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Effect of Plant Age and *Longidorus africanus* on the Growth of Lettuce and Carrot[1]

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Abstract: Needle nematodes, *Longidorus africanus,* were added to carrot and lettuce seedlings in a range of inoculum levels and at various times after seeding. The effects of inoculum density and delayed inoculation on plant growth were analyzed according to Seinhorst's damage function. Growth of both lettuce and carrot was severely affected by *L. africanus,* but delaying nematode inoculation until 10 days after seeding significantly increased estimated minimum yields in both crop species. Tolerance levels of lettuce and carrot for *L. africanus* were approximately 10 times lower than those reported for other longidorid-crop associations. We conclude that methods aimed at avoiding immediate exposure of germinating seeds to *L. africanus* may significantly reduce crop damage.

Key words: carrot, damage, lettuce, *Longidorus africanus,* needle nematode, tolerance.

The needle nematode, *Longidorus africanus* Merny 1966, causes damage to several vegetable crops, including carrot and head lettuce, in the Imperial Valley of southern California (Kolodge et al., 1986, 1987; Lamberti, 1969; Radewald et al., 1969a, 1969b). Field symptoms are usually most obvious in the seedling stage and include stunting and early wilting, ultimately leading to reduced yields (Radewald et al., 1969b). The root systems of affected plants exhibit root-tip galling and are generally reduced in size. Forking of the tap root in carrot further increases the economic importance of the nematode in this crop. Radewald et al. (1969a) reported yield losses in lettuce of 90 g per head in non-fumigated plots compared to fumigated controls and attributed this to the higher *L. africanus* populations in the non-fumigated plots. Although lettuce growth appears to be correlated with *L. africanus* densities, more detailed information on the damage function for this nematode is not available.

A general model describing the effect of increasing nematode pressures on plant growth was developed by Seinhorst (1965, 1998) and includes two biologically significant parameters: the tolerance limit "T," the lowest nematode level to affect plant growth; and the minimum yield "m," the yield remaining even at very high nematode levels. These parameters are influenced by many factors, including nematode species, crop cultivar, soil temperature, soil type, and plant age. Due to the variability of these parameters, better knowledge of the relationship between nematode levels and plant growth is essential for the prediction of crop losses caused by nematodes and for the employment of potential nematode management strategies. With the reduced availability of nematicides and resulting higher costs, growers will probably increase their reliance on soil sampling to assess the risk of nematode damage and to decide on cropping sequences and possible control tactics. The objective of this study was to establish the relationship between time and levels of *L. africanus* inoculum, and the growth of lettuce and carrot plants.

Materials and Methods

A culture of *L. africanus,* originally obtained from carrot field soil in the Imperial Valley, was maintained on tomato (*Lycopersicon esculentum* Mill. cv. Pixie) in 11 pots in a greenhouse at a constant soil temperature of 26 °C in steam-sterilized coarse sand. Subculturing took place every 2 to 3 months. Nematodes were extracted from the cultures with a modified sieving and decanting technique (Brown and Boag, 1988), and final separation was achieved by allowing the nematodes to migrate through a 100-μm-pore nylon sieve over a 12-hour period into a plastic saucer filled with enough water to touch the bottom of the sieve.

One hundred sixty-eight 200-ml plastic cones (Stuewe and Sons, Corvallis, OR) were filled with 250 g steam-sterilized sand and transferred to a growth chamber maintained at 26 ± 1 °C with a 16-hour lighting period. Three seeds of lettuce cv. Burpee's Iceberg (Burpee Seeds, Warminster, PA) were added to each of 42 cones at 10-day intervals. Soon after emergence, the seedlings were thinned to 1 seedling per cone. At the fourth seeding cycle, a shallow hole was made in the center of the soil surface of each of the 168 cones, and 2 ml of a suspension containing 10, 50, 100, 250, 500, or

