Effect of Crop and Weed Species on Development of a Minnesota Population of *Heterodera glycines* Race 5 After One to Three Growing Periods

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ABSTRACT

Sortland, M. E., and MacDonald, D. H. 1987. Effect of crop and weed species on development of a Minnesota population of *Heterodera glycines* race 5 after one to three growing periods. Plant Disease 71:23-27.

Heterodera glycines race 5 developed to high levels on soybean, medium levels on adzuki bean, low levels on pea, and did not develop on alfalfa, corn, lamb's-quarters, oat, pigweed, sugar beet, sunflower, or wheat. These crops and pea could be used in a crop rotation scheme to reduce this nematode population in the field, but the rotation, to be most effective, must extend through two seasons and preferably beyond three.

Additional key words: host range, soybean cyst nematode

Heterodera glycines Ichinohe, the soybean cyst nematode (SCN), is an important root pathogen of soybean (*Glycine max* (L.) Merr.) and has been reported to cause total crop loss in some soybean fields (2).

In Minnesota, the soybean cyst nematode was first discovered in August 1978 in a soybean field near Frost in the southern part of the state. The Frost isolate most closely resembles a race found in Japan (7) that was classified as race 5 (10). We will refer to the Frost isolate as SCN race 5.

Attempts to combat damaging SCN populations have used the following control measures and management practices: resistant cultivars, crop rotation, nematicides, sanitation, and good crop management (18). Currently, biological control is being investigated as a potential means of cyst nematode control (9). Once SCN is introduced into a field, it is very difficult to manage and has the potential of spreading to other areas. In Illinois, the first field infestation of SCN was found in 1959 in Pulaski County along the southern border of the state (11). By 1981, infestations had been detected in 58 counties (4). The female body wall, which forms a cyst, protects the eggs; therefore, control with soil fumigants and contact nematicides is not very effective and is costly (1,6). Also, many soils in northern areas of the United States are not suitable for fumigation because these materials do not diffuse adequately through the heavy

Paper 14,972, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul 55108.

Accepted for publication 15 September 1986 (submitted for electronic processing).

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soils in which soybeans are often grown. Soybean rotation with nonhost crops and the use of resistant and susceptible soybean cultivars are currently recommended as the most practical, economical, and ecologically feasible means of SCN control (12–14).

To plan a rotation scheme for control of SCN, crops need to be identified that inhibit or prevent the development of the nematode and are economical to grow. Soybeans grown in a 3- and 4-yr rotation with the nonhost crops cowpea, corn, and cotton resulted in increased soybean yields and protein content compared with a 2-yr rotation (15,16). Soybean yields were greater after 2 yr of corn than after 1 yr of corn in a SCN-infested field (17). Continuous soybean resulted in the lowest yields. In Japan, cropping soybeans every fifth and sixth year in rotation with garden pea, kidney bean, and red clover gave almost complete control of SCN (5). A 3- or 4-yr rotation was too short because of the survival of nematode eggs protected by the cyst (5). In Arkansas, the following 3-yr rotation slows the development of "new races" and is economical for SCN control: nonhost crop the first year, a resistant soybean cultivar the second year, and a susceptible soybean cultivar the third year (13,14). Numerous 4-yr crop rotation schemes have been tested by researchers in Illinois (12). They stress the importance of incorporating nonhost crops into a crop rotation scheme for fields infested with SCN because the economic return is ultimately greater from soybeans following nonhost crops than soybeans following resistant and susceptible soybeans. In addition, improved weed control is achieved through herbicide rotation.

The objectives of this study were to determine 1) if certain plants are hosts of SCN race 5, 2) the effects of these plants on initial nematode populations, and 3) the effects of these plants on the nematode populations through three growing seasons.

MATERIALS AND METHODS

The effects of adzuki bean (*Phaseolus angularis* L. 'Manoka'), alfalfa (*Medicago sativa* L. 'Agate'), corn (*Zea mays* L. 'Pioneer 3732'), lamb's-quarters (*Chenopodium album* L.), oat (*Avena sativa* L. 'Moore'), pea (*Pisum sativum* L. 'Little Marvel'), pigweed (*Amaranthus retroflexus* L.), soybean (*Glycine max* (L.) Merr. 'Hodgson 78'), sugar beet (*Beta vulgaris* L. 'Betaseed 1230'), sunflower (*Helianthus annuus* L. 'D.O.704XL82'), and wheat (*Triticum aestivum* L. 'Era') on the development of SCN race 5 were investigated. These crops are available to the Minnesota farmer, and the two weeds are commonly found in soybean fields. Soybean, adzuki bean, pea, and some species of pigweed are known hosts of SCN race 3 (12), whereas the other crops and the weeds used in this limited host range study are not.

