Journal of Nematology 31(4):407–417. 1999.


# Variations in Host Preference among and within Populations of *Heterodera trifolii* and Related Species

Shouhua Wang and Robert D. Riggs[1]

*Abstract:* Seven populations of *Heterodera trifolii* from Arkansas, Kentucky, Pennsylvania, and Australia plus 3 or 4 single-cyst isolates (SCI) from each population were tested for reproduction on seven species of plants to compare the host preferences among and within populations. Common lespedeza, *Kummerowia striata* cv. Kobe, was a good host for all populations and isolates. Therefore, a plant was considered to be a host if the number of females produced on it was 10% or more of the number on Kobe. All seven populations reproduced on *Trifolium repens* and *T. pratense.* None reproduced on *Beta vulgaris* or *Glycine max.* One single-cyst isolate from the Australian population produced a few females on *T. pratense.* The Australian population maintained on carnation, *Dianthus caryophyllus,* produced females on carnation but not on curly dock, *Rumex crispus.* However, its subpopulation maintained on *T. repens* produced females on *R. crispus* but not on carnation. Four of the other six populations produced females on *R. crispus,* and four produced females on carnation. Differences in host range were observed among seven of the mother populations and their SCI, and among isolates within each population. Five host range patterns were found in populations and SCI of *H. trifolii.* Significant quantitative differences occurred among populations in the numbers of females on most hosts, between isolates and their original populations, and among isolates from the same population. SCI selected from white clover produced fewer females on a series of test hosts and had host ranges the same as or narrower than those of the original populations. However, SCI selected from Kobe lespedeza had more females on some hosts and had host ranges the same as or wider than those of the original populations. The host ranges of all populations and SCI of *H. trifolii* were different from those of populations and SCI of race 3 of *H. glycines* and *H. lespedezae.*

*Key words:* clover cyst nematode, *Heterodera glycines, Heterodera lespedezae, Heterodera trifolii,* host-parasite interaction, host range, nematode.

The clover cyst nematode (*Heterodera trifolii* Goffart, 1932) occurs worldwide, including New Zealand (Yeates and RIsk, 1976), Australia (Mcleish et al., 1997), Canada (Kimpinski et al., 1994), United States (Singh and Norton, 1970), and The Netherlands (Seinhorst and Sen, 1966). Its host range includes species of Caryophyllaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae, Labiatae, Polygonaceae, and Scrophulariaceae (Maas and Heijbroek, 1982). Crops most commonly reported to be hosts are white clover (Yeates and Risk, 1976), red clover (Mercer and Campbell, 1986), carnation (Cuany and Dalmasso, 1973), other leguminous plants, and some vegetable crops (Inserra et al., 1993; Kimpinski et al., 1994; Sikora, 1977). In 1982, *H. trifolii* was reported as a serious pest of sugarbeet in certain areas of The Netherlands (Maas and Heijbroek, 1982). Damage to plants by *H. trifolii* is associated with early invasion of seedlings (Mercer and Campbell, 1986), interruption of nitrogen fixation (Yeates et al., 1977), interactions with other soil-borne fungal pathogens (Sikora, 1977), and influence on rhizosphere microbial biomass (Yeates et al., 1998). The yield losses of infected crops are generally significant and may be severe.

In early research, *H. trifolii* was referred to as a species complex that reproduced by parthenogenesis (Hirschmann and Triantaphyllou, 1979). The chromosome number of 24 to 35 indicates that this group of nematode is triploid or tetraploid in comparison to other *Heterodera* spp. that have a chromosome number of $n = 9$ (Triantaphyllou and Hirschmann, 1978). Separation of *H. trifolii* from other related species had been made by comparing morphometrics of eggs and second-stage juveniles (Steele and Whitehand, 1984) and cysts (Abawi et al., 1973), egg hatching (Steele et al., 1982), and ultrastructure by scanning electron microscopy (Hirschmann and Triantaphyllou, 1979; Stanger and Noel, 1996). Electrophoretic

Accepted for publication 4 February 1999.

Approved for publication by the director of the Arkansas Agricultural Experiment Station. This research was supported in part by a grant from the soybean growers of Arkansas through the Soybean Promotion Board.

[1] Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

E-mail: rdriggs@comp.uark.edu

This paper was edited by A. F. Robinson.

analysis of soluble proteins (Pozdol and Noel, 1984) and sequence comparison of spacer ribosomal DNA (Ferris et al., 1993) also have been employed to differentiate related species. These observations distinguished *H. trifolii* from several related species such as *H. lespedezae* and *H. galeopsidis,* which have been regarded as separate species.

