Meloidogyne incognita and *M. arenaria* Reproduction on Dwarf Hollies and Lantana¹

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Abstract: Meloidogyne incognita and M. arenaria reproduction and host plant tolerance were assessed in field and greenhouse experiments on seven holly cultivars including Ilex glabra 'Shamrock', I. vomitoria 'Schelling's Dwarf', I. cornuta 'Carissa', red holly hybrid (Ilex Little RedTM), and I. crenata 'Compacta', 'Green Luster', and 'Helleri' as well as Japanese boxwood (Buxus microphylla) and two lantana cultivars (Lantana camara 'Miss Huff' and 'New Gold'). Boxwood had the highest M. arenaria and M. incognita gall rating of any of the plants evaluated. Gall ratings from M. arenaria and M. incognita on I. crenata 'Green Luster' and 'Helleri' were not different from boxwood. Ilex crenata 'Compacta' had less root galling than boxwood, but the roots averaged up to 20% galling by M. incognita and 30% galling by M. arenaria. Ilex glabra 'Shamrock', I. vomitoria 'Schelling's Dwarf', I. cornuta 'Carissa', Ilex Little RedTM, and the two lantana cultivars had little or no root galling after 2 years of growth. Neither M. incognita was much lower than that of M. arenaria on the holly cultivars. Nematode reproduction in the greenhouse. Reproduction of M. incognita was much lower than that of M. arenaria on the holly cultivars. Nematode reproduction in the greenhouse was greatest on the three I. crenata cultivars, followed by Ilex Little RedTM and B. microphylla. Ilex glabra 'Shamrock', I. vomitoria 'Schelling's Dwarf', I. cornuta 'Carissa', and L. camara 'Miss Huff' and 'New Gold' could be useful as Meloidogyne-resistant landscape plants.

Key words: boxwood, Buxus microphylla, holly, Ilex, Lantana, Meloidogyne arenaria, Meloidogyne incognita, ornamental, perennial, resistance, root-knot nematode.

Meloidogyne spp., the most damaging nematodes to ornamental plants in the southeastern United States, are becoming increasingly common on landscape ornamentals as agricultural land is diverted into residential development. *Meloidogyne* spp. also can occur in container plant production if poor sanitation within a nursery allows their introduction into potting mixes or if containers contact infested native soil.

The most common species of *Meloidogyne* in Georgia are *M. incognita* (Kofoid and White) Chitwood and *M. arenaria* (Nell) Chitwood (Powell, 1990). Both species can cause serious plant decline in the landscape (Dunn, 1996). Root galling is the primary symptom of *Meloidogyne* infestation, but root elongation and differentiation also may be suppressed and interactions with other microorganisms may increase root rot incidence or severity (Dunn, 1996). *Meloidogyne*-infected plants grow slowly and may be chlorotic, wilt, or develop marginal browning or bronzing (Dunn, 1996).

No nematicides are labeled in the United States to control nematodes on plants in the landscape. Using non-hosts or plants that are resistant or tolerant to *Meloidogyne* spp. would be an effective management practice. Boxwood is a desired plant in Southern landscapes, but Japanese boxwood (*Buxus microphylla* Sieb. & Zucc.) is very susceptible and intolerant to *M. arenaria* (Benson and Barker, 1982). Infected plants usually show bronzing foliage, stunting, branch dieback, and root galling (Lehman, 1984). Dwarf holly cultivars

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that are less susceptible to *Meloidogyne* spp. may be suitable substitutes for boxwood in the landscape.

Numerous ornamental plant species have been screened for *M. hapla* Chitwood susceptibility (Bernard and Witte, 1987; Bernard et al., 1994; Heald, 1967; LaMondia, 1995, 1996; and McSorley and Frederick, 1994), but fewer have been evaluated for *M. incognita* or *M. arenaria* susceptibility (Barker et al., 1979; Benson and Barker, 1982; Nemec and Struble, 1968; Walker and Melin, 1998; and Walker et al., 1994). Select cultivars of some dwarf holly species including Japanese (*Ilex crenata* Thunb.), Chinese (*I. cornuta* Lindl. & Paxt.), and yaupon (*I. vomitoria* Ait.) also have been screened for *Meloidogyne* susceptibility (Barker et al., 1979; Bernard et al., 1994), but the susceptibility of many cultivars and newly cultivated holly species is unknown.

