

## Towards the development of a push-pull strategy for improving biological control of western flower thrips on chrysanthemum

J. Bennison<sup>1</sup>, K. Maulden<sup>1</sup>, S. Dewhurst<sup>2</sup>, E. Pow<sup>2</sup>, P. Slatter<sup>2</sup> and L. Wadhams<sup>2</sup>

<sup>1</sup>ADAS, Boxworth, Cambridge CB3 8NN, UK; <sup>2</sup>Rothamsted Research, Harpenden, Herts AL5 2JQ, UK.

E-mail: [Jude.Bennison@adas.co.uk](mailto:Jude.Bennison@adas.co.uk)

**Abstract:** A push-pull strategy is being developed to improve biological control of western flower thrips (WFT) on chrysanthemums. Using pot chrysanthemums as a 'model' crop, the strategy aims to repel (push) the WFT from the crop plants and attract (pull) them to a 'lure' or 'trap' chrysanthemum cultivar where thrips predators can be released or attracted for improved, cost-effective control of all WFT life stages. 'Swingtime' opening buds and flowers were highly attractive to WFT and this cultivar was selected as the 'lure' plant in the strategy. The plant volatile (*E*)- $\beta$ -farnesene (EBF) is attractive to WFT and EBF lures enhanced thrips attraction to 'Swingtime' plants. *Orius laevigatus* predatory bugs were attracted to WFT-damaged 'Swingtime' buds and to EBF, and both adults and third instar nymphs were effective predators of both WFT adults and larvae. Rosemary leaves and volatiles were repellent to WFT but were also repellent to *O. laevigatus*, so further research is planned to find a suitable 'push' component for the strategy. On 'Swingtime' plants, 97% of WFT second instar larvae pupated in the compost or substrate and were thus available for predation by the predatory mites *Stratiolaelaps (Hypoaspis) miles* and *Gaeolaelaps (Hypoaspis) aculeifer*, both of which showed equal potential against ground-dwelling WFT stages. In the first of a series of small-scale glasshouse experiments planned to test the principles of the push-pull strategy under high WFT pressure, 'Swingtime' with EBF lures were effective in attracting WFT away from a crop of cv. 'Charm' plants until the crop came into flower.

### Introduction

An increasing number of UK growers of protected ornamental crops are using biological control strategies for the control of western flower thrips (WFT), *Frankliniella occidentalis*, within Integrated Pest Management (IPM) programmes. However, there is currently very little use of IPM on chrysanthemums, as biological control of WFT is unreliable. The predatory mite *Amblyseius cucumeris* is the main biological control agent used against thrips, but this predated only first instar thrips larvae. Thus control is not always maintained during flowering, when WFT adults are attracted to the plants and the thrips population increases rapidly. For more robust biological control of WFT on chrysanthemums, all thrips life stages need to be targeted. Potential biological control agents to use in the strategy are the predatory bugs *Orius laevigatus* for control of WFT adults and larvae, and the predatory mites *Stratiolaelaps (Hypoaspis) miles* and *Gaeolaelaps (Hypoaspis) aculeifer* against WFT pupae. *O. laevigatus* are used commercially against WFT on sweet peppers where their establishment is

facilitated by pollen as a supplementary food source, but they are seldom used on ornamentals as little information is available on their behaviour and efficacy on the wide range of host plants grown. *S. miles* and *G. aculeifer* are commonly used within IPM on ornamentals against sciarid flies, and there is increasing interest in their potential against WFT pupae. However, there is little data on the comparative efficacy of the two species against WFT on different crops and their potential on chrysanthemums has not been previously determined.

Combinations of biological control agents could be made more cost-effective if used selectively on 'lure' plants attractive to WFT. Verbena flowers and their volatiles have been shown to attract WFT adults (Pow *et al.*, 1998b) and to be potential 'lure' plants in both flowering bedding plants and in pot chrysanthemum crops not yet in flower (Bennison *et al.*, 1998). Chrysanthemum cultivars are known to vary in their attractiveness or susceptibility to WFT and contributory factors have been shown to include flower colour (Broadbent *et al.*, 1990),

plant development stage (van Dijken *et al.*, 1993) and plant/flower morphology (de Jager *et al.*, 1995). The olfactory responses of WFT to chrysanthemum volatiles have been studied (Pow *et al.*, 1998a) and further knowledge on the chemical ecology of chrysanthemum cultivars and that of WFT and their natural enemies could be used to manipulate thrips behaviour and to improve biological control.

