

## Thrips Parasitic Nematode *Thripinema nicklewoodi* (Tylenchida: Allantonematidae) Reduces Feeding, Reproductive Fitness, and Tospovirus Transmission by Its Host, *Frankliniella occidentalis* (Thysanoptera: Thripidae)

S. ARTHURS AND K. M. HEINZ

Biological Control Facility, Department of Entomology, Texas A&amp;M University, College Station, TX 77843-2475

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**ABSTRACT** The parasitic nematode *Thripinema nicklewoodi* is a potential inoculative biological control agent of the western flower thrips, *Frankliniella occidentalis*. Laboratory studies were undertaken to assess the effect of *T. nicklewoodi* infection on: 1) host feeding, 2) host fecundity, and 3) viral competency of *F. occidentalis* coinfecting with a tospovirus. Individual thrips infected with nematodes as larvae and maintained on leaf discs in microcentrifuge vials showed a reduced feeding throughout the adult life span. This per capita reduction in feeding by parasitized individuals contributed to a total reduction in feeding of 87% on chrysanthemum petals and 91% on bean foliage relative to uninfected thrips. Parasitism also reduced the longevity of adult thrips by 3–5 d, although the preadult developmental time was unaffected. In a separate study, thrips infected with nematodes as larvae became reproductively sterile and appeared to have a reduced vector competency for tomato spotted wilt virus (TSWV). Thrips larvae were inoculated with both TSWV and nematodes in a factorial design, and adults were subsequently exposed to a petunia leaf disc assay to test for fecundity and virus transmission using enzyme-linked immunosorbent assay (ELISA). No eggs were recovered from thrips infected with nematodes, and dissections revealed that their embryos remained fully degenerate, which was not observed among healthy thrips. Although the proportion of thrips testing positive to a TSWV nonstructural protein (indicating systemic virus acquisition) was statistically similar between treatments, fewer viruliferous *F. occidentalis* coinfecting with *T. nicklewoodi* became virus transmitters. Moreover, the per capita frequency of virus transmission among nematode-infected thrips was reduced by  $\approx 50\%$  relative to nematode-free thrips. Our results suggest that *T. nicklewoodi* may help prevent thrips outbreaks and reduce direct feeding damage and secondary virus spread during the prelethal period of infection.

**KEY WORDS** *Thripinema nicklewoodi*, western flower thrips, tomato spotted wilt virus, tospovirus, virus-vector relations

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THE WESTERN FLOWER THRIPS, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), is a serious and widespread pest of over 200 vegetable and ornamental crops throughout North America, northern Europe, and elsewhere (Lewis 1997). Thrips feeding damage can reduce the esthetic quality of a crop, cause deformation of growing plants, and reduce yields (Childers and Achor 1995). Adult *F. occidentalis* can also cause greater problems as vectors of tospoviruses in the family Bunyaviridae, which are exclusively transmitted by thrips among the genera *Frankliniella* and *Thrips* (Wijkamp et al. 1995, Mound 1996). As a result of the spread of *F. occidentalis* through international trade, tomato spotted wilt virus (TSWV) now ranks among the top 10 of economically most important plant viruses affecting up to 900 plant species within 80 families (Goldbach and Peters 1994).

The spread of TSWV by *F. occidentalis* has prompted epidemiological studies with this vector. Only larval thrips (generally first instar) acquire the virus feeding on systemically infected plant material (Ullman et al. 1992, van de Wetering et al. 1996). An epithelial mid-gut barrier prevents virus acquisition by adult thrips (Ullman et al. 1992). Virus acquired by first instar larvae replicates in the vector tissue and may be transmitted by late second instars after a temperature-dependent latent period (Wijkamp and Peters 1993). However, the actual spread of TSWV among viruliferous *F. occidentalis* is probably mainly caused by the vagile and longer-lived adult stage, both sexes of which are efficient transmitters (van de Wetering et al. 1999).

