

## Influence of *Tylenchorhynchus ewingi* on growth of loblolly pine seedlings, and host suitability of legumes and small grains

Stephen W. FRAEDRICH<sup>1,\*</sup>, Michelle M. CRAM<sup>2</sup>, Zafar A. HANDOO<sup>3</sup> and Stanley J. ZARNOCH<sup>4</sup>

<sup>1</sup> Southern Research Station, USDA Forest Service Athens, GA 30602, USA

<sup>2</sup> Forest Health Protection, USDA Forest Service, Athens, GA 30602, USA

<sup>3</sup> USDA Agricultural Research Service, Nematology Laboratory, BARC-West, Beltsville, MD 20705, USA

<sup>4</sup> Southern Research Station, USDA Forest Service, Asheville, NC 28804, USA

Received: 10 May 2011; revised: 18 September 2011

Accepted for publication: 19 September 2011; available online: 5 December 2011

**Summary** – *Tylenchorhynchus ewingi*, a stunt nematode, causes severe injury to slash pine seedlings and has been recently associated with stunting and chlorosis of loblolly pine seedlings at some forest tree nurseries in southern USA. Experiments confirmed that loblolly pine is a host for *T. ewingi*, and that the nematode is capable of causing severe damage to root systems. Initial population densities as low as 60 nematodes (100 cm<sup>3</sup> soil)<sup>-1</sup> were sufficient to damage the root systems of loblolly pine seedlings. Populations of *T. ewingi* increased on pine from two- to 16-fold, depending on the initial population density. Evaluations of various cover crops used in southern forest tree nurseries indicated that legumes, rye and several varieties of sorghum were excellent hosts for *T. ewingi*. Other small grains such as ryegrass, oats and wheat were poorer hosts. A cultivar of pearl millet was a non-host for *T. ewingi*, and a cultivar of brown top millet appeared to be either a very poor host or a non-host. Nurseries that have seedling production losses caused by *T. ewingi* should consider rotating with non-host cover crops such as pearl millet or leaving fields fallow as part of their pest management programme.

**Keywords** – cover crops, forest tree nursery, nematode control, pest management, stunt nematode.

Stunt nematodes, *Tylenchorhynchus ewingi* and *T. claytoni*, feed on roots of pine seedlings in forest tree nurseries in southern USA and can greatly reduce the size of root systems (Hopper, 1958, 1959). Shoots of affected seedlings are frequently stunted with chlorotic foliage and reduced seedling quality. *Tylenchorhynchus ewingi* was first reported to cause damage to slash pine (*Pinus elliotii*) seedlings in forest tree nurseries in Louisiana and Texas, USA, in the late 1950s (Hopper, 1959). In more recent years, *Tylenchorhynchus* spp. have been reported to cause stunting of pine seedlings at several nurseries across southern USA (Carey & Bickerstaff, 1999). In 2001, we were contacted by a nursery manager in Texas because of stunting of loblolly pine (*P. taeda*) seedlings. Soil samples were obtained from affected areas of the nursery and nematodes were extracted and subsequently identified as *T. ewingi* (Handoo, 2000). Since the discovery of *T. ewingi* in 1956, very little information has been collected regarding the biology and host range of this nematode, or factors associated with the development of damaging population levels. Sporadic reports exist of the association of

*T. ewingi* with various other forest, agricultural and ornamental plants. In Bulgaria, *T. ewingi* has also been associated with roots of two pine species, *P. nigra* and *P. sylvestris* (Peneva & Choleva, 1994). In Arkansas, USA, *T. ewingi* has been found in fields with soybean (Robbins *et al.*, 1987), cotton (Robbins *et al.*, 1989a), wheat (Robbins *et al.*, 1989b), switchgrass (Cassida *et al.*, 2005) and blueberry (Clark & Robbins, 1994). The nematode has also been associated with roots of citrus in Greece (Koliopoulos & Vovlas, 1977), cotton in Australia (Knox *et al.*, 2006), and with coffee (Kumar, 1981) and roses (Pathak & Siddiqui, 1997) in India. The impact of *T. ewingi* on the production of these plants is not well understood.

The relationship between the population densities of specific plant-parasitic nematodes and damage to crops, and the suitability of cover crops as hosts for specific plant-parasitic nematodes, is valuable information for crop production managers. The objectives of this study were to: *i*) determine the effect of population densities of *T. ewingi* on root and shoot growth of loblolly pine seedlings; and *ii*) evaluate the suitability of various

\* Corresponding author, e-mail: sfraedrich@fs.fed.us

legumes and grasses used as cover crops in southern forest tree nurseries as hosts for *T. ewingi*.

## Materials and methods

### EFFECT OF POPULATION DENSITY ON LOBLOLLY PINE SEEDLINGS

*Tylenchorhynchus ewingi* was reared in growth chambers on roots of loblolly pine seedlings for all experiments. Soil containing *T. ewingi* was initially obtained from a forest tree nursery located in Texas, USA, and stock cultures were set up to increase the number of *T. ewingi* for experiments. Nematodes were extracted using Baermann funnels (Shurtleff & Avere, 2000), hand-picked, and approximately 30-60 adults and juveniles of *T. ewingi* were placed in containers with loblolly pine seedlings. The soil used in containers was a loamy sand (86% sand, 9% silt, 5% clay, 1.8% OM; pH 5.6) obtained from a site near Byromville, Georgia, USA. The soil was microwaved in 2000 g batches for 8 min prior to use. Each container (7 cm height × 10 cm diam.) had approximately 400 cm<sup>3</sup> of soil. Loblolly pine seedlings were produced by surface-sterilising seeds with hydrogen peroxide (30%) for 60 min (Barnett, 1976), rinsing with sterile distilled water, and stratifying the seeds in plastic bags at 6°C for 30-60 days. Seeds were germinated under sterile conditions and five germinated seeds were transplanted to each container with microwaved soil. Stock containers were placed in growth chambers at 25°C for 3-12 months prior to extraction of nematodes for experiments.