Seeds of each crop or weed species were planted in 1 l sand-filled (Mississippi River sand [97.5% sand and 2.5% silt]) clay pots (14 cm diameter). The plants that emerged were thinned to one per pot, and after 17 days, each plant was lifted from the sand and replaced after 58 cm^3 of field soil infested with about 5,000 juveniles and an unknown number of cysts of SCN race 5 was placed around the roots. The numbers of second-stage juveniles in the soil were estimated using the Cornell pie-pan extraction technique (8) following the processing procedure described by MacDonald et al (10). The SCN population, SCN race 5, was taken from an infested field site in the southwest quarter of section 26, Emerald Township, Faribault County, near Frost, MN. Infested field soil had been stored in a cold room at 4 C before use. The field soil, Canisteo clay loam (37.5% sand, 35% silt, and 27.5% clay), was sieved (6-mm^2 screen) to remove large pieces of organic material and thoroughly mixed in a cement mixer. The plants were watered immediately after inoculation to prevent wilting. A few days later, each plant was watered with 200 ml of a nutrient solution (11 g/4 L, 18-18-18). The plants were placed on a bench in a greenhouse with an average ambient temperature of 23 C. Based on results from previous research, the life cycle of the nematode (juvenile to egg) at this temperature was estimated to be 24 days (21).

The experiment consisted of six components, each of which had 11 treatments (nine crops and two weeds) replicated five times and arranged in a randomized block design. The components were designated by the letters and numerals A-1, A-2, B-1, B-2, C-1, and C-2. "A" was a one-season study represented by a 40-day growing period, "B" was a two-season study represented by two 40-day growing periods, and "C" was a three-season study represented by three 40-day growing periods. This amount of time was estimated to allow one or two generations of the nematode to develop. All plants were cut off at the soil surface between flowering and seed development, but the roots were not removed. There was a fallow period of about 30 days between growing periods. Alfalfa, lamb's-quarters, pigweed, and sugar beet, however, were cut down to just above the soil surface and allowed to continue growth. The infested sand/soil medium was kept moist during fallow to prevent desiccation of nematodes.

In components A-1, B-1, and C-1, infection by the nematode population was determined by staining root systems. Roots from these plants (after each designated growth period) were removed and the excess sand rinsed off. These roots were cut into sections about 2–3 cm long and stained following the procedures described by Byrd et al (3). Total numbers of nematodes present were not determined for soybean, adzuki bean, and pea because of the high degree of root infection by the nematode and the large root mass.

The sand from each pot in components A-1, B-1, and C-1 was also processed to extract any mature female nematodes or cysts that may have developed on the plants (but were detached from the roots during harvesting) and for cysts originally present in the inoculum. The contents of each pot were emptied into a bucket (4-L) one-third filled with water. The contents were agitated and the suspension poured on a set of sieves, a 25-mesh sieve (707-μm openings) to collect extraneous debris and a 60-mesh sieve (250-μm openings) to collect the female nematodes and cysts. This process was repeated twice. Material collected on the 60-mesh sieve was washed into a beaker. The liquid in each beaker was reduced to 100 ml, and the female nematodes in two 8-ml subsamples were counted. The stage of development of each female nematode recovered was recorded using the following descriptive categories: a = immature with pliable body wall, b = maturing with thickened, hardened body wall, c = mature with gelatinous matrix, d = mature with egg mass, and e = mature, containing eggs (21).

In components A-2, B-2, and C-2, soybean bioassays were done to determine how well the nematode could develop on a host after growing on a nonhost species. The plants in these components were clipped at the soil line. After the 30-day fallow period (after the designated growth periods), five soybean seeds (Hodgson 78) were planted in each pot and the plants were thinned to one plant per pot after the cotyledons emerged above the soil surface. The soybean plants were grown through flowering and initial pod development, then harvested by clipping at their primary leaf node. The plants were placed in paper bags and dried at 65 C. Female nematodes were extracted from the roots by kneading the roots 10 times in a bucket one-fourth filled with water. Female nematodes and cysts were collected and counted as previously described.

Because the inoculum used in all components of this study included cysts containing eggs in addition to the second-stage juveniles, the data on nematodes recovered from sand (A-1, B-1, and C-1) were divided into two categories: a = cysts containing eggs and b = younger stages of the female nematode (i.e., stages prior to cysts with eggs, including immature white females, maturing females, females producing gelatinous matrices, and females producing egg masses). This system provided a means for distinguishing between nematodes in the initial inoculum and most of those produced during the experiment. Because not all eggs hatch from cysts immediately, there is

some carryover of cysts with eggs from one growing period to the next. The development of the nematode on a given crop or weed would be indicated by the recovery of younger stages of the female nematode or by the increase in numbers of cysts with eggs the following season. Differences between initial inoculum concentration and subsequent development should be discernible following this procedure.

