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Influence of Selected Plant Species on Hatching of Eggs and Development of Juveniles of *Heterodera glycines*[1]

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Abstract: The influence of selected plant species on egg hatch and subsequent development of *Heterodera glycines* race 3 was investigated. Plants tested included four soybean cultivars, red clover, alfalfa, hairy vetch, field corn, sweet corn, cabbage, tobacco, cotton, and wheat. Soybean stimulated egg hatching more than any of the other plant species, with *H. glycines*-resistant cultivars being more stimulating than susceptible ones. Hairy vetch also increased hatch. Roots of cabbage, red clover, alfalfa, and hairy vetch were readily penetrated by juveniles of *H. glycines*. Maturation to adult occurred only on soybean and hairy vetch.

Key words: *Glycine max*, hatch stimulation, *Heterodera glycines*, soybean, soybean cyst nematode.

Hatching of nematode eggs is affected by environmental factors, including temperature, soil aeration, and moisture (4). Plant exudates stimulate hatch in certain host–parasite combinations (5,20,21). For example, eggs of *Heterodera schachtii* Schmidt, *H. carotae* Jones, *H. humuli* Filipjev, and *Globodera rostochiensis* (Wollenweber) Behrens hatch in exudates from hosts or plants closely related to hosts (21). Eggs of *H. trifolii* Goffart and *H. galeopsidis* Filipjev & Schurmans-Stekhoven did not respond to exudates of typical hosts but hatched readily in response to such compounds of pea, a nonhost (21).

Diapause of eggs of *Heterodera glycines* Ichinohe apparently is induced by conditions prevailing at the end of a growing season (15). Hatching does not occur until diapause is broken, presumably in response to a chilling requirement (11). Nevertheless, hatching of *H. glycines* often is enhanced by soybean root exudates (3,13,14,18,19), but not always (8,16,17). Hatch enhancement, or lack of it, may not be related to concentration of exudate or age of the soybean plant from which these materials originate. Hatch is stimulated more by exudates from roots of susceptible cultivars than by those from resistant plants (3). Diluting exudates sometimes enhances hatching of eggs (14). Hatch also is enhanced more when soybean root extracts are taken from plants with 4–7 leaves, small pods, or those beginning to senesce than from other stages of plant development (18). A hatching stimulant for eggs of *H. glycines*, Glycinoeclepin A from kidney bean, has been extracted and characterized (12). The objective of our research was to further clarify the effects of selected plant species on hatch of *H. glycines* eggs.

MATERIALS AND METHODS

Heterodera glycines race 3, cultured on soybean (*Glycine max* (L.) Merr. cv. Lee) in a greenhouse, was used for all laboratory and greenhouse experiments. Cysts were crushed with a glass tissue grinder to re-

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lease the eggs. Aliquants of 5,000 eggs per 7.5-cm-d clay pot filled with a fine sand from a river beach were used in all greenhouse experiments, and 500 eggs per hatching chamber were used in laboratory in vitro tests. Cysts, eggs, and second-stage juveniles (J2) were extracted from the soil in field and greenhouse experiments by a modified sugar flotation technique (10). Results were measured in terms of J2 extracted during the test periods.

Influence of various plant species: Several plant species were tested for hatch stimulation of eggs of *H. glycines.* These plants included soybean cultivars Bedford (resistant), Essex (susceptible), Lee (susceptible), and Forrest (resistant), cotton (*Gossypium hirsutum* L. cv. Deltapine 16), sweet corn (*Zea mays* L. cv. unknown), field corn (*Z. mays* L. cv. Funks 4740), cabbage (*Brassica oleracea* var. *capitata* L. cv. Flat Dutch Early), tobacco (*Nicotiana tabacum* L. cv. NC 95), and wheat (*Triticum aestivum* L. cv. Coker 747). Pots without plants served as controls. Seeds of each cultivar were germinated in vermiculite. Transplanting dates varied to assure that the roots of all plants would be approximately 1–2 cm long when the experiment was initiated. One corn seedling or two seedlings of each of the other plants were transplanted into each pot and replicated six times. Three replications were harvested at 9 days, and three at 18 days after transplanting. Shoots from all plants were removed, soil was gently washed from the roots, and the roots were stained (2). The test was repeated using only Flat Dutch Late cabbage, Coker 747 wheat, NC 95 tobacco, and Essex and Forrest soybean. The second test was harvested 15 days after transplanting. Experimental design was a randomized complete block design.

