Journal of Nematology 23(2):182–187. 1991.

Modification of Resistance Expression of *Phaseolus vulgaris* to *Meloidogyne incognita* by Elevated Soil Temperatures

B. A. MULLIN,[1] G. S. ABAWI,[1] AND M. A. PASTOR-CORRALES[2]

Abstract: The effect of temperature on the reaction of susceptible (Canario Divex) and resistant (A 211) bean pure lines to *Meloidogyne incognita* was studied with soil temperature tanks housed in a growth chamber at 22 or 24 C. Soil temperature remained constant at 16, 22, 24, 26, 30, or 32 C in several trials. Bean line A 211 was resistant at 16 and 22 C but was susceptible at 24 C and above. Resistance to root-knot nematode reproduction was affected by a lower temperature (24 C) than was resistance to root galling (26 C) in A 211. Incubation of A 211 at 30 C for 3 and 16 days after inoculation with *M. incognita* resulted in a significant increase in nematode reproduction and root galling, respectively. The resistant reactions of A 211 to nematode reproduction and root galling were retained when inoculated plants were incubated at 21 C for a minimum of 16 and 23 days, respectively, prior to high temperature treatment.

Key words: bean, host resistance, *Meloidogyne incognita*, *Phaseolus vulgaris*, reproduction, root galling.

The impact of temperature on the expression of host resistance to numerous plant pathogens, including nematodes, has been well documented (5,6,8,16). Holtzmann (8) noted that the tomato cultivars Anahu and Kalohi, as well as the accession No. 6586, were resistant to *Meloidogyne incognita* at 20 and 25 C but were susceptible at 30 C and above. He also reported a similar but less pronounced response in two additional resistant accessions. Dropkin (5) reported that the resistant tomato cultivar Nematex became more susceptible to *M. incognita*, *M. arenaria*, and *M. javanica* as the temperature was increased from 29 to 33 C. He also noted that elevated temperatures during only the first 2 to 3 days after inoculation affected the resistance exhibited at lower temperatures. The soybean cultivar Chief was shown to support significantly greater reproduction of *M. incognita* at 35 C than at 24 C (4). Root galling and nematode reproduction in the resistant bean accession PI 165426 increased as temperature was increased from 16 to 28 C (7). Furthermore, snap bean cultivar Alabama No. 1 and the bean accession PI 165435 lost their resistance to *M. incognita* and *M. hapla*, but not to *M. arenaria*, when the soil temperature was increased from 25 to 30 C (10). Resistance of snap bean cultivar Manoa Wonder (derived from Alabama No. 1) to *M. incognita* declined when soil temperature was held constant at 29 C but remained effective when temperatures fluctuated daily from 21 to 33 C (15).

Under greenhouse conditions with temperatures ranging from 18 to 23 C, bean germplasm resistant to root-knot nematodes can be selected (13). Results of previous evaluations indicated that the bean cultivar Canario Divex is susceptible and bean advanced line A 211 is resistant to several populations of *M. incognita* (11–13). However, when resistance evaluations were repeated in exceptionally warm weather, during which greenhouse temperatures remained at 30 C or above for several weeks, bean lines previously identified as resistant, including A 211, appeared highly susceptible (11). Subsequent evaluations at moderate greenhouse temperatures again demonstrated the resistant response, suggesting an interaction of temperature with resistance expression to root-knot nematodes in bean line A 211. The objectives of this investigation were to characterize the influence of soil temperature on the expression of resistance in bean breeding line A 211 to *M. incognita*, specifically to identify the critical temperatures at which resistance is modified, and to identify the length

Received for publication 15 December 1989.

[1] Graduate Research Assistant and Professor, Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456.

[2] Research Plant Pathologist, Bean Pathology Program, Centro Internacional de Agricultura Tropical, Apartado Aéreo 6713, Cali, Colombia.

of high temperature exposure necessary to cause this modification.

Materials and Methods

Incubation conditions: Three air-regulated temperature tanks were installed in a growth room, the temperature of which was maintained at 24 or 22 C (± 1 C). The temperature of each tank was maintained at 16, 22, 24, 26, 30, or 32 C (± 1 C) for the duration of the experiment and was monitored by a Taylor thermograph with soil probes attached. Open wells in the top of the tank permitted the insertion of pots into the tank so that soil temperature could be controlled by controlling air temperature within the tank. Soil temperature fluctuations coincided with watering events. Soil and air temperatures were regulated and allowed to stabilize for a week or longer before experiments were initiated. Light intensity in the growth room was approximately 11,000 lux of cool-white fluorescent light for 12 hours a day.