The influence of *T. ewingi* population density on the growth of loblolly pine seedlings was conducted by adding adult and juvenile nematodes at dosages of 0, 250, 500, 1000, 2000 and 3000 individuals to containers with soil. Four containers were established for each dose and the experiment was conducted twice. Container size, soil type, soil treatment and handling of loblolly pine seeds were as previously described. Nematodes were extracted from stock containers using Cobb's sieving and gravity technique (Shurtleff & Avere, 2000), and collected on sieves with a 44 µm aperture size. Nematodes were then separated from soil and other materials using Baermann funnels with Kimwipes® (Kimberly-Clark, Roswell, GA, USA) as filters (Shurtleff & Avere, 2000). Nematodes were removed from the Baermann funnels within 24 h and counts were performed on ten 1 ml samples to determine nematode densities. Stock solutions were adjusted to provide 25 nematodes per ml and nematodes were added to containers at designated dosages in 180 ml water

immediately after planting five germinated loblolly pine seedlings in each container. The containers were placed in a growth chamber at 25°C with a 14 h photoperiod, watered every 1-3 days, as needed, and fertilised every 4-6 weeks with 100 ml of Miracle Gro® (Scotts Miracle-Gro Products, Maryville, OH, USA) at a concentration of 0.5 g (1000 ml water)<sup>-1</sup>.

Seedlings were removed from containers at the end of 12 (Experiment 1) or 13 weeks (Experiment 2) and shaken gently to remove loosely attached soil on roots. The centrifugal flotation method (Shurtleff & Avere, 2000) was used to recover *T. ewingi* from 100 cm<sup>3</sup> soil of each container. Roots were soaked in 1000 ml water for approximately 20 min, and nematodes were then collected on a sieve with a 44 µm aperture size and further separated with Baermann funnels, after which the nematodes were counted. The total population of *T. ewingi* in containers at the end of the experiment was calculated as the total number of nematodes (cm<sup>3</sup> soil)<sup>-1</sup> multiplied by the total soil volume plus the number of nematodes obtained from roots of seedlings. Root systems were scanned using an Epson STD1600+ Transparency Scanner and evaluated using WINRHIZO (Regent Instrument, Ottawa, ON, Canada) to determine the number of root tips and total root lengths of seedlings. Roots and shoots were separated at the root collar, dried at 80°C for 48 h and weighed.

The experiment was established as a completely randomised design with six treatments (*T. ewingi* dose) and four replications (containers) per treatment. Relationships between the initial *T. ewingi* population and seedling morphological characteristics (*i.e.*, total root length, number of root tips, and root and shoot dry weights) were determined by regression analysis (Draper & Smith, 1981) using a non-linear, negative exponential model. The relationship between initial and final *T. ewingi* populations was characterised using an exponential growth model. Parameter estimates were determined with PROC NLIN (The SAS System for Windows, Version 8.01; SAS Institute, Cary, NC, USA) or with SigmaPlot (Version 8; Systat Software, San Jose, CA, USA). The criteria for fit of the models were based on the mean square error (MSE) and the significance of the overall regression. In addition, seedling morphological characteristics were also compared among nematode doses by an analysis of variance (ANOVA) and Tukey's HSD procedure (Steel & Torrie, 1980).

## HOST SUITABILITY

A series of three experiments were conducted with *T. ewingi* to evaluate the host suitability of plant species that have been used as cover crops by forest tree nurseries. Plant species tested in all experiments were: forage sorghum (*Sorghum bicolor* cvs ET-602 and Cane Sumac), sorghum-sudan (*Sorghum bicolor* cvs SG Ultra and Green Graze BMR), wheat (*Triticum aestivum* cv. VNS), rye (*Secale cereale* cv. Elbon), ryegrass (*Lolium multiflorum* cv. TAM 90), oats (*Avena sativa* cv. Mora), pearl millet (*Pennisetum americanum* cv. ET-300), brown top millet (*Panicum ramosum* cv. DW-01), cowpea (*Vigna unguiculata* cv. Pink Eye Purple Hull BVR), vetch (*Vicia villosa* cv. AU Early Cover) and alfalfa (*Medicago sativa* cv. Alfa-graze). Infested and non-infested loblolly pine treatments and a fallow treatment were also included in all experiments.

The soil used in containers was a loamy sand as previously described and microwaved for 8 min in 2000 g batches. In the first experiment, containers (7 cm height × 10 cm diam.) held approximately 400 cm<sup>3</sup> of soil, and in the second and third experiments, containers (15 × 12.5 × 12.5 cm) held approximately 1600 cm<sup>3</sup> of soil. In each experiment, there were four containers (replications) for each treatment and five plants were established in each container. Stunt nematodes were reared on loblolly pine seedlings and extracted from soil with Baermann funnels as previously described, and either 100 nematodes (Experiments 1, 2) or 500 nematodes (Experiment 3) were added to each container within 1-2 weeks after seed germination. Containers were placed in a growth chamber at 25°C with a 15 h photoperiod for 9 weeks (Experiment 1) or 12 weeks (Experiments 2, 3). Plants were watered and fertilised as previously described. Population densities of *T. ewingi* in 100 cm<sup>3</sup> of soil were determined at the end of experiments using the centrifugal flotation method (Shurtleff & Averre, 2000). In addition, the number of *T. ewingi* associated with roots in each container was determined by placing roots of plants from each container into approximately 1 l of water for a minimum of 15 min, and nematodes were subsequently extracted using Cobb's sieving and gravity technique followed by Baermann funnel extractions as previously described. Root dry weights were determined by placing roots in a drying oven at 80°C for 48 h before weighing. The numbers of nematodes extracted around roots were subsequently expressed on a g root dry weight basis.

