Supplement to the Journal of Nematology 29(4S):737–741. 1997.

Response of *Meloidogyne* spp., *Heterodera glycines*, and *Radopholus similis* to Tannic Acid[1]

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

Abstract: Tannins, which are water-soluble polyphenols, are toxic to numerous fungi, bacteria, and yeasts. Our objectives were to study the efficacy of tannic acid in control of *Meloidogyne arenaria* on tomato and its effects on the behavior of *M. arenaria, M. incognita, Heterodera glycines,* and *Radopholus similis.* Three concentrations of tannic acid, 0.1, 1.0, and 10 g/500 cm^3 of soil, were applied preplant (powder) and at-plant (powder and drench) into soil infested with *M. arenaria.* Tannic acid at the 1.0-g rate reduced galling compared with the untreated control, regardless of methods of application. The 0.1-g rate resulted in no reduction in galling when applied preplant but reduced galling when applied as a drench and in one of two experiments when applied at-plant. The 10-g rate was phytotoxic to tomato seedlings except when applied 7 days preplant. In the latter case, root galling was suppressed to very low numbers. In behavior studies on water agar, *Meloidogyne* second-stage juveniles were attracted to areas with an increasing tannic acid gradient. *Radopholus similis* was repelled from the tannic acid gradient in one of two experiments. There was no effect on *H. glycines.* The response of *M. arenaria* second-stage juveniles to different concentrations of tannic acid dissolved in alginate was tested. Movement behavior of the second-stage juveniles were observed at 1,000 and 10,000 µg/ml of tannic acid, but not at 10 and 100 µg/ml.

Key words: alginate, attractant, burrowing nematode, *Heterodera glycines, Meloidogyne arenaria, Meloidogyne incognita,* nematode, polyphenol, *Radopholus similis,* repellent, root-knot nematode, soybean cyst nematode, tannic acid.

Tannins are a group of water-soluble polyphenols that are able to precipitate proteins, such as gelatin, from solution. They are found in a large variety of higher plants of both herbaceous and woody types (Scalbert, 1991). Substantial accumulations of vegetable tannins may be found in almost any part of a plant (Haslam, 1989). Tannins protect some plants against herbivores (Feeny, 1976), and they are toxic to a wide variety of fungi, bacteria, and yeasts (Scalbert, 1991).

Studies on the effects of tannins on plant-parasitic nematodes are few. In an experiment where organic amendments with high polyphenol content were added to soil infested with *Meloidogyne arenaria,* tannic acid (used as a control) reduced nematode numbers but caused severe phytotoxicity to the host plant (Mian and Rodríguez-Kábana, 1982). Preliminary observations on the mode of action of tannic acid demonstrated that nematode behavior was altered by tannins on water agar (E. M. Hewlett, unpubl.). The objectives of our studies were to determine the efficacy of tannic acid on *M. arenaria* and its effects on the behavior of four species of plant-parasitic nematodes.

MATERIALS AND METHODS

The nematode populations used in these studies originated from greenhouse cultures maintained at the University of Florida, Gainesville, and from carrot tissue cultures from the USDA Laboratory in Orlando, Florida. *Meloidogyne arenaria* and *M. incognita* were cultured on tomato (*Lycopersicon esculentum* cv. Rutgers). Eggs of *Meloidogyne* spp. were extracted from roots treated with 0.5% sodium hypochlorite (Hussey and Barker, 1973), caught on a 25-µm-pore sieve, rinsed, and placed on a Baermann funnel for 3 days. *Heterodera glycines* was cultured on soybean (*Glycine max* cv. Cobb). Cysts extracted from soil and roots were placed on a modified Baermann funnel for 3 days in 4 mM $ZnCl_2$ (Chen et al., 1996). Second-stage juveniles (J2) of *Meloidogyne* spp. and *H. glycines* were no more than 3 days old when used in experiments. *Radopholus similis* was grown on monoxenic carrot disk cultures (Moody et al., 1973), and the nematodes

Received for publication 7 March 1997.
[1] Florida Agricultural Experiment Station Journal Series No. R-05656.
[2] Senior Biologist and Professor, Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611-0620.
[3] High School Student, Buchholz High School, Gainesville, FL 32606.
E-mail: hewlett@gnv.ifas.ufl.edu

were washed from 9-week-old culture tubes when needed. All nematodes were then washed in 100 ml of sterile distilled water onto a 25-µm-pore sieve before inoculation onto water agar.