The monosexual reproduction of *H. trifolii* may indicate that less genetic variation occurs than in other cyst-forming nematodes, such as *H. glycines* and *H. schachtii,* that reproduce by amphimixis. However, observations have shown significant variation in host ranges of populations of this species (Maas et al., 1982). The concept of "race" or "pathotype" for some populations of *H. trifolii* was proposed (Cuany and Dalmasso, 1973; Maas and Heijbroek, 1982; Maas et al., 1982). The most distinct host race proposed in *H. trifolii* is the yellow beet cyst nematode (YBCN), which has a distinct yellow phase; *H. schachtii* does not have a yellow stage during the change from white to brown and reproduces well on sugarbeet but poorly on white clover (Maas and Heijbroek, 1982). In addition to the variation in reproduction specific to different plant species, differences in cyst production on a series of clover cultivars also were observed in several geographically distinct populations (Mercer and Grant, 1993; Singh and Norton, 1970). The intraspecific variation in host-parasite relationships suggests that polyploid parthenogenetic species have much genetic variability and the capacity to adapt to host species or cultivars where populations are geographically dispersed. However, no research has been done on differences in host preference between individuals from the same population, which would be necessary for understanding the population genetic dynamics of a parthenogenetic species in a multihost planting system.

*Heterodera trifolii* has a very diverse host range. It is adapted to parasitism of sugarbeet, and R. A. Chapman (pers. comm.) identified populations that parasitized soybean. In this research, several isolates were developed from individual cysts of several geographical populations and tested for their reproduction on a series of test plants along with the original populations. The objectives of this research were to compare the reproductive abilities of geographical populations on a series of plant species and to investigate the variability in host preference among single-cyst isolates (SCI) derived from those populations of this parthenogenetically reproducing species.

## Materials and Methods

*Populations:* Populations of *H. trifolii* collected from Arkansas, Kentucky, Pennsylvania, and Australia were maintained on the host of origin and other selected hosts in a greenhouse (Table 1). A stock population of race 3 of *H. glycines* maintained on soybean cv. Lee 74 and a population of *H. lespedezae* maintained on common lespedeza cv. Kobe were included in this study for a comparison of the host ranges of different nematode species. All populations were allowed to propagate on the maintenance hosts for a generation or until enough cysts were obtained for the host test.

*Isolates:* Ten single cysts randomly selected from each stock population were picked out individually to a 1.5-cm-diam. watch glass. Each cyst was crushed to release eggs by pressing it with a glass bar. Each crushed cyst with more than 100 mature eggs was washed into the rhizosphere of a white clover or lespedeza plant (Table 1) growing in a 7.5-cm-diam. clay pot. After 35 to 40 days, mature females were extracted from roots of host plants by a rubbing-and-sieving method (Riggs and Schmitt, 1991). The four isolates from each stock population with the highest numbers of cysts were propagated on each maintenance host until enough cysts were obtained for host tests.

*Host test:* Plants included in the test were white clover (*Trifolium repens* cv. Ladino), red clover (*T. pretense* cv. Kenland), common lespedeza (*Kummerowia striata* cv. Kobe), curly dock (*Rumex crispus*), carnation (*Dianthus caryophyllus*), sugarbeet (*Beta vulgaris*), and soybean (*Glycine max* cultivars Essex and Hartwig). Each host was replicated

TABLE 1. The origin and maintenance hosts of parent populations, and their single-cyst isolates (SCI), of *Heterodera trifolii, H. glycines,* and *H. lespedezae.*

| Nematode population | Geographical area | Maintenance host | |
|---|---|---|---|
| | | Population | SCI[a] |
| *H. trifolii* | | | |
| A | Fayetteville, AR | White clover | White clover |
| E | Pine Tree, AR | White clover | White clover |
| F | Randoph Co., AR | Curly dock | White clover |
| B[b] | Australia | White clover | White clover |
| G | Australia | Carnation | White clover |
| C | Kentucky | Red clover | 'Kobe' lespedeza |
| D | Kentucky | Red clover | 'Kobe' lespedeza |
| H | Pennsylvania | White clover | White clover |
| *H. glycines* race 3 | Stock culture | 'Lee 74' soybean | 'Essex' soybean |
| *H. lespedezae* | Stock culture | 'Kobe' lespedeza | 'Kobe' lespedeza |

[a] Isolates were selected from and maintained on the host given.
[b] A sub-population of G; original host was carnation.