This paper presents initial work towards the development of a push-pull strategy for WFT using pot chrysanthemums as a 'model' crop. The strategy aims to repel (push) WFT from the crop plants and attract (pull) them to a chrysanthemum cultivar 'lure' that is used as a release and establishment site for thrips predators for improved, cost-effective biological control. A reliable and sustainable strategy for biological control of WFT on chrysanthemums would lead to further uptake of IPM and reduced reliance on pesticides.

## Materials and Methods

### *WFT culture*

A laboratory stock culture of WFT was maintained on flowering pot chrysanthemum plants (cv. 'Swingtime' and cv. 'Charm') in rearing cages kept at 21°C with a 16-hr photoperiod. For consistency, WFT females were used in the laboratory behavioural bioassays and were released in the glasshouse experiments.

### *Biological control agents*

*Orius laevigatus*, *Stratiolaelaps (Hypoaspis) miles* and *Gaeolaelaps (Hypoaspis) aculeifer* were obtained from commercial suppliers.

### *Experimental plants*

Pot chrysanthemum plants for the WFT culture and laboratory bioassays were either raised from cuttings in research glasshouses free from both WFT and pesticides, or were obtained from commercial growers. In the latter case, plants were free from residual pesticides that might affect the survival or behaviour of either WFT or the biological control agents used in the bioassays. Plants for the glasshouse experiments were raised from cuttings in research glasshouses free from both WFT and pesticides.

### *Identification of 'pull' components*

A pot chrysanthemum cultivar and a plant volatile attractive to both WFT and *Orius laevigatus* were identified using laboratory bioassays, and the results validated in glasshouse experiments. Using practical experience of WFT damage to commercial crops, eight commonly-grown pot chrysanthemum cultivars were selected, five being considered as attractive or susceptible to WFT. The cultivars included two of each of four flower colours, with one cultivar of each colour being a single, daisy-type and the other being a decorative-type flower (Table 1).

The olfactory responses of WFT adult females to uninfested opening leaf buds and flowers of each of the eight cultivars were tested in a modified Petersson star olfactometer (Pow *et al.*, 1998a). Ten replicate olfactometer bioassays were done for each cultivar and for each plant part. WFT responses to 'Charm' were also tested in the olfactometer, as this cultivar was used to maintain the WFT laboratory culture, to avoid any potential thrips preference for the test cultivars due to host plant conditioning.

Flower colour	Single-flowered cultivar	Decorative-flowered cultivar
Yellow	'Miramar'	'Yuba'*
Dark pink	'Lompoc'	'Delano'*
Bronze	'Swingtime'*	'Orange About Time'*
White	'Shasta'*	'Surf'

\*Flowers and/or leaves considered to be attractive or susceptible to WFT

Table 1. Pot chrysanthemum cultivars selected for testing for attractiveness to WFT

Similar olfactometer bioassays were used to identify the responses of *O. laevigatus* females to both infested and uninfested opening flower buds of each cultivar. Volatiles from the eight cultivars were collected between the opening bud and flowering stages using air entrainment techniques. Physiologically-active components in selected extracts were located by coupled gas-chromatography (GC)-electrophysiology and identified using GC and coupled GC-mass spectrometry (Pow *et al.*, 1998a). Selected volatiles were used in olfactometer bioassays to identify those attractive to both WFT and *O. laevigatus*.

