Journal of Nematology 29(1):112–116. 1997.

Importance of Temperature in the Pathology of *Meloidogyne hapla* and *M. chitwoodi* on Legumes[1]

G. D. Griffin[2] and K. B. Jensen[2]

Abstract: Effects of temperatures on the host-parasite relationships were studied for three legume species and four populations of root-knot nematodes from the western United States. The nematode populations were *Meloidogyne hapla* from California (MHCA), Utah (MHUT), and Wyoming (MHWY), and a population of *M. chitwoodi* from Utah (MCUT). The legumes were milkvetch (*Astragalus cicer*), alfalfa (*Medicago sativa*), and yellow sweet clover (*Melilotus officinalis*). All milkvetch plants survived inoculation with all nematode populations, while alfalfa and yellow sweet clover were more susceptible. On yellow sweet clover, MHCA was most pathogenic at 30 °C based on suppression of shoot growth while MHUT, MHWY, and MCUT were most pathogenic at 25 °C. All nematode populations suppressed growth of yellow sweet clover more than growth of milkvetch and alfalfa. The reproductive factor (Rf = final nematode population/initial nematode population) of MHCA was positively correlated ($r = 0.83$) with temperature between 15 °C and 30 °C. The greatest Rf occurred on alfalfa inoculated with MHCA at 30 °C. The Rf of MHUT, MHWY, and MCUT were positively correlated ($r = 0.76$, $r = 0.78$, and $r = 0.73$, respectively) with temperature between 15 °C and 25 °C. The Rf values of MHUT and MHWY were similar on all species and exceeded the Rf of MCUT at all temperatures ($P < 0.05$).

Key words: alfalfa, *Astragalus cicer*, legume, *Medicago sativa*, *Melilotus officinalis*, *Meloidogyne chitwoodi*, *Meloidogyne hapla*, milkvetch, pathogenicity, reproduction, temperature, thermal adaptation, yellow sweet clover.

The family Leguminosae, found worldwide, is second only to the grasses in economic importance, and several species that are adapted to rangeland conditions benefit rangeland ecology (Allen and Allen, 1981; Rumbaugh, 1982). Legumes make atmospheric nitrogen available for other plants, aid in land reclamation, and serve as feed for wildlife and livestock (Bergerson, 1977; Schmautz et al., 1980).

Legumes are hosts of several species of plant-parasitic nematodes, and resistance and susceptibility to nematodes differ between legume species (Griffin and Rumbaugh, 1996). Soil temperature affects the relationship between plant-parasitic nematodes and the growth of plants, including legumes (Griffin and Gray, 1995; Griffin et al., 1990). Some populations and races of plant-parasitic nematodes adversely affect survival, growth, and rhizobial nodulation of legumes (Goplen et al., 1959; Griffin and Gray, 1995; Griffin and McKenry, 1989; Griffin and Rumbaugh, 1996; Griffin and Thomson, 1988; Netscher and Taylor, 1979). This study was conducted to compare the effects of temperature on host-parasite relationship of the northern root-knot nematode *Meloidogyne hapla* Chitwood, and the Columbia root-knot nematode *M. chitwoodi* Golden, O'Bannon, Santo & Finley on selected legume species of economic importance in the western United States.

Materials and Methods

Nematode populations studied were *Meloidogyne hapla* (MHCA) from alfalfa (*Medicago sativa* L.) at Visalia, California (Griffin and McKenry, 1989); *M. hapla* (MHUT) from lettuce (*Lactuca sativa* L.) at Ogden, Utah; *M. hapla* (MHWY) from alfalfa at Laramie, Wyoming (Griffin and Gray, 1995); and *M. chitwoodi* race 2 (MCUT) from potato, *Solanum tuberosum* L., at Beryl, Utah (Griffin and Thomson, 1988). Nematodes were maintained on 'Rutgers' tomato (*Lycopersicon esculentum* Mill.) under greenhouse conditions, and eggs for use as inoculum were collected with an NaOCl method (Hussey and Barker, 1973).

