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The effect of fallow on *Longidorus americanus*, a nematode associated with stunting of loblolly pine seedlings in Georgia, USA

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Summary – Stunting of loblolly pine (*Pinus taeda* L.) seedlings, caused by *Longidorus americanus*, has been a problem at a Georgia (USA) nursery. Field and growth chamber studies were conducted to determine the survivability of the nematode in fallow nursery soil. The population density of *L. americanus* decreased rapidly in the upper 15 cm of soil in fallow field plots during the first 101 days and the nematode was not detected after 263 days. Bioassays subsequently failed to detect the nematode in the upper 30 cm of soil in fallow plots on days 263 and 365. Population densities also decreased rapidly in fallow soil during the initial 130 days of the growth chamber study and the nematode was not detected at days 334 and 427, or in subsequent bioassays. *Longidorus americanus* does not survive well in the upper 15-30 cm of nursery soil in the absence of a host, and the use of fallow may be an acceptable alternative to pesticides for the management of this nematode.

Keywords – forest-tree nursery, nematode control, pest management.

*Longidorus americanus*** has been found to damage loblolly pine (*Pinus taeda* L.) seedlings at a southern Georgia (USA) forest-tree nursery (Fraedrich & Cram, 2002; Handoo *et al.*, 2005). Root systems of affected seedlings were greatly reduced and lacked lateral and feeder roots, resulting in stunted seedlings. Portions of the field where the problem occurred were in constant production for pine and oak seedlings from 1990-2002 (Cram *et al.*, 2003). In sections of the affected field that were fumigated, the problem recurred during the second year of pine seedling production (Cram *et al.*, 2003).

Fallow is commonly practiced in some crop production systems to reduce nematode populations through starvation (Duncan, 1991). Individuals of some *Longidorus* species are thought to have long life spans with consequent slow rates of decline of populations under non-hosts and fallow (Cooke, 1993), and the effectiveness of fallow for control of these nematodes would be questionable. Other *Longidorus* species appear to decline relatively rapidly under fallow conditions (Lamberti, 1968; MacGuidwin, 1989) and periods of fallow may be beneficial for management of some *Longidorus* species (Radewald *et al.*, 1969). Although the use of fallow for nematode control has been rarely discussed for forest-tree nurseries in the southern United States (Ruehle, 1964,1972; Ruehle & Riffle, 1989), the practice is beneficial for nematode control in some Canadian nurseries (Sutherland & Webster, 1993).

Fields at most nurseries are not usually in continuous production for forest-tree seedlings and the use of fallow could be a viable management practice where there are nematode problems. Most nurseries typically rotate forest-tree seedling production with cover crops on a regular schedule. Many nurseries in the southern United States have relied on soil fumigation for pest control for the last 40 years and, as a result, information is

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** The specific name was originally published as *americanum* but must be modified to *americanus* to agree in gender with the genus (Ed.).

lacking on the biology of many soil-borne pests and alternative methods for their control. Methyl bromide, the most commonly used soil fumigant in the southern United States, is scheduled to be phased out in 2005 in accordance with the United States Clean Air Act and the Montreal Protocol, although exemptions are being considered on a yearly basis for some crops, including forest-tree seedlings, because of the lack of suitable alternatives. The development of alternative pest management practices is essential for maintaining long-term nursery production capabilities. The objective of this study was to examine the effect of fallow on population densities of the *L. americanus* through time in field plots and in containers under controlled conditions.

Materials and methods

Field study

Ten plots were established in a field where scattered patches of stunted pine seedlings had been noted during the 2000 and 2001 growing seasons. Plots were 1.2 m wide and lengths varied from 3.7 to 14 m. Pine seedlings were lifted from this field in early April 2002. Population densities of *L. americanus* were assessed initially in fallow plots on 29 April 2002, and assessments were continued at 4-8 week intervals until 29 April 2003. At each sample date, six to ten soil cores (2.5 cm diam.) were obtained to a 15-20 cm depth on each plot. *Longidorus americanus* was extracted from soil using the procedure of Flegg (1967), which is a modification of Cobb's sieving and gravity technique with final separation of nematodes from soil and organic debris using a Baermann funnel. Nematodes were collected on 90 μm aperture sieves instead of screens with a 150 μm aperture as described by Flegg (1967). The field was harrowed to a 15-20 cm depth during the first week in May, the first week in September and again during the first week of November 2002. Weeds were rarely observed in the plots during the spring, summer and autumn of 2002. In the spring of 2003, weeds were sparsely scattered throughout the field and were occasionally encountered in sample plots. Weeds noted in the field included species of *Linaria, Rumex, Crotalaria, Oenothera, Chenopodium* and *Cyperus*. Winter wheat (*Triticum aestivum* L.) was sown as a cover crop in early November 2002 but the crop was sparse (63 plants/m^2), was not irrigated or fertilised, and growth of plants was poor through the winter. In addition, wheat appears to be a non-host for *L. americanus* (Fraedrich *et al.*, 2003). For these reasons, we maintain the term 'fallow' when discussing the treatment in the field beyond November 2002.