In each of two research glasshouses, 60 WFT females reared on 'Charm' in the laboratory thrips culture were released to each of six replicate plots of the eight test pot chrysanthemum cultivars at the opening bud stage. Eight mated *O. laevigatus* females were released to each plot in one of the glasshouses two days after WFT release. Cultivar attractiveness was quantified by counting WFT and *O. laevigatus* per plant one day, one week and two weeks after release. A separate glasshouse experiment was done to validate the attraction of WFT to a plant volatile selected from the laboratory bioassays. Four replicate plots, each of four thrips-free plants of the selected attractive cultivar were arranged in each of two WFT-infested glasshouses. In each glasshouse, two plots were baited with a blank lure and two were baited with a field-stable formulation of the volatile. Numbers of WFT per plant were recorded one and four days after the plants were placed in the glasshouses.

#### *Identification of 'push' component*

The leaves of four poor host plants for WFT (tomato cv. Espero, zonal geranium cv. Salmon Satisfaction, plectranthus and rosemary) were tested in olfactometer bioassays to identify possible repellency to WFT. Any plants proving to be repellent to WFT were further tested in the olfactometer for repellency to *O. laevigatus* adults.

#### *Spatial distribution of WFT life stages on 'lure' pot chrysanthemum cultivar*

The spatial distribution of WFT adults, larvae,

prepupae and pupae on the selected WFT-attractive cultivar was determined, in order to identify whether the biological control agents to be used in the push-pull strategy occupied the same spatial niches as the target thrips life stages. Five WFT females per plant were released onto four replicate pot chrysanthemum plants at each of two growth stages: 3-wk old plants at the vegetative stage and 8-wk old plants at the opening bud stage. The plants at each growth stage were kept in separate thrips-proof mesh cages in the glasshouse, under standard commercial 'short day' environmental conditions for chrysanthemums, i.e. min. 18°C night, vent at 21°C, 11:13 hours L:D. Numbers of WFT of each life stage were recorded on the plants after one day, and one, two and three weeks. After one week, yellow sticky traps were placed around the base of the plant and left for a further two weeks to catch any second instar larvae dropping to the ground to pupate. After three weeks, the plants were cut off at compost level and the pots were incubated in separate Perspex boxes with a yellow sticky trap in each lid to catch emerging WFT adults, in order to determine how many had pupated in the compost.

#### *Potential of Orius laevigatus in push-pull strategy*

Replicated laboratory bioassays were carried out to determine the predation rates of individual *O. laevigatus* adult females and third instar nymphs on both WFT adults and second instar larvae over 24 hours on different plant parts of the selected WFT-attractive pot chrysanthemum variety. Five replicate bioassays were done for each life stage of both the predators and the thrips, on leaves, opening buds and newly-opened flowers. Additional bioassays were done with *O. laevigatus* adults on older flowers releasing pollen, to determine whether the WFT predation rate would be reduced on older lure plants with pollen available as an alternative food source. All bioassays were done at 21°C and at 11:13 hours L:D. Each predator was offered either five WFT adults or 20 second instar larvae and the numbers of live and predated thrips were recorded after 24 hours.

### Potential of ground-dwelling predatory mites in push-pull strategy

Laboratory bioassays were done with *Stratiolaelaps (Hypoaspis) miles* and *Gaeolaelaps (Hypoaspis) aculeifer* to determine their predation rates on WFT second instar larvae and pupae over 24 hours on damp compost or capillary matting. In addition, a glasshouse experiment was done to compare the population increase of the two predatory mite species on WFT-infested pot chrysanthemum plants of the selected thrips-attractive cultivar in replicated thrips-proof cages (Bennison *et al*, 2002).

### Glasshouse experiments to test push-pull strategy

The first of a series of small-scale glasshouse experiments to test the principles of the push-pull strategy under high WFT pressure was completed. In each experiment, 480 WFT females (a mean of 30 per plant) were released to a block of pot chrysanthemum plants consisting of eight 'lure' plants of the WFT-attractive variety, surrounded by 16 cv. 'Charm' plants representing the 'crop' plants. A field-stable formulation of the WFT-attractive semiochemical was added to the 'lure' plants. When the experiments were set up, four of the 'lure' plants were at the opening bud stage and the other four 'lure' plants and the 'Charm' plants were at the bud initiation stage. The first experiment tested whether the 'lure' plants with the semiochemical attracted WFT away from the 'crop' plants until these plants were at the marketing stage, i.e. the first few flowers open. Numbers of WFT per plant were recorded one day after WFT release and weekly thereafter for five weeks. On each assessment date, the plants were tapped over a large white plastic tray and after the WFT on the tray had been recorded, they were collected with an aspirator and specimen tube and returned to each plant by propping up the open tube within the plant canopy.

