Journal of Nematology 18(1):26–30. 1986.

Plant Parasitic Nematodes Associated with Leatherleaf Fern[1]

D. T. KAPLAN[2] AND LANCE S. OSBORNE[3]

Abstract: Seven species of plant parasitic nematodes were found to be associated with leatherleaf fern (*Rumohra adiantiformis*) in central Florida. Of these, *Pratylenchus penetrans, Tylenchorhynchus claytoni,* and *Criconemoides curvatum* were commonly encountered. Nematode communities generally included two or three species of plant parasitic nematodes, with greatest diversity in nematode species occurring in ferneries shaded by oak trees. Species diversity was not correlated with fernery age. Leatherleaf fern was tolerant of *P. penetrans* and *T. claytoni* in microplot tests.

Key words: species diversity, tolerance, survey, pathogenicity.

Florida leads the world in the production of leatherleaf fern (*Rumohra adiantiformis* (Forst.) Ching) with approximately 2,400 hectares (6,000 acres) concentrated primarily in three counties in central Florida. The current annual wholesale value of the crop is $63.2 million, and the volume is estimated to increase at a rate of 15% per year (2).

Leatherleaf fern is a host of the lesion nematode, *Pratylenchus penetrans* (8), with some populations causing more damage (7) to leatherleaf fern than others (3,7). *P. penetrans* infects both roots and rhizomes (3,7); it is dispersed by planting nematode-infected rhizomes (propagules) in new ferneries. Dipping infected propagules in nematicides reduces nematode densities (3,7,10); however, this procedure tends only to slow nematode establishment in ferneries (7). Application of nematicides to

Received for publication 26 February 1985.

[1] University of Florida, Journal Series No. 6888.

[2] Research Plant Pathologist, USDA ARS, U.S. Horticultural Research Laboratory, 2120 Camden Road, Orlando, FL 32803.

[3] Assistant Entomologist, University of Florida, IFAS, Agricultural Research and Education Center, Apopka, FL 32703.

We thank Dr. Ann Chase for plant material and Dr. Morgan Golden for confirmation of nematode identification. We also thank Beverly Hewett, Diana Johnson, Janice Kiel, and Margueritte Wettstein for technical assistance.

This paper reports the results of research only. Mention of a pesticide does not constitute a recommendation by the U.S. Department of Agriculture or the University of Florida, IFAS, nor does it imply registration under FIFRA.

fern beds has resulted in a variety of responses. Nematicide application can decrease nematode population densities and increase yield, decrease nematode densities without influencing yield, or have no significant effect on nematodes but increase yield (4,7,8,10). In one study, nematicide treatment reduced lesion nematode population densities within 3 to 6 months, but yield increases were not observed until 2 years after the initial nematicide treatment (3). The high cash value of leatherleaf fern enables growers to apply nematicides with little or no information on nematode-related yield losses in their ferneries.

In the field, we found that frequent harvesting of the crop (foliage) impeded identification of diseased areas within ferneries. Although nematodes were commonly encountered, nematode damage to roots was difficult to identify because leatherleaf fern roots are dark brown. In preliminary studies, we detected several plant-parasitic nematode species in ferneries which had not been previously associated with the crop.

Our objectives were to 1) identify plant-parasitic nematodes associated with leatherleaf fern in Florida, 2) determine population densities of nematode species in ferneries, 3) determine if soil sampling could adequately characterize the nematode community, and 4) evaluate the influence of *P. penetrans, Tylenchorhynchus claytoni,* and *Criconemoides curvatum* on growth of leatherleaf fern.

Materials and Methods

Identification and population densities of plant parasitic nematodes associated with leatherleaf fern: Samples collected from leatherleaf ferneries consisted of soil and roots in the top 30.5 cm of soil. Five samples, each a composite of five subsamples, were collected randomly in fern beds in 28 ferneries for a total of 140 samples. Samples were processed by first sifting out roots from soil with 3,540-μm-pore sieve. Sifted soil from each sample was thoroughly mixed and 600 cm^3 soil was processed (5) with nematodes recovered from 250-, 90-, 53-, 45-, and 38-μm-pore sieves. Roots were rinsed free of excess soil, and nematodes were extracted by jar incubation at 25 C for 7 days (12). Data are expressed as nematodes per 100 cm^3 soil or nematodes per gram root dry weight.

