

INFLUENCES OF BOTANICAL PESTICIDES AND BIOLOGICAL AGENTS ON *ORIUS LAEVIGATUS* – *FRANKLINIELLA OCCIDENTALIS* DYNAMICS UNDER GREENHOUSE CONDITIONS

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Abstract: We assessed the influence of nine biopesticides on adults and larvae of western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) and its predator, the anthocorid *Orius laevigatus* (Fieber) under Mediterranean greenhouse conditions. Trials were carried out in a strawberry crop where both species had naturally established. Foliar sprays were applied weekly for one month. Treatments did not provide sufficient control of larval and adult *F. occidentalis*. The negative effects on the dynamics of the predator were evident only with the use of some specific products. The botanical insecticides rotenone and neem, and the nematode *Steinernema feltiae* (Filipjev) reduced *O. laevigatus* numbers, and these effect are evident in the adult stage of *O. laevigatus*. Such products have determined a reduction of the population of the predator from the first treatments even if the incidence was not very high. We conclude that the use of some botanical pesticides and nematodes against WFT is uneconomical and possibly disadvantageous where there is an established predator-prey population.

Key words: biopesticide, neem, *Steinernema feltiae*, *Orius laevigatus*, predator-prey, biological control

INTRODUCTION

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) is widespread throughout the world. It infests more than 500 host plants belonging to about 50 families (Waterhouse and Norris 1989), including a number of economically important crops (Cho *et al.* 1989). WFT cause direct damage by feeding on the plant/flowers or fruit and indirect damage by vectoring plant viruses (Lewis 1997).

At present, pesticides are the main control measure used by growers to manage WFT depending on crop and geographic region. But, chemical control is not always effective due to resistance in WFT to insecticides used in greenhouse crops (Brødsgaard 1994; Zhao *et al.* 1995; Broadbent and Pree 1997). The cryptic behavior of WFT in flower buds also makes it less easy for insecticides to regulate the WFT population. In this context, biological control is highly desirable and provides economic and eco-toxicological benefits.

Parasitic wasps, predatory mites, entomopathogenic nematodes (Premachandra *et al.* 2003; Loomans 2003; Lim and Van Driesche 2004; Arthurs and Heinz 2006; Arthurs *et al.* 2009) and generalist predators including minute pirate bugs (*Orius* spp.) (Stoltz and Stern 1978; Waterhouse and Norris 1989; Bahsi and Tunc 2008), Nabidae (*Nabis* spp.) (Benedict and Cothran 1980) and lacewing larvae of *Chrysoperla carnea* Stephens have been used for biological

control of *Frankliniella* spp. (Waterhouse and Norris 1989; Obrist *et al.* 2005; Atakan 2006). Of the generalist predators found in the Mediterranean area, the anthocorid *Orius laevigatus* (Fieber) plays an important role in suppressing WFT (Vacante and Tropea Garzia 1993) as well as aphids, mites and whitefly populations (Alvarado *et al.* 1997; Montserrat *et al.* 2000) in unheated greenhouse conditions.

Different biopesticides, such as botanical pesticides, fungi, nematodes can contribute to WFT control. Biopesticides used to control pests include animals, plants, bacteria, and minerals. For each pest and its biological control agent, research is required to evaluate the biopesticide as part of an Integrated Pest Management (IPM) strategy. Laboratory, semifield, and field trials generally examine the activity and selectivity of substances on the pest and its biological control agents, in accordance with current International Organization for Biological Control (IOBC) protocols or suggestions (Bakker *et al.* 2000; Boller *et al.* 2005). However, field trials are required to fully evaluate the effects of biopesticides including their effects on predator-prey interactions. This aspect led to our study of *F. occidentalis* – *O. laevigatus* under Mediterranean greenhouse conditions. We assessed the efficacy of nine biopesticides that are currently used, or have potential for use in WFT control in glasshouses (Jackson *et al.* 1997; Copping 2001; Thoeming *et al.* 2003). This included six

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botanical pesticides (neem, rotenone, pyrethrum, *Derris elliptica* (Wallich) Benth., *Artemisia absinthium* Linnaeus, *Urtica dioica* Linnaeus), two microbials [fungi – *Beauveria bassiana* (Balsamo) Vuillemin, *Isaria fumosorosea* Wize] and a nematode (*Steinernema feltiae* Filipjev). Our aim was to verify the influence of these biopesticides on WFT and *O. laevigatus*.

