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Biology of *Thripinema nicklewoodi* (Tylenchida), an Obligate *Frankliniella occidentalis* (Thysanoptera) Parasite

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Abstract: *Frankliniella occidentalis,* a serious pest of agricultural crops, is difficult to manage because chemical and biological control measures frequently fail to affect *F. occidentalis* in their preferred microhabitats. Parasitism by the host-specific, entomopathogenic nematode *Thripinema nicklewoodi* may provide a much-needed alternative to current control strategies. Infection does not cause death of the host; rather, the result is sterilization that leads to suppression of *F. occidentalis* populations. We describe a simple rearing method and the results from studies aimed at providing details on its biology—both essential first steps to examining its biological control potential. All *F. occidentalis* life stages are susceptible to infection, but to varying degrees (most susceptible to least susceptible): female pupae, second instar larvae, first instar larvae, male pupae, adult females, adult males. Nematodes emerge from female and male *F. occidentalis* for approximately 15 and 9 days, with approximately 14 and 7 nematodes emerging per day, respectively. Females and males are short-lived outside of the host, with mean survival rates ranging between 7 and 86 hours. Transmission does not occur in the soil but rather on or within plant structures that are preferred microhabitats visited by *F. occidentalis.* Results from a dose-response study suggest that augmentative applications of *T. nicklewoodi* may be useful to generate increased infection rates and subsequent suppression of *F. occidentalis* populations.

Key words: biological control, entomopathogenic nematodes, *Thripinema nicklewoodi,* western flower thrips.

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is the most prevalent species of thrips attacking greenhouse crops (Robb, 1989). In floriculture crops, feeding damage reduces the aesthetic quality of plants, making the crop less valuable or even totally unmarketable (Parrella and Jones, 1987). Because thrips are small and because they feed within young, terminal foliage and developing flowers, early detection and subsequent control are difficult. While the ultimate damage from WFT is easily identified, the critical early stages of damage often go undiscovered until a large thrips population is present.

The thigmotactic behavior of WFT makes it difficult for most predators, parasitoids, and pathogens to locate this pest in its preferred microhabitats within floricultural crops Loomans et al., 1995; Loomans and van Lenteren, 1996; Parrella and Murphy, 1996). Entomopathogenic nematodes, however, have successfully controlled pests in situations that protect them from desiccation, radiation, and temperature extremes (Begley, 1990; Kaya, 1985), exactly the situations represented by tight flower buds and meristematic tissues where thrips control by insecticides is most difficult. Biological control research of thrips by entomopathogenic nematodes has focused on the families Steinernematidae and Heterorhabditidae (Chyzik et al., 1996; Helyer et al., 1995; Tomalak, 1991, 1994). Results from these studies suggest that thrips biological control using these nematodes is highly variable, with additional thrips mortality factors necessary to achieve acceptable levels of biological control.

The first record of parasitic nematodes of thrips (Thysanoptera) is from Europe when Uzel (1895) observed an unnamed nematode within the body cavity of *Thrips physapus* L. The first description was made by Sharga (1932) in the United Kingdom, who described a nematode from *Aptinothrips rufus* Gmelin as *Tylenchus aptini.* Since then, there have been a number of reports of nematodes from thrips (Lysaght, 1936; Nickle and Wood, 1964; Reddy et al., 1982; Wilson and Cooley, 1972). The genus *Thripinema* (Tylenchida: Allantonematidae) was erected by Siddiqi (1986) during a taxonomic revision of the species, and currently there are five described species of *Thripinema: T. reniraoi* (Reddy, Nickle and Rao, 1982) Siddiqi 1986; *T. aptini* (Sharga, 1932) Siddiqi 1986; *T. nicklewoodi* (Nickle and Wood, 1964) Siddiqi 1986; *T. khrustalevi* Chizhov, Subbotin & Zakharenkova 1995; and *T. fuscum* (Tipping et al., 1998). Despite the numerous reports of *Thripinema* species, little is known of the biology of these species. Most publications are either taxonomic or report the incidental finding of *Thripinema* species in a number of different thrips species. An exception is the first published study on *Thripinema* species (Lysaght, 1937), which reported observations on the biology and seasonal distribution of *T. aptini* (formerly *A. aptini*).

Thripinema species are considered obligate parasites of thrips, and our current understanding of *Thripinema* biology suggests that the life cycle of each species is similar. Following infection, the females develop within the abdominal cavity, although there are also reports of nematodes having been observed within the thorax (Reddy et al., 1982; Sharga, 1932). Following development and maturation, the female parasitic nematode oviposits eggs into the thrips haemocoel. Upon hatching, the vermiform juveniles feed on fluids within the thrips' abdominal cavity. When mature, both male and female nematodes penetrate into the lumen of the

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hosts' gut and leave the host via the anus in the frass (Greene and Parrella, 1993; Lysaght, 1936; Sharga, 1932). Little is known about the males and females on emergence from the host, although they are not considered to be true free-living stages.

