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# Plant-Induced Hatching of Eggs of the Soybean Cyst Nematode *Heterodera glycines*[1]

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*Abstract:* Root diffusate from soybean plants caused greater hatching of *Heterodera glycines* eggs during vegetative growth of the host, but the activity declined with plant senescence. Chelation of the root diffusate with ethylenediamine tetraacetic acid (EDTA) significantly increased hatching activity for *H. glycines* eggs. Diffusate from leafless plants caused little hatching, whereas treatment of intact plants with the growth regulators gibberellin and kinetin had no effect on the hatching activity of root diffusate. Treating *H. glycines* eggs with zinc chloride and root diffusate reduced egg hatching from zinc chloride alone. Levels of zinc in the root diffusate were insufficient to induce egg hatch, based on analysis by atomic absorption spectrophotometry. The enzymatic activity of leucine aminopeptidase in *H. glycines* eggs was not altered by treatment with chelated or nonchelated root diffusate.

*Key words: Glycine max*, soybean, Nematoda, root diffusate, chelation, zinc chloride, hatching factor, plant growth regulators.

Our understanding of hatching of eggs of cyst nematodes is incomplete. Calcium may bind to the eggshell of *Globodera rostochiensis* eggs and cause changes in permeability that lead to hatching (1). Synergism with the hatching factor from the roots of host plants may also occur. In contrast, zinc chloride stimulated more hatching of eggs of the soybean cyst nematode *Heterodera glycines* than did calcium (2,14,15). Zinc chloride induced little hatching of the *G. rostochiensis* eggs (2).

Diffusates from host roots increased hatching of *H. glycines* in one study (14), while those from nonhosts had no effect (9,17). Hatching activity was not found in another study of root diffusates from soybean plants (13). However, diffusates from the roots of plants such as potato stimulated hatching of *G. pallida* eggs, whereas diffusates from mustard roots, a nonhost, inhibited hatching (5). A hatching factor, glycinoeclepin A, for *H. glycines* eggs was recently isolated from the roots of kidney beans (8). The hatching factor from roots of soybean plants remains unidentified.

Our objective was to examine *H. glycines* egg hatch induced by root diffusate from soybean plants. Additional knowledge of plant-stimulated hatching may resolve whether a hatching factor exists for *H. glycines* in soybean and offer insight into the ecological regulation of egg hatching in this pest nematode.

## Materials and Methods

*Heterodera glycines* Race 3 was maintained in greenhouse-grown soybean (*Glycine max* cv. Union) plants (14). Physiological ages of soybean plants were determined according to the scheme of Fehr et al. (4). Cysts were recovered by a sieving procedure (14). The age of cysts used in experiments was 2–4 months, but cysts for any one trial were of the same age.

Cysts were opened manually with forceps in water and the suspension passed through a 250-μm-pore sieve to remove debris. Eggs were rinsed three times with 20-ml aliquots of reagent-grade water (Milli-Q, Millipore) on a 0.45-μm-pore filter and then recovered by rinsing the filter with water. Test substances were added to aliquots of the egg suspension and 200-μl volumes of suspension were placed in the wells of microtiter plates. A minimum of 12 wells with 15–25 eggs per well was used for each dosage of each treatment. All studies were done at 25 C, and reagent-grade water was used throughout the investigation.

Hatching was determined by counting eggs and juveniles five times in 2 weeks and expressed as the hatch rate, which is the change in number of juveniles in the treated group divided by the change in number

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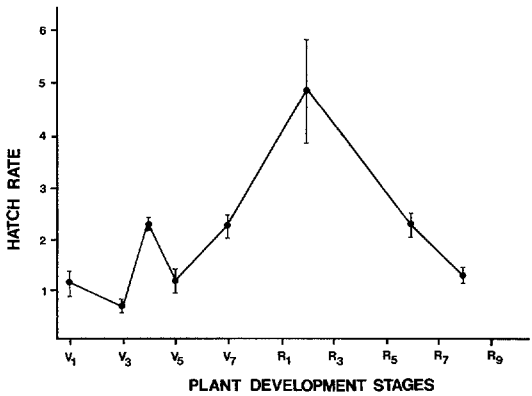