Comparison of two cabbage cultivars: This test focused on effects of Flat Dutch Late and Flat Dutch Early cabbage on hatching of *H. glycines* eggs and postinfection development of the nematode. Treatments were replicated four times and arranged in a randomized complete block design. Plants were harvested 20 and 47 days after transplanting.

Effects of selected legumes: Several legumes, with soybean as a standard, were tested to determine their effects on stimulation of hatching of eggs of *H. glycines.* Plants included 2-day-old seedlings of Essex soybean and 10-day-old seedlings of alfalfa (*Medicago sativa* L. cv. Cody), hairy vetch (*Vicia villosa* Roth), and red clover (*Trifolium pratense* L. cv. Kenland). Pots without plants served as controls. Treatments were arranged in a randomized complete block design with six replications. Three replications were harvested 14 days after transplanting and three 28 days after transplanting. The experiment was repeated with three replications, all harvested 14 days after transplanting.

In vitro hatch: Root leachates from several crop species were tested for hatch stimulation in vitro. Seedlings of sweet corn, Funks 4740 field corn, Flat Dutch Early cabbage, Deltapine 16 cotton, NC 95 tobacco, Coker 747 wheat, and Essex, Forrest, Bedford, and Lee soybean were transplanted into 15-cm-d clay pots. Four to five weeks after transplanting (depending on plant species), plants were allowed to wilt, then 500 ml water was poured into each pot. Leachate was collected from the bottom of each pot. Hatching chambers were made of 0.25-μm-pore plastic mesh placed in 50-cm-d petri dishes. Leachate was added to these dishes so that the liquid contacted the screen. Juveniles and unhatched eggs were counted after 12 days. Treatments were replicated twice and arranged in a randomized complete block design.

Effect of various crops under field conditions: Two experiments were conducted in fields naturally infested with *H. glycines* race 4, one at the Cotton Branch Experiment Station near Marianna, Arkansas, and another at the Pine Tree Experiment Station near Colt, Arkansas. Treatments at the Pine Tree Experiment Station were Coker 156, Bedford, and Essex soybean, cotton, and fallow. At Marianna, treatments were NC 95 tobacco, grain sorghum (*Sorghum vulgare* Persoon), Essex soybean, cotton, and

TABLE 1. Numbers of *Heterodera glycines* juveniles in soil and roots, 9 and 18 days after initiation of the experiment.

Plant	Unhatched eggs	Juveniles			Hatch (%)
		Soil	Root	Total	
		9 days			
None	797 ab	349 bc		349 d	30
Essex soybean	643 ab	163 d	809 b	972 b	60
Bedford soybean	717 ab	197 cd	1,099 a	1,296 a	64
Forrest soybean	632 ab	131 d	852 b	983 b	61
Flat Dutch Early cabbage	904 ab	507 ab	68 c	575 c	39
Deltapine 16 cotton	829 ab	544 a	1 c	545 cd	40
Sweet corn	1,285 a	669 a	0 c	669 c	34
Funks 4740 corn	784 ab	600 a	0 c	600 c	43
Coker 747 wheat	570 b	667 a	0 c	667 c	54
NC 95 tobacco	947 ab	544 a	0 c	544 cd	36
		18 days			
None	504 a	627 a		627 c	55
Essex soybean	133 b	96 b	766 bc	862 bc	87
Bedford soybean	101 b	85 b	1,189 b	1,274 b	93
Forrest soybean	104 b	75 b	2,348 a	2,423 a	96
Flat Dutch Early cabbage	416 ab	333 ab	314 cd	647 c	61
Deltapine 16 cotton	379 ab	371 ab	9 d	380 c	50
Sweet corn	376 ab	405 ab	0 d	380 c	50
Funks 4740 corn	216 ab	371 ab	0 d	371 c	62
Coker 747 wheat	392 ab	445 a	0 d	445 c	53
NC 95 tobacco	507 a	488 a	0 d	488 c	49

Means followed by the same letter are not different ($P = 0.05$) according to the Waller-Duncan k-ratio *t*-test (k-ratio = 100).

fallow. The experimental design at each location was a 5 × 5 latin square. Plots were 6 m long and four rows wide (92-cm row spacing at Pine Tree and 97-cm spacing at Cotton Branch Experiment Station). Plots at both locations were sampled 16 June 1986, planted 17 June, and resampled to determine hatch 30 June.

