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Evaluation of the nematodes *Steinernema feltiae* and *Thripinema nicklewoodi* as biological control agents of western flower thrips *Frankliniella occidentalis* infesting chrysanthemum

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Abstract
In greenhouse studies, we evaluated a commercial formulation of the entomopathogenic nematode *Steinernema feltiae* and the inoculative release of the thrips-parasitic nematode *Thripinema nicklewoodi* against western flower thrips (WFT), *Frankliniella occidentalis* Pergande infesting potted chrysanthemums. Foliar sprays of *S. feltiae* applied at $1.25–2.5 \times 10^3$ IJ mL^{-1} and 1000 – 2000 L ha^{-1} at 3-day intervals alone (targeting feeding stages) or in combination with soil applications (simultaneously treating non-feeding stages in the soil at the same rates) decreased but did not provide adequate control of thrips in flowering plants artificially infested with a dense population. Similar nematode treatments applied for four to five applications at 6-day intervals in two batches of initially clean chrysanthemums failed to prevent unacceptable damage to flowers and leaves from a dense natural infestation within the greenhouse. Although some IJ survived up to 48 h within flowers and flower buds, few nematode-infected thrips (larvae and adults) were recovered. In studies with *T. nicklewoodi* (which is not amenable for mass production), the inoculative releases of two parasitized hosts per plant enabled the nematode to become established within existing WFT populations under greenhouse conditions. However, relatively poor transmission and slow speed of kill (nematode primarily suppresses populations through host sterilization) prevented low level inoculations being effective over a single crop cycle. Further studies showed that transmission of *T. nicklewoodi* persisted for nine host generations, infected up to 83% of adult thrips and provided long-term suppression of discrete caged populations, but only after uneconomically high thrips densities had been reached.

Keywords: *Biological control, chrysanthemums, entomopathogenic nematodes, host inoculation,* Frankliniella occidentalis, *parasitic nematodes,* Steinernema feltiae, Thripinema nicklewoodi, *western flower thrips*

Introduction

The western flower thrips (WFT), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) has become a serious and widespread pest of over 200 species of vegetables

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and ornamental crops due to direct feeding damage, disease vectoring and control costs (Lewis 1997). In glasshouses, the routine use of chemical insecticides as a management strategy against WFT has come under increasing scrutiny due to emerging problems of direct and cross resistance to commonly used insecticides (Immaraju et al. 1992; Brodsgaard 1994; Zhao et al. 1995; Broadbent & Pree 1997) and the desire to integrate biological control tactics for concurrent pest problems (Parrella 1995; van Lenteren & Loomans 1998).

To date, most thrips biological control strategies have focused on inundative releases of predatory mites and bugs. Several species, such as *Amblyseius cucumeris* (Acari: Phytoseiidae) and *Orius* spp. (Hemiptera: Anthorcoridae), have been shown to effectively suppress WFT populations in protected vegetable crops and are commercially marketed for this purpose (Gilkeson et al. 1990; Chambers et al. 1993; Gabarra et al. 1995; Castañé et al. 1996; Jacobson et al. 2001). However, releases of predators do not provide sufficient control of WFT in high value ornamental crops because of their low economic thresholds (Hessien & Parrella 1990; Parrella & Murphy 1996; Castañé et al. 1999). Two parasitoids, *Ceranisus menes* and *C. americensis* (Hymenoptera: Eulophidae), have been investigated for their potential to suppress WFT in greenhouse crops. However, both species' development times are long (relative to WFT) and releases failed to provide effective control (Loomans et al. 1995; Loomans & van Lenteren 1996).

The cryptic behavior of flower thrips in many ornamental crops may hinder biological control using predatory arthropods or parasitoids. Larvae and adults (feeding stages) tend to accumulate in tightly enclosed feeding areas, such as apical meristems and flower buds, which may be difficult for arthropod natural enemies to penetrate. Foliar dwelling predators may also be disrupted by insecticides and cannot sufficiently control the soil-dwelling stages of thrips; although predatory mites are being investigated for this latter role (Premachandra et al. 2003; Berndt et al. 2004).

Pathogens may be vectored and transmitted in cryptic areas and are more easily integrated with traditional insecticides. However, few studies have assessed microbial control of thrips. No effective protozoan, viral or bacterial pathogens are known for thrips (Brownbridge 1995), although entomopathogenic fungi appear to hold some promise against soil stages (see Butt & Brownbridge 1997). Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are potential biological control agents of thrips soil stages (Helyer et al. 1995; Chyzik et al. 1996; Ebssa et al. 2004a,b). Unfortunately, there has been little reported on the use of EPNs as foliar sprays which target the feeding stages that comprise the initial infestation. We evaluated the efficacy of a commercial formulation of *Steinernema feltiae* Filipjev (Rhabditida: Steinernematidae) as a high volume foliar spray against all WFT stages infesting potted chrysanthemums. We also report on studies evaluating inoculative releases of the parasitic nematode, *Thripinema nicklewoodi* Siddiqi (Tylenchida: Allantonematidae), which naturally infects feeding stages residing within the flower buds and foliar terminals. Infection by *T. nicklewoodi*, although non-lethal, results in sterility for female hosts. Further details of the taxonomy and biology of *Thripinema* spp. are presented elsewhere (Siddiqi 1986; Loomans et al. 1997; Lim et al. 2001; Mason & Heinz 2002; Arthurs & Heinz 2003).