FIG. 1. Hatch rate (treated/nontreated) of *Heterodera glycines* eggs in root diffusate containing 2 root-gram-hours (2 g of root per hour in 1 ml of water) from plants of various physiological age ($V_1$ = unrolled leaf at unifoliate node; $V_3$, $V_4$, $V_5$ = number of nodes on main stem; $R_1$ = one flower; $R_3$ = 0.5 cm pod; $R_5$ = bean development; $R_7$ = yellowing pods; $R_9$ = harvest maturity) as predicted by regression analysis (± SEE).

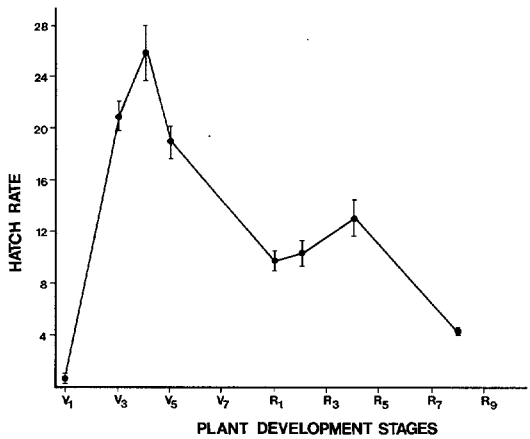


FIG. 2. Hatch rate (treated/nontreated) of *Heterodera glycines* eggs in root diffusate containing 2 root-gram-hours (2 g of root per hour in 1 ml of water) from plants of various physiological ages (see Fig. 1) after addition of EDTA as predicted by regression analysis (± SEE).

of juveniles in the nontreated group. Thus, a hatch rate of one indicates no difference between treated and nontreated groups.

Plant roots were cleaned of debris and rinsed, and the root system of the intact plant was placed in reagent-grade water to prepare root diffusate. After 24 hours at 22–24 C, roots were blotted, removed from the plant, and weighed. Hatching activity in the root diffusate is expressed as root-gram-hours per milliliter (RGH). One RGH is the amount of hatching activity from 1 gram of root per hour in 1 ml of water. Various concentrations of RGH were obtained by diluting root diffusate with reagent-grade water.

The hatching activity of root diffusate from plants of different physiological ages was examined at a concentration of 2 RGH. Eggs were exposed to the root diffusate for 2 weeks for comparison to water-only controls. Another experiment determined any involvement of divalent ions with the hatching activity of root diffusate. The chelator ethylenediamine tetraacetic acid (EDTA) was added at a final concentration of 6 mM to root diffusate from plants of various ages. Eggs were incubated in root diffusate with and without EDTA to determine its influence on the hatching activity of root diffusate. Eggs were also incubated in 6 mM EDTA as a control.

To locate the source of hatching activity, plants were partitioned into roots, stems and leaves, or roots and stems which were immediately placed in water. Diffusate equivalent to 2 RGH was collected from the partitioned plants. Three plants at the same physiological age ($R_2$; flower below uppermost node) were used for each test. Diffusate from these plants was also treated with 6 mM EDTA.

Plants were treated with growth regulators to examine their influence on production of hatching activity in root diffusate. Plants grown singly in sterile sand and peat moss (9:1) were treated on day 3 or day 10 after emergence. Plants were irrigated with 90 ml water containing gibberellic acid $GA_3$ or kinetin (Sigma) at 0.01 mM concentration. Diffusate was collected from roots of intact plants by the above procedures to compare treated and nontreated plants at the $V_4$ stage (four nodes on main stem).

The interaction of zinc and hatching activity in diffusate was studied because zinc stimulates hatching of *H. glycines* eggs (2,14). Eggs were exposed to zinc chloride at 0–500 $\mu$M with and without 2 RGH of root diffusate. The zinc content of root diffusate was analyzed by atomic absorption spectrophotometry (Perkin-Elmer 103) to determine if sufficient zinc was released from roots to stimulate hatching of eggs.