Four non-fallow plots (10 m long and 1.2 m wide) were also established in a section of the field where *L. americanus* was present and loblolly pine seedlings were established. Seeds were sown on 17 April 2002, and seedlings were irrigated and fertilised on the same schedule as seedlings in production areas of the nursery. Soil samples were collected just before seed sowing, and then at 3-10 week intervals until 15 January 2003. The soil samples were collected and nematodes extracted as described for the fallow plots.

Growth chamber study

Soil was collected from areas of a field known to be infested with *L. americanus*. The soil was mixed in a small mixer (Model 59015B, CF Gilco, Inc., Port Washington, WI, USA) for 4 min, and approximately 2000 cm^3 of soil was placed in each of 54 containers (12.7 × 12.7 × 14 cm). Six of the containers were selected at random, and *L. americanus* was extracted from a 100 cm^3 sample from each container to provide an initial estimate of the nematode population density. The remaining containers were divided into two treatments where 24 containers were left fallow and 24 containers were each planted with five germinated loblolly pine seeds. Containers were placed in a growth chamber at 25°C with a 14 h photoperiod, and all containers were watered every 2-3 days. At 5-14 week intervals, four containers of each treatment were randomly selected and population densities of the *L. americanus* were determined. After 130 days, ten additional germinated pine seeds were planted in each remaining non-fallow container to increase the availability of roots for the nematode. Fallow containers were used to monitor *L. americanus* population densities through time in the absence of a host. A primary purpose of the non-fallow containers planted with pine seedlings was to ensure that conditions in the growth chamber were at all times conducive to the survival of *L. americanus*.

Soil bioassays

After *L. americanus* was no longer detected in fallow plots of the field study, additional soil was collected on two sample dates (days 263 and 365) for bioassays to enhance nematode detection. Three soil cores (15 cm diam. and 30 cm deep) were collected from each of the ten plots. Soil was gently but thoroughly mixed within plastic bags for each core and 100 cm^3 of soil was used to determine population densities of *L. americanus* as previously described. Containers (12.7 × 12.7 × 14 cm) were filled with approximately 2000 cm^3 of soil from each core (three containers per plot; 30 containers per bioassay). Five germinated loblolly pine seeds were planted in each container. Containers were placed in a growth chamber at 25°C with a 14 h photoperiod for 22-24 weeks. In order to verify that conditions were maintained for nematode survival during bioassays, soil was also obtained from three areas of the field containing loblolly pine seedlings and known to be infested with *L. americanus*. This soil also was mixed, potted (three containers per area; nine containers per bioassay) and each container was planted with five germinated loblolly pine seeds. At the end of each bioassay, the soil in each container was mixed and 100 cm^3 samples of soil were used to determine the *L. americanus* population densities.

In the growth chamber study, soil was also repotted for bioassays on two sample dates (days 334 and 427) when *L. americanus* was no longer detected in fallow containers. The bioassays were conducted as described for the field study and *L. americanus* population densities were assessed for all containers at the end of the bioassays as previously described.

STATISTICAL ANALYSES

A negative exponential model ($y = ae^{-b*x}$) was used to characterise the relationship between *L. americanus* population densities (y) and duration of fallow (x) in both the field and growth chamber studies. Parameter estimates were determined using PROC NLIN (SAS System for Windows, Version 8.01, SAS Institute, Cary, NC, USA). Parameter estimates for the negative exponential models were compared for field and growth chamber studies by calculating the 95% confidence interval for each study and determining if there was overlap for the estimates. In the field study, the relationship between *L. americanus* population densities and days of non-fallow in plots with pine seedlings was characterised by a simple linear regression model using PROC REG (SAS System for Windows, Version 8.01). In the growth chamber study, the relationship between *L. americanus* population densities and days in non-fallow containers with pine seedlings was characterised with a linear quadratic relationship using PROC REG.

