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BUILDING A "STANDING ARMY" OF BENEFICIALS: A REALITY IN GREENHOUSE CROPS

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ABSTRACT

In this study, we provide some examples of applications of the "standing army" concept in commercial greenhouses with the focus on the use of cattail pollen (*Typha angustifolia*), marketed as Nutrimite™. This product has been the first commercially available food supplement for predatory mites, allowing growers to build up and maintain predatory mite populations in the absence of pests. Nowadays, in greenhouse roses, it is no longer necessary to introduce mites weekly or hang sachets of predatory mites monthly, which are both labor intensive practices. The pollen allows growers to reduce both labor and costs while realizing a strong and persisting "standing army" of the generalist predatory mites *Euseius gallicus*, *Amblyseius swirskii* or *Iphiseius degenerans*. This concept is a breakthrough in biological pest control as it makes it possible to establish predatory mites preventatively, that is before the pest arrives.

Keywords: predatory mites, Euseius, thrips, roses, greenhouse.

RÉSUMÉ

FORMATION D'UNE ARMÉE ACTIVE D'AUXILLIAIRES: UNE RÉALITÉ EN CULTURES SOUS SERRE

Dans cet article, nous citons quelques applications du concept d'«armée active» en serres de production, et en particulier, l'utilisation du pollen de massette (*Typha angustifolia*), commercialisé sous le nom Nutrimite™. Nutrimite™ a été le premier complément alimentaire disponible dans le commerce pour les acariens prédateurs. Il aide les producteurs à former et maintenir les populations de phytoséides en l'absence de proies. En culture de roses sous serre, il n'est maintenant plus nécessaire d'introduire les auxilliaires en vrac chaque semaine ou d'accrocher les sachets d'acariens prédateurs chaque mois. Le pollen permet un gain de travail et de coûts, tout en réalisant une «armée active» d'acariens prédateurs tels que *Euseius gallicus*, *Amblyseius swirskii* ou *Iphiseius degenerans*. Le concept d'armée active fait une percée en lutte biologique car il permet d'établir les prédateurs de façon préventive, c'est-à-dire avant que le ravageur n'apparaisse.

Mots-clés : acarien prédateur, Euseius, thrips, rose, serre.

INTRODUCTION

In greenhouse crops most generalist biological agents are used in a preventative way on young clean plants. However, population build-up is limited due to absence of prey or pollen due to the lack of flowers. If an insufficient number of natural enemies is present at the time of pest arrival, pest outbreaks and resulting plant damages is likely to occur. Furthermore, the chemical sprays required to counter the pests' outbreaks jeopardize the persistence of the beneficials. To address this issue, biological control strategies are mostly relying on repeated introductions of high numbers of natural enemies. But the last decade, the interest in the concept of building "standing armies" of natural enemies is growing. A "standing army" is a population of biological control agents which is maintained through the presence of alternative food sources. Despite the many well-documented examples, only a few reached practical applications. Some conservation biological control (CBC) techniques for biological agents are increasingly implemented to prevent pest outbreaks, others seem to get gradually abandoned. The older techniques like the introductions of pests ("pest-in-first") and banker plants (Starý, 1993) are no longer being widely used. The "pest-in-first" strategy is one of the oldest "standing army" strategies, where an early introduction of spider mites has to reinforce the settlement of the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Hussey *et al*., 1965 ; Markkula & Tiittanen, 1976 ; Havelka & Kindlmann, 1984 ; Waite, 2001). This concept is still being used by some sweet pepper and cucumber growers, but the majority of the growers are nowadays reluctant to release pests due to the risks of causing crop damage. The banker plant systems against aphids also used to be broadly implemented in greenhouses to controle *Myzus persicae* Sulzer. The bankers consist of barley or wheat seedlings infested with aphids *Rhopalosiphum padi* Linnaeus or *Sitobion avenae* Fabricius which are harmless to greenhouse crops. The parasitoids *Aphidius colemani* Viereck and *Aphidius ervi* Haliday could reproduce and emerge continuously on the bankers until the crop aphids appeared. The use of banker plants is decreasing in the Netherlands because they are labor intensive are thought to facilitate early hyperparasitism by *Dendrocerus aphidum* Rondani which is disturbing biological control of aphids (Messelink, 2013).

