Running Head: Mirids and *A. swirskii* against sweet pepper pests

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Combined use of predatory mirids with *Amblyseius swirskii* to enhance pest management in sweet pepper

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Abstract

The combined release of *Orius laevigatus* with *Amblyseius swirskii* provides effective control of sweet pepper key pests, such as thrips and whiteflies. However, the management of the aphids can still be improved. Recently, the predatory mirids *Nesidiocoris tenuis* and *Macrolophus pygmaeus* have been found to be effective in the control of aphids, thrips and whiteflies when tested alone. Hence, integrating one of these two mirids with *A. swirskii* might enhance sweet pepper pest management. In this work, we began by investigating the co-occurrence of both mirid species when released together with *A. swirskii*. This was compared to the standard release of *O. laevigatus* with *A. swirskii*. *Nesidiocoris tenuis* and *A. swirskii* were involved in a bidirectional intraguild predation (IGP). On the contrary, this interaction (IGP) was apparently unidirectional in the case of *M. pygmaeus* with *A. swirskii* and *O. laevigatus* with *A. swirskii.* Both, *M. pygmaeus* and *O. laevigatus* significantly reduced the abundance of *A. swirskii*. Secondly, in a greenhouse experiment, where the same release combinations were tested (either *N. tenuis*, *M. pygmaeus* or *O. laevigatus* combined with *A. swirskii*), IGP seemed to be neutralized. Mirids with *A. swirskii* significantly suppressed thrips, whitefly and aphid infestations. Contrarily, the combined use of *O. laevigatus* with *A. swirskii* did not reached a satisfactory control for aphids, despite the reduction in thrips and whitefly densities. Therefore, our results suggest that the use of mirids combined with *A. swirskii* could result in more efficient and robust biological control programs in sweet pepper crops.

Keywords: Intraguild predation, *Nesidiocoris tenuis*, *Macrolophus pygmaeus*, *Orius laevigatus,* biological control.

Introduction

Biological control (BC) programs, especially in greenhouse crops, are increasingly based on releases of several species of generalist predators against common greenhouse pests (Calvo et al. 2009, 2012a; van Lenteren 2012, van Lenteren et al. 2017). One of the most impressive success stories of BC was observed is sweet pepper crops in southeast Spain (Calvo et al. 2015). This success was achieved thanks to the combined release of the anthocorid bug *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae), and the predatory mite, *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae) which can control two important sweet pepper pests, the thrips species, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). The release of *O. laevigatus* is mainly focused for controlling thrips, but when well-established it can also contribute to the management of whiteflies (Arnó et al. 2008), aphids (Alvarado et al. 1997), and spider mites (Venzon et al. 2002). Complementary, *A. swirskii* is released for controlling whiteflies (Nomikou et al. 2002; Calvo et al. 2009) but it can also manage thrips (Messelink et al. 2006), broad mites (van Maanen et al. 2010) and to a lesser extent, spider mites (Messelink et al. 2010). In spite of the broad diet range of both predators, the BC system is still challenged due to aphids. The management of aphids requires the release of a combination of specialized parasitoids and predators (Blom 2008; Messelink et al. 2011, 2013). However, the abundance of hyperparasitoids in southeastern Spain (Belliure et al. 2008, Sanchez et al. 2011) and the interference occurring among generalist thrips predators and specialist aphid natural enemies (Messelink et al. 2011), have negatively affected the outcomes of this BC program in sweet pepper. To make matters worse, the program can end up being extremely expensive (Messelink et al. 2011).

In recent years a line of research to improve the biological control of aphids has been focusing on identifying the generalist zoophytophagous predators able to be established in the sweet pepper crop prior to aphid arrival. This could result in rapid responses to new aphid infestations and would prevent aphid establishment (Messelink et al. 2011). The effectiveness of zoophytophagous mirid predators (Hemiptera: Miridae) such as *Macrolophus pygmaeus* (Rambur), *Nesidiocoris tenuis* (Reuter), *Dicyphus maroccanus* (Wanger) and *D. tammaninii* (Wanger) has been explored in sweet pepper crops. Either, their inoculation or their release after pest outbreaks resulted in effective aphid management in this crop (Messelink et al. 2015; Pérez-Hedo and Urbaneja 2015). In addition, some of these mirid species, such as *N. tenuis* and *M. pygmaeus* can also be effective to control whiteflies and thrips in sweet pepper under different temperature conditions (same authors in preparation). Therefore, the next step would be to know whether the pre-pest establishment of both species of mirids in sweet pepper could manage the populations of the three pests, whiteflies, thrips and aphids. For this, the combined use of either *N. tenuis* or *M. pygmaeus* and *A. swirkii* to reduce the populations of *F. occidentalis*, *B. tabaci* and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) was evaluated under greenhouse conditions. The contribution of *A. swirskii*, in the end, is currently so notably positive for biological control in sweet pepper that a hypothetical use of mirids would inexorably be linked to the release of *A. swirskii*. Both alternative strategies were compared with the standard release of *O. laevigatus* and *A. swirskii.*

However, generalist zoophytophagous predators do not only feed on pests or plant-provided food, but also on other natural enemies (Rosenheim et al. 1995; Rosenheim 1998). This feeding on other natural enemies can be classified as intraguild predation (IGP) when the competitors share prey and thus contend for it (Polis et al. 1989; Holt and Polis 1997; Rosenheim et al. 1995). Therefore, the combined release of a pair of generalist predators could result in a negative outcome on BC of the target pest (Rosenheim et al. 1995; Rosenheim and Harmon 2006; Janssen et al. 2006). As mentioned above, both predatory mirids and *A. swirskii* are true omnivores (Coll and Guershon 2002) feeding on several trophic levels such as plant materials (pollen, nectar, plant sap), herbivores and other natural enemies, which can increase the probability of IGP. Indeed, Messelink et al. (2011) showed that the predatory mite *A.*

swirskii can seriously disrupt BC of aphids by preying on the eggs of the predatory midges, *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae). *Orius* bugs can also prey on eggs and larvae of *A. aphidimyza*, and therefore act as an intraguild predator (Christensen et al. 2002). However, this outcome could also results positive for BC. Messelink and Janssen (2014), observed that IGP between *M. pygmaeus* and *O. laevigatus* did not affect BC of thrips and aphids, at contrary the combined augmentative release of both predator increased pest suppression.

Therefore, a laboratory experiment was conducted in order to study the interaction between predatory mirids and *A. swirskii* in combined release, in comparison to the standard release of *O. laevigatus* with *A. swirskii*. In this caged-experiment whether the intensity of IGP (if any) affects the abundance of the predatory bugs (*N. tenuis*, *M. pygmaeus* and *O. laevigatus*) or the predatory mite (*A. swirskii*) when co-occurring on sweet pepper plants provided or deprived of eggs of the shared prey, *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) was studied. Then, under greenhouse conditions we evaluated the efficacy of each the mixed releases (*N. tenuis* with *A. swirskii*; *M. pygmaeus* with *A. swirskii* and *O. laevigatus* with *A. swirskii*) in reducing *F. occidentalis*, *B. tabaci* and *M. persicae* infestation in sweet pepper. Implications of these results for the future of BC of sweet pepper pests are discussed.

Materials and Methods

Plants and insects

The plants used in all experiments were pesticide free-sweet pepper seedlings [*Capsicum annuum* (Solanaceae)] *cv* (‘Lipari’) (Dulce italiano, Mascarell semillas S.L, Valencia, Spain). Two weeks after germination seedlings were transplanted in plastic pots (8 × 8 × 8 cm) containing a mixture of natural soil with local peat moss and were housed in a growth chamber at 25 ± 2 °C, 60-80% RH and 14:10 h (L:D) photoperiod at the Instituto Valenciano de Investigaciones Agrarias (IVIA).

Nesidiocoris tenuis, *M. pygmaeus*, *O. laevigatus* and *A. swirskii* adults, were obtained from a commercial supplier (NESIBUG®, MYRICAL®, THRIPOR®, SWIRSKI-MITE®, Koppert Biological Systems, S.L., Águilas, Murcia, Spain). Each predatory bug was provided in plastic bottles containing 500 adult individuals approximately 3-days-old (FJ Calvo, Koppert BS; Personal Communication). *Amblyseius swirskii* was supplied in bottle or in sachet according to the experimental requirements. Frozen *Ephestia kuehniella* (Entofood®; Koppert B.S.) eggs were used as additional food to enhance mirids establishment in sweet pepper. *Frankliniella occidentalis* adults were obtained from a colony established at IVIA initiated in 2010 with individuals originally collected from Campo de Cartagena (Murcia, Spain). The thrips colony was reared on bean plants (*Phaseolus vulgaris* L.; Fabales: Fabaceae) and housed in a growth chamber at IVIA, at the same conditions mentioned above. The *M. persicae* (green phenotype) colony was obtained from a laboratory insect culture established on sweet pepper plants in IVIA, as described in Pérez-Hedo and Urbaneja (2015).

