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Tannic Acid Effects on Hatching of *Heterodera glycines* in Vitro[1]

S. CHEN,[2] D. W. DICKSON,[3] AND T. E. HEWLETT[3]

Abstract: Effects of tannic acid on hatching of *Heterodera glycines* eggs were determined in vitro using three batches of eggs obtained from greenhouse cultures in Florida or from naturally infested field soil in Minnesota. A quadratic model fits the percentage egg hatch. Hatch increased with increasing tannic acid concentrations from 0 to about 39 mg/liter, then declined with further increases in concentration. Tannic acid did not induce hatching of dormant eggs obtained from the field.

Key words: cyst, dormancy, egg, hatch, *Heterodera glycines*, soybean cyst nematode, tannic acid.

Many factors influence hatching of nematode eggs, including temperature, soil aeration, moisture, pH, exudates from host plants and other organic compounds, and inorganic ions (Clarke and Perry, 1977). Numerous studies have been done on hatching of the soybean cyst nematode, *Heterodera glycines*, that include both inhibitors and stimulants of hatching. Generally, exudates from host plants stimulate hatching of the soybean cyst nematode (Caballero et al., 1986; Okada, 1971a, 1971b; Schmitt and Riggs, 1991; Takasugi et al., 1974; Tefft and Bone, 1985; Tefft et al., 1982; Tsutsumi and Sakurai, 1966), whereas nonhosts do not stimulate hatching (Schmitt and Riggs, 1991; Tsutsumi and Sakurai, 1966). Attempts have been made to find natural or synthesized products that may be applied in fields to stimulate nematode hatching and reduce population densities when the host crops are absent. Five herbicides and zinc fertilizers were tested for their potential to stimulate hatching, but none were effective at practical concentrations (Behm et al., 1995; Wong et al., 1993). In preliminary studies, tannic acid attracted root-knot nematodes but not soybean cyst nematode (Hewlett et al., 1995). This compound reduced infection of tomato by the root-knot nematode, *Meloidogyne arenaria*, in the greenhouse and microplots (unpublished; Hewlett et al., 1995). Tannic acid and its analogues are abundant in many plant products, and tannins have been reported to have antimicrobial properties (Scalbert, 1991). Our objectives were to evaluate effects of tannic acid on hatching of the soybean cyst nematode and to determine the possibility of using the compound to induce nematodes to hatch.

Materials and Methods

Test 1: The source of nematodes used for this test was a population of *H. glycines* race 3 that had been maintained for 4 years on the susceptible soybean (*Glycine max*) cultivar Cobb in a greenhouse at the University of Florida.

Soybean seeds were sown in pots on 11 January 1995. Fifty-seven days after sowing, females and cysts were extracted from roots and soil by a procedure described previously (Chen et al., 1996). The females and cysts were crushed in a tissue grinder to release eggs. The eggs were separated from debris by centrifuging them in a 0% to 55% (w/v) sucrose linear gradient. The band containing eggs and second-stage juveniles (J2) was transferred to a 38-μm-pore sieve and rinsed with sufficient water to separate J2 from eggs. The eggs retained on the sieve were transferred onto another 38-μm-pore sieve that had been autoclaved, rinsed with sterile water, and treated with a solution containing 100 mg of streptomycin, 50 mg of chlortetracycline, and 30 mg of quinolinol per liter for 24 hours at 10 °C. The eggs were

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[2] University of Minnesota, Southern Experiment Station, 35838 120th Street, Waseca, MN 56093-4521.
E-mail: chenx099@gold.tc.umn.edu

[3] Entomology and Nematology Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611-0620.
E-mail: dwd@gnv.ifas.ufl.edu

rinsed with sterile water and treated again with 0.5% chlorhexidine diacetate salt for 15 minutes, and then rinsed with sterile water. Eggs were suspended in sterile deionized water at a concentration of 3,000 eggs/ml. One milliliter of the egg suspension was placed on each 1-cm-diam. sieve with 35-μm openings. The sieves with eggs were placed in a 24-well tissue culture plate.

Tannic acid was tested at concentrations of 0, 39, 156, 625, 2,500, and 10,000 mg/liter in sterile deionized water. A solution of 4 mM $ZnCl_2$, a hatching stimulant of the soybean cyst nematode (Tefft et al., 1982), was used to determine viability and degree of dormancy of the nematode eggs. The solutions were passed through a 0.45-μm filter to remove possible contaminants such as bacteria and fungal spores. Sufficient solution was added into each tissue culture well so as to reach the bottom of the sieves. Four replicates of each concentration were used. The eggs were incubated in the solutions at 23 to 24 °C. The solutions were replaced by freshly prepared solutions on days 3 and 7. The numbers of nematodes hatched by days 3, 7, and 14 were counted, and percent hatch was calculated. The data was subjected to analysis by regressing mean percent hatch of the four replicates against concentration using a quadratic model.