Materials and methods

Source of plants and thrips

Chrysanthemum plants (*Dendranthema grandiflora* Tzvelev) obtained as rooted cuttings from Yoder Brothers (Barberton, OH) were grown under a thrips-proof screen in an 8×8-m bay of an experimental greenhouse at TAMU. Cuttings were transplanted individually in 12.5-cm pots filled with multipurpose compost (Sunshine Mix #1®, SunGro Horticulture Canada Ltd., Bellevue, WA) and watered with 350 ppm Peter's Pot Mum Special 10-10-30 NPK (The Scotts Co., Maryville, OH). Terminal meristems were pinched 12–14 days post transplanting to encourage lateral branching in accordance with grower practices. Because the chrysanthemum is a qualitative short day plant, studies were conducted during the fall through early spring (2001–2003) when flowering was naturally initiated. Fertilizer was not used during flowering to delay senescence. A colony of *F. occidentalis*, originating from a field population collected in alfalfa at the University of California Davis campus in 1998, were maintained on chrysanthemums in a growth chamber at 26–28°C, 60% relative humidity (r.h.) and 14L:10D photoperiod.

Studies with Steinernema feltiae

Nematode applications. S. feltiae were obtained as a commercial strain 'Nemasys® F' formulated in a calcium alginate-based gel in packets containing 250 million infective juveniles (IJ). Nematodes were kept refrigerated and used within 2 weeks of shipment. Nematodes were applied as aqueous suspensions in reverse osmosis water using a Solo® 10-L hand-pressurized backpack sprayer (Newport News, VA) fitted with a hollow cone nozzle applying 600 mL min^{-1} at $\approx$40 psi (276 kPa). During treatments, the filter and gasket were removed from the spray wand to protect the IJ and the sprayer was agitated to prevent settling.

Nematodes were applied as foliar sprays alone (targeting feeding stages) or as foliar plus soil combination treatments (also targeting pre-pupal and pupal soil stages). Each treatment was applied at two concentrations according to label recommendations; 1.25×10^3 IJ mL^{-1} (preventative rate) and 2.5×10^3 IJ mL^{-1} (recommended for heavy infestations). There were thus four treatments, (1) low rate on foliage (12.5 IJ cm^{-2}), (2) low rate on both foliage and soil (25 IJ cm^{-2}), (3) high rate on foliage (25 IJ cm^{-2}), and (4) high rate on both foliage and soil (50 IJ cm^{-2}). Foliar sprays were applied at a volume application rate (VAR) of 1000 L ha^{-1} and directed from above ($\approx$30 cm) to ensure good coverage of foliage and flowers with limited run-off. For combination treatments, the soil surface was also sprayed at the same rates as the foliage; i.e., VAR of 2000 L ha^{-1} overall. The wetting agent 'Kinetic' (Setre Chemical Co., Memphis, TN) was included at 0.07% (v/v).

Tests against heavy thrips infestations. In the first greenhouse trial, nematodes were applied against a dense artificially established WFT infestation. Prior to treatments, 60 chrysanthemums cv. Charm (selected 61 days post transplanting at early bud burst) were individually placed in plastic buckets (40 cm high, 30 cm diameter) and infested with six adult female WFT removed from the colony. Eight 10-cm diameter holes in the bucket side and a tightly fitting lid both screened with thrips-proof polyester mesh (32 holes mm^{-1}) prevented escape and reduced condensation. Cages were maintained in the greenhouse and monitored daily.

Nematodes applied as foliar sprays alone or in combination with soil applications (described above), were applied 12 days post infestation; 1 day after prepupae were observed in pots and when some feeding damage (scarring of leaves and flowers) had already occurred. A second equivalent application was made 3 days later. Plants were sprayed *in situ* (lid removed) to minimize disturbance. Control plants for both foliar and combination treatments were sprayed with water and wetting agent only. Cages were randomly allocated among the six treatments with 10 cage replicates. Soil was irrigated prior to treatments and to provide most favorable conditions for the survival of IJ, applications were timed shortly before dusk (5–8 pm). For the assessments, plants were harvested 3–4 days after the second spray and the number of thrips life stages determined through destructive sampling in the laboratory. Sub-samples of thrips ($\approx$100 per treatment) were dissected to look for nematodes. Plants were placed individually in sealable plastic bags and maintained at 4°C until processing.