## Results and Discussion

### Identification of 'pull' components

In the olfactometer bioassays, WFT females were not attracted to the leaves of any of the cultivars, but were significantly attracted to the opening buds of 'Swingtime' and 'Orange About Time' (Fig.1) and to 'Swingtime' open flowers. The glasshouse experiment comparing the attractiveness of the eight selected cultivars showed good agreement with the olfactometer studies, with consistently higher numbers of WFT found on 'Swingtime' than on the other cultivars on each assessment date following thrips release. In the olfactometer, *O. laevigatus* females did not respond to uninfested opening buds from any of the cultivars, but 'Swingtime' buds infested with five WFT adults for 24 hours were highly attractive ( $P < 0.01$ , Fig.2). In the glasshouse experiment, *O. laevigatus* showed no cultivar preference and both adults and nymphs were found on all cultivars during the four weeks following release. In the glasshouse, both WFT and *O. laevigatus* might be responding to both visual and olfactory cues whereas in the olfactometer, they would be responding only to olfactory cues.

In the olfactometer, the mixture of volatiles collected from 'Swingtime' by air entrainment attracted WFT adults, but the only identified electrophysiologically-active compound which

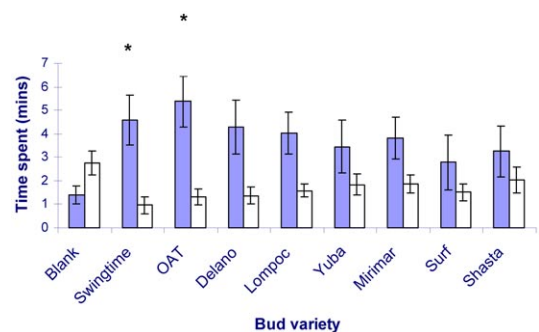


Fig.1 Responses of WFT females to opening buds of pot chrysanthemum cultivars: time spent (mins) in olfactometer. \* =  $P < 0.05$ . OAT = cv. 'Orange About Time'.

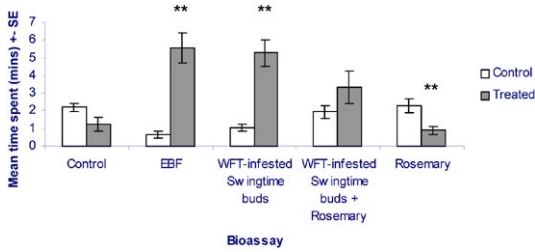


Fig. 2 Responses of *Orius laevigatus* females to EBF, to WFT-infested ‘Swingtime’ buds with and without rosemary, and to rosemary: time spent (mins) in olfactometer. \*\* =  $P < 0.01$ .



Fig. 3 Numbers of WFT adults per ‘Swingtime’ plant, with or without EBF lures, one and four days after placing plants at the opening bud stage in WFT-infested glasshouses. \*\* =  $P < 0.01$ , \*\*\* =  $P < 0.001$

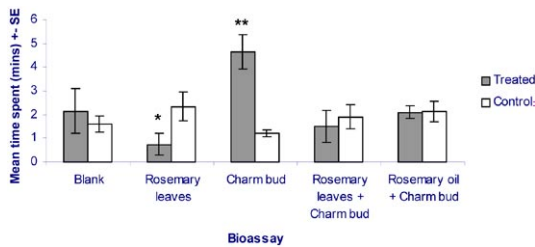


Fig. 4 Responses of WFT females to rosemary leaves and to cv. ‘Charm’ opening buds, with and without rosemary leaves and rosemary oil. \* =  $P < 0.05$ , \*\* =  $P < 0.01$ .

attracted WFT when tested individually in the olfactometer was (*E*)- $\beta$ -farnesene (EBF). This result is consistent with previous studies where EBF was shown to be attractive to WFT adults in both olfactometer bioassays and in glasshouses where sticky traps were baited

with an EBF lure (Pow *et al.*, 1998a). EBF was also highly attractive to *O. laevigatus* females in the olfactometer ( $P < 0.01$ , Fig. 2).

