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Nicotine Content of Tobacco Roots and Toxicity to *Meloidogyne incognita*[1]

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Abstract: The motility of *Meloidogyne incognita* second-stage juveniles (J2) and their ability to induce root galls in tomato were progressively decreased upon exposure to nicotine at concentrations of 1–100 μg/ml. EC_{50} values ranged from 14.5 to 22.3 μg/ml, but J2 motility and root-gall induction were not eliminated at 100 μg/ml nicotine. Nicotine in both resistant NC 89 and susceptible NC 2326 tobacco roots was increased significantly 4 days after exposure to *M. incognita*. The increase was greater in resistant than in susceptible tobacco. Root nicotine concentrations were estimated to be 661.1–979.1 μg/g fresh weight. More *M. incognita* were detected in roots of susceptible than in roots of resistant tobacco. Numbers of nematodes within resistant roots decreased as duration of exposure to *M. incognita* was increased from 4 to 16 days. Concentrations of nicotine were apparently sufficient to affect *M. incognita* in both susceptible and resistant tobacco roots. Localization of nicotine at infection sites must be determined to ascertain its association with resistance.

Key words: *Nicotiana tabacum*, root-knot nematode, resistance, alkaloid, *Meloidogyne incognita*, nicotine, tobacco.

Development of flue-cured tobacco, *Nicotiana tabacum* L., cultivars resistant to *Meloidogyne incognita* (Kofoid and White) Chitwood has significantly reduced tobacco losses to root-knot disease (1). The histological basis of this resistance was first described by Powell (15) for NC 95 tobacco (12). He observed that giant cells and cells surrounding infection loci exhibited a hypersensitive reaction (HR) 7 days after juveniles (J2) of *M. incognita* were injected into tobacco leaf midribs. In a similar study, significantly greater numbers of *M. incognita* J2 occurred in roots of susceptible tobacco than in resistant tobacco (16). Most juveniles apparently ceased development 14 days after penetration of resistant tobacco roots, and roots exhibited localized regions of necrotic tissue where J2 had penetrated.

Inducible biochemical defense mechanisms of plant origin, termed phytoalexins, have been implicated in the HR of some plant cultivars challenged by plant-parasitic nematodes (19). To date, no biochemical defense mechanism has been associated with the resistance of tobacco to *M. incognita*. Tobacco plants produce the alkaloid nicotine in growing root tips and readily translocate it to the shoots via xylem (18). The toxicity of nicotine to insects and other animals is well documented (13,14), but little is known about nicotine toxicity to nematodes. When *Rhabditis* spp. were exposed to 100 ppm aqueous nicotine tartrate for 48 hours, 21% died (5). *Panagrellus* spp. were totally inactivated 4–6 hours after immersion in 1,000 ppm nicotine sulfate solution (17).

Since nicotine is produced in the region of the root where *M. incognita* J2 infect (3), it may be associated with the resistance of tobacco to this nematode. In one study, the nicotine content in resistant and susceptible tobacco roots challenged by *M. incognita* was 42% and 62% greater, respectively, than in noninoculated controls (7). In a second study (8), nicotine increased 77% in resistant and 56% in susceptible tobacco roots exposed to 64 *M. incognita* eggs per 1.5 cm^3 soil. In both studies, nicotine content was determined 55 or more days after exposure to *M. incognita*. An association between nicotine and the resistance of tobacco to *M. incognita* cannot be extrapolated from these data.

Our objectives were to determine the dosage response of *M. incognita* to nicotine and to quantify nicotine in response to in-

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fection and the onset of resistance to *M. incognita* in tobacco.

Materials and Methods

A population of *M. incognita* (Race 3) isolated from a tobacco field near Live Oak, Florida, was maintained on tomato, *Lycopersicon esculentum* Mill. 'Rutgers'. A 0.5% sodium hypochlorite solution was used to extract nematode eggs from infected roots (10). Eggs were suspended on a Baermann pan and hatched at room temperature. J2 collected within 24 hours after hatching were used in these experiments.