Nematode counts were transformed using a square root transformation (19). An analysis of variance was made on data from each component of this study, and differences among treatment means were tested for significance using Tukey's procedure (22). Only differences significant at $P = 0.05$ will be discussed. An abstract of this experiment has been published (20).

RESULTS

Effect of one growing period on development of female nematodes (A-1). Younger stages (categories a–d) of the female nematode were recovered only from sand in which soybean, adzuki bean, pea, pigweed, or wheat had grown (Table 1). More nematodes were recovered from sand in which soybean and adzuki bean had grown than from sand in which the other plants had grown. There were no differences in the numbers of cysts with eggs recovered (Table 1). Stained soybean, adzuki bean, and pea roots contained many nematodes; all developmental stages were present (Fig. 1). Also, 16 juveniles and two third-stage nematodes were observed in alfalfa roots and seven juveniles and two third-stage nematodes were observed in sunflower roots.

Effect of two growing periods on female nematode development (B-1). Younger stages of the nematode were recovered only from sand in which soybean and adzuki bean had grown (Table 1). Many nematodes were present in soybean, adzuki bean, and pea roots. Two juveniles were observed in wheat roots. The number of cysts with eggs recovered from sand in which adzuki bean, corn, lamb's-quarters, oat, pigweed, or wheat had grown was not significantly different from the number recovered from sand in which soybean had grown (Table 1).

Effect of three growing periods on development of female nematodes (C-1). After the third growing period, the number of younger stages of the nematode was significantly greater from sand in which soybean had grown than from sand in which pea had grown but was not significantly greater than the number recovered from sand in which adzuki bean had grown (Table 1). The number of cysts containing eggs recovered from sand in which soybean or adzuki bean had grown was significantly greater than the number recovered from sand in which other plant species had grown (Table 1).

Stained soybean roots were infected with a large number of nematodes at the end of each growing period. There was a large decrease in the number of nematodes in the roots of pea and a slight decrease in adzuki bean from the first to the last growing period.

Bioassay of inoculum present after one, two, or three growing periods. Numbers of female nematodes recovered from soybean bioassay roots did not differ after the first growing period (A-2) (Table 2). However, after the second growing period (B-2), the number of nematodes recovered from the soybean bioassay roots following soybean was greater than the number recovered from soybean bioassay roots grown after the other plant species. There were no female nematodes recovered from soybean bioassay roots following corn and lamb's-quarters (Table 2).

The number of female nematodes recovered from soybean bioassay roots following soybean at the end of the second growing period (C-2) was significantly greater than the number of female nematodes recovered from soybean bioassay roots following alfalfa, corn, oat, pea, pigweed, sunflower, and wheat, but was not significantly different from the number of female nematodes recovered from soybean bioassay roots following adzuki bean, lamb's-quarters, and sugar beet (Table 2).

Effect of previous crop and resulting SCN population on growth of soybean. Dry weight of soybean at the end of the first growing period (A-2) following sunflower and pea was significantly greater than the dry weight of soybean following pigweed. Dry weight of soybean at the end of the second growing period (B-2) following pea was significantly greater than the dry weight of soybean following pigweed, wheat, sugar

Table 1. Effect of nine crop and two weed species on development of *Heterodera glycines* race 5 after one (A-1), two (B-1), and three (C-1) growing periods

	No. female nematodes					
	A-1[w]		B-1[w]		C-1[w]	
Crop or weed	Younger stages[x]	Cysts with eggs[y]	Younger stages[x]	Cysts with eggs[y]	Younger stages[x]	Cysts with eggs[y]
Adzuki bean	8.9 a	7.3 a	5.3 b	10.7 ab	3.9 ab	11.4 b
Alfalfa	–[z]	6.7 a	–	–	–	1.6 c
Corn	–	5.3 a	–	5.8 abc	–	3.0 c
Lamb's-quarters	–	6.5 a	–	4.7 abc	–	2.0 c
Oat	–	5.7 a	–	5.7 abc	–	1.7 c
Pea	2.2 b	5.0 a	–	3.8 bc	0.5 b	1.2 c
Pigweed	0.6 b	6.3 a	–	6.6 abc	–	3.7 c
Soybean	9.3 a	4.7 a	9.7 a	11.4 a	7.8 a	23.2 a
Sugar beet	–	5.6 a	–	1.5 c	–	3.6 c
Sunflower	–	4.8 a	–	2.8 c	–	2.2 c
Wheat	0.5 b	5.8 a	–	7.1 abc	–	2.2 c

[w] Means followed by the same letter within a column are not significantly different ($P = 0.05$) according to Tukey's HSD. A square root transformation was applied to raw data.
[x] Mean number of female nematodes (immature, maturing, with gelatinous matrix, and producing egg masses) recovered from sand in which five plants were grown.
[y] Mean number of cysts with eggs recovered from sand in which five plants were grown.
[z] – = No recovery of the nematode.