Alternative like non-pest foods, such as pollen, honeydew, nectar or harmless arthropods are increasingly being used to reinforce the biological control programmes. Several studies have recognized the benefit of using alternative food for the persistence of predators in biological control (Collyer, 1964 ; McMurtry and Scriven, 1966 ; Ramakers, 1990 ; Hagen, 1986 ; Bakker & Klein, 1992 ; Wäckers & Swaans, 1993 ; Karban *et al*., 1994 ; Hanna *et al*., 1997 ; Walde *et al*. 1997 ; van Rijn & Tanigoshi, 1999 ; van Rijn *et al*., 2002 ; Nomikou *et al*. 2002 ; Matsuo *et al*., 2003 ; Wäckers *et al*., 2005 ; Liu *et al*. 2006 ; Nomikou *et al*., 2010 ; Janssen & Sabelis, 2015 ; Pijnakker *et al*., 2016 ; Muñoz-Cárdenas *et al*., 2017).
The most established example of the "standing army" concept with using of alternative food in greenhouses is the supply of eggs of the flour moth *Ephestia kuehniella* Zeller and cysts of the brine shrimp *Artemia* spp. to enhance the settlement of the predatory bug *Macrolophus pygmaeus* in tomato crops (Castañé *et al*., 2006 ; Riudavets *et al*., 2006 ; Bonte & de Clercq, 2008 ; Vandekerkhove *et al*., 2009 ; Put et *al*., 2012). Nowadays, most Dutch tomato growers release *M. pygmaeus* two to four times per crop (in total generally 1 and sometimes 2 bugs/m^2) with alternative food. The following strategy is given as an example. During 2 to 4 weeks, 50 g of *E. kuehniella*/ha are given on the plants at which the predator is released. Afterwards, the alternative food is supplied on the entire row in which *M. pygmeaus* was released to obtain a better distribution of the predator. Growers subsequently supply 50 g of *E. kuehniella*/ha/week during 6 weeks or 500 g *Artemia* sp./ha/week as long as needed to have a sufficient number of predators. Some growers only use *Artemia* sp. to reduce the costs of the strategy.