MATERIALS AND METHODS

Study site and experimental design

Trials were carried out on a strawberry crop in an unheated greenhouse (N 38° 49' 49" – E 16° 16' 37"), 10 m above sea level, and approximately 100 meters from the sea, in Pizzo Calabro (Calabria, South Italy). The greenhouse was constructed from plastic and iron, and covered an area of 0.25 ha. Strawberry plants (Camarosa cultivar) were planted in October 2005. Plants were arranged 0.2 by 0.3 m in double rows, with a distance of 1.4 m between each pair of rows. The length of the rows was 50 m and the width of the area was approximately 50 m. The area was divided into 100 experimental units. Each experimental unit was 5 m long and 4.5 m wide, with three parallel double rows of 150 plants. A Latin square experimental design was used with 10x10 plots randomly assigned to 10 treatments with 10 replicates per treatment. Following planting, harmful fungal pathogens and mites were treated with pesticides, but no pesticides were applied the month before the experiment. The presence of WFT and *O. laevigatus* was verified at the end of March. Pre-treatment data on the population dynamics of *O. laevigatus* – WFT were collected in April, to evaluate any difference in their density and distribution in the experimental units. Trials were conducted from May-June 2006, when plants were on full vegetation and there were no differences in thrips and predator densities between experimental units. The keys of Pèricart (1972) were used for taxonomic identification of *Orius* spp. Thrips species were identified using Mound and Kibby (1998).

Botanical pesticides and biological agents

Each experimental unit was treated weekly with the same product, for four weeks. Details of the products applied and the application rates are given in table 1.

Products were applied with a mechanical sprayer atomizer. For each experimental area, five liters of biopesticide suspension or emulsion were applied. To ensure that no spray drift occurred, each experimental unit was isolated from adjacent units with a plastic cover.

Sampling of *O. laevigatus* and WFT

For each experimental unit, each week five open flowers were randomly collected from the central double row. Flowers were examined within 24 hours of collection under a stereo microscope. The nymphal and adult stages, of pest and predator, were recorded for pre-treatment data and for trials.

Statistical analyses

The pre-treatment data collected to evaluate possible different predator-prey densities in the blocks before the treatments, were subjected to one way ANOVA.

The effect of treatments on WFT (adults and larvae) and on *O. laevigatus* (adult and nymphal stages) was evaluated using a multivariate analysis of variance (MANOVA) in general linear model (GLM) (SPSS 14.1 for Windows). Variables included in the model were treatment (botanical pesticide, nematodes, etc.), flower harvesting dates, blocks and their interactions. To correctly determine the effects of the treatments on the population of WFT, the juvenile and adult *O. laevigatus* density were used as covariates. The use of the predator as a covariate explains part of the variability of the dependent variable and allows a more precise calculation of the effects of the treatments reducing the casual variability. Likewise, larval and adult WFT were used as covariates to evaluate the effects of insecticides on the *O. laevigatus* population. The multivariate analysis was conducted in two directions. The purpose of the analysis was to evaluate the effects of the regressive variables on the dependent variables. To