The number of female parasitic nematodes per thrips host is generally only one (Tipping et al., 1998), although there are reports of up to 10 per host (Chizhov et al., 1995). Parasitized thrips appear unable to produce viable eggs. Examination of parasitized thrips reveals that their ovaries are generally much reduced or absent (Nickle and Wood, 1964; Sharga, 1932; Tipping et al., 1998). Lysaght (1936) proposed that sterilization may result either from protein deprivation to the thrips caused by the developing nematodes or perhaps from a toxin released by the nematodes that damages the thrips reproductive organs. It is unclear if the behavior of parasitized thrips is affected, although Sharga (1932) did note that heavily infected thrips displayed sluggish behaviour. Teulon et al. (1997) suggested that the longevity of parasitized *Thrips obscuratus* (Crawford) was not affected; however, the sample size of parasitized thrips was small.

In a survey for natural enemies of *F. occidentalis* (Pergande) (WFT) in California floriculture and nursery crops, Heinz et al. (1996) reported that *T. nicklewoodi* was the numerically dominant natural enemy of this thrips species. Given the severity of WFT as a pest of many different crops, the onset of pesticide resistance as an emerging problem (Brodsgaard, 1994; Robb et al., 1995), and a lack of effective WFT biological control options (Jacobson, 1997), the use of *T. nicklewoodi* may provide a biologically interesting potential alternative control measure for WFT. However, if *T. nicklewoodi* is to be examined as a potential biological control agent of WFT, it is imperative that its basic biology be examined and quantified. Here, we provide the first detailed quantitative studies on the biology of *T. nicklewoodi* and details on a simple rearing method for *T. nicklewoodi* in WFT.

Materials and Methods

Plant material: Kidney bean plants *(Phaseolus vulgaris)* were grown four per pot (15-cm-diam. 15-cm-depth) filled with Sunshine #1 potting mix (Sun Gro Horticulture, Bellevue, WA) and were watered as needed. Plants were 14 to 21 days old at time of use in the *F. occidentalis* culture. Chrysanthemum plants (*Dendranthema grandiflora* cv. Golden Polaris) were grown individually in 10-cm-diam. pots filled with Sunshine #1 potting mix and watered as needed. Plants were approximately 12 weeks old at time of use in experiments and were in full bloom.

Frankliniella occidentalis culture: An *F. occidentalis* colony, established from a collection made from alfalfa *(Medicago sativa)* on the campus of the University of California at Davis in September 1998, was maintained on kidney bean. The rearing method was as follows. Each rearing chamber consisted of a polypropylene container (15-cm square by 5-cm high) containing four bean leaves supported in a non-deodorant feminine napkin saturated with purified water. Streaking with honey and adding bee pollen to their surface supplemented the bean leaves. Twenty female and five male adult WFT were then added to each rearing chamber. The container was sealed with a tight-fitting lid, which had a hole cut in the center and was covered with a fine mesh polyester material. The WFT were maintained at 27 °C with a 14-hour-light: 10-hour-dark photoperiod. Using this rearing system, second instar larvae and pupae were available on approximately days 9 and 13 after inoculation, respectively.

Rearing of Thripinema nicklewoodi: A population of WFT infected with *T. nicklewoodi* was collected in September 1998 from alfalfa at the University of California at Davis campus. *Thripinema nicklewoodi* is maintained in WFT, as this nematode is an obligate parasite of thrips. The culturing method was as follows. After adding approximately 60 second instar and (or) female pupae of WFT to a standard infection vial, three nematode-infected WFT from which nematodes were actively emerging (with nematode emergence having begun no more than 5 days previously) were also added to the vial. After 3 days' incubation at 23 °C, all WFT were removed from the vial and placed in a polypropylene container (as used to maintain the WFT colony) that contained one kidney bean leaf streaked with honey and pollen added to the surface. These were then maintained at 23 °C for 9 days to allow the WFT to mature to adults. The emergence of nematodes from WFT was monitored as follows. On day 12 post-infection, all WFT were placed individually in standard infection vials. Every second day, the WFT were removed and added to a fresh vial. The used vials were filled with water and the contents of the water checked under a dissecting microscope for the presence of *T. nicklewoodi* females and males.

Standard infection method/vial: The standard infection vial consisted of a 1.5-ml vial with the central area of the lid removed. A small piece (ca. 1 × 4 cm) of moist tissue was placed in the bottom of each vial to maintain humidity. As a food source, one 11-mm-diam. kidney bean leaf disk streaked with honey was added to the vial. Infected adult female WFT were housed individually in each vial, with uninfected WFT then added as required. A piece of fine mesh material was placed over the top of the tube and held in place by closing the lid of the vial. Vials were then stored in a polypropylene container (as used to maintain the WFT colony). The adult WFT were transferred to fresh vials every second to third day, as required.

