Journal of Nematology 21(2):173–178. 1989.

Effects of Temperature on Pathogenicity and Population Development of Two Isolates of *Heterodera lespedezae*[1]

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Abstract: Population development of isolates of *Heterodera lespedezae* from Illinois and North Carolina was compared on Korean, sericea, and striate lespedezas and red clover at soil temperatures of 14, 18, 22, 26, 30, 34 C (± 1 C) and in a greenhouse where temperatures ranged from 19 to 40 C (av. 25 C). Cyst production on all plants, but not at all temperatures, was significantly different between the two isolates. Males of the Illinois isolate were recovered from red clover and striate lespedeza at 22 and 26 C and at greenhouse temperatures. No males of the North Carolina isolate were found on any host. Both isolates retarded growth of striate lespedeza but had no effect on growth of the other species tested.

Key words: biotype, *Heterodera lespedezae*, lespedeza, lespedeza cyst nematode, pathogenicity, population dynamics, red clover, temperature.

Variation in morphology, developmental rate, pathogenicity, reproductive potential, and host preference within nematode species is well known. Physiological variation within several cyst nematode species is manifested by differences in host range. These differences are commonly used to identify races or pathotypes (14,17,20). Discrepancies in published results of host-range tests with populations of *Heterodera lespedezae* Golden & Cobb from Illinois (IL) and North Carolina (NC) indicate the possibility of physiological variation within the species (6,13,19). Variations in host range could be caused by differences in greenhouse conditions, since the tests were conducted at different locations; however, comparison of populations reared concurrently in the same greenhouse showed significant differences in host status of four plant species (7).

Temperature affects many aspects of the life history of cyst nematodes. Populations of the same species may differ in temperature requirements for hatch and emergence from cysts (12,21), seedling penetration (10,12), and reproduction (10). Host range and the effects of environmental factors on the NC population of *H. lespedezae* (2,3,11,13,19) and on the IL population (5–8) have been reported. The populations differ in cyst production on selected hosts (7). They also differ in hatch response to temperature and root leachate and in effect of temperature on root penetration by second-stage juveniles (8). This study compared the pathogenicity and population development of the IL and NC populations of the lespedeza cyst nematode at different temperatures.

Materials and Methods

Isolates of *H. lespedezae* were obtained from populations collected from striate lespedeza, *Lespedeza striata* (Thumb.) Hook & Arn., in Hamilton County, Illinois, and in Union County, North Carolina. The isolates, designated IL and NC, respectively, were increased and maintained separately on 'Kobe' striate lespedeza in a greenhouse.

Juveniles were collected by comminuting root fragments containing cysts for 3 minutes at high speed in a blender. Released eggs and juveniles were separated from coarse plant debris by differential sieving. The eggs and juveniles retained by a 45-μm-pore sieve were backwashed onto a double layer of Whatman #1 filter paper

Received for publication 22 February 1988.

[1] Mention of trademark, warranty, proprietary product or vendor does not constitute a guarantee by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

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TABLE 1. Shoot heights (cm) of striate lespedeza inoculated with either of two isolates of *Heterodera lespedezae*, one from Illinois (IL) and one from North Carolina (NC), at seven temperatures, 6 and 8 weeks after inoculation.

Temperature (C)	Shoot height					
	6 weeks			8 weeks		
	IL	NC	Control	IL	NC	Control
14	26.4	23.1*	30.2	32.4	27.8	36.8
18	29.2	27.2	30.5	36.6	35.0	38.4
22	34.0	29.0*	35.6	37.6	29.2*	46.0
26	33.5	26.4*	40.4	34.6*	27.8*	47.6
30	45.7	44.7	45.7	51.2	51.6	52.0
34	41.3	37.3	42.7	47.4	42.0	46.6
Greenhouse†	33.8	30.0	34.3	38.8	33.6	38.8

Each value is the mean of five observations. Pi was 550 J2/pot. Means separated by Duncan's multiple-range test.
* Differs significantly ($P = 0.05$) from the control.
† Greenhouse temperature averaged 25 C (19–40 C).

in a Buchner funnel. The top layer of filter paper was placed on cheesecloth in an extraction ring (4) in a dish of tap water at room temperature. Emerged juveniles were collected daily, stored at 5 C, and used within 4 days.