Protection against natural thrips infestations. In two further tests, nematode treatments (described above) were applied as a preventative measure against 'natural infestations'. Source populations likely comprised adult WFT from outside that entered through greenhouse vents and others migrating from adjacent bays that previously housed infested plant material. Ninety pest-free chrysanthemums cv. Charm were placed on two adjacent uncovered benches (each 3×1.5 m) at 25-cm spacing and were randomly allocated among the same *S. feltiae* treatments described above. Initial applications were made 52 days post transplanting when buds were still green and plants still unattractive to WFT (checked through inspections of plants and yellow sticky cards above the bench). Subsequent treatments were made at 6-day intervals, for four applications. Plants were moved to a separate area of the greenhouse for applications. During this time the bench surfaces were also sprayed with the high rate of nematodes. Soil was irrigated prior to treatments and to provide high humidity within the plant canopy, applications were timed shortly before dusk (5–8 pm) and both benches were covered overnight with a large sheet of black plastic suspended over a PVC frame.

For the assessments, plants were harvested 3–4 days following the final application and the number of flowers, buds and thrips life stages determined in the laboratory. The diameter of the three largest flowers and the proportions of the 10 largest leaves and flowers with visible thrips scarring damage were also recorded. Plants were placed individually in sealable plastic bags and maintained at 4°C until processing. The study was repeated with another cohort of plants. On this occasion, five applications were made starting slightly earlier (44 days post transplanting), and only plant damage was recorded. Temperature and relative humidity were logged hourly throughout the study period using a Hobo H8 Pro Series (Onset Corp; Pocasset, MA) datalogger.

IJ persistence studies. Ten additional plants in full bloom were sprayed once (high concentration combination tratment described above) to test for the survival of IJ. Shortly after spraying and at intervals of 2, 4, 12, 24, 48, 84, 96, 120, and 144 h (0–6 days), one plant was randomly selected and the leaves and flowers were removed and a sample of topsoil taken. Plants were covered for the first 12 h (as described above) and the soil (but not foliage) was irrigated daily during this time. Separately, leaves, flowers/open buds and soil were rinsed in water + 0.07% wetting agent and serially decanted to trap nematodes, which were assessed in counting wells under a dissecting

microscope. A minimum of 300 IJs were examined and survival determined by nematode movement. The study was conducted twice.

Studies with Thripinema nicklewoodi

A colony of *T. nicklewoodi*, originating from parasitized WFT collected as previously described, was cultured *in vivo* as previously described (Arthurs & Heinz 2002). No *in vitro* production techniques for *T. nicklewoodi* are currently available. However, greenhouse studies were conducted to evaluate nematode transmission and the ability of *T. nicklewoodi* to suppress thrips populations following the inoculative release of nematodes in caged WFT populations on chrysanthemums.

Population dynamics of WFT following low-level inoculation. In the first study, 12 pots (20 cm diameter) each containing four plants cv. Golden Polaris were each infested with WFT taken from the colony at rate of 38 larvae (both instars) and four adult females per plant. Plants were $\approx$8 weeks post-transplanting and at the first open bud stage. In half of the pots (randomly selected) nematode-parasitized adult female thrips were introduced at a rate of two per plant, forming 'nematode' and 'control' lines. Parasitized thrips were 14 days post infection and confirmed to be actively releasing infective stages of nematodes the day prior to use. Because parasitized WFT remain sterile (Arthurs & Heinz 2003), no additional thrips were added to controls to standardize initial host populations. There were six cages per treatment. Following infestations, plants were placed in thrips-proof cages (previously described) and maintained in the greenhouse. Plants were watered every 48 h and destructively harvested in the laboratory after 25 days when in full bloom, approximately two nematode generations (Arthurs & Heinz 2002; Mason & Heinz 2002), to determine the numbers of thrips life stages as previously described.

Because there was no reduction in host populations in the first cohort of plants, we investigated host–parasite population dynamics (at least in a simplified system that prevents dispersal) over further generations. To do this, the study was repeated with the exception that all WFT harvested from each cage (with the exception of eggs and soil stages which could not be reliably collected) were used as starting populations for the next cohort. Replacement plants were also at the first open bud stage. The process of using the final population as a starting source in new cages was repeated at approximately 3-week intervals for 21 weeks; representing an estimated nine thrips generations (Katayama 1997) and 12 nematode generations (Arthurs & Heinz 2002; Mason & Heinz 2002) under the greenhouse conditions. On each sampling occasion a sub-sample up to 30 adult female WFT from each cage (depending on availability) were randomly selected and dissected to estimate nematode-infection rates. To minimize bias resulting from sub-sampling, thrips used to determine infection rates were replaced, i.e., equivalent numbers of infected and healthy females were obtained from the laboratory cultures. To investigate the importance of plant phenology, the study was repeated with plants of equivalent age but in the vegetative state. To prevent flowering, plants were initially maintained under a long light regime (16 h) in a growth chamber and additional buds produced in the greenhouse were removed.