The objective of this study was to evaluate *M. incognita* and *M. arenaria* reproduction on selected cultivars of dwarf holly species and assess host plant tolerance. The herbaceous perennial *Lantana camara* L. also was evaluated because of its widespread use in southern landscapes.

MATERIALS AND METHODS

Field study: Seven holly cultivars including inkberry (*I. glabra* [L.] A. Gray 'Shamrock'), yaupon holly (*I. vomitoria* 'Schelling's Dwarf'), Chinese holly (*I. cornuta* 'Carissa'), red holly hybrid (*Ilex* Little RedTM), and Japanese holly (*I. crenata* 'Compacta', 'Green Luster', and 'Helleri') as well as Japanese boxwood (*B. microphylla*) and two lantana cultivars (*L. camara* 'Miss Huff' and 'New Gold') were obtained from a commercial nursery. All plants were grown in 3.8-liter plastic containers, except *I. glabra*, which was grown in 11.4-liter plastic containers.

Plants were transplanted into two geographically separated field sites in April 1998 at the University of

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Georgia Bamboo Farm and Coastal Gardens, Savannah, Georgia (soil type Ocilla Complex; loamy fine sand [92% sand-6% silt-2% clay, 1.5% OM]).

Each field site used a randomized complete block design with six replications. Each replication consisted of two plants of each of the 10 ornamental cultivars in the test randomly planted in two rows spaced on 1.5-m centers both within and between rows. Within each replication, one of the two plants per cultivar was designed as nematode-infested.

One week after planting (27 April 1998), the soil surrounding each plant designated as nematodeinfested was inoculated with 8,000 *M. arenaria* race 1 eggs per plant in 8 ml of water at field site 1. Plants at field site 2 were inoculated with 8,000 *M. incognita* race 3 eggs per plant in 8 ml of water. Nematode eggs for inoculum were extracted from greenhouse-grown tomato plants (Hussey and Barker, 1973). Plants designed as uninfested were not inoculated with nematodes. Nematode populations were minimized on the uninfested plants by applying Nemacur 10G (fenamiphos; Bayer Corporation, Kansas City, KS) at a 18.36kg/ha rate in a 1.5-m² area surrounding each plant on 9 April 1999. Nematode-infested and uninfested plants were randomized within the same block.

Plants were not fertilized after planting. Irrigation was applied as needed through rotating sprinkler heads that applied 3.8 cm of water per irrigation. Weeds were controlled by hand-pulling and application of a 20.8 ml/liter rate of Roundup PRO (41% glyphosate; Monsanto Company, St. Louis, MO). Plants were dug on 1 December 1999 and rated for root-knot nematode galling using a linear 0-to-10 scale where 0 = no root galling, 1 = 1–10%, 2 = 11–20%, 3 = 21–30%, 4 = 31–40%, 5 = 41–50%, 6 = 51–60%, 7 = 61–70%, 8 = 71–80%, 9 = 81–90%, and 10 = 91–100% of the root system galled. Data were subjected to analysis of variance and Tukey's HSD means separation test ($P \le 0.05$).

Soil samples (three 2.5-cm-diam. soil cores to a depth of 20 cm) were collected near the crown of each plant in December 1998 and 1999, and nematodes were extracted from 100-cm³ subsamples (Jenkins, 1964). The number of root-knot juveniles per sample was recorded, and data were subjected to analysis of variance and Fisher's Protected LSD means separation test ($P \leq 0.05$).

Greenhouse study: The same holly, boxwood, and lantana cultivars used in the field study were obtained from a commercial nursery as rooted cuttings. Plants were transplanted into 15-cm-diam. plastic pots containing field soil (88% sand, 8% silt, 4% clay; pH 6.4) that was fumigated with methyl bromide (0.6 kg/m³ for 36 hours) prior to use. Night/day temperatures ranged from 18 to 30 °C within the greenhouse. Plants were watered daily, and no supplemental fertilization was provided. This study was conducted twice from June to November 1999.

One week after planting, each pot was inoculated

with 8,300 *M. incognita* race 3 or 8,100 *M. arenaria* race 1 eggs in 8 ml of water by making two holes in the soil near the plant roots and dispensing the egg-suspension. Pots were placed on greenhouse benches in a randomized complete block design with six replications per experiment. Nematode-infested and uninfested plants were placed within the same block.