Influence of P. penetrans and T. claytoni on leatherleaf fern: Nematode-free leatherleaf ferns were grown from spores (6). Plants were grown for 2 years in steamed potting media before use in the experiment. Polyvinyl chloride microplots (15 cm d × 35 cm deep) were filled with steamed sandy loam (pH 6.0) obtained from a fernery in Seville, Florida, randomized, placed in two greenhouse soil tanks (1) at 24 ± 2 C, and maintained under saran shade cloth (13–20 microwatts/cm^2). Ferns were watered semiweekly and top dressed bimonthly with 10 cm^3 slow release fertilizer (19-6-12). Two, 10-cm rhizome segments with growing points intact were planted in each microplot and grown for 12 months before the microplot soil was infested with nematodes. Nematodes for inoculum were extracted from soil (5), quantified, and treated with aqueous 1.0% 8-hydroxyquinoline sulfate for 10 minutes before introduction into microplot soil.

Pratylenchus penetrans was collected from a fernery in DeLand, Florida, and 10 microplots were each inoculated with 0, 12.5, 25.0, or 50.0 nematodes per 100 cm^3 soil. *Tylenchorhynchus claytoni* and *Criconemoides curvatum* were obtained from fernery soil collected in DeLeon Springs, Florida. Nematodes were extracted and surface sterilized as described earlier, and microplot soil was inoculated with 0, 5, 10, or 20 nematodes per 100 cm^3 soil. Commencing 6 months after microplot inoculation, microplot yields (fronds) and nematode population densities were determined at 3-month intervals for 1 year. At each harvest, five fronds were left intact. Yield data included the number and dry weight of fronds harvested from each microplot. To determine nematode population densities, soil samples (100 cm^3) consisting of two composite 2.5-cm-d cores removed from each microplot were extracted by Baermann funnel for 24 hours, nematodes recovered were counted, and nematode days (9) were determined for each plot. Nematode days facilitate damage estimates attributed to nematodes over time when plotted against average frond dry weight and the number of fronds produced in each microplot.

TABLE 1. Relative frequencies (%) and population densities of seven plant parasitic nematodes in root and soil samples from 28 leatherleaf ferneries located in central Florida.

Nematode	Soil		Roots	
	%	Density*	%	Density†
Pratylenchus penetrans	71	24.6 (21.3)	68	174 (196)
Tylenchorhynchus claytoni	32	34.8 (49.9)	43	21 (17)
Criconemoides curvatum	71	25.4 (22.6)	25	45 (71)
Hemicycliophora sp.	14	15.6 (6.4)	4	9 (0)
Trophonema floridensis	18	14.1 (5.9)	7	9 (1)
Paratrichodorus minor	4	30.2 (8.0)	0	—
Xiphinema americanum	4	7.8 (0.0)	0	—

* Nematodes per 100 cm³ soil followed by standard deviation in parentheses.
† Nematodes per gram root dry weight followed by standard deviation in parentheses.

RESULTS

Seven species of plant parasitic nematodes were detected in 28 leatherleaf ferneries located in central Florida (Table 1). *Pratylenchus penetrans, T. claytoni,* and *C. curvatum* were most commonly encountered (Table 1, Fig. 1). Soil samples in general provided a better qualitative estimate of the plant-parasitic nematode community in ferneries than did root samples (Table 1, Fig. 1).

A greater diversity of plant-parasitic nematode species was detected in ferneries under oak tree canopies than in those covered with shade cloth (Fig. 2). *Trophonema floridensis* and *Hemicycliophora* sp. were detected only in ferneries under oak trees. Ferneries sampled were 2–13 years old. Nematode species diversity was not correlated with fernery age ($r = 0.02$).