Dose response study. To determine whether higher inoculation rates of *T. nicklewoodi* would provide better initial control, a dose–response study was conducted. Sixty first

instar WFT (<2 days old) were released onto chrysanthemums cv. Golden Polaris grown individually in 12.5-cm pots. Plants were at the first flower open stage (four to nine open buds per plant). Nematodes were inoculated at four rates; one, five, 10 or 30 adult female WFT actively releasing nematode progeny were released per plant and plants were maintained in cages in the greenhouse as previously described. There were five replicates per treatment. Host infection rates were assessed after 14 days as previously described. Non-flowering plants were not included.

Data analysis

All analysis was performed using SPSS 12.0.1 for Windows. For tests with *S. feltiae*, treatment effects were compared using one- and two-way univariate ANOVA. Significant F-ratio means were further separated with Fisher's LSD for multiple comparisons, at $P < 0.05$. In *T. nicklewoodi* inoculation tests, populations from each plant cohort were also compared using independent samples t-tests. All proportional and count data were normalized via arcsine and $\log(n+1)$, respectively, prior to analysis.

Results

Steinernema feltiae *studies*

In tests of *S. feltiae* sprays against an established thrips infestation, two-way univariate ANOVA revealed nematode concentration (control, low, high; $F_{2,54} = 6.2$, $P < 0.001$) but not application strategy (foliar or combination sprays; $F_{1,54} = 0.13$, $P = 0.72$) were significant in the overall numbers of thrips recovered. Because the different application strategies tested (foliar and combination) confounded both application rate (IJ plant^{-1}) and volume (L ha^{-1}), one-way ANOVA followed by mean separations was used to compare treatments side by side. Compared to control plants, significant reduction in thrips numbers were observed in two of the four nematode treatments (Figure 1a). However, there were no significant differences between these treatments, thus no clear benefits of using a higher rate or combination treatments were demonstrated. When thrips life stages were compared individually, one-way ANOVA revealed a significant effect of treatment on the numbers of pre-pupae/pupae ($F_{2,54} = 2.5$, $P = 0.04$) and adult females ($F_{2,54} = 2.9$, $P = 0.02$) but not larvae ($F_{2,54} = 1.9$, $P = 0.11$) or males ($F_{2,54} = 1.4$, $P = 0.25$).

When *S. feltiae* was applied as a preventative measure against natural infestations, two-way univariate ANOVA revealed neither nematode concentration ($F_{2,84} = 1.5$, $P = 0.22$) nor application strategy ($F_{1,84} = 1.1$, $P = 0.31$) affected the final number of thrips recovered. One-way ANOVA comparisons also showed no differences in the number of thrips between individual treatments (Figure 1b). Moreover, there were no differences when thrips life stages were compared individually; larvae ($F_{5,84} = 0.77$, $P = 0.58$), pre-pupae/pupae ($F_{5,84} = 1.15$, $P = 0.34$), females ($F_{5,84} = 1.0$, $P = 0.42$) or males ($F_{5,84} = 1.25$, $P = 0.29$). In assessments of plant phenology (including additional tests from a second batch), there was no overall effect of nematode treatments on the number of flowers and buds (first batch; $F_{5,85} = 1.5$, $P = 0.2$, second batch; $F_{5,85} = 0.9$, $P = 0.49$) or diameter of the three largest flowers (first batch; $F_{5,85} = 0.9$, $P = 0.51$, second batch; $F_{5,85} = 1.37$, $P = 0.24$). Although damage was highest in the

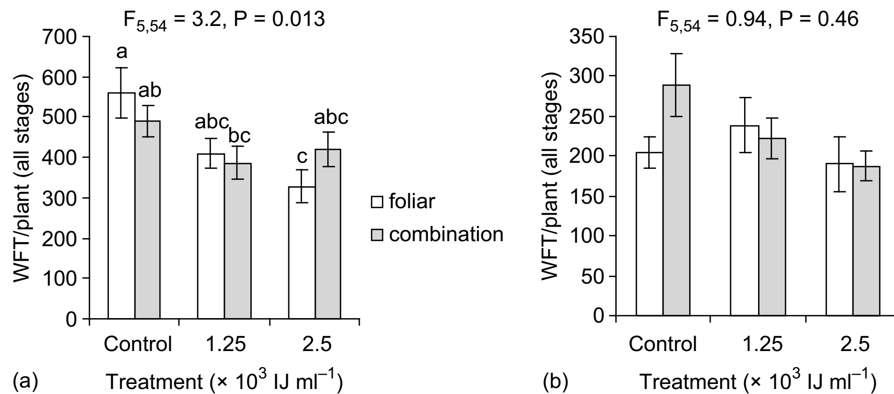

Figure 1. Number of WFT recovered from potted chrysanthemums cv. Charm following applications of *S. feltiae* at two rates and application strategies (foliar sprays alone or in combination with soil applications at the same rates) compared with controls. (a) Nematodes were applied twice (3-day intervals) against a heavy infestation when plants were in full bloom; (b) nematodes were initially applied at the green bud stage for four to five applications (6-day intervals) against 'natural infestations' within the greenhouse. Data show mean ± SEM for (a) 10 and (b) 15 plants, letters indicate Fishers LSD at $P < 0.05$.

control treatments in the second batch, there was also no overall treatment effect on the proportion of flowers or leaves with thrips scarring (Figure 2).