Eight to 9 weeks after inoculation, nematode eggs were extracted from inoculated roots (Hussey and Barker, 1973) and counted. Plant foliage and roots were harvested, dried at 80 °C for 3 days, and weighed. Data were pooled from the two runs of the experiment testing *M. arenaria* and also from the two runs testing *M. incognita*. Foliage and root dry weights were subjected to analysis of variance and Fisher's Protected LSD means separation test ($P \le 0.05$). Nematode egg counts were subjected to square-root transformation prior to statistical analysis.

RESULTS

Field study: Japanese boxwood, included in the study as a susceptible control, had the highest *M. arenaria* and *M. incognita* gall rating of any of the plants evaluated (Table 1). Gall ratings from *M. arenaria* and *M. incognita* on *I. crenata* cultivars 'Green Luster' and 'Helleri' were not different ($P \le 0.05$) from boxwood. Almost all of the *I. crenata* 'Helleri' plants in the *M. incognita* field site died in 1999. *Ilex crenata* 'Compacta' had less root galling than boxwood, but the roots averaged up to 20% galling from *M. incognita* and 30% galling with *M. arenaria. Ilex glabra* 'Shamrock', *I. vomitoria* 'Schelling's Dwarf', *I. cornuta* 'Carissa', *Ilex* Little RedTM (Red Holly Hybrid), and the two lantana cultivars had no galls or very minor root galling after 2 years of growth in the *M. arenaria* or *M. incognita* infested sites.

The number of M. incognita juveniles extracted from soil surrounding boxwood plants was higher ($P \le 0.05$) than for all plant cultivars except *Ilex* Little Red[™] and I. crenata cultivars 'Green Luster' and 'Helleri' in 1998 (Table 1). In 1999, lower ($P \le 0.05$) densities of juveniles were found in all cultivars than for boxwood. No M. incognita juveniles were found in soil surrounding I. glabra 'Shamrock', I. vomitoria 'Schelling's Dwarf', or L. camara 'Miss Huff' in 1998 or 1999. The number of M. arenaria juveniles was greatest from boxwood followed by *Ilex* Little Red[™] in 1999. Although not significantly different, the plant heights of the holly cultivars grown in M. incognita-infested soils for 2 years were numerically lower than the uninfested plants, especially for 'Helleri,' 'Compacta', *Ilex* Little Red[™], and Japanese boxwood (Fig. 1).

Greenhouse study: The presence of either *M. incognita* or *M. arenaria* did not affect plant growth of any of the plant cultivars evaluated compared to the uninfested plants. There were no differences between foliage and root dry weights of nematode-inoculated and uninfested plants from either the *M. incognita* or *M. arenaria* tests (Tables 2 and 3).

TABLE 1. Root gall ratings and mean nematode counts from dwarf holly and lantana cultivars grown for 2 years in root-knot nematodeinfested field soil.

Plant species and cultivar	Meloidogyne incognita race 3			Meloidogyne arenaria race 1		
	Mean root gall rating ^a	Mean nematode count ^b			Mean nematode count ^b	
		Dec 98	Dec 99	Mean root gall rating ^a	Dec 98	Dec 99
Buxus microphylla (Japanese Boxwood)	6 b	304 c	414 c	7 d	328 b	397 с
Ilex glabra 'Shamrock'	0 a	0 a	0 a	0 a	0 a	1 a
I. vomitoria 'Schelling's Dwarf'	0 a	0 a	0 a	0 a	0 a	3 a
I. cornuta 'Carissa'	0 a	3 a	1 a	1 ab	21 a	0 a
<i>Ilex</i> Little Red TM (Red Holly Hybrid)	1 a	71 abc	81 b	1 ab	227 b	84 b
I. crenata 'Compacta'	2 a	50 ab	0 a	3 bc	27 a	0 a
I. crenata 'Green Luster'	5 b	90 bc	10 ab	5 cd	42 a	3 a
I. crenata 'Helleri'	5 b	68 abc	23 ab	5 cd	230 b	7 a
Lantana camara 'Miss Huff'	0 a	0 a	0 a	0 a	8 a	0 a
Lantana camara 'New Gold'	0 a	37 ab	0 a	0 a	3 a	0 a

^a Gall rating based on a scale of 0 to 10 where 0 = no root galling, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100% of the root system galled. Means followed by the same letter are not different based on Tukey's HSD mean separation test ($P \le 0.05$).