Co-occurrence between predatory bugs and *A. swirskii*

Under laboratory conditions, we studied the co-occurrence of predatory bugs *N. tenuis, M. pygmaeus*, *O. laevigatus* and the predatory mite, *A. swirskii* on sweet pepper plants provided or deprived of *E. kuehniella* eggs. Four different treatments were assayed per predatory bug: 1) Predatory bug alone, 2) Predatory bug + *E. kuehniella*, 3) Predatory bug + *A. swirskii* and 4) Predatory bug + *A. swirskii* + *E. kuehniella*. In addition two treatments with *A. swirskii* were also conducted 1) *A. swirskii* alone and 2) *A. swirskii* + *E. kuehniella*. There were four replicates of each treatment. For this, 56 screened plastic cages (30 x 30 x 30 cm) (BugDorm-1 insect cages; MegaView Science Co., Ltd., Taichung, Taiwan) were maintained in a climatic chamber at IVIA (same conditions mentioned above). In each cage (replicate), one sweet pepper plant with 6 fully-developed leaves (approximately 15 cm in height) was placed inside. Two couples (male and female) of each predatory bug (*N. tenuis*, *M. pygmaeus* or *O. laevigatus*) and 50 *A. swirskii* individuals were released at the same time. The number of adults and nymphs of each predatory bug species and the number of *A. swirskii* individuals (adults, protonymphs, deutonymphs and larvae) per plant was weekly recorded using a manual magnifying glass. The experimental period lasted six consecutive weeks.

Greenhouse efficacy experiment

The experiment was carried out in a greenhouse equipped with drip irrigation system located at IVIA in Moncada (Valencia, Spain). The greenhouse was divided into 24 experimental cages with access through an isolating double mesh door. Cages were screened with "anti-thrips" polyethylene mesh of 220 x 331 µm interstices and had concrete floors. Each experimental compartment measured 4 x 3 x 3 m (length × height × width) and were equipped with five hydroponic substrate containers (growbag). Each cage was accessed by a separate door secured with a zipper.

The climatic conditions including temperature and relative humidity were recorded using a data-logger (model TESTO 175-H2, Amidata S.A. Pozuelo de Alarcón, Madrid), that was placed in the central cage. The average temperature during the experiment ranged between 24.9°C on May 4th, 2016 and 32.7°C on June 9, 2016 with a minimum and maximum temperature of 21.1°C and 43.5°C, respectively. The average relative humidity varied between 55.4% on May 4th, 2016 and 98.6% on June 9, 2016 with absolute minimum and maximum values of 33.7% and 99.7%, respectively.

Four different treatments were considered: 1) *N. tenuis* + *A. swirskii*, 2) *M. pygmaeus* + *A. swirskii*, 3) *O. laevigatus* + *A. swirskii* and 4) control, without any predators released. Four replicates per treatment were made. Each replicate was assigned to one cage, hence 16 cages were used. The experiment started by inoculating sweet pepper seedlings in the nursery with either *N. tenuis* or *M. pygmaeus* at a release rate of one predator couple per plant (Fig. 1). To help predator establishment and oviposition, frozen eggs of the factitious prey *E. kuehniella,* approximately 0.1 g per plant were supplied as alternative food (Urbaneja-Bernat et al. 2015). One week later, either inoculated or non-inoculated sweet pepper plants (depending on the treatment) were transplanted and moved to the greenhouse. In each cage, 20 plants were transplanted to hydroponic substrates at a rate of 4 plants per growbag. The day following transplantation one sachet of *A. swirskii,* containing around 125 mites of mixed ages, was hung on the first plant node on every three sweet pepper plant. One week after transplantation the infestation of the plants with weekly releases of *F. occidentalis* (5 adults/m^2, 40 adults/cage), *B. tabaci* (10 adults/m^2, 80 adults/cage) and *M. persicae* (30 aphid of mixed age/plant) was initiated. Six releases were conducted for *F. occidentalis* and for *B. tabaci*. In the case of *M. persicae* only three releases were made due to the rapid proliferation of aphids in the control treatment. These release rates and their frequency were chosen to simulate a strong and early pest attack. Four weeks after transplantation, sweet pepper plants began flowering and *O. laevigatus* individuals were released in the cages corresponding to "*O. laevigatus* + *A. swirskii*" treatment at the rate of 1 adult/plant. Two releases of *O. laevigatus* were done at an interval of 7 days. One week later the pest infestation samplings were initiated and continued until the end of the experiment which was 6 weeks later. For this, five random plants per cage were selected and the number of *F. occidentalis*, *B. tabaci*, *M. persicae* and the number of predators (immature and adults) per plants (referring to one leaf and one flower) were recorded. Special care was always taken to count the replications in the control compartment first and then the cages with the predators to reduce risk of accidental contamination among treatments. The full experiment ran for nine weeks, from beginning of May to the end of June 2016. The experiment was stopped after the sixth data collection date due to the high attack in the control cages which resulted in the total collapse of the control plants. Plants were irrigated twice a week throughout the experiment. A graphical scheme summarizing the release calendar is shown in Fig. 1.

Statistical analysis

In the co-occurrence experiment, the accumulated number of predatory bugs (adults + nymphs) and predatory mites (nymphs, protonymphs, deutonymphs and adults) during the course of the trial were calculated. The resulting estimates of insect per days (=area under the weekly incidence curve) was log transformed and then subjected to a one-way analysis of variance (ANOVA) joined with a Tukey's test for mean separation ($P < 0.05$). Treatments with zero values during the 6 weeks of the analysis were excluded from the analysis. Therefore, in *N. tenuis* treatment, one-tailed Student's *t*-test ($P < 0.05$) was conducted since only two treatments were compared. For the greenhouse experiment, the total number of *F. occidentalis*, *B. tabaci*, *M. persicae* and predators per sweet pepper plants were log (x+1) transformed prior to analysis using the generalized Linear Mixed Models (GLMM). Treatment was considered to be a fixed factor and time (weeks) as a random one. Each GLMM used a normal distribution and identity link function. Untransformed value are presented in figures. Whenever a significant difference was found, pairwise comparisons of the fixed factor levels were performed with the least significant difference (LSD) post-hoc test ($P<0.05$). To calculate *F. occidentalis*, *B. tabaci* and *M. persicae* percentage of reduction, Abbott's formula was applied, *100 × [1- (treated/control)]* (Abbott, 1925) using the accumulated number of *F. occidentalis*, *B. tabaci* and *M. persicae*. To know whether differences between the percentages of reduction existed, data were log transformed and then a one way ANOVA followed by comparison of means (Tukey's test) at $P < 0.05$ was conducted. The results were expressed as the means ± standard error.

Results

Dynamics of predatory bugs when co-occurred with *A. swirskii*

The population dynamics of the three predatory bugs was significantly different among the four release treatments (released alone, released together with *A. swirskii*, released with the addition of *E. kuehniella* eggs and released together with *A. swirskii* with the addition of *E. kuehniella* eggs (*N. tenuis*: $t_{1,7}$= 2.51, P= 0.046; *M. pygmaeus*: $F_{3, 15}$= 296.8, P< 0.001 and *O. laevigatus*: $F_{2,11}$= 230.7 P< 0.001) (Fig. 2). The highest population density for the three predators was observed in treatments receiving *E. kuehniella* eggs as additional food. Both *N. tenuis* and *O. laevigatus* were unable to survive and reproduce in the absence of *E. kuehniella* eggs. Nevertheless, *M. pygmaeus* was able to survive 7 weeks after its release on sweet pepper plants deprived from alternative prey, although at a very low density (Table 1; Fig. 2). When predatory bugs were released together with the predatory mite on those plants provided with the addition of *E. kuehniella* eggs, *N. tenuis* significantly decreased its population density (Fig. 2a). However, the density of *O. laevigatus* and *M. pygmaeus* was not negatively affected when released with *A. swirskii* in the presence of *E. kuehniella* eggs (Figs. 2b, c).

Dynamics of *A. swirskii* when co-occurred with predatory bugs

The number of *A. swirskii* per plant was not different when comparing the population dynamics of the treatment where *A. swirkii* was released alone with the population dynamics of those treatments where *A. swirskii* was released with one of the three predatory bugs tested ($F_{3, 15}$= 0.52, P = 0.68) (Fig. 3a). In spite the general trend, the populations of *A. swirksii* decreased from the date they were released. This predatory mite was able to survive in the sweet pepper plants without access to prey during the 7 weeks of the experiment, independent of the presence or absence of a predatory bug. Nevertheless, the population dynamics of *A. swirskii* were significantly different between the four treatments where the releases of *A. swirskii* were supplemented with eggs of *E. kuehniella* ($F_{3, 15}$ = 24.02, P< 0.001)

(Fig. 3b). The combined release of any of the three predatory bugs with *A. swirskii* in the presence of *E. kuehniella* significantly reduced *A. swirskii* populations when compared to the treatment when *A. swirskii* was released with *E. kuehniella* alone. Indeed, the combined release with *O. laevigatus* was the one that was significantly more detrimental for the phytoseiid populations (Fig. 3b).

Predators' abundance under greenhouse conditions

The three predatory bugs established themselves on sweet pepper plants when released together with *A. swirskii* and remained active until the end of the experiment, although significant differences were observed among their abundances ($F_{2, 69}$ = 13.10, P< 0.001) (Fig. 4a) (Table 2). *Nesidiocoris tenuis* was significantly the most abundant predator in comparison to the number reached by *M. pygmaeus* and *O. laevigatus* (Table 2; Fig. 4a). On the contrary, the number of *A. swirskii* per plant was similar when released in a mixed treatment with either *N. tenuis*, *M. pygmaeus* or *O. laevigatus* ($F_{2,69}$= 1.90*;* P= 0.16) (Table 2; Fig. 4b). However, it should be mentioned that the number of *A. swirskii* per plant was abruptly reduced after the release of *O. laevigatus* (Fig. 4b), although when compared to the number of *A. swirskii* in the other two predatory bug treatments, no differences were found.

