Hatch and Emergence of *Heterodera glycines* in Root Leachate from Resistant and Susceptible Soybean Cultivars

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Abstract: Egg hatch and emergence of second-stage juveniles (J2) of *Heterodera glycines* races 3 and 4 from cysts exposed to soybean root leachate of cv. Fayette (resistant to *H. glycines*) and *H. glycines*-susceptible cultivars A2575, A3127, and Williams 82 were determined in three sets of experiments. In the first experiment, cysts of both race 3 and race 4 were exposed to leachate of 8-week-old plants for a 2-week period. In the second experiment, cysts from populations of races 3 and 4 were raised on cultivars A2575, A3127, and Williams 82. Cysts then were exposed to leachate from 8-week-old plants for a 2-week period in all possible race-per-cultivar combinations. In the third experiment, cysts of races 3 and 4 were exposed at 4-day intervals to leachate from plants as the plants developed 7 to 59 days after planting. In experiments 1 and 2, leachate from 8-week-old Williams 82 and A3127 stimulated more hatch and emergence of *H. glycines* than leachate from A2575, Fayette, or the control. In the first experiment, cumulative hatch and emergence were greater for race 3 than for race 4. In experiment 2, no apparent relationship developed between leachate from a cultivar and the population developed on that cultivar in terms of stimulation of hatch and emergence. In the third experiment, A2575 stimulated more hatch and emergence of both race 3 and race 4 than A3127, Fayette, and Williams 82. Leachate from Fayette stimulated less hatch and emergence of both race 3 and race 4. Hatch and emergence were greatest during the initial 12 days of the experiment.

Key words: diapause, emergence, *Glycine max*, hatch, *Heterodera glycines*, nematode race, resistance, soybean, soybean cyst nematode.

Reports concerning stimulation of egg hatch of second-stage juveniles (J2) of soybean cyst nematode, *Heterodera glycines* Ichinohe, by soybean root leachate have been contradictory. Most workers during the 1950s and 1960s reported a lack of stimulation (1,10,19,20). More recent studies have indicated that hatch is stimulated by soybean (17,22,23). Stimulation of hatch was reported to increase with plant age, with the greatest stimulation by root leachate from plants in early reproductive stages (R1–R2) of growth (6,8,22,26). More eggs hatched from cysts collected during August on field-grown soybean than eggs obtained during the rest of the year (8,26). However, numbers of J2 in soil were highest in September, lowest in November, and increased gradually during the spring (16).

The research described herein was initiated to determine egg hatch and emergence of J2 from cysts of two populations of *H. glycines* classified as either race 3 or race 4 (15). Experiments were established to determine (i) the effect of root leachate from resistant and susceptible soybean on hatch and emergence during the early reproductive phase of soybean growth, (ii) the influence of resistant and susceptible soybean on subsequent hatch and emergence of races 3 and 4 raised on susceptible soybean, and (iii) the effect of soybean phenology on hatch and emergence of these races.

**Materials and Methods**

*Experiment 1*: In vitro tests were conducted under laboratory conditions in November 1988 and February 1989. Three seeds of public cv. Fayette, belonging to Maturity Group (MG) III and resistant to...
races 3 and 4, and three seeds of susceptible cvs. A2575 (MG II), A3127 (MG III) (Asgrow Seed Co., Des Moines, IA), and public cv. Williams 82 (MG III) were planted separately in 500 cm$^3$ autoclaved builder’s sand in 10-cm-diam. pots and maintained in a greenhouse for 8 weeks. Plants were watered daily and fertilized with a 23-19-17 (N:P:K) liquid fertilizer at 2 and 4 weeks after planting. Techniques and procedures for obtaining soybean root leachate were modified from those used by Beane and Perry (J. Beane & R. Perry, pers. comm.). Root leachate was obtained from three pots of each cultivar 8 weeks after planting (R2-R3 developmental stage). Pots were not watered during the 24 hours prior to collection of leachate. Double-distilled water (100 ml) was poured into each pot. After 1 hour, an additional 300 ml was poured into each pot and leachate was collected in 600-ml beakers positioned beneath each pot. Leachates from pots of the same cultivar were combined, filtered twice through No. 1 Whatman filter paper, and stored at 4 °C.