Juveniles were surface disinfected with 100 μg/ml phenyl mercuric acetate ("Phix," active ingredients 22% phenyl mercuric acetate) for 15 minutes, then alternately centrifuged and washed three times in sterile distilled water. Numbers of nematodes per milliliter of solution were determined by averaging counts from three 1-ml samples. Dilutions were made to obtain the desired inoculum levels.

The soil was a steam-pasteurized, 3:1 mixture of loamy sand (83% sand, 6% clay, 11% silt) and silica sand. Seeds of red clover (*Trifolium pratense* L. cv. Dollard), striate lespedeza (*L. striata* cv. Kobe), Korean lespedeza (*Lespedeza stipulacea* Maxim. cv. Summit), and sericea lespedeza (*Lespedeza cuneata* (Dumont) G. Don.) were inoculated with commercial *Rhizobium* inoculant and sown in 10.5-cm-d plastic pots. Plants were thinned to two per container 2 weeks after seeding. Nematode inoculum in an aqueous suspension was applied to the soil by pipetting an aliquant into each of two holes, approximately 4 cm deep, one on either side of the plants. The holes were filled with soil and the soil was watered. Thirty-five pots of each plant species were inoculated with each nematode isolate; inoculum density was 550 juveniles in a 1.5-ml water suspension. Thirty-five noninoculated pots of each plant species served as controls. All pots were placed on a greenhouse bench for 4 days to allow time for the juveniles to penetrate plant roots. Each pot was then nested in a 950-cm^3 plastic cup containing 150 cm^3 of a 2:1 mixture of moist silica sand and coarse gravel to provide weight and drainage space. Five pots of each treatment were arranged in randomized complete blocks in each of six Cornell type constant temperature tanks (9) maintained at 14, 18, 22, 26, 30, or 34 C (± 1 C) or were retained on a greenhouse bench, where the temperature ranged from 19 to 40 C (av. 25 C). Drainage water was discarded periodically from the plastic cups.

Plant heights were recorded at 6 and 8 weeks after inoculation. Ten weeks after inoculation, plants were harvested and fresh weights of shoots and roots were recorded. Cyst-infested roots were placed in 110 ml water in a blender. The blender was run at high speed for 30 seconds. Cysts from roots and from soil were washed through 250-μm-pore and 150-μm-pore sieves. Numbers of cysts retained on both sieves were determined by direct count, if fewer than 300, and by dilution if more than 300 were present.

Analysis of variance was performed on

TABLE 2. Fresh weights (g) of striate lespedeza plants inoculated with either of two isolates of *Heterodera lespedezae*, one from Illinois (IL) and one from North Carolina (NC), at seven temperatures, 10 weeks after inoculation.

Temperature (C)	Root weight			Shoot weight		
	IL	NC	Control	IL	NC	Control
14	2.10*	1.18*	3.38	2.22*	1.22*	3.50
18	2.32*	1.64**	3.46	3.66	2.46	3.96
22	1.68*	0.70*	4.16	1.78*	0.78*	6.00
26	0.68*	0.72*	4.40	1.36*	0.52*	6.36
30	3.70	3.58	3.70	7.98	8.24	9.20
34	3.98	3.84	4.48	9.26	8.36	9.42
Greenhouse†	4.90*	3.02*	6.02	7.64	4.84*	10.06

Each value is the mean of five observations. Pi was 550 J2/pot. Means separated by Duncan's multiple-range test.
* Differs significantly ($P = 0.05$) from control.
** Differs significantly ($P = 0.01$) from the IL isolate.
† Greenhouse temperature averaged 25 C (19–40 C).

the data and Duncan's multiple-range test was used to separate means. Unless otherwise indicated significance at $P = 0.05$ is reported.

