

# Single and combined releases of biological control agents against canopy- and soil-dwelling stages of *Frankliniella occidentalis* in cyclamen

Alberto Pozzebon · Andrea Boaria · Carlo Duso

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**Abstract** *Frankliniella occidentalis* is a major pest in agriculture. Problems with its control are mainly due to pupation in the soil and resistance to insecticides. Biological control agents (BCA) can be used against thrips pests as an alternative to conventional insecticides. We evaluated the effectiveness of a number of BCA for control of *F. occidentalis* in cyclamen under greenhouse conditions. Three BCA (*Amblyseius swirskii*, *Neoseiulus californicus* and *Orius laevigatus*) were applied to the canopy and two (*Macrocheles robustulus* and *Steinernema feltiae*) to the soil in single and combined releases. Results showed that applications of predatory mites and nematodes achieved good control of *F. occidentalis*. Unsatisfactory control was obtained using *O. laevigatus*. The use of nematodes determined the extinction of thrips, but with a delay. The combination of nematodes in the soil and predatory mites in the canopy caused a more prompt reduction of thrips in flowers.

**Keywords** *Frankliniella occidentalis* · Biological control · *Amblyseius swirskii* · *Neoseiulus californicus* · *Macrocheles robustulus* · *Steinernema feltiae*

## Introduction

*Frankliniella occidentalis* Pergande is one of the most economically important pests of greenhouse ornamentals worldwide (Tommasini and Maini 1995). It causes serious direct damage by feeding on flowers and leaves, leading to a reduction in economic value of various ornamental crops (Cloyd 2009). It is also of importance as a vector of plant viruses: Tomato Spotted Wild Virus, Tobacco Streak Virus and Impatiens Necrotic Spot Virus can be transmitted to susceptible crops (e.g., Riley et al. 2011). *Frankliniella occidentalis* larvae acquire viral particles from infected plants and adults transmit them (Whitfield et al. 2005).

The life cycle of *F. occidentalis* is characterized by a cryptic phase: first and second instars and adults are canopy-inhabiting stages while pupation occurs in the soil or hidden within flowers (Tommasini and Maini 1995; Broadbent et al. 2003; Berndt et al. 2004). The choice between soil or flowers for pupation depends on relative humidity and the availability of sites to hide in (Buitenhuis and Shipp 2008; Steiner et al. 2011; Holmes et al. 2012). Together with insecticide resistance, this behaviour makes the insect difficult to

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A. Pozzebon (✉) · A. Boaria · C. Duso  
Department of Agronomy, Food, Natural Resources,  
Animals and Environment (DAFNAE), University of  
Padua, Viale dell'Università 16, Legnaro, 35020 Padua,  
Italy  
e-mail: alberto.pozzebon@unipd.it

A. Boaria  
e-mail: andrea.boaria@studenti.unipd.it

C. Duso  
e-mail: carlo.duso@unipd.it

control (e.g., Brødsgaard 1994; Jacobson et al. 2001). Pesticide resistance has promoted a strong interest in biological control agents (BCA) also for ornamental crops (van Lenteren 2000; Cloyd 2009). Most of the proposed biocontrol strategies in ornamentals use the BCA against canopy-dwelling stages (e.g., van de Veire and Degheele 1992; Riudavets 1995; Vänninen and Linnamäki 2002; van Driesche et al. 2006). More recently, attention has been paid to the control of soil-dwelling stages (e.g., Buitenhuis and Shipp 2005; Arthurs and Heinz 2006; Ansari et al. 2008; Brownbridge et al. 2014).

Pest damage in ornamental crops is often related to their aesthetic value (Parrella and Jones 1987; Cloyd 2009). Some studies on the biological control of *F. occidentalis* on ornamentals were performed by combining the predatory mites *Neoseiulus cucumeris* (Oudemans) in the canopy and *Hypoaspis aculeifer* (Canestrini) in the soil or by integrating above- and below-ground microbial BCA applications (e.g., Linnamäki et al. 1998; Wiethoff et al. 2004; Brownbridge et al. 2011). More recently, the combined use of a wide range of BCA in the canopy and soil has been proposed, in particular in Australasia (e.g., Manners et al. 2013).