Nematodes were obtained from infested soil collected from microplots at the University of Illinois Pell farm in Urbana, Illinois, and stored for 1 month at 4 °C. Soil was collected in October 1988 for test 1 (November test) and January 1989 for test 2 (February test). The race 3 and race 4 populations were reared on cv. Williams 82. Cysts were extracted by wet-sieving (2) and then soaked in double-distilled water for 48 hours at 25 °C. Each experimental unit consisted of 20 cysts in a 3.5-cm-diam. petri dish. Cysts were divided into groups of 15 and transferred into hatching dishes. Leachates of Fayette, A2575, A3127, and Williams 82 were obtained from 8-week-old plants (R2–R3 developmental stage). Fresh and dry root weights were obtained. Cysts were incubated as described in experiment 1 for 2 weeks in 2 ml of either root leachate or control leachate. Treatments were replicated four times and arranged in a completely randomized design. Percentage hatch and emergence were calculated at the conclusion of the experiment.

Experiment 2: An experiment was conducted in February 1989 to compare effects of leachate on the race 3 and race 4 populations reared on A2575, A3127, or Williams 82. Seeds of A2575, A3127, or Williams 82 were potted directly into soil infested with either race collected from microplots in November 1988. The six populations were allowed to complete one generation, which required 35 days, and then the infested soil was stored at 4 °C for 2 months. Cysts were divided into groups of 15 and transferred into hatching dishes. Leachates of Fayette, A2575, A3127, and Williams 82 were obtained from 8-week-old plants (R2–R3 developmental stage). Fresh and dry root weights were obtained. Cysts were incubated as described in experiment 1 for 2 weeks in 2 ml of either root leachate or control leachate. Treatments were replicated four times and arranged in a completely randomized design. Percentage hatch and emergence were calculated at the conclusion of the experiment.

Experiment 3: Two tests were conducted to determine effects of plant age on hatch and emergence of J2. The first was from 10 October 1989 to 1 December 1989 and the second from 16 March 1990 to 7 May 1990. Three seeds of Fayette, A2575, A3127, or Williams 82 were planted in 500 cm$^3$ autoclaved sand in 10-cm-diam. pots. Root leachate was obtained from three pots of each cultivar at 4-day intervals beginning 7 days (V1 developmental stage) after planting and continued for a total of 52 days (R3 developmental stage). Collection was similar to that of experiment 1 except that 60 ml of double-distilled water was poured into each pot and an additional 160 ml was poured after 1 hour. Leachates were added to hatching dishes
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on the same day they were obtained. Leachate obtained from pots containing only the sand medium served as the control.

Soybean growth stage was recorded at each leachate extraction date. One pot of each cultivar, grown concurrently with the leachate extraction plants, was removed at each 4-day interval to determine fresh and dry root weight at the time of leachate collection. Fresh and dry root weights for leachate extraction plants were determined at the end of the experiment.

Soil infested with either race 3 or race 4 was obtained from microplots in April 1989 for test 1 (October test) and in January 1990 for test 2 (March test) and stored at 4 °C. The race 3 and race 4 populations were reared on Williams 82. Cysts were extracted from the soil by wet-sieving and soaked in double-distilled water for 48 hours at 25 °C. Cysts were divided into groups of 20 and transferred to hatching dishes. Treatments were replicated five times and arranged in a completely randomized design. Experimental conditions were as described for experiment 1. Emerged J2 were counted and removed at 4-day intervals. Leachate was removed, and 2 ml of leachate obtained from plants that were 4 days older was added. All cysts were crushed after 52 days, and total J2 and eggs were counted. Percentage emergence of J2 at each 4-day interval, total emergence, and total hatch were calculated.

Data from all tests were analyzed by analysis of variance (SAS Institute, Cary, NC). Means were separated by Fisher's least significant difference.

**RESULTS**

Experiment 1: In test 1, leachate from Williams 82 stimulated more hatch and emergence of the race 3 population than did the other four treatments (*P* < 0.05) (Fig. 1). There was no significant difference in hatch between A2575 and A3127 nor were there significant differences in hatch or emergence between Fayette and...
the control. A3127 stimulated significantly more emergence than A2575, Fayette, or the control. In test 2, Williams 82 stimulated significantly more hatch than the control and more emergence of the race 3 population than the other four treatments (Fig. 1). There were no differences among the other four treatments.