WFT stage susceptibility: The susceptibility of each WFT life stage to infection by *T. nicklewoodi* was assessed.

Sixty first instar, second instar, female pre-pupae and pupae, male pre-pupae and pupae, adult females, or adult males were placed as single-stage cohorts in a standardized infection arena. Three infected adult female WFT from which nematodes were emerging were then added to each vial. After 72 hours, all WFT were dissected and the status of nematode infection assessed. There were two replicates per WFT life stage. The nematode-infected adult WFT used in this study were those from which nematodes had been emerging for approximately the same length of time (approximately 3 days) prior to use. This allows the general assumption that the mean number of nematodes emerging from each WFT was approximately the same, allowing comparison of infection between the different WFT life stages.

Development of T. nicklewoodi in WFT: The development of *T. nicklewoodi* in female WFT was examined at 23 °C. Second instars were infected with *T. nicklewoodi* as follows. A 5–µl droplet of water was placed on an 11-mm-diam. kidney bean leaf disc. Approximately 40 female and 5 male *T. nicklewoodi* were then added to the water droplet. The leaf disk was then transferred to a 1.5-ml vial, and 60 larvae were added to the vial. To allow development to be assessed every 24 hours until nematode emergence from the WFT began (on approximately day 12 when maintained at 23 °C), 14 infection vials were set up. After 24 hours, the WFT from each vial were placed in a polypropylene rearing box containing two kidney bean leaves. Approximately 60 WFT were dissected every 24 hours and the stage of nematode development assessed. In addition, changes in development of the host's internal organs also were assessed.

Emergence of T. nicklewoodi from female and male WFT: Thirty adult female and 14 adult male WFT, infected as late second instars using the standard infection method, were housed individually in 1.5-ml vials containing a small piece of moist tissue and an 11-mm-diam. kidney bean leaf disk streaked with honey. Controls consisted of 30 adult females and 14 adult males sampled from the same initial WFT colony as the larvae used for infection, placed individually in 1.5-ml vials. Thus all WFT were of approximately the same age. The vials were maintained at 23 °C. Every 24 hours, until nematode emergence ceased, each WFT was removed and placed in a fresh vial. The used vials were rinsed with water and the contents examined for the presence of *T. nicklewoodi*.

Survival of T. nicklewoodi following emergence: The survival of males and females of *T. nicklewoodi* following emergence was examined at a range of temperatures: 3, 7, 9, 14, 23, and 30 °C. Male and female nematodes were collected within 2 hours of emergence from infected WFT. Approximately 20 to 30 females or 5 to 10 males were placed in small watch glasses containing water (to prevent desiccation) and incubated at the assigned temperature, with two to three replicates per temperature. Following incubation, nematodes were removed and the number living recorded. Nematodes not responding to mechanical stimulation were considered dead.

Site of transmission: The site of transmission of *T. nicklewoodi* on WFT-infested chrysanthemum plants was examined at 23 °C. All plants used were in full bloom. To prevent WFT from pupating in the soil mix, a thin layer of plaster of paris, approximately 3 mm deep, was poured onto the top of the compost in each pot to seal the interface. Then, a thin layer of clean moist sand (8% v/v), approximately 3 mm deep, was placed on top of the plaster of paris once it had set. The plants were then infested with WFT by transferring approximately 160 first/second instars randomly onto the flowers and leaves and approximately 50 male and female pupae randomly onto the surface of the sand. The plant was then placed in a thrips-proof cage (27-cm diam. by 32-cm height ventilated by one 14.5-cm and two 5.5-cm-diam. openings covered with very fine mesh cloth on the sides. Approximately 2 hours post-infestation, 10 female WFT from which nematodes were emerging were then randomly placed on the plant. Four plants were inoculated with *T. nicklewoodi*-infected WFT and two plants acted as controls, with the exception that 10 uninfected adult female WFT were used. After 4 days, all WFT were aspirated from the flowers and leaves of each plant. Thrips larvae were not kept separate as it was noted that many of the WFT that had been placed on the leaves migrated to the flowers as these were the majority of WFT recovered. Thus, we would have been unable to differentiate if infection had occurred in the flower or on the leaf. However, the adult WFT, having emerged from those pupae placed on the sand surface, were kept separate. All WFT collected were dissected and the status of nematode infection assessed. Each plant was then destructively sampled to determine on which plant structures nematodes were to be found. The leaves, flowers, and stems were separated and placed in plastic containers (13 cm square by 7 cm high). Each plant structure was soaked in water for approximately 4 hours, removed, washed with water, and then discarded. The water in each extraction container was then checked for the presence of nematodes. To check for the presence of nematodes in the sand, the sand was removed from each pot and placed in a 500-ml glass beaker, and 100 ml of water was added and the beaker agitated for approximately 20 seconds. The supernatant was then decanted and the procedure repeated. The combined supernatants were then checked for the presence of nematodes.

