Journal of Nematology 18(3):347–352. 1986.

Differential Reaction of Alfalfa Cultivars to *Meloidogyne hapla* and *M. chitwoodi* Populations[1]

G. D. Griffin,[2] R. N. Inserra,[3] N. Vovlas,[4] and D. V. Sisson[5]

Abstract: *Meloidogyne hapla* reproduced and suppressed growth ($P < 0.05$) of susceptible Lahontan and Moapa alfalfa at 15, 20, and 25 C. At 30 C, resistant Nevada Syn XX lost resistance to *M. hapla*. *M. hapla* invaded and reproduced on *Rhizobium meliloti* nodules of Lahontan and Moapa, inducing giant cell formation and structural disorder of vascular bundles of nodules without disrupting bacteroids. At 15, 20, and 25 C a *M. chitwoodi* population from Utah reproduced on Lahontan, Moapa, and Nevada Syn XX alfalfa, suppressing growth ($P < 0.05$). Final densities of the Utah *M. chitwoodi* population were greater ($P < 0.05$) than those of Idaho and Washington State populations on Lahontan at 15 and 25 C and on Nevada Syn XX at 15 C, but were less consistent and smaller ($P < 0.05$) than those of *M. hapla* on Lahontan and Moapa at 20 and 25 C. Inconsistent reproduction of the Utah *M. chitwoodi* population on alfalfa suggests the possible existence of nematode strains revealed by variability in alfalfa resistance. No reproduction or inconsistent final nematode population densities with no damage were observed on Lahontan, Moapa, and Nevada Syn XX plants grown in soil infested with Idaho and Washington State *M. chitwoodi* populations.

Key words: Columbia root-knot nematode, northern root-knot nematode, *Medicago sativa*, Lahontan, Moapa, Nevada Syn XX, *Rhizobium meliloti*, histopathology, pathogenicity, reproduction, resistance, temperature.

The Columbia root-knot nematode *Meloidogyne chitwoodi* Golden et al. attacks potato (*Solanum tuberosum* L.) and cereals in the Pacific Northwest (8). Alfalfa (*Medicago sativa* L.) is usually rotated with potato and cereals in this part of the United States. Recent studies have shown that alfalfa, which is a very susceptible host for the northern root-knot nematode *M. hapla* Chitwood (3), is not a suitable host for *M. chitwoodi* (8). However, root-knot infections on alfalfa have been observed in fields of southern Utah and Idaho with records of *M. chitwoodi* infections on potato, suggesting that some *M. chitwoodi* populations are able to attack alfalfa. To obtain more detailed information on the parasitic behavior of *M. chitwoodi* on alfalfa, a series of experiments was conducted at Logan, Utah, to compare pathogenicity and reproduction rate of four populations of *M. chitwoodi* and one population of *M. hapla* on *M. hapla*-susceptible and *M. hapla*-resistant alfalfa cultivars at different temperatures.

Received for publication 1 April 1985.

[1] Cooperative investigation by USDA ARS and Utah State University Agricultural Experiment Station, Journal Paper.

[2] Nematologist, USDA ARS, Crops Research Laboratory, Utah State University, UMC 63, Logan, UT 84322.

[3] Nematologist, Bureau of Nematology, Division of Plant Industry, P.O. Box 1269, Gainesville, FL 32602.

[4] Nematologist, Istituto Nematologia Agraria, C.N.R., 70126, Bari, Italy.

[5] Professor, Department of Applied Statistics, Utah State University, UMC 42, Logan, UT 84322.

Materials and Methods

Meloidogyne chitwoodi populations from Bel Rapid, Idaho; Beryl, Utah; Fort Hall, Idaho; and Prosser, Washington; and a *M. hapla* population from Ogden, Utah, were used in all experiments. The *M. chitwoodi* and *M. hapla* populations were reared on wheat (*Triticum aestivum* L. em Thell cv. Nugaines) and tomato (*Lycopersicon esculentum* Mill. cv. California Pack), respectively, in a greenhouse to obtain inocula for experiments. Inocula were collected using NaOCl (5).