Table 1. Botanical pesticides and biological agents used for four weeks

Products	Concentrations	Doses hl – hectoliters	Trade names and producers
Fungus <i>Beauveria bassiana</i>	2.3x10 ⁷ CFU	200 g/hl	Naturalis® Intrachem Bio Italia
Nematode <i>Steinernema feltiae</i>		2 millions/(0.5 hl)	Nemasys Becker Underwood
Fungus <i>Paecilomyces fumosoroseus</i> Apopka Strain 97	2x10 ⁹ CFU	200 g/hl	Biobest
Rotenone <i>Derris elliptica</i> , <i>Lonchocarpus</i> sp. and <i>Tephrosia</i> sp.	(4.0% liter)	300 g/hl	Bioroten® Intrachem Bio Italia
Botanical <i>Artemisia absinthium</i> (Absinthe)	powder – areal part (150 µm)	300 g/hl	Fitofarmaceutica Medica srl Italia
Pyrethrum <i>Chrysanthemum cinerariifolium</i>	(8.0%)	300 g/hl	Biopiren plus® Intrachem Bio Italia
Botanical <i>Derris elliptica</i> and <i>Lonchocarpus</i> spp. (Origin Brasil)	powder – roots parts (150 µm)	300 g/hl	Fitofarmaceutica Medica srl, Italia
Botanical <i>Urtica dioica</i> (Origin Italy)	powder – areal part (150 µm)	300 g/hl	Fitofarmaceutica Medica srl, Italia
Neem <i>Azadirachta indica</i>	(1.0%)	300 g/hl	Neemazal-t/s® Intrachem Bio Italia
Control		water	

meet the assumption of normality in the data, a square root transformation was used $[(X+1)^{1/2}]$. A pairwise comparison test, based on Student's *t* statistics ($p = 0.05$) with Bonferroni correction, was used to test for significant differences amongst treatments. Wilks' lambda test was also performed. This test is a direct measure of the proportion of variance in the combination of dependent variables, that is unaccounted for by the independent variable. If a large proportion of the variance is accounted for by the independent variable, then it suggests that there is an effect from the grouping variable, and that the groups have different mean values (Everitt and Dunn 1991).

RESULTS

Pre-treatments study

In the pre-treatment analysis, no differences among experimental units were detected in numbers of adults ($F = 1.031$, d.f. = 9, 90; $p = 0.42$) and larvae ($F = 1.392$, d.f. = 9, 90; $p = 0.20$) of WFT, as well as in numbers of adults ($F = 0.814$, d.f. = 9, 90; $p = 0.61$) and nymphs ($F = 1.012$, d.f. = 9, 90; $p = 0.44$) of *O. laevigatus*.

The density (max number found) of WFT adult per flower was 3, and 12 for larvae. The density of *O. laevigatus* adults was 1 and 3 for the nymphal stages.

F. occidentalis

Table 2 indicates the factors that affected the density of WFT (larvae and adults). The variables that did not affect the dependent variables are blocks, and dates, and for the covariate - the adults of *O. laevigatus*. Although the biopesticides in the model influence the density both of the larvae and of the adults of WFT (Table 2) there were no differences in the untreated control for the larvae and adults (Table 3). The only significant mean difference was for the *U. dioica* and *D. elliptica* (mean difference = 0.513; $p = 0.04$) for larvae (*U. dioica* > *D. elliptica*) and *D. elliptica* and rotenone for adults (mean difference = 0.49; $p = 0.049$), (rotenone > *D. elliptica*). The Wilks' multivariate test parameter which ranges between 0 and 1, has a value of 0.909 ($F = 2.342$; $p = 0.01$) with values close to 1, indicating that the means are not very different. None of the different interactions examined in the analysis showed effects in the dependent variables (Table 2). For covariates, WFT adult numbers were affected by the juvenile stages of *O. laevigatus* (Table 2).