Pest management

The number of *F. occidentalis* per plant sampled was continuously suppressed at significant levels in the three treatments which received releases of predators (*Nt*+*As, Mp*+*As* and *Ol*+*As*) when compared to the control ($F_{3, 92}$= 13.66*;* P< 0.001) (Table 3; Fig. 5a). The infestation by *F. occidentalis* was significantly reduced to 97%, 95% and 75%, in the *Nt*+*As, Mp*+*As* and *Ol*+*As* treatments, respectively, without differences between them (Fig. 5b). In a similar way, *B. tabaci* was significantly reduced in the three treatments with predator releases (*Nt*+*As, Mp*+*As* and *Ol*+*As*) when compared to the control ($F_{3,92}$=22.475; P< 0.001) (Table 3; Fig. 6a). The percentage of whitefly reduction was not significantly different between treatments and was 95%, 82% and 89% in *Nt+As, Mp+As* and *Ol+As* treatments, respectively (Fig. 6b). Aphid densities significantly differed among treatments ($F_{3,92}$= 15.66*;* P< 0.001) (Table 3). Sweet pepper plants managed with either *Nt+As* or *Mp+As*, harbored significantly lower density of aphids when compared to *Ol+As* and to the control. The abundance of aphids was also significantly lower in *Ol+As* when compared to the control (Fig. 7a). Aphid infestation was significantly reduced in *Nt+As* (90%) and *Mp+As* (77%) in comparison to *Ol+As* (35%) (Fig. 7b).

Discussion

Under laboratory conditions, sweet pepper plants maintained *M. pygmaeus* at a very low developmental level during approximately two months. In contrast, *N. tenuis* and *O. laevigatus* were not able to build up a significant population when prey was absent on the plant. The ability of *M. pygmaeus* to continue its immature development by feeding exclusively on plant tissue is already known (Perdikis and Lykouressis 2000; Portillo et al. 2012), while *N. tenuis* is prey dependent (Urbaneja et al. 2005) and *O. laevigatus* survival and reproduction are dependent on the presence of either prey or pollen (Cocuzza et al. 1997). Interestingly, we observed that even when one of the three predatory bugs co-existed on the same plant with *A. swirskii*, despite the need of *N. tenuis* and *O. laevigatus* for a protein source, the three predators ignored the predatory mite and *vice versa.* On the contrary, when *E. kuehniella* eggs were provided as a shared prey, only *N. tenuis* was negatively affected by *A. swirskii*. The population levels of *A. swirskii* were negatively affected by the three predatory bugs and significantly more affected by the presence of *O. laevigatus* than that the presence of mirids (Fig. 3b). Urbaneja et al. (2003) previously observed *O. laevigatus* preying on *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae) in sweet pepper greenhouses. Because *N. cucumeris* was released in large quantities in a period of prey scarcity, prior to the release of *O. laevigatus*, these authors pointed out that the availability of *N. cucumeris* (intraguild prey) facilitated the establishment of *O. laevigatus* (intraguild predator). In our experiment, it seems that when *E. kuehniella* eggs were provided as shared prey, which is a high quality food for both of the predatory bugs (Cocuzza et al. 1997; Castañé et al. 2006) and the predatory mite (Nguyen et al. 2014), competition occurred. However, reasons why *A. swirskii* only negatively affected the stability of *N. tenuis* is still an open question.

Intraguild predation among predators is widespread and both unidirectional and bidirectional. IGP appears to be common and associated with natural enemies used in greenhouse production systems (Rosenheim et al. 1995). Based on theory, IGP is not expected to benefit BC (Rosenheim et al. 1995), and could be a determining factor in the abundance and distribution of BC agents (Lucas and Alomar 2001; Perdikis et al. 2014). However, in practice, results are diverse and the potential risk of IGP disrupting biological control appears to be low in many cases (Janssen et al. 2006; Messelink and Janssen 2014). This is also the case of the current study, where, despite being involved in IGP, the mixed release of *N. tenuis* and *A. swirskii*, which were involved in a bidirectional IGP in the laboratory experiment, was virtually the best combination in the suppression of thrips, whiteflies and aphids under greenhouse conditions. Availability and variability of prey (thrips, whiteflies and aphids) and food provided by the plant (pollen, nectar, plant sap) might be the reason for this non-aggressive coexistence.

Indeed, an increase in extraguild prey density has been suggested to decrease the likelihood of predation events occurring among members of the predator guild (Polis et al. 1989, Lucas et al. 1998; Holt and Huxel 2007). The within plant distribution and patch occupation by both predators, might have also reduced the encounter rate. *Amblyseius swirskii* on sweet pepper is generally found on the tuft domatia of the vein axils, a special structure of the sweet pepper leaves, which constitute a refuge for this mite and therefore might reduce the intensity of IGP under field conditions (Walter 1996; Schmidt 2014). The treatment involving *M. pygmaeus* with *A. swirskii* was as effective in pest suppression as *N. tenuis* with *A. swirskii*, however *M.*

pygmaeus was significantly less abundant than *N. tenuis*. It is well known that *M. pygmaeus* is less thermophilous than *N. tenuis* (Sánchez et al. 2009), hence the high temperatures registered (mean of 30°C) during the experimental period could have hampered the development of this predator. The combined release of *O. laevigatus* with *A. swirskii* successfully reduced *F. occidentalis* and *B. tabaci* populations at levels similar to those that provided treatments based on predatory mirids. However, in the cages that received the combined release of *O. laevigatus* and *A. swirskii*, aphids were far from being controlled, which resulted in the collapse of all the plants.

In this experiment, we adopted the pre-plant release strategy for mirids suggested by Calvo et al. (2012b) for tomatoes. This strategy is widely used today in greenhouse tomato crops in Southeastern Spain (Pérez-Hedo and Urbaneja 2016; Pérez-Hedo et al. 2017). At transplantation, mirids had already laid eggs and the establishment was easier thanks to their ability to feed on plant tissue along with the addition of *E. kuehniella* eggs.

Overall, in order to extrapolate our experimental conditions to field conditions the availability of prey must be high. This high availability of prey is necessary to maintain the population of the mirids after their pre-plant release. In this sense, this strategy could be valid for summer plantings when the level of prey is high when the transplant occurs. However, in late winter plantations where pray availability is low, it is necessary to supplement the pre-plant release of mirids with the alternative food source. This could possibly make the system more expensive. In any case all these conditions have to be evaluated before making a decision regarding the use of mirids under true field conditions.

In conclusion, our study provides further evidence that the release of natural enemies involved in IGP does not necessary have negative effects on BC. Additional success by mirids in managing sweet pepper pests was confirmed throughout this study. Together with the newly discovered ability of predatory mirids to induce plant defence (Bouagga et al. 2017, 2018), we expect the future BC of sweet pepper in commercial greenhouse could rely on the release of

A. swirskii with predatory mirids. What is clear is, the use of mirids in sweet pepper is possible and can be more effective than the current system based on *O. laevigatus*, which motivates us to suggest that mirids deserve more attention in the BC of sweet pepper pests.

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References

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Alvarado, P., O. Balta, and O. Alomar. 1997. Efficiency of four Heteroptera as predators of *Aphis gossypii* and *Macrosiphum euphorbiae* (Hom.: Aphididae). Entomophaga. 42: 215-226.

Arnó, J., J. Roig, and J. Riudavets. 2008. Evaluation of *Orius majusculus* and *O. laevigatus* as predators of *Bemisa tabaci* and estimation of their prey preference. Biol. Control. 44: 1-6.

Belliure, B., P. Pérez, M. Marcos, J. Michelena, and A. Hermoso de Mendoza. 2008. Control biológico de pulgones, pp. 209-238. In J. Jacas and A. Urbaneja (eds.), Control biológico de plagas agrícolas. Phytoma-España*,* Valencia, Spain.

Bouagga, S., A. Urbaneja, J. L. Rambla, V. Flors, A. Granell, J. A. Jaques, and M. Pérez-Hedo. 2017. Zoophytophagous mirids provide pest control by inducing direct defences, antixenosis and attraction to parasitoids in sweet pepper plants. Pest. Manag. Sci. DOI: 10.1002/ps.4838.

Bouagga, S., A. Urbaneja, J. L. Rambla, A. Granell, and M. Pérez-Hedo. 2018. *Orius laevigatus* strengthens its role as a biological control agent by inducing plant defences. J. Pest. S ci. 91: 55-64.

Blom, J. 2008. Pimiento bajo abrigo. pp. 399-409. In J. Jacas and A. Urbaneja (eds.), Control biológico de plagas agrícolas. Phytoma-España*,* Valencia, Spain.

Calvo, J., K. Bolckmans, and J. E. Belda. 2009. Development of a biological control-based integrated pest management method for *Bemisia tabaci* for protected sweet pepper crops. Entomol. Exp. Appl. 133: 9-18.