The thrips parasitic nematode *Thripinema nicklewoodi* Siddiqi (Tylenchida: Allantonematidae) is under investigation for use in inoculative release strat-

egies against *F. occidentalis* infesting greenhouse floricultural crops (Lim et al. 2001, Arthurs and Heinz 2002, Mason and Heinz 2003). A possible factor in the success of this nematode is that thrips are naturally infected feeding on the plant within cryptic areas such as buds, open flowers, and foliar terminals, areas in which larger hard-bodied arthropods may have difficulty penetrating. After host infection, parasitic female nematodes reproduce via a single heterosexual generation within the host's abdominal cavity. When mature, male and female nematode progeny burrow into the lumen of the host gut and pass out of the anus over a period of time as short-lived free-living forms (Lim et al. 2001, Mason and Heinz 2003).

Unlike entomopathogenic rhabditid species among the genera *Steinernema* and *Heterorhabditis*, *T. nicklewoodi* does not release pathogenic bacteria that kill infected hosts quickly. In practical terms, however, control is often reflected in the degree of protection given to cropping areas, rather than the rate at which pests die. Thus, there may be the potential for substantial crop protection if sublethal effects of the nematode are significant. The current paper reports on laboratory studies investigating sublethal aspects of the host/nematode association. Specifically, we tested the effect of *T. nicklewoodi* infection on: 1) host feeding, 2) host fecundity, and 3) viral competency of *F. occidentalis* coinfecting with a tospovirus.

### Materials and Methods

**Source of Thrips and Nematodes.** A virus-free *F. occidentalis* colony, originating from a field population collected in alfalfa at the University of California Davis campus in 1998, was maintained on excised food-grade red kidney bean leaves, *Phaseolus vulgaris* L. (Kroger Co., Cincinnati, OH), using a modification to the method of Doanne et al. (1995). Colony boxes consisted of polypropylene containers (15 × 15 × 5 cm) containing four excised primary, nontrifoliolate bean leaves rooted in the cotton of a nondeodorant feminine napkin (Kroger Co.) saturated with 200 ml of reverse-osmosis-treated tap water and seven drops of 10-15-10 Schultz-Instant Liquid Fertilizer/500 ml (Schultz Co., St. Louis, MO). To ensure a regular supply of all *F. occidentalis* life stages, new rearing boxes were set up five times per week and maintained at 26–28°C; 60% RH (r.h.); 14L:10D photoperiod. A population of *T. nicklewoodi*, originating from infected *F. occidentalis* collected as above, was cultured in vivo within *F. occidentalis* using the methods outlined in Arthurs and Heinz (2002).

**Virus Isolate and Test Plants.** The isolate of TSWV used in our studies originated from field tobacco collected in North Carolina (Wilson County) in 2002. The isolate was mechanically transmitted to *Nicotiana benthamiana* Domin. twice before being transmitted by thrips to *Emilia sonchifolia* L. (lilac tassel flower), which served as a virus source plant for acquisition feeding. New *E. sonchifolia* were inoculated 2–3 wk after sowing by confining viruliferous adult *F. occidentalis* to foliage for 24 h. Plants were subsequently

maintained in a growth chamber at 25°C and 14L:10D photoperiod for symptom development. *Petunia × hybrida* Vilm. cultivar Summer Madness was used for virus transmission studies. Although not systemically infected, petunia is a highly sensitive local lesion host to detect TSWV (Wijkamp and Peters 1993).

**Feeding Rate Effects.** Feeding rates of individual *T. nicklewoodi* parasitized and nonparasitized adult *F. occidentalis* were compared. Thrips were infected by exposing second instar larvae (susceptible stage) to adult thrips releasing nematodes (infective stage) in 1.5-ml microcentrifuge vials. Fifty susceptible stage and four infective stage thrips were transferred to each vial using a small aspirator. Each vial contained one bean leaf disc (8-mm diameter) and a small piece of Kimwipe tissue paper (Kimberly-Clark, Roswell, NM) at the bottom to adsorb excess moisture. The central area of the vial lid was replaced with polyester fabric (32 holes/mm) for ventilation; vials were placed upright in plastic vial holders with clear tops and maintained at 100% r.h. for a 48-h nematode inoculation period. It is not known how many nematodes were exposed to thrips, although the procedure described routinely infects ≈50% of exposed thrips with nematodes (Arthurs and Heinz 2002). Equivalent vials containing only susceptible stages of thrips served as controls.