O. laevigatus

As in WFT, the density of *O. laevigatus* (nymphs and adults) is affected by different factors (Table 4). The variables that did not affect the dependent variables are block, and for covariate the larvae of *F. occidentalis*. Date affected only the juvenile stages of *O. laevigatus* and the biopesti-

Table 2. Results of MANOVA evaluating the effect of different biopesticides on *F. occidentalis* adults and larvae

Source	Dependent variable <i>F. occidentalis</i>	d.f.	F value	p-value
Intercept	adults	1	112.117	< 0.001
	larvae	1	358.341	< 0.001
Blocks	adults	9	0.777	0.638
	larvae	9	1.628	0.105
Biopesticides	adults	9	2.759	0.004
	larvae	9	1.968	0.042
Date	adults	4	1.196	0.312
	larvae	4	0.475	0.754
Adults of <i>O. laevigatus</i> (a)	adults	1	0.954	0.329
	larvae	1	0.721	0.396
Nymphal stages of <i>O. laevigatus</i> (a)	adults	1	5.149	0.024
	larvae	1	0.557	0.456
Date * Juvenile stages of <i>O. laevigatus</i>	adults	4	1.019	0.397
	larvae	4	0.431	0.786
Date * Adults of <i>O. laevigatus</i>	adults	4	0.566	0.687
	larvae	4	1.036	0.388
Biopesticides * date	adults	36	0.803	0.786
	larvae	36	1.224	0.180
Error	adults	431		
	larvae	431		
Total	adults	500		
	larvae	500		

(a) Covariate: density of *O. laevigatus* (nymphs and adults) at the time of flower harvesting
d.f. – degrees of freedom; * – interaction between variables

Table 3. Mean difference (\pm SE) in *F. occidentalis* numbers between the control and different biopesticides (pairwise comparison test)

Adults of WFT				Larvae of WFT			
biopesticides	mean difference	SE	p-value	biopesticides	mean difference	SE	p-value
<i>Artemisia absinthium</i>	-0.020	0.129	1.000	<i>Artemisia absinthium</i>	0.186	0.153	1.000
Pyrethrum	-0.249	0.128	1.000	Pyrethrum	0.108	0.152	1.000
<i>Urtica dioica</i>	-0.168	0.130	1.000	<i>Urtica dioica</i>	-0.174	0.154	1.000
Neem	-0.003	0.132	1.000	Neem	0.320	0.157	1.000
<i>Derris elliptica</i>	0.045	0.130	1.000	<i>Derris elliptica</i>	0.338	0.154	1.000
<i>Beauveria bassiana</i>	-0.318	0.131	0.702	<i>Beauveria bassiana</i>	0.200	0.155	1.000
Rotenone	-0.359	0.130	0.272	Rotenone	0.194	0.154	1.000
<i>Isaria fumosorosea</i>	-0.266	0.130	1.000	<i>Isaria fumosorosea</i>	0.140	0.154	1.000
<i>Steinernema feltiae</i>	-0.045	0.131	1.000	<i>Steinernema feltiae</i>	0.268	0.155	1.000

WFT – Western flower thrips

Table 4. Results of MANOVA (a) evaluating the effect of different biopesticides on *O. laevigatus* adults and nymphs

Source	Dependent variables <i>O. laevigatus</i>	d.f.	F value	p-value
Intercept	adults	1	128.814	<0.001
	nymphs	1	255.010	<0.001
Blocks	adults	9	0.767	0.648
	nymphs	9	1.631	0.104
Biopesticides	adults	9	4.074	<0.001
	nymphs	9	1.230	0.274
Date	adults	4	1.215	0.304
	nymphs	4	2.554	0.038
Larvae of <i>F. occidentalis</i>	adults	1	1.973	0.161
	nymphs	1	0.742	0.389
Adults of <i>F. occidentalis</i>	adults	1	1.197	0.275
	nymphs	1	6.976	0.009
Date * Larvae of <i>F. occidentalis</i>	adults	4	1.324	0.260
	nymphs	4	0.640	0.634
Date * Adults of <i>F. occidentalis</i>	adults	4	0.669	0.614
	nymphs	4	0.859	0.489
Treatments * date	adults	36	0.895	0.646
	nymphs	36	1.277	0.136
Error	adults	431		
	nymphs	431		
Total	adults	500		
	nymphs	500		