Generalist predatory mites are increasing importance in biological pest control. Research long focused on finding the most effective or suitable generalist predatory mites for diverse crops. The first experiments with predatory mites in the Netherlands date from the 70's using *Neoseiulus cucumeris* (Oudemans), *Amblyseius barkeri* (Hughes) and *Amblyseius aurescens* Athias-Henriot against thrips (*Thrips tabaci* Linderman) (Ramakers, 1980; Ramakers & van Lieburg, 1982). *N. cucumeris* remained long the main predatory mite species used in greenhouses especially in cucumbers and sweet pepper crops where it establishes well with pollen from the flowering crop and thrips. Breeding sachets of *N. cucumeris*, operating as open rearing systems, were a first attempt to increase resilience of predatory mites on the plants. Sachets aim at limiting repeated introductions of predatory mites by providing a continuous release for 4 up to 8 weeks. But the astigmatid prey mites generally fail to settle on the plants in order that sachets have to be renewed regularly. In 2005, *Amblyseius swirskii* (Athias-Henriot) was launched and has become the most commonly used phytoseiid species against thrips and whitefly. Later, three other generalist species, namely, *Transeius* montdorensis (Schicha) (2011), *Amblydromalus limonicus* Garman & McGregor (2011) and *Euseius gallicus* Kreiter & Tixier (2014), reached the Dutch market of commercial acarine biocontrol agents (ABA's). *N. cucumeris, A. swirskii, T. montdorensis* and *A. limonicus* are usually repeatedly introduced with a carrier material (e.g. bran, vermiculite) or in breeding sachets because their persistence in crops without pollen depends on the presence of pests. Pest presence needed to be monitored among other crops in roses, where the predators could only sustain themselves when sufficient pests were present (Pijnakker & Leman, 2012). Therefore, biocontrol was mainly curative. Until 2013, the concept of achieving a "standing army" of predatory mites with alternative food was mainly a focus of researchers, not of commercial growers. Researchers insufficiantly succeeded in their attempts to motivate growers to use pollen and nectar providing banker plants to enhance predatory mites population levels (Ramakers & Voet, 1995). *Ricinus communis* was advocated for this purpose as it is a suitable host plant for generalist phytoseiid mites, such as *Iphiseius degenerans* (Berlese), due to the continuous presence of pollen and extra-floral nectar (Van Rijn & Tanigoshi, 1999). However, this banker plant requires a lot of maintenance by its rapid growth. Additionally, it can become a host plant for pests, like thrips and mealybugs. Their usefulness was rapidely contested in sweet pepper crops which produce pollen themselves. Castor bean plants are only being used by a limited number of ornamental growers. In 2002, Van Rijn *et al*. showed that supplying *Typha latifolia* Linneaus pollen to a crop significantly enhanced the biological control of thrips. However, the "standing army" concept for predatory mites was only implemented by growers when *Typha angustifolia* Linneaus pollen and later *E. gallicus* became commercially available in 2013. Since then, other "standing army" strategies, like the supply of factitious astigmatid prey mites or eggs (e.g. eggs of *Aleuroglyphus ovatus*) have been developed (Ferrero *et al*., 2016). In this study, we assessed the suitability of *T. angustifolia* pollen as an alternative food source for three phytoseiid mites in roses under field conditions.

MATERIALS AND METHODS

Field experiments - Colonization of commercial rose crops by *E. gallicus, A. swirskii* and *I. degenerans* with pollen of *T. angustifolia* (Nutrimite^{TM})

In August 2013, *E. gallicus* and *A. swirskii* were introduced in a greenhouse of 8 ha with diverse rose cultivars where roses were harvested daily. The temperature was set at 20 ± 2 °C and the relative humidity at 80 ± 10% RH. Rose plants cv. Gladiator grown on 4 gutters per row (plant density of 8 plants/m^2) were selected for the experiment. Before the trial, sprays of flonicamid (24/07) and abamectine (31/07) were achieved to control outbreaks of greenhouse whitefly *Trialeurodes vaporariorum* Westwood, *Frankliniella occidentalis* (Pergande) and *Echinothrips americanus* Morgan. At the moment of introduction of the predatory mites, a low number of spider mites and greenhouse whiteflies were still present in the crop. *P. persimilis* (28/m^2/4 weeks) and *Encarsia formosa* (8/m^2/2 weeks) were released to control these pests.

The predatory mite *E. gallicus* was tested in three replicates in a block design experiment in comparison with two strategies implying the standard used predator *A. swirskii*. *E. gallicus* was introduced only once in week 33 at the dosage of 100 mites/m^2 in a saw dust substrate and was biweekly supplied with pollen. *A. swirskii* was released in week 33 in breeding sachets containing bran (1 sachet/2m^2) either with or without cattail pollen Nutrimite™, which was supplied by Biobest Belgium N.V. Sachets of *A. swirskii* were released a second time in week 39 in the plots without pollen. Pollen was provided biweekly with a blower at a concentration of 500 g/ha.
No sulphur was used against powdery mildew. Fungicides like dodemorf, penconazole, fluopyram and boscalid + kresoxim-methyl, which are compatible with natural enemies, were sprayed weekly one day before the pollen application (Put *et al*., 2015). Potassium hydrogen carbonate was used in weeks 42 and 44. Methoxyfenozide was sprayed against caterpillars in weeks 35, 41 and 45 and flonicamid against aphids in weeks 40 and 45. In week 43 lufenuron and *Beauveria bassiana* were sprayed in the benched stems against *E. americanus*. Thirty leaves were randomly collected in each plot in weeks 35, 39, 40, 42, 45 and 47. The number of predators was counted under a stereo-microscope. All mites were mounted and cleared in a Marc André medium for identification. The results were analysed with regression analyses, using a generalised linear model (GLM) accounting for a Poisson distribution of the data.