After inoculation, infective stage thrips were discarded, and thrips larvae were transferred to rearing boxes identical to those used for maintaining the thrips colony to complete their development. Rearing boxes were checked daily, and adults eclosing (8–10 d later) were immediately isolated in new microcentrifuge vials with a piece of Kimwipe at the bottom to assess feeding rates. Every 48 h until death, each thrips was supplied with a fresh 8-mm-diameter leaf disc removed from an 8- to 12-d-old bean plant, and the area of feeding damage on exposed leaf discs was assessed using image-analysis software (Jandal Scientific, SigmaScan 2.0, Chicago, IL). Leaf discs were mounted on glass slides and photographed at ×10 with a digital camera attached to the microscope. Dead thrips were individually dissected and checked for developing nematodes. Parasitic female nematodes reproduce continuously throughout the host lifetime (Arthurs and Heinz 2002), and dissections proved a reliable method to confirm the infection status of thrips. Thrips were maintained at 25°C and 14L:10D photoperiod and 100% r.h. throughout the assessments.

The experiment was repeated using a subculture of thrips maintained on chrysanthemum plants, *Dendranthema grandiflora* Tzvelev cultivar Charm, for ≈12 generations, to test for host plant effects. In this case, excised flowers and discs cut from flower petals were substituted for foliage in rearing boxes and microcentrifuge vials, respectively. Plants were 8–12 wk old and in full bloom at the time of use. Each treatment was replicated at least 40 times, with each microcentrifuge vial acting as a replicate.

**Fecundity and Viral Competency Effects.** Previous studies illustrate that *F. occidentalis* transmits TSWV when larvae are allowed short acquisition periods on

TSWV-infected leaves and subsequent inoculation periods with a susceptible host (Wijkamp and Peters 1993, Wijkamp et al. 1996, van de Wetering et al. 1999). In this study for virus acquisition,  $\approx 100$  thrips larvae ( $< 24$  h old) were confined to a leaf section of *E. sonchifolia* removed from a 4- to 5-wk-old plant showing recent symptoms of TSWV. Thrips were transferred to leaf sections ( $\approx 3$  cm<sup>2</sup>) housed in a previously described 1.5-ml microcentrifuge vial. Vials were incubated at 100% r.h. for a 24-h acquisition-feeding period. Larvae similarly caged on healthy *E. sonchifolia* served as virus controls. After acquisition feeding, thrips were removed and exposed to *T. nicklewoodi* for 48 h in new microcentrifuge vials with equivalent nematode-free vials serving as nematode controls, as described in the previous study. Thrips larvae thus exposed to two-way factorial treatments, namely infection with virus/nematode alone or in combination, were transferred to rearing boxes to complete their development.

Adult female thrips eclosing in rearing boxes were isolated in microcentrifuge vials containing one petunia leaf disc (8-mm diameter) removed from a 2- to 4-wk-old plant to assay for both fecundity and virus transmission. Wijkamp and Peters (1993) developed this petunia leaf disc method and found no difference in virus transmission rates when using the leaf discs versus whole plants. Each thrips was supplied with a fresh leaf disc every day until death. Leaf discs exposed to each 24-h inoculation access period were incubated by floatation on sterile water for 3 d in 24-well plates to develop local lesions. To assess thrips fecundity, a subsample of 20 leaf discs per treatment was mounted on a microscope slide and checked for developing eggs or newly emerged larvae. Virus transmission was then confirmed using DAS-ELISA (double Ab sandwich enzyme-linked immunosorbent assay), according to the method outlined in Wijkamp and Peters (1993). Thrips were maintained at 25°C and 14L:10D photoperiod and 100% r.h. throughout the assessments.