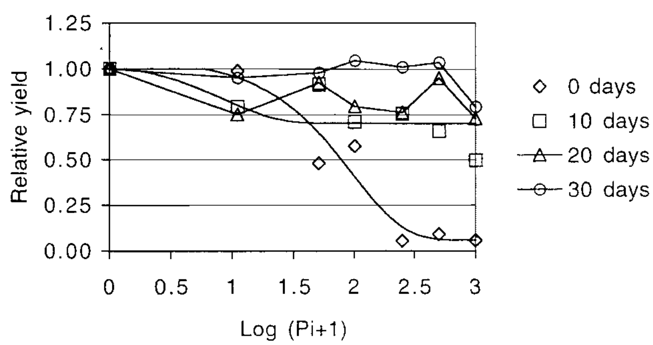

Fig. 1 Effect of time of inoculation of *Longidorus africanus* (0, 10, 20, 30 days after seeding) on the relationship between the relative fresh top weight of lettuce seedlings 30 days after inoculation and inoculum density (Pi; per 250 g soil). Datapoints are means of 6 replicates.

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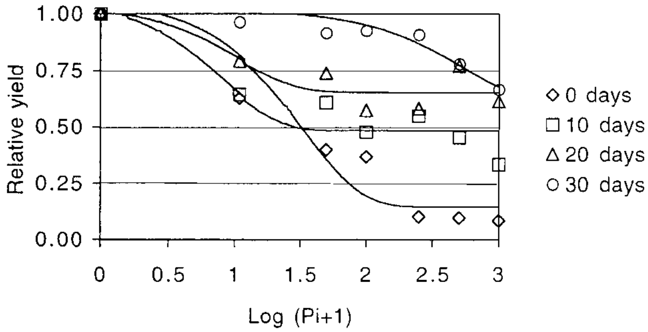

FIG. 2 Effect of time of inoculation of *Longidorus africanus* (0, 10, 20, 30 days after seeding) on the relationship between the relative dry top weight of lettuce seedlings 30 days after inoculation and inoculum density (Pi; per 250 g soil). Datapoints are means of 6 replicates.

1,000 *L. africanus* was pipeted into the hole of each cone. For inocula with 10 nematodes, *L. africanus* were handpicked from the suspension using a fine needle. Higher inoculum densities were obtained by concentrating or diluting the nematode suspension to the required inoculum density. Negative controls received 2 ml of water only. Six replicates were made for each combination of inoculum density (0, 10, 50, 100, 250, 500, 1,000 *L. africanus* per cone) and seedling age at time of inoculation (0, 10, 20, 30 days old). The cones were then placed in randomly assigned positions in their holding trays and the plants were grown for another 30 days.

Experiments using carrot cv. Triumph (Petoseed, Saticoy, CA) were similarly designed, but with *L. africanus* inoculum levels of 0, 10, 20, 50, 100, and 200 nematodes per cone and seedling ages at time of inoculation of 0, 5, 10 and 15 days. Each combination of inoculum density and seedling age also was replicated 6 times to give a total of 144 cones. The plants were grown for 6 weeks after inoculation.

Plants were watered daily and fertilized weekly with full-strength liquid fertilizer (Miracle-GRO; N-P-K: 15-30-15). At harvest, all plants were carefully washed from the cones and blotted dry on filter paper. Plant growth was evaluated by recording the fresh and dry weights of lettuce tops and roots, and the fresh root weights and tap root lengths of the carrot plants.

The relationships between inoculum density and relative plant growth were fitted, where possible, to Seinhorst's (1998) model:

$y' = 1$ for $Pi \leq T$ and $y' = m + (1 - m) \times 0.95^{(PI/T)-1}$ for $Pi > T$ using SAS statistical software (SAS Institute, Cary, NC). In this equation, y' = plant growth relative to the no-nematode control, Pi = inoculum density, T = tolerance limit, and m = the minimum yield relative to the no-nematode control.

RESULTS

There was a strong effect of *L. africanus* on the top growth of lettuce when the plants were inoculated at seeding time (Figs. 1, 2). Maximum reductions in fresh and dry top weights were estimated at 96% and 85%, respectively. Estimates of minimum top weights increased significantly when inoculation was delayed from 0 to 10 days after seeding, but a further increase in time between seeding and nematode inoculation affected only the minimum top weight in a minor way (e.g., top dry weight at 10 days vs. at 20 days) (Table 1). There was no clear negative correlation between increased inoculum levels and fresh top weight when plants were 20 or 30 days old at inoculation (Fig. 1). Consequently, tolerance levels and minimum yields could not be reliably estimated from these data. Tolerance levels for the fresh top weight for the other two treatments (0, 10 days) were estimated at fewer than 5 *L. africanus* per 250 g soil (Table 1). Tolerance levels for the dry top weight also were low (<2 nematodes/250 g soil) for the 0, 10, and 20-day treatments, but much greater (28 nematodes/250 g soil) for plants inocu-

TABLE 1. Estimated tolerance levels (T) as nematodes per 250 g soil, and minimum yields (m) proportional to non-inoculated controls, of lettuce plants inoculated with *Longidorus africanus* at different times after seeding.