It was determined in microplots that 1) leatherleaf fern was a host of *P. penetrans* and *T. claytoni* (Table 2), 2) *C. curvatum* did not persist under our experimental conditions, and 3) neither *P. penetrans* (Fig. 3a, b) nor *T. claytoni* (Fig. 4a, b) adversely influenced leatherleaf fern yield.

DISCUSSION

Although seven species of plant parasitic nematodes were associated with leatherleaf ferns, only *P. penetrans, T. claytoni,* and *C. curvatum* were recovered frequently. *Paratrichodorus minor* was detected in only one fernery which was located in Zellwood, Florida. This fernery differed from others in that it was geographically isolated from other ferneries and was situated in muck soils. Most leatherleaf ferns are grown in sandy loam soils. *T. floridensis* and *Hemicycliophora* spp. appear to be primarily associated with roots of oak trees which provide shade for many ferneries.

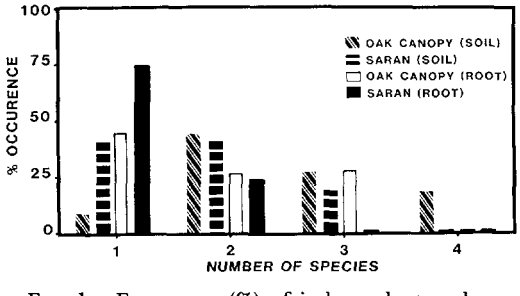

FIG. 1. Frequency (%) of independent and concomittant detection of plant parasitic nematode species in root and soil samples from 28 leatherleaf ferneries located in central Florida. PP = *Pratylenchus penetrans.* TY = *Tylenchorhynchus claytoni.* C = *Criconemoides curvatum.* H = *Hemicycliophora* sp. TP = *Trophonema floridensis.* X = *Xiphinema americanum.* PM = *Paratrichodorus minor.*

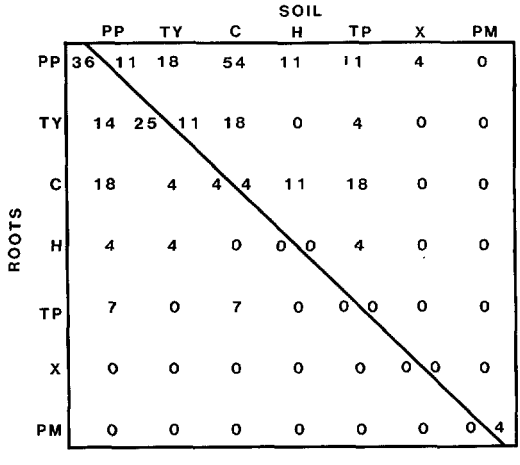

FIG. 2. Numbers of plant parasitic nematode species found in leatherleaf ferneries shaded by shade cloth or by oak trees.

TABLE 2. Initial (Pi, 15 July 1983) and final Pf, 15 November 1984) population densities of *Pratylenchus penetrans* and *Tylenchorhynchus claytoni* per 100 cm^3 soil in leatherleaf fern greenhouse microplots.

	Pi	Pf
P. penetrans	12.5	60.0
	25.0	103.0
	50.0	105.0
T. claytoni	5.0	14.0
	10.0	30.0
	20.0	56.0

Leatherleaf ferns are grown in shade provided by overstories of large oak trees or under shade cloth (approximately 73% shade). Greater nematode species diversity in ferneries under oak trees may be related to site preparation. In ferneries under oak trees, oak roots limit tillage and preplant treatment of soil. In contrast, sites under shade cloth received extensive preparative tillage which may require several months imposing a fallow period on indigenous nematode populations and thereby reducing their densities. These sites are occasionally fumigated for weed control which would also reduce nematode population densities.