five times. About 50 to 70 seeds of white clover, red clover, and lespedeza and five to 10 seeds of sugarbeet were planted into fine sand in 7.5-cm-diam. clay pots 3 weeks before inoculation. Seeds of curly dock and carnation were planted in vermiculite in metal flats 4 weeks before transplanting. Four to five small seedlings were transplanted into heated fine sand in each 7.5-cm-diam. clay pot and allowed to grow for an additional 3 weeks before inoculation with nematodes. Seeds of soybean were germinated in vermiculite, and seedlings were transplanted into heated fine sand in 7.5-cm-diam. clay pots 48 hours before inoculation. Cysts or gravid females of each population and SCI were extracted from maintenance hosts following the procedure of Riggs and Schmitt (1991). The resulting suspension was centrifuged at 1,130*g* for 6.5 minutes to remove nematodes and debris from the water. The pellet was then suspended in a 2 M sucrose solution and centrifuged at 1,130*g* for 2.5 minutes. The supernatant containing the cysts and females was poured onto a 180-µm-pore sieve (80 mesh), after which the nematodes were rinsed free of sucrose and crushed with a ground glass homogenizer to release eggs. The egg suspension was adjusted to a final concentration of 1,500 eggs + second-stage juveniles (J2)/ml, which was determined by counting three 1-ml aliquots of the sample. One milliliter of egg + J2 suspension was injected into each of two holes near the roots of a plant with a Gilson pipet (Rainin Instrument, Inc., Woburn, MA) to ensure that the same volume of eggs + J2 suspension (3,000) was placed around the roots of each plant. Nematodes were allowed to develop on test plants for 35 to 40 days in a greenhouse with a temperature fluctuation of 25 to 35 °C and 12-hour day length. Females and cysts were extracted from each pot and counted with a stereomicroscope. For qualitative comparison, a female index (number of females on test host/number females on lespedeza × 100) was calculated. Plants on which the number of females produced was 10% or more of the number on lespedeza were considered efficient hosts, those with less than 10% of the number on lespedeza were considered poor hosts, and plants with no females were nonhosts. For quantitative analysis, the numbers of females produced on each host were compared statistically among populations and SCI with ANOVA (SAS Institute, Cary, NC).

## RESULTS

*Interspecific variation: Heterodera trifolii, H. glycines,* and *H. lespedezae* were easily separated by their significantly different host ranges (Table 2). All populations of *H. trifolii* produced numerous females on lespe-

TABLE 2. Comparison of production of females by *Heterodera trifolii, H. glycines,* and *H. lespedezae* populations on eight test plants.[a]

| Nematode population | Number of females on test plants[b] | | | | | | | | Host range pattern based on female indices[c] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | KL | WC | RC | CD | CN | SB | ES | HS | KL | WC | RC | CD | CN | SB | ES | HS |
| *H. trifolii* | | | | | | | | | | | | | | | | |
| A | 788 b | 694 d | 337 a | 380 a | 198 b | 12 a | 0 | 0 | + | + | + | + | + | – | – | – |
| B | 434 d | 602 de | 115 c | 173 b | 28 c | 2 a | 0.6 | 0 | + | + | + | + | – | – | – | – |
| C | 180 e | 206 f | 82 c | 72 c | 31 c | 9 a | 0 | 0 | + | + | + | + | + | – | – | – |
| D | 438 d | 452 e | 114 c | 43 c | 1.2 c | 0 a | 0 | 0 | + | + | + | – | – | – | – | – |
| E | 617 c | 725 d | 234 b | 14 c | 248 b | 0.6 a | 0.2 | 0 | + | + | + | – | + | – | – | – |
| F | 1,187 a | 918 bc | 317 ab | 354 a | 56 c | 14 a | 0 | 0 | + | + | + | + | – | – | – | – |
| G | 812 b | 1,046 b | 308 ab | 54 c | 182 b | 9 a | 0 | 0 | + | + | + | – | + | – | – | – |
| H | 906 b | 1,297 a | 268 ab | 142 b | 496 a | 10 a | 0.4 | 0 | + | + | + | + | + | – | – | – |
| *H. glycines* | 599 | 0 | 0 | 0 | 0.2 | 0 | 774 | 0 | + | – | – | – | – | – | + | – |
| *H. lespedezae* | 196 | 29 | 35 | 13 | 20 | 21 | 248 | 21 | + | + | + | – | + | + | + | + |

[a] KL = 'Kobe' lespedeza, WC = white clover, RC = red clover, CD = curly dock, CN = carnation, SB = sugarbeet, ES = 'Essex' soybean, HS = 'Hartwig' soybean.

[b] Data are means of five replications. Means in a column followed by a common letter are not significantly different ($P = 0.05$) according to Fisher's Least Significant Difference Test.

[c] "+" indicates an efficient host on which the population produced at least 10% as many females as the average number produced on 'Kobe' lespedeza; "–" indicates a poor host or nonhost on which the population produced fewer females than 10% of the average number produced on 'Kobe' lespedeza.

deza, white clover, and red clover, with fewer on all other hosts and few or none on the two soybean cultivars. The two soybean cultivars and sugarbeet were poor hosts or nonhosts of *H. trifolii*. Essex soybean and lespedeza were the only good hosts of *H. glycines,* and all plant species tested except curly dock were good hosts of *H. lespedezae*. However, *H. lespedezae* produced fewer females (10–20% of number on lespedeza) on white clover, red clover, carnation, sugarbeet, and Hartwig soybean. Only lespedeza was a good host for all three species.

*Interpopulation variation:* Based on female indices (FI), seven populations and one subpopulation had four different host range patterns (Table 2). Populations A, B, C, F, and H produced numerous females on white clover, red clover, lespedeza, and curly dock, but only A, C, and H reproduced well on carnation. Populations E and G produced numerous females on white clover, red clover, lespedeza, and carnation but not on curly dock. Population D produced numerous females only on white clover, red clover, and lespedeza.