		Plant age at inoculation (days)							
		0		10		20		30	
top fresh weight									
	m	0.06 ± 0.08	a	0.70 ± 0.06	b	n.a.		n.a.	
	T	4.90 ± 1.30	a	0.45 ± 0.55	b	n.a.		n.a.	
top dry weight									
	m	0.15 ± 0.07	a	0.48 ± 0.04	b	0.65 ± 0.04	c	0.61 ± 0.10	bc
	T	1.77 ± 0.64	a	0.43 ± 0.21	a	0.58 ± 0.37	a	27.94 ± 12.71	a
root fresh weight									
	m	0.06 ± 0.04	a	0.34 ± 0.05	b	0.48 ± 0.02	c	0.64 ± 0.03	d
	T	2.38 ± 0.46	a	1.88 ± 0.58	a	2.14 ± 0.34	a	3.09 ± 0.84	a
root dry weight									
	m	0.07 ± 0.03	a	0.31 ± 0.03	b	0.36 ± 0.02	b	0.41 ± 0.03	b
	T	0.38 ± 0.08	a	0.59 ± 0.15	a	0.91 ± 0.17	a	2.37 ± 0.51	b

Values within a row followed by the same letter are not significantly different ($P > 0.05$; *t*-test).

lated 30 days after seeding. Due to the high standard error associated with the latter estimate, tolerance levels were not significantly different among the four inoculation dates (Table 1).

Longidorus africanus also dramatically influenced the growth of lettuce roots (Figs. 3, 4). Estimates for maximum reductions in root weight decreased with plant age at inoculation but, even with plants inoculated 30 days after seeding, fresh and dry root weights were reduced by 36% and 59%, respectively, compared to the non-inoculated controls (Table 1). Simultaneous seeding and nematode inoculation significantly increased maximum root weight loss over that found with delayed exposure of the roots to the nematodes (Table 1). Tolerance levels for root growth were always less than 4 nematodes per 250 g soil, and were hardly affected by age of the plant at time of inoculation (Table 1).

Longidorus africanus caused obvious symptoms on carrot, including stunting of tops and small, stubby carrots. The data for the effects of increased *L. africanus* inoculum levels on the weight of the carrots closely fitted the Seinhorst model, which accounted for more than 95% of the variation observed (Fig. 5). The age of the seedlings at the time of inoculation significantly affected the estimated values of the parameters m and T in the Seinhorst model. When inoculated at seeding, maximum reductions in carrot fresh weight were 81% compared to non-inoculated controls (Table 2). Delaying inoculation by 5 or 10 days did not significantly affect the estimated maximum reduction in carrot weight, but delaying nematode inoculation until the plants were 15 days old significantly increased the estimated minimum yield to 70% of the non-inoculated controls (Table 2). In contrast, an initial 5-day delay in exposure of the seedlings to the nematodes significantly increased the tolerance to the nematodes from ca. 0.4 to 0.9 *L. africanus* per 250 g soil. Further delay in exposure of the plants to the nematodes did not significantly increase their tolerance.

The carrot fresh weights were strongly correlated with the carrot length (Fig. 6). Consequently, the effects on carrot length of increased *L. africanus* inoculum levels and delayed nematode inoculation were very similar to the effects on carrot fresh weight, as described previously.