RESULTS AND DISCUSSION

Influence of various plant species: More *H. glycines* eggs hatched in pots with soybean than in fallow soil or pots with nonlegumes by 9 days after planting. Hatching was greater at 9 days in pots with the resistant cultivar Bedford and at 18 days with the resistant cultivar Forrest than with the susceptible cultivar Essex (Table 1). Total hatch at 9 days in pots with Bedford was 3.7 times greater than the hatch in soil without plants. Forrest and Essex stimulated 2.8 times greater hatch and nonlegumes stimulated 1.6–1.9 times greater hatch than in control pots. The greatest hatch occurred through 18 days in soil in which Forrest was growing; it was 3.9 times the hatch in fallow soil. Hatch in pots with Bedford increased very little after 9 days, and some juveniles in the roots may have died and disintegrated by 18 days. Hatch in the fallow soil almost doubled between 9 and 18 days. Fewer ($P = 0.05$) unhatched eggs remained in pots with soybean at 18 days than in those with tobacco or fallow.

Cabbage did not stimulate hatch even though its roots were readily penetrated by *H. glycines* J2. Occasionally a J2 penetrated the roots of cotton, but not sweet corn, field corn, wheat, or tobacco.

Comparison of two cabbage cultivars: Roots of Flat Dutch Late and Flat Dutch Early cabbage were infected readily by *H. glycines* J2. Fourth-stage juveniles (J4) were found in both cultivars. Development proceeded more readily in Flat Dutch Early where several J4 were found, including males coiled inside the fourth-stage cuticle. Only one J4 was found on Flat Dutch Late. No

TABLE 2. Hatch of *Heterodera glycines* eggs as influenced by selected legumes.

Plant	Unhatched eggs	Juveniles			Hatch (%)
		Soil	Root	Total	
		Test 1			
None	1,016 a	123 a		123 b	11
Kenland red clover	872 a	107 a	180 b	287 b	25
Cody alfalfa	939 a	80 a	88 b	168 b	15
Hairy vetch	621 a	48 a	263 b	311 b	33
Essex soybean	711 a	40 a	749 a	799 a	53
		Test 2			
None	1,795 a	543 a		543 c	23
Kenland red clover	1,515 ab	192 bc	528 c	720 c	32
Cody alfalfa	1,209 ab	235 b	305 cd	540 c	31
Hairy vetch	1,064 ab	48 c	1,783 b	1,831 b	63
Essex soybean	676 b	77 c	2,627 a	2,704 a	80

Means within a column followed by the same letter are not different ($P = 0.05$) according to the Waller-Duncan k-ratio *t*-test (k-ratio = 100).

females or cysts were detected on either cultivar.

Effects of selected legumes: Soybean effected greater hatch than the other legumes tested (Table 2). Hairy vetch also stimulated hatch, particularly in the second test, but not as much as soybean. Hatching of *H. glycines* eggs in soil planted to red clover and alfalfa was not different ($P = 0.05$) from hatch in fallow soil.

A few *H. glycines* developed slowly to maturity on hairy vetch. *Heterodera glycines* did not develop beyond the vermiform second-stage in red clover and developed to the swollen second stage in alfalfa.

TABLE 3. In vitro hatch of eggs of *Heterodera glycines* in root leachates.