Calvo, F., K. Bolckmans, and J. E. Belda. 2012a. Biological control-based IPM in sweet pepper greenhouses using *Amblyseius swirskii* (Acari: Phytoseiidae). Biocontrol. Sci. Technol. 22: 1398-416.

Calvo, F. J., K. Bolckmans, and J. E. Belda, 2012b. Release rate for a preplant application of *Nesidiocoris tenuis* for *Bemisia tabaci* control in tomato. BioControl. 57: 809-817.

Calvo, F.J., M. Knapp, Y. M. van Houten, H. Hoogerbrugge, and J. E. Belda, 2015. *Amblyseius swirskii*: What made this predatory mite such a successful biocontrol agent? Exp. Appl. Acarol. 65: 419-433.

Castañé, C., R. Quero, and J. Riudavets. 2006. The brine shrimp *Artemia* sp as alternative prey for rearing the predatory bug *Macrolophus caliginosus*. Biol. Control. 38: 405-412.

Christensen, R. K., A. Enkegaard, and H. F. Brodsgaard. 2002. Intraspecific interactions among the predators *Orius majusculus* and *Aphidoletes aphidimyza*. IOBC/WPRS. Bull. 25: 57-60.

Cocuzza, G. E, P. De Clercq, M. Van de Veire, A. De Cock, D. Degheele, and V. Vacante. 1997. Reproduction of *Orius laevigatus* and *Orius albidipennis* on pollen and *Ephestia kuehniella* eggs. Entomol. Exp. Appl. 82: 101-104.

Coll, M. and M. Guershon. 2002. Omnivory in terrestrial arthropods: mixing plant and prey diets. Annu. Rev. Entomol. 47: 267-297.

Crawley, M. J. 2002. Statistical computing. An introduction to data analysis using S-plus. Wiley&Sons Press, Chichester, UK.

Holt, R. D. and G. A. Polis. 1997. A theoretical framework for intraguild predation. Am. Nat. 149: 745-764.

Holt, R. D. and G. R. Huxel. 2007. Alternative prey and the dynamics of intraguild predation: Theoretical perspectives. Ecology. 88: 2706-2712.

Janssen, A., M. Montserrat, R. HilleRisLambers, A. M. de Roos, A. Pallini, and M. W. Sabelis. 2006. Intraguild predation usually does not disrupt biological control, pp. 21-44. In J.

Brodeur and G. Boivin (eds.), Trophic and Guild Interactions in Biological Control. Springer, Netherlands, Dordrecht.

Lucas, É., D. Coderre, and J. Brodeur. 1998. Intraguild Predation among aphid predators: characterization and influence of extraguild prey density. Ecology, 79: 1084-1092.

Lucas, É. and O. Alomar. 2001. *Macrolophus caliginosus* (Wagner) as an Intraguild Prey for the Zoophytophagous *Dicyphus tamaninii* Wagner (Heteroptera: Miridae). Biol. Control. 20: 147-152.

Messelink, G. J., S. E. F. van Steenpaal, and P.M.J. Ramakers. 2006. Evaluation of phytoseiid predators for control of Western flower thrips on greenhouse cucumber. BioControl. 51: 753-768.

Messelink, G. J, R. van Maanen, R. van Holstein-Saj, M. W. Sabelis, and A. Janssen. 2010. Pest species diversity enhances control of spider mites and whiteflies by a generalist phytoseiid predator. BioControl. 55: 387-398.

Messelink, G. J., C. M. J. Bloemhard, L. Kok, and A. Janssen. 2011. Generalist predatory bugs control aphids in sweet pepper. IOBC/WPRS Bull. 68: 115-118.

Messelink, G. J., C. M. J. Bloemhard, M. W. Sabelis, and A. Janssen. 2013. Biological control of aphids in the presence of thrips and their enemies. BioControl. 58: 45-55.

Messelink, G. and A. Janssen. 2014. Increased control of thrips and aphids in greenhouses with two species of generalist predatory bugs involved in intraguild predation. Biol. Control. 79: 1-7.

Messelink, G., C. M. J. Bloemhard, H. Hoogerbrugge, J. van Schelt, B.L. Ingegno, and L. Tavella. 2015. Evaluation of mirid predatory bugs and release strategy for aphid control in sweet pepper. J. Appl. Entomol. 139: 333-41.

Nomikou, M., A. Janssen, R. Schraag, and M. W. Sabelis. 2002. Phytoseiid predators suppress populations of *Bemisia tabaci* on cucumber plants with alternative food. Exp. Appl. Acarol. 27: 57-68.

Nguyen, D. T., D. Vangansbeke, and P. De Clercq. 2014. Artificial and factitious foods support the development and reproduction of the predatory mite *Amblyseius swirskii*. Exp. Appl. Acarol. 62: 181-94.

Perdikis, D. C. H. and D.P. Lykouressis. 2000. Effects of various items, host plants, and temperatures on the development and survival of *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae). Biol. Control. 17: 55-60.

Perdikis, D. C. H., E. Lucas, N. Garantonakis, A. Giatropoulos, P. Kitsis, D. Maselou, S. Panagakis, P. Lampropoulos, A. Paraskevopoulos, D. P. Lykouressis, and A. Fantinou .2014. Intraguild predation and sublethal interactions between two zoophytophagous mirids, *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. Biol. Control. 70: 35-41.

Pérez-Hedo, M., and A. Urbaneja. 2015. Prospects for predatory mirid bugs as biocontrol agents of aphids in sweet peppers. J. Pest. Sci. 88: 65-73.

Pérez-Hedo, M. and A. Urbaneja. 2016. The zoophytophagous predator *Nesidiocoris tenuis*: A successful but controversial biocontrol agent in tomato crops, pp. 121-138. In A.R. Horowitz and I. Ishaaya (eds.), Advances in Insect Control and Resistance Management. Springer, Netherlands, Dordrecht.

Pérez-Hedo, M., R. Suay, M. Alonso, M. Ruocco, M. Giorgini, C. Poncet, and A. Urbaneja. 2017. Resilience and robustness of IPM in protected horticulture in the face of potential invasive pests. Crop. Prot. 97: 119-127.

Polis, G. A., C. A. Myers, and R.D. Holt. 1989. The ecology and evolution of intraguild predation: Potential competitors that eat each other. Annu. Rev. Ecol. Syst. 20: 297-330.

Portillo, N., O. Alomar, and F. Wackers. 2012. Nectarivory by the plant-tissue feeding predator *Macrolophus pygmaeus* Rambur (Heteroptera: Miridae): Nutritional redundancy or nutritional benefit? J. Insect. Physiol. 58: 397-401.

Rosenheim, J. A., H. K. Kaya, L. E. Ehler, J.J. Marois, and B. A. Jaffee. 1995. Intraguild predation among biological-control agents: theory and evidence. Biol. Control. 5: 303-335.

Rosenheim, J. A. 1998. Higher order predators and the regulation of insect herbivore populations. Annu. Rev. Entomol. 43: 421-447.

Rosenheim, J. A. and J. Harmon. 2006. The influence of intraguild predation on the suppression of a shared prey population: an empirical reassessment, pp. 1-20. In J. Brodeur and G. Boivin (eds.), Trophic and guild interactions in biological control. Springer, New York, USA.

Sánchez, J. A., A. Lacasa, J. Arnó, C. Castañé, and O. Alomar. 2009. Life history parameters for *Nesidiocoris tenuis* (Reuter) (Het, Miridae) under different temperature regimes. J. Appl. Entomol. 133: 125-132.

Sánchez, J., M. La-Spina, J.M. Michelena, A. Lacasa, and A. Hermoso de Mendoza. 2011. Ecology of the aphid pests of protected pepper crops and their parasitoids. Biocontrol. Sci. Technol. 21: 171-88.

Schmidt, R. 2014. Leaf structures affect predatory mites (Acari: Phytoseiidae) and biological control: A review. Exp. Appl. Acarol. 62: 1-17.

Urbaneja, A., F. J. Leon, A. Giménez, E. Aran, and J. van der Blom. 2003. The interaction of *Neoseiulus* (*Amblyseius*) *cucumeris* (Oudemans) (Aca.: Phytoseiidae) on the establishment of *Orius laevigatus* (Fieber) (Hem.: Anthocoridae) in sweet pepper crops. Bol. Sanid. Veg., Plagas. 29: 1-12.

Urbaneja, A., G. Tapia, and P.A. Stansly. 2005. Influence of host plant and prey availability on developmental time and survival of *Nesidiocoris tenuis* Reuter (Het: Miridae). Biocontrol. Sci. Technol. 15: 513-518.

Urbaneja-Bernat, P., O. Mollá, M. Alonso, K. Bolckmans, A. Urbaneja, and A. Tena. 2015. Sugars as complementary alternative food for the establishment of *Nesidiocoris tenuis* in in greenhouse tomato. J. Appl. Entomol. 139: 161-167.

van Lenteren, J. 2012. The state of commercial augmentative biological control: plenty of natural enemies but a frustrating lack of uptake. BioControl, 57: 1-20.

van Lenteren, J., K. Bolckmans, J. Kohl, W. J. Ravensberg, and A. Urbaneja. 2017. Biological control using invertebrates and microorganisms: plenty of new opportunities. BioControl. (in press). doi:10. 1007/s10526-017-9801-4.

van Maanen, R., E. Vila, M. W. Sabelis, and A. Janssen. 2010. Biological control of broad mites (*Polyphagotarsonemus latus*) with the generalist predator *Amblyseius swirskii*. Exp. Appl. Acarol. 52: 29-34.