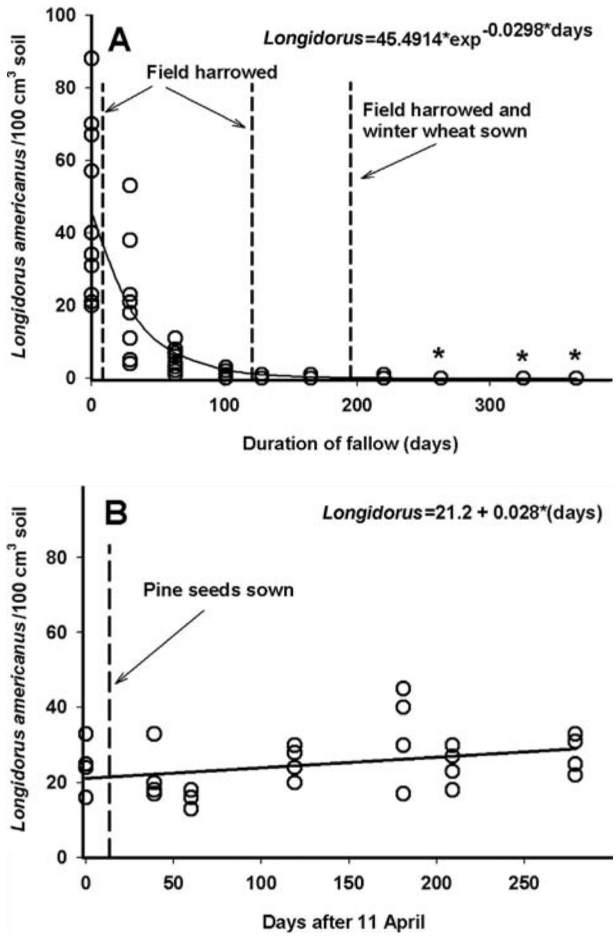

Fig. 1. *A: Relationship between* Longidorus americanus *population densities and days of fallow in field plots; B: Population densities of* L. americanus *and days after 11 April 2002 in field plots with loblolly pine seedlings. Asterisks (*) at sample days indicate that* L. americanus *was not detected in any field plot. Data were recorded from ten fallow and four non-fallow field plots.*

Results

FIELD STUDY

The population density of *L. americanus* decreased exponentially with respect to days of fallow (Fig. 1A; MSE = 74.98; $P \leqslant 0.0001$). Parameter estimates were $a = 45.49$ and $b = 0.0298$ (Table 1). *Longidorus americanus* population density decreased greatly during the spring and early summer months (days 0-101), and the nematode was found only occasionally in some plots during the late summer and autumn months (days 128-220). The nematode was not detected on any fallow plot

Table 1. *Parameter estimates, standard errors of estimates, and confidence intervals (95%) of estimates for a negative exponential model ($y = ae^{-b*x}$) characterising the relationship between population densities of* Longidorus americanus *and days of fallow in field and growth chamber studies.*

Study	Parameter	Estimate	Standard error	Confidence interval (95%)
Field	α	45.4914	2.6986	40.1-50.8
	β	0.0298	0.00392	0.0221-0.0376
Growth chamber	α	67.6692	2.5172	62.5-72.8
	β	0.0214	0.00206	0.0172-0.0256

on 15 January (day 263), 18 March (day 325) or 29 April (day 365).

In the non-fallow field plots with pine seedlings, population densities of *L. americanus* remained somewhat static throughout the growing season (Fig. 1B; $R^2 = 0.1181$; MSE = 55.4, $P = 0.0734$). Seedlings in these field plots were stunted with poorly developed root systems.

Growth chamber study

The population density of *L. americanus* again decreased exponentially with respect to days of fallow (Fig. 2A; MSE = 39.0; $P \leqslant 0.0001$). The parameter estimate for '*a*' in the growth chamber study differed significantly from the estimate in the field study (Table 1). However, the estimate of the negative exponential decay parameter (*b*) did not differ significantly from the estimate in the field study. In the non-fallow containers with pine seedlings, population densities followed a quadratic relationship with respect to days after planting pine seedlings (Fig. 2B; $R^2 = 0.5668$; MSE = 2090.9, $P < 0.0001$). Population densities initially decreased during the first 130 days of the study, but then increased during the later sampling dates after ten additional loblolly pine seedlings were established in each container.

