and providing supplementary food helps establishing populations in time (Messelink et al., 2011b, 2015).

This study found that the population build-up of an introduced *M. pygmaeus* population in greenhouse sweet pepper crops happens more swiftly when food is supplemented. *Macrolophus pygmaeus*, like *O. laevigatus*, is a zoophytophagous predator that feeds on plant tissue and pollen in the absence of prey (Perdikis & Lykouressis, 2000; Lykouressis et al., 2001; Ingegno et al., 2011; Portillo et al., 2012). Although *O. laevigatus* can reach sufficient numbers by feeding on sweet pepper nectar and pollen without the need for food supplementation (Cocuzza et al., 1997; Hulshof & Jurchenko, 2000), we found that this is not the case for *M. pygmaeus*.

Female bugs oviposit near food sources (Put et al., 2012; Moerkens et al., 2017). Supplementing food locally might limit the need for dispersal of these females. Moreover, when food sources are abundant on the plants of hatching, young bugs are not forced to disperse in search for more food. Our results indicated that dispersal rate was indeed significantly lower when applying the local food strategy. When applying the full field strategy, a significantly larger proportion of the second generation was found in the dispersal plots. This was also the case when no food was supplemented. Limited food availability on release or hatching plants clearly encourages bugs to disperse.

Aside from improving the dispersal rate, the full field strategy also causes the population to reach higher numbers compared to the local food strategy. Various factors may be involved. For example, females may have to compete more for oviposition spots near the locally supplemented food sources. *Macrolophus pygmaeus* is known to exhibit cannibalism (Hamdi et al., 2013) and too high densities on release plants due to limited dispersal rates may trigger cannibalism. Another relevant difference may be the total amount of food added – this was higher in the full-field strategy.

The results of this study indicate that the optimal release strategy for *M. pygmaeus* in sweet pepper greenhouse crops is similar to that in tomato crops (Put et al., 2012), requiring full field food supplementation to ensure sufficient population build-up and dispersal levels at the start of the season. Food supplementation should last 6–8 weeks, which is the time required to complete one generation (Moerkens et al., 2017).

*Macrolophus pygmaeus* is used extensively in tomato crops against a number of pests, but has yet to find its way into general sweet pepper management practices. Pest control was achieved in all compartments and no pest outbreaks were registered during the trial. Later in the season thrips invaded each greenhouse compartment. A quick and easy control was achieved in both compartments with the full field strategy, although one of these compartments has a yearly, severe thrips outbreak. In the other compartments thrips were also controlled, but often only after they reached very high numbers. This confirms that *M. pygmaeus* is able to control thrips outbreaks. Messelink et al. (2011b, 2014) also found that *M. pygmaeus* can be used against thrips in sweet pepper, either alone or together with *O. laevigatus*.

The past 10–20 years, the tobacco whitefly, *Bemisia tabaci* Gennadius, has become a major problem in Western European greenhouse crops (Oliveira et al., 2001). Originally a pest in subtropical regions across the world, it has spread to temperate regions with a mild climate or with a greenhouse culture (Kirk et al., 2000). This highly resistant whitefly species (Elbert & Nauen, 2000; Palumbo et al., 2001; Horowitz et al., 2002; Nauen & Denholm, 2005) cannot be eradicated by any insecticide currently on the market. Growers rely on biological control by the predatory mite *Amblyseius swirskii* Athias-Henriot (Bolckmans et al., 2005) and parasitoid wasps of the genus *Eretmocerus* (Stansly et al., 2005; Urbaneja et al., 2007). Releasing *M. pygmaeus* might be a solution for this emerging pest in sweet pepper, as it can feed and reproduce on *B. tabaci* nymphs (Sylla et al., 2016) and is already used successfully in tomato greenhouses.

*Macrolophus pygmaeus* is zoophytophagous and thus also capable of plant or fruit damage. The bugs will feed on tomato plants which provide essential nutrients not found in prey (Moerkens et al., 2016). Severe damage occurs at high population densities and especially when there is an interaction with the Pepino mosaic virus (PepMV) in tomato (Moerkens et al., 2016). As all Belgian and Dutch tomato crops are infected with PepMV, either naturally of vaccinated, this is an important issue. Despite the risks, both the literature and our study suggest to incorporate *M. pygmaeus* in sweet pepper pest management programs as well.

Possible complications should not be neglected, however, and more research must be done on determining the risks in sweet pepper. Growers who already use *M. pygmaeus* in sweet pepper crops gave notice of splitting in the heads of the plants at high population densities and they sometimes reported small spots on the fruits. This was also observed in some of our study plants and should be investigated further.

In practice, several different application rates are used when providing supplementary food for *M. pygmaeus*.

The best program in tomato was found to be a weekly application for 6–8 weeks (i.e., *M. pygmaeus* generation time) (Moerkens et al., 2017). Supplementary food was provided for 7 weeks in this experiment, which yielded good results, but additional experiments should be conducted to optimize application rates and determine the most cost-efficient one. The choice of food source is also a topic of interest. We opted for the cheaper *A. franciscana* cysts in contrast to the more expensive *E. kuehniella* eggs. However, the latter might produce better results or may require fewer applications as it has a higher nutritional value (Vandekerkhove et al., 2009). Our limited number of greenhouse compartments did not allow to test these factors during the same growing season, but it will be a topic of future work.

Another subject of research could be the release method of *M. pygmaeus*. In our study, bugs were released from four locations of five consecutive plants in each greenhouse compartment. Applying a full-field strategy for bug release might also enhance dispersal rate and population growth, but will also increase labor costs.

We found that the way supplemental food is distributed in the greenhouse can have a significant impact on the population build-up and dispersal levels of the beneficial released. Most studies on supplemental food start with laboratory tests and end with cage experiments, consisting of only a few plants in a limited space. Trials in whole greenhouses or greenhouse compartments are very rare. Our findings demonstrate that testing food applications on a more realistic scale and with different distribution strategies is important and can contribute to a more successful and cost-efficient release of beneficials in greenhouses.

## Conclusion

To successfully release *M. pygmaeus* in greenhouse sweet pepper crops, food must be supplemented homogenously across the greenhouse to ensure both quick dispersal and large populations. *Artemia franciscana* cysts proved to be a good food source for this. With this strategy a sufficiently large population of *M. pygmaeus* may be obtained early in the season to prevent outbreaks of aphids, thrips, whiteflies, and other pests.

## Acknowledgements

This research was financed by Research Centre Hoogstraten and the Agency Flanders Innovation & Entrepreneurship (VLAIO) in the context of research project 140948. The research project 140948 was granted to Research Centre Hoogstraten (RM) in cooperation with Research Station for Vegetable Production and University of Antwerp.

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