The persistence of IJ stages on different structures (leaves, flowers/open buds and soil) following two tests are shown in Figure 3. Estimated nematode LT_{50} (tests combined) were 3.1 h (leaves), 15 h (flowers/buds) and 79 h (soil). Conditions in the greenhouse ranged from 11 to 35°C and 30 to 100% r.h. during the studies. However, during the first 12 h post spraying (plants were covered with plastic approximately from 21:00 through 09:00 h), average temperature was 19.5°C (range 13–23°C) and r.h. 97.5% (range 87–100%).

Thripinema nicklewoodi *studies*

The impact of *T. nicklewoodi* inoculations on WFT densities in potted chrysanthemums is shown in Figure 4. In flowering plants, nematodes established in all cases following low-level inoculation. Although infection rates of adult female thrips reached 11%, *T. nicklewoodi* failed to significantly reduce thrips densities within a single cropping cycle (i.e., on the plant that was inoculated). However, when plants were periodically replaced to allow population dynamics to be observed over multiple generations, the proportion of infected hosts increased asymptotically, reaching 83% after an estimated 7.5 host generations and *T. nicklewoodi* provided long-term suppression. Although proportionally fewer females were infected at the end of the study, nematode introductions reduced WFT populations by 87.4% compared with control plants after nine host generations. There was also a trend towards more developed stages of nematodes in later cohorts of hosts, with a higher proportion releasing parasitic stages (Figure 5). Significant reductions in thrips numbers occurred after six host generations when thrips densities were high (≈600 per plant).

Plant phenology was critical for nematode establishment. At the end of our studies, an average of 905 ± 138 nematodes per plant were recovered from flowers compared with 33 ± 8 from leaves and none from soil. *T. nicklewoodi* was unable to establish in

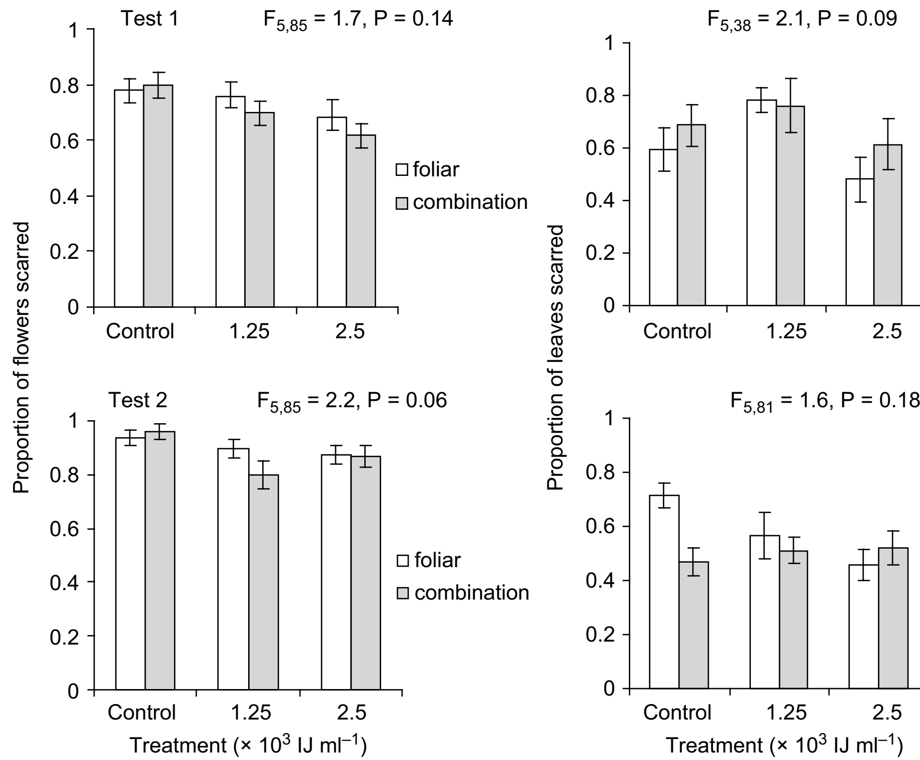

Figure 2. Assessments of thrips damage in potted chrysanthemums cv. Charm following applications of *S. feltiae* at two rates and application strategies compared with controls. Data show mean ± SEM for two tests.

vegetative (non-flowering) plants and died out within ≈3 generations, concurrently with a decline in host density (Figure 5). In the dose response study, higher release rates of *T. nicklewoodi* increased rates of infection in adults of the larval generation exposed logarithmically, with 60% infected at the highest release rate (Figure 6).