Experiment 1: M. hapla-susceptible alfalfa cultivars Lahontan and Moapa and *M. hapla*-resistant Nevada Syn XX (160 seedlings of each) were grown singly in 1,600-cm^3 plastic pots containing methyl bromide-fumigated sandy loam soil (72% sand, 18% silt, 10% clay). *Rhizobium meliloti* Dang. was applied to the alfalfa seeds as described by Somasegan and Hoben (9). Each 4-day-old seedling was inoculated with either 0 or 8,000 eggs and second-stage juveniles (J2), an initial population level (Pi) of five nematodes/cm^3 soil. Eggs and J2 in an aqueous suspension were poured into five holes 10 cm deep in the soil around the hypocotyl base and tap root of each seedling. Pots were maintained at 10, 15, 20, and 25 C in water baths; plants received 19 hours light per day supplemented with high-output fluorescent lamps. Each treatment was replicated four times.

To further evaluate the effect of high

TABLE 1. Effect of *Meloidogyne hapla* and *M. chitwoodi* populations† on the dry shoot weight of three alfalfa cultivars grown for 100 days at four temperatures.

Nematode species and population	Temperature (C)	Dry shoot weight (g)					
		Lahontan		Moapa		Nev. Syn XX	
		N	I	N	I	N	I
M. chitwoodi—Beryl, Utah	10	0.65	0.34*	0.81	0.62	0.85	0.92
	15	1.29	0.77*	1.47	0.80*	1.63	1.00*
	20	1.77	0.68*	2.43	1.68*	2.05	2.50
	25	2.82	1.26*	3.17	3.11	3.91	2.52*
M. chitwoodi—Bel Rapid, Id.	10	0.75	0.67	0.65	0.55	0.71	0.85
	15	2.90	1.90*	3.47	2.10*	2.58	2.11
	20	3.66	3.88	3.60	3.00	3.12	2.60
	25	3.30	2.51	3.31	3.22	2.63	2.85
M. chitwoodi—Fort Hall, Id.	10	0.65	0.55	0.81	0.56	0.85	0.71
	15	1.29	0.76*	1.47	1.10*	1.63	1.33
	20	1.77	1.65	2.40	2.00	2.05	1.70
	25	2.82	3.00	3.17	2.60	3.91	2.95
M. chitwoodi—Prosser, Wash.	10	0.68	0.65	0.75	0.69	0.78	0.70
	15	2.87	1.73*	3.47	1.61*	2.58	1.85
	20	3.02	2.40	3.60	2.33*	3.12	3.12
	25	3.30	2.60	3.31	3.44	2.63	2.91
M. hapla—Ogden, Utah	10	0.73	0.69	0.70	0.65	0.56	0.60
	15	2.87	1.62*	3.47	1.60*	2.58	3.22
	20	3.12	2.28*	3.66	2.60*	3.12	2.73
	25	2.51	1.52*	3.31	2.22*	2.63	3.21

Values are means of four replications.
N = noninfected. I = infected.
* Significant ($P < 0.05$) growth suppression of infected plants compared to noninfected controls according to the LSD test.
† Initial inoculation, five eggs and second-stage juveniles/cm³ soil.

temperature on the resistance of Nevada Syn XX to *M. hapla,* an additional group of *M. hapla*-infected and noninfected seedlings of this cultivar was maintained at 30 C.

All plants were harvested 100 days after nematode inoculation and dried at 70 C for 24 hours, and dry shoots were weighed. Nematode final population densities (Pf) were determined by extracting eggs from roots using NaOCl and reporting them as eggs/cm³ soil. All data were analyzed using a split-plot in time analysis of variance.

