Reproduction of Criconemoides simile, Helicotylenchus pseudorobustus, and Paratylenchus projectus on Soybean[1]

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Abstract: Reproduction of *Criconemoides simile, Paratylenchus projectus,* and *Helicotylenchus pseudorobustus,* alone and in certain combinations, was characterized during 50 days on the soybean cultivars Custer and Hood. Custer was the more suitable host for all three nematodes and *C. simile* was the most vigorous parasite on both cultivars. *C. simile* and *H. pseudorobustus* reproduced as well in combination as they did alone; addition of *P. projectus* to this combination did not alter results. Reproduction by *P. projectus* was repressed on both cultivars when it was combined with *C. simile* and *H. pseudorobustus.* The relative importance of time was assessed, as it relates both to the establishment of parasitism by applied nematodes and to the rate of development of larvae in the various host-parasite combinations. The necessity of characterizing as well as enumerating populations when evaluating population development of ectoparasitic nematodes was demonstrated. *Key words:* ring nematode, pin nematode, spiral nematode, concomitant nematodes.

Journal of Nematology 15(1):87-91. 1983.

In recent years, considerable attention has been focused on nematode-nematode interactions within polyspecific communities of phytoparasitic nematodes associated with various crops. Examinations of soil from fields of soybean (*Glycine max* L.) in Kentucky revealed that *Criconemoides simile* ([Cobb, 1918] Chitwood, 1949), *Helicotylenchus pseudorobutus* ([Steiner, 1914] Golden, 1956), and *Paratylenchus projectus* (Jenkins, 1956) occurred in many fields, often concomitantly. The wide range of numbers in samples of soil collected from fields of different cultivars at various stages of growth provided little information about the development of populations of these parthenogenetic ectoparasites on this host. The objectives of this investigation were to determine the reproductive capabilities of these nematodes, alone and in combination, on relatively suitable and unsuitable soybean cultivars during the first 50–60 days after inoculation. An abstract on some of this work has been published (4).

METHODS

Nematodes obtained from soybean fields in western Kentucky and maintained on the soybean cultivar Clark in a greenhouse were used as inoculum. Nematodes were recovered from soil by the centrifugal-sugar flotation technique (3) with a 37-μm (400 mesh)

Received for publication 16 July 1981.

[1]Portion of a Ph.D. dissertation submitted by the senior author to the University of Kentucky at Lexington. The investigation reported here was connected with a project of the Kentucky Agricultural Experiment Station and is published as Paper No. 80-11-54 with the approval of the Director.

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sieve at the bottom of a stack of nested sieves. A controlled vacuum pipette (2) was used to pick up selected numbers of active, nongravid females and/or larvae. Suspensions (5 ml) containing 100 nematodes were placed into holes, 4 cm deep x 2 cm d, in the center of 10-cm-d clay pots containing a mixture of silt loam soil and Weblite (Weblite Corp., Roanoke, Virginia). The planting medium was previously fumigated with methyl bromide.

Soybean seeds were dusted with nodulating bacteria and sown in flats containing fumigated planting medium. Uniform seedlings were selected and individually transplanted into the nematode-infested holes 10 days after planting. Pots were arranged in randomized blocks on greenhouse benches. Fluorescent and incandescent lamps provided 16 h of supplemental light each day (2,000 lux, 45 cm above bench surface).

Nematode reproduction was monitored by counting the numbers of females and larvae recovered from each plant. Nematode counts were used to calculate total nematode density (number of females plus number of larvae), female-to-larva ratio (F:L), and rate of population increase (R) (5), where $R = P_F/P_I$ and P_I equals the number of nematodes in the inoculum).

Experiments contained 3–5 replicates of each treatment and were repeated at least once. Two sets of controls, one with 5 ml of suspending fluid without nematodes and the other with 5 ml of distilled water, were included in each experiment. Growth of plants was measured as fresh weights of tops plus weights of washed, blotted roots. Root systems were periodically washed and hung in a mist chamber to determine if nematodes had entered the roots (1). The experiments were conducted during all seasons of the year, and the duration of most experiments was 50–60 days.