Efficacy of tannic acid: The soil (500 cm^3 for each treatment) was an Arredondo fine sand (94% sand, 1% silt, 5% clay; <1% organic matter). The tannic acid used was extracted from tara (*Caesalpinia spinosa*) and nut galls (species unknown) (Fisher Scientific, Fair Lawn, NJ). Tannic acid was added preplant and at-plant in a powder form, and at-plant as a drench (powder dissolved in 100 ml of water) at 0.1, 1.0, and 10 g/pot. The preplant application was made 1 day before planting in experiment 1 and 7 days before planting in experiment 2. Powdered tannic acid was thoroughly mixed into the soil before adding nematodes, whereas the drench treatment was added directly to the soil surface after the soil had been infested with nematodes and a tomato seedling added. The soil for each treatment was infested with 1,000 J2, placed in 10-cm-diameter clay pots, and a 5-week-old Rutgers tomato seedling was added. An untreated control with no tannic acid was included for each test. All treatments were replicated five times, and each experiment was repeated.

The experiments were conducted in a growth room at 26 °C, and pots were arranged in a randomized complete-block design. Plants were watered with 100 ml of water when the soil surface was dry. Plant height and number of root galls per plant were recorded 20 days after planting. Data were subjected to analysis of variance and when F values were significant means were separated with Duncan's multiple-range test.

Nematode behavior: Nematode movement and orientation on tannic acid gradients were studied on 1.7% water agar (15 ml/dish) in 100-mm-diameter disposable petri dish. Circular wells (2-cm diam.) were cut out of the agar on opposite sides near the edge of each petri plate. The plates were stored at room temperature (24 °C) for 3 days before either 0.3 ml of a 10% tannic acid solution or 0.3 ml deionized water (control) was pipetted into a well. Two days later, after the water or the tannic acid solution had been absorbed into the water agar and gradients had formed, nematodes were placed at the center of each plate in 0.05 ml of water to deliver 20 to 30 nematodes per plate. Inoculated plates were placed in a box in the dark at room temperature (24 °C). After 24 hours, nematode position on the plates was recorded with the use of a counting template (Fig. 1). The number of nematodes in the attractive and repellent zones was recorded. All four nematode species were tested twice on plates with tannic acid or water, and treatments were replicated 10 times. Data from the two zones were compared with a Chi-square test. Nematode tracks formed on the agar with tannic acid gradients were observed and compared to the sinusoidal tracks formed on water agar.

Tannic acid detection: The concentrations of tannic acid that *M. arenaria* can detect were studied in 10% Gelcrin (FMC, Philadelphia, PA) in 100-mm-diameter petri dishes. The alginate Gelcrin was used instead of water agar because tannic acid pre-

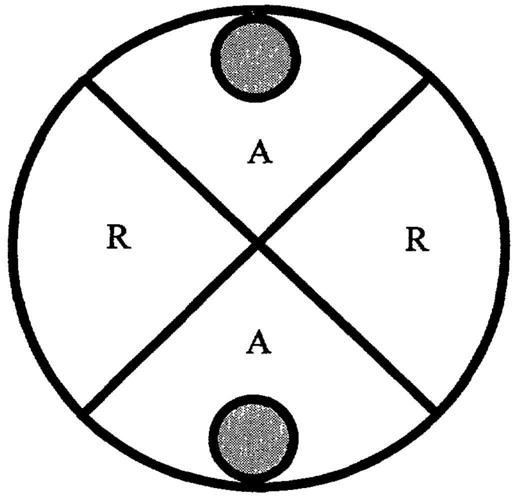

FIG. 1. Diagram of a 100-mm-diameter petri dish showing division of attractive (A) and repellent (R) zones. Circular wells, 2-cm-diameter, were cut out of the agar (1.7%, 15 ml per plate) on opposite sides near the edge of the dish, and a 10% tannic acid solution (0.3 ml) or deionized water (0.3 ml) was placed in a well and left for 48 hours to allow gradients to form. Twenty to thirty nematodes were placed in the middle of each plate, and after 24 hours their position on the plate was recorded as either in the A or R zone.