Venzon, M., A. Janssen, and M. W. Sabelis. 2002. Prey preference and reproductive success of the generalist predator *Orius laevigatus*. Oikos. 97: 116-24.

Walter, D. E. 1996. Living on leaves: mites, tomenta, and leaf domatia. Annu. Rev. Entomol. 41: 101-114.

Tables

Table 1

Average of predatory bugs (*N. tenuis*, *M. pygmaeus, O. laevigatus*) (±SE) and predatory mites (±SE) accumulated per day when released alone or when combined on plant deprived of or provided with eggs of the alternative shared prey, *E. kuehniella*. Means in the same row followed by the same letter are not significant different (Tukey, *P*< 0.05; **t*-test, *P*< 0.05).

	Nesidiocoris tenuis*	***Macrolophus pygmaeus***	***Orius laevigatus***
Predatory bug			
Predatory bug alone	-	3.7 ± 0.2 b	-
Predatory bug + *A. swirskii*	-	5.2 ± 0.6 b	0.3 ± 0 b
Predatory bug + *E. kuehniella*	45.7 ± 5.5 a	38.0 ± 1.6 a	6.7 ± 0.5 a
Predatory bug + *E. kuehniella* + *A. swirskii*	26.5 ± 5.3 b	38.5 ± 1.4 a	7.4 ± 0.8 a
Predatory mite	**Without *E. kuehniella***		**With *E. kuehniella***
A. swirskii	24.2 ± 2.5 a		91.5 ± 5.4 a
A. swirskii* + *N. tenuis	23.1 ± 2.1 a		46.1 ± 8.8 b
A. swirskii* + *M. pygmaeus	21.5 ± 2.1 a		48.7 + 3.4 b
A. swirskii* + *O. laevigatus	20.5 ± 2.0 a		20.6 ± 2.1 c

Table 2

P-values for the pairwise comparison of the number of *A. swirskii* (*As*) and predatory bug (*O. laevigatus*, *N. tenuis*, *M. pygmaeus*) per sweet pepper plant in the following treatments (*N. tenuis* + *A. swirskii* (*Nt*+*As*), *M. pygmaeus* + *A. swirskii* (*Mp*+*As*) and *O. laevigatus* + *A. swirskii* (*Ol*+*As*)). Values in bold correspond to significant differences between treatments.

Treatments	**Predatory mite**		**Predatory bug**	
	t_{69}	P	t_{69}	P
Nt+As vs Mp+As	0.31	0.76	**3.50**	**0.001**
Nt+As vs Ol+As	1.50	0.14	**4.98**	**<0.001**
Mp+As vs Ol+As	1.82	0.07	1.48	0.14

Table 3

P-values for the pairwise comparison of the number of *F. occidentalis, B. tabaci* and *M. persicae* per sweet pepper plant that received a release of *N. tenuis* + *A. swirskii* (*Nt+As*), *M. pygmaeus* + *A. swirskii* (*Mp+As*), *O. laevigatus* + *A. swirskii* (*Ol+As*) and control without release (C). Values in bold correspond to significant differences between treatments.

Treatments	***Frankliniella occidentalis***		***Bemisia tabaci***		***Myzus persicae***	
	t_{92}	*P*	t_{92}	*P*	t_{92}	*P*
C *vs Nt+As*	**5.37**	**<0.001**	**6.54**	**<0.001**	**6.36**	**<0.001**
C *vs Mp+As*	**5.55**	**<0.001**	**6.80**	**<0.001**	**4.60**	**<0.001**
C *vs Ol+As*	**4.54**	**<0.001**	**6.76**	**<0.001**	**2.03**	**0.04**
Nt+As vs Mp+As	0.18	0.85	0.26	0.80	1.77	0.08
Nt+As vs Ol+As	0.82	0.41	0.23	0.82	**4.33**	**<0.001**
Mp+As vs Ol+As	1.00	0.312	0.03	0.97	**2.55**	**0.01**

Fig. legends

Fig. 1. Timeline representing mirid inoculation, *A. swirskii* and *O. laevigatus* introduction, pest infestation and insect abundance, data collection during the greenhouse experiment.

Fig. 2. Variation in the abundance of predatory bug **(a)** *N. tenuis* (*Nt*), **(b)** *M. pygmaeus* (*Mp*) and **(c)** *O. laevigatus* (*Ol*) (adults + nymphs, mean number ±SE) per sweet pepper plant in single species treatment and in mixed treatment with *A. swirskii* (*As*) when the shared prey *E. kuehniella* (*Ek*) eggs were and were not provided. Different letters indicate significant differences of predator bug numbers of the same species between treatments.

Fig. 3. Variation in the abundance of *A. swirskii* (*As*) (adults + deutonymph + protonymph + nymphs, mean number ±SE) per sweet pepper plant in single specie treatment and in mixed treatment with predatory bugs when the shared prey *E. kuehniella* (*Ek*) eggs were **(a)** and were not provided **(b)**. Different letters indicate significant differences of *A. swirskii* numbers between treatments.

Fig. 4. (a) Dynamics of the predatory bugs *N. tenuis* (*Nt*), *M. pgymaeus* (*Mp*) and *O. laevigatus* (*Ol*) (adults + nymphs, mean number ±SE) per sweet pepper plant when released combined with *A. swirskii*. **(b)** Dynamics of *A. swirskii* (*As*) (adults + deutonymph + protonymph + nymphs, mean number ±SE) per sweet pepper plant in different combined treatments with predatory bugs under greenhouse experimental conditions. Different letters indicate significant differences of predators' numbers between treatments.

Fig. 5. (a) Number (mean ± SE) of *F. occidentalis* (adults + larvae) per sweet pepper plant, **(b)** percentage reduction (Abbott) (mean ± SE) of *F. occidentalis* individuals in a greenhouse experiment comparing the effectiveness of predatory bug (*N. tenuis* (*Nt*), *M. pgymaeus* (*Mp*) and *O. laevigatus* (*Ol*)) when released in mixed treatment with *A. swirskii* (*As*) each, at different

time intervals. Bars with different letters are significantly different (ANOVA, Tukey's multiple comparison test $P < 0.05$).

Fig. 6. (a) Number (mean ± SE) of *B. tabaci* (adults + nymphs) per sweet pepper plant, **(b)** percentage reduction (Abbott) (mean ± SE) of *B. tabaci* individuals in a greenhouse experiment comparing the effectiveness of predatory bug (*N. tenuis* (*Nt*), *M. pgymaeus* (*Mp*) and *O. laevigatus* (*Ol*)) when released in mixed treatment with *A. swirskii* (*As*) each, at different time intervals. Bars with different letters are significantly different (ANOVA, Tukey's multiple comparison test $P < 0.05$).

Fig. 7. (a) Number (mean ± SE) of *M. persicae* (adults + nymphs) per sweet pepper plant, **(b)** percentage reduction (Abbott) (mean ± SE) of *M. persicae* individuals in a greenhouse experiment comparing the effectiveness of predatory bug (*N. tenuis* (*Nt*), *M. pgymaeus* (*Mp*) and *O. laevigatus* (*Ol*)) when released in mixed treatment with *A. swirskii* (*As*) each, at different time intervals. Bars with different letters are significantly different (ANOVA, Tukey's multiple comparison test $P < 0.05$).

Fig. 1.