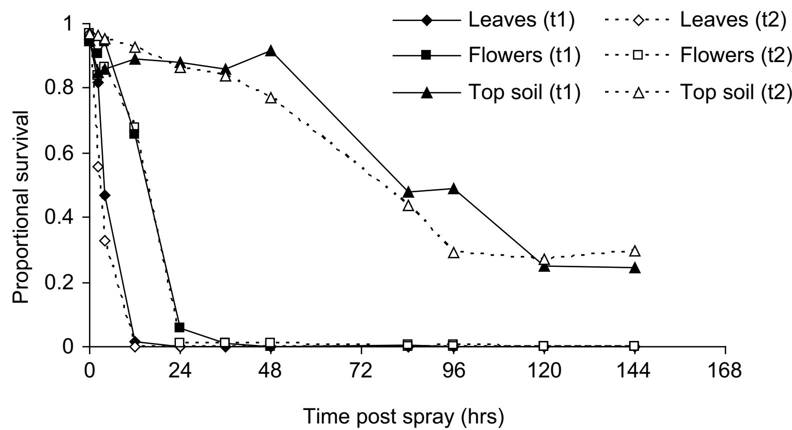

Figure 3. Survival of *Steinernema feltiae* infective juveniles on leaves, flowers/open buds and moist soil following application to potted chrysanthemums. Data shown for two tests (t1 and t2).

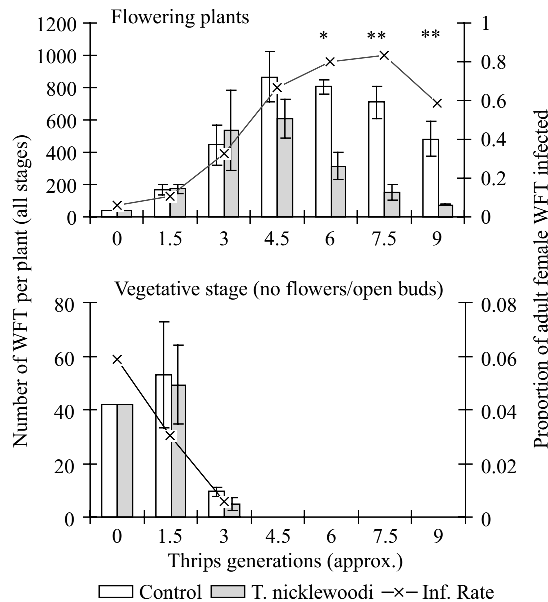

Figure 4. Population densities and nematode infection rates of WFT infesting chrysanthemums following inoculation with *T. nicklewoodi*. Additional plants were provided at 3-week intervals ($\approx$1.5 thrip s generations) and the study conducted with both flowering and non-flowering (vegetative) plants. Data show mean $\pm$ SEM from five replicate cages. Differences between treatments indicated by $^{*}P<0.005$ and $^{**}P<0.001$.

Greenhouse conditions over the study period (measured inside cages) were variable reaching daytime highs $>30°C$ and nighttime lows $<5°C$ with an average of 88% r.h. (Figure 7). Conditions were hotter and drier towards the end of the study when the greenhouse cooling system malfunctioned (coinciding with the decline in infection rates in Figure 5).

Discussion

The natural environment of EPNs is the soil, where they infect a broad range of insects (Kaya & Gaugler 1993). In the majority of WFT, the prepupae and pupae remain in the soil for approximately one-third of its development (Pickett et al. 1988;

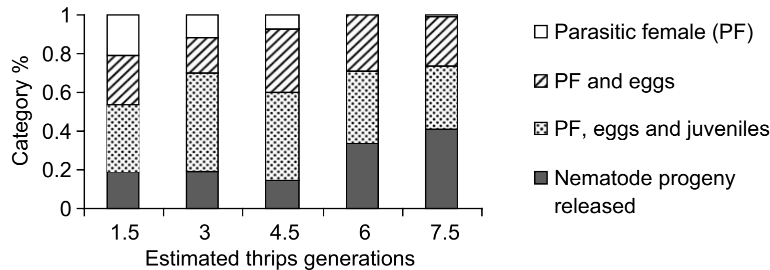

Figure 5. Proportional distribution of *T. nicklewoodi* developmental stages monitored from successive host cohorts.

