

Variability in Host-Parasite Relationships of *Heterodera trifolii*

N. D. Singh and D. C. Norton

Department of Botany and Plant Pathology, Iowa State University, Ames 50010. Present address of senior author: Lenora, West Demerara, Guyana.

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ABSTRACT

Significant differences in cyst production existed among *Heterodera trifolii* isolates from Canada, Illinois, Kentucky, Oregon, and Story and Union counties, Iowa, when propagated on *Trifolium repens* in a greenhouse. Quantitative differences in cyst production were greatest on *Trifolium pratense*, *T. fragiferum*, and one cultivar of *Melilotus officinalis*. Differences in cyst production were as great among eight isolates from western Iowa as were differences among isolates obtained from the more widely distributed localities. Cysts of the Canadian isolate were significantly longer than those from Illinois

and from Story and Union counties, Iowa. The length: breadth cyst ratio of the Union County isolate was significantly less than from Canada, Illinois, Kentucky, Oregon, and Story County. Differences in pathogenicity of three isolates were correlated with histopathological differences and cyst production. Histological studies indicated that cyst production was high only when stelar syncytia were formed. When either hypersensitive cell necrosis or syncytial abortion or both occurred, cyst production was relatively low. *Phytopathology* 60:1834-1837.

The ability of *Heterodera trifolii* (Goffart) Oostenbrink to infect and produce cysts on several hosts varies. *Trifolium pratense* was considered a poor host for *H. trifolii* by Mankau & Linford (8) and Raski & Hart (14), but Chapman (1) found it to be a good one; Norton & Isely (13) found that red clover accessions varied in susceptibility. Mulvey (11) found that red clover was susceptible to an English, but not to a Canadian, population of *H. trifolii*. There are also inconsistencies concerning the susceptibility of *T. hybridum*, *T. fragiferum*, and *T. incarnatum* to *H. trifolii* (3, 4, 8, 11, 12, 13).

Mankau & Linford (8) found that stelar syncytial size increased rapidly in highly susceptible hosts such as Ladino white clover and spinach, and that cyst multiplication was extensive. In resistant hosts, such as red clover and pea, syncytial abortion or necrosis caused many premature nematode deaths, resulting in limited cyst production. *Trifolium* spp. were reported as not being damaged by *H. trifolii* (2, 16), although others reported or suggested that certain hosts including *Trifolium* spp. can be damaged seriously (1, 5, 6, 8, 9, 12, 14).

The primary purposes of these investigations were (i) to study the variability in cyst production of *H. trifolii* isolates from both widely and narrowly distributed geographic locations; and (ii) to study the host-parasite relationships of certain populations on a variety of plants in an attempt to explain why previous reports are conflicting.

MATERIALS AND METHODS.—*Varietal susceptibility.*—Populations of *Heterodera trifolii* from Canada (C), Illinois (I), Kentucky (K), Oregon (O), and Story (S) and Union (U) counties, Iowa, were started from single cysts and increased on Ladino white clover in the greenhouse. Twenty-seven cultivars (Table 1) were selected for testing on the basis of varied reports of host susceptibility. Cross-fertilizing plant species were propagated by clones, and self-fertilizing species were grown

from seed. Plants were grown in a steamed soil mixture in 3-inch pots, and were inoculated 4 weeks after seedling or transplanting.

Approximately 200 active juveniles of each population of *H. trifolii* were pipetted into a 1-inch deep hole centered between two spaced plants in each pot. The experiments were performed in a greenhouse maintained at 24 to 26 C. There were four replications of two plants/pot for each treatment. The soil was processed for cysts 80 to 90 days after inoculation by wet-washing and sieving through 35 and 60 mesh sieves. Total cysts/pot were counted up to 500 cysts/pot.

The variability in cyst production among isolates obtained from a smaller geographical area, i.e., western Iowa, was investigated. Procedures similar to those described previously were used, employing seven cultivars and *H. trifolii* collected from white clover in eight pastures from seven counties in western Iowa.