Soil bioassays

Longidorus americanus was not detected in soil from fallow plots at the beginning or the end of the bioassays for days 263 and 365 of the field study (Table 2). The nematode was present in containers with soil from non-fallow field plots at the beginning and the end of the bioassays.

In the growth chamber study, *L. americanus* was not detected at the beginning or end of bioassays of fallow containers from days 334 and 427. The nematode was present at the beginning and end of the bioassays in non-fallow containers.

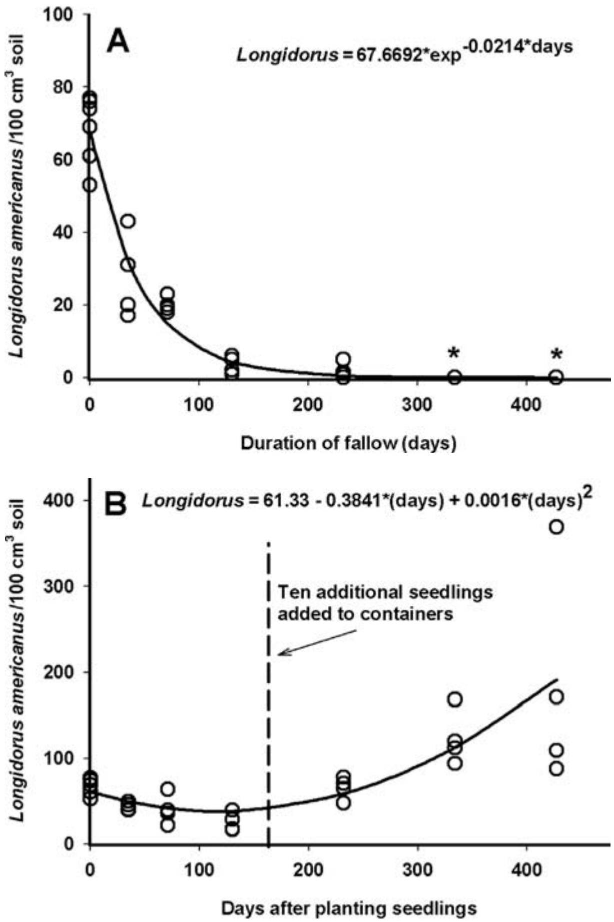

Fig. 2. *A: Relationship between population densities of* Longidorus americanus *and days in fallow containers; B: Population densities of* L. americanus *and days after planting loblolly pine seedlings in non-fallow containers. The study was conducted in a growth chamber at 25°C. Asterisks (*) at sample days indicate that the* L. americanus *was not detected in soil from any fallow container. Data were recorded from four fallow and four non-fallow containers at each sample date.*

Discussion

Population densities of *L. americanus* decreased rapidly under fallow, and nematodes apparently did not survive for extended periods in the upper 15 cm of soil in fallow field plots (>263 days) or in fallow containers under controlled conditions (>334 days). The results of our study are similar to findings in other studies that assessed the

Table 2. *Mean population densities of* Longidorus americanus *at the beginning and end of soil bioassays for fallow and non-fallow plots (field study) and containers (growth chamber study) at sample dates when the nematode was not detected in fallow soil.*

Study	Sample date (day)	Treatment[1)]	*Longidorus*/ 100 cm^3 soil	
			Initial	Final[2)]
Field	263	Fallow	0	0
		Non-fallow	12.7	20.5
	365	Fallow	0	0
		Non-fallow	5.5	15.9
Growth chamber	334	Fallow	0	0
		Non-fallow	124.0	47.0
	427	Fallow	0	0
		Non-fallow	184.0	37.5

[1)] In the field study there were 30 containers total in the fallow treatment (three containers for each of ten plots) for each bioassay and in the non-fallow treatment there were nine containers total (three containers from each of three infested, non-fallow areas). In the growth chamber study there were four containers in the fallow and non-fallow treatments for each bioassay.