^b Mean number of root-knot nematodes recovered from 100 cm³ of field soil. Means followed by the same letter are not significantly different based on Fisher's Protected LSD mean separation test ($P \le 0.05$).

Nematode reproduction data are presented in Tables 2 and 3. Analysis of variance of the *M. incognita* experiments indicates a difference between the two experiments in nematode reproduction (P = 0.016). The two experiments differed because of *Ilex* Little RedTM. In the first trial, the red holly hybrid supported the greatest nematode reproduction with an average of 29,999 eggs per plant, whereas, in the second trial, it supported only an average of 39 eggs per plant. Nematode reproduction for all other plant species and cultivars is consistent between the two experiments.

Overall, reproduction of *M. incognita* was much lower than that of *M. arenaria* on the holly and lantana cultivars. An *M. incognita* reproductive factor (Rf = final egg

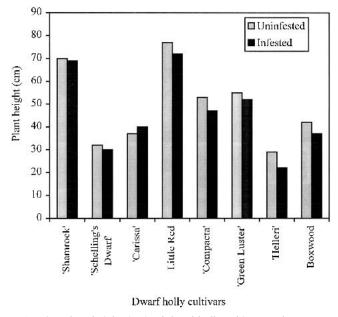


FIG. 1. Plant height (cm) of dwarf holly cultivars and Japanese boxwood 2 years after infesting a field site with 8,000 *Meloidogyne incognita* race 3 eggs per plant. Heights of infested and uninfested plants were not different at $P \le 0.05$.

count/inoculum level) greater than 1.0 occurred on the *I. crenata* cultivars 'Compacta' and 'Helleri', as well as *Ilex* Red holly hybrid (Table 2). All other cultivars, including *B. microphylla*, supported little nematode reproduction resulting in an Rf less than 1.0.

Analysis of variance for the *M. arenaria* experiments shows that plant species significantly affected nematode reproduction (P < 0.0001). Nematode reproduction was greatest on the three *I. crenata* cultivars, followed by *Ilex* Little RedTM and *B. microphylla* (Table 3). Nematode reproduction was lowest on the two lantana cultivars, *I. glabra* 'Shamrock', and *I. vomitoria* 'Schelling's Dwarf'.

DISCUSSION

Data from the field and greenhouse studies led to similar conclusions. Overall, *I. glabra* 'Shamrock', *I. vomitoria* 'Schelling's Dwarf', and *L. camara* 'Miss Huff' and 'New Gold' supported the least *M. incognita* and *M. arenaria* reproduction.

Japanese boxwood was included in this study as a Meloidogyne-susceptible control. It was the most susceptible plant in the field study, having significantly greater root galling by both nematode species than any of the plant species and cultivars evaluated except I. crenata. Boxwood supported M. arenaria reproduction in the greenhouse trial, but it was not the most susceptible plant species evaluated. Little M. incognita reproduction was detected on boxwood in the greenhouse. Susceptible cultivars should have an Rf greater than 1.0 so that more eggs will be recovered at the end of the experiment than were inoculated onto the plant. The low number of M. incognita eggs recovered from boxwood roots in the greenhouse study is not consistent with the galling and recovery of juveniles in the field study. The low numbers of eggs extracted from boxwood could be due to egg hatch prior to egg extraction or due to very

Plant species and cultivar	Uninfested				
	Foliage dry wt. ^a	Root dry wt. ^b	Foliage dry wt.	Root dry wt.	Nematode eggs ^c
Buxus microphylla					
Japanese Boxwood Ilex glabra	4.1	1.4	6.2	1.9	520 с
'Shamrock' Ilex vomitoria	7.8	3.3	7.1	3.2	164 c
'Schelling's Dwarf' Ilex cornuta	2.9	1.0	4.2	1.4	5 c
'Carissa' Ilex crenata	4.4	1.4	5.1	2.4	41 c
'Green Luster' Ilex crenata	10.4	2.4	13.9	3.7	5,586 b
'Compacta' Ilex crenata	12.1	2.3	12.2	3.1	17,999 a
'Helleri' <i>Ilex</i> Little Red [™]	7.1	1.4	6.6	1.3	9,076 b
Red holly hybrid Lantana camara	10.0	2.1	10.8	2.3	15,021 b
'New Gold' Lantana camara	13.2	4.1	13.3	5.2	0 c
'Miss Huff'	13.7	6.0	15.6	6.5	0 c

TABLE 2. Mean foliage, root dry weights, and egg counts from greenhouse screening of dwarf holly and lantana cultivars for *Meloidogyne incognita* race 3 resistance.