Higher numbers of females were produced by populations A and F of *H. trifolii* on lespedeza than on any other host and by populations B, C, D, E, G, and H on white clover. However, significant differences in female numbers on these two favorable hosts were found among populations. Red clover was a host of all populations but always had lower numbers of females than white clover or lespedeza. Curly dock and carnation were favorable hosts of some populations and poor hosts of others.

Populations B and G were derived from a population from carnation in Australia. The original population had been split so that part was maintained on white clover (B) and part on carnation (G). The number of females produced on curly dock by population G was much lower than the number produced by population B and less than 10% of the number of females on lespedeza, whereas the number of females produced on carnation by population B was much lower than that produced by population G and also less than 10% of the number of females on lespedeza. Both populations produced high numbers of females on lespedeza, white clover, and red clover, but the numbers were significantly different.

*Intrapopulation variation:* Three different host range patterns were observed among population A and its SCI based on FI values (Table 3). No A-SCI had the same pattern as population A. All A-SCIs produced few females on carnation and (or) curly dock. All A-SCIs had at least one host plant on which it produced significantly fewer females than did population A. Significant differences in numbers of females on all plant species except soybean were observed among the four A-SCIs.

Population B had the same host range pattern as all B-SCIs derived from this population except one that produced very few females on red clover. Numbers of females produced on white clover were not significantly different among B-SCIs nor between B-SCIs and population B. B-SCI-2 and B-SCI-3 produced significantly more females on lespedeza than did population B. B-SCI-1 and B-SCI-4 produced the same number of females as population B on curly dock, B-SCI-4 produced the same number as population B on carnation, and none produced the same number as population B on sugarbeet. Differences in number of females among B-SCIs were found on lespedeza, red clover, carnation, and curly dock.

Based on FI, C-SCI-3 was similar to population C, but the other three were different (Table 3). On carnation, all C-SCIs except C-SCI-3 produced less than 10% of the number of females on lespedeza and had different host range patterns from population C. All C-SCIs produced significantly more females on lespedeza and white clover than did population C. No differences in female numbers were found on red clover, curly dock, and carnation.

The FI patterns for all three D-SCIs were different from population D (Table 3). All D-SCI isolates had higher FI on curly dock than did population D; other FI were similar for all hosts. All D-SCIs produced significantly more females on lespedeza and curly dock than did population D. D-SCI-2 and

TABLE 3. Comparison of the number of females produced by single-cyst isolates (SCI) and the parent populations of *Heterodera trifolii, H. glycines,* and *H. lespedezae* on eight test hosts.[a]

| Nematode population[d] | Number of females on test plants[b] | | | | | | | | Host range pattern based on female indices[c] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | KL | WC | RC | CD | CN | SB | ES | HS | KL | WC | RC | CD | CN | SB | ES | HS |
| *H. trifolii* A | 788 a | 694 a | 337 a | 380 a | 258 a | 12 a | 0 | 0 | + | + | + | + | + | − | − | − |
| SCI-1 | 940 a | 665 a | 182 b | 326 a | 50 b | 2.2 ab | 0 | 0 | + | + | + | + | − | − | − | − |
| SCI-2 | 364 b | 592 ab | 71 c | 26 c | 7 b | 0.4 b | 0.2 | 0 | + | + | + | − | − | − | − | − |
| SCI-3 | 283 b | 272 b | 55 c | 32 bc | 10 b | 0 b | 0 | 0.2 | + | + | + | + | − | − | − | − |
| SCI-4 | 1014 a | 850 a | 311 a | 109 b | 25 b | 5 a | 0 | 0 | + | + | + | + | − | − | − | − |
| *H. trifolii* B | 434 c | 602 a | 115 ab | 173 a | 28 a | 2 a | 0.6 | 0 | + | + | + | + | − | − | − | − |
| SCI-1 | 561 bc | 353 a | 46 b | 173 a | 4.2 b | 0.6 b | 0 | 0 | + | + | − | + | − | − | − | − |
| SCI-2 | 643 ab | 461 a | 199 a | 80 b | 2 b | 0 b | 0 | 0 | + | + | + | + | − | − | − | − |
| SCI-3 | 716 a | 486 a | 199 a | 104 b | 1.6 b | 0 b | 0 | 0 | + | + | + | + | − | − | − | − |
| SCI-4 | 544 bc | 576 a | 168 a | 149 a | 34 a | 0 b | 0 | 0 | + | + | + | + | − | − | − | − |
| *H. trifolii* C | 180 c | 206 c | 82 a | 72 a | 31 a | 9 b | 0 | 0 | + | + | + | + | + | − | − | − |
| SCI-1 | 662 a | 367 b | 137 a | 107 a | 13 a | 0 b | 0 | 0 | + | + | + | + | − | − | − | − |
| SCI-2 | 508 ab | 509 a | 143 a | 88 a | 30 a | 0.2 b | 0 | 0 | + | + | + | + | − | − | − | − |
| SCI-3 | 476 b | 491 ab | 115 a | 91 a | 67 a | 0 b | 0 | 0 | + | + | + | + | + | − | − | − |
| SCI-4 | 625 a | 560 a | 85 a | 98 a | 41 a | 0.4 b | 0 | 0 | + | + | + | + | − | − | − | − |
| *H. trifolii* D | 438 c | 452 b | 114 b | 43 c | 1.2 c | 0 a | 0 | 0 | + | + | + | − | − | − | − | − |
| SCI-1 | 947 ab | 701 ab | 221 a | 289 a | 42 ab | 5.2 a | 0.4 | 0 | + | + | + | + | − | − | − | − |
| SCI-2 | 1266 a | 984 a | 199 ab | 229 b | 64 a | 0.2 a | 0.4 | 0 | + | + | + | + | − | − | − | − |
| SCI-3 | 920 b | 988 a | 246 a | 194 b | 23 bc | 0.2 a | 0.2 | 0 | + | + | + | + | − | − | − | − |
| *H. trifolii* E | 617 ab | 725 a | 234 ab | 14 a | 248 a | 0.6 a | 0.2 | 0 | + | + | + | − | + | − | − | − |
| SCI-1 | 708 ab | 920 a | 284 a | 31 a | 49 b | 0.4 a | 0.6 | 0 | + | + | + | − | − | − | − | − |
| SCI-2 | 436 bc | 823 a | 149 b | 19 a | 33 b | 0 a | 0.2 | 0 | + | + | + | − | − | − | − | − |
| SCI-3 | 315 c | 475 b | 178 b | 13 a | 11 b | 0 a | 0 | 0 | + | + | + | − | − | − | − | − |
| SCI-4 | 750 a | 785 a | 161 b | 31 a | 110 b | 0.8 a | 0.2 | 0 | + | + | + | − | + | − | − | − |