Planting: Seeds of the bean (*Phaseolus vulgaris* L.) cultivar Canario Divex (susceptible) and bean line A 211 (resistant) were germinated in a peat mix in cell packs for 1–3 days, rinsed, and transplanted into a steam-sterilized sand : soil mixture (1:1, v:v) in 10-cm-d pots. The pots were set into 16-cm-d pots filled with sand, which in turn were inserted into the wells of the soil temperature tanks.

Egg collection and soil infestation: Populations of *Meloidogyne incognita* race 3, collected from Popayán, Colombia, or from North Carolina (supplied by Dr. K. R. Barker) were maintained on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) in a greenhouse. Nematode eggs were extracted with NaOCl (9). At the time bean seedlings were transplanted, the soil was infested with the appropriate *M. incognita* population (10,000 eggs per pot) by pipetting the egg suspension into two depressions made close to the germinated seed. Inoculum was covered with fresh soil and pots were watered immediately. Plants were maintained with appropriate watering and were fertilized with a complete fertilizer solution (16-32-16).

Effect of soil temperature on expression of resistance in A 211 to M. incognita: Germinated seedlings of susceptible bean cultivar Canario Divex and resistant bean line A 211 were transplanted into soil which was then infested with the Popayán population of *M. incognita* as described and incubated at soil temperatures of 16, 24, or 32 C. Ambient temperature was maintained at 24 C, and treatments were replicated eight times. Plants were grown for 6 or 8 weeks in either of two trials and were then harvested by discarding plant tops and washing root systems. Roots were rated for galling and nematode reproduction (egg mass production and total egg production) on scales of 1 to 9. Gall rating: 1 = no galling, 2 = 1–5% roots galled, 3 = 6–10, 4 = 11–18, 5 = 19–25, 6 = 26–50, 7 = 51–65, 8 = 66–75, and 9 = > 75% roots galled. Egg mass rating: 1 = no egg masses produced, 2 = 1–2, 3 = 3–6, 4 = 7–10, 5 = 11–20, 6 = 21–30, 7 = 31–60, 8 = 61–100, and 9 = > 100 egg masses per root system. Total egg production was assessed by extracting and enumerating eggs from each root system (9). Data from the two trials were combined for analyses.

Effect of soil temperature on resistance expression in A 211 to two populations of M. incognita: Seedlings of A 211 were inoculated at planting with either *M. incognita* population and incubated at 22, 26, or 30 C. Ambient temperature was maintained at 22 C, and treatments were replicated four times. At 6 weeks after planting, root galling and nematode reproduction were evaluated.

Effect of length of high-temperature incubation on expression of resistance in A 211 to M. incognita: Seedlings of A 211 were inoculated at the time of transplanting with the Popayán population of *M. incognita* and incubated at a high temperature (30 C) for 3, 7, 14, 21, or 28 days before transferring plants to a growth chamber maintained at a lower temperature (22 C). Two treatments were incubated at either 22 or 30 C for the duration of the experiment. Treat-

TABLE 1. Effect of soil temperature on the reaction of bean cultivar Canario Divex (CD) (susceptible) and advanced bean line A 211 (resistant) to *Meloidogyne incognita*.

Incubation temperature	Root galling			Egg mass production			Egg production (× 10^4)		
	CD	A 211	LSD	CD	A 211	LSD	CD	A 211	LSD
16 C	4.7	1.7	1.0	1.4	1.4	NS	0.01	0.01	NS
24 C	6.7	1.4	1.1	8.6	6.7	0.9	7.7	1.0	4.1
32 C	8.2	4.9	1.9	8.0	7.9	NS	2.9	2.3	NS
LSD	0.8	1.1		0.6	0.9		2.4	1.0	

Root galling evaluated on scale of 1 = no galls to 9 = > 75% of roots galled.
Egg mass production evaluated on scale of 1 = no egg masses to 9 = > 100 egg masses per root system.
Egg production = total number of eggs recovered per root system.
LSD = least significant difference ($P = 0.05$) among bean germplasm within temperatures, or among temperatures within germplasm, or no significant difference (NS).

ments were replicated three times and were evaluated 42 days after inoculation for root galling and nematode reproduction.

Effect of length of low-temperature incubation on expression of resistance in A 211 to M. incognita: Seedlings of A211 were inoculated with *M. incognita* at the time of transplanting and were then incubated at a low temperature (21 C) for varying lengths of time (5, 7, 13, 16, 19, or 23 days) prior to incubation at higher temperatures (26–35 C) for the duration of the experiment (47 days). Control treatments were incubated at only 21 C or at 26–35 C. Treatments were replicated five times, and the experiment was repeated once.