Plant selections used in the study were milkvetch (*Astragalus cicer* L.), alfalfa (*M. sa-*

Received for publication 25 March 1996.

[1] Cooperative investigation by USDA Agricultural Research Service and the Utah Agricultural Experiment Station. Journal Paper No. 4885. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or Utah State University.

[2] Nematologist and Geneticist, USDA ARS, Forage and Range Research, Utah State University, Logan, UT 84322-6300.
E-mail: Griffin@cc.usu.edu

tiva L.), and yellow sweet clover (*Melilotus officinalis* Willd.). Seeds were scarified and germinated on filter paper. Plants were grown in individual 4-cm-diam. plastic containers containing 240 g steam-sterilized Kidman fine sandy loam soil (coarse-loamy mixed mesic Calcic Haploxeroll; 84% sand, 8% silt, 8% clay; pH 7.4, 1% OM). After 28 days in a greenhouse at 24 ± 4°C, an aqueous suspension of eggs (2 eggs/cm^3/soil) of one of the root-knot nematode populations was poured into four holes 10 cm deep in the soil around the hypocotyl base of each plant. A control treatment receiving no nematodes also was included for each plant species at each temperature tested. Plants were grown in four chambers at temperatures of 15, 20, 25, and 30 °C ± 2 °C, and received 19 hours of light per day supplemented with high-output fluorescent lamps. The experiment was a 3 × 3 × 2 factorial (3 nematode populations × 3 plant cultivars × 2 inoculum densities) per temperature in a randomized complete block design with 12 replications (containers), with one plant per container. Plants were watered daily and fertilized every 2 weeks with a complete (10 × 10 × 10) nutrient solution. After 90 days, plants were harvested and the dry shoot weight, a root galling index (1 = no galling, 2 = 1–10%, 3 = 11–20%, 4 = 21–50%, 5 = 51–80%, 6 = 81–100% root tissue galled), and percentage plant survival were recorded. Nematode eggs were extracted from each root system by the NaOCl method (Hussey and Barker, 1973), and the nematode reproductive factor (Rf = final nematode population/initial nematode population) and resistance classifications (Rf < 1) were calculated for each plant (Cook and Evans, 1987). Percentage data on plant survival were transformed with arcsine ($\sqrt{x}$) before analysis, and differences among means were compared at $P < 0.05$ using the LSD. Each parameter measured was regressed against nematode population.

Results and Discussion

Plant selections were chosen because of differences in their relationships to *M. hapla*