(a) Covariate: density of *F. occidentalis* larvae and adults at the time of flower harvesting

* – interaction between variables

Table 5. Mean difference (\pm SE) in *O. laevigatus* numbers between the control and different biopesticides (pairwise comparison test)

Adults of <i>O. laevigatus</i>				Nymphal stages of <i>O. laevigatus</i>			
biopesticides	mean difference	SE	p-value	biopesticides	mean difference	SE	p-value
<i>Artemisia absinthium</i>	0.108	0.076	1.000	<i>Artemisia absinthium</i>	0.035	0.093	1,000
Pyrethrum	0.019	0.076	1.000	Pyrethrum	0.105	0.093	1,000
<i>Urtica dioica</i>	0.031	0.076	1.000	<i>Urtica dioica</i>	0.226	0.093	0.692
Neem	0.271(*)	0.076	0.020	Neem	0.071	0.093	1.000
<i>Derris elliptica</i>	0.068	0.076	1.000	<i>Derris elliptica</i>	0.145	0.093	1.000
<i>Beauveria bassiana</i>	0.234	0.077	0.107	<i>Beauveria bassiana</i>	0.135	0.093	1.000
Rotenone	0.259(*)	0.076	0.035	Rotenone	0.002	0.093	1.000
<i>Isaria fumosorosea</i>	0.160	0.076	1.000	<i>Isaria fumosorosea</i>	0.025	0.093	1.000
<i>Steinernema feltiae</i>	0.259(*)	0.076	0.038	<i>Steinernema feltiae</i>	0.117	0.094	1.000