In tests 1 and 2, Williams 82 stimulated significantly more hatch of the race 4 population than either Fayette or the control and significantly more emergence than the control (Fig. 1). In test 2, A3127 stimulated significantly more hatch than Fayette and the control. There were no differences among A2575, Fayette, or the control.

Combined percentage hatch and emergence for the race 3 population was significantly greater than race 4 in both tests. In test 1, percentage hatch and emergence for race 3 were 26% and 13%, respectively, compared to 17% and 6% for race 4. In test 2, percentage hatch and emergence were 24% and 9%, respectively, for race 3 and 16% and 5%, respectively, for race 4. More J2 hatched (44%) and more hatched J2 emerged (19%) in test 1 than in test 2 (39% and 14%, respectively).

Experiment 2: A3127 stimulated more hatch and emergence than A2575, Fayette, or the control for the three race 3 per-cultivar populations ($P \leq 0.05$) (Fig. 2). There were no differences in either hatch or emergence between A3127 and Williams 82, regardless of the cultivar on which the nematodes were raised. Differences between Williams 82 and both A2575 and Fayette varied among populations, although Williams 82 always stimulated more hatch and emergence than the control. There were no differences in either hatch or emergence between A2575 and Fayette.

Leachate from Williams 82 stimulated significantly more hatch and emergence of race 4 populations than did A2575, Fayette, or the control except for hatch of race 4 reared on A2575 (Fig. 2). There were no

![Figure 2](https://example.com/figure2.png)

**Fig. 2.** Percentage egg hatch and emergence of second-stage juveniles of *Heterodera glycines* races 3 and 4 reared on different soybean cultivars and exposed to root leachate of 8-week-old (R2-R3 developmental stage) plants. Subheadings under each set of columns indicate race (R3, R4) and cultivar (25 = A2575, 31 = A3127, W = Williams 82). Legend refers to source of root leachate. Columns beneath similar letters within sets are not significantly different according to Fisher's least significant difference ($P \leq 0.05$).
differences in either hatch or emergence between Williams 82 and A3127, regardless of the cultivar on which race 4 was cultivated. Differences between A3127, A2575, and Fayette varied among populations selected on A2575, A3127, and Williams 82. More hatch and emergence occurred when nematodes were reared on Williams 82 and placed in leachate of A3127, when compared to nematodes in leachate of A2575 and Fayette. Nematodes reared on A3127 and A2575 and placed in leachate of A3127, A2575, or Fayette did not differ in hatch or emergence except for hatch or nematodes raised on A2575 and placed in leachate of A3127 and Fayette. There were no differences between A2575 and Fayette. In all cases, except for hatch of J2 reared on A2575, leachate treatments stimulated more hatch and emergence than the control.

Experiment 3: In both tests, leachate from A2575 stimulated more hatch (Fig. 3) and emergence of J2 (Fig. 4) from cysts of both races than did leachate from the other cultivars, with the exception of the race 4 population in leachate from A3127 (P ≤ 0.05). In test 1, fewer J2 of both races hatched and emerged when cysts were in leachate obtained from Fayette. In test 2, hatch and emergence of J2 from cysts of both races in leachate from Fayette were less than that observed for A2575. Hatch and emergence of J2 in leachate from Fayette did not differ from the other treatments except for race 4 in leachate obtained from A3127. More J2 hatched (47%) and more J2 emerged (70%) from cysts in test 1 than in test 2 (25% and 56%, respectively). In both tests, emergence of J2 from cysts was greatest during the initial 12 days when plants were in the vegetative stage of development.