The final soil-borne population density of *T. ewingi* (100 cm<sup>3</sup> soil)<sup>-1</sup> and the number of *T. ewingi* (g dry

weight root)<sup>-1</sup> were compared among treatments within each experiment by an ANOVA and Tukey's HSD test (Steel & Torrie, 1980; The SAS System for Windows, Version 8.01, SAS Institute). Data were transformed with the  $\log_{10}(x+1)$  transformation prior to analyses and the back-transformed mean values are presented in tables and text.

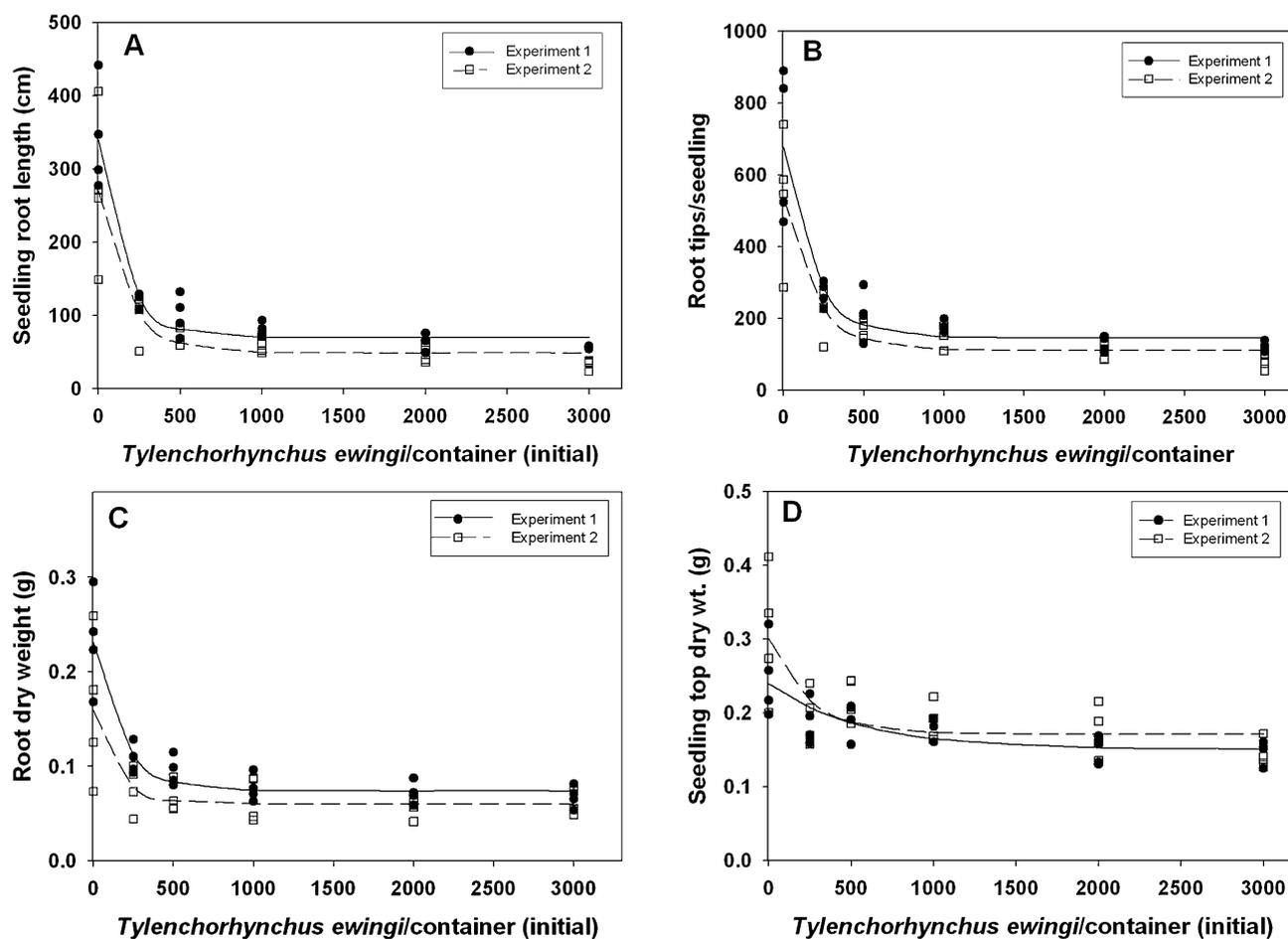
## Results

### EFFECT OF POPULATION DENSITY ON LOBLOLLY PINE SEEDLINGS

Root systems of loblolly pine were greatly reduced by *T. ewingi*. Total root lengths (Fig. 1A), number of root tips (Fig. 1B), root dry weights (Fig. 1C) and shoot weights (Fig. 1D) of seedlings decreased with respect to increasing initial population densities of *T. ewingi*. Parameter estimates for the negative exponential model to characterise the relationship between *T. ewingi* population density and the seedling variables were similar for the two experiments (Table 1). Based on the ANOVA and Tukey's HSD test, doses of *T. ewingi* at 250 nematodes per container and greater significantly reduced root dry weight, root length and number of root tips compared with controls in both experiments ( $P < 0.05$ ). In Experiment 2, seedling top weights were also significantly reduced at doses as low as 250 nematodes per container compared with seedlings in containers without nematodes ( $P < 0.05$ ), but in Experiment 1, only doses at 1000 nematodes and greater significantly reduced seedling top weights. Parameter estimates for the exponential growth model to characterise the relationship between initial and final nematode populations were also similar for the two experiments (Fig. 2). Populations of *T. ewingi* increased greatly in containers during the course of the experiments, and these increases were particularly noticeable at lower initial population levels. For example, an initial population of 250 nematodes per container increased to over 5000 nematodes in some containers in 12 weeks, although initial populations of 3000 nematodes increased only two-fold.