Effect of initial T. nicklewoodi dosage on infection of WFT: The effect of initial nematode dosage on the number of WFT infected was examined at 23 °C. The method used to set up infections is described previously. The number of female and male nematodes added to each water

droplet varied, with the numbers recorded, which ranged from 12 to 46 females and 0 to 18 males. Approximately 60 late second instars were added to each vial. After 48 hours, the WFT were dissected to assess status of infection.

Statistical analyses: All statistical analyses were performed using generalized linear modelling in the statistical package GLIM 3.77 (Numerical Algorithms Group Ltd., Oxford, U.K.) The error structures used during analyses were used in conjunction with their default link functions: binomial errors—logit link, normal errors—identity link, Poisson errors—log link (Crawley, 1993). Mortality data from the stage susceptibility study were analyzed by ANOVA, using a binomial error structure. Intensity of infection data also was analyzed by ANOVA but using a Poisson error structure. The data for survival of male and female *T. nicklewoodi* following emergence from WFT were analyzed by ANCOVA, using a binomial error structure. The data are presented in the figures as the line of best fit from the model obtained during analyses and were prepared using TableCurve 2D (SPSS Inc., Chicago, IL). Estimates of the survival times for 50% of females and males were calculated using Fieller's theorem, and are presented along with their 95% confidence intervals (Crawley, 1993). The dose response data was analyzed by linear regression using a normal error structure. All other data were analyzed by ANOVA using a normal error structure. Significance is reported at the 5% level.

Results

WFT stage susceptibility: Thripinema nicklewoodi was found to infect all six WFT life stages tested, namely, first and second instars, female pre-pupae and pupae, male pre-pupae and pupae, adult females and adult males (Table 1). This is the first report of *T. nicklewoodi* infecting male thrips. Significant differences in the numbers of infected WFT were observed between the different life stages (Table 1), with the highest level of infection observed in female pupae and the lowest in adult male WFT, with 54.0% and 4.4% infected, respectively. Conversely, the mean intensity of infection was found not to differ significantly between the life stages (Table 1). One or two infective nematodes were commonly recovered from infected WFT, with the upper maxima tending to be greater in the immature stages of WFT compared to the adults.

Development of T. nicklewoodi in WFT: Details on the development of *T. nicklewoodi* within WFT at 23 °C are given in Table 2. Following infection of the second instars, the first eggs of *T. nicklewoodi* were observed 5 days later within the abdomen. Egg production continued until after the first nematode emergence from the WFT. The mean number of eggs produced by each parasitic female at any one time was five eggs per female (SEM = 0.41) nematode. The second-stage juveniles (J2s) were observed within the abdomen on day 9, third-stage juveniles (J3s) on day 10, and fourth-stage (J4) immature females and males on day 11. Nematodes were observed only within the WFT abdomen, even in heavily parasitized WFT (the highest number of parasitic females observed was four). Emergence of the mature females and males from the host began on day 12. Both female and male nematodes were observed within the gut of WFT, with emergence from the host via the frass. The generation time for *T. nicklewoodi* in WFT, from initial infection to emergence of progeny, is approximately 12 days at 23 °C. Based on these life-cycle measurements, *T. nicklewoodi* completes only one heterosexual generation within WFT.

Table 1. Mean percentage of 60 WFT by life stage infected and the mean number of infective *T. nicklewoodi,* the intensity of infection, recovered per infected WFT.

WFT life stage	Mean WFT infected (%)	Mean (± 1 s.e.) intensity of infection[a,b]
female pupae	54.0 a	1.57 (±0.23)a (1–8)
L2	36.4 ab	1.79 (±0.26)a (1–6)
L1	36.2 ab	1.57 (±0.21)a (1–5)
male pupae	28.8 b	2.15 (±0.30)a (1–6)
female adults	13.8 c	1.33 (±0.19)a (1–3)
male adults	4.4 c	1.25 (±0.25)a (1–2)

[a] The range is given in parentheses on the second line.
[b] *n* = two replicates per life stage.

Table 2. The developmental cycle of *T. nicklewoodi* within WFT at 23 °C.