In the glasshouse, blocks of ‘Swingtime’ plants at the opening bud stage attracted high numbers of WFT adults within 1-4 days and plants baited with the stabilised EBF lure attracted significantly more WFT than unbaited plants (Fig. 3). There were 63% and 61% more WFT recorded on baited plants one and four days after placing the plants in the WFT-infested glasshouses ( $P < 0.01$  and  $P < 0.001$  respectively). This clearly demonstrates the potential of ‘Swingtime’ as a ‘lure’ plant and that attraction can be enhanced using the semiochemical, EBF. ‘Swingtime’ was selected as the ‘lure’ cultivar to use in the rest of the research project and EBF was selected as the semiochemical lure to enhance attraction to ‘Swingtime’ in the push-pull strategy.

#### Identification of ‘push’ component

In olfactometer studies, of the four poor host plants for WFT, tomato and plectranthus leaves were attractive to WFT adults and zonal geranium leaves were neither repellent nor attractive. Although rosemary leaves and volatiles were repellent to WFT and reduced the attractiveness of WFT-infested pot chrysanthemum cv. ‘Charm’ buds (Fig. 4), they were also repellent to *O. laevigatus* adults and reduced the attractiveness of WFT-infested chrysanthemum buds to the predator (Fig. 2). Thus rosemary is not a suitable ‘push’ component of the overall strategy since it would interfere with *O. laevigatus* foraging on the crop plants. Further research is planned to identify a suitable semiochemical with activity against WFT but not against biological control agents.

#### Spatial distribution of WFT life stages on ‘lure’ pot chrysanthemum cultivar

Three weeks after WFT females had been released onto the ‘Swingtime’ plants, there was a mean of 13.3 adults, 49 larvae and two pupae per plant at the opening bud to flowering stages

and only 0.5 adults and 1.5 larvae per plant at the vegetative to bud initiation stages. These results were consistent with those of de Jager *et al.* (1993), who reported that 30-60 times as many WFT were found on chrysanthemum plants with flowers than on those without. Of the WFT adults and larvae found on the plants, 99% were found in buds or flowers if available, rather than on the leaves. This result indicated that the majority of WFT adults and larvae will occupy the same spatial niche as the flower bugs *O. laevigatus*. Counts of prepupae and pupae on the plants, larvae on sticky traps under the plants and adults emerging from the compost indicated that 97% of the second instar larvae fell to the compost in the pots or to the capillary matting on the floor of the cages to pupate. Of the few pupae found on the plants, 88% were in buds or flowers rather than on the leaves. This result indicated that most of the late second instar larvae, prepupae and pupae would be available for the ground-dwelling predatory mites, *Stratiolaelaps (Hypoaspis) miles* and *Gaeolaelaps (Hypoaspis) aculeifer*.

#### *Potential of Orius laevigatus in push-pull strategy*

The mean predation rates of *O. laevigatus* females and third instar nymphs on WFT adults and second instar larvae over 24 hours on ‘Swingtime’ leaves, opening buds, newly opened flowers and older flowers with pollen are shown in Table 2. Mean predation rates by *O. laevigatus* females were statistically higher ( $P < 0.05$ ) on older flowers with pollen than on newly-opened flowers. Thus *O. laevigatus* has good potential for control of

both WFT adults and larvae on ‘Swingtime’ lure plants, even on older plants where the flowers are releasing pollen, as the predators do not prefer pollen to WFT. This result is consistent with those of Hulshof *et al.* (2000 and 2002 respectively) who reported that *O. laevigatus* adults preferred WFT to sweet pepper pollen and that the presence of pine pollen enhanced the predation rate of *O. laevigatus* third instar nymphs on WFT larvae.