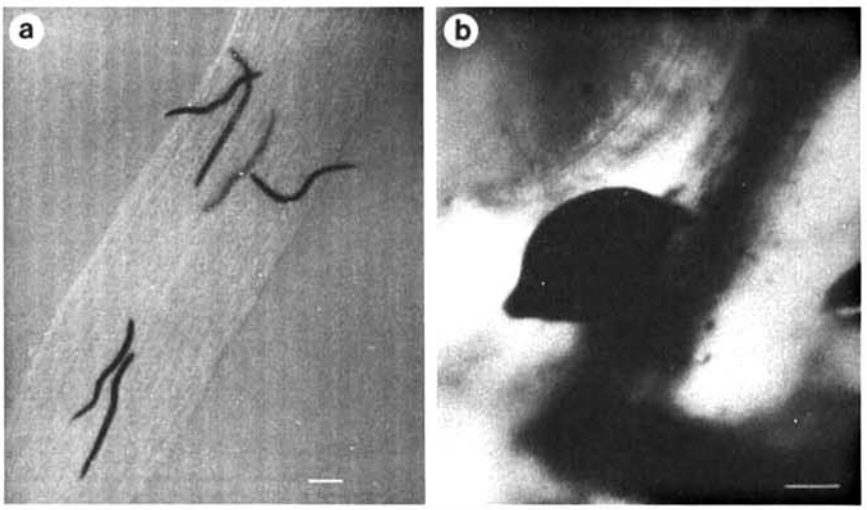

Fig. 1. (A) Stained *Heterodera glycines* race 5 juveniles in roots of soybean after 4 days and **(B)** a cyst with eggs in an adzuki bean root after one growth period.

beet, and lamb's-quarters. Dry weight of soybean at the end of the third growing period (C-2) following pea was significantly greater than the dry weight of soybean following pigweed and sugar beet (Table 3).

DISCUSSION

The results obtained in this study support the hypothesis that three distinctly different phenomena may be components of a scheme to control the SCN race 5 by cultural means.

The first and most commonly measured phenomenon is the effect of nonhost crops and uncontrolled weeds on infection, development, and maturation of the SCN. Our results suggest that two factors may be affecting this process. If the inoculum concentration is high, 5,000 juveniles plus cysts with eggs per 58 cm^3 of soil, then at least a few SCN may mature as parasites of roots of plants that under other circumstances may be nonhosts. In this study, this phenomenon, although not tested, was observed on pea, pigweed, and wheat. It is also possible that the physiology of the plant can determine if an infection of a questionable suscept will succeed. In this study, a few females matured on pigweed and wheat only when both the inoculum concentration was high (the original inoculation level) and the plants were well fertilized (only during the first growing period). Limited and erratic maturation of SCN females occurred on pea when it was absent on pigweed and wheat. Nitrogen fixation by a *Rhizobium* species may have contributed positively to infection and maturation by the nematode in the pea roots. This phenomenon, however, was not necessarily manifested in alfalfa. Although third-stage juveniles were found in stained alfalfa and sunflower roots, development of mature females on those plants did not occur. It would be interesting to test the hypothesis with these plants to see if a higher concentration of inoculum and/or better host nutrition might allow a few individuals to complete their life cycles.

