Cyst Production by Two Geographical Isolates of *Heterodera lespedezae* on Selected Legumes

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ABSTRACT

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Cyst production by isolates of *Heterodera lespedezae* from Illinois and North Carolina populations was compared on 14 species of legumes. Twelve species were hosts for both isolates, ranging from good to very poor. There were significant differences between isolates on four species. The Illinois isolate produced more cysts on striate lespedeza and red clover and fewer cysts on alsike clover. The North Carolina isolate produced a few egg-bearing cysts on soybean, which was a nonhost for the Illinois isolate. Only common vetch was a nonhost for both isolates.

Additional key words: biotypes, host range, lespedeza cyst nematode

Heterodera lespedezae Golden & Cobb, the lespedeza cyst nematode, has been reported from Illinois (2), North Carolina (5), and Tennessee (8) and may be an important factor in stand decline of annual lespedezas (1). *H. lespedezae* can be found on volunteer striate lespedeza (*Lespedeza striata* (Thunb.) Hook & Arn.) in fields of soybean (*Glycine max* (L.) Merr.) and thus can be mistaken for *H. glycines* Ichinohe, the soybean cyst nematode (3).

Discrepancies in published results of host range tests with populations of *H. lespedezae* from Illinois and North Carolina indicate the possibility of at least two biotypes in the species. Soybean has been reported as a poor (7) or a nonhost (3,6), yellow sweet clover (*Melilotus officinalis* (L.) Lam.) as a good (3) or a poor host (7), and vetch (*Vicia* sp.) as a good (3) or a nonhost (6). This study was undertaken to determine if the Illinois and North Carolina populations of *H. lespedezae* differ in their host ranges among certain legumes or in their reproductive capacities on those plants.

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MATERIALS AND METHODS

Isolates of *H. lespedezae* were obtained from populations collected from striate lespedeza in Hamilton County, IL, and Union County, NC, and were increased separately on striate lespedeza cultivar Kobe, the type host, in a greenhouse. Fourteen leguminous plant species were screened for susceptibility to the two isolates in the first of two tests. Reported host species included were striate lespedeza cultivar Kobe, Korean lespedeza (*L. stipulacea* Maxim. cv. Summit and unimproved), yellow sweet clover, red clover (*Trifolium pratense* L. cv. Dollard), common white clover (*T. repens* L. cv. Dutch White), alsike clover (*T. hybridum* L.), hairy vetch (*Vicia villosa* Roth), adzuki bean (*Phaseolus angularis* (Willd.) W. F. Wight), green bean (*P. vulgaris* L. cv. GU-50), mung bean (*P. aureus* Roxbg.), and garden pea (*Pisum sativum* L. cv. Freezonian). Reported nonhost species tested were sericea lespedeza (*L. cuneata* (Dumont) G. Don) and soybean cultivar Clark 63. Common vetch (*V. sativa* L.) also was included because its host status was unclear. Seed was obtained from various commercial sources and USDA cooperators. Plants were grown in 10.5-cm clay pots of steam-pasteurized loamy fine sand amended with silica sand, one plant per pot for large-seeded species and two plants per pot for small-seeded species. Seeding dates were staggered so that all plants emerged within a 1-wk period.

Cysts from isolate cultures were ground for 3 min at high speed in a blender. Eggs and second-stage juveniles were separated from coarse debris by passing suspensions through 425-, 150-, 75-, and 45-μm sieves. Eggs and juveniles retained on the 45-μm sieve were backwashed onto a double layer of no. 1 filter paper in a Büchner funnel. The top layer was placed on cheesecloth in an extraction ring in a dish of tap water. Juveniles were collected daily, stored at 5 C, and inoculated within 4 days. Nematodes were surface-disinfested with 100 μg/ml phenyl mercuric acetate for 15 min, then alternately centrifuged and rinsed three times in sterile distilled water.

Three to 4 wk after seeding, each pot was inoculated with a 5-ml suspension of 700 juveniles of the appropriate isolate. Inoculum was pipetted into two 4-cm-deep holes on opposing sides of the plants. The holes were filled with soil, and the soil surface was watered lightly. Treatments were replicated four times. Pots were arranged in a completely randomized design on a greenhouse bench, where ambient temperatures averaged 24 C (20–38 C). Thirty days after inoculation, soil was processed for cysts by wet-sieving through 1,180- and 250-μm sieves. Large roots of reportedly poor hosts were teased apart and examined for deeply embedded females. Numbers of "cysts" (including swollen white females) per pot were determined by direct or dilution counts.