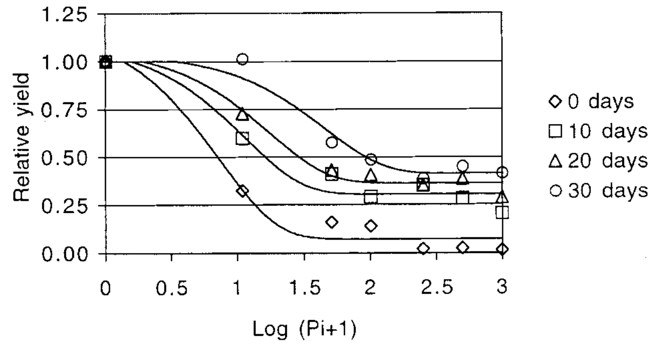

FIG. 4 Effect of time of inoculation of *Longidorus africanus* (0, 10, 20, 30 days after seeding) on the relationship between the relative dry root weight of lettuce seedlings 30 days after inoculation and inoculum density (Pi; per 250 g soil). Datapoints are means of 6 replicates.

Discussion

Our results confirm earlier reports on the pathogenicity of *L. africanus* to lettuce and carrot (Kolodge et al., 1986, 1987; Lamberti, 1969; Radewald et al., 1969a, 1969b). Growth reductions in both lettuce (top dry weight) and carrot (tap root fresh weight) were most severe when plants were infected at a very early stage, with maximum reductions of 85% and 81%, respectively (Tables 1, 2). Increasing inoculum levels also reduced root weights in lettuce (Table 1). This confirms observations from in vitro studies that indicate that root tip growth ceased soon after initiation of feeding by *Longidorus* species (Bleve-Zacheo et al., 1977; Huang and Ploeg, 2001; Robertson et al., 1984). A similar strong impact on root growth was reported for *Xiphinema index* feeding on grape (Di Vito et al., 1985). In contrast, increasing inoculum levels of *Meloidogyne incognita* caused an increase in melon root weights due to the severe root-galling response of melon to *M. incognita* infestation (Ploeg and Phillips, 2001). The strong

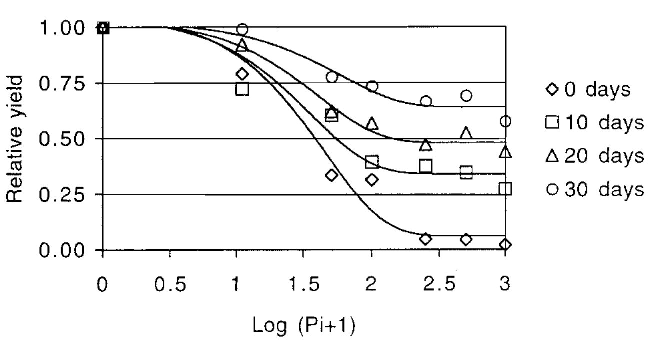

FIG. 3 Effect of time of inoculation of *Longidorus africanus* (0, 10, 20, 30 days after seeding) on the relationship between the relative fresh root weight of lettuce seedlings 30 days after inoculation and inoculum density (Pi; per 250 g soil). Datapoints are means of 6 replicates.

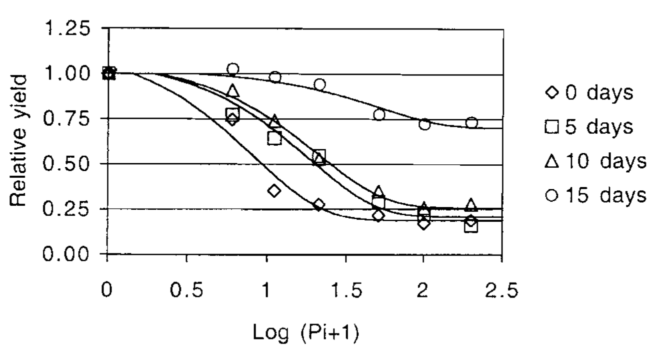

FIG. 5 Effect of time of inoculation of *Longidorus africanus* (0, 5, 10, 15 days after seeding) on the relationship between the relative fresh tap root weight of carrot seedlings 6 weeks after inoculation and inoculum density (Pi; per 250 g soil). Datapoints are means of 6 replicates.

TABLE 2. Estimated tolerance levels (T) as nematodes per 250 g soil, and minimum yields (m) proportional to non-inoculated controls, of carrot plants inoculated with *Longidorus africanus* at different times after seeding.