### HOST SUITABILITY

Based on the analyses of variance, the final soil-borne population densities of *T. ewingi* and the number of *T. ewingi* (g dry weight root)<sup>-1</sup> varied significantly among crop and fallow treatments in all experiments ( $P < 0.0001$ ). Loblolly pine, the legumes (*i.e.*, cowpeas, alfalfa and vetch), and rye were excellent hosts for *T.*



**Fig. 1.** Relationship between initial population of *Tylenchorhynchus ewingi* and loblolly pine seedling root length (A), root tips (B), and root (C) and shoot (D) weight, 12 (Experiment 1) or 13 (Experiment 2) weeks after inoculation. The relationships are based on the negative exponential model ( $y = a + b(\exp^{-lx})$ ) where  $x$  is the initial population of *T. ewingi*,  $y$  is the seedling variable, and  $a$ ,  $b$  and  $l$  are model parameter estimates.

*ewingi* in all experiments (Table 2). Cultivars of sorghum and sorghum-sudan were also excellent hosts in most experiments, although in Experiment 1, the final soil-borne population densities of nematodes in containers with cvs such as Ultra and Sumac were exceptionally low and not significantly different from fallow. Oats, wheat, and ryegrass were generally poor hosts when compared to the legumes, pine and most sorghum cultivars. Brown top millet appeared to be a very poor host or a non-host because final soil-borne population densities of *T. ewingi* were less than initial populations in all experiments and soil-borne population densities did not differ from fallow in two of the three experiments. The pearl millet cultivar had the best performance in all experiments and this species was a nonhost for *T. ewingi*. Final soil-borne

population densities of *T. ewingi* in containers with pearl millet did not differ from fallow containers in any of the experiments and there was no evidence of reproduction on this grain, and the nematode was rarely recovered from the rhizosphere around roots.

## Discussion

A previous investigation in southern pine nurseries indicated that slash pine is a host for *T. ewingi* (Hopper, 1959), and results of the present study determined that loblolly pine is also an excellent host. In the past it was thought that *Tylenchorhynchus* spp. damaged seedlings only when populations reached high levels (Ruehle, 1964;

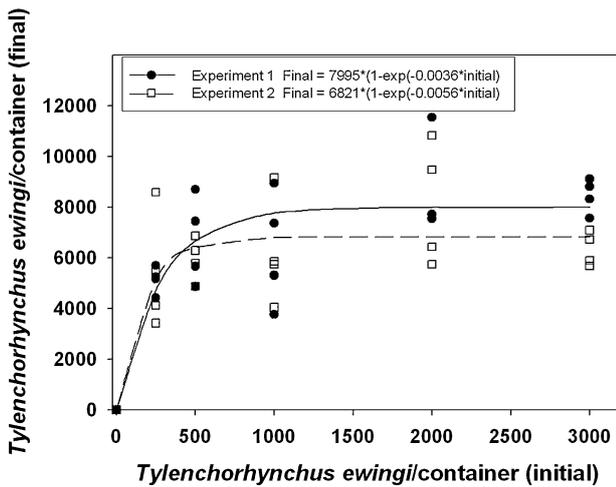
**Table 1.** Mean square errors (MSE) with degrees of freedom (df), probability levels and parameter estimates with 95% confidence limits for the negative exponential model ( $y = a + b(\exp^{-lx})$ ) characterising the relationships between initial population densities ( $x$ ) of *Tylenchorhynchus ewingi* and seedling variables ( $y$ ) including root length, root tips, root dry weight and shoot dry weight of loblolly pine seedlings.

| Seedling variable  | Experiment no. | MSE (df)      | Probability level | Parameter estimates*<br>(with 95% confidence limits)  |
|--------------------|----------------|---------------|-------------------|---|
| Root length (cm)   | 1              | 1074.5 (21)   | <0.0001           | $a = 69.52$ (50.65-88.40)<br>$b = 270.7$ (232.0-309.4)<br>$l = 0.0063$ (0.0036-0.0090)          |
|                    | 2              | 1887.4 (21)   | <0.0001           | $a = 48.6$ (23.1-74.1)<br>$b = 222.3$ (170.9-273.6)<br>$l = 0.0056$ (0.0020-0.0092)             |
| Root tips (number) | 1              | 8065.6 (21)   | <0.0001           | $a = 146.2$ (93.1-199.4)<br>$b = 532.0$ (425.7-638.2)<br>$l = 0.0054$ (0.0024-0.00829)          |
|                    | 2              | 6598.4 (21)   | <0.0005           | $a = 111.0$ (62.5-159.5)<br>$b = 426.6$ (330.6-522.8)<br>$l = 0.0051$ (0.0021-0.00820)          |
| Root dry wt. (g)   | 1              | 0.000569 (21) | <0.0001           | $a = 0.0739$ (0.0599-0.0879)<br>$b = 0.1574$ (0.1292-0.1856)<br>$l = 0.0056$ (0.0028-0.0084)    |
|                    | 2              | 0.00116 (21)  | 0.0002            | $a = 0.0601$ (0.0408-0.0794)<br>$b = 0.0994$ (0.0593-0.1395)<br>$l = 0.0068$ (-0.0019-0.0155)   |
| Shoot dry wt. (g)  | 1              | 0.000893 (21) | 0.0002            | $a = 0.1503$ (0.1254-0.1752)<br>$b = 0.0893$ (0.0536-0.1249)<br>$l = 0.00178$ (0.00001-0.00356) |
|                    | 2              | 0.00230 (21)  | 0.0005            | $a = 0.1704$ (0.1401-0.2007)<br>$b = 0.1297$ (0.0728-0.1866)<br>$l = 0.0039$ (-0.00026-0.00808) |

\*Parameter 'a' is defined as the lower asymptotic value for the seedling variable (e.g., root length) ( $y$ ) as nematode density ( $x$ ) approaches infinity (that is, the minimum possible for the seedling variable as nematode density gets very large); 'b' is the maximum decrease in the seedling variable ( $y$ ) as nematode density ( $x$ ) varies from 0 to infinity; 'a + b' is the maximum for the seedling variable ( $y$ ) at nematode density of 0; 'l' is the negative exponential rate of decrease (for example, when 'l' = 0 then there is no decrease in root length but as 'l' increases, the rate of decrease in root length becomes faster, that is, the decline in root length is faster for 'l' = 0.5 than for 'l' = 0.1).