Thrips that were exposed to TSWV were assayed within 2 days of death, to verify thrips that were potential transmitters rather than having only fed on the virus. Ag-coated plate (ACP)-ELISA was used to identify viruliferous thrips by detecting a small nonstructural protein encoded by the small RNA of TSWV that is only present if the virus has replicated within the host (Ullman et al. 1993, Bandla et al. 1994). Dead thrips were ground up individually in 120  $\mu$ l of PBS using a microcentrifuge pestle, and the suspension was pipetted to a 96-well ELISA plate (Nunc MaxiSorp, PGC Scientifics, Gaithersburg, MD) and incubated overnight at 4°C. The samples were checked for developing nematodes to confirm the infection status of thrips. Next, antiserum directed against the TSWV nonstructural protein was used to identify viruliferous thrips, according to the method outlined in Bandla et al. (1994). For the ELISAs, reagents and antisera were purchased from Agdia (Elkhart, IN). For DAS-ELISA, a positive (from Agdia) and negative (extraction buffer) procedural

check was used. Because of the high rate of thrips mortality during exposures, the study was repeated so that each treatment was replicated at least 30 times, with a microcentrifuge vial acting as a replicate.

**Data Analysis.** The effect of nematode and host plant on the cumulative plant area damaged was assessed using a between-subjects two-way analysis of variance (ANOVA) with data normalized via a log ( $n + 1$ ) transformation. Thrips longevity and development rate (first instar to adult) were calculated using Kaplan-Meier survival analysis (SPSS for Windows 10.1, Chicago, IL), with differences between treatments tested for significance with a log rank test employing a Bonferroni correction. Fecundity was compared using a Tukey's test after a two-way ANOVA, with confirmed nematode and virus infection representing independent variables and data normalized via a log ( $n + 1$ ) transformation. Among thrips exposed to TSWV as larvae, the proportions of adults acquiring virus (i.e., testing viruliferous) as well as becoming actual virus transmitters (i.e., inoculating petunia leaf discs with TSWV at least once) were compared between nematode-infected and healthy thrips using Pearson  $\chi^2$  tests for independence (Phi and Cramer's V). Furthermore, among thrips testing viruliferous, the per capita frequency of virus transmission was analyzed using an independent sample *t*-test with arcsine-transformed data.

## Results

**Feeding Rate Effects.** Analysis of leaf discs from different feeding treatments revealed a significant effect of nematode infection ( $F_{1,89} = 168.6, P < 0.0001$ ) and host plant ( $F_{1,89} = 37.2, P < 0.001$ ) on the cumulative feeding damage per insect. The plant/nematode interaction term was not significant ( $F_{1,89} = 0.66, P = 0.42$ ). In thrips infected with *T. nicklewoodi*, a clear reduction in feeding consistent across both host plants was observed throughout the adult life span (Fig. 1). This reduced feeding among infected thrips contributed to a total per capita reduction in feeding by 87% on chrysanthemum petals and 91% on bean foliage relative to controls. The average survival of thrips among treatments is shown in Table 1. Thrips fed chrysanthemum flowers survived longer than equivalent treatments maintained on bean foliage. On both host plants, thrips infected with *T. nicklewoodi* died at a faster rate compared with paired controls, although the reduction in longevity in infected hosts was only statistically significant with thrips maintained on chrysanthemum flowers.

**Fecundity and Viral Competency Effects.** The developmental periods of thrips larvae infected by TSWV and *T. nicklewoodi* alone or in combination were unaffected, although the average longevity of adult thrips was again reduced by nematode infection (Table 2). In addition, thrips infected with nematodes were reproductively sterile. Dissections revealed that their embryos remained degenerate and no eggs were recovered from petunia leaf discs. By comparison, nematode-free thrips continued to oviposit through-