		Plant age at inoculation (days)							
		0		5		10		15	
relative fresh tap root weight									
	m	0.19 ± 0.03	a	0.21 ± 0.03	a	0.26 ± 0.02	a	0.70 ± 0.04	b
	T	0.42 ± 0.07	a	0.93 ± 0.12	b	1.05 ± 0.07	b	2.51 ± 0.75	b

Values within a row followed by the same letter are not significantly different ($P > 0.05$; *t*-test).

reduction in carrot and lettuce root growth, even at low numbers of *L. africanus,* is probably what restricted top growth.

Tolerance levels for carrot and lettuce exposed to *L. africanus* at seeding were less than 5 nematodes per 250 g soil (Tables 1, 2). Reported tolerance levels per 250 g soil for other longidorid species were ca. 25 for *L. elongatus* on strawberry (Seinhorst, 1966) and ca. 40 for *Xiphinema index* on grape (Di Vito et al., 1985). Thus, compared to these other longidorid-crop associations, the tolerance of lettuce and carrot for *L. africanus* is about 10-fold less. At high inoculum densities, *X. index* damage to grape (95% reduction in top fresh weight) is similar to *L. africanus* damage to lettuce (94% reduction in top fresh weight) and more severe than *L. elongatus* damage to strawberry (70% reduction in top fresh weight) (Di Vito et al., 1985; Seinhorst, 1966).

A delay of 10 days between seeding of lettuce and nematode inoculation significantly increased the minimum yield but only marginally affected tolerance levels (Table 1). Similar findings were reported for *Heterodera avenae* on oats (Seinhorst, 1995a) and for *M. incognita* on melon (Ploeg and Phillips, 2001). Several studies have shown that the root tips are the preferred feeding sites for longidorid nematodes (Bleve-Zacheo et al., 1977; Huang and Ploeg, 2001; Robertson et al., 1984). Older plants have more root tips, so the density of nematodes per root tip is less, which may contribute to the increase in minimum yield when plants are older at time of inoculation. However, Seinhorst (1995b) concluded from comparisons of growth curves of plants inoculated at different time intervals that the strong effect on minimum yield of delayed nematode attack could not be explained simply by differences in plant size at inoculation. In our study, lettuce seedlings had just started to emerge 10 days after seeding and had very small root systems, which also suggests that the observed increase in minimum yield cannot be attributed solely to a dilution of the nematodes over the available root tips, but rather resulted from a decreased sensitivity to damage of 10-day-old seedlings.

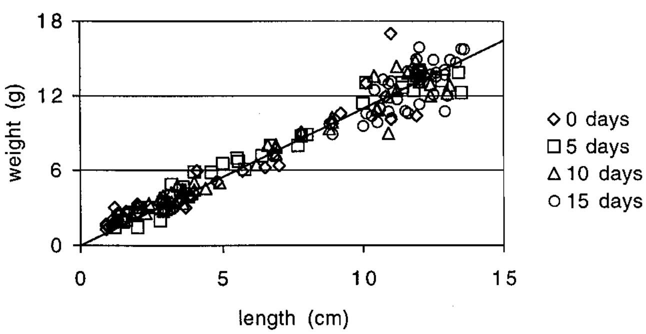

FIG. 6 The relationship between the fresh weight and the length of carrot tap roots 6 weeks after inoculation of 0, 5, 10 or 15-day-old seedlings with *Longidorus africanus.*

Carrots were very sensitive to damage up to at least 10 days after seeding. However, when plants remained nematode-free for 15 days, minimum yields increased significantly. The significant difference in tolerance levels between carrots inoculated at seeding and those inoculated 5 days later—when seeds had just started to germinate—remains unexplained. Tap root length was strongly correlated with fresh weight and thus also strongly affected by the nematodes.

The strong effect of plant age on minimum yields complicates comparison of results from different studies. Thus, comparison of minimum yields from our study, where nematodes were added at seeding, with those from Di Vito et al. (1985), who inoculated 45 day-old grape plants, or with those from Seinhorst (1966), who inoculated transplanted strawberry seedlings (age not reported), is probably not very meaningful.

This study shows that *L. africanus* can cause severe growth reductions in both carrot and lettuce, especially when nematode attack occurs within 10 days of seedling, and that tolerance levels are very low. Therefore, cultural practices or management strategies that temporarily prevent access of the nematodes to germinating seedlings may have a large impact on yields, but this remains to be validated in field trials.

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