In this paper, we focus on the use of BCA against canopy- and soil-dwelling life stages of *F. occidentalis*. We selected commercially available BCA species of importance in Europe. More specifically, we used the two predatory mites, *Amblyseius swirskii* Athias-Henriot and *N. cucumeris* (Acari: Phytoseiidae) and the predatory bug *Orius laevigatus* (Fieber) (Heteroptera: Anthracoridae) in the canopy, and the predatory mite *Macrocheles robustulus* (Berlese) (Acari: Macrochelidae) and the entomoparasitic nematode *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) in the soil. The predators used in the canopy are well-known *F. occidentalis* antagonists for augmentative biocontrol strategies in various crops, including ornamentals (e.g., Tavella et al. 1991; Jacobson 1997; Skirvin et al. 2006; Messelink et al. 2008; Buitenhuis et al. 2014). The entomoparasitic nematode *S. feltiae* can be used against *F. occidentalis* in ornamental crops through foliar or soil applications, but soil applications are considered most cost-effective (e.g., Ebssa et al. 2001; Buitenhuis and Shipp 2005; Arthurs and Heiz 2006). Fewer studies are available on *M. robustulus*, a soil inhabiting mite, used against *F. occidentalis* on chrysanthemum (Messelink and van Holstein-Saj 2008).

This study was performed on potted cyclamen, which is considered a suitable crop for the application of biological control due to its long production cycle (de Courcy Williams 2001). *Frankliniella occidentalis* can cause feeding damage and vector viruses in cyclamen (Allen and Matteoni 1988). We compared thrips densities and thus their control obtained by single and combined releases of BCA in the canopy and the soil in an experiment under greenhouse conditions.

## Materials and methods

### Experimental design

The experiment was conducted in a greenhouse during 2012 using potted cyclamen plants (cv. Halios). Four potted plants with well-developed flowers (pot diameter 20 cm) were placed in an insect-proof cage (1 × 1 × 1 m, 105 µm mesh). Within cages, plants were spaced to avoid contact of the canopies. Each plant was infested with about ten adult and 50 juvenile thrips two weeks before BCA application. Plants were placed on the cage floor and regularly watered and fertilized using drip irrigation. Climatic conditions in the greenhouse were kept at 18 ± 8 °C and 63 ± 15 % RH, a regime similar to commercial greenhouse conditions.

We compared 12 treatments characterized by different combinations of soil-dwelling and canopy-dwelling BCA (Table 1). Each treatment comprised four replicates with four plants per replicate. Before BCA release, the population of *F. occidentalis* was estimated by shaking the plant canopy onto a sheet of white paper and counting fallen individuals. A sample of commercial product from each packaging unit was analysed under a dissecting microscope to assess predator density and mobility prior to the release. For nematodes, a sample of the commercial product was mounted on a slide (in aqueous medium) to assess their density and mobility. All BCA were released once at the beginning of the experiment. Predatory mites were released by dispersing the material in the canopy or on the soil. The number of *O. laevigatus* insects was assessed under a dissecting microscope before release. Nematode release was performed by a drench application of 2 l m<sup>-2</sup> of nematode suspension.