Day	Developmental stages observed within WFT abdomen
1	Parasitic females—no visible change.
2	Parasitic females starting to swell—appear slightly wider but not shorter.
3	Parasitic females are visibly shorter and wider.
4	Parasitic females are approximately five times as long as wide. Gut appears non-functional (i.e., mouth and oesophagus appear detached).
5	Parasitic females appear mature—approximately four times as long as wide. Eggs, of different developmental stages, visible in parasitic females. Eggs being laid into WFT abdomen. Each parasitic female lays a mean (± s.e.) of 5 (0.41) eggs at any one time.
6	Mature parasitic females and eggs. Mean of 19.5 (2.12) eggs within the abdomen. Eggs contain J1s.
7	Mature parasitic females and eggs (J1 and J2). Mean of 75.6 (4.96) eggs.
8	Mature parasitic females and eggs (J1 and J2). Mean of 97.0 (10.87) eggs.
9	Mature parasitic females, eggs (J1 and J2), J2.
10	Mature parasitic females, eggs (J1 and J2), J2, J3.
11	Mature parasitic females, eggs (J1 and J2), J2, J3, immature J4 males and females.
12	Mature parasitic females, eggs (J1 and J2), J2, J3, immature J4 males and females, mature males and females. Emergence from WFT begins.
13	Mature parasitic females, eggs (J1 and J2), J2, J3, immature J4 males and females, mature males and females.
14	Mature parasitic females, eggs (J1 and J2), J2, J3, J4 immature males and females, mature males and females.

[a] Numeric values represent means ± 1 SEM.
[b] The developmental stages of *T. nicklewoodi* juveniles are depicted as J1–J4.

In each of the nematode-infected WFT examined, the ovaries of the host were severely stunted, or absent, compared with uninfected WFT. However, in a few of the infected hosts, WFT eggs were observed, although only one egg per WFT was ever observed. The viability of these eggs is unknown. No other morphological differences were noted.

Emergence of T. nicklewoodi from female and male WFT: Significantly higher numbers of females than males of *T. nicklewoodi* emerged from female WFT (Fig. 1A), with a mean sex ratio of 5.5 females for every male. In general, the numbers emerging decreased over time, with frequently a large number emerging just prior to death of the WFT host. This is especially obvious in Figure 1A on day 16 and through days 20 to 22. The mean number of days over which emergence occurred was 15.4 (Table 3), with an overall mean of approximately 14 nematodes per day. The total number of nematodes emerging ranged from 77 to 345, with the number of females ranging from 57 to 302 (Table 3). Under the optimal conditions provided during this study, no difference in overall mean survival was noted between infected and uninfected WFT at 15.40 (± 0.98) and 16.97 (± 1.27) days, respectively.

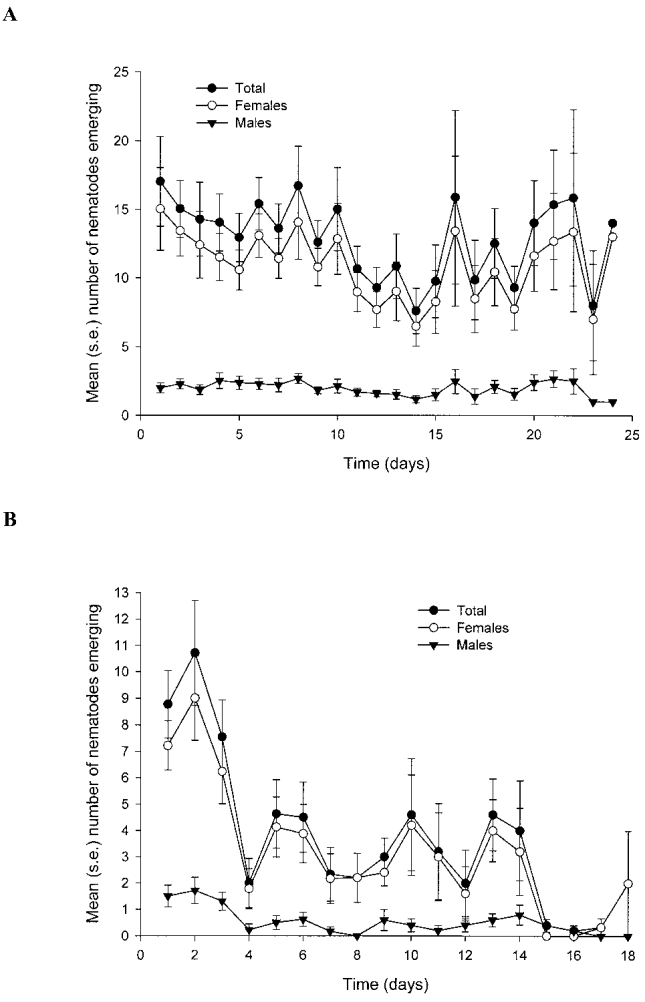

FIG. 1. The mean (± 1 SEM) numbers of females (○) and males (▼), and the total (●) mean numbers of *T. nicklewoodi* emerging from female (A) and male (B) WFT from time of first emergence though death of the host (n = 30 female host WFT and 14 male host WFT).