In March 2014, *E. gallicus* and *I. degenerans* were introduced with pollen applications in a greenhouse of 4 ha roses cv. Red Naomi. They were released only once in week 12 at a rate of 37 and 3.7 individuals per m^2 respectively in a experimental plot of 532 m^2. Pollen (Nutrimite™) was provided every two weeks in each row with a blower at a concentration of 500 g/ha. Climate conditions were kept at 20 ± 2°C and 80 ± 10 % RH. The crop was dense and only few spider mites and whiteflies were present with their natural enemies *P. persimilis* and *A. swirskii*, respectively. Fungicides (dodemorf or boscalid + kresoxim-methyl) were sprayed weekly against powdery mildew. Bupirimate was only sprayed in the summer. Heptamethyltrisiloxan was sprayed in the woody heart of the plants, through the benched stems, against rose scale *Aulacaspis rosae* Bouché. Prior to the release of the two tested predatory mite species, an introduction of *A. swirskii* in breeding sachets was performed by the grower in week 8 and was accidentally renewed in week 17. Hundred eighty leaves were randomly collected in weeks 15, 18, 23, 26, 29, 32, 34 and 36. The number of predators was counted under a stereo-microscope and the mites were identified in the same way as the first field experiment.

RESULTS AND DISCUSSION

Figure 1: Population dynamics of *E. gallicus* and *A. swirskii* (all mobile stages) in a rose crop cv. Gladiator where pollen is blown every two weeks in comparison with the population dynamics of *A. swirskii* applied in breeding sachets every 6 weeks without pollen.
(Dynamique des populations d'*E. gallicus* et d'*A. swirskii* (stades mobiles) dans une culture de rose cv. Gladiator où du pollen est appliqué toutes les deux semaines comparée à une dynamique des populations d'*A. swirskii* introduit en sachets toutes les 6 semaines sans pollen.)