In preliminary tests the soybean cultivars Calland, Custer, Cutler 71, Dare, Essex, Hood, Kent, Pickett, and York were screened for their suitability as hosts for the three nematodes. In a second type of test, *C. simile* was used to investigate the effect of inoculum composition (F:L ratio) on reproduction by inoculating Custer and Hood seedlings with 100 females, or 50 females plus 50 larvae, or 100 larvae.

Population development of the three nematodes, singly and in combination, was characterized by inoculating Custer and Hood seedlings with 100 females of each species alone or 100 females of each nematode in the combination under investigation. Nematodes were collected at intervals of 13, 20, 27, 34, 41, and 50 days after inoculation.

RESULTS AND DISCUSSION

Of the nine soybean cultivars tested, Custer was the most and Hood the least suitable host for *C. simile*, *H. pseudorobustus*, and *P. projectus* (Table 1). At 50 days after inoculation, R values for each nematode were significantly greater on Custer than on Hood and the amount of reproduction by *C. simile* was significantly greater than that of either *H. pseudorobustus* or *P. projectus*. Although the level of inoculum in the cultivar-screening tests was the same for each nematode, the F:L ratios were different and the resultant influence on the rate of population increase was significant (Table 2). Inocula having F:L ratios of 100:0, 50:50, and 0:100 yielded corresponding significantly different 60-day R values

Table 1. Reproduction of *Criconemoides simile*, *Helicotylenchus pseudorobustus*, and *Paratylenchus projectus*, each alone, on nine soybean cultivars. Inoculum contained 100 nematodes; female-to-larvae ratios were 30:70, 90:10, and 50:50 for *C. simile*, *H. pseudorobustus*, and *P. projectus*, respectively. Nematodes were collected 50 days after inoculation. Data are means of three replicates.

	$R = \frac{\text{final population}}{\text{initial population}}$		
Cultivar	*C. simile*	*H. pseudorobustus*	*P. projectus*
Custer	16.8a*	5.1a	1.8a
Cutler 71	14.8ab	3.7b	1.4b
Pickett	14.4ab	2.2b	1.3b
York	8.8c	3.0b	0.9b
Essex	7.3cd	1.4b	1.2b
Calland	5.5de	3.3b	1.6b
Dare	4.0ef	4.4a	1.5b
Hood	3.1efg	2.3b	0.8b
Kent	3.0efg	3.3b	1.6b

*Data analyzed by Duncan's new multiple-range test; numbers within columns followed by common letters are not significant at $P = 0.05\%$.

Table 2. Effect of inoculum composition on the rate of population increase (R) of *Criconemoides simile* on the soybean cultivars Custer and Hood. Nematodes were collected 60 days after inoculation. Data are means of three replicates.

Inoculum composition (No. females:No. larvae)	$R = \frac{\text{final population}}{\text{initial population}}$	
	Custer	Hood
100:0	30.5a*	16.8a
50:50	15.6b	8.1b
0:100	9.0c	2.6c

*Data analyzed by Duncan's new multiple-range test; numbers within columns followed by different letters are significantly different at $P = 0.01\%$.

of 30.5, 15.6, and 9.0 and 16.8, 8.1, and 2.6 from Custer and Hood, respectively.

Reproduction by single species: Total populations of *C. simile* declined during the first 13 days on both cultivars (Table 3). Thereafter, except for the 20-day collection, significantly greater numbers of *C. simile* were recovered from Custer at each interval. Values of R at 50 days after inoculation were 25.5 on Custer and 11.2 on Hood.

The total *C. simile* population increased slightly on Custer between 13 and 20 days because the number of larvae produced during this period offset the decline (55% during the first 20 days) in the number of females (Table 3). However, the larvae produced on Hood did not compensate for the 65% loss of females that occurred during the same period, and the total population did not increase until some time between 20 and 27 days. Populations of females declined at approximately the same rate in fallow soil, but few larvae were produced (Table 4), indicating that approximately half the applied inoculum failed to survive 3 weeks after introduction into soil and that the presence of soybean roots did not enhance survival. During the last 30 days, populations of females increased arithmetically at a 4-per-day rate on Hood and at a 5-fold greater rate, 21 per day, on Custer. Larval populations increased at the rate of 31 per day on Hood and at a substantially greater rate, 60 per day, on Custer during the last 30 days.