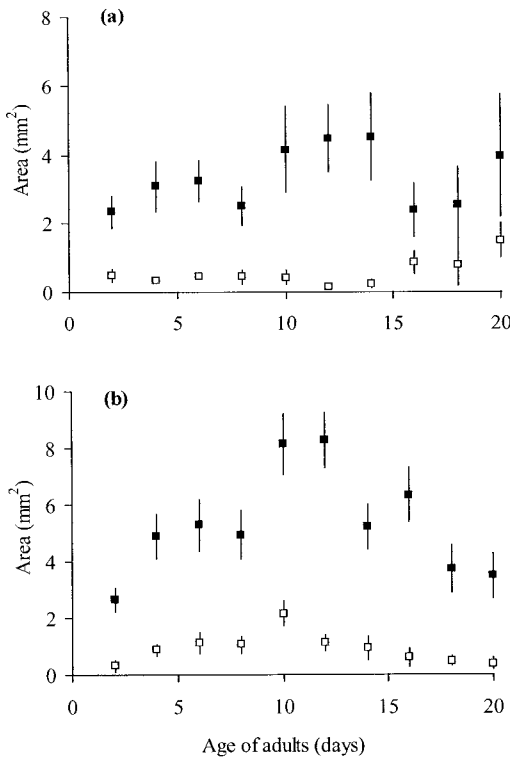


Fig. 1. Per capita feeding rates ( $\pm$ SE) of adult female *F. occidentalis* infected with *T. nicklewoodi* ( $\square$ ) and healthy controls ( $\blacksquare$ ) and maintained on (a) foliage of bean leaves and (b) chrysanthemum flowers. Feeding damage on 8-mm leaf discs was assessed at 48-h intervals using image analysis software. Data not shown after 20 d when  $>90\%$  thrips had died.

out their lifetime, with mean daily oviposition rates similar between viruliferous and nonviruliferous thrips (Table 2). Nematode infection also reduced the vector competency of thrips for TSWV (Table 3). Although the overall proportion of thrips testing positive to a TSWV nonstructural protein (i.e., indicating systemic virus acquisition) was statistically similar between treatments, fewer viruliferous *F. occidentalis* coinfecting with *T. nicklewoodi* ever became actual virus transmitters. Moreover, assessments of petunia leaf discs revealed that the per capita frequency of virus transmission among nematode-infected thrips

Table 1. Survival (days postinfection  $\pm$  SEM) of *F. occidentalis* parasitized with *T. nicklewoodi* while second instar larvae on two host plants, together with healthy controls

	Chrysanthemum flower (n = 45)	Bean foliage (n = 48)
Nematode infected	25.5 $\pm$ 0.7Aa <sup>a</sup>	20.9 $\pm$ 0.9Ab
Control	30.7 $\pm$ 1.2Ba	25.6 $\pm$ 1.3Ab

<sup>a</sup> Capital letters show difference (log rank test employing a Bonferroni correction,  $P < 0.05$ ) between nematode-infected and control thrips for a given food plant (i.e., compared vertically). Lower case letters show differences between food plants.

was reduced by  $\approx 50\%$  relative to thrips free of nematodes.

## Discussion

Given that the eradication of thrips is not always practicable, tactics that limit or prevent development of populations may be useful in thrips/tospovirus management strategies. Results from the present studies demonstrate that while *F. occidentalis* parasitized by *T. nicklewoodi* develop normally, adult female thrips infected as larvae were reproductively sterile, had substantially reduced rates of feeding, and appeared less competent vectors for TSWV. In combination, such sublethal effects suggest that introductions of *T. nicklewoodi* may help regulate thrips population growth and reduce direct damage and virus spread in existing populations. Because of the very low numbers of adult males in our cultures, they were not included in our assessments, although males may be infected by *T. nicklewoodi* (Lim et al. 2001, Mason and Heinz 2003). Field surveys over 2 yr by Funderburk et al. (2002) suggest that natural populations of *Thripinema fuscum* Tipping and Nguyen operating through host density-dependent factors suppress mid to late season populations of *Frankliniella fusca* and prevent significant secondary spread of TSWV in field-grown peanut.

The mechanisms underlying the type of physiological changes observed from *Thripinema*-infected hosts are unknown. The observation that *F. occidentalis* infected with *T. nicklewoodi* have greatly reduced or degenerate embryos was noted previously (Greene and Parrella 1995, Heinz et al. 1996, Mason and Heinz 2003), although in these studies lifetime fecundity was not specifically measured. The reduced feeding and TSWV vector competency observed among infected thrips has not been previously reported. Lysaught (1937) proposed that the preclusion of egg generation in parasitized *Aptinothrips rufus* Gmelin might be the result of protein deprivation or the release of a toxin, which damages the thrips reproductive organs. Another hypothesis is that stretch receptors in the thrips abdomen, which normally regulate oogenesis, may respond to increasing numbers of nematodes by signaling the ovaries to halt oogenesis as if maximum egg capacity had been attained (Greene and Parrella 1995). Sensory structures in the alimentary canal may respond to nematode pressure in a similar manner, which would explain the reduced feeding rates among infected hosts. Such a resource limitation may in turn explain the reduction in longevity of infected hosts observed in both studies. Thrips mortality may have also been increased by the method of confinement in microcentrifuge vials and daily handling.