Cages were sampled before BCA release and weekly until 35 days thereafter. Plants and cages were

**Table 1** Experimental design with treatments identified by the combinations of biological control agents (BCA) released in the canopy and the soil

Treatments	Release in the canopy <sup>a</sup>	Release density (n m <sup>-2</sup> )	Release on the soil <sup>b</sup>	Release density (n m <sup>-2</sup> )
1	<i>A. swirskii</i>	100	No soil-dwelling BCA	–
2	<i>N. cucumeris</i>	100	No soil-dwelling BCA	–
3	<i>O. laevigatus</i>	10	No soil-dwelling BCA	–
4	No canopy-dwelling BCA	–	No soil-dwelling BCA	–
5	<i>A. swirskii</i>	100	<i>S. feltiae</i>	500,000
6	<i>N. cucumeris</i>	100	<i>S. feltiae</i>	500,000
7	<i>O. laevigatus</i>	10	<i>S. feltiae</i>	500,000
8	No canopy-dwelling BCA	–	<i>S. feltiae</i>	500,000
9	<i>A. swirskii</i>	100	<i>M. robustulus</i>	250
10	<i>N. cucumeris</i>	100	<i>M. robustulus</i>	250
11	<i>O. laevigatus</i>	10	<i>M. robustulus</i>	250
12	No canopy-dwelling BCA	–	<i>M. robustulus</i>	250

<sup>a</sup> All BCA released in the canopy were supplied by Koppert B.V., The Netherlands

<sup>b</sup> *Macrocheles robustulus* was supplied by Koppert B.V., The Netherlands. *S. feltiae* was supplied by Becker Underwood Inc., UK

inspected visually for 1 min to evaluate the abundance of predatory bugs. The abundance of *F. occidentalis* and predatory mites was assessed by weekly samples of eight leaves and four flowers per plant. Samples were observed under a dissecting microscope to count the thrips and identify life stages. To determine the presence and persistence of *S. feltiae* and *M. robustulus*, c. 15 ml of soil was collected weekly from each pot, for a total of 60 ml of soil per cage. The collected soil was divided into two sterilized vials to obtain samples with 30 ml of soil per vial. One sample from each replicate was used to evaluate the presence of entomoparasitic nematodes using the “*Galleria* bait method” (Zimmerman 1986), i.e., three–four *Galleria mellonella* larvae were introduced in each soil sample and their mortality was checked after two weeks. Dead larvae were surface-sterilised in 1.0 % sodium hypochlorite for 3 min, then washed in sterile distilled water and placed individually on a wet filter paper in a sealed Petri dish and incubated for 15 days, then the symptoms of nematodes infection were assessed. To determine the persistence of *S. feltiae* in the soil, we evaluated the infection rate as the proportion of *G. mellonella* larvae showing symptoms of *S. feltiae* infection. The other half of each sample was observed under a dissecting microscope for the presence of *M. robustulus*. Mites were mounted on slides using Hoyer’s medium and their identity was confirmed using Karg (1993) and Tixier et al. (2013).

### Statistical analysis

Data on *F. occidentalis* densities on the plants before BCA releases were analysed using analysis of variance (ANOVA) with the MIXED procedure of SAS (v 9.2, SAS Institute, Cary, NC, USA) and differences among treatments were evaluated with an *F* test ( $\alpha = 0.05$ ). Numbers of *F. occidentalis* and predators observed in flowers and on leaves after BCA releases were analysed with a repeated measures ANOVA model with the MIXED procedure of SAS (v 9.2, SAS Institute, Cary, NC, USA). Densities of *F. occidentalis* and predators observed in flowers and on leaves were analysed separately and considered as response variables with repeated measures, i.e., sampling dates. Using an *F* test ( $\alpha = 0.05$ ), we evaluated the effect of treatments, time and their interactions. Differences among treatments were evaluated using a *t*-test to the least-square means with Tukey adjustment of the *p* values ( $\alpha = 0.05$ ). The slice option was used to perform a partitioned analysis of the least-square means for a significant interaction. With the slice option, an *F* test was applied to evaluate the effect of one factor for a given level of the other factors (Littell et al. 2006). The Kenward-Roger method was used for degrees of freedom estimation (Littell et al. 2006). Data were checked for analysis assumptions. Numbers of thrips in flowers were not transformed whereas numbers of thrips found on leaves and predators in flowers and on leaves were  $\log(x + 1)$  transformed.