Moapa alfalfa roots with *R. meliloti* nodules infected with *M. hapla* were selected for histological examination. Root segments were fixed in FAA, dehydrated in a tertiary butyl alcohol series, and embedded in paraffin. Sections 10 μm thick were stained with safranin and fast-green, mounted in Dammar xylene (6), and examined with a microscope.

Experiment 2: Because preliminary field and greenhouse observations of the *M. chitwoodi* population from Beryl, Utah, showed it to be more pathogenic than other nematode populations to alfalfa, the ability of this population to infect alfalfa was tested again in a second experiment on Apollo, Lahontan, Moapa, and Nevada Syn XX alfalfa cultivars. Six 4-day-old seedlings of each cultivar were grown in 400-cm³ plastic pots and inoculated by the same procedures used in experiment 1. Seven eggs and J2/cm³ soil were used as the initial population (Pi) level. Plants were maintained on a greenhouse bench at 25 ± 4 C. Sixty days after inoculation plants were harvested, eggs were extracted as in experiment 1, and nematode population densities (eggs/cm³ soil) were determined. Roots were stained with acid fuchsin in hot lactoglycerol, and nematode numbers and life stages were determined under a dissecting microscope.

Experiment 3: To obtain further confirmation of the pathogenicity of the *M. chitwoodi* Beryl, Utah, population to alfalfa, an experiment was conducted using the *M. hapla*-resistant Nevada Syn XX cultivar. Forty-eight 4-day-old Nevada Syn XX alfalfa seedlings were inoculated with 0 or 12 eggs and J2/cm³ soil and grown in 1,600-

TABLE 2. Final population densities and Pf/Pi ratios of *Meloidogyne hapla* and *M. chitwoodi* egg populations† recovered from three alfalfa cultivars grown for 100 days at three different temperatures.

Nematode species and population	Temperature (C)	Lahontan	Moapa	Nev. Syn XX
M. chitwoodi—Beryl, Utah	15	12.8 (2.6) a	0.1 (< 0.1) a	11.9 (2.4) a
	20	4.5 (0.9) a	5.7 (1.1) a	1.1 (0.2) a
	25	18.2 (3.6) a	0.2 (< 0.1) a	2.0 (0.4) a
M. chitwoodi—Bel Rapid, Id.	15	0.5 (0.1) b	0.0 (0.0) a	0.1 (< 0.1) b
	20	0.0 (0.0) a	0.0 (0.0) a	0.0 (0.0) a
	25	2.7 (0.5) b	0.1 (< 0.1) a	0.0 (0.0) a
M. chitwoodi—Fort Hall, Id.	15	0.0 (0.0) b	0.0 (0.0) a	0.0 (0.0) b
	20	0.1 (< 0.1) a	0.0 (0.0) a	0.0 (0.0) a
	25	0.1 (< 0.1) b	0.0 (0.0) a	0.0 (0.0) a
M. chitwoodi—Prosser, Wash.	15	1.0 (0.2) b	0.1 (< 0.1) a	8.5 (1.7) a
	20	0.1 (< 0.1) a	0.1 (< 0.1) a	0.1 (< 0.1) a
	25	0.0 (0.0) b	0.0 (0.0) a	0.0 (0.0) a
M. hapla—Ogden, Utah	15	15.0 (3.0) a	2.7 (0.5) a	0.0 (0.0) b
	20	140.0 (28.0) b	61.7 (12.3) b	0.1 (< 0.1) a
	25	162.2 (32.4) c	156.0 (31.2) b	0.1 (< 0.1) a

Values are means of four replications.
Column means followed by same letters are not different according to a split plot in time analysis of variance ($P < 0.05$). LSD ($P < 0.05$) = 9.18 and 1.83 for the differences, respectively, of Pf and Pf/Pi values among cultivars at same temperature and nematode, and 11.33 and 2.26 for the differences of Pf and Pf/Pi values among temperature for the same cultivar and nematode population.
† Initial inoculation, five eggs and second-stage juveniles/cm³ soil.

cm³ plastic pots in water baths at 15, 20, and 25 C as in experiment 1. Each treatment was replicated eight times. Ninety days after inoculation shoots were dried and weighed and nematode population densities (eggs/cm³ soil) were determined.