May, 1984b), but much of this perception arose from the manner in which observations were made and studies conducted. For instance, Hopper (1958, 1959) observed that damage to slash pine seedlings caused by *T. ewingi* occurred at population densities greater than 525 nematodes ( $100 \text{ cm}^3 \text{ soil}^{-1}$ ); however, these soil samples were not obtained in the spring at seed sowing, but rather during the growing season (i.e., summer and autumn months) after stunting in seedling shoots was observed. Growth and yield of annual crops such as pine seedlings is pri-

marily a function of pre-plant densities of plant-parasitic nematodes (Barker & Olthof, 1976). Furthermore, many pathogenicity tests in the past evaluated the effects of *Tylenchorhynchus* spp. on conifers using seedlings with established root systems, and in some studies seedlings were 1 or more months old at the time of inoculation (Sutherland & Adams, 1964; Ruehle, 1966, 1973; Gowen, 1971). These pathogenicity tests for *Tylenchorhynchus* spp. often did not take into account that young seedlings, attacked from the time of seed germination, are affected by plant-



**Fig. 2.** Relationship between initial population and final population of *Tylenchorhynchus ewingi*, 12 (Experiment 1) or 13 (Experiment 2) weeks after the inoculation of loblolly pine seedlings. The relationship is based on the exponential growth model ( $y = a + b(1 - \exp^{-lx})$ ) where  $x$  is the initial population of *T. ewingi*,  $y$  is the final population, and  $a$ ,  $b$  and  $l$  are model parameters estimates.

parasitic nematodes and are more prone to injury even at low population levels. Studies on agricultural crops with various plant-parasitic nematodes have found that delaying nematode attacks on plants for even 7-10 days after seed germination can greatly increase the size of plants or their yield compared with plants inoculated at seed germination (Wong & Mai, 1973; Seinhorst, 1995; Ploeg & Phillips, 2001).

Results of the present study with *T. ewingi* indicate that population densities as low as 250 nematodes per container (60 nematodes  $(100 \text{ cm}^3 \text{ soil})^{-1}$ ) at seed sowing were capable of damaging pine root systems and reducing seedling quality. We cannot rule out the possibility that even lower initial population densities could be a problem for pine seedling production, but this hypothesis has not yet been tested. The relatively low damage threshold for *T. ewingi* in the present study is consistent with previous reports for *Tylenchorhynchus* spp. on other crops. Damage threshold levels for *T. claytoni* have varied from more than 20 individuals  $(100 \text{ cm}^3 \text{ soil})^{-1}$  for azalea (Barker & Nusbaum, 1971) to 60 individuals  $(100 \text{ cm}^3 \text{ soil})^{-1}$  for soybean (Rickard & Barker, 1982).

The results of our experiments indicate that populations of *T. ewingi* can increase very rapidly on the roots of loblolly pine, even on very young seedlings. The density of pine seedlings in a nursery is typically in the range of 220 to 270  $\text{m}^{-2}$ , which provides abundant food

for nematodes. Although information on the life cycle of *T. ewingi* has not been developed, generation times range from 31 to 38 days for *T. claytoni* (Wang, 1971), a nematode similar to *T. ewingi* and also found in southern pine nurseries. Based on the rapid population increase of *T. ewingi* in our experiments, we would expect that this nematode has an equally short generation time, and capable of increasing very rapidly in pine nursery beds.

Many forest tree nurseries in southern USA grow cover crops in their fields when they are not producing seedlings (Boyer & South, 1984; Cram & Fraedrich, 1997). Pine seedling crops are typically alternated with cover crops on a rotation of 1:1 (number of years in seedling crop/number of years in cover crops), 2:1, 2:2, or similar rotation (Boyer & South, 1984; Cram & Fraedrich, 1997). The use of cover crops that are suitable hosts for *T. ewingi* can have detrimental effects on subsequent pine seedlings crops because nematode population densities increase rapidly when host crops are continuously cultivated (Dropkin, 1989). At one time, legumes such as cowpeas and vetch were preferred by nursery managers because they added nitrogen to the soil (Wakeley, 1954). More recently, nursery managers have favoured various species of grasses such as sorghum varieties (Boyer & South, 1984; May, 1984a). In our study, cowpeas, vetch, and alfalfa were excellent hosts for *T. ewingi* and greatly increased populations of this nematode. The use of these cover crops was likely a factor in the damage to loblolly pine seedlings at the Texas nursery that experienced problems with *T. ewingi*. Nursery personnel indicated that cowpeas had been grown in fields prior to the production of pine seedlings and the cowpeas could have increased greatly the populations of *T. ewingi*. Nursery personnel also indicated that several sorghum varieties and rye had also been used routinely. Although these grains are currently favoured by many nursery managers as summer and winter cover crops, they can also be excellent hosts for *T. ewingi*.