The effect of root diffusate on activity of the zinc metalloenzyme, leucine ami-

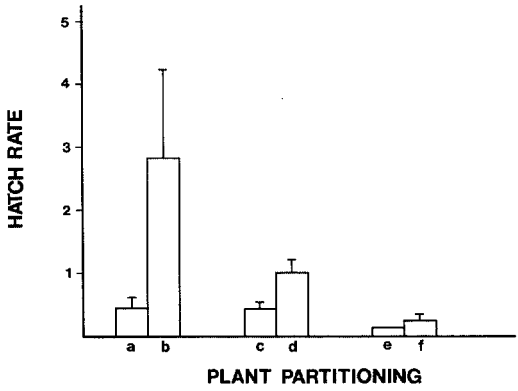


FIG. 3. Hatch rate (treated/nontreated) of *Heterodera glycines* eggs in root diffusate containing 2 root-gram-hours (2 g of root per hour in 1 ml of water) from severed roots only (a), severed roots only after addition of EDTA (b), severed stems and leaves (c), severed stems and leaves after addition of EDTA (d), leafless plants (e), and leafless plants after addition of EDTA (f) (± SEM).

nopeptidase (LAP), was studied also, because this enzyme was implicated in the hatching process of nematode eggs (12). LAP in a supernatant from homogenized eggs of *H. glycines* was determined colorimetrically according to Green et al. (6) as modified by Rogers (11). Enzyme activity in eggs was examined after treatment with chelated and nonchelated root diffusate from intact plants. The biological activity of the root diffusate was confirmed by parallel testing on other *H. glycines* eggs. LAP was recovered for assay as reported previously (16).

Data were analyzed by linear regression and analysis of variance. Hatch rates are given as the predicted value from regression analysis for a 2-week period ± standard error of estimate.

## RESULTS

Hatching activity of the root diffusate from intact plants increased during the vegetative and reproductive stages of host development and declined with plant senescence (Fig. 1). The highest level of hatching activity was found in root diffusate from early reproductive plants at the $R_2$ (flowering) stage. Twofold higher hatching activity occurred in root diffusate from plants at this stage, compared with younger or older plants.

Adding EDTA to root diffusate from plants of various ages greatly stimulated the rate of egg hatch (Fig. 2). The hatch rate of *H. glycines* eggs in root diffusate with EDTA was different ($P < 0.05$) from root diffusate without EDTA ($F = 111.4$). Treating eggs with only EDTA gave a 0.1 hatch rate.

Diffusate from severed roots and stems was less active than diffusate from intact plants (Fig. 3). Diffusate from severed roots treated with EDTA caused a hatch rate 2.9 times that of no EDTA, whereas EDTA added to diffusate from severed stems caused no stimulation of hatch. Diffusate from roots of leafless plants stimulated little hatching of *H. glycines* eggs. Treatment of the plants with the growth regulators gibberellin or kinetin caused no increase in hatch of eggs in root diffusate from treated plants (Table 1).

TABLE 1. Mean hatch rate (treated/nontreated) of *Heterodera glycines* eggs incubated in root diffusate (2 root-gram-hours*) from 'Union' soybean plants treated at 3 and 10 days after emergence with plant growth regulators.

| Plant age when treated | Growth regulator (0.01 mM) | Mean hatch rate (SEM) |
|---|---|---|
| Nontreated | None | 4.44 (1.61) |
| 3 days | Kinetin | 1.88 (0.43) |
| 3 days | Gibberellin | 1.8 (0.48) |
| 10 days | Kinetin | 3.08 (0.10) |
| 10 days | Gibberellin | 3.14 (1.42) |

* Root-gram-hour is the diffusate from 1 g of root per hour in 1 ml of water.

The addition of various concentrations of zinc chloride to 2 RGH of root diffusate caused a significant reduction ($F = 11.6$) in hatching of *H. glycines* eggs (Fig. 4). The level of zinc detected in root diffusate from plants of four ages was below levels that stimulate *H. glycines* egg hatching (Table 2).