## Results

### *Frankliniella occidentalis*

Before BCA releases, an average density of  $3.75 \pm 0.75$  *F. occidentalis* per plant was found with no differences among treatments ( $F_{11, 36} = 0.32$ ,  $p = 0.975$ ). The density of *F. occidentalis* was influenced by BCA applications in the canopy and in the soil (Table 2; Fig. 1). A significant effect of time and an interaction between treatment and time was also observed (Table 2; Fig. 1). In all treatments with BCA release, except where *O. laevigatus* was released alone, the number of thrips in flowers was lower than that in the treatment not receiving BCA releases (Table 3; Fig. 1). The combined use of *A. swirskii* with both BCA released in the soil reduced thrips densities in flowers compared to *O. laevigatus* treatment (Fig. 1; Table 3). The variation in time was significant where *S. feltiae* was used alone or in combination with *O. laevigatus* since a dramatic thrips decline was observed from 28 days after release onwards (Fig. 1). Fewer *F. occidentalis* were found on leaves than in flowers (Fig. 2). A significant effect of treatment was observed,

with fewer thrips on leaves where BCA were released than in the treatment with no BCA releases (Tables 2, 3; Fig. 2). A significant interaction between treatment and time was also found because of the increase in *F. occidentalis* numbers on leaves in the treatment with no BCA releases (Table 2; Fig. 2).

### Biological control agents

No predators were observed prior to BCA release. The presence of *N. cucumeris* and *A. swirskii* was continuously observed in flowers and on leaves (Fig. 3). A single *O. laevigatus* nymph was found seven days after the experiment began. The presence of predatory mites in flowers was observed only in the respective release treatments and *A. swirskii* reached higher population levels than *N. cucumeris* (Tables 2, 3; Fig. 3a, c). No predatory mites were observed in the no canopy-dwelling BCA and *O. laevigatus* treatments. No effect of BCA released on the soil was observed on predatory mites abundance (Table 3; Fig. 3a, c).

Among predators, only *N. cucumeris* and *A. swirskii* were observed on leaves, but at lower levels than in flowers (Fig. 3). Their presence was higher in the respective treatments compared to other treatments (Tables 2, 3). No differences were observed between the density of the two predatory mites (Table 3). Their abundance was not influenced by the combination with BCA released in the soil (Table 3; Fig. 3b, d). No predatory mites were observed in the no canopy-dwelling BCA and *O. laevigatus* treatments.

*Macrocheles robustulus* was observed in soil samples until the end of the experiment in the respective release treatment with an average density of three mites per 15 ml of soil. Its abundance was not influenced by BCA released in the canopy. *Steinernema feltiae* was detected in soil during the entire experiment using the “*Galleria* bait method” with an average infection rate of 85–100 % of *G. mellonella* larvae.