[2)] The duration of bioassays for the field study were 22 weeks for day 263 and 24 weeks for day 365. The duration of the bioassays for the growth chamber study were 15 weeks at day 334 and 24 weeks at day 427.

effects of fallow on longidorids. For example, Lamberti (1968) found that population densities of *L. africanus* Merny, 1966 decreased greatly over a 3 month period, and the nematode was only occasionally detected in soil after 4 months. Similarly, MacGuidwin (1989) reported that *L. breviannulatus* Norton & Hoffman, 1975 overwintered successfully in Wisconsin (USA) agricultural fields but population densities of the nematode declined rapidly during the spring in fallow plots and the nematode did not persist into the summer months.

Soil temperature and moisture can greatly affect the survival of longidorids in fallow soil. Sutherland and Sluggett (1974) found that survival of *Xiphinema bakeri* Williams, 1961 was favoured by moist soil and low soil temperatures (*i.e.*, 4 and 15°C) during fallow but nematode longevity was shortened by a higher soil temperature (*i.e.*, 30°C) and drier soil conditions. Radewald *et al.* (1969) believed that periods of fallow for a month or more during hot and dry conditions played an important role in reducing populations of *L. africanus*. In the present study, the greatest decline in *L. americanus* population densities during the field study coincided with a period of moisture deficits and greatest soil temperatures. A review of records at several weather stations within 40 km of the nursery indicated that daily maximum air temperatures frequently exceeded 30°C during summer months and reached as high as 37.5°C. Monthly rainfall ranged from 29-118 mm between May and August of 2002 and these levels were well below historical averages for this time period. Nevertheless, the similarity of the decay parameter estimates (b) for the negative exponential models in our field and growth chamber studies suggests that the rate of decrease of *L. americanus* population densities under fallow were similar for the two studies. In contrast to the periods of higher temperatures and moisture deficits in the field study, containers in the growth chamber study were maintained at a constant 25°C and regularly watered. Thus, declines in population densities in the field and growth chamber studies may have been due primarily to nematode starvation rather than desiccation and heat.

A limited food supply for *L. americanus* also may have contributed to declines in nematode population densities in other portions of these studies. For example, the decline in population densities in non-fallow containers during the first 130 days of the growth chamber study was possibly due to the limited number of available roots. Immediately after the sampling on day 130 we established ten additional seedlings in each container and population densities subsequently increased from day 130 through to day 427. In addition, the decrease in population densities of *L. americanus* in the non-fallow treatment during bioassays for the growth chamber study may have been due to a limited food supply. Population densities were initially high (>124 individuals/100 cm^3 soil) in the non-fallow treatment in both bioassays from the growth chamber study but only five seedlings were established in these containers. At the end of the bioassays, seedlings in this treatment were very stunted and the poorly developed root systems lacked lateral and feeder roots.

At this time we do not know if *L. americanus* can survive below 30 cm soil depth for extended periods in the absence of a suitable host. Sutherland (1974) found that populations of *Xiphinema bakeri* were confined primarily to the upper 20 cm of soil in a Canadian forest-tree nursery, although a small percentage of the population was found at soil depths greater than 40 cm. Vertical migration with season or with ambient temperature changes was not evident for *X. bakeri* (Sutherland, 1974). Some *Longidorus* species have been documented at soil depths greater than 60 cm in natural ecosystems and agricultural fields (Flegg, 1968; Ploeg, 1998) and some species are believed to migrate vertically through soil (MacGuidwin, 1989; Rössner, 1991). In the present study our sampling was confined to the upper 30 cm of soil and we cannot rule out the possibility that some individuals may have survived below this depth. Nonetheless, the absence of *L. americanus* at soil depths up to 30 cm in the fallow field during the spring of 2003 when pine seeds would normally be sown suggests that the nematode would not have been a threat to the development of pine seedlings during the first year of production in this field.

Most nurseries in the southern United States rotate fields to cover crops when they are not in production for forest-tree seedlings, and crop rotation cycles can vary greatly among nurseries (Boyer & South, 1984). Many nurseries produce two consecutive pine crops in fields before rotating to cover crops for 2 years, although other nurseries prefer sequences such as yearly rotations of pine seedlings and cover crops. Periods of fallow often occur in nurseries for varying lengths of time depending on production practices. For example, some nurseries fumigate in the autumn and fields are then fallow until spring when pine seeds are sown. The ability of nursery managers to rotate crops on a normal schedule and to leave fields fallow at selected times may provide the means to manage *L. americanus* and possibly other nematodes without the use of costly pesticides. Periods of fallow during the spring and summer, and the use of non-host cover crops (Fraedrich *et al.*, 2003) may provide the greatest benefit for reduction in nematode population densities.

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