TABLE 3. *Continued*

| Nematode population[d] | Number of females on test plants[b] | | | | | | | | Host range pattern based on female indices[c] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | KL | WC | RC | CD | CN | SB | ES | HS | KL | WC | RC | CD | CN | SB | ES | HS |
| *H. trifolii* F | 1187 a | 918 ab | 317 ab | 354 a | 56 a | 14 a | 0 | 0 | + | + | + | + | − | − | − | − |
| SCI-1 | 1172 a | 1086 a | 361 a | 377 a | 38 a | 4 b | 0.2 | 0 | + | + | + | + | − | − | − | − |
| SCI-2 | 492 b | 635 bc | 176 bc | 106 b | 15 a | 1 b | 1.2 | 0 | + | + | + | + | − | − | − | − |
| SCI-3 | 336 c | 538 c | 118 c | 97 b | 7 a | 0.6 b | 0.6 | 0.2 | + | + | + | + | − | − | − | − |
| SCI-4 | 606 b | 793 abc | 178 bc | 31 b | 15 a | 0.6 b | 0.6 | 0 | + | + | + | − | − | − | − | − |
| *H. trifolii* G | 812 a | 1046 ab | 308 a | 54 a | 182 ab | 9 a | 0 | 0 | + | + | + | − | + | − | − | − |
| SCI-1 | 246 b | 539 c | 181 b | 11 b | 102 ab | 0 a | 0 | 0 | + | + | + | − | + | − | − | − |
| SCI-2 | 737 a | 785 bc | 203 ab | 34 ab | 81 b | 0 a | 0.2 | 0 | + | + | + | − | + | − | − | − |
| SCI-3 | 756 a | 1019 ab | 203 ab | 27 ab | 75 b | 0 a | 0 | 0.6 | + | + | + | − | + | − | − | − |
| SCI-4 | 910 a | 1117 a | 275 ab | 56 a | 247 a | 0.4 a | 0.2 | 0 | + | + | + | − | + | − | − | − |
| *H. trifolii* H | 906 a | 1297 a | 268 a | 142 a | 496 a | 10 a | 0.4 | 0 | + | + | + | + | + | − | − | − |
| SCI-1 | 404 c | 502 cd | 200 a | 27 b | 36 b | 1.4 a | 0 | 0 | + | + | + | − | − | − | − | − |
| SCI-2 | 606 b | 673 bc | 89 b | 28 b | 30 b | 0.4 a | 0.4 | 0 | + | + | + | − | − | − | − | − |
| SCI-3 | 641 b | 707 b | 94 b | 17 b | 86 b | 0.2 a | 0.8 | 0 | + | + | + | − | + | − | − | − |
| SCI-4 | 558 bc | 413 d | 101 b | 26 b | 86 b | 0.2 a | 0 | 0.2 | + | + | + | − | + | − | − | − |
| *H. glycines* | 599 a | 0 | 0 | 0 | 0.2 b | 0 | 774 a | 0 | + | − | − | − | − | − | + | − |
| SCI-1 | 269 b | 0 | 0 | 0 | 2.4 ab | 0.6 | 371 c | 0 | + | − | − | − | − | − | + | − |
| SCI-2 | 342 b | 0 | 0 | 0 | 0.2 b | 0 | 325 c | 0 | + | − | − | − | − | − | + | − |
| SCI-3 | 379 b | 0 | 0 | 0 | 18 a | 0 | 588 ab | 0 | + | − | − | − | − | − | + | − |
| SCI-4 | 302 b | 0 | 0 | 0.4 | 0.8 b | 0 | 498 bc | 0 | + | − | − | − | − | − | + | − |
| *H. lespedezae* | 196 c | 29 a | 35 a | 13 a | 20 a | 21 a | 248 b | 21 a | + | + | + | − | + | + | + | + |
| SCI-1 | 941 a | 0 b | 0.4 b | 0.2 b | 18 a | 0.6 b | 911 a | 0 b | + | − | − | − | − | − | + | − |
| SCI-2 | 671 b | 10 b | 5.2 b | 3 b | 2.6 a | 0 b | 1043 a | 0 b | + | − | − | − | − | − | + | − |