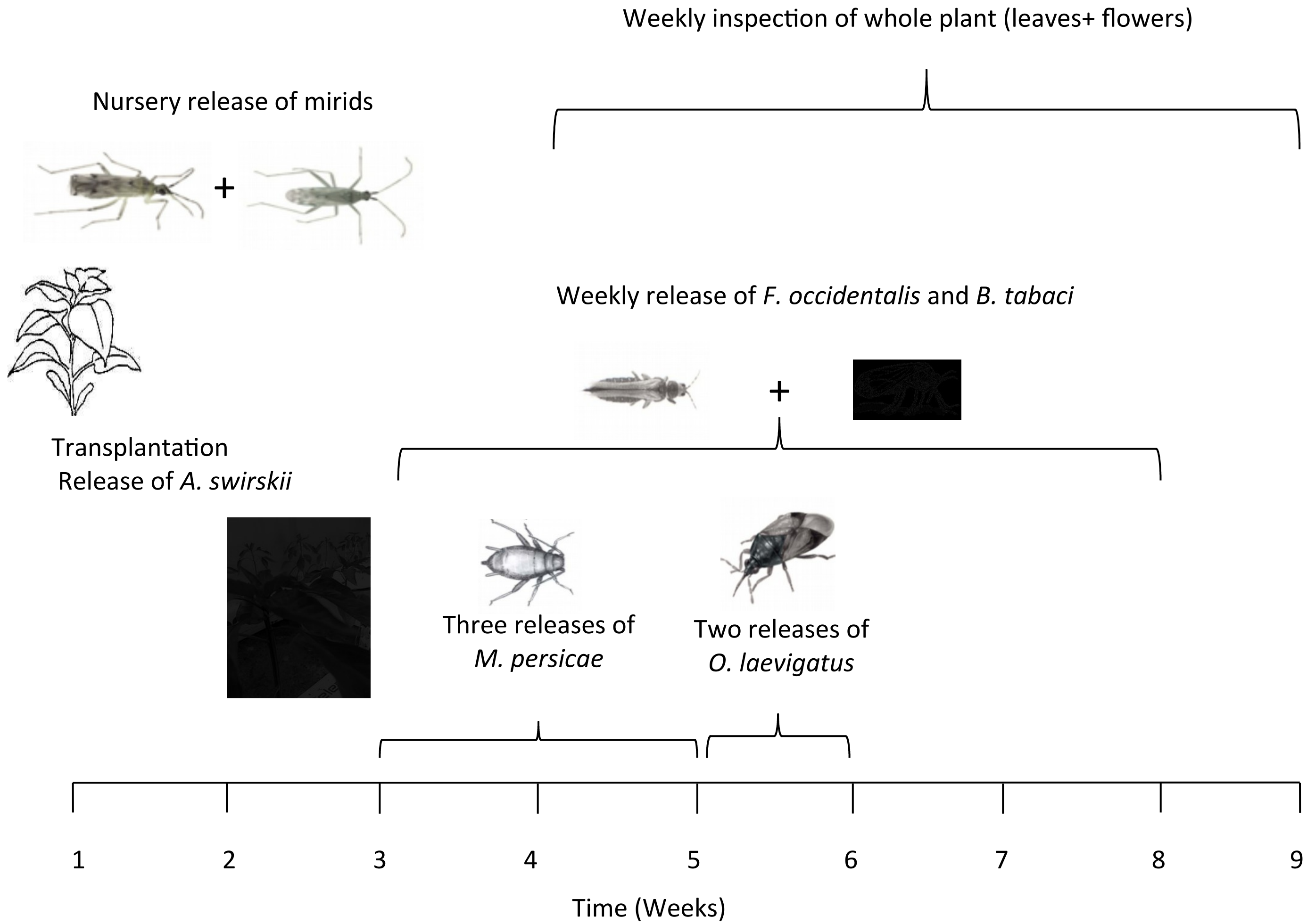

Weekly inspection of whole plant (leaves+ flowers)
Nursery release of mirids
+
Weekly release of *F. occidentalis* and *B. tabaci*
+
Transplantation
Release of *A. swirskii*
Three releases of
M. persicae
Two releases of
O. laevigatus
1
2
3
4
5
6
7
8
9
Time (Weeks)

Fig. 2.

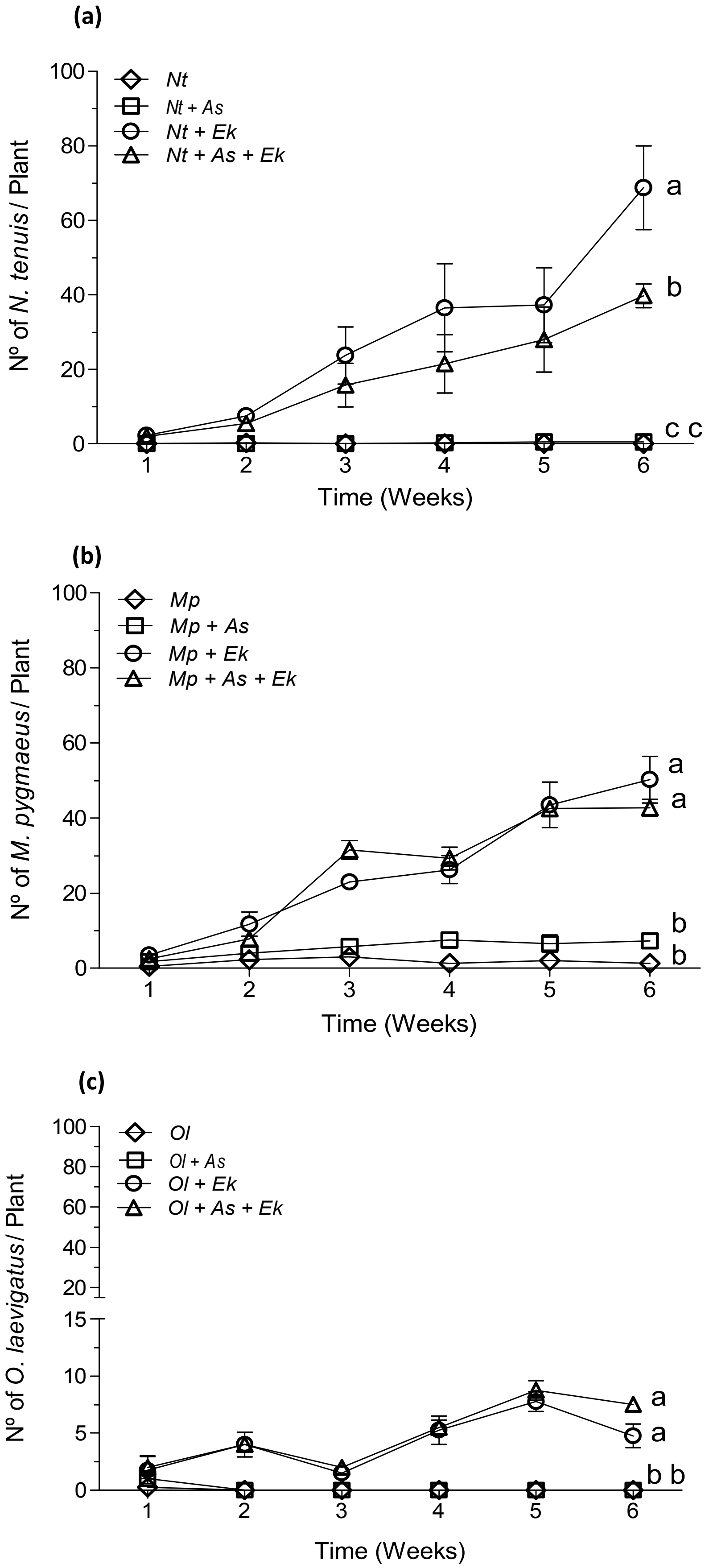
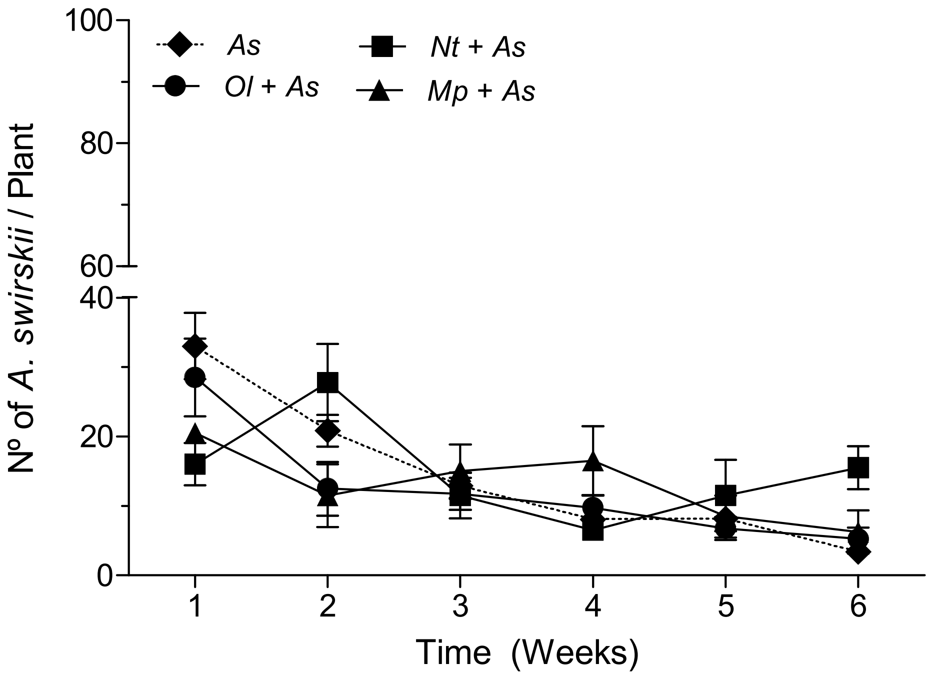

(a)
Nt
Nt + As
Nt + Ek
Nt + As + Ek
Nº of N. tenuis/ Plant
0
20
40
60
80
100
1
2
3
4
5
6
Time (Weeks)
a
b
c c
(b)
Mp
Mp + As
Mp + Ek
Mp + As + Ek
Nº of M. pygmaeus/ Plant
0
20
40
60
80
100
1
2
3
4
5
6
Time (Weeks)
a
a
b
b
(c)
Ol
Ol + As
Ol + Ek
Ol + As + Ek
Nº of O. laevigatus/ Plant
0
5
10
15
20
40
60
80
100
1
2
3
4
5
6
Time (Weeks)
a
a
b b