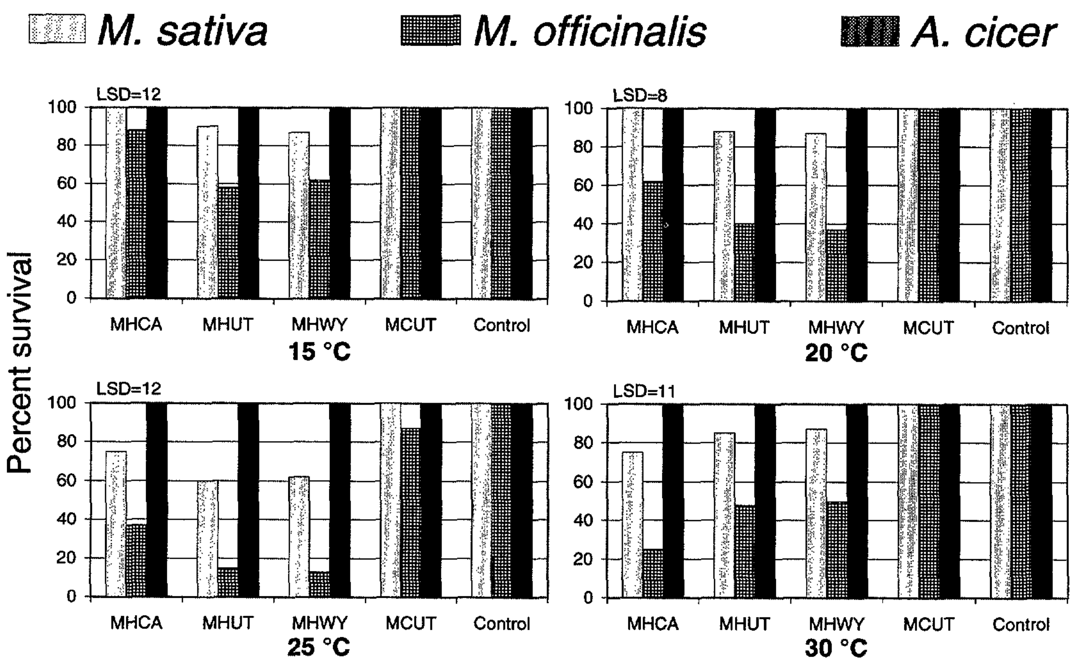

FIG. 1. Survival of milkvetch (*Astragalus cicer*), alfalfa (*Medicago sativa*), and yellow sweet clover (*Melilotus officinalis*) infected with populations of *Meloidogyne hapla* from California (MHCA) and Wyoming (MHWY), and with *M. chitwoodi* from Utah (MCUT) at four temperatures. Plants inoculated with 0 or 2 eggs/cm^3 soil and grown for 90 days after soil infestation. (LSD for nematode populations).

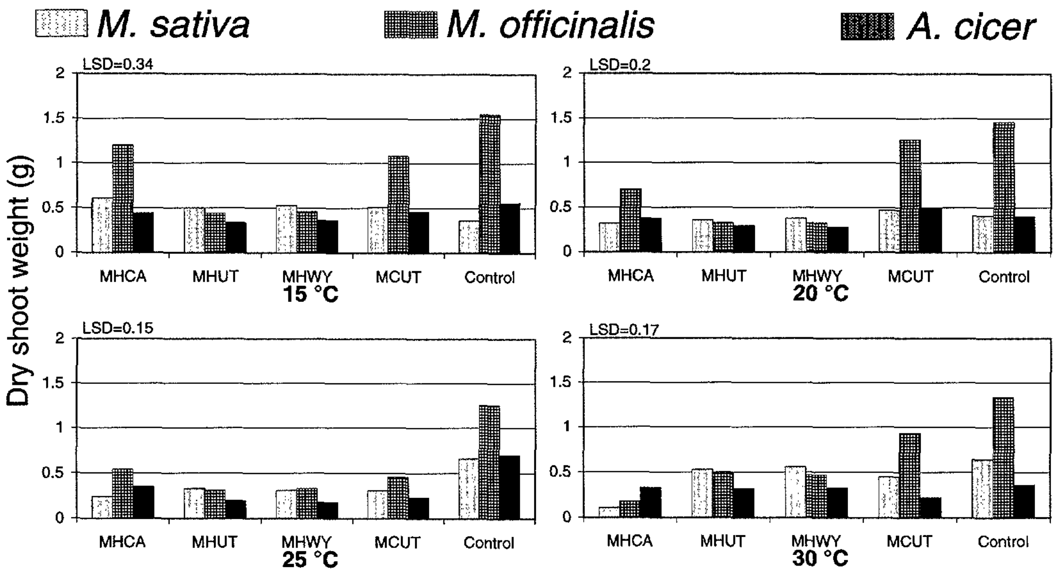

FIG. 2. Effect of *Meloidogyne hapla* populations from California (MHCA) and Wyoming (MHWY), and *M. chitwoodi* from Utah (MCUT) on dry shoot weight of milkvetch (*Astragalus cicer*), alfalfa (*Medicago sativa*), and yellow sweet clover (*Melilotus officinalis*) at four temperatures. Plants inoculated with 0 or 2 eggs/cm^3 soil and grown for 90 days after soil infestation. (LSD for nematode populations).