In none of the three experiments did either fresh or dry root weights differ significantly among cultivars. In experiments 1 and 2, leachate was collected when plants were at the R2-R3 growth stage. In exper-

![Graph](image-url)

Fig. 3. Total percentage egg hatch of *Heterodera glycines* races 3 and 4 exposed to soybean root leachates for 52 days (V1-R3 developmental stages), tests 1 and 2. Columns beneath similar letters are not significantly different according to Fisher's least significant difference (P ≤ 0.05).
Fig. 4. Percentage emergence of second-stage juveniles (J2) of Heterodera glycines races 3 and 4 exposed to soybean root leachate of 7- to 59-day-old plants (V1-R3 developmental stages), test 1 and 2. After counting the J2 at 4-day intervals, nematodes and leachates were removed and fresh leachate from 4-day-older plants was added to each hatching dish. Letters in common at each age of leachate indicate cultivar differences are not significantly different according to Fisher's least significant difference ($P \leq 0.05$).

DISCUSSION

The stimulatory effect of soybean root leachate on hatch and emergence of H. glycines J2 was observed in all experiments.
Although results varied among cultivars and among experiments, root leachate from the susceptible cultivars A2575, A3127, and Williams 82 stimulated hatch and emergence of the nematode during one or more of the experiments when compared to the control. Among the three experiments, there were no consistent differences in hatch and emergence related to expected maturity under field conditions. Leachate from different cultivars of potato had different effects on hatch of Globodera rostochiensis and G. pallida (4). Root leachate from most of 25 potato cultivars tested stimulated hatch of G. rostochiensis eggs from 20.5% to 67.4%, whereas hatching activity on G. pallida ranged from 4.4% to 33.0%.

Differences in hatch of cyst nematodes in response to leachates from soybean and potato cultivars of varying root weights and root lengths has been reported (14, 22). In this study, differences in fresh and dry root weights among cultivars on days of leachate extraction were not significantly different and did not affect the results.

Lack of oxygen was reported to inhibit emergence of juveniles from cysts of Heterodera schachtii and G. rostochiensis (24, 25). In the present study, length of cyst exposure varied among experiments. In experiments 1 and 2, cysts were exposed to leachate for a 2-week period, whereas in experiment 3 the leachate was changed at 4-day intervals. Changing the leachate at shorter intervals may have replenished the oxygen content of the leachate, thus increasing hatch and emergence. The 2-week exposure period in experiments 1 and 2 may have been suitable for the buildup of microorganism development in the leachate, leading to oxygen depletion and inhibition of hatch and emergence. However, fungal growth and cloudiness of the leachate due to bacterial growth were not observed.

Differences in percentage hatch and emergence among the experiments reported in our studies were due, in part, to the soybean developmental stage in which leachate was obtained. In experiments 1 and 2, root leachates were obtained from 8-week-old plants at the reproductive (R2–R3) developmental stage, whereas leachates in experiment 3 were obtained periodically from soybeans beginning at the V1 and concluding with collection through the R3 developmental stage. Greater stimulation of hatch and emergence of J2 in leachate from vegetative plants disagrees with reports that indicated hatch of J2 from eggs in water was greater when cysts develop on reproductive plants (8, 26). However, those studies attempted to elucidate factors that led to induction of diapause and did not evaluate effects of leachate on hatch and emergence. An additional study demonstrated that reproductive plants stimulated more hatch when eggs from greenhouse-cultured cysts were exposed to excised roots placed in water for 24 hours (22). However, in that study, treatment of leachate with ethylenediamine tetraacetic acid caused more hatch to occur at the vegetative (V3–V5) stage of development. Potato root diffusate production has been associated with the vegetative phase of plant growth (14). Stimulation of G. rostochiensis hatch by potato root diffusate peaked at 3 weeks after plant emergence and declined afterwards. Root diffusate from 4- and 6-week-old pea plants stimulated more egg hatch of Heterodera goettingiana than did leachate from 2- or 10-week-old plants (13).

Diapause appears to have affected hatch and emergence of H. glycines in this study. Diapause in H. glycines (8, 11, 16) and other cyst nematodes (4, 18, 25) is induced in part by low temperature and, in the case of G. rostochiensis, may also be initiated by signals passed to the nematode from the potato plant during the growing season (9). The role of soybean phenology in initiation of diapause in H. glycines was inconclusive (7). In a study done in Missouri, a sharp decline occurred in the number of J2 hatching from eggs freed from cysts obtained from microplot soil in August (26). In North Carolina a decline in numbers of J2 obtained from field soil occurred in Sep-
tember and was followed by low recovery of J2 in November and a gradual increase in numbers of J2 through mid-April (7). Cysts used in this study were obtained from the field for experiment 1 in October (test 1) and January (test 2). For experiment 3, cysts were obtained in April (test 1) and January (test 2). Cysts used in experiment 2 were cultured in a greenhouse. Hatch and emergence were greater when cysts were obtained in October or April than in January, indicating that a diapause had been induced that inhibited hatch and emergence of the nematode.