RESULTS AND DISCUSSION

Experiment 1: Growth of *M. hapla*-infected Lahontan and Moapa alfalfa plants was suppressed ($P < 0.05$) after 100 days at 15, 20, and 25 C (Table 1). Dry shoot weights of *M. hapla*-inoculated Nevada Syn XX did not differ from those of noninoculated plants at all temperatures (Table 1). The *M. chitwoodi* Beryl population suppressed growth ($P < 0.05$) of Lahontan plants at 10, 15, 20 and 25 C, and Moapa and Nevada Syn XX at 15 and 20 C and 15 and 25 C, respectively (Table 1). The *M. chitwoodi* Bell Rapids and Fort Hall populations suppressed shoot growth ($P < 0.05$) of Lahontan and Moapa at 15 C. The *M. chitwoodi* Prosser population suppressed shoot growth ($P < 0.05$) of Lahontan at 15 C and Moapa at 15 and 20 C.

Meloidogyne hapla reproduction occurred on Lahontan and Moapa at 15, 20, and 25 C, but not at 10 C (Table 2). *M. hapla* final populations on Nevada Syn XX were less than 0.1 eggs/cm³ soil. Reproduction of *M. hapla* on Lahontan and Moapa was significantly greater ($P < 0.05$) than reproduction of any *M. chitwoodi* population at 20 and 25 C (Table 2).

There was no reproduction of any *M. chitwoodi* population at 10 C, and no reproduction was observed on *Rhizobium* nod-

TABLE 3. Effect of soil temperature on parasitism and final population densities (Pf) of *Meloidogyne hapla*† on resistant Nevada Syn XX alfalfa seedlings grown for 100 days.

Temperature (C)	Dry shoot weight (g)		Pf (eggs/cm³ soil)
	N	I	
10	0.56	0.60	0.0 a
15	2.58	3.22	< 0.1 a
20	3.12	2.73	< 0.1 a
25	2.63	3.21	< 0.1 a
30	2.76	1.88*	228.0 b

Values are means of four replications. Means followed by common letters are not different according to LSD test ($P < 0.01$).
N = noninfected. I = infected.
* Significant ($P < 0.05$) growth suppression of infected plants compared with noninfected controls according to the LSD test.
† Initial inoculation, five eggs and second-stage juveniles/cm³ soil.

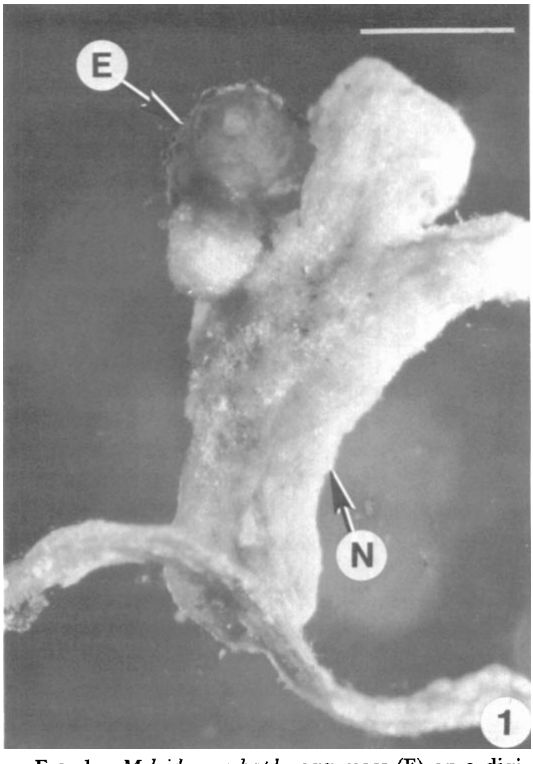


FIG. 1. *Meloidogyne hapla* egg mass (E) on a digitiform *Rhizobium meliloti* nodule (N) on a Moapa alfalfa root. Scale bar = 0.95 mm.

ules. The greatest nematode reproduction occurred from the Beryl population on Lahontan and Nevada Syn XX, whereas all alfalfas were poor hosts for the other *M. chitwoodi* populations (Table 2).