## Discussion

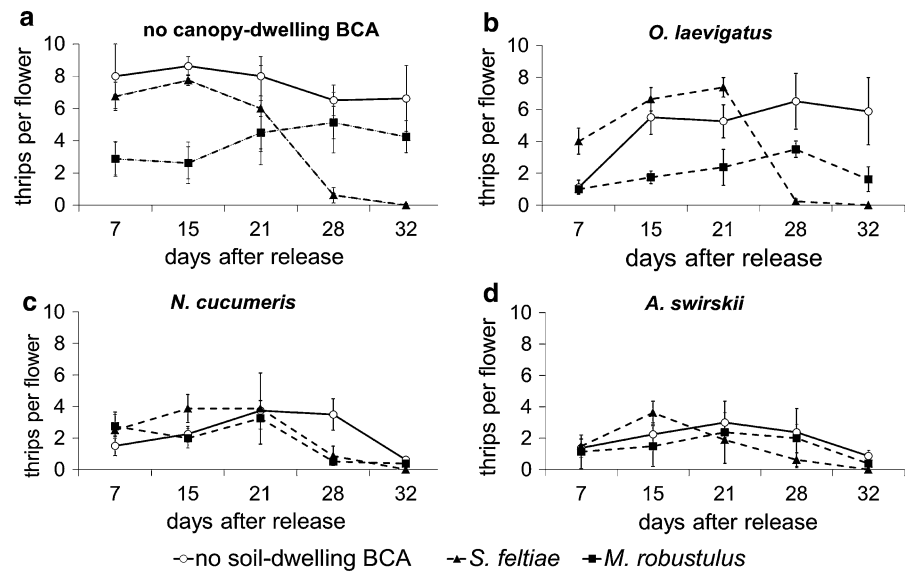
The presence of *F. occidentalis* was higher in flowers than on leaves, probably because the thrips found optimal food resources (i.e., pollen) and oviposition sites in flowers (Cloyd 2009). Flowers are of primary importance for marketing cyclamen. Feeding of *F.*

**Table 2** Results of repeated measures ANOVA performed on *F. occidentalis* and predatory mite abundance observed in flowers and on leaves after biological control agents release

Effects	F	df	p
<i>Frankliniella occidentalis</i> in flowers			
Treatment	7.54	1, 152.2	<0.001
Time	8.01	4, 137.0	<0.001
Treatment × Time	1.67	44, 138.0	0.013
<i>Frankliniella occidentalis</i> on leaves			
Treatment	5.22	1, 171.1	<0.001
Time	1.52	4, 133.0	0.199
Treatment × Time	1.65	44, 139.0	0.015
Predatory mites in flowers			
Treatment	11.96	1, 171.0	<0.001
Time	0.79	4, 140.0	0.537
Treatment × Time	1.01	44, 142.0	0.471
Predatory mites on leaves			
Treatment	1.95	1, 162.1	0.048
Time	1.24	4, 136.0	0.295
Treatment × Time	1.35	44, 139.0	0.096

Degrees of freedom in all models were calculated using the Kenward-Roger method

**Fig. 1** *Frankliniella occidentalis* population (mean  $\pm$  SE) observed in cyclamen flowers after biological control agents (BCA) release. The experimental design consisted of combinations of releases of natural enemies on the soil (no soil-dwelling BCA, the release of *S. feltiae* or of *M. robustulus*) and in the canopy [no canopy-dwelling BCA (a), release of *O. laevigatus* (b), release of *N. cucumeris* (c), and release of *A. swirskii* (d)]



*occidentalis* can cause flower discoloration and deformation, with a large drop in value of the crop. The use of predatory mites reduced *F. occidentalis* numbers in flowers. Among BCA released in the canopy, *A. swirskii* gave better control than *O. laevigatus* when combined with soil-dwelling BCA. Considering BCA released in the soil, both natural enemies provided equal control of *F. occidentalis* in flowers. No differences in the control of thrips on leaves were found among BCA released in the canopy or in the soil. At the end of the experiment all flowers were damaged by *F. occidentalis* in treatments without BCA release, 30 % of the flowers were damaged in the treatment with *O. laevigatus* alone, while less than 10 % of the flowers showed symptoms of infestation where other BCA were released. No damage was observed on leaves. *Amblyseius swirskii* was more abundant in flowers than *N. cucumeris*, but no differences in thrips control were found for the two predatory mites. It should be stressed that both these mites feed primarily on first instar larvae (Bakker and Sabelis 1989; Wimmer et al. 2008). Contrasting results have been found in other studies where the two predatory mites were compared against various thrips pests (van Houten et al. 2005; Messelink et al. 2006; Arthurs et al. 2009; Buitenhuis et al. 2010; Cuthbertson et al. 2012). Wimmer et al. (2008) categorized the thrips *F. occidentalis* as a suboptimal (e.g., compared to pollen and whiteflies) food source for *A. swirskii* suggesting that on this prey, the

population growth capacity is not higher than that of *N. cucumeris*. It should be noted that the authors came to this conclusion not by a direct experimental comparison but through a comparison with previously published data. These two predatory mites can feed on various food sources, including pollen (McMurtry et al. 2013). Possible explanations for differences in the control efficacy of the two mites may be related to availability of pollen or other foods (e.g., Wimmer et al. 2008; Goleva and Zebitz 2013; Ranabhat et al. 2014).