Data analyses: Analysis of variance was used to determine treatment significance, and treatment means were separated by least significant difference (LSD). Regression analysis was used to evaluate the effect of duration of high-temperature or low-temperature incubation on resistance expression. Where necessary, data were transformed using natural logarithm.

RESULTS

Effect of soil temperature on expression of resistance in A 211 to M. incognita: Soil temperature and bean germplasm alone and their interaction affected ($P < 0.01$) all measured parameters of resistance. The susceptible cultivar Canario Divex exhibited moderate root galling and low nematode reproduction parameters at the low temperature of 16 C, but all values were high at 24 C and above (Table 1). Little or no root galling was observed on A 211 at 16 and 24 C, but significant ($P < 0.01$) root galling occurred at 32 C. In contrast, a significant increase in nematode reproduction (as measured by both egg mass production and total egg production) occurred on A 211 at 24 C and above. Thus, the critical temperature range at which resistance to *M. incognita* in A 211 was modified was between 24 and 32 C for the parameter of root galling and between 16 and 24 C for nematode reproduction.

Effect of soil temperature on expression of resistance in A 211 to two populations of M. incognita: In this experiment, root galling, egg mass production, and total egg production increased with increasing incubation temperature (Table 2). Higher values for all measured parameters were usually obtained with the North Carolina population of *M. incognita* than with the Popayán population. Root galling was moderate or greater when soil temperature was maintained at a minimum of 26 C. Egg mass production was intermediate, high, and high, at 22, 26, and 30 C, respectively, which apparently overestimated actual reproduction (low, high, and high, respectively) at the lowest temperature in this experiment. Both root galling and egg production were increased ($P < 0.01$) at a minimum temperature between 22 and 26 C.

Effect of length of high-temperature incubation on expression of resistance in A 211 to M. incognita: Both root galling and nematode reproduction increased linearly with increasing length of high-temperature incubation (Fig. 1). The increase in root gall-

TABLE 2. Effect of soil temperature on the reaction of A 211 to populations of *Meloidogyne incognita* collected from Popayán, Colombia (P), and North Carolina, USA (NC).

Incubation temperature	Root galling			Egg mass production			Egg production (× 10^4)		
	P	NC	LSD	P	NC	LSD	P	NC	LSD
22 C	1.0	1.3	NS	4.8	3.3	NS	0.4	0.6	NS
26 C	3.8	5.0	1.2	8.5	9.0	NS	40.5	104.9	61.6
30 C	4.8	7.3	NS	8.8	9.0	NS	33.7	93.3	25.1
LSD	1.0	2.1		1.6	0.9		9.0	49.2	

Root galling evaluated on scale of 1 = no galls to 9 = > 75% of roots galled.
Egg mass production evaluated on scale of 1 = no egg masses to 9 = > 100 egg masses per root system.
Egg production = total number of eggs recovered per root system.
LSD = least significant difference ($P = 0.05$) among nematode populations within temperatures, or among temperatures within nematode populations, or no significant difference (NS).

ing with incubation length was best described by the following equation: $Y = 0.74X + 0.1$ ($P < 0.01$, $R^2 = 0.85$). Significant root galling was incurred by A 211 after a minimum incubation period of 16 days at the higher temperature. Egg production increased almost linearly with increasing incubation length, with significant ($P < 0.01$) "susceptible" levels of reproduction occurring after only 3 days of incubation at the higher temperature. The response of egg production to incubation length was best described by the equation: $Y = 7{,}751X + 7{,}430$ ($P < 0.001$, $R^2 = 0.88$).

Effect of length of low-temperature incubation on expression of resistance in A 211 to M. incognita: Both root galling and egg mass production were inversely related to length of low-temperature incubation (Fig. 2). After a minimum of 23 days of low-temperature incubation, there was no effect of subsequent incubation temperature on root galling, and after only 16 days there was no effect of subsequent incubation temperature on egg mass production. Natural log transformation of root galling data resulted in the equation $Y = -0.0006X^2 - 7.8773X + 1.3614$ ($P < 0.001$, $R^2 = 0.68$). Egg mass production (not transformed) decreased according to the equation: $Y = 0.0046X^2 - 0.3657X + 8.2313$ ($P < 0.001$, $R^2 = 0.75$).