Nevada Syn XX resistance to *M. hapla* was lost at 30 C which agrees with the results of other *M. hapla*–alfalfa (3,4) and *M. incognita*–tomato (2) studies. Seedlings were heavily infected and dry shoot weights of nematode-infected seedlings were less ($P < 0.05$) than noninfected control seedlings (Table 3), which agrees with previous findings (3,4).

M. hapla parasitized both nodulated and nonnodulated roots of Lahontan and Moapa plants, and egg masses protruded from the nodule surfaces (Fig. 1). Histological examination of Moapa-nodulated roots revealed that *M. hapla* established a permanent feeding site in the vascular bundles of the nodules (Fig. 3), and several nematodes could attack a single nodule (Fig. 3). Vascular bundles of infected nodules were abnormal and fragmented by the nematode body and by giant cells induced by the

TABLE 4. Numbers of life stages of *Meloidogyne chitwoodi*† from Beryl, Utah, per gram of fresh roots of four alfalfa cultivars grown for 60 days.

	Eggs	J2	J3 + J4	Females	Males
Apollo	1,406	96	12	33	5
Lahontan	1,537	108	31	58	4
Moapa	106	25	3	2	1
Nevada Syn XX	424	96	9	21	1

Values are means of six replicates.
† Initial inoculation, seven second-stage juveniles/cm³ soil.

nematode feeding (Fig. 4). Nematode-infected portions of the nodules appeared to enlarge, possibly from hyperplasia of the nodule cortex (Fig. 3). There was no evidence of direct invasion or disruption of bacteroids by *M. hapla*, but bacteroids were mechanically compressed by enlarging nematode bodies (Fig. 5). Plant tissue alterations induced by *M. hapla* in susceptible alfalfa roots were similar to those induced by *M. incognita* in nitrogen-fixing root nodules in soybean (1).

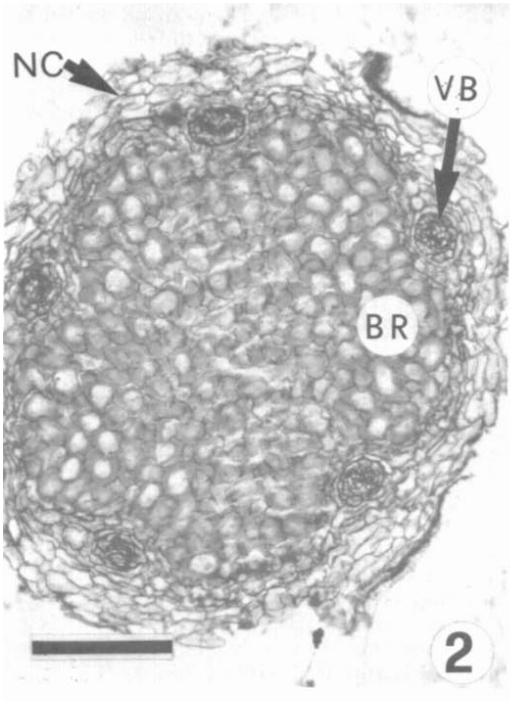
Experiment 2: The *M. chitwoodi* Beryl population infected, parasitized, and reproduced on all four alfalfa cultivars tested, and no differences occurred in the number of nematode life stages observed in any cultivar (Table 4). High variability, however, was observed in the Pf on any one cultivar.