[a] KL = 'Kobe' lespedeza, WC = white clover, RC = red clover, CD = curly dock, CN = carnation, SB = sugarbeet, ES = 'Essex' soybean, HS = Hartwig soybean.

[b] Data are means of five replications. Data followed by a common letter are not significantly different ($P = 0.05$) according to Fisher's Least Significant Difference Test.

[c] "+" indicates an efficient host on which the population produced at least 10% as many females as the average number produced on 'Kobe' lespedeza; "–" indicates a poor host or nonhost on which the population produced fewer females than 10% of the average number produced on 'Kobe' lespedeza.

[d] Nematode populations are designated by species names and uppercase letters (A–H), and single-cyst isolates are designated SCI-1, etc.

D-SCI-3 produced more females on white clover than did population D, as did D-SCI-3 on red clover and all three D-SCIs on curly dock.

Two host range patterns were observed in population E and its SCI (Table 3). All E-SCI produced significantly fewer females on carnation than did population E. E-SCI-2, E-SCI-3, and E-SCI-4 produced fewer females on red clover than E-SCI-1, and E-SCI-3 had fewer females on lespedeza and white clover than did population E as well as E-SCI-1 and E-SCI-4. All populations were the same on curly dock and sugarbeet, both poor hosts of population E.

Isolate F-SCI-4 produced fewer females on curly dock and had a different host pattern from F-SCI-1 and the parent population (Table 3). All F-SCIs and population F produced few females on carnation, and differences were not significant. In addition, all F-SCIs had lower numbers of females on sugarbeet than population F. The female numbers produced by F-SCI-2, F-SCI-3, and F-SCI-4 on lespedeza and curly dock were significantly lower than was produced by population F and F-SCI-1, as was the case with F-SCI-3 on white and red clover.

Population G and its SCIs had the same host range pattern, but significantly fewer females were produced by G-SCI-1 on white clover, red clover, lespedeza, and curly dock than by population G. Some variation in numbers of females produced also was found among the four G-SCIs.

Three host patterns were observed in population H. All SCIs from population H produced significantly fewer females on white clover, red clover, lespedeza, curly dock, and carnation except H-SCI-1 on red clover and had a narrower host range than population H. Variation in female production among H-SCIs was observed on three hosts but not on the other hosts.

The population of *H. glycines* and its SCI produced females efficiently on lespedeza and Essex soybean only, and all had the same host pattern. However, significantly fewer females were produced by Hg-SCIs on lespedeza and by Hg-SCI-1, Hg-SCI-2, and Hg-SCI-4 on soybean than was produced by the parent population. Some females were produced by population Hg and all Hg-SCIs on carnation, and even though numbers were low, differences among SCI were observed.

The parent population had a different host pattern from that of the H1-SCI. Few females were produced by SCI of *H. lespedezae* on white clover, red clover, curly dock, sugarbeet, and Hartwig soybean, but many females were produced on lespedeza and Essex soybean.

## Discussion

In a polyploid parthenogenetic species, genetic variation is not expected to be as great as in species that reproduce by amphimixis. However, the variations observed in earlier studies and in this study are considerable. For example, Maas et al. (1982) reported significant differences in host range of five populations of *H. trifolii* from different localities. Significant differences in cyst production were observed on 27 cultivars or species of *Trifolium* inoculated with geographically distinct populations of *H. trifolii* (Singh and Norton, 1970). Results of the present research support those earlier observations, and the intrapopulation variation in host preference suggests that variation among populations of *H. trifolii* is common.

The isolates of *H. trifolii* used in this research should have exhibited maximum variation because all SCIs were selected from the most reproductive individuals on a given host and considerable variation was observed. However, some individuals that were chosen for development of an isolate reproduced poorly and did not increase enough to be used in these experiments. Therefore, individuals that might have had different host preferences to the selection host were excluded in these tests.