We observed relatively low control of thrips in flowers when *O. laevigatus* was released compared with some BCA combinations. In contrast, *F. occidentalis* infestation on leaves was similar in *O. laevigatus* and other BCA treatments. We suggest that predatory bugs were active in the first week of the experiment and then became extinct. The lack of differences in leaf damage between the treatment with *O. laevigatus* and predatory mites could have been caused by the relatively low thrips densities. In contrast to the present study, *O. laevigatus* controls thrips infestation of sweet pepper flowers better than *A. swirskii* (Skirvin et al. 2006; Weintraub et al. 2011). The differences between these and the current study could be due to the architecture of cyclamen flowers providing a refuge for *F. occidentalis* from *O. laevigatus*. Indeed, host plant features are known to influence predation by anthocorids by providing refuge for prey (e.g., Coll and Ridgway 1995; Norton

**Table 3** Estimated least-square means of *F. occidentalis* and predatory mite abundance observed in different treatments in flowers and on leaves of cyclamen after biological controlagents (BCA) release. Values within a column bearing different letters were significantly different at according to a Tukey test ( $\alpha = 0.05$ )

Treatments	BCA released in the canopy	BCA released on the soil	<i>F. occidentalis</i> in flowers <sup>a</sup>	<i>F. occidentalis</i> on leaves <sup>*,a</sup>	Predatory mites in flowers <sup>*,a</sup>	Predatory mites on leaves <sup>*,a</sup>
1	No canopy-dwelling BCA	No soil-dwelling BCA	7.55a	0.41a	0.00b	0.00b
2	<i>A. swirskii</i>	No soil-dwelling BCA	1.97bc	0.12b	0.55ab	0.05a
3	<i>N. cucumeris</i>	No soil-dwelling BCA	2.32bc	0.07b	0.17b	0.06a
4	<i>O. laevigatus</i>	No soil-dwelling BCA	4.85ab	0.06b	0.00b	0.00b
5	No canopy-dwelling BCA	<i>S. feltiae</i>	4.22bc	0.08b	0.00b	0.00b
6	<i>A. swirskii</i>	<i>S. feltiae</i>	1.35c	0.01b	1.00a	0.05a
7	<i>N. cucumeris</i>	<i>S. feltiae</i>	1.77bc	0.10b	0.35b	0.06a
8	<i>O. laevigatus</i>	<i>S. feltiae</i>	3.65bc	0.03b	0.00b	0.00b
9	No canopy-dwelling BCA	<i>M. robustulus</i>	3.87bc	0.05b	0.00b	0.00b
10	<i>A. swirskii</i>	<i>M. robustulus</i>	1.48c	0.01b	1.05a	0.03a
11	<i>N. cucumeris</i>	<i>M. robustulus</i>	1.78bc	0.02b	0.01b	0.50a
12	<i>O. laevigatus</i>	<i>M. robustulus</i>	2.57bc	0.08b	0.00b	0.00b

\* Numbers in the table were calculated by back-transformation of least-square means estimated by the model

<sup>a</sup> SE of least-square means are 0.70 for *F. occidentalis* in flowers, 0.05 for *F. occidentalis* on leaves, 0.12 for predatory mites in flowers and 0.01 for predatory mites on leaves