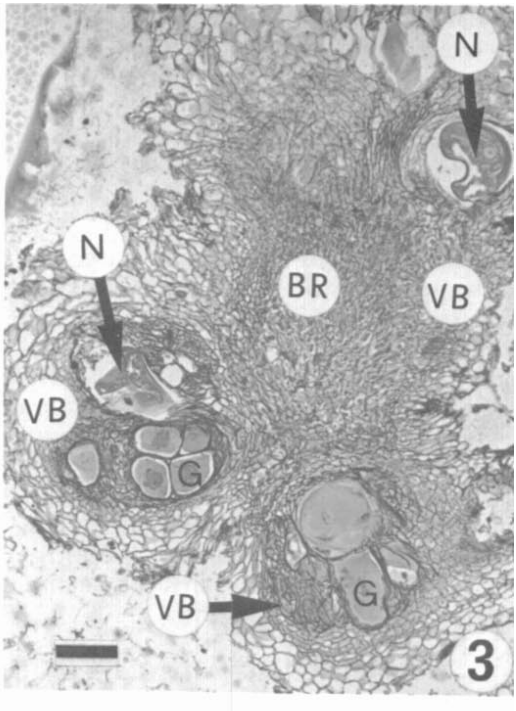
Experiment 3: The *M. chitwoodi* Beryl population infected Nevada Syn XX plants, but final nematode population densities were not consistent among the replications at any one temperature as experienced in

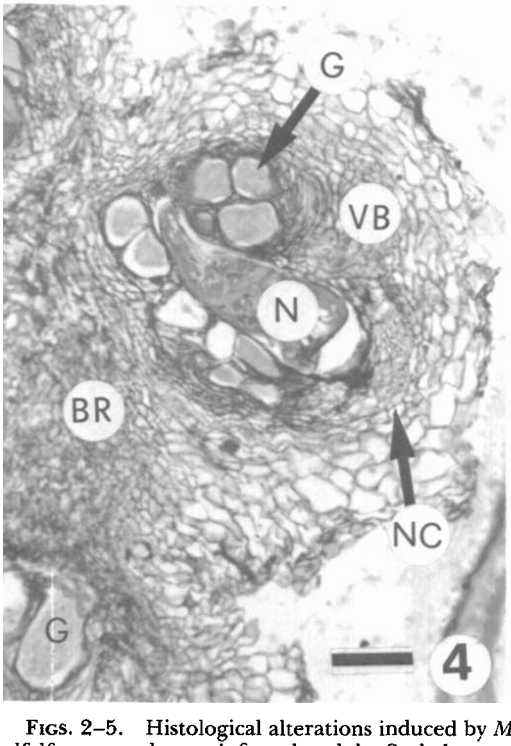
TABLE 5. Effect of *Meloidogyne chitwoodi*† Beryl, Utah, population on the growth of Nevada Syn XX alfalfa seedlings grown for 90 days at three different temperatures and nematode final densities (Pf) and Pf/Pi ratios.

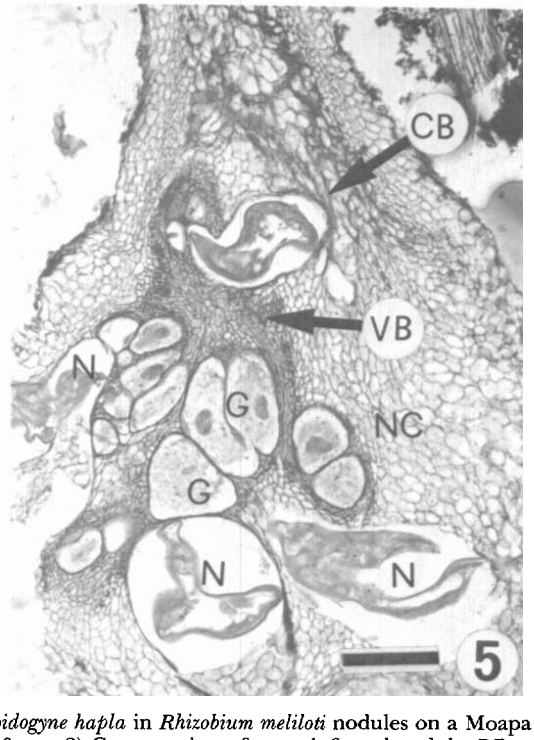
Temperature (C)	Dry shoot weight (g)		Pf (eggs/cm³ soil)	Pf/Pi
	N	I		
15	0.44†	0.34	2.0	0.16
20	0.85	0.63*	4.2	0.35
25	0.36	0.39	0.03	0.0