The emergence of nematodes from male WFT began on approximately day 12, the same as observed for female WFT. The numbers of nematodes emerging per day was much lower than from female WFT (Fig. 1). Again, higher numbers ($P < 0.05$) of females than males emerged from male WFT (Fig. 1B), with the overall sex ratio also the same as noted for female WFT. The total number of nematodes emerging from male WFT ranged from 15 to 87, with an average of approximately 7 (± 1.00) nematodes per day (Table 3), lower ($P < 0.05$) than from female WFT. Emergence occurred for a mean of approximately 9 (SEM = 1.79) days; in addition, it was common that no nematodes emerged for 2 to 3 days at a time. Survival of infected and uninfected males was not different ($P > 0.05$), with mean life spans (± 1 SEM) of 8.79 (± 1.79) and 12.71 (± 1.64) days, respectively.

Survival of T. nicklewoodi following emergence: Survival of *T. nicklewoodi* females decreased ($P < 0.05$) with increasing temperature (Fig. 2A). At 3 °C, survival was higher ($P < 0.05$) than at the other temperatures, with 100% survival even after approximately 38 hours. A comparison of survival after 38 hours at the other temperatures demonstrated that 100% mortality had occurred at both 22 and 30 °C. Survival at 30 °C was poorer ($P < 0.05$) than at the other test temperatures, with 100% mortality observed within approximately 20 hours. The above trends are also reflected in the estimated S_{50} values (Table 4), with a value of 86.4 hours at 3 °C, which is about 2.5 times higher than the value of 33.7 hours at 7 °C.

The survival of male *T. nicklewoodi* (Fig. 2B) followed the same general trends as observed for the females. Survival of the males was generally higher than for females, with the only exception being at 3 °C. This is again reflected in the estimated S_{50} values (Table 4).

Site of transmission: Of the 640 larvae added to the flowers/leaves and 200 pupae added to the sand in four replicate plants, approximately 450 larvae/pupae (primarily from flowers; none in sand) and 158 adults (not including recovery of 31 of the originally infected females) were subsequently recovered in total. Dissection of all recovered WFT revealed that only four pupae and one larva were infected with *T. nicklewoodi,* with each harboring only one nematode. However, examination of each of the plant structures for *T. nicklewoodi* revealed large numbers of nematodes, especially in the flowers. Pooling across replicates, 729 nematodes were recovered from flowers, 48 from leaves, and zero from the stems and sand. In other words, approximately 93.7% (± 3.7) of the nematodes were recovered from the flowers.

Effect of initial T. nicklewoodi dosage on infection of WFT: The mean number of WFT infected by *T. nicklewoodi*

TABLE 3. The emergence of female and male of *T. nicklewoodi* from individual female and male WFT.

Parameter	Female WFT (n = 30)			Male WFT (n = 14)		
	Female nematodes	Male nematodes	Total nematodes	Female nematodes	Male nematodes	Total nematodes
Mean (±s.e.) number	12.20 ± 0.84	2.24 ± 0.20	14.37 ± 0.98	5.86 ± 0.83	1.07 ± 0.19	6.92 ± 1.00
Emerging/day[1]	(0–80)	(0–10)	(0–93)	(0–24)	(0–5)	(0–29)
Mean (±s.e.) number of	15.40 ± 0.98	15.40 ± 0.98	15.40 ± 0.98	8.79 ± 1.79	8.79 ± 1.79	8.79 ± 1.79
Emergence days	(7–24)	(7–24)	(7–24)	(2–18)	(2–18)	(2–18)
Mean (±s.e.) total number emerging	174.27 ± 11.41 (57–302)	31.47 ± 1.89 (12–47)	205.07 ± 12.89 (77–345)	36.50 ± 4.42 (15–87)	6.57 ± 1.06 (1–13)	43.07 ± 5.31 (15–87)

[1] The range is given in parentheses.

females (males do not infect WFT) generally increased as the initial nematode dosage was increased (Fig. 3A). However, the relationship was not strongly linear, with a correlation coefficient of r = 0.331. In contrast, the relationship between initial nematode dosage and the subsequent mean number of nematodes establishing in WFT are strongly linear, with r = 0.817 (Fig. 3B). Furthermore, examination of the regression line equation (Fig. 3) suggests that approximately 16% of the initial dosage of females infect the WFT.