The test was repeated with two additional cultivars, red clover cultivar Kenland and ladino white clover (*T. repens* unimproved). All data were analyzed statistically using Duncan's multiple range test after transformation of cyst counts to log $(x + 1)$.

RESULTS AND DISCUSSION

Results of the two tests were similar and are combined in Table 1. Twelve of the 14 species were hosts for both isolates of *H. lespedezae*. Suitability of hosts ranged widely from good (mean cyst production $\geqslant 400$) to very poor (mean $\leqslant 25$). A partial second generation of nematodes developed on the more favorable hosts. Egg-bearing cysts were recovered from all hosts, but cysts from unfavorable hosts generally were poorly developed, were largely or totally embedded within roots, and contained few if any eggs.

Striate lespedeza, yellow sweet clover, Dutch White clover, and hairy vetch were good hosts for both isolates. The Illinois isolate produced significantly more cysts on striate lespedeza and red clovers but fewer cysts on alsike clover than the North Carolina isolate. One cyst of the

Table 1. Numbers of cysts produced within 30 days by Illinois (IL) and North Carolina (NC) isolates of *Heterodera lespedezae* on 14 species of legumes

Plant species and cultivar	Isolate[a]			
	IL		NC	
	Mean	SE	Mean	SE
Striate lespedeza (*Lespedeza striata* 'Kobe')	1,172*[b]	253	651	52
Korean lespedeza (*L. stipulacea* 'Summit')	18	9	55	9
Korean lespedeza (*L. stipulacea* unimproved)	89	61	332	166
Sericea lespedeza (*L. cuneata*)	29	21	34	17
Yellow sweet clover (*Melilotus officinalis*)	631	384	426	240
Red clover (*Trifolium pratense* 'Dollard')	446*	91	118	28
Red clover (*T. pratense* 'Kenland')	482*	121	120	39
Common white clover (*T. repens* 'Dutch White')	1,694	414	1,177	440
Ladino white clover (*T. repens*)	552	328	178	60
Alsike clover (*T. hybridum*)	32*	21	366	162
Hairy vetch (*Vicia villosa*)	808	162	678	240
Common vetch (*V. sativa*)	0	0	0	0
Adzuki bean (*Phaseolus angularis*)	63	6	109	30
Green bean (*P. vulgaris* 'GU-50')	48	7	102	26
Mung bean (*P. aureus*)	17	3	13	3
Garden pea (*Pisum sativum* 'Freezonian')	34	14	12	2
Soybean (*Glycine max* 'Clark 63')	0*	0	12	2

[a] Each value is the mean or standard error for eight replicates, except four for Kenland red clover and ladino white clover; inoculum level was 700 J2 per pot.
[b] * = Significantly different ($P \leq 0.05$) from the NC isolate according to Duncan's multiple range test.

North Carolina isolate was recovered from the soil in each of two pots and three to 15 egg-bearing cysts were found in taproots in four of eight pots of soybean. These cysts were verified as *H. lespedezae* by A. M. Golden. No cysts were produced by the Illinois isolate on soybean. Summit Korean lespedeza, sericea lespedeza, adzuki bean, green bean, mung bean, and garden pea were poor to very poor hosts for both isolates. Neither isolate developed cysts on common vetch.

Closely related plant species within the same genus (viz. *Lespedeza, Trifolium, Vicia*) showed extreme differences in ability to support reproduction of both isolates of *H. lespedezae*. Interpot variation in cyst production within species also was extreme with both isolates. Genetic heterogeneity of seed-grown plants, particularly in unimproved cultivars, probably was the major contributor to the intraspecific host variation. With mutually inclusive plant species, host status results agree with those of Hung (6). The unspecified species of vetch in that study apparently was *V. sativa* (common vetch), which indeed is a nonhost, whereas *V. villosa* (hairy vetch) is a good host. The reaction of the Illinois isolate was similar to that reported by Edwards and Malek (3), except on sericea lespedeza, which they listed as a nonhost. Because they used brown cysts as inoculum, the number of juveniles emerging may have been so low that no infection occurred and/or the few new cysts produced were impossible to differentiate from inoculum. Differences in cyst production on striate lespedeza probably were based on differential pathogenicity of the isolates rather than on reproductive rates on this host. In this and related research (4), the North Carolina isolate consistently suppressed growth of striate lespedeza more than the Illinois isolate, apparently limiting population development of the former. The two isolates of *H. lespedezae* represent relatively distinct biotypes, which may be differentiated on the basis of reproduction primarily on alsike clover and secondarily on Dollard or Kenland red clover and soybean.

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