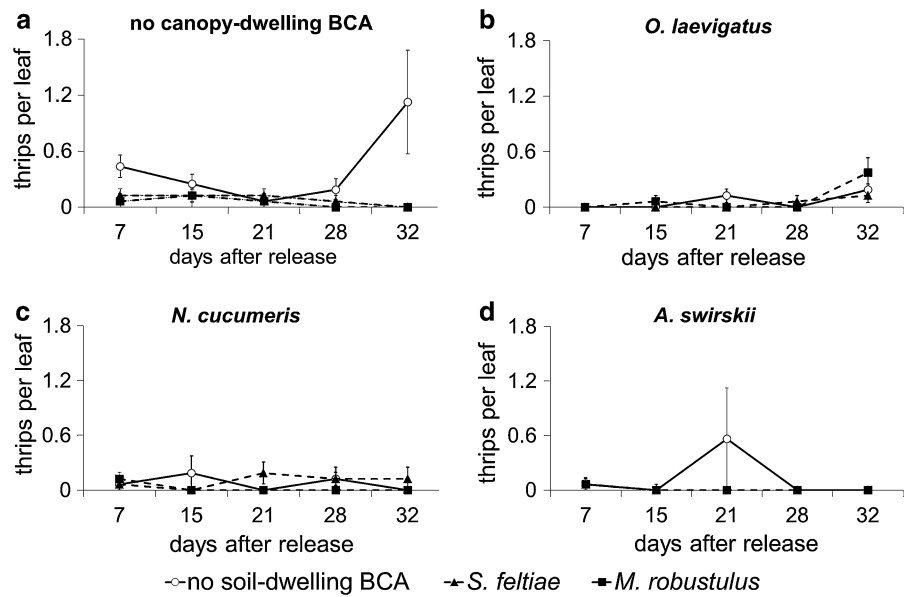
et al. 2001). Previous studies found that predation on *F. occidentalis* by anthocorids can be related to the structural complexity of flowers, resulting high in relatively simple flowers (e.g., rose, Chow et al. 2008, 2010) and low in relatively complex flowers (e.g., chrysanthemum, Brødsgaard and Enkegaard 2005). These studies and the results obtained here suggest that structural complexity of flowers is a host plant feature that can influence the success of biological control strategies based on the release of anthocorids in ornamental crops. Moreover, host plant features are probably involved in the lack of establishment of *O. laevigatus*. Anthocorids lay their eggs on plants (e.g., Cocuzza et al. 1997; Lundgren 2011) preferring plants with the thinnest external tissues (Lundgren et al. 2008). Cyclamen leaves and petiole epidermis are relatively coriaceous and probably unsuitable as oviposition sites for predatory bugs.

Both BCA released on the soil provided satisfactory control of *F. occidentalis*. This indicates a consistent pupation of *F. occidentalis* in the soil where soil-dwelling antagonists were active. A single soil application of the entomoparasitic nematode led to the complete annihilation of *F. occidentalis* by the end of the experiment. The efficacy of *S. feltiae* against soil-dwelling stages of *F. occidentalis* has previously been