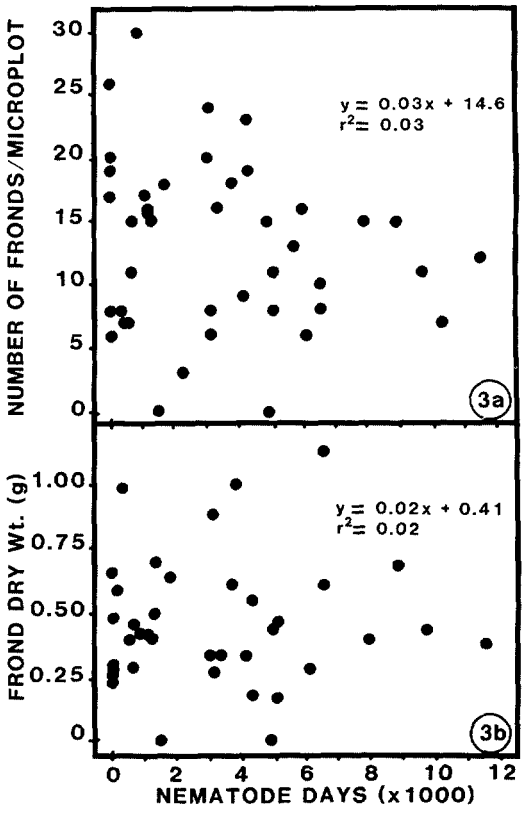

FIG. 3. Influence of *Pratylenchus penetrans* (nematode days) on a) the number of leatherleaf fern fronds per microplot and b) dry weight in grams of fronds produced by leatherleaf ferns growing in microplots.

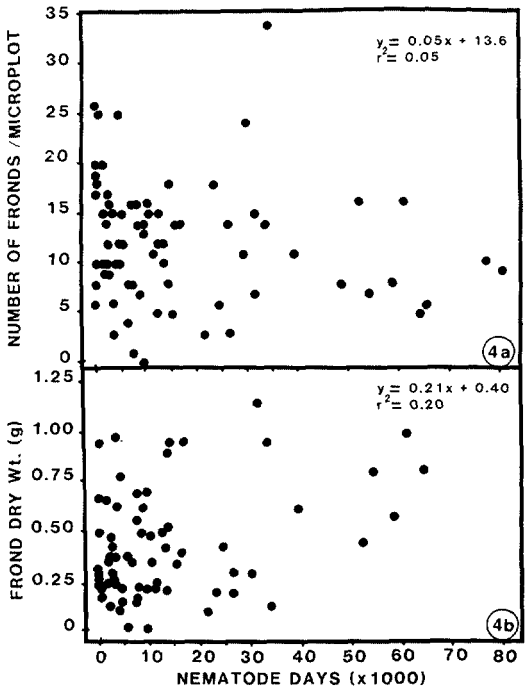

FIG. 4. Influence of *Tylenchorhynchus claytoni* (nematode days) on a) the number of leatherleaf fern fronds per microplot and b) dry weight in grams of fronds produced by leatherleaf fern growing in microplots.

Evaluation of soil samples extracted by Baermann funnel appeared adequate to characterize nematode communities in ferneries. Lesion nematodes on other crops are not readily found in soil whereas high population densities occur in roots, such as *P. coffeae* on citrus (5). While not addressed in this study, factors responsible for the preponderance of lesion nematodes in fernery soils may be related to the unusual roots of leatherleaf fern. From a practical standpoint, nematodes in leatherleaf ferneries can be adequately determined by extracting soil samples. This is helpful in that extracting nematodes from root samples requires more handling and time than extracting nematodes from soil with Baermann funnels. Lesion nematode popula-

tion densities in microplot soil were similar to those observed in the field suggesting that microplots may be used to evaluate the influence of plant parasitic nematodes on the growth of leatherleaf fern.

In our study, leatherleaf fern yield was not suppressed by *P. penetrans* or *T. claytoni* in contrast to the report of Rhoades (8), who observed suppressed growth within several months of inoculation with *P. penetrans.* This difference may be attributed to the following: 1) inoculum source—variation in the pathogenicity of lesion nematode isolates (11); 2) leatherleaf fern source—leatherleaf fern from different sources may respond differently to nematode parasitism; 3) treatment of propagules—in our study, leatherleaf fern propagules were raised from spores and maintained in sterilized potting media before being used in microplots, whereas in the previous research propagules were taken from the field; 4) treatment of inoculum—nematodes were surface sterilized prior to use in our study, but not in previous studies; and 5) plant condition—ferns in our study were well established prior to inoculation.