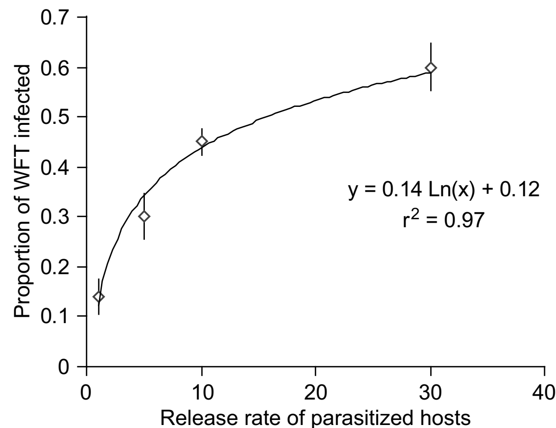

Figure 6. Proportion of WFT parasitized by *T. nicklewoodi* as a function of the release rates of infected hosts. Thrips were exposed to *T. nicklewoodi* as larvae and assessed as adults. Data shows mean ± SEM per plant from five replicate cages.

Higgins 1992) providing a window for control with EPNs. A number of studies show some level of thrips control can be achieved by applying EPNs against the soil stages. For example, laboratory studies showed strains of *H. bacteriophora* caused 36–49% thrips mortality at 40–200 IJ cm^{-2} (Chyzik et al. 1996). In other laboratory trials, the effectiveness of 100 strains of *S. feltiae* against WFT soil stages varied between strains and ranged between 3.7 and 72.6% (Tomalak 1994). In other tests against mixed soil-dwelling life stages, strains of *Heterorhabditis* spp. and *Steinernema* spp. caused 2.6–60% mortality at 200 IJ cm^{-2}, although in general, *Heterorhabditis* spp. were the more virulent (Ebssa et al. 2004a). Results from these and other studies vary widely, and thus far only a few species have been shown to cause high rates of mortality in WFT and only when applied at comparatively high application rates during favorable conditions. Recent studies suggest that the effectiveness of some nematodes against thrips soil stages may be improved through careful post application irrigation maintaining high substrate moisture (Ebssa et al. 2004b).

Although soil applications may be useful within integrated thrips management programs, an inherent problem is that this strategy only targets a small portion of the WFT population. Moreover, because adult thrips are vagile and initial pest stages occur on the plant, infestations may spread and cause damage to foliage or flowers

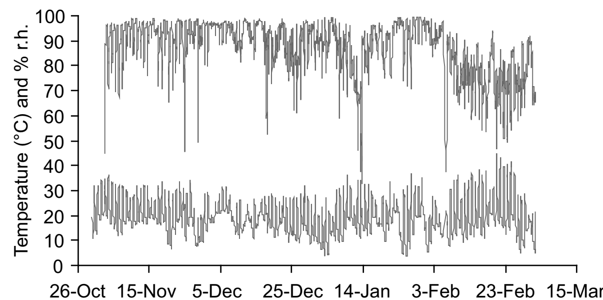

Figure 7. Greenhouse conditions during the *Thripinema* inoculation trials.

before thrips can be controlled. Such limitations suggest little prospect of these nematodes being highly effective, at least not without very frequent use. In crops with low aesthetic thresholds, foliar sprays of nematodes targeting the feeding stages may allow an infestation to be controlled at an earlier stage before thrips breeding has occurred.

With that background, we assessed foliar sprays of a commercial strain of *S. feltiae* previously marketed in the UK and North America for use against WFT and leafminers infesting protected ornamental and bedding plants. In the first experiment, foliar sprays of *S. feltiae* applied at 3-day intervals alone or in combination with soil applications failed to control a heavy WFT infestation in plants at full bloom (Figure 1a). Although thrips numbers were statistically reduced in some of the nematode treatments, few cadavers or confirmed infected hosts were recovered from flowers, where the vast majority of thrips were recovered. In the second study, although plants were initially clean and efforts were made to maintain favorable environmental conditions following spraying, similar treatments repeated at 6-day intervals also failed to reduce thrips populations or prevent severe aesthetic damage resulting from a natural infestation in the greenhouse (Figure 1b and 2). Although some thrips economic injury thresholds have been published (Schmidt & Frey 1995; Shipp et al. 1998), these are very sensitive to particular conditions, including the crop variety, local market standards and whether tospoviruses are present. It should be noted that routine pest control practiced in many commercial operations would maintain pests at low populations and thus heavily infested plants would most likely be sanitized.

The reasons for the lack of success are unclear. To achieve good control with EPNs, the ecology of the nematode strain and target should be matched; in practice, targets are protected from environmental extremes, and applications are timed to coincide with susceptible host stages and favorable environmental conditions (Klein 1990; Kaya & Gaugler 1993). In a research review, it was shown that foliage feeding pests were generally poor targets for EPNs due to exposure to air movement, sunlight, and low r.h. resulting in rapid desiccation and death of IJs, although some success has been demonstrated in cryptic locations when IJs are to some degree protected in their target site (Arthurs et al. 2004). In the present studies, IJs rapidly died on leaves, but some survived up to 48 h within flowers and flower buds (Figure 3). Although theoretically IJs survived at the target site long enough to locate and infect hosts, feeding stages of thrips are likely too small to support nematode reproduction and may be intrinsically unsuitable hosts, although host recognition behaviour studies for thrips appear lacking (Lewis et al. 1996). Optimum movement and infectivity of EPNs requires films of free water around soil particles (Glazer 2002; Grant & Villani 2003), which may have limited the ability of IJ to locate and penetrate thrips within flowers.

