Reducing spread of TSWV on ornamentals by biological control of western flower thrips

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Abstract: In a glasshouse experiment, *Amblyseius cucumeris* gave excellent control of western flower thrips (WFT) on Impatiens and reduced the spread and severity of *Tomato spotted wilt virus* (TSWV). In the untreated glasshouse, mean numbers of WFT adults increased to 17.4 per plant over a 6-week period and all the plants showed severe TSWV symptoms on most of the leaves. In the glasshouse treated with weekly releases of *A. cucumeris*, mean numbers of WFT remained very low, with only 0.3 adults per plant after six weeks. Although 47% plants were infected with TSWV in the treated glasshouse at the end of the experiment, symptoms were only visible on one or two leaves per infected plant. There was good consistency between the numbers of plants with visual symptoms of TSWV and those testing positive in ELISA assays. Using the TaqMan assay, 50% WFT in the untreated glasshouse and 30% WFT in the glasshouse treated with *A. cucumeris* were confirmed to be viruliferous. The results indicated that *A. cucumeris* can give excellent control of WFT on Impatiens but only very low numbers of viruliferous thrips (0.1 per plant) are needed to spread TSWV on this host when virus pressure is high. Further work is needed on tospovirus epidemiology and on the development of effective integrated control strategies.

Introduction

Western flower thrips (WFT), *Frankliniella occidentalis*, is a major pest of protected crops and is resistant to many available pesticides. WFT causes direct plant damage and is also a vector of the tospoviruses *Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot virus* (INSV). Until recently in the UK, TSWV has been the most prevalent tospovirus on ornamentals, but INSV is now occurring more frequently and both viruses have been confirmed on a range of ornamental plant hosts (Bennison *et al*, 2001).

Biological control methods for WFT within Integrated Pest Management (IPM) programmes are now used on an increasing number of ornamental crops. The main biological control agent used against WFT on ornamentals in the UK is the predatory mite *Amblyseius cucumeris*, which can give reliable control on many host crops including Impatiens. Anecdotal evidence from commercial nurseries in the UK indicates that tospovirus incidence on many ornamental hosts is much lower on crops where biological control of WFT is used, than on crops receiving routine pesticide programmes. However, it has not been demonstrated how many WFT are needed to spread either TSWV or INSV, nor whether biological control can reduce or prevent virus spread.

TSWV and INSV are acquired by first instar WFT larvae feeding on infected plants, and spread to other plants by adult thrips. An important step in control of virus spread is to prevent viruliferous larvae from reaching the adult stage. *A. cucumeris* predates only first instar larvae and this stage in the thrips life cycle often lasts for only one or two days, depending on temperature and host crop (Loomans *et al*, 1995). Thus for effective biological control, it is essential that the young

thrips larvae are predated very soon after hatching.

The experiment described in this paper aimed to determine the number of WFT needed to spread TSWV on Impatiens and to quantify reduction of virus spread by biological control of the thrips vector using *A. cucumeris*. Impatiens was selected as the model experimental crop as it is a good host for both WFT and TSWV, shows obvious virus symptoms, and can be easily mechanically-infected with the virus. In addition, as *A. cucumeris* is usually effective against WFT on commercial Impatiens crops, the results should demonstrate the relationship between thrips pressure and virus spread.

Materials and methods

WFT culture

A laboratory stock culture of virus-free WFT, set up in 1996 from thrips collected on commercial nurseries, was maintained at ADAS Boxworth on pot chrysanthemum plants (c.v. 'Swingtime' and c.v. 'Charm'). The plants were kept in rearing cages in a controlled-environment room at 21ºC and with a 16-hr photoperiod. Synchronised-age first instar WFT larvae for infesting plants in the glasshouse experiment were reared on French bean (*Phaseolus vulgaris*) pods by taking female thrips from the stock culture and allowing them to oviposit on bean pods kept in perspex sandwich boxes, using the standard laboratory culture method (Loomans and Murai, 1997).

Test plants

Impatiens c.v. 'Accent white' were obtained as plugs from a commercial propagator. The plugs had been grown in a thrips-free glasshouse and were free from pesticide residues. A sample of 25 representative plants were tested by ELISA to confirm they were free from tospoviruses before the glasshouse experiment was set up. The young test plants for the glasshouse experiment were potted into 12 cm pots in Levington 'M2'® compost, consistent with that used in commercial practice.