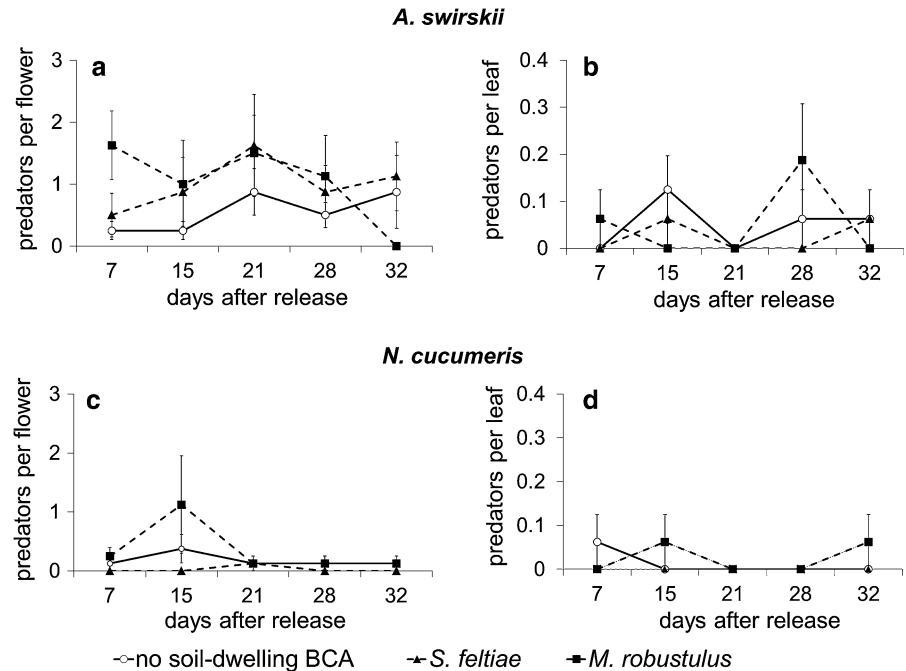
shown under laboratory conditions (Ebssa et al. 2001; Premachandra et al. 2003). In these studies, a higher effect of nematode application was obtained as compared to *S. feltiae* applied against above-ground stages (Buitenhuis and Shipp 2005). On chrysanthemum under greenhouse conditions, repeated foliar applications of *S. feltiae* alone, or in combination with an entomopathogenic fungus, are deemed necessary to improve thrips control (Beerling 2008; Brownbridge et al. 2011), but thrips pupation can occur in chrysanthemum flowers (Buitenhuis and Shipp 2008). In our study, *M. robustulus* maintained thrips numbers at low levels throughout the experiment, and no differences emerged in overall infestation levels as compared to the nematode. Only one study is available on the efficacy of *M. robustulus* against *F. occidentalis* (Messelink and van Holstein-Saj 2008). They found that *M. robustulus* was more effective than other soil-dwelling predatory mites in the control of thrips on chrysanthemum. The results obtained here confirm that the release of *M. robustulus* is a valid option for the improvement of biocontrol strategies on ornamental plants.

We have demonstrated, in a simulated commercial cultivation scenario, that applications of predatory mites and nematodes can achieve good control of *F.*

**Fig. 2** *Frankliniella occidentalis* population (mean ± SE) observed on cyclamen leaves after biological control agents (BCA) release. See legend to Fig. 1 for further explanation



**Fig. 3** Predatory mites abundance (mean ± SE) observed in flowers (a, c) and on leaves (b, d) after biological control agents (BCA) release. Shown are data obtained by combining predatory mites release in the canopy with the different soil treatments (no soil-dwelling BCA, release of *S. feltiae* or release of *M. robustulus*). See legend to Fig. 1 for further explanation



*occidentalis* on cyclamen. The release of *O. laevigatus* did not provide satisfactory control of *F. occidentalis* on cyclamen. The use of nematodes caused the extinction of thrips, but their effect was delayed. A prompt reduction in thrips infestation was obtained by

the combined release of nematodes on the soil and predatory mites in the canopy. Such combined releases should be considered for the development of biological control strategies in ornamental crops, where thrips damage should be minimized.

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**Alberto Pozzebon** is a post-doc at the Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE)—University of Padua, Italy. Research interests include integrated pest management in protected ornamentals.

**Andrea Boaria** was a Ph.D. student at DAFNAE—University of Padua, Italy, dealing with integrated pest management of phytophagous thrips.

**Carlo Duso** is a full professor in agricultural entomology at DAFNAE—University of Padua, Italy. Research topics include biological and integrated control of phytophagous insects and mites in vineyards, fruit orchards and protected crops; biology and ecology of predatory mites; effects of pesticides on plant mites. He is convenor of IOBC working group “Integrated protection in viticulture; sub-group integrated pest management”.