Cyst measurements.—Cyst length and breadth of 100 randomly collected mature brown cysts were measured from each of six nematode (C, I, K, O, S, U) populations propagated on Ladino white clover. Measurements were made from camera lucida drawings.

Pathogenicity.—Seed of Ladino white clover, and of Kenland, Lakeland, and Dollard red clover were sown in steamed sandy clay loam soil contained in 4-inch pots. Seedlings were thinned to three evenly spaced plants/pot. Three to 4 weeks after germination, 150 washed juveniles of the C, K, and S isolates were pipetted into the center of the pots. There were three replications of four pots for each variety with each nematode isolate. Plants growing in similar noninfested soil served as controls. The foliage was cut 2.5 months after inoculation and at the conclusion of the experiment when dry top and root wt were recorded.

Host-parasite relationships.—The C, K, and S isolates were used to study the host-parasite relationships in 12 cultivars. Seed were germinated on wet blotting paper in a petri dish. After radicle emergence, the seedlings

were transferred to moist, fine sand in other petri dishes. Forty-eight hr later, 200 washed juveniles of each isolate were poured on the sand around the roots in each dish. Twenty-four hr after inoculation, the seedlings were carefully lifted, washed to remove any unattached juveniles, and transplanted in a steamed soil mixture of 2 parts sand and 1 part soil in 3-inch pots. At 10, 20, and 30 days after inoculation, selected seedlings were carefully washed, and those portions of the root exposed at inoculation were fixed, dehydrated, embedded in paraffin, sectioned, and stained with safranin and fast green (15).

RESULTS.—Varietal susceptibility.—The greatest differential cyst production was obtained on red clover, yellow sweet clover, and strawberry clover (Table 1). The K isolate produced numerous cysts on all varieties

of red clover, whereas, the O isolate produced numerous cysts only on Lakeland red clover. All other pairings resulted in few cysts even though plant growth was good. Yellow sweet clover (M204) was highly susceptible to the K and U isolates, but highly resistant to the other isolates. Yellow Blossom and Madrid sweet clovers supported few cysts of any isolate. Strawberry clover was highly susceptible to the C isolate, but resistant to the others. There were no major differences in cyst production on the remaining 24 plant entries. When growth of clover was poor with a poorly developed root system, especially with white clover, cyst counts were low.

When eight isolates of *H. trifolii* from seven counties in western Iowa were compared for cyst production, there were significant differences (.01) among the iso-

TABLE 1. Cyst production by six isolates of *Heterodera trifolii* on 27 cultivars^a

Cultivars	Sources of isolate					
	Canada	Illinois	Kentucky	Oregon	Story County	Union County
<i>Trifolium pratense</i> L. ^b						
Dollard	12	10	500+	45	24	1
Kenland	9	18	500+	32	19	6
Lakeland	9	13	500+	221	15	10
<i>Trifolium repens</i> L. ^b						
Merit Ladino	500+	116 ^c	500+	500+	500+	103 ^d
Regal	500+	120 ^c	61 ^c	500+	500+	500+
(P.I. 166371)	500+	52 ^c	65 ^c	61 ^c	91 ^c	500+
<i>Melilotus officinalis</i> (L.) Lam. ^b						
Madrid	6	1	39	5	0	32
Yellow (M204)	7	3	500+	6	5	500+
Yellow Blossom (M38)	23	11	14	8	0	4
<i>Trifolium hybridum</i> L. ^b						
Italy (P.I. 258448)	3	0	5	7	5	0
Turkey (P.I. 204524)	18	32	9	6	2	2
<i>Trifolium fragiferum</i> L. ^b						
Turkey (P.I. 204640)	500+	1	2	18	9	12
<i>Trifolium incarnatum</i> L. ^b						
	11	4	3	3	3	2
<i>Lotus corniculatus</i> L. ^b						
Empire	3	2	9	9	0	1
Mansfield	2	1	3	11	2	8
Viking	5	0	0	4	0	6
<i>Dianthus caryophyllus</i> L.						
	14	20	7	122	3	27
<i>Rumex crispus</i> L.						
	41	42	49	33	36	36
<i>Pisum sativum</i> L.						
Laxton's Progress ^d	13	2	2	2	2	2
Perfection ^d	22	1	1	2	9	12
<i>Phaseolus vulgaris</i> L.						
Top Crop	3	2	3	3	3	1
Tender Long	6	1	1	3	3	1
Golden Wax Bean	5	3	14	4	2	2
Stringless Green Bean	7	3	4	3	1	4
<i>Cucurbita sativa</i> L.						
Triumph	10	2	3	13	1	5
American	5	2	5	17	1	0
Ashley	8	3	10	16	2	3