Altered feeding behavior may explain the lower frequency of TSWV transmission to petunia leaf discs among viruliferous thrips coinfecting with *T. nicklewoodi*. Tospoviruses are persistently transmitted in a propagative manner in the saliva of thrips (Nault 1997). Thrips have piercing/sucking mouthparts comprising a single mandibular stylet, which extends to

**Table 2.** Effects of TSWV and *T. nicklewoodi* (alone or in combination) on *F. occidentalis* development, longevity, and reproductive output (mean  $\pm$  SEM reported for females)

Treatment <sup>a</sup>	(n)	Development period: first instar to adult (days)	Thrips adult longevity (days)	Mean daily fecundity (eggs/female) <sup>b</sup>
Nematode infected				
Viruliferous	30	9.9 $\pm$ 0.14a <sup>c</sup>	14.6 $\pm$ 0.29a <sup>c</sup>	0.0 $\pm$ 0.0a <sup>d</sup>
Nonviruliferous	57	10.3 $\pm$ 0.12a	15.4 $\pm$ 0.34a	0.0 $\pm$ 0.0a
Control				
Viruliferous	35	10.1 $\pm$ 0.1a	19.7 $\pm$ 0.59b	1.9 $\pm$ 0.1b
Nonviruliferous	46	10 $\pm$ 0.17a	18.7 $\pm$ 0.5b	1.6 $\pm$ 0.11b

<sup>a</sup> Thrips larvae (<24 h old) were sequentially exposed to TSWV-infected *E. sonchifolia* and nematodes in microcentrifuge vials.

<sup>b</sup> Observed from a daily subsample of leaf discs (n = 20).

<sup>c</sup> Letters show differences between treatments (log rank test employing a Bonferroni correction,  $P < 0.05$ ).

<sup>d</sup> Tukey's honestly significant difference,  $P < 0.05$ .

punch feeding holes, and paired maxillary stylets containing a feeding/salivary channel, which independently extend or retract during feeding (Hunter and Ullman 1992). Successful TSWV transmission requires the injection of replicated viral particles in the salivary glands into cells, which are not extensively destroyed (Wijkamp et al. 1996).

There is other evidence that TSWV transmission may depend on vector feeding rates. Van de Wetering et al. (1999) showed that the efficiency of TSWV transmission by both sexes of *F. occidentalis* dropped with age, simultaneously with the consumption rate. In field and greenhouse experiments, the reduced probing and feeding by viruliferous *F. fusca* Hinds on imidacloprid-treated pepper, tomato, and tobacco were also linked to a lower TSWV incidence (Groves et al. 2001). However, other studies suggest that thrips feeding damage alone may not accurately predict the risk of TSWV infection. Broadbent et al. (1990) reported a low correlation between the number of *F. occidentalis* feeding scars and the occurrence of TSWV among cultivars of florists chrysanthemum, indicating that the feeding activity of the vector was epidemiologically less significant than cultivar susceptibility. Moreover, despite their smaller size and reduced feeding activity, male *F. occidentalis* may transmit TSWV with a higher efficiency than females, possibly because of their higher mobility and probing frequency (van de Wetering et al. 1998, van de Wetering et al. 1999). Until the amount of saliva injected or preferably the number of infectious units entering intact plant cells can be adequately quantified, the exact relationship between host susceptibility, the

feeding behavior of the vector, and TSWV transmission may remain unclear.