Although the routine use of methyl bromide for soil fumigation is being phased out worldwide, most forest tree nurseries in southern USA continue to fumigate with this chemical through critical use and quarantine pre-shipment exemptions. Fumigation with methyl bromide and methyl isothiocyanate products can provide excellent control of nematodes (Ruehle, 1972; Hildebrand & Dinkel, 1988; Barnard *et al.*, 1994; Fraedrich & Dwinell, 2005) but fumigation does not eradicate nematodes in fields (Lembright, 1990). Nematodes can survive below the zone of fumigant placement and in soil clods where fumigants fail

**Table 2.** Backtransformed log means of the population densities of *Tylenchorhynchus ewingi* ( $100\text{ cm}^3\text{ soil}^{-1}$ ) from containers with various crop species or fallow, and number of *T. ewingi* (g root dry weight) $^{-1}$  obtained around roots, and root dry weights of the crop species.

| Experiment no. <sup>1)</sup> | Plant species 'variety' | <i>Tylenchorhynchus ewingi</i>         |                          | Root dry weight (g) |
|------------------------------|-------------------------|--|--------------------------|---------------------|
|                              |                         | 100 cm <sup>3</sup> soil <sup>2)</sup> | g dw roots <sup>2)</sup> |                     |
| 1                            | Cowpea                  | 582 a                                  | 781 ab                   | 2.58                |
|                              | Loblolly pine           | 273 ab                                 | 1443 a                   | 0.33                |
|                              | Vetch                   | 144 abc                                | 538 abc                  | 0.19                |
|                              | Rye                     | 83 abcd                                | 203 abcd                 | 1.61                |
|                              | Sorghum-sudan 'BMR'     | 27 abcde                               | 22 def                   | 2.05                |
|                              | Sorghum 'ET-602'        | 22 bcde                                | 50 bcde                  | 1.73                |
|                              | Alfalfa                 | 17 bcde                                | 54 bcde                  | 1.26                |
|                              | Sorghum 'Cane Sumac'    | 10 cde                                 | 15 def                   | 0.72                |
|                              | Wheat                   | 5 cde                                  | 16 def                   | 1.56                |
|                              | Oats                    | 6 cde                                  | 5 ef                     | 1.66                |
|                              | Brown top millet        | 6 cde                                  | 4 ef                     | 0.62                |
|                              | Sorghum-sudan 'Ultra'   | 5 de                                   | 41 cdefg                 | 1.07                |
|                              | Ryegrass                | 4 de                                   | 31 def                   | 1.25                |
|                              | Pearl millet            | 4 de                                   | 2 f                      | 1.21                |
|                              | Fallow                  | 1 e                                    | –                        | –                   |
|                              | 2                       | Cowpea                                 | 1135 a                   | 1003 ab             |
| Alfalfa                      |                         | 287 a                                  | 132 abc                  | 9.92                |
| Sorghum 'ET-602'             |                         | 204 ab                                 | 23 cdef                  | 5.92                |
| Sorghum-sudan 'BMR'          |                         | 175 ab                                 | 65 bcd                   | 5.00                |
| Rye                          |                         | 170 ab                                 | 37 cde                   | 3.31                |
| Loblolly pine                |                         | 168 ab                                 | 1306 a                   | 0.39                |
| Vetch                        |                         | 142 ab                                 | 1020 ab                  | 0.24                |
| Sorghum 'Cane Sumac'         |                         | 115 abc                                | 28 cdef                  | 3.59                |
| Sorghum-sudan 'Ultra'        |                         | 16 bcd                                 | 7 cdefg                  | 1.74                |
| Oats                         |                         | 13 bcde                                | 1 fg                     | 1.95                |
| Ryegrass                     |                         | 8 cde                                  | 4 defg                   | 4.34                |
| Wheat                        |                         | 6 de                                   | 1 efg                    | 3.96                |
| Brown top millet             |                         | 5 de                                   | 3 defg                   | 1.54                |
| Pearl millet                 |                         | 1 de                                   | 0 g                      | 1.50                |
| Fallow                       |                         | 0 e                                    | –                        | –                   |
| 3                            |                         | Cowpea                                 | 3594 a                   | 2748 ab             |
|                              | Alfalfa                 | 1139 ab                                | 179 cde                  | 8.78                |
|                              | Vetch                   | 1032 ab                                | 3354 a                   | 0.44                |
|                              | Sorghum-sudan 'BMR'     | 822 ab                                 | 291 bcd                  | 8.33                |
|                              | Rye                     | 756 ab                                 | 480 abcd                 | 3.82                |
|                              | Sorghum 'Cane Sumac'    | 650 ab                                 | 499 abc                  | 5.12                |
|                              | Sorghum 'ET-602'        | 636 ab                                 | 167 cde                  | 7.88                |
|                              | Loblolly pine           | 562 b                                  | 3701 a                   | 0.49                |
|                              | Sorghum-sudan 'Ultra'   | 305 bc                                 | 140 cdef                 | 3.70                |
|                              | Wheat                   | 63 cd                                  | 37 def                   | 2.77                |
|                              | Oats                    | 42 d                                   | 29 def                   | 1.58                |
|                              | Ryegrass                | 32 d                                   | 23 ef                    | 8.18                |
|                              | Brown top millet        | 14 d                                   | 13 fg                    | 1.33                |
|                              | Pearl millet            | 1 e                                    | 0 g                      | 5.66                |
|                              | Fallow                  | 1 e                                    | –                        | –                   |

The variety is given in quotation marks where applicable.

<sup>1)</sup>Experiment 1, containers had 400 cm<sup>3</sup> of soil and were inoculated with 100 nematodes; Experiment 2, containers had 1600 cm<sup>3</sup> of soil and were inoculated with 100 nematodes; Experiment 3, containers had 1600 cm<sup>3</sup> of soil and were inoculated with 500 nematodes.

<sup>2)</sup>Means followed by the same letter do not differ significantly according to Tukey's HSD procedure ( $P < 0.05$ ); means based on four replications per plant species.

to penetrate and, thus, populations can quickly rebound on host crops (Sipes & Schmidt, 1998). Fallow and non-host cover crops are additional practices which could be used to control nematode populations and possibly increase the time interval between fumigations.