^a Average cysts per 4-inch pot; 200 juveniles were placed in each pot.

^b Cuttings were used in the experiment.

^c Growth of plants was relatively poor, and in some instances they died.

^d Plants died 40-50 days after inoculation.

TABLE 2. Cyst production of eight Iowa isolates of *Heterodera trifolii* on seven cultivars^a

Source of isolate (county)	Hosts						<i>Trifolium pratense</i>	<i>Trifolium pratense</i>
	<i>Melilotus alba</i>	<i>Melilotus officinalis</i>	<i>Trifolium ambiguum</i>	<i>Trifolium fragiferum</i>	<i>Trifolium michelianum</i>	var. Kenland	var. Lakeland	
Cherokee	10	10	7	466	215	332	231	
Clay	43	18	151	500+	308	259	120	
O'Brien (isolate 1)	14	6	361	500+	422	273	175	
O'Brien (isolate 2)	1	1	62	294	445	33	5	
Ringgold	1	1	70	340	6	189	35	
Sioux	2	1	137	453	340	182	141	
Story	1	3	267	500+	364	152	59	
Union	7	21	14	407	256	276	214	
LSD = .05	16	NS	194	NS	192	203	19	
LSD = .01	23	NS	267	NS	265	NS	26	

^a Cysts per 4-inch pot of soil.

lates on a given host (Table 2), but all isolates did not behave similarly on different hosts. For example, cyst production was similar with the two isolates from O'Brien County on four plant entries, but differed significantly on three entries. Differences in cyst production could not be attributed to an insufficient root system based on root dry wt. The cyst production experiment was repeated with similar results.

Cyst measurements.—Significant differences in mature cyst length (including neck) occurred when six nematode isolates were propagated on Merit Ladino white clover (Fig. 1). The C isolate was significantly longer than the I, S, and U isolates, when grown under similar conditions. There were no differences in treatment means among the other nematode isolates. The U isolate had a significantly smaller length:breadth ratio than other 5 nematode isolates when propagated on Merit Ladino white clover. There were no significant differences in length:breadth ratios among the other nematode isolates.

Pathogenicity.—When the data were analyzed by Tukey's w-procedure test, there was a significant (.01) reduction in yield with the K isolate when compared with either the S or C isolate. The K isolate reproduced better on red clover than the other isolates. Plants inoculated with the C isolate consistently yielded significantly more than plants not inoculated, a phenomenon demonstrated before, in instances of light infection by cyst nematodes (7, 12).

Host-parasite relationships.—When the histopathology of the S, K, and C isolates was investigated on 12 cultivars from seven plant species, it was found that root penetration occurred in all cultivars except Vernal alfalfa. Only the C and K isolates invaded Grimm alfalfa. The few nematodes that did invade alfalfa died within a few days. Most juveniles that invaded cucumber, pea, sweet clover, and birdsfoot trefoil died within 10-20 days. In these instances, hypersensitive cell necrosis, in which the syncytia never really developed, usually occurred, or the stelar or cortical syncytia that formed usually aborted and few nematodes reached maturity.