TABLE 1. Effect of tannic acid on galling of tomato root by *Meloidogyne arenaria* in two greenhouse experiments.

Tannic acid (g/500 cm^3 of soil)	Average number of root galls per root system					
	Preplant powder		At-plant powder		At-plant drench	
	Exp. 1[a]	Exp. 2[b]	Exp. 1	Exp. 2	Exp. 1	Exp. 2
0.1	57.6 a	19.2 ab	58.2 a	31.4 b	27.7 b	35.2 b
1.0	7.0 b	9.2 bc	3.6 b	30.2 b	22.8 b	33.2 b
10.0	—[c]	0.2 c	—	—	—	—
0	59.0 a	33.4 a	59.0 a	58.8 a	59.0 a	58.8 a

Tannic acid was applied at three different rates and application methods to 500 cm^3 of soil infested with 1,000 *Meloidogyne arenaria* second-stage juveniles and transplanted with tomato seedlings.

Data are means of five replicates. Means within columns with the same letter are not different according to Duncan's multiple-range test ($P \geq 0.05$).

[a] Experiment 1 preplant application was 1 day before planting.

[b] Experiment 2 preplant application was 7 days before planting, including the untreated control.

[c] Phytotoxic reaction to tannic acid killed plants.

cipitates protein in liquid agar but does not react with Gelcrin. Tannic acid was added to the Gelcrin solution after it was heated to 75 °C and allowed to cool to 45 °C to form concentrations of 0, 10, 100, 1,000, and 10,000 µg/ml. Petri plates were poured with 15 ml of the different tannic acid concentrations. Plates were stored for 48 hours at 24 °C. Fifteen to twenty *M. arenaria* J2 were added to the center of each plate in 0.05 ml of water. Plates were stored in the dark in a box at room temperature. Twenty-four hours later, nematode tracks on plates with tannic acid were compared with tracks made on control plates with no tannic acid. Treatments were replicated five times, and the test was repeated.

RESULTS AND DISCUSSION

Efficacy of tannic acid: The 10-g rate of tannic acid was phytotoxic to tomato in pot tests except when applied 7 days preplant in experiment 2 (Table 1). No differences in plant height occurred among the remaining treatments ($P > 0.05$) (data not shown). The 1-g rate resulted in reductions in root galling with all application methods ($P \leq 0.05$). Preplant and at-plant powder, and drench applications with the 1-g rate, resulted in 88%, 94%, and 61% reduction in root galling in experiment 1, and 72%, 49%, and 44% reduction in root galling in experiment 2, respectively. The 0.1-g rate reduced galling in the drench application in experiment 1 by 53% and by 40% in experiment 2.

Nematode behavior: In the behavior study, 87% and 77% (each an average of the two experiments) of *M. arenaria* and *M. incognita* J2, respectively, were present in the attractive zone as compared to 13% and 23% in the repellent zone, respectively, after 24 hours on plates with tannic acid gradients ($P \leq 0.05$) (Table 2). Eighty-one percent of *R. similis* (average of two experiments) accumulated in the repellent zone on plates with tannic acid gradients, but only in experiment 2 were there significantly more nematodes in the repellent zone than in the attractive zone ($P \leq 0.05$). No differences in the numbers of *H. glycines* J2 occurred in either zone. Control plates with water were

TABLE 2. Response of plant-parasitic nematodes to a tannic acid gradient.

Nematodes tested	Percentage of nematodes in attractive zone	
	Exp. 1	Exp. 2[a]
Meloidogyne arenaria race 1	84*	90*
M. incognita race 1	81*	72*
Radopholus similis	12	26*
Heterodera glycines	45	42

Numbers represent percentage of nematodes, placed on water agar plates with tannic acid gradients, found near the source of tannic acid attractant zone after 24 hours.

Numbers of nematodes with asterisks are significantly different from the numbers of nematodes found in the repellent zone, based on a Chi-square test ($P \leq 0.05$).

[a] Experiment 2 was a repeat of experiment 1.

consistent in that no differences were observed with any of the species tested in the number of nematodes present in either zone.