In the second study, the thrips pressure was relatively high (sticky cards hung above the benches indicated a steady immigration of WFT adults during the treatment period). The comparatively short residual activity in flowers would have thus allowed significant oviposition and feeding damage to occur between treatments. The combination foliar/soil treatments did not significantly improve control compared with foliar sprays alone, despite the longer survival of IJs in the soil (Figure 3). Because we did not specifically assess parasitism of WFT soil stages, it is unclear to what extent they were controlled, although the rates of nematodes applied were lower compared with previously mentioned studies. It is unclear whether applications of

S. feltiae would be more efficacious in cases of low thrips pressure, or if applied more frequently or in combination with additional interventions.

In contrast to *S. feltiae*, the parasitic nematode *T. nicklewoodi* naturally infects WFT residing within the flower and open buds and reproduces through one heterosexual generation (Lim et al. 2001; Arthurs & Heinz 2002; Mason & Heinz 2002). In our greenhouse studies with *T. nicklewoodi*, the inoculative releases of two parasitized hosts per plant enabled the nematode to become established within existing WFT populations under greenhouse conditions. However, relatively poor transmission and slow speed of kill (nematode primarily suppresses populations through host sterilization) prevented low level inoculations being effective over a single crop cycle i.e., plant inoculated (Figure 4). Further experiments showed that transmission of *T. nicklewoodi* persisted for nine host generations, infected up to 83% of adult female thrips and provided long-term suppression of discrete caged populations (provided plants were in flower), but only after high thrips densities had been reached.

In many flowering ornamental crops, the short production cycle and low pest infestation levels tolerated by commercial nurseries limits the value of *T. nicklewoodi* as a biological control agent. Because chrysanthemums are only produced for several weeks prior to shipment from the nursery, the long developmental time for *T. nicklewoodi* (12–14 days from host infection to release of nematode progeny) (Arthurs & Heinz 2002; Mason & Heinz 2002), and relatively low rates of transmission even at relatively high release rates (Figure 6) appear to prohibit the effective use of an inoculation strategy.

In similar studies to ours, Lim and Van Driesche (2004a) evaluated a single inoculative release of *T. nicklewoodi* in caged impatiens plants. Nematode transmission persisted for the length of the study (seven host generations) and populations of healthy female thrips were reduced up to 79% after four generations (800 degree days) and parasitism rates of adult female thrips reached 52%. No differences were found between two initial release rates, three and seven parasitized thrips per cage. The author considered introductions not practicable, considering the delay in thrips control and short production cycle. In follow up studies, multiple releases of nematode were tested in an attempt to reduce thrips populations more quickly. Nematode-infected thrips released 7 times over 43 days reduced thrips populations up to 56% for second instars, 72% for adult females and 62% for males compared with controls (Lim & Van Driesche 2004b). Although initial reductions in thrips (adult females) occurred more quickly (400 degree days), level of thrips control and protection of flowers was still considered ineffective compared with a spinosad treatment.

It should be noted that the present study and those of Lim and Van Driesche (2004a,b) were based on caged, isolated populations that did not allow for dispersal and thus do not necessarily predict the ability of *T. nicklewoodi* to prevent the spread of thrips infestations within the greenhouse. In thrips release studies, we observed parasitized adult thrips vectored *T. nicklewoodi* between adjacent chrysanthemums grown at a commercial bench spacing. In flight tests using yellow sticky cards suspended 6 m from a source population, we also recaptured adult female thrips infected with *Thripinema* at an equal rate to controls (unpublished data).

The natural population dynamics or seasonal abundance of species of *Thripinema* have been studied in WFT (Heinz et al. 1996) and other thrips species, *F. fusca* Hinds (Funderburk et al. 2002b), *F. australis* Morgan (Funderburk et al. 2002a), *Aptinothrips*

rufus Gmelin (Sharga 1932; Lysaught 1937) and *Megaluriothrips* sp. (Reddy et al. 1982). In the majority of these studies, high infection rates and significant suppression of thrips populations are only reported during the mid or late season, once relatively high host densities have been reached. Such cyclical fluctuations are consistent with host density-dependent transmission rates, which support the theory that inoculative releases of *T. nicklewoodi* are not appropriate for crops grown for high aesthetic qualities. More success may be achieved if mass production methods were available to support inundative applications of *T. nicklewoodi*. Inoculations may still be useful to regulate thrips populations in field crops or refugia as part of longer-term thrips management strategies (Funderburk et al. 2002a,b). Natural suppression of thrips populations by species of *Thripinema* may be widespread and deserves further attention.

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