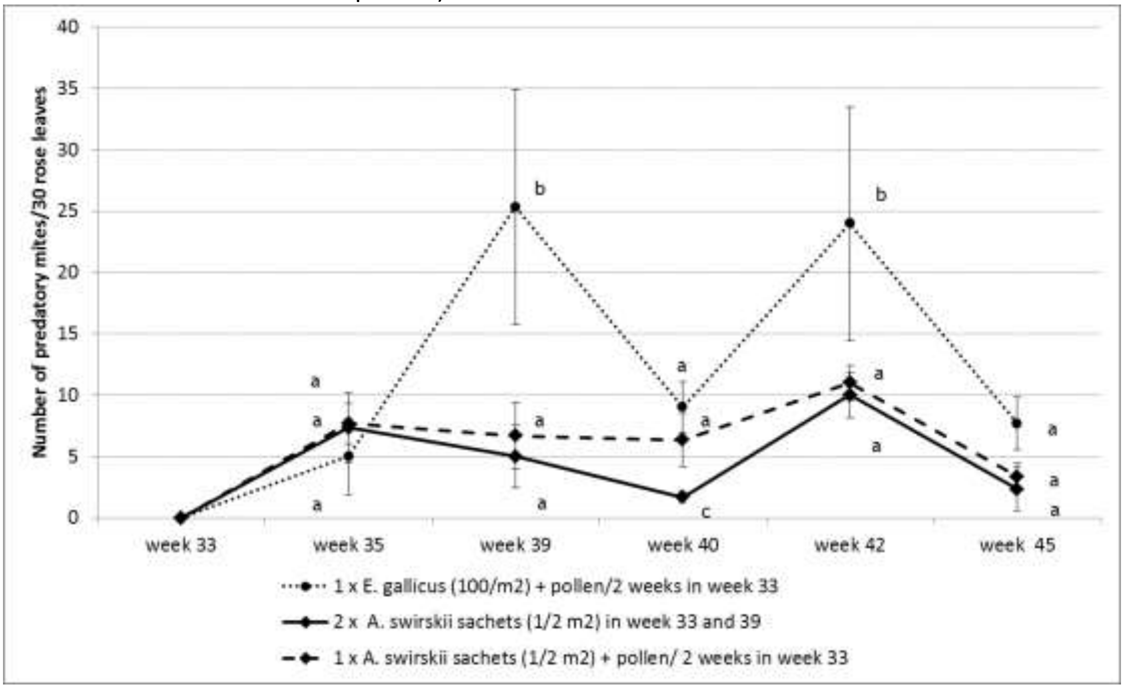
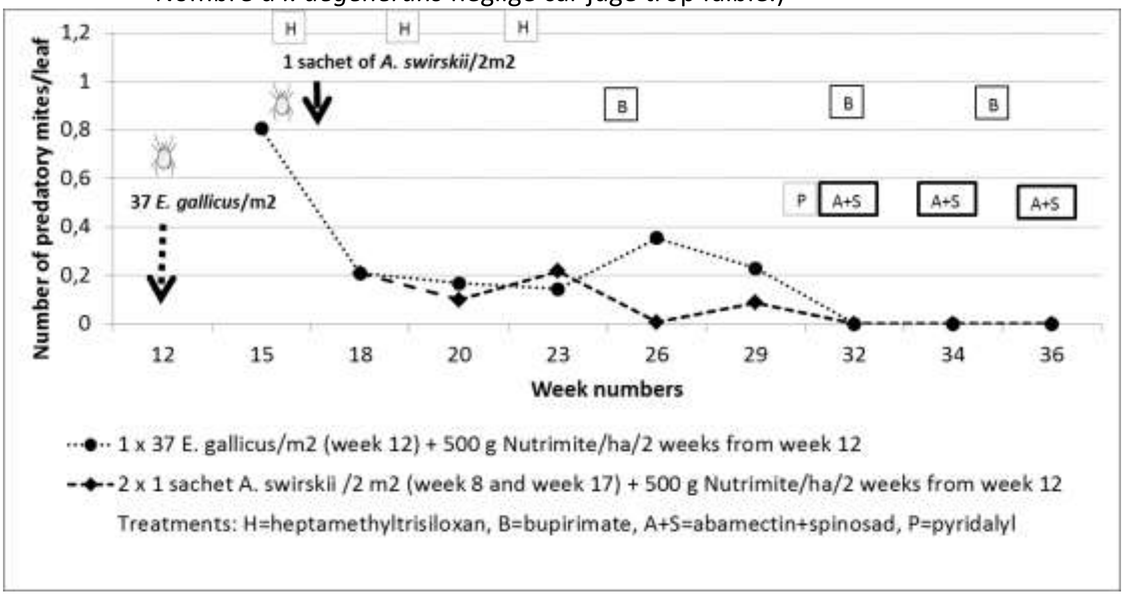


Figure 2: Population dynamics of *E. gallicus* and *A. swirskii* (all mobile stages) in a rose crop cv. Red Naomi where pollen is blown every two weeks. Due to the low number of *I. degenerans* found, the predator is not shown in this figure.
(Dynamique des populations d'*E. gallicus* et d'*A. swirskii* (stades mobiles) dans une culture de rose cv. Red Naomi où du pollen est appliqué toutes les deux semaines. Nombre d'*I. degenerans* négligé car jugé trop faible.)



Results of the trials performed in cv. Gladiator (Figure 1) suggest that the settlement of *A. swirskii* and *E. gallicus* is significantly enhanced in greenhouse crops when pollen of *T. angustifolia* is added to the biological strategy. These results are consistent with previous studies showing that pollen, and especially cattail pollen, favours the performance of phytoseiids in terms of intrinsic rates of population growth (Van Rijn & Tanigoshi, 1999 ; Van Rijn *et al*., 1999 ; Broufas & Koveos, 2000 ; Goleva & Zebitz, 2013 ; Lorenzon *et al*., 2012 ; Vangansbeke *et al*., 2014a).