The variation in ability of populations of *H. trifolii* to reproduce on a given host revealed, to some extent, the specificity in host-parasite association. This moderate degree of specificity might have been the result of many biotic and abiotic factors in the soil

(Sidhu and Webster, 1981). For *H. trifolii,* however, the most important factor might have been the adaptation of nematode individuals or populations to the host species that occurred in the specific geographical location where these populations were found. For example, the Australian population had become adapted to carnation in its native location and it reproduced well on carnation in the current study. It did not reproduce well on curly dock, perhaps because it may never have been exposed to curly dock before these tests. However, its subpopulation that was maintained for a time on white clover lost the ability to reproduce on carnation but produced numerous females on curly dock, even though it had not been exposed to curly dock before the test. The population apparently has two genotypes for reproductive preference: one for carnation and one for curly dock. The loss of ability to reproduce on carnation after a period of reproduction on white clover could indicate that the white clover selects against reproduction on carnation. This suggestion is supported by the low numbers of females produced on carnation by most of the SCI that had been selected and maintained on white clover. The lack of ability to reproduce on carnation and the ability to reproduce on curly dock could even be linked to the ability to reproduce on white clover. However, populations A, E, and H (all from white clover in either Arkansas or Pennsylvania) produced numerous females on carnation, and two of them (A and H) produced numerous females on curly dock. In contrast, SCI from the same three populations produced few females on carnation.

The adaptation of populations to a host indicates the genetic diversity among individuals of a population, as shown by the variations observed among single-cyst isolates from the same population. For example, some isolates developed from single cysts on white clover did not reproduce as well on carnation or curly dock as their parent population did. It appears that isolates selected from white clover have reduced ability to produce females on some hosts. In contrast, isolates selected from lespedeza (population D) had increased ability to produce females on most hosts. One explanation for this phenomenon may be that the ability to parasitize white clover may be controlled by the same genetic factors as ability to parasitize the other host species tested. Because Kobe lespedeza is a "universal" susceptible host of lemon-shaped *Heterodera* species, the same genetic factor may not control the ability to parasitize it.

The production of low numbers of females showed the potential for some populations to parasitize sugarbeet. However, no population or isolate of *H. trifolii* was found that reproduced as prolifically on sugarbeet as the population reported from The Netherlands by Maas and Heijbroek (1982) or that reported by Steele et al. (1983). However, four populations of *H. trifolii* produced 8–14 mature females/pot, and up to 40 cysts were counted in some pots of sugarbeet. The production of low numbers of females showed the potential of some populations to parasitize sugarbeet. In most populations, SCI produced significantly fewer females on sugarbeet than did their parent populations. Some individuals capable of reproducing on sugarbeet may not reproduce well on white clover and would have been excluded in the selection of single cyst isolates.

The race of *H. trifolii* parasitic on sugarbeet (YBCN) was found to have 35–36 chromosomes and reproduce by mitotic parthenogenesis (Steele and Whitehand, 1984). The quite different host range of YBCN from those of populations in our experiments indicates that YBCN is a unique host race. Earlier research suggested that the sugarbeet race may have evolved very recently from *H. schachtii* in Europe based on the similarity of its host range to that of *H. schachtii* (Maas and Heijbroek, 1982; Steele et al., 1983). Cuany and Dalmasso (1973) also reported two races of *H. trifolii*: one that was a diploid that reproduced by amphimixis and had a wide host range; and another that was triploid, reproduced by parthenogenesis, and had a host range limited to carnation and clover. The proposed races reflect significant parasitic associations between population and host species. How-

ever, comparatively less variation was observed in the association between population and host cultivar, which has been used to characterize races of amphimictic species. One reason may be the lack of resistant cultivars for the selection of resistance-breaking individuals. Also, parthenogenetic reproduction may provide more physiological adaptation to hosts rather than frequent genetic change, as occurs in amphimictic species.

Geographically dispersed populations may have distinct genetic diversity, some of which may enable virulent individuals or strains to break resistance in a host. For example, three of 16 populations of *H. schachtii* from several countries were found to produce relatively high numbers of females on resistant sugarbeet lines, demonstrating a potential for breaking resistance (Porte et al., 1997). The differences in host range among intraspecific populations may complicate the crop rotation systems. A nonhost of some populations may be a good host for other populations. For example, carnation was reported to be a good host for *H. trifolii* (Cuany and Dalmasso, 1973), but in the present tests, three of seven populations could not produce females on carnation efficiently. Also, curly dock was a good host for five geographical populations of *H. trifolii* (Maas et al., 1982), but three populations in our collection could not produce females efficiently on curly dock. Genotype differences in *H. trifolii* related to ability to parasitize different host species may originate from long-term host-parasite evolution in isolated geographical areas.

## Literature Cited


Abawi, G. S., J. Foster, and W. F. Mai. 1973. Differentiation of cysts of *Heterodera schachtii* and *H. trifolii* by fenestral length. Journal of Nematology 5:74–75.

Cuany, A., and A. Dalmasso. 1973. Characters and specificity of two biological races of *Heterodera* on *Dianthus caryophyllus*. Nematologia Mediterranea 3:11–21.

Ferris, V. R., J. M. Ferris, and J. Faghihi. 1993. Variation in spacer ribosomal DNA in some cyst-forming species of plant-parasitic nematodes. Fundamental and Applied Nematology 16:177–184.