The pattern of development of female and larval populations during these experiments suggested three factors, or some combination of them, which could account for the significant difference between populations of *C. simile* on the two cultivars: 1)

Table 4. Survival of *Criconemoides simile* in fallow soil infested with 100 females. Data are means of three replicates.

Days after infestation	Number recovered		
	Females	Larvae	Total
5	76a*	0a	76a
10	71a	0a	71a
22	52b	3b	55b
50	18c	9c	27c

*Data analyzed by Duncan's new multiple-range test; numbers within columns followed by common letters are not significant at $P = 0.05\%$.

Table 3. Reproduction of *Criconemoides simile, Helicotylenchus pseudorobustus,* and *Paratylenchus projectus,* each alone, on the soybean cultivars Custer (C) and Hood (H). Plants were inoculated with 100 females. Data are means of three replicates.

Days after Inoculation	No. *C. simile*				No. *H. pseudorobustus*				No. *P. projectus*			
	Females		Larvae		Females		Larvae		Females		Larvae	
	C	H	C	H	C	H	C	H	C	H	C	H
13	79	47	0	0	...	...	...	...	...	...	...	...
20	45	35	63**	16	75	65	0	0	68	41	0	0
27	60	52	331**	54	56	56	0	0	52	31	61	17
34	191**	89	798**	401	56	56	47	0	107*	48	79*	39
41	312**	127	1,055**	564	28	47	159*	103	144**	73	111**	60
50	672**	168	1,876**	952	103*	9	417*	264	245**	124	140*	87

*Difference between numbers of larvae or numbers of females on Custer and Hood significant at $P = 0.05$.

**Difference between numbers of larvae or numbers of females on Custer and Hood significant at $P = 0.01$.

the original females deposited fewer eggs on Hood than did their counterparts on Custer; 2) the eclosion process was slower in the presence of Hood than in the presence of Custer; and 3) larvae were not maturing and/or surviving as well on Hood as they were on Custer. Attempts to obtain sufficient quantities of eggs to work with were unsuccessful. However, a partial assessment of these possibilities were achieved when Custer and Hood were inoculated with 100 of the smallest larvae which could be found, the majority of which were presumably second stage (Table 5). The number of larvae recovered from Custer at 10 days after inoculation was significantly greater than the number recovered from Hood, indicating that larvae survived better on the former than on the latter. Also, at 20 days after inoculation a significantly greater number of females was recovered from Custer than from Hood. Larvae survived as well in fallow soil as they did on Hood during the first 10 days, which suggested that they did not have to feed on the roots of this cultivar during this time to survive. These data suggest that a major factor responsible for the significant difference between populations of *C. simile* on the two cultivars is failure of many larvae to survive and mature into females on Hood.

Table 5. Maturation of *Criconemoides simile* on the soybean cultivars Custer and Hood and in fallow soil 10 and 20 days after inoculation with 100 larvae. Data are means of five replicates.

Cultivar	Number		
	Larvae	Females	Total
Custer			
10 days	78a*	0a	78a
20 days	67a	12c	79a
Hood			
10 days	45b	0a	45b
20 days	28c	6b	34b
Fallow			
10 days	37bc	0a	37b
20 days	7d	0a	7c

*Data analyzed by Duncan's new multiple-range test; numbers within columns followed by the same letter are not significantly different at $P = 0.05\%$.

There were no significant differences in the total *H. pseudorobustus* populations recovered from the two cultivars during the first 41 days (Table 3). At 50 days after inoculation, however, a significantly greater number of *H. pseudorobustus* were recovered from Custer. Fifty-day R values for *H. pseudorobustus* were 5.2 on Custer and 2.7 on Hood. Larvae were first found at 34 and 41 days after inoculation on Custer and Hood, respectively, after which they increased arithmetically at rates of 24 and 9 per day. Numbers of females declined 72% on Custer and 53% on Hood during the first 41 days (Table 3). Larvae were first detected on both cultivars at 27 days after inoculation and they increased at a 3-per-day rate on both cultivars during the next 23 days (Table 3).