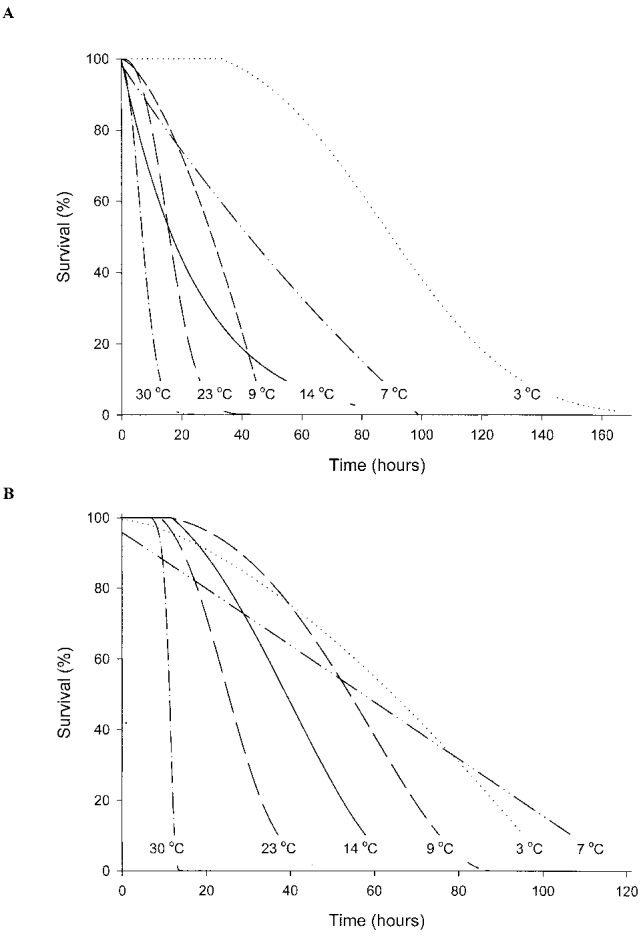

FIG. 2. The effect of temperature on the survival of female (A) and male (B) *T. nicklewoodi* following emergence from WFT. Shown are individual survivorship lines for each of the six temperature regimes used in the studies.

DISCUSSION

A simple infection and rearing method for *T. nicklewoodi,* an obligate parasite of WFT was developed. Using our rearing method, we can routinely infect 60 to 85% of WFT. Furthermore, we have successfully maintained *T. nicklewoodi* continuously in culture for more than 17 months, representing approximately 34 generations to date, assuming a generation time of approximately 2 weeks at 23 °C. The development of this simple rearing method has allowed us to proceed to further examine the biology of *T. nicklewoodi.* This is a crucial step as this nematode may have potential use for the biological control of WFT.

The females of *T. nicklewoodi* were shown to infect all WFT life stages, although differences in stage susceptibility were noted, with female pupae the most susceptible to infection. The order of stage susceptibility (from most to least susceptible) was female pupae > second instars > first instars > male pupae > adult females > adult males. In addition, this is the first species noted to infect all thrips life stages. *Thripinema fuscum* has been recovered only from adults of *Frankliniella fusca* (Hinds) and, rarely, from second instar larvae (Tipping et al., 1998), while *T. khrustalevi* was found parasitizing larvae of *Thrips trehernei* and *Thrips physopus*

TABLE 4. Mean times to attain 50% cohort survival (S_{50}) for *T. nicklewoodi* females and males emerging from WFT in water at six different constant temperatures.

Temperature (°C)	S_{50}^{a}	
	Females	Males
3	86.4 (78.5–94.4)	61.6 (45.5–78.8)
7	33.7 (30.2–38.2)	56.1 (37.6–77.3)
9	32.7 (26.1–39.4)	49.1 (38.0–65.3)
14	27.9 (22.4–32.5)	40.6 (35.2–50.0)
22	17.7 (13.2–19.8)	27.6 (25.6–28.5)
30	7.3 (6.6–8.0)	10.3 (8.9–12.0)

[a] Hours, 95% confidence interval.