The pearl millet cv. ET-300 performed as well as fallow for control of *T. ewingi* and could be an excellent alternative to sorghum and sorghum-sudan as a cover crop in fields where *T. ewingi* is a problem. The lack of reproduction by *T. ewingi* on pearl millet cv. ET-300 suggests that this crop is a non-host (Nusbaum & Barker, 1971). Pearl millet cultivars have been shown to be resistant to various types of plant-parasitic nematodes, including *Paratrichodorus minor*, *Meloidogyne* spp., *Belonolaimus longicaudatus* and *Pratylenchus brachyurus* (Timper et al., 2002; Timper & Hanna, 2005). Additional testing of pearl millet cultivars is needed under field conditions at nurseries that have problems with *T. ewingi*. Although brown top millet did not always reduce *T. ewingi* populations to the levels of fallow, the use of this cover crop could be a viable alternative to other grasses such as sorghum and sorghum-sudan varieties. Oats and ryegrass also performed better than other crops for control of *T. ewingi* and should be considered in areas where this stunt nematode is a problem to restrict the build-up of nematode populations.

Crop rotations with non-hosts such as pearl millet or leaving fields fallow can reduce *T. ewingi* in fields at forest nurseries, and the use of these practices could be integrated into current pest management programmes. Additional research is needed to increase understanding of the basic biology of *T. ewingi*, and the host suitability of weeds, other nursery crops, and various cultivars of pearl millet and other cover crops that could be used in southern forest tree nurseries.

## Acknowledgements

We gratefully acknowledge the expert laboratory assistance of Susan Best during the study. We also greatly appreciate the insightful comments and suggestions of two anonymous reviewers. The use of trade names or firm names in this publication is for reader information and does not imply recommendations or endorsement by the US Department of Agriculture of any product or service.

## References

BARKER, K.R. & NUSBAUM, C.J. (1971). Diagnostic and advisory programs. In: Zuckerman, B.M., Mai, W.F. &

- Rohde, R.A. (Eds). *Plant parasitic nematodes, Vol. I*. New York, NY, USA, Academic Press, pp. 281-301.
- BARKER, K.R. & OLTHOF, T.H.A. (1976). Relationships between nematode population densities and crop responses. *Annual Review of Phytopathology* 14, 327-353.
- BARNARD, E.L., GILLY, S.P. & ASH, E.C. (1994). An evaluation of dazomet and metam-sodium soil fumigants for control of *Macrophomina phaseolina* in a Florida forest nursery. *Tree Planters' Notes* 45, 91-95.
- BARNETT, J.P. (1976). Sterilizing southern pine seeds with hydrogen peroxide. *Tree Planters' Notes* 27, 17-24.
- BOYER, J.N. & SOUTH, D.B. (1984). Forest nursery practices in the South. *Southern Journal of Applied Forestry* 8, 67-75.
- CAREY, W.A. & BICKERSTAFF, G. (1999). *Assessing stunt nematodes on pine seedling roots*. Research report 99-9, Auburn University Southern Forest Nursery Management Cooperative, Auburn, AL, USA, 4 pp.
- CASSIDA, K.A., KIRKPATRICK, T.L., ROBBINS, R.T., MUIR, J.P., VENUTO, B.C. & HUSSEY, M.A. (2005). Plant-parasitic nematodes associated with switchgrass (*Panicum virgatum* L.) grown for biofuel in the South Central United States. *Nematopica* 35, 1-10.
- CLARK, J.R. & ROBBINS, R.T. (1994). Phytoparasitic nematodes associated with three types of blueberries in Arkansas. *Journal of Nematology* 26, 761-766.
- CRAM, M.M. & FRAEDRICH, S.W. (1997). Survey of southern forest nurseries: fumigation practices and pest management concerns. In: Landis, T.D. & South, D.B., (Eds). *National Proceedings: Forest and Conservation Nursery Associations-1996. Proceedings PNW-GTR-389*. Portland, OR, USA, USDA Forest Service, Pacific Northwest Research Station, pp. 19-27.
- DRAPER, N.R. & SMITH, H. (1981). *Applied regression analysis*, 2nd edition. New York, NY, USA, John Wiley & Sons, 709 pp.
- DROPKIN, V.H. (1989). *Introduction to plant nematology*, 2nd edition. New York, NY, USA, Wiley & Sons, 304 pp.
- FRAEDRICH, S.W. & DWINELL, L.D. (2005). Effects of dazomet, metam sodium, and oxamyl on *Longidorus* populations and loblolly pine seedling production. *Southern Journal of Applied Forestry* 29, 117-122.
- GOWEN, S.R. (1971). *Tylenchus emarginatus* and *Tylenchorhynchus dubius* associated with Sitka spruce (*Picea sitchensis*) seedlings. *Plant Pathology* 20, 69-72.
- HANDOO, Z.A. (2000). A key and diagnostic compendium to the species of the genus *Tylenchorhynchus* Cobb, 1913 (Nematoda: Belonolaimidae). *Journal of Nematology* 32, 20-34.
- HILDEBRAND, D.M. & DINKEL, G.B. (1988). Basamid and solar heating effective for control of plant parasitic nematodes at Bessey Nursery, Nebraska. In: Landis, T.D. (Ed.). *Proceedings, Combined meeting of the Western Forest Nursery Associations. General technical report RM-167*. Fort Collins,