Source of leachate	Unhatched (n)	Hatched (n)	Hatch (%)
Lee soybean	74 a	73 ab	50
Bedford soybean	74 a	110 a	60
Forrest soybean	84 a	120 a	59
Essex soybean	130 a	64 abc	33
Sweet corn	198 a	36 bc	15
Deltapine 16 cotton	211 a	12 bc	5
NC 95 tobacco	292 a	20 bc	6
Flat Dutch Early cabbage	353 a	24 bc	6
Coker 747 wheat	392 a	20 bc	5
Funks 4740 corn	406 a	22 bc	5
Water (control)	390 a	38 bc	9

Means within a column followed by the same letter are not different ($P = 0.05$) according to the Waller-Duncan k-ratio *t*-test (k-ratio = 100).

In vitro hatch: Hatching of eggs in leachate from Bedford and Forrest soybean was greater ($P = 0.05$) than from nonlegumes or the water control (Table 3). Leachates from Bedford and Forrest soybean induced more hatch than Lee or Essex soybean, but the differences were not significant. In vitro hatch in the water control was similar to hatch in leachate from the nonleguminous plants (Table 3).

Effect of various crops under field conditions: At the Cotton Branch Experiment Station, the numbers of *H. glycines* eggs were 32% fewer with soybean and 41% fewer with tobacco at 2 weeks after planting, relative to numbers at planting. Egg numbers changed little with cotton (11%) and sorghum (4%) in 2 weeks. These differences were not significant.

Numbers of *H. glycines* eggs at the Pine Tree location decreased more with soybean than with other treatments. Decrease in egg numbers was least in fallow soil and with cotton (Table 4). The egg decrease was 69–71% on the three soybean cultivars, 43% on cotton, and 38% in the fallow soil. Numbers of J2 recovered from soil were greater ($P = 0.05$) from the soybean cultivars than from fallow or cotton. These numbers were probably greater in the soybean plants because many J2 would have infected the roots (numbers in roots were not determined).

TABLE 4. Numbers of eggs and second-stage juveniles of *Heterodera glycines* recovered from soil 2 weeks after planting in two locations.

Crops	Eggs		Juveniles	
	At planting	2 weeks postplant	At planting	2 weeks postplant
	Cotton Branch Experiment Station			
Fallow	1,688 a	1,960 a	22 a	21 a
Essex soybean	1,488 a	1,020 a	21 a	144 a
Cotton (cv. unknown)	1,544 a	1,370 a	14 a	18 a
NC 95 tobacco	2,252 a	1,190 a	11 a	35 a
Sorghum (cv. unknown)	1,528 a	1,460 a	16 a	21 a
	Pine Tree Experiment Station			
Fallow	12,200 a	7,560 ab	91 a	62 c
Essex soybean	12,640 a	3,460 b	74 a	816 ab
Coker 156 soybean	14,840 a	4,600 ab	77 a	907 a
Bedford soybean	17,240 a	5,200 ab	56 a	493 b
Cotton (cv. unknown)	15,760 a	8,960 a	53 a	64 c

Means within a column followed by the same letter are not different ($P = 0.05$) according to the Waller-Duncan k-ratio *t*-test (k-ratio = 100).

In our experiments, resistant cultivars tended to stimulate more egg hatching than susceptible ones, which contradicts the results of others (18). Cultivars of soybean and populations of the nematode were different; both factors may be important in the hatching response and thus explain the divergent results.

Several reasons may explain the lack of hatching stimulation reported in previous studies (8,16,17). Eggs may differ in development and physiological state which would affect hatch (1,6). Day length and temperature may influence host stimulant concentration. Exudates may be more stimulatory at some phenological stages of the plant than at others (19). Leachates collected from 30-day-old soybean plants and plants in early pod development were effective in stimulating hatch (18); however, between those two stages and late in the growth of the plants, little or no stimulation of hatching activity was found (18).

Nonhosts and poor hosts probably do not influence egg hatching of *H. glycines*. The absence of a hatching stimulant under field conditions would prolong survival of eggs. For example, eggs have survived at least 9 years in the absence of any plant (9).

Glycinoeclepin A, a hatching stimulant for *H. glycines* extracted from kidney beans (12) or similar materials (7), may have potential for management of *H. glycines* if it is economically feasible. Such a product would be especially useful if applied when soil is fallow or a nonhost is growing.

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