In conclusion, inoculative releases of *T. nicklewoodi* may be useful as part of a thrips/virus management strategy for ornamentals in which other tactics, such as use of resistant cultivars, predatory mites, and bio-rational insecticides, are employed. As Heinz et al. (1996) consistently recovered *T. nicklewoodi* from commercial floriculture greenhouse and field operations, this thrips natural enemy appears to be compatible with existing crop production and thrips management practices. However, because *T. nicklewoodi* sterilizes, but does not kill its host, time lags in measurable thrips suppression are probable. Hence, integration of other thrips management strategies together with *T. nicklewoodi* introductions may be required to provide an effective and economical strategy for controlling *F. occidentalis* within nursery, floriculture, or field crops. Tests to evaluate the compatibility of periodic inoculations of *T. nicklewoodi* and spinosad to prevent outbreaks of *F. occidentalis* in a simulated chrysanthemum-production system are planned.

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**Table 3.** Effect of *T. nicklewoodi* on the vector competency of adult female *F. occidentalis* for TSWV

	Proportion of TSWV-exposed thrips acquiring virus (n = 168) <sup>a</sup>	Proportion of viruliferous thrips becoming transmitters (n = 65) <sup>b</sup>	Per capita frequency of transmission
Nematode infected	0.34a <sup>c</sup>	0.53a <sup>c</sup>	0.22 $\pm$ 0.05a <sup>d</sup>
Control	0.43a	0.77b	0.42 $\pm$ 0.06b

<sup>a</sup> Thrips exposed to TSWV-infected *E. sonchifolia* as larvae were assayed with ACP-ELISA at death

<sup>b</sup> Petunia leaf discs exposed to thrips at 24-h intervals were assayed with DAS-ELISA.

<sup>c</sup> According to a Pearson  $\chi^2$  test for independence ( $P < 0.05$ ).

<sup>d</sup> Mean  $\pm$  SEM; letters according to *t*-test on arcsine-transformed frequency data ( $P < 0.05$ ).