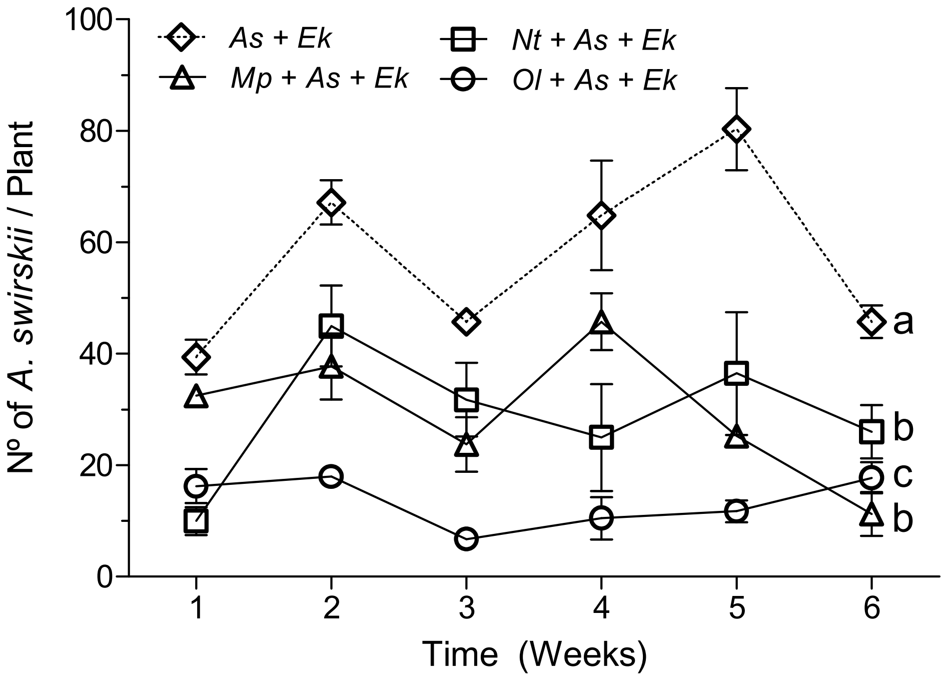
Fig. 3.

(a)



As
Nt + As
Ol + As
Mp + As
Nº of *A. swirskii* / Plant
100
80
60
40
20
0
1
2
3
4
5
6
Time (Weeks)

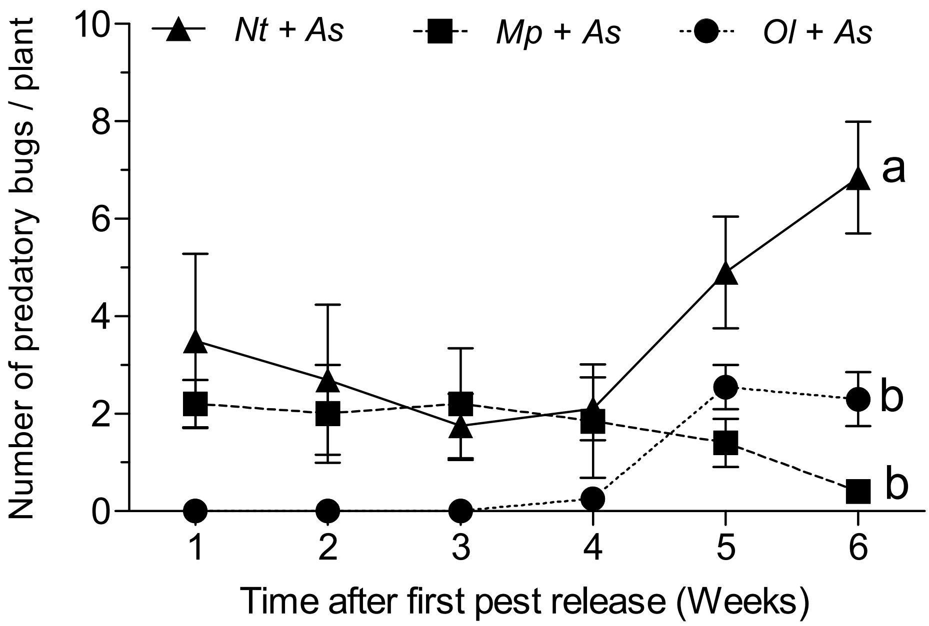
(b)



As + Ek
Nt + As + Ek
Mp + As + Ek
Ol + As + Ek
Nº of *A. swirskii* / Plant
100
80
60
40
20
0
a
b
c
b
1
2
3
4
5
6
Time (Weeks)

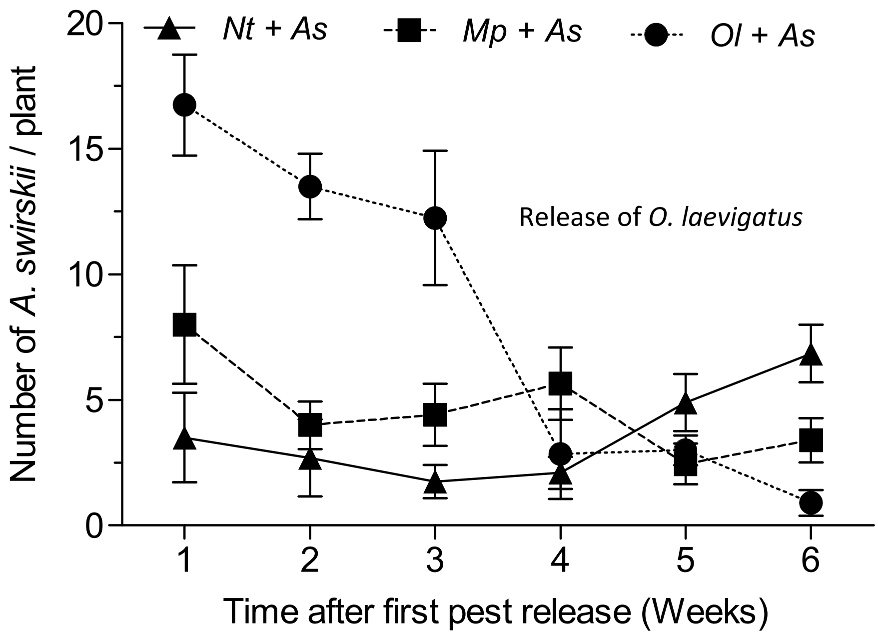
Fig. 4.

(a)



Nt + As
Mp + As
Ol + As
Number of predatory bugs / plant
10
8
6
4
2
0
a
b
b
1
2
3
4
5
6
Time after first pest release (Weeks)

(b)



Nt + As
Mp + As
Ol + As
Number of A. swirskii / plant
20
15
10
5
0
Release of O. laevigatus
1
2
3
4
5
6
Time after first pest release (Weeks)

Fig. 5.

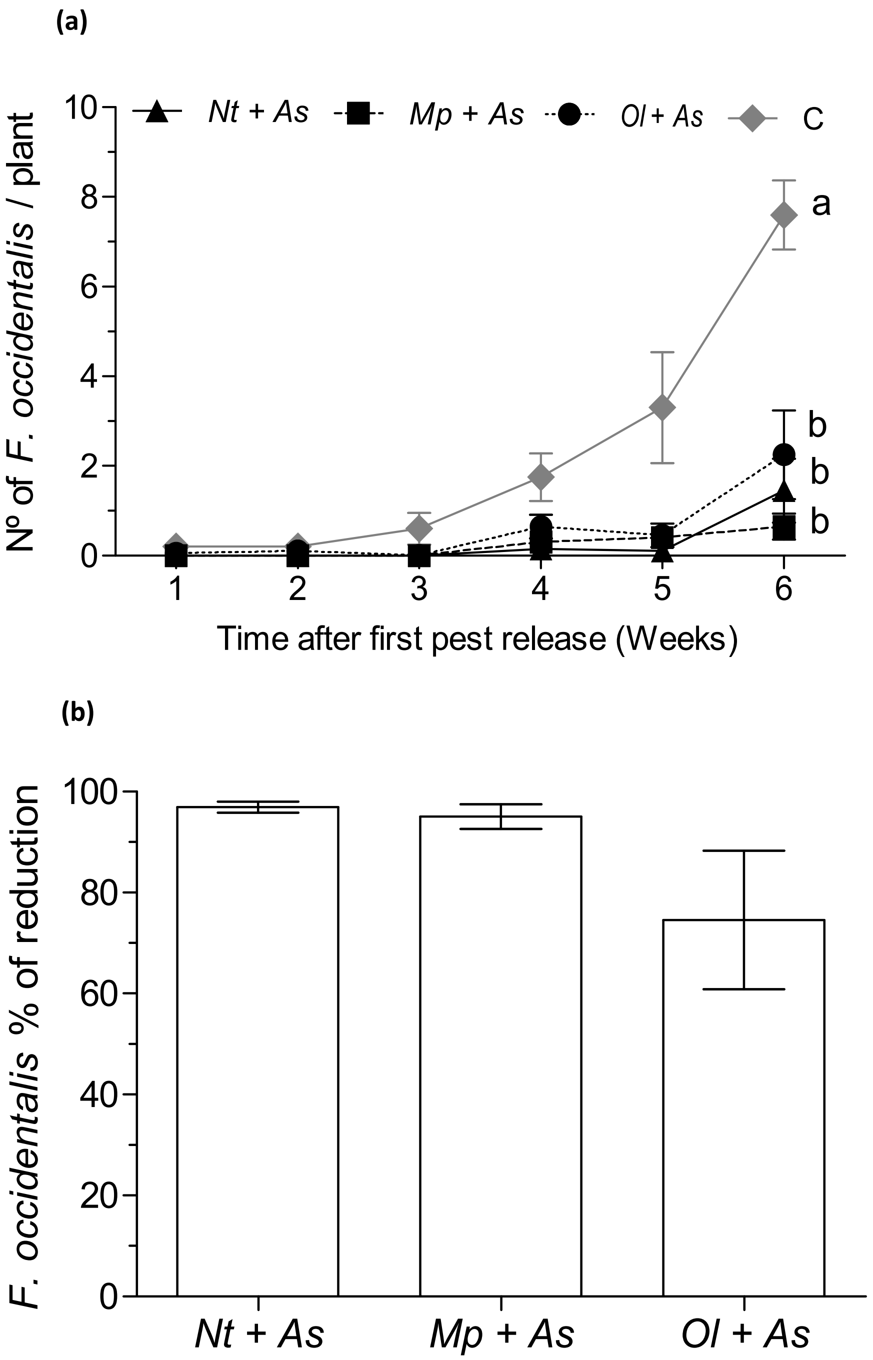
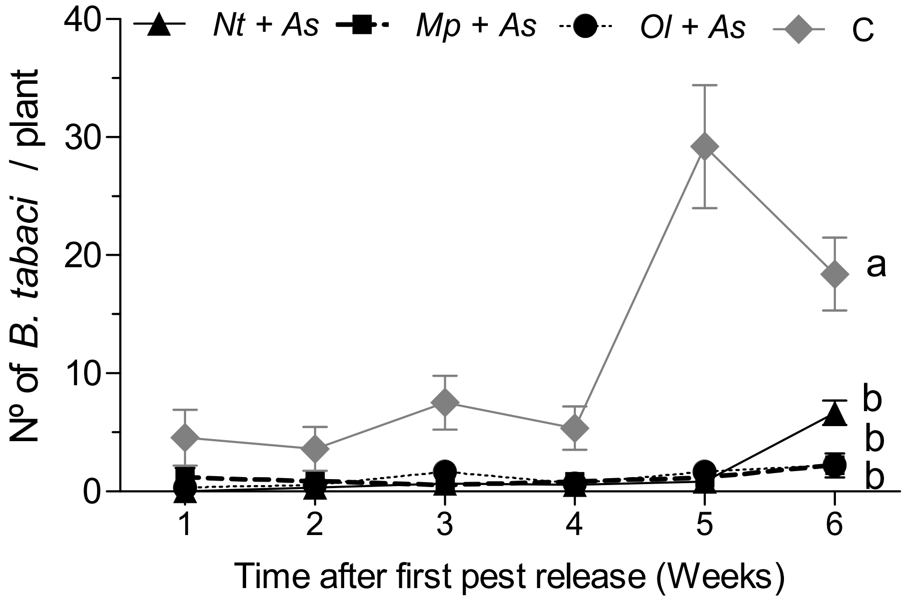