The accumulation of *Meloidogyne* J2 in the attractive zone on plates with tannic acid gradients indicates that tannic acid is an attractant to this nematode. Observations of nematode movement and tracks showed that *Meloidogyne* J2 moved up the tannic acid gradient toward the tannic acid wells. Upon reaching areas near the well, where the higher concentrations of tannic acid were present (ca. 1 cm from the well), J2 began moving in circular or spiral patterns (Fig. 2A,B) or continuously moved their bodies without moving forward or backward (Fig. 2C). Tracks left in the gradients on the agar by *Meloidogyne* J2 were deeper and wider than the sinusoidal tracks found on control agar plates (Fig. 2D) or in the repellent zone. Nematodes that formed the deeper and wider tracks were observed to move their heads side to side while turning or rolling their bodies. Sinusoidal body waves moved the bodies forward a short distance and then the head movements and body rolling repeated. These motions are similar to the shock behavior described by Green (1971), which is considered a type of orientation behavior. However, in our studies nematode movement was continuous. Klingler (1963) observed similar agar tracks made by *Ditylenchus dipsaci* with CO_2 as an attractant, and Rode and Starr (1961) illustrated wide, spiral-shaped nematode tracks

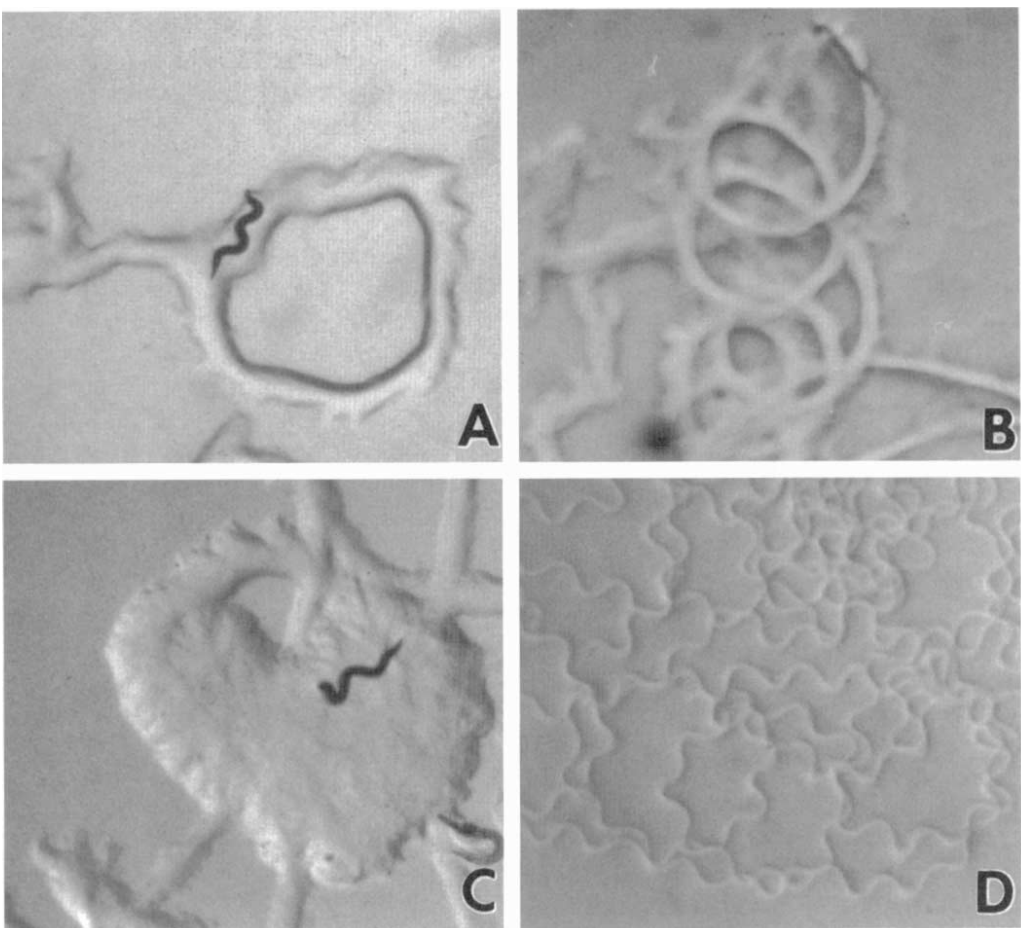

FIG. 2. Response of *Meloidogyne arenaria* race 1 to a tannic acid gradient on water agar. A) Circular tracks. B) Spiral tracks. C) Tracks formed by continuous changes in body position. D) Sinusoidal tracks on water agar without tannic acid.

on agar in an attractant study. Habituation to tannic acid was not observed on plates; however, once nematodes were in the gradient they remained in the gradient. Tannic acid caused a repellent reaction for *R. similis* in one test, and no orientation behavior tracks were observed. *Heterodera glycines* J2 were not affected by a tannic acid gradient, and their tracks were sinusoidal in the gradients.