TSWV-infected plants

Impatiens plants to be used as 'inoculum' plants in the glasshouse experiment were mechanically-infected with TSWV using the CSL strain 'TSWV GB103'. The inoculum plants were selected for use in the glasshouse experiment when they showed early virus symptoms e.g. leaf bubbling, leaf arcs or ring-spots.

ELISA assay for testing plants for TSWV

The plants were tested for TSWV using the standard ELISA assay (Clarke & Adams, 1977) and commercially-available antisera specific to TSWV (Adgen).

TaqMan assay for testing WFT for TSWV

WFT adults and second instar larvae collected from the plants in the glasshouse experiment were tested for TSWV using a real-time PCR (TaqMan) assay (Boonham *et al*, 2002).

Glasshouse experiment

The glasshouse experiment was set up on 19 July 2000 at ADAS Boxworth. Two identical glasshouse compartments were used for the experiment, each measuring 23m^2. One compartment was used for each of the two treatments:

1. *Amblyseius cucumeris* at 180 per m^2 (20 per plant) per week.
2. Untreated control.

In each compartment there were eight replicate plots, each plot consisting of eight young virus-free Impatiens plants. One 'inoculum' TSWV-infected Impatiens plant was added to the middle of each plot as a source of virus. The ratio of inoculum plants to test plants was high, in order to provide high virus pressure. Twenty first instar WFT larvae, 0-1 days old, were added to each inoculum plant on 19 July, using a fine paintbrush to transfer the larvae from the bean pods to a young leaf showing early virus symptoms. The plants were spaced so that they were not touching, in order to avoid thrips larvae being able to walk from the inoculum plants to the adjacent test plants. *A. cucumeris* releases were made every week to all plants in the 'treated' glasshouse compartment between 19 July and 31 August, by evenly broadcasting the predators in a vermiculite carrier over the plants. Before adding to the plants, the mean number of predators per gram of carrier was checked in the laboratory, to ensure that accurate numbers were released. The rate of *A. cucumeris* used was higher than the standard commercially-used rate (50 per m^2), as the inoculum plants were put under high thrips pressure and thus sufficient predators were needed to achieve the experiment objectives.

Assessments of thrips numbers and virus symptoms were made on each test plant and innoculum plant at weekly intervals, on 2, 9, 16, 23 August and on 1 September. Thrips numbers were assessed by tapping each plant over a large white plastic tray and counting thrips adults and larvae which had fallen onto the tray. The thrips were returned to each plant immediately after assessment, by collecting them in a tube with an aspirator and leaving the open tube supported within the foliage, so that the thrips could fly or crawl out onto the plant. TSWV symptoms recorded included leaf arcs, ring-spots, necrotic spots and stem blackening. After the final assessment, one upper and one lower leaf (with early virus symptoms if any present) and all thrips adults and second instar larvae detected on each plant were tested for TSWV using ELISA and TaqMan assays respectively.

Data analysis

Numbers of WFT and percentage of plants with TSWV symptoms were analysed using the Two sample T-test, assuming that environmental conditions in the two glasshouse compartments were identical.

Results

Control of WFT

In the untreated glasshouse, mean numbers of WFT per plant increased from 0.4 adults two

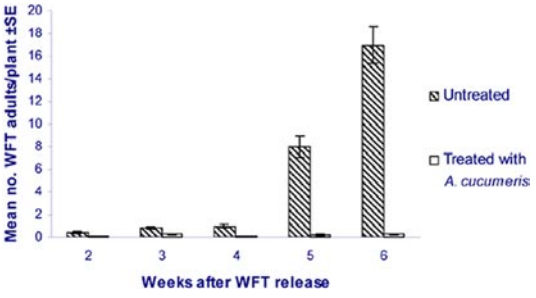

Fig. 1. Mean number of WFT adults per plant in untreated glasshouse, and glasshouse treated with *A. cucumeris*, 2-6 weeks after WFT infestation.

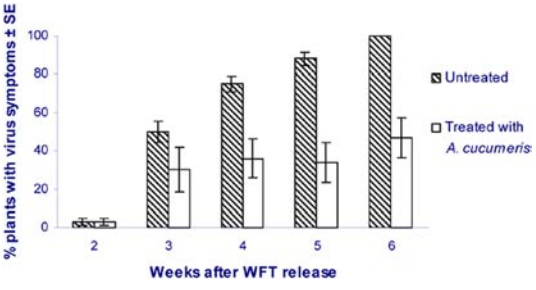

Fig. 2. Mean percentage test plants with virus symptoms in untreated glasshouse, and glasshouse treated with *A. cucumeris*, 2-6 weeks after WFT infestation. (No error bar given for untreated plants six weeks after WFT release as 100% plants had virus symptoms).