(a)
Nt + As
Mp + As
Ol + As
C
Nº of F. occidentalis / plant
Time after first pest release (Weeks)
a
b
b
b
(b)
F. occidentalis % of reduction
Nt + As
Mp + As
Ol + As

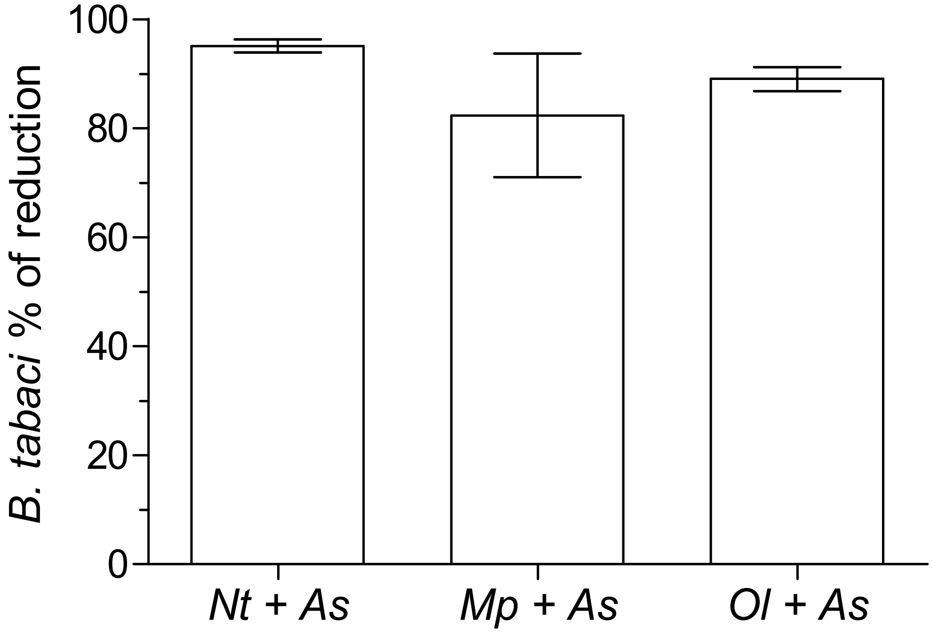
Fig. 6.

(a)



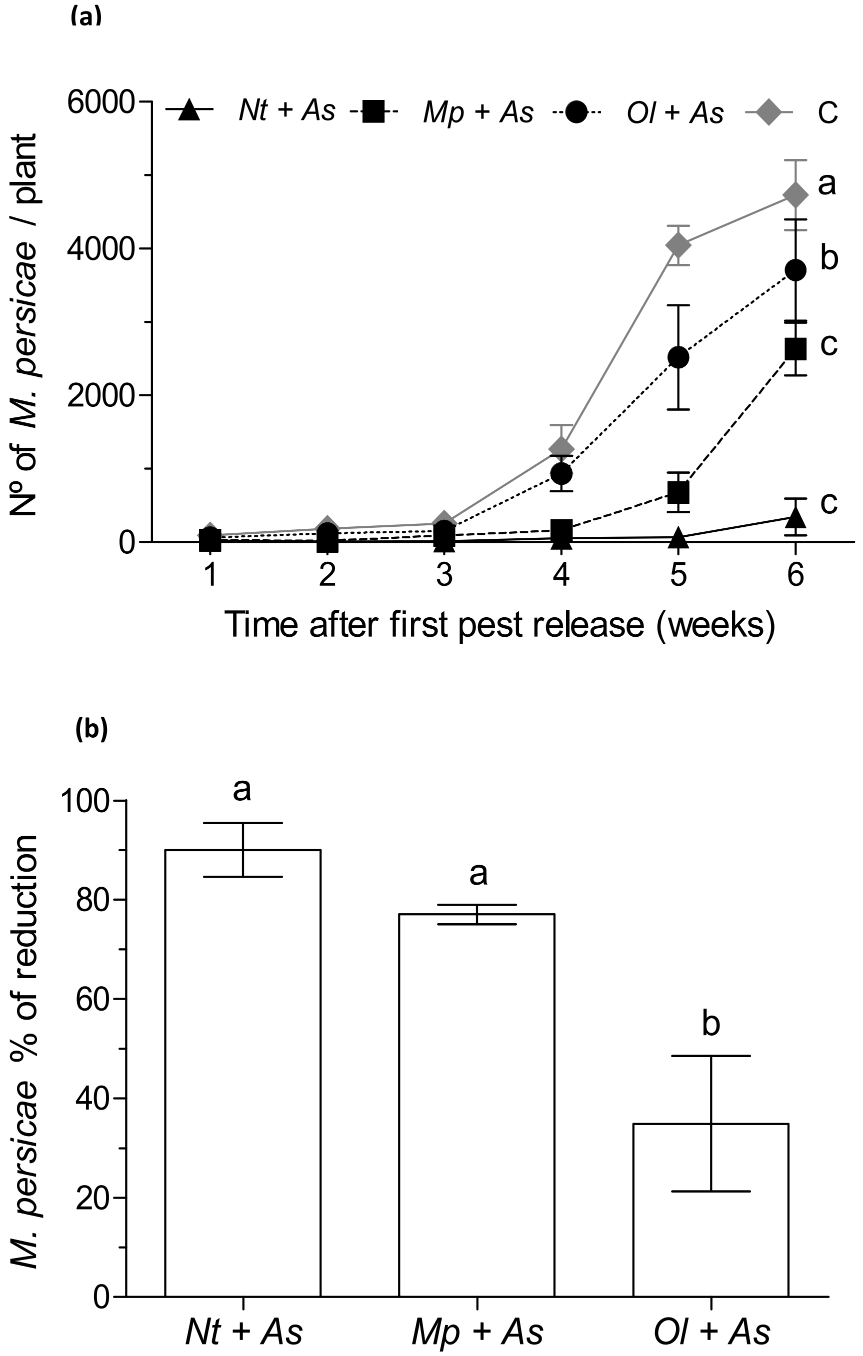
Nt + As
Mp + As
Ol + As
C
Nº of B. tabaci / plant
0
10
20
30
40
a
b
b
b
1
2
3
4
5
6
Time after first pest release (Weeks)

(b)



B. tabaci % of reduction
0
20
40
60
80
100
Nt + As
Mp + As
Ol + As

Fig. 7.



(a)
Nt + As
Mp + As
Ol + As
C
Nº of M. persicae / plant
6000
4000
2000
0
a
b
c
c
1
2
3
4
5
6
Time after first pest release (weeks)
(b)
M. persicae % of reduction
100
80
60
40
20
0
a
a
b
Nt + As
Mp + As
Ol + As