Test 2: The same nematode population used in the Test 1 was cultured in the greenhouse from 9 March to 20 May 1995. All the procedures were the same as Test 1. However, tannic acid concentrations of 0, 0.15, 0.6, 1.2, 2.4, 4.9, 9.8, 39, 156, 625, 2,500, and 10,000 mg/liter in sterile deionized water were used.

Test 3: The source of nematodes used for this test was a population of *H. glycines* race 3 collected from soybean rhizosphere in a naturally infested field site in Minnesota on 12 October 1995. The soil was stored at room temperature (23 to 24 °C) until 7 November 1995, when the cysts were extracted. Eggs and juveniles were released from the cysts by a modified mechanical method (Niblack et al., 1993) and separated from debris by centrifugation in a 35% (w/v) sucrose solution. The eggs and J2 were transferred onto a 38-μm-pore sieve and rinsed with sufficient water to remove J2. The eggs were stored in a solution containing 100 mg of streptomycin, 50 mg of chlortetracycline, and 30 mg of quinolinol/liter water at 4 °C for 3 weeks before they were tested. The concentrations of tannic acid were the same as in Test 2, and all other procedures were the same as in Test 1.

Results and Discussion

In all tests, low concentrations of tannic acid increased nematode hatching. Percent hatch increased with an increase of tannic acid concentration up to 35 to 40 mg/liter and then declined with increasing concentration (Fig. 1A–C). When compared at similar concentrations, the percent egg hatch differed among the three tests. In Test 1 (Fig. 1A), when eggs were incubated in 39 mg/liter tannic acid solution for 14 days, 32% of the eggs hatched (60% increase of hatch compared to hatch in water). In contrast, eggs in tannic acid at 10,000 mg/liter (1% w/v) had only 6.8% cumulative hatch at day 14 (61% decrease compared to hatch in water). Hatch in $ZnCl_2$ at 4 mM increased by 15% compared to hatch in water at day 14, which indicated that the eggs were not dormant. In Test 2, 42% of eggs hatched in the 39 mg/liter tannic acid solution at day 14, which represented a 13% increase compared to hatch in water. Cumulative egg hatch decreased 56% in the 10,000 mg/liter tannic acid solution at day 14 compared to hatch in water. Incubation in $ZnCl_2$ did not increase hatch of eggs compared to water in Test 2. Hatch of nematode eggs collected from the field site in Minnesota was very low. In water, only 0.9% of the eggs had hatched by day 14, and in tannic acid solution cumulative hatch reached a maximum of only 1.7% at 39 mg/liter by day 14. Hatch decreased to 0.5% in the 10,000 mg/liter tannic acid solution at day 14, or 55% of hatch in water. When these eggs were incubated in 4 mM $ZnCl_2$, 77% of the eggs hatched by day 14, indicating that egg viability was high but that the eggs were dormant.