- CO, USA, USDA Forest Service, Rocky Mountain Forest & Range Experiment Station, pp. 139-144.
- HOPPER, B.E. (1958). Plant-parasitic nematodes in soils of southern forest nurseries. *Plant Disease Reporter* 42, 308-314.
- HOPPER, B.E. (1959). Three new species of the genus *Tylenchorhynchus* (Nematoda: Tylenchida). *Nematologica* 4, 23-30.
- KNOX, O.G.G., ANDERSON, C.M.T., NEHL, D.B. & GUPTA, V.V.S.R. (2006). Observation of *Tylenchorhynchus ewingi* in association with cotton soils in Australia. *Australasian Plant Disease Notes* 1, 47-48.
- KOLIOPANOS, C.N. & VOVLAS, N. (1977). Records of some plant parasitic nematodes in Greece with morphometrical descriptions. *Nematologia Mediterranea* 5, 207-215.
- KUMAR, A.C. (1981). Studies of nematodes in coffee soil in south India. 5. Descriptions of three new species of *Tylenchorynchus* and occurrence of four other tylenchid species. *Journal of Coffee Research* 11, 88-99.
- LEMBRIGHT, H.W. (1990). Soil fumigation: principles and application technology. *Journal of Nematology* 22, 632-644.
- MAY, J.T. (1984a). Basic concepts of soil management. In: Lantz, C.W. (Ed.). *The southern pine nursery handbook*. Atlanta, GA, USA, United States Department of Agriculture, Forest Service, pp. 1-9.
- MAY, J.T. (1984b). Insects and other animal pests: life cycles and control. In: Lantz, C.W. (Ed.). *The southern pine nursery handbook*. Atlanta, GA, USA, United States Department of Agriculture, Forest Service, p. 11.
- NUSBAUM, C.J. & BARKER, K.R. (1971). Population dynamics. In: Zuckerman, B.M., Mai, W.F. & Rohde, R.A. (Eds). *Plant parasitic nematodes, Volume I, morphology, anatomy, taxonomy and ecology*. New York, NY, USA, Academic Press, pp. 303-323.
- PATHAK, M. & SIDDIQUI, A.U. (1997). One new and five known species of *Tylenchorhynchus* Cobb, 1913 from ornamental crops in Udaipur region of Rajasthan. *Indian Journal of Nematology* 27, 99-103.
- PENEVA, V. & CHOLEVA, B. (1994). Plant-parasitic nematodes associated with pine trees in Bulgaria. *EPPO Bulletin* 24, 459-466.
- PLOEG, A.T. & PHILLIPS, M.S. (2001). Damage to melon (*Cucumis melo* L.) variety Durango by *Meloidogyne incognita* in southern California. *Nematology* 3, 151-157.
- RICKARD, D.A. & BARKER, K.R. (1982). Nematode assays and advisory services. In: Riggs, R.D. (Ed.). *Nematology in the southern region of the United States. Southern Cooperative series Bulletin 276*. Fayetteville, AR, USA, University of Arkansas, pp. 8-20.
- ROBBINS, R.T., RIGGS, R.D. & VON STEEN, D. (1987). Results of annual phytoparasitic nematode surveys of Arkansas soybean fields, 1978-1986. *Annals of Applied Nematology* 1, 50-55.
- ROBBINS, R.T., RIGGS, R.D. & VON STEEN, D. (1989a). Phytoparasitic nematode surveys of Arkansas cotton fields, 1986-88. *Journal of Nematology* 21, 619-623.
- ROBBINS, R.T., RIGGS, R.D. & VON STEEN, D. (1989b). Phytoparasitic nematode surveys of Arkansas wheat fields, 1986-88. *Journal of Nematology* 21, 624-628.
- RUEHLE, J.L. (1964). Plant parasitic nematodes and their significance in forest nursery production. In: Belcher, E. (Ed.). *Proceedings of the region 8 forest nurserymen's conference*, Atlanta, GA, USA, USDA Forest Service, pp. 92-98.
- RUEHLE, J.L. (1966). Nematodes parasitic on forest trees. I. Reproduction of ectoparasites on pines. *Nematologica* 12, 443-447.
- RUEHLE, J.L. (1972). Nematodes of forest trees. In: Webster, J.H. (Ed.). *Economic nematology*. London, UK, Academic Press, pp. 313-334.
- RUEHLE, J.L. (1973). Influence of plant-parasitic nematodes on longleaf pine seedlings. *Journal of Nematology* 5, 7-9.
- SEINHORST, J.W. (1995). The effect of delay of attack of oats by *Heterodera avenae* on the relation between initial nematode density and plant growth, plant weight, water consumption, and dry matter content. *Nematologica* 41, 487-504.
- SHURTLEFF, M.C. & AVERRE, C.W., III, (2000). *Diagnosing plant diseases caused by nematodes*. St Paul, MN, USA, The American Phytopathological Society, 187 pp.
- SIPES, B.S. & SCHMITT, D.P. (1998). Nematode-pesticide interactions. In: Barker, K.R., Pederson, G.A. & Windham, G.L. (Eds). *Plant nematode interactions*. Madison, WI, USA, American Society of Agronomy Publishers, pp. 173-185.
- STEEL, R.G.D. & TORRIE, J.H. (1980). *Principles and procedures of statistics: a biometrical approach*. New York, NY, USA, McGraw-Hill, 633 pp.
- SUTHERLAND, J.R. & ADAMS, R.E. (1964). The parasitism of red pine and other nursery crops by *Tylenchorhynchus claytoni*. *Nematologica* 10, 637-643.
- TIMPER, P. & HANNA, W.W. (2005). Reproduction of *Belonolaimus longicaudatus*, *Meloidogyne javanica*, *Paratrichodorus minor*, and *Pratylenchus brachyurus* on pearl millet (*Pennisetum glaucum*). *Journal of Nematology* 37, 214-219.
- TIMPER, P., WILSON, J.P., JOHNSON, A.W. & HANNA, W.W. (2002). Evaluation of pearl millet grain hybrids for resistance to *Meloidogyne* spp. and leaf blight caused by *Pyricularia grisea*. *Plant Disease* 86, 909-914.
- WAKELEY, P.C. (1954). *Planting the southern pines*. Washington, DC, USA, United States Department of Agriculture, 233 pp.
- WANG, L.H. (1971). Embryology and life cycle of *Tylenchorhynchus claytoni* Steiner, 1937 (Nematoda: Tylenchoidea). *Journal of Nematology* 3, 101-107.
- WONG, T.K. & MAI, W.F. (1973). Pathogenicity of *Meloidogyne hapla* to lettuce as affected by inoculum level, plant age at inoculation and temperature. *Journal of Nematology* 5, 126-129.