(*) The mean difference is significant at the 0.05 level

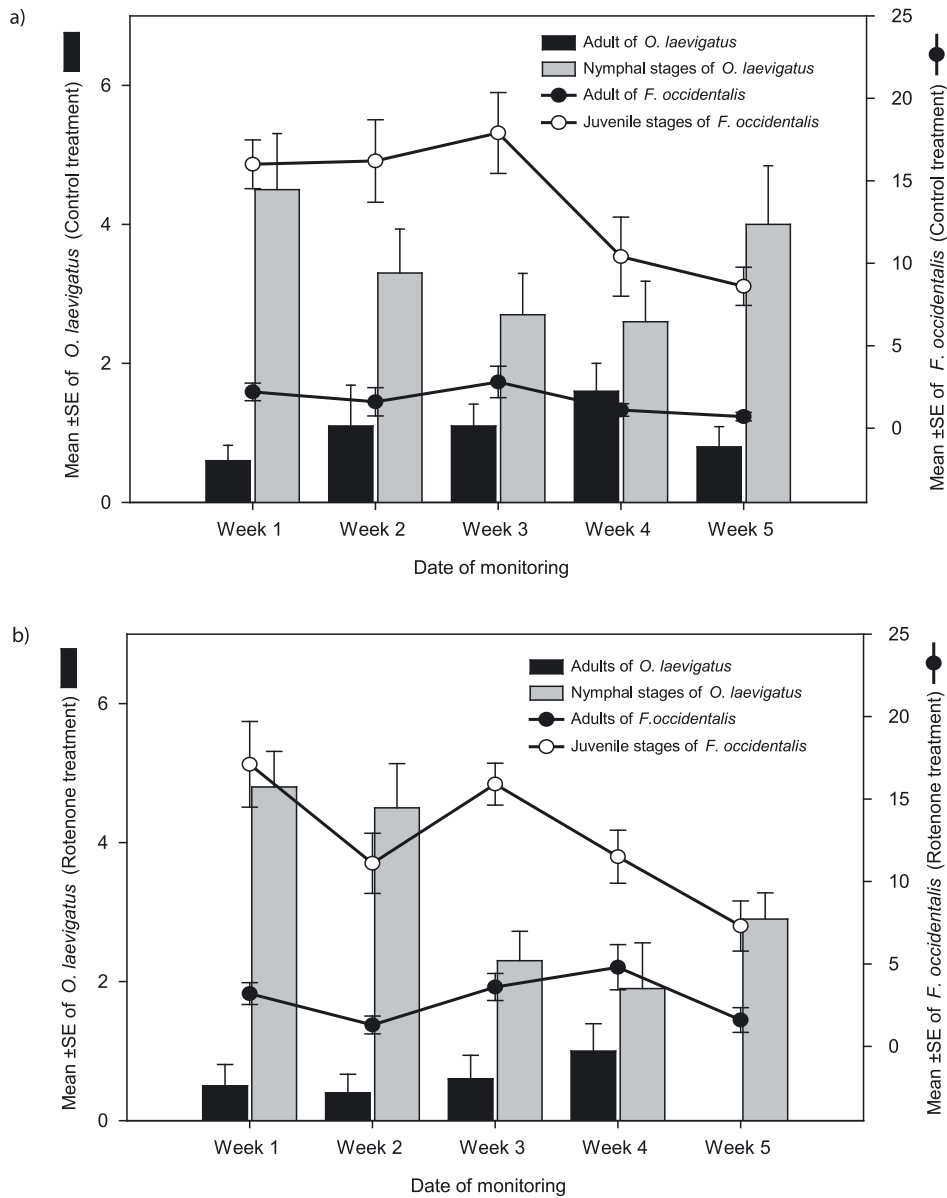


Fig. 1. *O. laevigatus* and *F. occidentalis* dynamics for different significant treatments (mean \pm SE per experimental units n = 5 flowers): control (a); Rotenone (b); Neem (c); *S. feltiae* (d)