Leucine aminopeptidase from supernatants of homogenized eggs treated with root diffusate hydrolyzed 0.045 (± 0.003) nmoles of substrate, whereas supernatant from nontreated eggs hydrolyzed 0.048 nmoles. LAP in egg supernatant treated with both EDTA-supplemented and nonsupplemented root diffusate hydrolyzed 0.035 (± 0.002) nmoles of substrate. Thus, root diffusate had no effect on the enzymatic activity of LAP in the supernatant from homogenized of eggs of *H. glycines*.

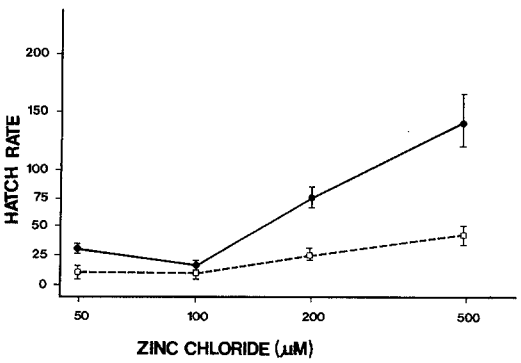


FIG. 4. Hatch rate (treated/nontreated) of *Heterodera glycines* eggs at several concentrations of zinc chloride (●) and zinc chloride with 2 root-gram-hours (2 g of root per hour in 1 ml of water) of root diffusate (○) (± SEM).

TABLE 2. Zinc content of root diffusate from 'Union' soybean plants at various stages of development.

| Plant developmental stage* | Zn (μg)/root-gram-hour† |
|---|---|
| $V_3$ (three nodes on main stem) | Not detected |
| $V_5$ (five nodes on main stem) | 0.038 |
| $R_1$ (one flower on any node) | 0.015 |
| $R_4$ (2-cm pod at uppermost nodes) | 0.017 |

* Stages from Fehr et al. (4).
† Root-gram-hour is the diffusate from 1 g of root per hour in 1 ml of water.

## DISCUSSION

The stimulatory effect of diffusate from plant roots for hatching *H. glycines* eggs was established previously (9,14,17). Little information is available, however, on the physiological status of the soybean plant affecting the biological activity of root diffusate on *H. glycines* eggs. The increased activity of root diffusates from the different aged plants in this study agreed with reports for other cyst nematodes. Widdowson (18) found that root diffusate from 2–3-week-old potato plants stimulated more hatching of *G. rostochiensis* eggs than did diffusate from older plants. Perry et al. (10) reported that root diffusate from 2–6-week-old plants stimulated greater hatch of *H. goettingiana* eggs than did diffusate from older or younger plants. However, qualitative and quantitative comparisons of biological activity among host and nematode species is confounded by the absence of data on root mass, differences in methodology, variable water quality among the studies, and other factors.

The increased biological activity of the root diffusate after addition of EDTA suggests that ions interfering with activity in the diffusate are removed by EDTA. The negative interaction of zinc chloride and hatching factor in this study indicates that ions may inhibit the hatching activity of root diffusate. The reported chemistry of the hatching factors in root diffusate, such as lactone rings (7) and polar hydroxyl groups (8) which contribute to ligand formation, also supports the concept of ionic interference. However, Evans (3) proposed that EDTA directly affected permeability of the eggshell. The absence of enhanced LAP activity in eggs after treatment with root diffusate suggests that root diffusate does not directly increase the activity of this enzyme in *H. glycines* eggs.

Hatching induced by root diffusate was not affected by treatment of the plants with the plant growth regulators gibberellin or kinetin. In contrast, Evans (3) reported increased emergence of juveniles from cysts of *G. rostochiensis* from gibberellin-treated plants as compared to nontreated controls. Further research is needed to clarify this difference in the effects of growth regulators on cyst nematodes and to isolate in host plants the site of production of hatching activity for nematode eggs.

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