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### References Cited

- Arthurs, S., and K. M. Heinz. 2002. In vivo rearing of *Thripinema nicklewoodi* (Tylenchida: Allantonematidae) and prospects as a biological control agent of *Frankliniella occidentalis* (Thysanoptera: Thripidae). *J. Econ. Entomol.* 95: 668–674.
- Bandla, M. D., D. M. Westcot, D. E. Ullman, T. L. German, and J. L. Sherwood. 1994. Use of monoclonal antibody to the nonstructural protein encoded by the small RNA of the tomato spotted wilt tospovirus to identify viruliferous thrips. *Phytopathology* 84: 1427–1431.
- Broadbent, A. B., J. A. Matteoni, and W. R. Allen. 1990. Feeding preferences of the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera, Thripidae), and incidence of tomato spotted wilt virus among cultivars of florists' chrysanthemum. *Can. Entomol.* 122: 1111–1117.
- Childers, C. C., and D. S. Achor. 1995. Thrips feeding and oviposition injuries to economic plants, subsequent damage and host response to infestation, pp. 31–51. In B. L. Parker, M. Skinner, and T. Lewis (eds.), *Thrips biology and management*, vol. 276. NATO ASI Series A: Life Sciences, New York.
- Doanne, E. N., B. L. Parker, and Y. Pivot. 1995. Method for mass rearing even-aged western flower thrips on beans, pp. 587–593. In B. L. Parker, M. Skinner, and T. Lewis (eds.), *Thrips biology and management*, vol. 276. NATO ASI Series A: Life Sciences, New York.
- Funderburk, J., J. Stavisky, C. Tipping, D. Gorbet, T. Momol, and R. Berger. 2002. Infection of *Frankliniella fusca* (Thysanoptera: Thripidae) in peanut by the parasitic nematode *Thripinema fuscum* (Tylenchida: Allantonematidae). *Environ. Entomol.* 31: 558–563.
- Goldbach, R., and D. Peters. 1994. Possible causes of the emergence of tospovirus diseases. *Semin. Virol.* 5: 113–120.
- Greene, I. D., and M. P. Parrella. 1995. Two new natural enemies of western flower thrips in California, pp. 277–283. In B. L. Parker, M. Skinner, and T. Lewis (eds.), *Thrips biology and management*, vol. 276. NATO ASI Series A: Life Sciences, New York.
- Groves, R. L., C. E. Sorenson, J. F. Walgenbach, and G. G. Kennedy. 2001. Effects of imidacloprid on transmission of tomato spotted wilt tospovirus to pepper, tomato and tobacco by *Frankliniella fusca* Hinds (Thysanoptera: Thripidae). *Crop Prot.* 20: 439–445.
- Heinz, K. M., L. M. Heinz, and M. P. Parrella. 1996. Natural enemies of western flower thrips indigenous to California ornamentals. *IOBC WPRS Bull.* 19: 51–54.
- Hunter, W. B., and D. E. Ullman. 1992. Anatomy and ultrastructure of the piercing-sucking mouthparts and paraglossal sensilla of *Frankliniella occidentalis* (Pergande) (Thysanoptera, Thripidae). *Int. J. Insect Morphol. Embryol.* 21: 17–35.
- Lewis, T. 1997. Pest thrips in perspective, pp. 1–13. In T. Lewis (ed.), *Thrips as crop pests*. CAB International, Wallingford, United Kingdom.
- Lim, U. T., R. G. Van Drieche, and K. M. Heinz. 2001. Biological attributes of the nematode, *Thripinema nicklewoodi*, a potential biological agent of western flower thrips. *Biol. Contr.* 22: 300–306.
- Lysaught, A. M. 1937. An ecological study of a thrips (*Aptinothrips rufus*) and its nematode parasite (*Anguillulina aptini*). *J. Anim. Ecol.* 6: 169–192.
- Mason, J. M., and K. M. Heinz. 2002. Biology of *Thripinema nicklewoodi* (Tylenchida), an obligate *Frankliniella occidentalis* (Thysanoptera) parasite. *J. Nematol.* 34: 332–339.
- Mound, L. A. 1996. The Thysanoptera vector species of tospoviruses. *Acta Hort.* 431: 299–309.
- Nault, L. R. 1997. Arthropod transmission of plant viruses: a new synthesis. *Ann. Entomol. Soc. Am.* 90: 521–541.
- Ullman, D. E., J. J. Cho, R. F. L. Mau, D. M. Westcot, and D. M. Custer. 1992. A midgut barrier to tomato spotted wilt virus acquisition by adult western flower thrips. *Phytopathology* 82: 1333–1342.
- Ullman, D. E., T. L. German, J. L. Sherwood, D. M. Westcot, and F. A. Cantone. 1993. Tospovirus replication in insect vector cells: immunocytochemical evidence that the nonstructural protein encoded by the S RNA of tomato spotted wilt virus is present in thrips vector cells. *Phytopathology* 83: 456–463.
- Van de Wetering, F., R. Goldbach, and D. Peters. 1996. Tomato spotted wilt tospovirus ingestion by first instar larvae of *Frankliniella occidentalis* is a prerequisite for transmission. *Phytopathology* 86: 900–905.
- Van de Wetering, F., J. Hulshof, K. Posthuma, P. Harrewijn, R. Goldbach, and D. Peters. 1998. Distinct feeding behavior between sexes of *Frankliniella occidentalis* results in higher scar production and lower tospovirus transmission by females. *Entomol. Exp. Appl.* 88: 9–15.
- Van de Wetering, F., M. van der Hoek, R. Goldbach, and D. Peters. 1999. Differences in tomato spotted wilt virus vector competency between males and females of *Frankliniella occidentalis*. *Entomol. Exp. Appl.* 93: 105–112.
- Wijkamp, I., N. Almarza, R. Goldbach, and D. Peters. 1995. Distinct levels of specificity in thrips transmission of tospoviruses. *Phytopathology* 85: 1069–1074.
- Wijkamp, I., and D. Peters. 1993. Determination of the median latent period of two tospoviruses in *Frankliniella occidentalis* using a novel leaf disk assay. *Phytopathology* 83: 986–991.
- Wijkamp, I., F. van de Wetering, R. Goldbach, and D. Peters. 1996. Transmission of tomato spotted wilt virus by *Frankliniella occidentalis*; median acquisition and inoculation access period. *Ann. Appl. Biol.* 129: 303–313.

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