The red and white clovers were the most susceptible

of all the plants tested, but the nematode isolates developed differently in red clover. All three nematode isolates developed abundantly in Ladino clover, but only the K isolate did so in red clover. When the C and S isolates were inoculated on three red clover varieties, the syncytia were stelar, but they frequently aborted. The results of host-parasite interactions are summarized in Table 3.

DISCUSSION.—These results help explain some of the variability in cyst production as previously reported in the literature, especially on red clover. Although stelar syncytia are formed in red clover, they frequently abort, resulting in few cysts being produced. When syncytial abortion does not occur, many cysts are produced. The variability in host reactions to different isolates of *H. trifolii* indicates different biological forms within the nematode, a phenomenon surmised from the study by Mulvey (11) and the generally conflicting reports. Our work agrees with that of Chapman (1) and Mulvey (11) in that the Kentucky isolate was highly pathogenic to red clover and the Canadian isolate was not. These differences can be related to the abortion of the syn-

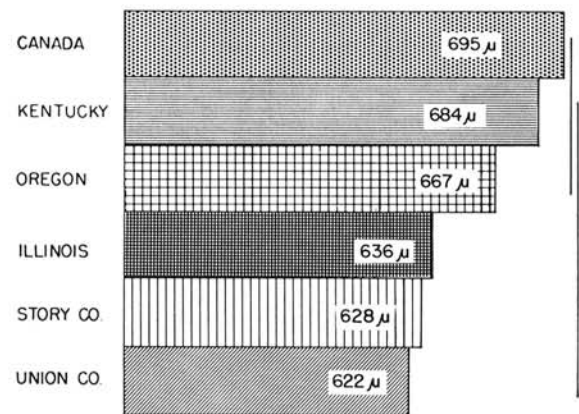


Fig. 1. Mean length, in μ , of 100 mature cysts from six populations of *Heterodera trifolii* produced on Ladino white clover. Any two means underscored by the same line are not significantly different.

TABLE 3. Summary of host-parasite interactions of 12 cultivars inoculated with three isolates of *Heterodera trifolii*

Cultivars	Root penetration			Syncytia									Cyst production/pot			
	K ^a	C	S	Stelar			Cortical			Abortion			K	C	S	
				K	C	S	K	C	S	K	C	S				
White clover																
Ladino white	+ ^b	+	+	+	+	+	-	-	-	-	-	-	500+	500+	500+	
Red clover																
Dollard	+	+	+	+	+	+	-	-	-	-	+	+	500+	8	22	
Kenland	+	+	+	+	+	+	-	-	-	-	+	+	500+	6	19	
Lakeland	+	+	+	+	+	+	-	-	-	-	+	+	500+	13	84	
Sweet clover																
Madrid	+	+	+	+	+	+	+	+	+	+	+	+	39	6	0	
Yellow Blossom	+	+	+	+	+	+	+	+	+	+	+	+	14	23	0	
Birdsfoot trefoil																
Mansfield	+	+	+	+	+	+	+	+	+	+	+	+	3	2	2	
Peas																
Laxton's progress	+	+	+	+	+	+	+	+	+	+	+	+	2	13	2	
Cucumber																
Pritchard	+	+	+	+	+	+	+	+	+	+	+	+	3	3	3	
Triumph	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	
Alfalfa																
Grimm	+	+	-	-	-	-	-	-	-	-	-	-	0	0	0	
Vernal	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	

^a K = Kentucky; C = Canada; S = Story County, Iowa.
^b + = Positive; - = negative.

cytia with the Canadian isolate (Table 3). Presumably, the same isolates were used in this study as were used by Chapman (1) and Mulvey (11).

Since differences in cyst production were as great among eight isolates of *H. trifolii* from western Iowa as among isolates obtained from more widely distributed localities, one may question how representative any given isolate is to a region. Miller (10) reported that isolates of *H. glycines* from the same or adjoining states produced different numbers of egg-bearing females on the same plant species.

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