The mechanism involved in increased

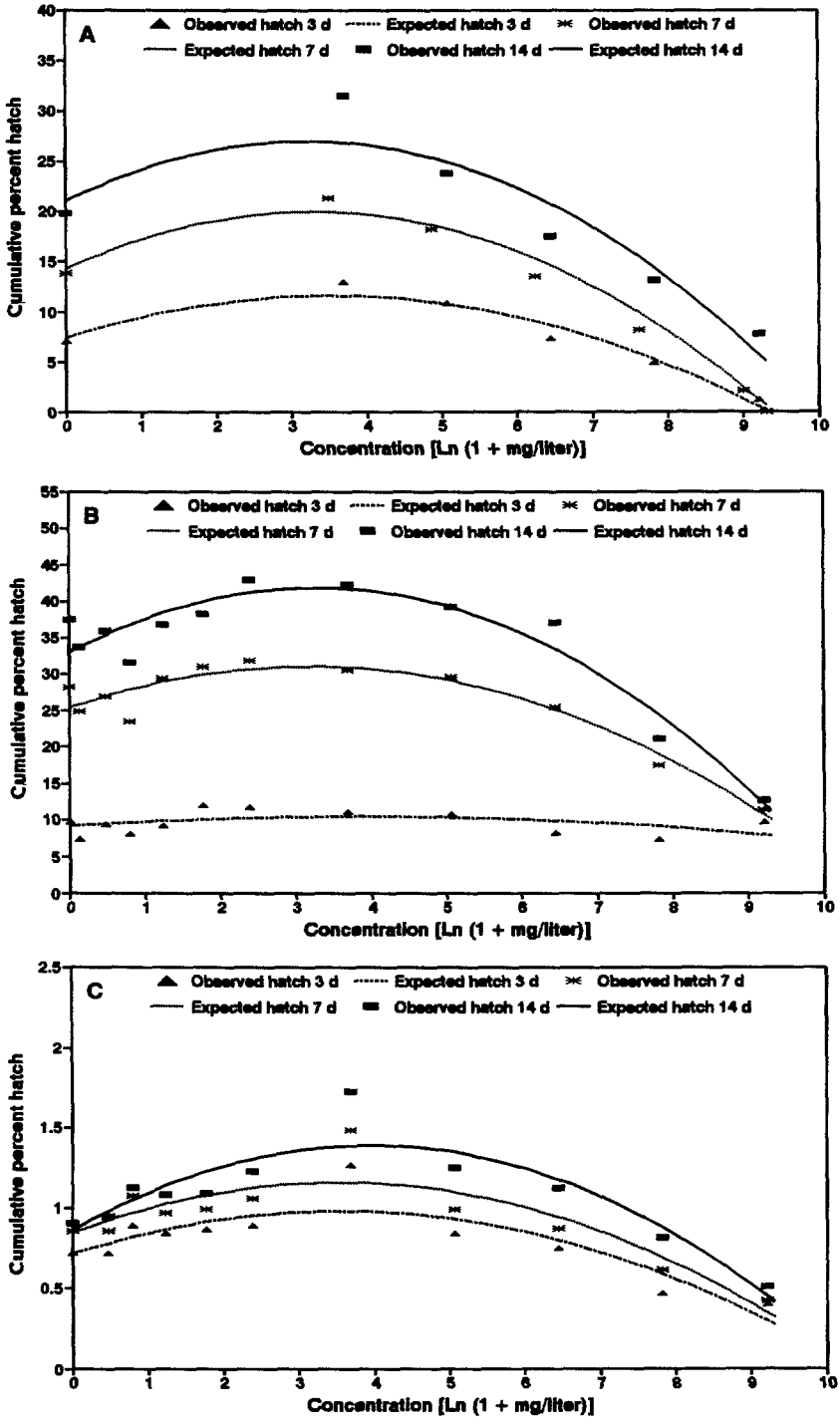

FIG. 1. The effect of different concentration of tannic acid on hatching of *Heterodera glycines*. A quadratic model, $Y = a + bx + cx^2$, was used to predict percentage hatch in different concentrations of tannic acid solutions with incubation periods of 3, 7, and 14 days. (3d, 7d, 14d). A) Test 1, using nematode eggs obtained from a greenhouse culture in Florida during 11 January through 9 March 1995. Curve 3 d: $r^2 = 0.95$, $P = 0.01$. Curve 7 d: $r^2 = 0.98$, $P = 0.003$. Curve 14 d: $r^2 = 0.88$, $P = 0.04$. B) Test 2, using nematode eggs obtained from a greenhouse culture in Florida during 9 March through 20 May 1995. Curve 3 d: $r^2 = 0.21$, $P = 0.35$. Curve 7 d: $r^2 = 0.91$, $P < 0.0001$. Curve 14 d: $r^2 = 0.90$, $P < 0.001$. C) Test 3, using nematode eggs collected from a Minnesota soybean field 12 October 1995. Curve 3 d: $r^2 = 0.75$, $P = 0.004$. Curve 7 d: $r^2 = 0.77$, $P = 0.003$. Curve 14 d: $r^2 = 0.81$, $P < 0.001$.

hatch of the soybean cyst nematode in low concentrations of tannic acid solutions is not understood. The results of Test 3 indicate that tannic acid did not break the dormancy of the eggs. Nevertheless, concentration of 35–40 mg/liter of tannic acid will increase hatching of nondormant eggs. High concentrations of tannic acid inhibit hatching. Further tests are needed to determine if the J2 within the egg is killed or just inhibited. Several researchers have reported that the concentration effect on egg hatching is critical (Clarke and Perry, 1977; Clarke and Shepherd, 1967; Okada, 1971b). Some nematicides, such as aldicarb and carbofuran, stimulate hatching at low concentrations but inhibit hatching at high concentrations (Hough and Thomason, 1975; Steele, 1983; Steele and Hodges, 1975). The stimulatory effects of low concentrations of nematicides on nematode hatching may be attributed to increased activity of juveniles in the egg (Hough and Thomason, 1975; Steele and Hodges, 1975). Tannic acid may act in a similar way.

Because tannic acid cannot break dormancy of the nematode eggs, we think that it may be unrealistic to use either tannic acid or plant products containing tannic acid to induce the soybean cyst nematode to hatch and reduce nematode population in fields during periods without host plants.

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