Another important effect of the presence of nonhost crops and weeds is their effect on the hatching of eggs protected within cysts. The presence of actively growing host and nonhost plants did not significantly affect the number of cysts with eggs that were present until the second growing period. Perhaps, the presence of numerous juveniles in the inoculum inhibited hatching during the first growth period. As is commonly known from field observations, one growing period with a nonhost or poor host for the SCN does not significantly affect infection and development of the pathogen in the succeeding planting of susceptible soybeans. Although alfalfa, pea, sugar beet, and sunflower all had a significant negative effect on the number of cysts with eggs that were present at the end of the second growing period compared with soybeans, the presence of those plants did not significantly affect the numbers of nematodes that developed on a succeeding bioassay crop of soybean when compared with most of the other crops including adzuki beans. When all comparisons dealing with the maintenance of infective inoculum of the SCN race 5 made during the last two growing periods are considered, pea and sunflower tended to be least like soybean and adzuki bean and lamb's-quarters were most similar. These findings at least partially agree with those of Ichinohe (5), who described red clover, alfalfa, and pea as being trap crops of SCN that effectively control the nematode. In this study, all of the crops except adzuki bean appeared effective in reducing a population of SCN race 5 over three growing periods. The results, which took 210–250 days to obtain, agree with those from field trials lasting 3–4 yr (12, 13,16,17). It would still be interesting, however, to see if a particular sequence of nonhost crops (e.g., pea, corn, and sunflower) might be more effective than a straight pea or sunflower monoculture, which is agronomically unacceptable because of disease, insect, and weed management.

A third phenomenon, in addition to multiplication of the nematode on and maintenance of inoculum concentrations by various nonhost crops and weeds that needs to be considered in the development of a rotation scheme for controlling the SCN race 5, is the possibility that growth-inhibiting compounds are released from the residues of the previous crop. Such allelopathiclike effects were observed in this study. The culture of pea and sunflower, which was proposed earlier as a strong candidate for inclusion in a rotation to control the SCN race 5, was associated with maximum dry weight of bioassay soybeans. The culture of sugar beets and pigweed, on the other hand, was associated with minimum dry weight of such soybeans. These results appeared to be due to more than just growth-promoting effects resulting from nitrogen production by leguminous plants. The fact that the cysts-with-eggs populations remaining in the sand after three growing periods with these four plants did not differ significantly and the fact that soybeans after three growing periods of soybeans or adzuki beans, which were exposed to significantly more SCN inoculum, weighed more than soybeans after sugar beets or pigweed, support the idea that factors other than the SCN or nutrition are involved and deserve consideration. All of the abovementioned phenomena require testing to further examine their role in SCN race 5 control.

Table 2. Development of *Heterodera glycines* race 5 on soybean bioassay plants after one (A-2), two (B-2), and three (C-2) growing periods of nine crop and two weed species

Crop or weed	No. female nematodes[y]		
	A-2	B-2	C-2
Adzuki bean	6.3 a	2.3 b	4.4 ab
Alfalfa	4.4 a	2.3 b	0.6 c
Corn	1.6 a	–[z]	0.5 c
Lamb's-quarters	2.9 a	–	2.6 abc
Oat	3.9 a	0.7 b	0.7 c
Pea	3.6 a	1.4 b	0.5 c
Pigweed	3.3 a	1.0 b	1.5 bc
Soybean	6.2 a	7.5 a	5.7 a
Sugar beet	2.4 a	0.8 b	2.3 abc
Sunflower	2.8 a	0.5 b	0.5 c
Wheat	3.2 a	1.2 b	1.8 bc

[y] Means followed by the same letter within a column are not significantly different ($P = 0.05$) according to Tukey's HSD. A square root transformation was applied to raw data. Mean number of female nematodes (immature, maturing, with gelatinous matrix, producing egg masses, and cysts with eggs) recovered from five soybean bioassay root systems.
[z] No recovery of the nematode.

Table 3. Dry weight of soybean bioassay plants grown in sand after one (A-2), two (B-2), and three (C-2) growing periods of crops or weeds

Crop or weed	Dry weight (g)				
	A-2[z]	B-2[z]	C-2[z]	Mean total	Rank[z]
Adzuki bean	1.25 ab	0.79 ab	0.71 ab	0.92 ab	3
Alfalfa	1.12 ab	0.89 ab	0.61 ab	0.87 ab	4
Corn	1.26 ab	0.76 ab	0.55 ab	0.86 ab	5
Lamb's-quarters	1.08 ab	0.65 b	0.66 ab	0.80 ab	7
Oat	1.39 ab	0.77 ab	0.59 ab	0.92 ab	3
Pea	1.56 a	1.17 a	0.75 a	1.16 a	1
Pigweed	0.79 b	0.45 b	0.51 b	0.58 b	9
Soybean	1.14 ab	0.68 ab	0.60 ab	0.81 ab	6
Sugar beet	1.00 ab	0.55 b	0.51 b	0.67 b	8
Sunflower	1.59 a	0.79 ab	0.52 ab	0.97 ab	2
Wheat	1.26 ab	0.53 b	0.63 ab	0.81 ab	6

[z] Mean dry weight of five soybean plants (g). Means followed by the same letter within a column are not significantly different ($P = 0.05$) according to Tukey's HSD.

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