Hirschmann, H., and A. C. Triantaphyllou. 1979. Morphological comparison of members of the *Heterodera trifolii* species complex. Nematologica 25:458–481.

Inserra, R. N., N. Vovlas, S. Walker, and R. A. Dunn. 1993. The clover cyst nematode, *Heterodera trifolii,* a potential pest of clover and vegetable crops in Florida. Nematology Circular 204:4. Division of Plant Industry, Gainesville, FL.

Kimpinski, J., G. Plumas, and M. C. MacDonald. 1994. Occurrence of the clover cyst nematode, *Heterodera trifolii,* in Prince Edward Island soils. Supplement to the Journal of Nematology 25:876–879.

Maas, P. W. T., E. D. Bois, and J. Dede. 1982. Morphological and host range variation in the *Heterodera trifolii* complex. Nematologica 28:263–270.

Maas, P. W., and W. Heijbroek. 1982. Biology and pathogenicity of the yellow beet cyst nematode, a host race of *Heterodera trifolii* on sugarbeet in The Netherlands. Nematologica 28:77–93.

Mcleish, L. J., G. N. Berg, J. M. Hinch, L. V. Nambiar, and M. R. Norton. 1997. Plant-parasitic nematodes in white clover and soil from white clover pastures in Australia. Australian Journal of Experimental Agriculture 37:75–82.

Mercer, C. F., and B. D. Campbell. 1986. Early invasion of over-drilled red clover seedlings by nematodes. New Zealand Journal of Agricultural Research 29:495–499.

Mercer, C. F. and J. L. Grant. 1993. Reproduction of *Meloidogyne hapla* and *Heterodera trifolii* from several sites in New Zealand of resistant and susceptible lines of white clover. Nematologica 39:312–321.

Porte, C., A. Mahfoud, and G. Caubel. 1997. Reaction of geographically dispersed populations of *Heterodera schachtii* and *H. trifolii* to resistant lines of sugarbeet. Nematologia Mediterranea 25:113–120.

Pozdol, R. F., and G. R. Noel. 1984. Comparative electrophoretic analyses of soluble proteins from *Heterodera glycines* races 1–4 and three other *Heterodera* species. Journal of Nematology 16:332–340.

Riggs, R. D., and D. P. Schmitt. 1991. Optimization of the *Heterodera glycines* race test procedure. Journal of Nematology 23:149–154.

Seinhorst, J. W., and A. K. Sen. 1966. The population density of *Heterodera trifolii* in pastures in The Netherlands and its importance for the growth of white clover. Netherlands Journal of Plant Pathology 72:169–183.

Sidhu, G. S., and J. M. Webster. 1981. The genetics of plant-nematode parasitic systems. Botanical Review 47: 387–419.

Sikora, R. A. 1977. *Heterodera trifolii* (nematode) associated with *Fusarium* root rot of *Trifolium subterraneum* (subterranean clover) in northern Tunisia. Nematologia Mediterranea 5:319–321.

Singh, N. D., and D. C. Norton. 1970. Variability in host-parasite relationships of *Heterodera trifolii*. Phytopathology 60:1834–1837.

Stanger, B. A., and G. R. Noel. 1996. Scanning electron microscopy of cephalic regions of *Heterodera glycines* and three related cyst nematode species. Nematropica 26:1–8.

Steele, A. E., H. Toxopeus, and W. Heijbroek. 1982. A comparison of the hatching of juveniles from cysts of *Heterodera schachtii* and *H. trifolii*. Journal of Nematology 14:588–592.

Steele, A. E., H. Toxopeus, and W. Heijbroek. 1983. Susceptibility of plant selections to *Heterodera schachtii* and a race of *H. trifolii* parasitic on sugarbeet in The Netherlands. Journal of Nematology 15:281–288.

Steele, A. E., and L. Whitehand. 1984. Comparative morphometrics of eggs and second-stage juveniles of *Heterodera schachtii* and a race of *H. trifolii* parasitic on sugarbeet in The Netherlands. Journal of Nematology 16:171–177.

Triantaphyllou, A. C., and H. Hirschmann. 1978. Cytology of the *Heterodera trifolii* parthenogenetic species complex nematodes. Nematologica 24:418–424.

Yeates, G. W., and W. H. Risk. 1976. Annual cycle of root nematodes on white clover in pasture. III. *Heterodera trifolii* in a yellow-brown earth. New Zealand Journal of Agricultural Research 19:393–396.

Yeates, G. W., D. J. Ross, B. A. Bridger, and T. A. Visser. 1977. Influence of the nematodes *Heterodera trifolii* and *Meloidogyne hapla* on nitrogen fixation by white clover under glasshouse conditions. New Zealand Journal of Agricultural Research 20:401–413.

Yeates, G. W., S. Saggar, C. S. Denton, and C. F. Mercer. 1998. Impact of clover cyst nematode (*Heterodera trifolii*) infection on soil microbial activity in the rhizosphere of white clover (*Trifolium repens*) — a pulse-labeling experiment. Nematologica 44:81–90.