Reproduction by concomitant species: Populations of *C. simile* and *H. pseudorobustus* reproduced as well in combinations as when alone on both cultivars (Table 6). Furthermore, the sequence of development

Table 6. Reproduction (R) by *Criconemoides simile* (C), *Helicotylenchus pseudorobustus* (H), and *Paratylenchus projectus* (P), alone and in various combinations, during 50 days on Custer and Hood soybean. Inocula contained 100 females of each species. Data are means of three replicates.

Host	Inoculum	*C. simile*		*H. pseudorobustus*		*P. projectus*	
		R*	F:L†	R	F:L	R	F:L
Custer	C or H or P	24.0	25:75	5.3	26:74	3.9	64:36
	C + H	25.7	25:75	5.0	17:83	...	...
	C + H + P	22.8	27:73	5.1	23:77	1.5	67:33
Hood	C or H or P	10.2	16:84	2.4	10:90	2.1	59:41
	C + H	10.3	16:84	2.8	13:87	...	...
	C + H + P	9.8	17:83	2.7	14:86	1.0	60:40

*R = number of females plus number of larva infinal population/100.
†F:L = ratio of females to larvae in final population.

and composition of populations for each species was essentially the same as that in Table 3. However, in the presence of both *C. simile* and *H. pseudorobustus,* reproduction by *P. projectus* was drastically curtailed (Figure 1 and Table 3). Final populations of *P. projectus* were approximately 50% of those obtained repeatedly when *P. projectus* was alone; furthermore, most of the larvae recovered during the last 16 days were preadults. The preadult larvae represent a survival mechanism which occurs in response to conditions unfavorable for reproduction (6, 7). Cortical tissue is the preferred feeding site for *C. simile* and *H. pseudorobustus,* the former near root tips and the latter throughout the piliferous zone, and their activity could be expected to reduce the quality and/or quantity of epidermal and root hair cells which are primary substrates for *P. projectus* (7, 8).

Ectoparasitism, root weights, and R values. The nematodes remained strictly ectoparasitic in these studies. None was ever recovered from washings collected from roots hung in a mist chamber. The fresh weights of roots, measured 50 days after inoculation, varied from midwinter lows of 7.5 g for Custer and 8.9 g for Hood to midsummer highs of 26.3 g for Custer and 32.8 g for Hood. Nematode reproduction was not directly proportional to root weights. Seasonal variation in 50-day R values was greater with *C. simile* than it was with either *H. pseudorobustus* or *P. projectus,* and it ranged from 22.1 to 26.9 and from 9.8 to 12.4 on Custer and Hood, respectively. Also, there were no significant differences in root weights between controls and plants inoculated with the nematodes singly or in combination. Hence, reproduction by the nematodes was not limited by quantity of roots, and differences in reproduction by the single and concomitant populations were due primarily to inherent characteristics of the nematodes as parasites and the plants as hosts.

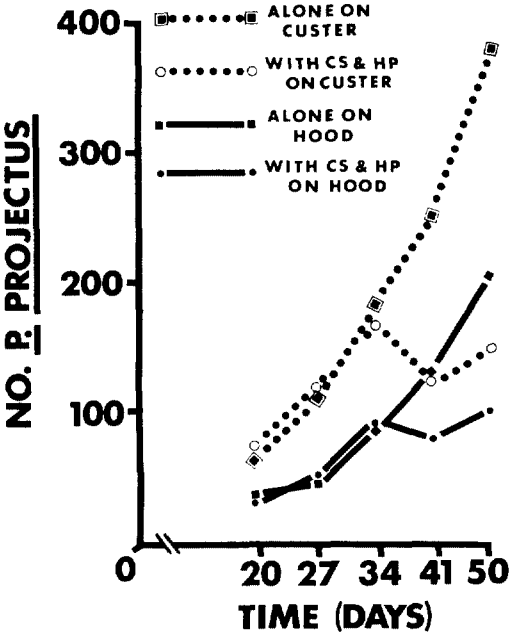

Fig. 1. Population development of *Paratylenchus projectus* (PP) alone and in combination with both *Criconemoides simile* (CS) and *Helicotylenchus pseudorobustus* (HP) during 50 days on Custer and Hood soybeans. Inoculum contained 100 females of PP or 100 females each of the three species together. Data are means of three replicates.

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