DISCUSSION

Results of this investigation indicated that the resistant reaction of bean line A 211 to *M. incognita* was temperature dependent. Resistance in A 211 to *M. incognita*

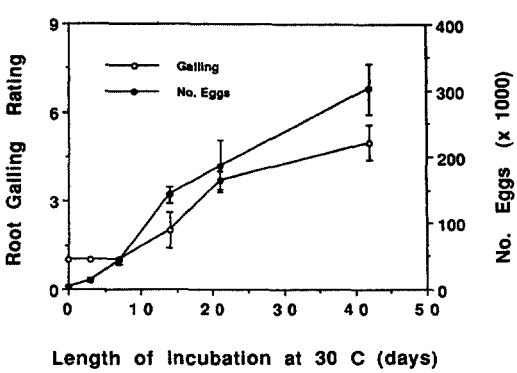

FIG. 1. Influence of length of incubation at 30 C on root galling and number of eggs produced on bean line A 211 inoculated with *Meloidogyne incognita*. Bars refer to standard errors.

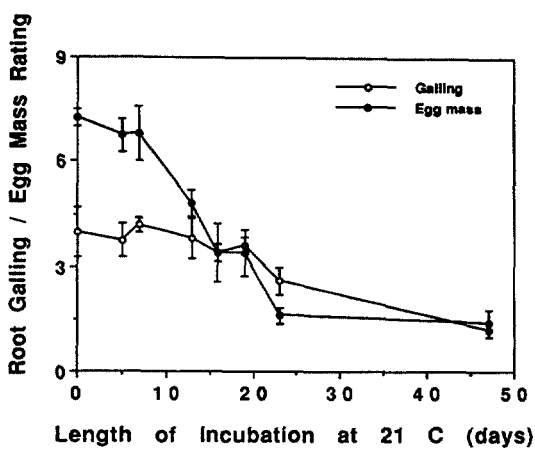

FIG. 2. Influence of length of incubation at 21 C prior to high-temperature incubation on root galling and nematode reproduction on bean line A 211 inoculated with *Meloidogyne incognita*. Bars refer to standard errors.

was not expressed at high soil temperatures. Interestingly, the critical temperature at which this effect occurred was between 24 and 26 C for the root galling response, and between 22 and 24 C for the nematode reproduction response. These temperature ranges are much lower than those previously considered to be critical for influencing resistance to *Meloidogyne* spp. in other crops (4,5,7). Additionally, it was found that a shorter minimum incubation length was sufficient to modify resistance to nematode reproduction than that required to affect resistance to root galling. However, the reaction of A 211 to *M. incognita* was not modified when the inoculated plants were initially incubated at a lower temperature for a minimum period prior to the high temperature treatment. Thus, it appears that temperature plays an important role in resistance expression, particularly so during the infection process. A similar effect in tomato was noted by Dropkin (5), who suggested that only a short exposure to high temperature was sufficient for the loss of resistance to root-knot nematodes to occur. A mechanism for temperature dependence of resistance to root-knot nematodes in tomato has been postulated (17). Oxygen-free radicals may be important in the resistant response to root-knot nematodes in tomatoes, as their production increased following nematode infection in resistant tomato roots. Because high-temperature treatment inhibited the production of oxygen-free radicals (correlating to the susceptible plant response), the mechanism for loss of resistance to root-knot nematodes in tomato may be due to the corresponding lack of oxygen-free radicals. However, the process is at best poorly understood and has not been studied in beans. Additional bean pure lines that have exhibited temperature-dependent resistance to *Meloidogyne* spp. include Nemasnap, Kabanima, PI 313709, Carioca, Manoa Wonder, BAT 1297, A 55, A 56, A 322, A 439, and AB 136 (Mullin and Abawi, unpubl.).

Root galling and nematode reproduction are separate host responses and may not occur concurrently, although both are measured components of root-knot nematode resistance (2,4). The minimum incubation temperature, as well as its duration, that was necessary to significantly influence root galling and nematode reproduction on A 211 were different. Preliminary genetic analysis of A 211 indicated that resistance to root galling and nematode reproduction are under separate genetic control (11), suggesting a genetic basis for these differences. Study of the separate mechanisms of root-knot nematode resistance independently may be possible, through the control of incubation temperature.

There were differences in the response of A 211 to infection by the two populations of *M. incognita* used in this study. Generally, resistance was less effective against the North Carolina population than the Popayán population. This suggests that the characterization of the host races of *Meloidogyne incognita* populations may not always reflect differences in the pathogenicity of such populations to beans. Further work is needed to determine the utility and significance of *Meloidogyne* host race specifications for *Phaseolus vulgaris* germplasm.