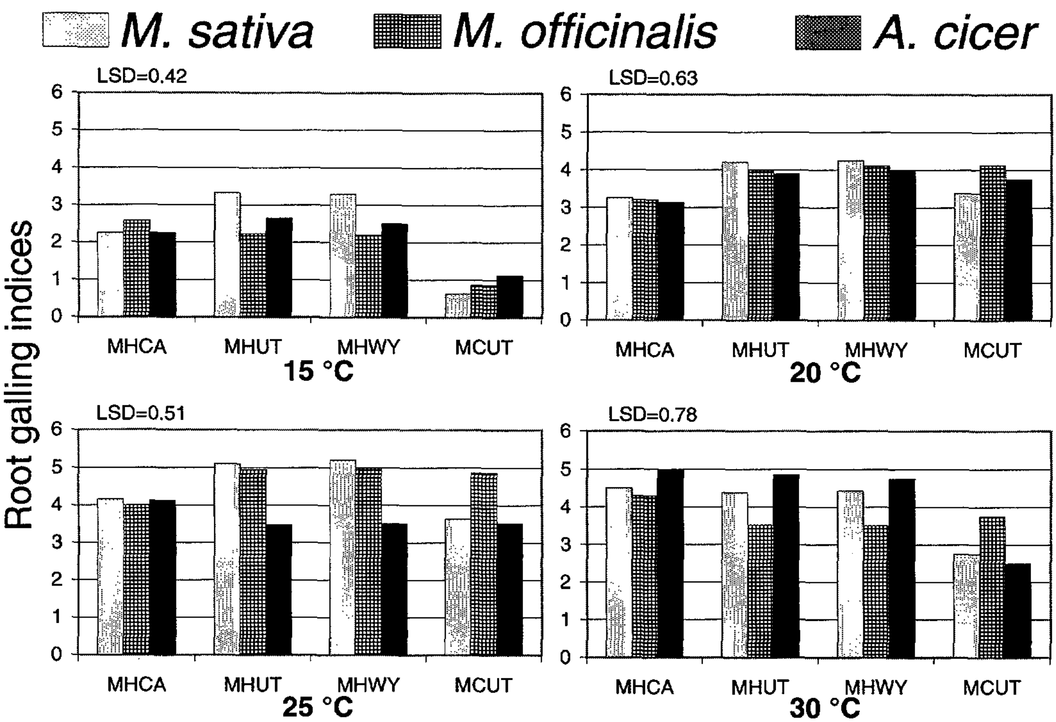

FIG. 3. Root galling indices of milkvetch (*Astragalus cicer*), alfalfa (*Medicago sativa*), and yellow sweet clover (*Melilotus officinalis*) by *Meloidogyne hapla* populations from California (MHCA) and Wyoming (MHWY), and *M. chitwoodi* (MCUT) from Utah at four temperatures. Plants inoculated with 0 or 2 eggs/cm^3 soil and grown for 90 days after soil infestation. (LSD for nematode populations).

and *M. chitwoodi* (Griffin and Rumbaugh, 1996), and survival rates differed between plant cultivars and nematode populations (Fig. 1). Milkvetch was the most tolerant, and yellow sweet clover was the most sensitive to nematode invasion. Milkvetch plants survived at all temperatures. There was a negative relationship in the survival of yellow sweet clover and alfalfa with MHUT and MHWY populations between 15 °C and 25 °C ($r = -0.64$) and yellow sweet clover and alfalfa at 15 °C and 30 °C when inoculated with MHCA ($r = -0.72$). Survival of yellow sweet clover and alfalfa also was affected at 30 °C by MHUT and MHWY, but the decline was less than at 25 °C. MCUT was less pathogenic than *M. hapla*, whereas only yellow sweet clover succumbed to MCUT at 25 °C.

Plant growth differed between cultivars and nematode populations, and yellow sweet clover was more susceptible ($P < 0.05$) to nematodes than were milkvetch and alfalfa (Fig. 2). Suppression of shoot growth of yellow sweet clover was significantly greater from MHUT and MHWY than from MHCA and MCUT. The suppression of plant growth was negatively correlated with temperature ($r = -0.77$), and nematode populations differed with temperature. The greatest shoot growth suppression of plants inoculated with MHUT, MHWY, and MCUT occurred at 25 °C, whereas MHCA was more virulent at 30 °C.