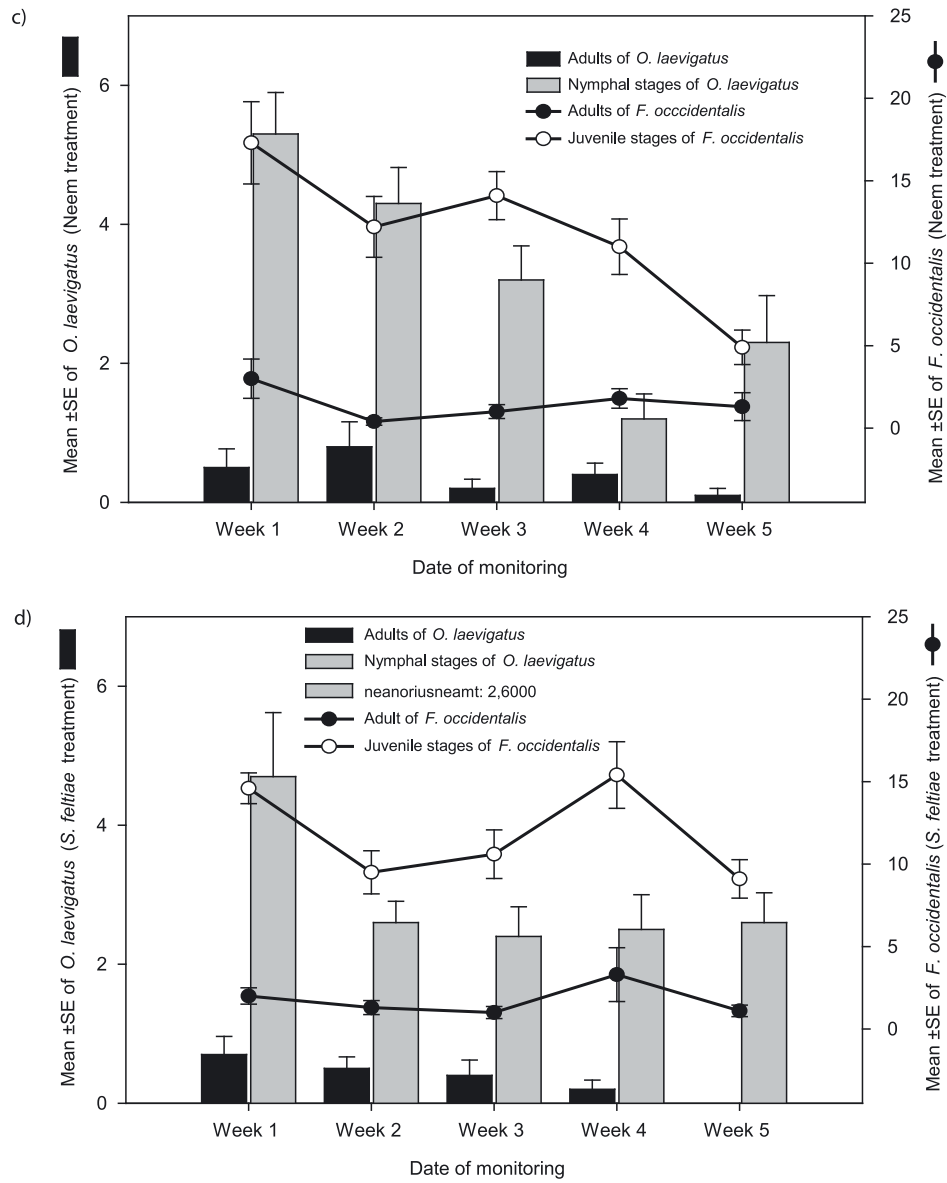


Fig. 1. *O. laevigatus* and *F. occidentalis* dynamics for different significant treatments (mean \pm SE per experimental units n = 5 flowers): control (a); Rotenone (b); Neem (c); *S. felitiae* (d)

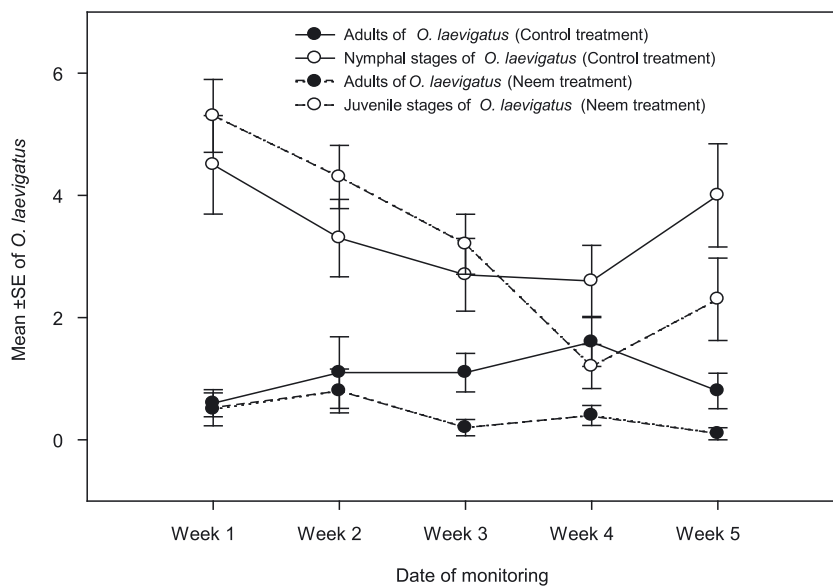


Fig. 2. *O. laevigatus* (adult and nymphal stages) dynamics for Neem vs the control (mean \pm SE per experimental units n = 5 flowers)

cides influenced the density of *O. laevigatus* adults (Table 4). Rotenone, neem and nematodes (*S. feltiae*) reduced adult numbers of *O. laevigatus* in comparison to the control (Table 5). The Wilks' multivariate test yielded a value of 0.890 ($F = 2.342$; $p = 0.01$) indicating that the effect of treatments on the means is not high. Moreover, no significant interactions were detected. The adult of WFT as a covariate, affected the juvenile stages of *O. laevigatus* (Table 4). The numbers of adult *O. laevigatus* in week 4 were between 1.5 and 8 times lower than those of the control (Figs. 1a, b, c, d). For neem this dynamic is evident vs the control after the third monitoring (Fig. 2).