Greater hatch and emergence in experiment 2 compared to experiment 1 may have resulted from differences in how nematodes were obtained. In the second experiment, white females were obtained from greenhouse-grown soybeans after completion of one generation at soil temperatures of 25–30 °C. Subsequent storage of the newly developed females at 4 °C for 2 months probably did not induce diapause and may explain the high level of hatch and emergence in this experiment, thus indicating that once diapause is induced, hatch and emergence will be low until conditions that overcome diapause are met.

Root leachate from the resistant cultivar Fayette inhibited or had no effect on hatch and emergence when compared to the control in all experiments except for race 4 in experiment 2. Regardless of which race was evaluated, Fayette stimulated less hatch and less emergence except for race 4 in experiment 1, test 2. In experiment 3, Fayette leachate stimulated less hatch of the race 3 population and less emergence of both populations when compared to the control, indicating that inhibition by Fayette leachate may occur after diapause is interrupted in the spring. Hatch of both *G. rostochiensis* and *G. pallida* was greatest when eggs were exposed to cv. Bintje susceptible to both species (3). However, when eggs of both species were exposed to cv. Elkana resistant to *G. rostochiensis* and clone ZB35-29 resistant to *G. pallida*, percentage hatch was reduced (3). It is possible that the resistant mechanism in Fayette to *H. glycines*, which involves a hypersensitive reaction at the nematode feeding site (12), also may include inhibition of hatch and emergence of certain populations of the nematode. However, it is reported that *H. glycines*-resistant cultivars Bedford and Forrest tended to stimulate more hatch of eggs liberated from cysts than susceptible cultivars in greenhouse pot studies (17). Fayette is the progeny of a Williams × PI88.788 cross; resistance from PI88.788 also was incorporated into Forrest to produce Bedford. Steele et al. (21) noted that hatch in leachates of plants either susceptible or resistant to *H. schachtii* was not significantly different, indicating that the factor(s) affecting nematode hatch is not linked to resistance in sugarbeet.

During experiment 3, J2 emerged at a high rate (84%) during the first 12 days of vegetative growth and then emergence decreased over the last 40 days. A study to determine the rate of emergence of *H. glycines* in distilled water over a 12-week period found that, of the total J2 emerged, 43% emerged by 2 weeks, 72% by 4 weeks, and 87% by 6 weeks (20). Few J2 emerged during the last 6 weeks of that study. Results of this study indicate that soybean root leachate can have a greater stimulatory effect on emergence of J2 during early periods of plant growth. However, the stimulus appears to weaken as plants age. Cysts of *H. cajani* produced on senescing plants were more dependent on root diffusate to hatch (7).

Population-specific differences in hatch and emergence were not observed in experiment 2 among the three race 3 and three race 4 populations developed on A2575, A3127, and Williams 82 and exposed to leachate of these three susceptible cultivars and resistant Fayette. Although both A3127 and Williams 82 in general stimulated more hatch and emergence, culturing *H. glycines* on these two cultivars caused no apparent increase in hatch and emergence when cysts were placed in leachate of either A2575 or Fayette.

Hatch and emergence of J2 from cysts is
a complex biological process involving diapause, plant developmental stage, and other factors. The present study also demonstrated that hatch and emergence are affected by the source of leachate. The susceptible cultivars A2575, A3127, and Williams 82 stimulated more hatch and emergence than resistant Fayette. Differences in the abilities of soybean cultivars to stimulate hatch and emergence may aid in decisions for long-term management of H. glycines. Additional research to determine stimulation of hatch and emergence of many soybean cultivars, as has been done for potato to G. rostochiensis (5), would be advantageous in selecting cultivars that are less stimulatory, and produce smaller residual populations of H. glycines.

LITERATURE CITED