RESULTS

Striate lespedeza inoculated with *H. lespedezae* NC isolate exhibited foliar chlorosis after 25 days at 26 C and after 32 days at 22 C. The IL isolate caused similar symptoms at 26 and 22 C, but they appeared 7–14 days later. No symptoms occurred on red clover, sericea lespedeza, or Korean lespedeza.

Six weeks after inoculation with the NC isolate, striate lespedeza had lower shoot heights at 14, 22, and 26 C, but shoot heights were unaffected by the IL isolate at any temperature (Table 1). At 8 weeks, growth of plants inoculated with the NC isolate and incubated at 22 and 26 C had ceased. Although shoot heights tended to be lower in plants inoculated with the IL isolate, they were significantly different from the control plants only at 26 C. At 26 C plants inoculated with either isolate had ceased to grow and defoliation had begun at 10 weeks.

Root weights of striate lespedeza inoculated with the NC isolate were lower than those inoculated with the IL isolate, but the differences were significant only at 18 C (Table 2). Greatest inhibition of root growth occurred at 26 C with the IL isolate and at 22 and 26 C with the NC isolate. Shoot weights with both isolates were less than controls at 14, 22, and 26 C and with the NC isolate were less at greenhouse temperatures. Root and shoot weights of the other plant species tested were not affected by the nematodes.

Highest numbers of cysts were recovered from striate lespedeza inoculated with the IL isolate at 22 and 26 C and greenhouse temperatures (Table 3). There were no significant differences among the three temperatures. More cysts were produced by the NC isolate than by the IL isolate at 14 C, whereas the reverse was true at 22 C. No cysts of either isolate were found on striate lespedeza at 34 C. With both isolates, many small, thin-walled cysts that passed through the 250-μm-pore sieve were produced at 22 and 26 C and greenhouse temperatures. Most of the cysts retained by the 150-μm-pore sieve were brown and contained only 3–15 eggs and juveniles per cyst.

The IL isolate produced significantly more cysts on red clover than did the NC isolate at all temperatures except 34 C, where only an occasional cyst was present, and 26 C (Table 3). This was the only host species on which females matured at 34 C. Maximum cyst production by both isolates occurred at 22 and 26 C and greenhouse temperatures. When cyst numbers were high, numerous small cysts passed through

TABLE 3. Numbers of cysts produced by Illinois (IL) and North Carolina (NC) isolates of *Heterodera lespedezae* on selected hosts at seven temperatures.

Temperature (C)	Striate lespedeza		Red clover		Sericea lespedeza		Korean lespedeza	
	IL	NC	IL	NC	IL	NC	IL	NC
14	381 a	572* ab	1,111 a	32* a	8 a	9 a	17 a	32 ab
18	885 b	1,977 bc	8,948 b	228* bc	5 a	2 b	8 ab	50* bc
22	5,618 c	3,656* c	11,027 c	1,439* d	25 b	27 a	15 a	244* de
26	6,398 c	3,882 c	8,240 c	3,228 d	101 c	2* b	9 ab	367* e
30	320 a	294 a	2,884 b	140* b	3 a	0* c	1 b	20* a
34	0 d	0 d	3 d	1 e	0 d	0 c	0 c	0 f
Greenhouse†	6,800 c	7,349 c	8,270 c	747* cd	22 a	1* bc	5 ab	111* cd

Each value is the mean of five observations. Pi was 550 J2/pot. Means separated by Duncan's multiple-range test. Means within the same column and followed by the same letter do not differ significantly ($P = 0.05$).

* Differs significantly ($P = 0.05$) from the IL isolate on the same host.

† Greenhouse temperature averaged 25 C (19–40 C).

the 250-μm-pore sieve and were collected on the 150-μm-pore sieve.