DISCUSSION

Information about the side effects of pesticides on beneficials is used to appraise the possibility of their use in IPM programs (Way and van Emden 2000; Tedeschi *et al.* 2001; Cameron *et al.* 2009).

The general negative effects of botanical pesticides and biological agents on predators have been evaluated for different species (Schmutterer 1997; Hilbeck *et al.* 1999; Peveling and Ould El 2006; Thungrabeab and Tongma 2007; Nadimi *et al.* 2008; Nawrocks 2008), and their effect on biological diversity and epigeic fauna by Pekàr (1999). The present study demonstrates that the biopesticides rotenone and neem, and the nematode *S. feltiae* reduced *O. laevigatus* adult numbers. Thus, the use of these biopesticides affected the *O. laevigatus* – WFT dynamics in strawberry, although their effect does not interrupt their relationship. The nematode *S. feltiae* is used to control a range of pests including whitefly *Bemisia tabaci* (Gennadius) (Cuthbertson *et al.* 2007), and is an alternative biological agent for WFT.

S. feltiae generally induces mortality in the first larval stage and prepupae of WFT (Buitenhuis and Shipp 2005). Arthurs and Heinz (2006) reported that nematodes did not provide adequate control of thrips in flowering plants of chrysanthemum. An antagonistic interaction between entomogenous nematodes and *B. bassiana* is reported by Barberchek and Kaya 1990. The application of *S. feltiae* had a significant effect on *O. laevigatus* in our trial. We found complete absence of adult predators where *S. feltiae* had been applied.

Neem showed high direct toxicity and was harmful to the mirid *Macrolophus caliginosus* Wagner, when nymphal stages were exposed to fresh dry residues (Tedeschi *et al.* 2001). However, 5 days after treatment, neem had no significant effect on mortality or fecundity of surviving females. Similarly, neem had no effect on survival and fecundity of *O. laevigatus* exposed via direct contact or by ingestion of infected eggs of *Ephesia kuehniella* Zeller (Van de Veire *et al.* 1996; Angeli *et al.* 2005). Qi *et al.* (2001) found that neem treatments were not toxic to adult ladybird [*Harmonia conformis* (Boisduval) (Coleoptera: Coccinellidae)] and *Mallada signatus* (Schneider) (Neuroptera: Chrysopidae) larvae exposed via feeding. However, the metamorphosis and pupal survival of *M. signatus* were negatively affected. These studies confirm that neem compounds act in different ways on insects, disrupting the developmental processes, inducing adult sterility, dis-

turbing adult behavior, or by repelling adults and larvae (Schmutterer 1990; Mordue and Blackwell 1993).

However, few studies have examined the effect of biopesticides on the predator-prey system. The present study suggests that *O. laevigatus* is effective in suppressing *F. occidentalis* populations in strawberry. The number of thrips were kept low for the whole trial period under the economic thresholds of WFT/flower, as esteemed by Coll *et al.* (2006). They found an economic threshold of 24 WFT/flowers in the spring period.

In greenhouses in the Mediterranean area, the use or the natural control of *O. laevigatus* in spring and summer should suppress WFT populations. However, throughout the winter, temperatures of around 10°C induce a winter diapause in *O. laevigatus* (Alauzet *et al.* 1994). WFT continues to reproduce through winter (Coll *et al.* 2006) and at the beginning of spring, and chemical control measures are sometimes required. In this situation, biopesticides may be useful. Biopesticides may also be useful when applied in the first phases of thrips colonization or if no beneficials are present. However, the efficacy of these biopesticides against WFT needs to be re-evaluated. Further studies should focus on their efficacy against WFT for use in other greenhouse crops.

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