Results obtained from this study underscore the importance of evaluating potentially resistant germplasm in target areas. Ideally, germplasm should be evaluated during the normal crop production periods utilized by the local farmers. In this way, testing procedures would best represent the prevailing conditions to which the adapted germplasm would later be subjected. In the tropics, temperature may be a critical factor in the expression of resistance, as soil temperatures may reach 24 C or higher for a week or longer during the growing season and possibly during the critical stages of infection. Consequently, germplasm with otherwise adequate resistance may be rendered ineffective in certain environments. Evaluation of root-knot nematode resistance in tomato at high temperatures (1) suggests the existence of resistance genes that are not temperature de-

pendent. It is not known if such genes exist in *Phaseolus*.

Literature Cited

1. Ammati, M., I. J. Thomason, and H. E. McKinney. 1986. Retention of resistance to *Meloidogyne incognita* in *Lycopersicon esculentum* genotypes at high soil temperatures. Journal of Nematology 18:491–495.

2. Canto-Saenz, M. 1985. The nature of resistance to *Meloidogyne incognita* (Kofoid & White) Chitwood, 1949. Pp. 225–231 *in* J. N. Sasser and C. C. Carter, eds. An advanced treatise on *Meloidogyne*, vol. 1. Biology and control. Raleigh: North Carolina State University Graphics.

3. Carter, W. W. 1982. Influence of soil temperature on *Meloidogyne incognita* resistant and susceptible cotton. Journal of Nematology 14:343–346.

4. Dropkin, V. H. 1963. Effect of temperature on growth of root-knot nematodes in soybeans and tobacco. Phytopathology 53:663–666.

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

6. Durbin, R. D., and Z. Klement. 1977. High temperature repression of plant hypersensitivity to bacteria: A proposed explanation. Pp. 239–242 *in* Z. Kiraly, ed. Proceedings of the Symposium of the Hungarian Academy of Sciences. Budapest: Akademia Kiado.

7. Fassuliotis, G., J. R. Deakin, and J. C. Hoffman. 1970. Root-knot nematode resistance in snap beans: Breeding and nature of resistance. Journal of the American Society of Horticultural Science 95:640–645.

8. Holtzmann, O. V. 1965. Effect of soil temperature on resistance of tomato to root-knot nematode (*Meloidogyne incognita*). Phytopathology 55:990–992.

9. 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.

10. Irizarry, H., W. R. Jenkins, and N. F. Childers. 1971. Interaction of soil temperature and *Meloidogyne* spp. on resistance of the common bean, *Phaseolus vulgaris* L., to the root-knot disease. Nematropica 1:41–42.

11. Mullin, B. A. 1990. Ecology and host-parasite relationships of root-knot nematodes (*Meloidogyne* spp.) affecting dry edible beans (*Phaseolus vulgaris* L.) in Colombia and Peru. Ph.D. thesis, Cornell University, Ithaca, NY.

12. Mullin, B. A., and G. S. Abawi. 1991. Influence of elevated soil temperatures on resistance expression in *Phaseolus vulgaris* lines to *Meloidogyne incognita*. Phytopathology 81, in press (Abstr.).

13. Mullin, B. A., G. S. Abawi, and J. L. Kornegay. 1988. Host suitability of bean germplasm to *Meloidogyne incognita* and *M. hapla*. Phytopathology 78: 1566–1567 (Abstr.).

14. Mullin, B. A., M. A. Pastor-Corrales, and G. S. Abawi. 1991. Field evaluation of bean germplasm for resistance to root-knot nematodes (*Meloidogyne javanica* and *M. incognita*). Phytopathology 81, in press (Abstr.).

15. Santoso, I. 1973. The effect of various factors on the expression of genetic resistance to root-knot nematodes (*Meloidogyne incognita* [Kofoid and White] Chitwood) in snap bean (*Phaseolus vulgaris* L.), tomato (*Lycopersicon esculentum* Mill.), soybean (*Glycine max* Merr.), and lima bean (*Phaseolus lunatus* L.). Ph.D. thesis, University of Hawaii, Honolulu.

16. Sule, S., and Z. Klement. 1971. Effect of high temperature and the age of bacteria on the hypersensitive reaction of tobacco. ACTA Phytopathologica et Entomologica Hungarica 6:119–122.

17. Zacheo, G., B. Zacheo, and F. Lamberti. 1982. Role of peroxidase and superoxide dismutase activity in resistant and susceptible tomato cultivars infested by *Meloidogyne incognita*. Nematologia Mediterranea 10:75–80.