^a Pooled mean of foliage dry weight (g) from two experiments.

^b Pooled mean of root dry weight (g) from two experiments.

^c Pooled mean egg counts from inoculated plots in two experiments. Analysis of variance was conducted on square-root transformed data. Values presented are not transformed. Numbers followed by the same letter are not different based on Fisher's Protected LSD ($P \le 0.05$).

slow root growth of boxwood, which slowed nematode reproduction during the experiments. Juveniles were not extracted from the soil as part of the greenhouse study; therefore, the influence of egg hatching on nematode recovery in this study is unknown.

Yaupon holly (*I. vomitoria*) is often suggested as a boxwood replacement in nematode-infested soils because of its tolerance to *Meloidogyne* species. In this study, it showed very little response in galling or plant growth to *Meloidogyne* inoculation. Some *I. vomitoria* cultivars including 'Nana' and 'Stokes Dwarf' have been classified as resistant to *M. hapla* and *M. arenaria* (Barker et al., 1979; Bernard et al., 1994). The 'Schelling's Dwarf' cultivar evaluated in this study appears to be resistant to both *M. arenaria* and *M. incognita*. No *I. vomitoria* cultivar has been previously screened against *M. incognita*.

Ilex glabra 'Compacta' and 'Nordic' have been

screened and found to be susceptible to M. incognita and one M. hapla isolate from Tennessee, but resistant to another M. hapla isolate from North Carolina (Bernard et al., 1994). Bernard et al. (1994) speculated that the difference in susceptibility might be attributed to preferential host response because the isolate from Tennessee was collected from commercial woody ornamental nursery beds where the nematodes may have become selectively adapted to hollies. The potential of isolate-dependent resistance in hollies should be investigated further. Our study indicates that I. glabra 'Shamrock', a previously unscreened cultivar, is very resistant to both *M. arenaria* and *M. incognita* and that it could be used successfully as a boxwood replacement plant in Meloidogyne-infested sites. 'Shamrock' was the most promising boxwood replacement holly in this study. Although it is relatively new to the ornamental industry, its growth characteristics of dark-green, small leaves

TABLE 3. Mean foliage, root dry weights, and egg counts from greenhouse screening of dwarf holly and lantana cultivars for *Meloidogyne* arenaria race 1 resistance.

Plant species and cultivar	Uninfested			Infested	
	Foliage dry wt. ^a	Root dry wt. ^b	Foliage dry wt.	Root dry wt.	Nematode eggs ^c
Buxus microphylla					
Japanese Boxwood Ilex glabra	3.3	1.7	3.3	1.6	15,518 bo
'Shamrock' Ilex vomitoria	8.7	5.0	8.5	4.7	337 d
'Schelling's Dwarf' Ilex cornuta	4.2	2.2	3.0	1.7	1,788 d
'Carissa' Ilex crenata	4.7	1.8	4.5	1.4	5,224 cd
'Green Luster' Ilex crenata	15.7	3.9	13.9	4.0	70,786 a
'Compacta' Ilex crenata	13.6	4.1	10.1	3.8	98,658 a
'Helleri' <i>Ilex</i> Little Red [™]	4.7	1.2	8.3	1.3	79,096 a
Red holly hybrid Lantana camara	12.9	2.9	12.6	2.8	27,697 b
'New Gold' Lantana camara	9.0	2.0	9.4	1.7	12 d
'Miss Huff'	21.3	9.7	19.0	7.8	7 d

^a Pooled mean of foliage dry weight (g) from two experiments.

^b Pooled mean of root dry weight (g) from two experiments.

^c Pooled mean egg counts from inoculated plots in two experiments. Analysis of variance was conducted on square-root transformed data. Values presented are not transformed. Numbers followed by the same letter are not different based on Fisher's Protected LSD ($P \le 0.05$).

with little to no spines make it a viable alternative to boxwood in *Meloidogyne*-infested sites.