Values are means of eight replicates.
N = noninfected. I = infected.
* Significant ($P < 0.01$) growth suppression of infected plants compared with noninfected controls according to split plot analysis of variance.
† Initial inoculation, 12 second-stage juveniles/cm³ soil.









FIGS. 2–5. Histological alterations induced by *Meloidogyne hapla* in *Rhizobium meliloti* nodules on a Moapa alfalfa root and a noninfected nodule. Scale bars = 200 μm. 2) Cross section of a noninfected nodule, BR = bacteroids. NC = nodular cortex. VB = vascular bundle. 3) Cross section of a nodule with nematodes (N) and giant cells (G) in the vascular bundles (VB) that are enlarged. BR = bacteroids. 4) Cross section of a nodule showing an enlarged and fragmented vascular bundle (VB) by a nematode (N) and giant cells (G). Note hyperplastic nodular cortical parenchyma (NC). BR = bacteroids. 5) Cross section of a nodule with an enlarged and fragmented vascular bundle (VB) by nematodes (N) and giant cells (G). Note senescent bacteroids compressed (CB) by a nematode body. NC = hyperplastic nodular cortex.

the other temperature experiments (Table 5). Growth of infected Nevada Syn XX seedlings was suppressed ($P < 0.01$) at 20 C; nematode infection was more uniform among replicates at 20 C than at other temperatures. There were no differences among Pf and Pf/Pi values at different temperatures.

The results of our experiments indicated that some populations of *M. chitwoodi* can infect and damage alfalfa seedlings, and continuous exposure of alfalfa to such populations may increase the virulence of the nematode population to alfalfa by selection pressure. This suggests the possible existence of nematode strains revealed by the variability in alfalfa resistance. Information on the geographical distribution of *M. chitwoodi* alfalfa races would be useful for developing rotation programs for *M. chitwoodi*-infested fields.

Literature Cited

1. Barker, K. R., and R. S. Hussey. 1976. Histopathology of nodular tissues of legumes infected with certain nematodes. Phytopathology 66:851–855.

2. Dropkin, V. H. 1969. The necrotic reaction of tomatoes and other hosts resistant to *Meloidogyne*: Reversal by temperature. Phytopathology 59:1632–1637.

3. Griffin, G. D. 1969. Effect of temperature on *Meloidogyne hapla* in alfalfa. Phytopathology 59:599–602.

4. Griffin, G. D., and J. H. Elgin, Jr. 1977. Penetration and development of *Meloidogyne hapla* in resistant and susceptible alfalfa under differing temperatures. Journal of Nematology 9:51–56.

5. Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter 57:1025–1028.

6. Johansen, D. A. 1940. Plant microtechnique. New York: McGraw-Hill.

7. O'Bannon, J. H., and R. N. Peaden. 1982. Host suitability and reproductive potential of *Meloidogyne chitwoodi* to *Medicago sativa*. Journal of Nematology 14:462–463 (Abstr.).

8. O'Bannon, J. H., and G. S. Santo. 1981. Effect of soil temperature on reproduction of *Meloidogyne chitwoodi* and *M. hapla* alone and in combination on potato and *M. chitwoodi* on rotation plants. Journal of Nematology 16:309–312.

9. Somasegaran, P., and H. J. Hoben. 1985. Methods in legume-*Rhizobium* technology. USAID Document. Washington, D.C.: GPO.