Weeks after inoculum plants infested with WFT	Untreated – mean no. WFT adults + larvae per plant	Untreated - mean % plants with virus symptoms	Treated – mean no. WFT adults + larvae per plant	Treated - mean % plants with virus symptoms
2	0.4 + 0	3%	0.1 + 0*	3%
3	0.8 + 0.2	50%	0.3 + 0**	30%
4	1.0 + 1.0	75%	0.1 + 0***	36%**
5	8.1 + 0.7	88%	0.2 + 0***	34%**
6	17.4 + 6.1	100%	0.3 + 0***	44%***

* Significantly different from the untreated value ($P<0.05$)
** Significantly different from the untreated value ($P<0.01$)
*** Significantly different from the untreated value ($P<0.001$)

Table 1. Mean numbers of WFT adults and larvae per plant, and mean percentage plants with TSWV symptoms in 'treated' and 'untreated' compartments, 2-6 weeks after plants were infested with WFT larvae.

weeks after thrips infestation, to 17.4 adults and 6.1 larvae six weeks after infestation (Table 1 and Fig.1). In the glasshouse treated with *A. cucumeris*, mean numbers of WFT were significantly lower than in the untreated glasshouse, with only 0.3 adults per plant recorded six weeks after infestation ($P<0.001$).

Reduction in spread of TSWV

In the untreated glasshouse, mean percentage plants with virus symptoms increased from 3% to 100%, two and six weeks after thrips infestation respectively (Table 1 and Fig. 2). Virus symptoms were severe and were present on most of the leaves on each plant at the end of the experiment. In the glasshouse treated with *A. cucumeris*, mean percentage plants with virus symptoms were significantly lower than in the untreated glasshouse four, five and six weeks after thrips infestation, with 44% plants showing symptoms at the final assessment ($P<0.001$). Virus symptoms in the treated glasshouse were very slight at the end of the experiment and were present on only one or two leaves per infected plant. In ELISA assays on the leaf samples taken after the final assessment, 95% and 47% plants in the untreated and treated compartments respectively proved positive for TSWV.

Proportion of viruliferous WFT

In TaqMan assays, 50% and 30% WFT collected from plants after the final assessment in the untreated and treated compartments respectively were positive for TSWV.

Discussion

The results indicate that *A. cucumeris* successfully reduced the survival of thrips larvae introduced to the inoculum plants in the treated glasshouse. Consequently, numbers of viruliferous adult thrips and the spread of TSWV were also reduced. At the end of the experiment, marked visual differences in virus symptom expression and severity were evident between untreated plants and those treated with *A. cucumeris*. At the final assessment, the results of visual recording of virus symptoms and confirmation of virus infection by ELISA were highly consistent.

Of the 0.3 WFT per plant recorded on the final assessment in the treated glasshouse, only 30% (i.e. 0.1 per plant) were confirmed by TaqMan to be viruliferous. These results indicate that very few viruliferous WFT adults are needed for spread of TSWV in Impatiens. The results might also indicate that despite TSWV being highly systemic in Impatiens, the severity and incidence of virus symptoms on infected plants could be dependent on the number of viruliferous WFT adults present. Alternatively, it is possible that the control of viruliferous WFT by *A. cucumeris* in this experiment led to delayed plant infection, resulting in some degree of mature plant resistance.

Further research is needed in order to understand the epidemiology of both TSWV and INSV, and to improve biological control methods for the vectors and tospoviruses on differerent ornamental crops. In this experiment, the plants were put under high WFT and virus pressure in order to achieve the experiment objectives. On most commercial nurseries using *A. cucumeris* for control of WFT within IPM on bedding plants including Impatiens, thrips pressure is usually maintained at a very low level. Further research is planned on evaluating biological control of TSWV on ornamental plants under lower thrips pressures and also on improving control of virus spread by using a combination of biological control agents, effective against all life stages of WFT. Future research will also include chrysanthemum, which is highly susceptible to TSWV and a more difficult host plant for biological control of WFT.

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