In cv. Red Naomi (Figure 2), *I. degenerans* was released at a low rate (3.7/m^2) due to a higher cost price. This mite was only found sporadically from week 20, with a maximum of 8 mites on the 180 leaves in week 29. Intraguild competition between the predators could explain this low number. *E. gallicus* and *A. swirskii* both colonized the crop and reached high population levels. Between week 18 and 23, the density of the mobile stages of *E. gallicus* and *A. swirskii* was similar while the number of *E. gallicus* introduced was 30 times less important (37 *E. gallicus*/m^2 against 0.5 sachet of *A. swirskii*/m^2). *E. gallicus* became the most numerous species (0.35 mites/leaf) once the sachets of *A. swirskii* stopped producing mites. The accumulated "standing army" of phytoseiids could not totally prevent plant damage caused by thrips at the end of July (week 31), but allowed the grower to reduce the number of chemical applications with 50% in comparison with previous years. The grower's pest threshold is 6 thrips per sticky trap per week or the observation of more than 50 damaged harvested buds per week at the sorting machines. None of the three predatory mite species survived the sprays of abamectine and spinosad. The grower reintroduced *E. gallicus* in October 2015 and managed to maintain his "standing army" thanks to the pollen application until the following summer. From week 35, only four non selective chemical treatments were still necessary to prevent thrips damages during the summer. The grower didn't have to spray the rest of the year against thrips. In an integrated biological control program, a "standing army" approach is most advantageous when it is combined with the correct choice of needed pesticides. Thus, the possibilities of using more selective insecticides (e.g. azadirachtine or pyridalyl) rather than broad spectrum products (e.g. abamectin and spinosad) will be tested to avoid flower injuries. Further, the most optimal timing to stop the pollen application before the summer period is also being discussed by the growers and the crop protection advisors.

OUTLOOKS

At present, only few approaches of the "standing army" concept reached practical use in greenhouse crops, but many research projects are already launched like the provision of additional floral resources through insectary plants (Pineda *et al*., 2008 ; Parolin *et al*., 2012 ; Pinheiro *et al*., 2013 ; Amorós-Jiménez *et al*., 2013), the practical application of artificial and factitious foods (Riddick, 2009 ; Nguyen *et al*., 2014 ; Vangansbeke *et al*., 2014 a and b, Muñoz-Cárdenas, 2016) like food sprays (Hagen *et al*., 1971 ; Wade *et al*., 2008 ; Ogawa & Osakabe, 2008), the development of artificial shelter habitats and oviposition substrates (Pekas *et al*., 2015), dispersion tools for predators and alternative food, plant manipulation or the growing substrates. It might take time to uncover the benefit of these future challenges when integrated in different greenhouse crops.

Other promising approaches implying different types of conservation techniques are already implemented at a few growers on a experimental basis like the addition of yeast or astigmatid mites in mulches to boost predatory mites or the addition of poultry feed in the growing media to enhance *Atheta coriaria* Kraatz. Their efficacy in the field and their practical aspects will be determining for the adoption of these approaches by growers.

CONCLUSION

The last decade, attempts have been undertaken to standerdize biological control strategies in Dutch greenhouse with varying success. However, new methodologies have been developed by researchers, biocontrol companies, distributors and growers to maintain natural enemies populations with alternative food in crops aiming to enhance pest control. Considerable progress in this field has been made during recent years. The application of pollen is now used routinely by an increasing number of rose and cucumber growers. In 2016, 661 kg of Nutrimite™ were used in North-Amerika, 656 kg in the Netherlands and 136 kg in France. Due to the supplementation of latter alternative food, one or two introductions of predatory mites are now sufficient to obtain an early establishment of the beneficials before pest arrival.

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