The optimum temperature for cyst production on sericea lespedeza was 26 C for the IL isolate and 22 C for the NC isolate (Table 3). The IL isolate produced significantly more cysts than did the NC isolate at 26 and 30 C and greenhouse temperature. Cyst numbers were low and no small cysts were collected on the 150-μm-pore sieve.

On Korean lespedeza the NC isolate produced significantly more cysts than the IL isolate at all temperatures except 14 and 34 C (Table 3). Maximum cyst production by the NC isolate occurred at 22 and 26 C. The few cysts produced by the IL isolate on this host were insufficient to show any trend in temperature effect. No cysts were collected on the 150-μm-pore sieve.

Seventeen males were recovered from red clover and striate lespedeza inoculated with the IL isolate. They were found only in pots containing high numbers of cysts. No males were associated with the NC isolate.

DISCUSSION

Both isolates were pathogenic on striate lespedeza but had no effect on growth of the other plant species. Symptom expression on striate lespedeza occurred earlier and growth inhibition was greater in plants inoculated with the NC isolate. The NC isolate was more pathogenic than the IL isolate at all temperatures except 30 and 34 C. The data from the NC isolate are similar to those of Bhatti (2), who reported stunting 2 weeks after inoculation with whole cysts from a NC population. The severity of damage by either isolate to striate lespedeza was greater at 14 C than at 18 C. Since the number of cysts produced at 18 C was higher than at 14 C, the growth inhibition may be due to the response of the plant to the nematodes rather than to the effect of temperature on the nematodes directly. Damage threshold density on striate lespedeza varied between isolates and among temperatures.

Cyst production on test plants commonly is based on the number collected on 250–300-μm-pore sieves, which retain normal size cysts of several *Heterodera* spp. Under conditions optimum for cyst production, numerous small cysts developed and were retrieved on a finer sieve. Although this dwarfing has been reported in *H. lespedezae* with poor hosts (6), it may occur in other *Heterodera* species. Differences between total cyst numbers and those retained by a 300-μm-pore sieve alone may not significantly change the results of an experiment; however, erroneous conclusions could be drawn from the loss of the dwarf cysts.

A few IL juveniles developed into males under certain conditions. Males were found more often on red clover than on striate lespedeza and only at 22 and 26 C and bench temperature, where large numbers

of cysts were produced. No males were found in the NC isolate. The presence of males in a population of the lespedeza cyst nematode may be significant if they occur in the same field with *H. glycines*. Hybridization with the soybean cyst nematode may be possible, resulting in progeny capable of infecting soybeans. Interspecific crosses between *Heterodera* species (17,18) and *Globodera* species (17) have been produced, with some of the resulting hybrids differing from the parents in host range.

Related species of nematodes vary in their response to temperature (1,15,16). Such variation may be correlated not only with species, isolates, or races of nematodes, but also with the host plant. On striate lespedeza, the NC isolate of *H. lespedezae* was tolerant to a wider range of temperatures than was the IL isolate. The reverse was true on red clover. Differences in cyst numbers on striate lespedeza at 22 and 26 C probably resulted from the smaller size of the root systems of plants inoculated with the NC isolate rather than from a difference in reproductive ability of the two isolates.

The effect of a fluctuating temperature on population development approximates those of a constant 26 C, which was equal to the mean of the bench temperature. This was not true, however, of the pathogenic effects of the nematodes; plant growth in controls was greater at greenhouse temperature and plant growth inhibition by the nematodes was less than at a constant 26 C. During the frequent periods of temperatures exceeding 30 C, the nematodes may have been inactive and not feeding as efficiently as at lower temperatures. The effect of fluctuating temperatures also appears to favor the growth of the plant, which enables it to support a larger population of nematodes.

Differences in cyst production by these two isolates occur on selected hosts (7). These isolates also vary in hatch and emergence of juveniles from cysts and in penetration of striate lespedeza roots (8). This study shows that there are also significant differences in pathogenicity, reproductive ability, and tolerance to a range of temperatures between the IL and the NC isolates of the lespedeza cyst nematode.

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