All plant species were moderately to heavily galled by all nematode populations (Fig. 3). More root galling occurred with *M. hapla* populations than with MCUT ($P < 0.05$). Root galling by MHCA increased with temperature between 15 °C and 30 °C ($r = 0.70$), as did galling by MHUT ($r = 0.73$), MHWY ($r = 0.72$), and MCUT ($r = 0.68$) and 15 °C and 25 °C.

The Rf of MHCA increased with a rise in temperature from 15 °C to 30 °C ($r = 0.83$); this effect was especially pronounced on alfalfa at 30 °C (Fig. 4). The Rf of MHUT ($r =$

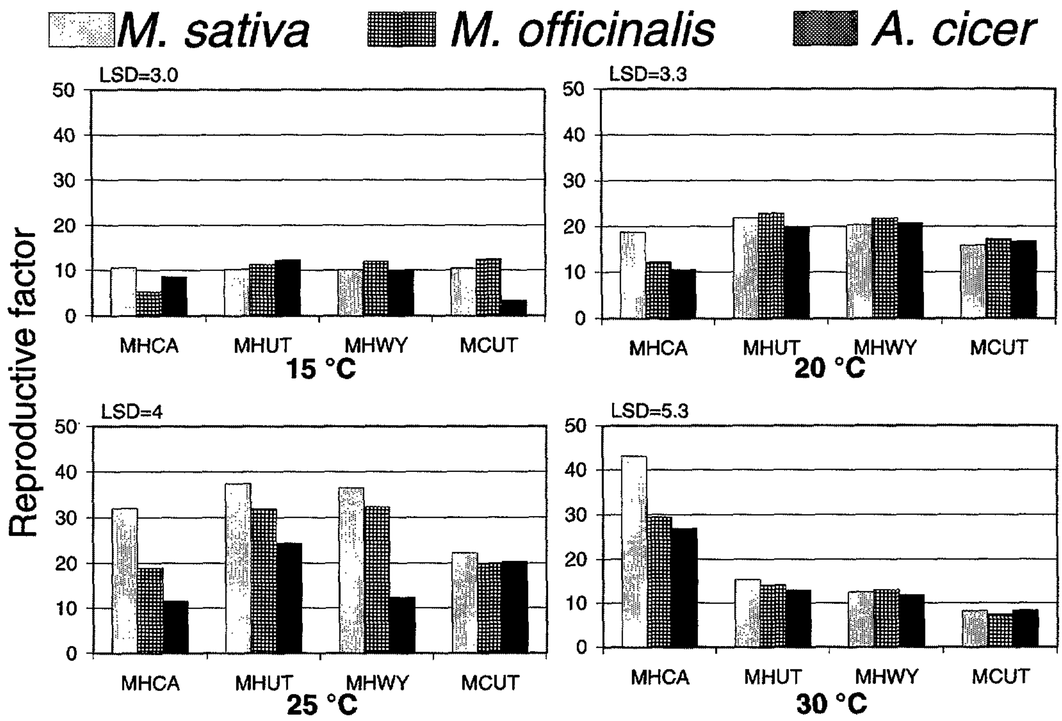

FIG. 4. Reproductive factor (Rf = final nematode population/initial nematode population) of *Meloidogyne hapla* populations from California (MHCA) and Wyoming (MHWY), and *M. chitwoodi* (MCUT) from Utah on milkvetch (*Astragalus cicer*), alfalfa (*Medicago sativa*), and yellow sweet clover (*Melilotus officinalis*) at four temperatures. Plants inoculated with 0 or 2 eggs/cm^3 soil and grown for 90 days after soil infestation. (LSD for nematode populations).

0.76), MHWY (r = 0.78), and MCUT (r = 0.73) increased with temperatures between 15 °C and 25 °C. The Rf of MHUT and MHWY were comparable on all species and exceeded ($P < 0.05$) the Rf of MCUT at all temperatures.