Ilex crenata cultivars including 'Compacta', 'Convexa', 'Helleri', and 'Rotundifolia' are highly susceptible to *M. incognita, M. arenaria,* and *M. hapla* (Benson and Barker, 1982; Bernard et al., 1994; Heald, 1967). This study further documents that *I. crenata* cultivars, including 'Green Luster', which has not been previously evaluated against *M. arenaria*, are highly susceptible to both *M. incognita* and *M. arenaria*. These holly cultivars supported the greatest nematode reproduction of any of the holly species and cultivars evaluated in the greenhouse study and also sustained significant root galling in the field study. Reproduction of *M. arenaria* on the holly cultivars was greater overall than that of *M. incognita*.

The only other holly species that supported high nematode reproduction in the greenhouse study was *Ilex* Little RedTM. This holly is an open pollinated seedling variety of 'Mary Nell' (a controlled cross of [I. cornuta 'Burfordii' × I. pernyi Franchet 'Red Delight'] × I. latifolia Thumb.) (Galle, 1997). Little evaluation has been conducted on these relatively new hybrids, and their current use in landscapes is limited. Of the holly cultivars evaluated, *Ilex* Little RedTM gave the most conflicting results. In the first M. incognita greenhouse study, an average of almost 30,000 eggs were recovered from inoculated plants; in the second trial, less than 40 were recovered per plant. In the field study Ilex Little RedTM showed little root galling (generally less than 15%), but *Meloidogyne* juveniles were consistently recovered from the soil surrounding the plants at levels second only to boxwood in 1999 for both M. arenaria and M. incognita. The lack of resistance to root-knot nematodes may be derived from the cultivar's parentage. Neither I. pernyi nor I. latifolia have been screened against Meloidogyne species, but I. cornuta has been reported to range from resistant to highly susceptible (Barker et al., 1979; Bernard et al., 1994; Nemec and Struble, 1968).

Barker et al. (1979) found I. cornuta 'Burfordii' to be resistant and I. cornuta 'Rotunda' to be susceptible to M. arenaria. The cultivar 'Burfordii' also was reported as highly susceptible to *M. incognita* (Nemec and Struble, 1968). Another cultivar, 'Needlepoint', was susceptible to *M. incognita* and highly susceptible to one isolate of *M. hapla* but resistant to another isolate of *M*. hapla, the same as reported for I. glabra (Bernard et al., 1994). From the previous studies, it appears that I. cornuta cultivars differ greatly in their susceptibility to species of Meloidogyne. The I. cornuta cultivar evaluated in this study, 'Carissa', appears to be resistant to M. incognita and M. arenaria. 'Carissa' supported some M. arenaria and very little M. incognita reproduction in the greenhouse study, and virtually no root galling or recovery of root-knot juveniles was observed in the field study. Although 'Carissa' may be a good choice to use in root-knot nematode-infested landscape sites, it is not a suitable substitute for boxwood in traditional gardens because of its leaf characteristics (longer leaves, lightgreen color, and larger leaf spines typical of some holly species).

Lantana camara is an increasingly popular plant in southern United States landscapes, where it is considered an annual or tender perennial suitable for USDA Hardiness Zones 7b-10. Two of the most popular cultivars are 'New Gold' and 'Miss Huff'. This study represents the first screening of lantana cultivars for *Meloidogyne* susceptibility. Both 'Miss Huff' and 'New Gold' appear to be highly resistant to *M. arenaria* and *M. incognita.* Plants in the field study had no visible root galling, and their growth was not hindered by nematode inoculation. Virtually no nematode reproduction occurred with either nematode species, making lantana a good plant option in *Meloidogyne*-infested landscapes.

Nematode control within landscapes is probably the most challenging problem for gardeners and commercial landscape managers. No nematicides are labeled in the United States for use on ornamentals in landscapes, and pre-plant fumigation of landscape beds within residential areas is not legally permitted or is severely restricted. The use of host resistance is therefore the most viable and cost-effective long-term solution to nematode control within landscapes. The results from this study suggest I. cornuta 'Carissa', I. glabra 'Shamrock', and L. camara 'Miss Huff' and 'New Gold' would be suitable in landscape plantings infested with M. incognita and (or) M. arenaria. Additional studies should be conducted to evaluate more woody and herbaceous ornamental species and cultivars for nematode susceptibility.

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