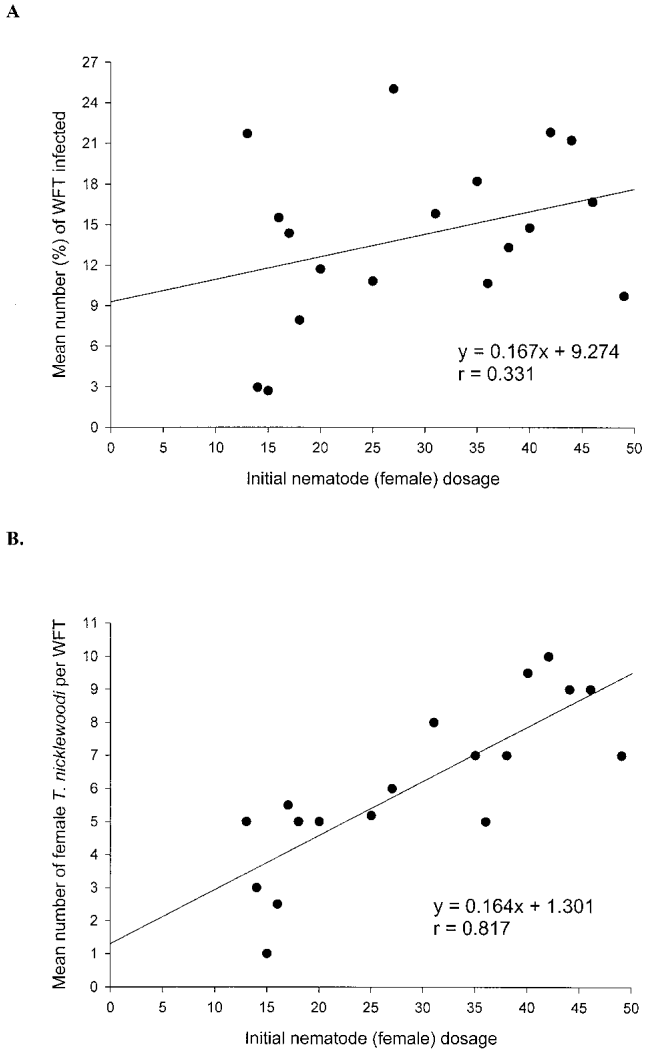

FIG. 3. The effect of initial nematode dosage on the mean percentage of WFT infected (A) and on the mean number of female *T. nicklewoodi* infecting WFT (B).

but was not found in adults (Chizhov et al., 1995). That *T. nicklewoodi* infected male WFT was somewhat surprising as it was generally considered that male WFT are not infected, as infected males had never been recovered from field collections (Lysaght, 1937; Nickle and Wood, 1964; Mason, pers. obs.). Furthermore, we have shown that, following infection, the parasitic females can establish, develop, and reproduce within male WFT. The numbers of nematodes subsequently emerging from male WFT are significantly lower than from females. For example, a mean total of approximately 205 nematodes from females compared with 43 from males. However, whether or not *T. nicklewoodi* infection of males occurs in natural populations of WFT is unknown, with none recovered to date. Nevertheless, the implications for biological control are positive, with all life stages as potential hosts.

Using a simple dose-response assay, we have shown that approximately 16% of females of *T. nicklewoodi* can infect WFT, irrespective of the initial dosage of nematodes. Following infection, females of *T. nicklewoodi* develop only within the haemocoel of the WFT host and not in the thorax as suggested by Sharga (1932). By examining the development of *T. nicklewoodi* within WFT on a daily basis, we confirmed that there is one heterosexual generation within the host and that both females and males exit the host with the frass. Indeed, both females and males have been observed within the gut lumen (Mason, pers. obs.). We have further shown that the life cycle of *T. nicklewoodi* in WFT takes approximately 12 days at 23 °C. This rate of development will undoubtedly be temperature dependent. Indeed, Lysaght (1937), who recorded springtime temperatures of between approximately 1 to 6 °C in the United Kingdom, suggested a developmental time of approximately 6 weeks.

The survival of both females and males of *T. nicklewoodi* following emergence is short. Typically, survival of females was observed for up to approximately 160 hours at 3 °C, compared with only 20 hours at 30 °C, with similar values for males. The only other species for which survival times are available is *T. reniraoi,* with males observed to survive for up to 36 hours and females for more than 3 days in distilled water, although storage temperature was not stated (Reddy et al., 1982). Nickle and Wood (1964) and Siddiqi (1986) have proposed that both *Thripinema* fertilization and transmission occur outside of the host, an extremely harsh habitat for nematodes. Furthermore, they suggest that both would occur within plant structures with prevailing high humidity. Indeed, the preferred feeding sites of WFT, within the tightly confined habitats of flowers and meristematic tissues, would presumably provide such areas of elevated humidity. However, that the survival of both females and males was brief in water, a supposedly optimum medium, suggests that their survival on the plant surface, even within the more protected structures, must be even shorter. The results from our study to examine sites of transmission confirmed that transmission does occur on the plant, and not in the soil, although the numbers of infected WFT recovered was low. However, we have shown that more than 93% of the nematodes recovered were from flowers, with none recovered from the sand. In the absence of such protected plant structures, transmission readily occurs on the leaf surface (Mason, unpubl.). Many reasons could be attributable for this overall poor level of infection in the chrysanthemum flowers, but the obvious one is poor contact between the infective females and the most susceptible hosts due to, for example, differences in feeding behavior between the WFT life stages (Kirk, 1997). Next would be low infectivity of the females (approximately 16%, from the dose response study) or poor nematode survival within in the flowers, although approximately 37% of the nematodes recovered from the flowers were still alive.

Overall, the results presented here have clarified

many aspects of the biology of *T. nicklewoodi* and provide preliminary quantitative information. They have also highlighted that further studies are required to fully elucidate the potential of this nematode for the biological control of WFT.

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