Leatherleaf fern tolerance to lesion nematodes may explain lack of response to treatments where relatively nonphytotoxic nematicides reduced lesion nematode soil population densities, but did not increase yields (3,7,8), as well as those in which treatment of soil or propagules did not reduce nematode population densities, but did cause increases in yield (4).

This is the first report that leatherleaf fern is a host of *T. claytoni.* Its feeding habit on fern roots has not been determined.

In this study, nematode days were used in a manner similar to insect days (9), which provide a single measure of the intensity of insect attack. In this case, nematode days give a measure of damage caused by nematodes over time by incorporating both time and population density data into a single variable. Calculation of nematode days represents the area under the curve when nematode numbers are plotted against time. They provide a single expression of the intensity and duration of nematode infestation.

Our findings suggest that *P. penetrans*-infection or *T. claytoni*-infection of well-established leatherleaf fern did not suppress yield. Pathogenicity of *P. penetrans* or *T. claytoni* to leatherleaf fern could require that fern propagules be infested at planting or that they be planted in infested soil. Nematodes may also interact with rhizosphere microorganisms to impair plant growth. These conditions were not investigated in our study. However, this would suggest that leatherleaf fern production could be improved through the use of nematode-free propagules in conjunction with the use of noninfested fernery sites or with preplant fumigation. It appears that nematicide usage could be greatly reduced if leatherleaf ferns were well established prior to the introduction of these nematodes.

Literature Cited

1. Feder, W. A., and J. Feldmesser. 1956. Starting and maintaining burrowing nematode infected citrus under greenhouse conditions. Proceedings of the Florida State Horticultural Society 69:29–30.

2. Fieser, S., and E. F. Scarborough. 1984. Marketing Florida ornamental crops, fresh flowers, and ferns, summary 1983–1984 season. Winter Park, Florida Federal–State Market News Service.

3. Hamlen, R. A. 1978. Suppression of *Pratylenchus penetrans* in leatherleaf fern by nematicides. Plant Disease Reporter 62:899–902.

4. Hart, W. H., and A. R. Maggenti. 1970. Granular and soil-spray nematicidal treatments for the control of root-lesion nematode, *Pratylenchus penetrans,* on leatherleaf fern. Plant Disease Reporter 54:1016–1017.

5. Kaplan, D. T., and L. W. Timmer. 1982. Effects of *Pratylenchus coffeae—Tylenchulus semipenetrans* interactions on nematode population dynamics in citrus. Journal of Nematology 14:368–373.

6. Knauss, J. F. 1976. A partial tissue culture method for pathogen-free propagation of selected ferns from spores. Proceedings of the Florida State Horticultural Society 89:363–365.

7. Overman, A. J. 1975. Control of *Pratylenchus penetrans,* endoparasitic nematode of leatherleaf fern. Proceedings of the Florida State Horticultural Society 88:573–578.

8. Rhoades, H. L. 1968. Pathogenicity and control of *Pratylenchus penetrans* on leatherleaf fern. Plant Disease Reporter 52:383–385.

9. Ruppel, R. F. 1983. Cumulative insect days as an index of crop protection. Journal of Economic Entomology 76:375–378.

10. Stokes, D. E., and C. W. Laughlin. 1970. Control of *Pratylenchus penetrans* on leatherleaf fern transplants. Plant Disease Reporter 54:287–288.

11. Townshend, J. L., R. Tarte, and W. F. Mai. 1978. Growth response of three vegetables to smooth- and crenate-tailed females of three species of *Pratylenchus.* Journal of Nematology 10:259–263.

12. Young, T. W. 1954. An incubation method for collecting migratory endoparasitic nematodes. Plant Disease Reporter 38:794–795.