#### *Potential of ground-dwelling predatory mites in push-pull strategy*

*Stratiolaelaps (Hypoaspis) miles* and *Gaeolaelaps (Hypoaspis) aculeifer* females had statistically similar predation rates on WFT pupae and second instar larvae over 24 hours. *S. miles* and *G. aculeifer* predated a mean of 1.5 and 0.7 WFT pupae respectively on compost and a mean of 0.9 and 1.3 pupae respectively on capillary matting. On compost, *S. miles* and *G. aculeifer* predated a mean of 1.7 and 1.3 WFT second instar larvae respectively and these values were statistically similar (Bennison *et al.*, 2002). Five weeks after release to WFT-infested ‘Swingtime’ plants in replicated cages in the glasshouse, *S. miles* and *G. aculeifer* multiplied by 5.2 and 4.3 times respectively and these population increases were statistically similar. Mean numbers of WFT adults at the end of the experiment were 59% and 66% lower in cages treated with *S. miles* and *G. aculeifer* respectively than in control cages and these reductions were statistically similar (Bennison *et al.*, 2002). Thus either *S. miles* or *G. aculeifer* might contribute equally to control of WFT on pot chrysanthemums if released to ‘lure’ plants in the push-pull strategy.

‘Swingtime’ plant part	Mean WFT adults (second stage larvae) predated by <i>O. laevigatus</i> adult females	Mean WFT adults (second stage larvae) predated by <i>O. laevigatus</i> third instar nymphs
Leaf	4.8 (13.2)	1.4 (16.4)
Opening bud	1.6 (16.0)	2.0 (17.6)
New flower	1.6 (13.8)	1.4 (14.9)
Older flower with pollen	3.2 (17.4)	2.4 (17.0)

Table 2. Mean predation rates of *O. laevigatus* females and third instar nymphs on WFT females and second instar larvae on ‘Swingtime’ plant parts over 24 hrs

### Glasshouse experiments to test push-pull strategy

The first of the series of glasshouse experiments to test the components of the push-pull strategy under high WFT pressure demonstrated that flowering ‘Swingtime’ with the EBF semiochemical were effective lure plants in a crop of ‘Charm’. Significantly more WFT were found on the ‘Swingtime’ from the opening bud to flowering stages, than on the ‘Charm’ from green bud to flowering stages (Fig. 5). Three weeks after thrips release, when the ‘Charm’ were at the opening bud stage, 84% of the WFT adults were on the ‘Swingtime’ and only 16% were on the ‘Charm’. However, when the ‘Charm’ were in flower at the marketing stage, five weeks after thrips release, 69% of WFT adults were on the ‘Swingtime’ (a mean of 51 per plant) and 31% were on the ‘Charm’ (a mean of 23 per plant). This result indicates that the differential attractiveness between the ‘lure’ plants and the crop plants needs to be increased when the crop plants come into flower. Further experiments are planned to test adding a ‘push’ semiochemical to the ‘Charm’ plants and to control the WFT by releasing *O. laevigatus* and *G. aculeifer* or *S. miles* onto the ‘Swingtime’ plants.

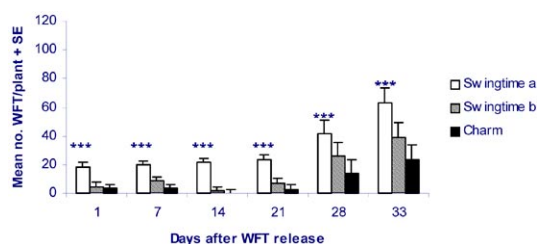


Fig. 5. Mean numbers of WFT adults per plant on ‘Swingtime’ and ‘Charm’ during the 5-wk period following WFT release. Swingtime (a) = plants at opening bud stage when experiments set up. Swingtime (b) = plants at same stage as ‘Charm’ (bud initiation) when experiments set up. \*\*\* =  $P < 0.001$ .

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