The variability in the reaction of alfalfa to *M. hapla* and *M. chitwoodi* is consistent with a previous report (Griffin and Rumbaugh, 1996). All populations of *M. hapla* were more pathogenic and had a higher Rf than *M. chitwoodi* on all plant species. The relationship between root galling and nematode reproduction was usually consistent (Griffin and Rumbaugh, 1996).

Plants differed in their reactions to *M. hapla*. The reproduction of MHCA increased significantly with temperature up to 30 °C, whereas the reproduction of MHUT and MHWY was greatest at 25 °C and suppressed at 30 °C. The MHUT and MHWY populations are from ca. 42°N latitude, whereas the MHCA population is from near Visalia, California, at 37°N latitude at a much lower elevation and in soil with an average annual temperature lower than that in Utah and Wyoming.

As noted in a previous study (Griffin et al., 1993), population or race differences should be considered in plant breeding and plant-parasite relationship studies. An accurate determination of the susceptibility, resistance, or tolerance of a plant to some nematode aids crop species selection for use in a given environment, particularly since nematodes predispose plants to other pathogens (Griffin et al., 1993).

Literature Cited

Allen, O. N., and E. K. Allen 1981. Leguminosae. Madison: University of Wisconsin Press.

Bergerson, F. J. 1977. Factors controlling nitrogen fixation by rhizobia. Pp. 151–165 *in* A. Ayanaba and P. J. Dart, eds. Biological nitrogen fixation in farming systems of the tropics. New York: John Wiley.

Cook, R., and K. Evans. 1987. Resistance and tolerance. Pp. 179–231 *in* R. H. Brown and B. R. Kerry, eds. Principles and practice of nematode control in crops. New York: Academic Press.

Goplen, B. P., E. H. Stanford, and M. W. Allen. 1959. Demonstration of physiological races within three root-knot nematode species attacking alfalfa. Phytopathology 49:653–656.

Griffin, G. D., and F. A. Gray. 1995. Biological relationship of *Meloidogyne hapla* populations to alfalfa cultivars. Journal of Nematology 27:353–361.

Griffin, G. D., F. A. Gray, D. A. Johnson, and D. L. Crebs. 1993. Pathological relationship of *Meloidogyne hapla* and *Phytophthora megasperma* f. sp. *medicaginis* in *Medicago sativa* L.: Importance of inoculation timing, soil texture, and temperature. Nematropica 23:183–193.

Griffin, G. D., and M. V. McKenry. 1989. Susceptibility of Nevada Synthetic XX germplasm to a California race of *Meloidogyne hapla*. Journal of Nematology 21: 292–293.

Griffin, G. D., and M. D. Rumbaugh. 1996. Host suitability of twelve Leguminosae species to populations of *Meloidogyne hapla* and *M. chitwoodi*. Journal of Nematology 28:400–405.

Griffin, G. D., M. D. Rumbaugh, and D. L. Crebs. 1990. Northern root-knot nematode populations and soil temperature effect on alfalfa. Crop Science 30:541–544.

Griffin, G. D., and S. V. Thomson. 1988. The Columbia root-knot nematode, *Meloidogyne chitwoodi*, discovered in the state of Utah. Plant Disease 72:363.

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.

Netscher, C., and D. P. Taylor. 1979. Physiologic variations with the genus *Meloidogyne* and its implications on integrated control. Pp. 269–294 *in* F. Lamberti and C. E. Taylor, eds. Root-knot nematodes (*Meloidogyne* species). Systemics, biology, and control. London: Academic Press.

Rumbaugh, M. D. 1982. Legumes—their use in wildland plantings. Pp. 115–122 *in* S. B. Monsen and N. Shaw, compilers. Managing intermountain rangelands-improvement of range and wildlife habitats. Symposia Proceedings, Elko, Nevada. June 22–24.

Schmautz, J. E., M. D. Belinger, and R. W. Harris. 1980. Range. Pp. 246–315 *in* An assessment of the forest and range land situation in the United States. U.S. Department of Agriculture, Forest Service, FS-345. Washington, DC U.S. Government Printing Office.