Tannic acid detection: The tracks produced by *Meloidogyne arenaria* J2 in Gelcrin agar with 1,000 and 10,000 µg/ml of tannic acid were similar to those observed in the behavior test with tannic acid gradients on water agar (Fig. 2A-C), whereas on plates with 10 and 100 µg/ml of tannic acid only sinusoidal nematode tracks were observed. Orientation behavior of *M. arenaria* J2 was initiated at concentrations between 100 and 1,000 µg/ml of tannic acid. Studies using smaller gradations in concentrations would likely give a more accurate estimate of root-knot nematode response to tannic acid.

Few attractants for *Meloidogyne* spp. have been identified (Bird, 1959; Bird, 1960; Oteifa and Elgindi, 1961). The use of attractants or repellents as a control tactic for plant-parasitic nematodes may be a possible complement or alternative to the repeated use of nematicides. Initiation of J2 orientation behavior before planting could cause nematodes to waste energy needed for locating and parasitizing root systems. At-plant applications of attractants or repellents may serve to disorient nematodes, making it difficult for them to locate root systems. Post-plant applications via a drench or applied continuously via drip irrigation could have similar effects. Also, attraction of nematodes into areas treated with nematicides might increase the efficacy of some nematicides.

Tannins are found in a wide variety of plants. The behavioral response of *Meloidogyne* J2 to tannic acid indicated that tannins and other polyphenolic compounds may be chemical cues that *Meloidogyne* spp. use to navigate toward roots, recognize plant hosts, or locate areas for root penetration. Research to determine the specific polyphenolic compounds responsible for this orientation behavior may create new possibilities for future nematode control strategies.

Literature Cited

Bird, A. F. 1959. The attractiveness of roots to the plant-parasitic nematodes *Meloidogyne javanica* and *M. hapla*. Nematologica 4:322–335.

Bird, A. F. 1960. Additional notes on the attractiveness of roots to plant-parasitic nematodes. Nematologica 5:217.

Chen, S. Y., D. W. Dickson, and D. J. Mitchell. 1996. Population development of *Heterodera glycines* in response to mycoflora in soil from Florida. Biological Control 6:226–231.

Feeny, P. 1976. Plant apparency and chemical defense. Recent Advances in Phytochemistry 10:1–40.

Green, C. D. 1971. Mating and host finding behavior of plant nematodes. Pp. 247–266 *in* B. M. Zuckerman, W. F. Mai, and R. A. Rohde, eds. Plant-parasitic nematodes, vol. II. New York: Academic Press.

Haslam, E. 1989. Plant polyphenols: Vegetable tannins revisited. Cambridge, UK: Cambridge University Press.

Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57: 1025–1028.

Klingler, J. 1963. Die Orientierung von *Ditylenchus dipsaci* in gemessenen künstlichen und biologischen CO_2-Gradienten. Nematologica 9:185–199.

Mian, I. H., and R. Rodríguez-Kábana. 1982. Organic amendments with high tannin and phenolic contents for control of *Meloidogyne arenaria* in infested soil. Nematropica 12:221–234.

Moody, E. H., B. F. Lownsbery, and J. M. Ahmed. 1973. Culture of the root-lesion nematode *Pratylenchus vulnus* on carrot disks. Journal of Nematology 5:225–226.

Oteifa, B. A., and D. M. Elgindi. 1961. Physiological studies on host-parasite relationship of the root-knot nematode, *Meloidogyne javanica*. Plant Disease Reporter 45:928–929.

Rode, H., and G. Starr. 1961. Die photographische Darstellung der Kriechspuren (Ichnogramme) von Nematoden und ihre Bedeutung. Nematologica 6:266–271.

Scalbert, A. 1991. Antimicrobial properties of tannins. Phytochemistry 30:3875–3883.