Nicotine toxicity: Stock solutions of pure nicotine (Eastman Chemical Co., Rochester, New York) were prepared in deionized water at concentrations of 2, 10, 50, 100, and 200 μg/ml. These solutions were diluted 1:1 (v/v) with suspensions of J2 in deionized water for each bioassay. Thus, final nicotine concentrations were 1, 5, 10, 25, 50, and 100 μg/ml plus a deionized water control.

The effect of nicotine on J2 motility was examined by laboratory bioassay (11). Four milliliters nicotine stock solution and 4 ml water containing 400 ± 10 *M. incognita* J2 were added to individual glass scintillation vials (25 ml). Each vial was covered with a 17-μm-pore Nitex screen (Tobler, Ernst, and Traber, New York, New York) and incubated at room temperature for 24 hours. Vials were then inverted over petri dishes containing 8 ml solution of the same nicotine concentration. The number of juveniles that had migrated into each dish was recorded 24 hours later. Each treatment was replicated 10 times with vials arranged in a completely randomized design. Treatment values were expressed as a percentage of control values. The experiment was repeated once.

The effect of nicotine on the gall-inducing ability of *M. incognita* was examined by a modification of a technique described by Bunt (2). Glass vials (18.5 ml) were filled with 5 cm^3 quartz sand (99.3% sand, 0.3% silt, 0.3% clay). One milliliter of the appropriate nicotine solution and 1 ml water containing 500 ± 10 *M. incognita* juveniles were added to each vial. The liquid barely saturated the sand within each vial. Vials were placed in a growth chamber in the dark at 25 C. Each treatment was replicated 10 times with vials arranged in a completely randomized design.

After 24 hours, a 6–8-cm-tall Rutgers tomato seedling was transplanted into each vial. Seedling roots were washed thoroughly and trimmed to 1 cm from the base of the stem before transplanting. Vials with plants were returned to a growth chamber set for a photoperiod of 14 hours of fluorescent (260 lux) light per day and a temperature of 25 C. Ten days after transplanting, the sand was washed from each seedling root system and galls were counted at 40× magnification. Treatment values were expressed as a percentage of control values. The experiment was repeated once.

Nicotine content of tobacco roots: Two closely related flue-cured tobacco cultivars, NC 89 (resistant to *M. incognita*) and NC 2326 (susceptible to *M. incognita*), were inoculated in two greenhouse experiments by injecting aqueous suspensions of juveniles into the soil at the base of each plant.

In the first test, 6-, 8-, 10-, and 12-week-old NC 89 and NC 2326 tobacco seedlings were transplanted into 10-cm-d plastic pots containing 400 cm^3 steam-sterilized soil. Plants of different ages were used because nicotine content increases with plant age (18). Forty plants of each cultivar and age were grown in a greenhouse at 27–29 C. Two weeks after transplanting, half of the NC 89 and NC 2326 plants were inoculated each with 4,000 *M. incognita* J2. Plants were arranged in a completely randomized design on a greenhouse bench. The experiment was a 2 × 2 × 2 × 4 factorial with two cultivars, two inoculation treatments, two durations of exposure to *M. incognita*, and four plant ages.

Four days after inoculation, 10 inoculated and 10 noninoculated plants of each cultivar and age were selected at random. From these, five plants from each treatment were chosen at random for analysis of root nicotine content. Roots were ex-

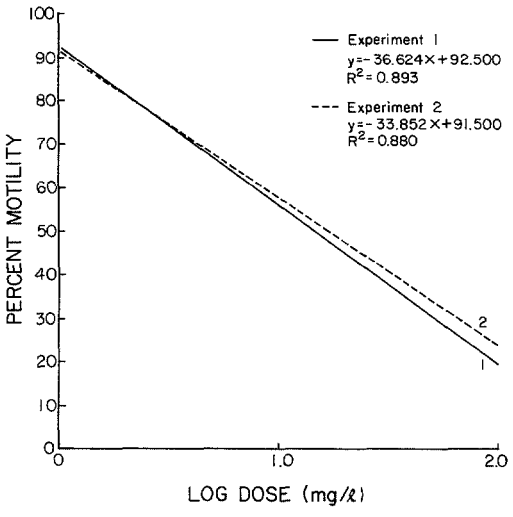

FIG. 1. Dosage-response relationship between nicotine and the motility of juveniles of *Meloidogyne incognita* in two experiments.

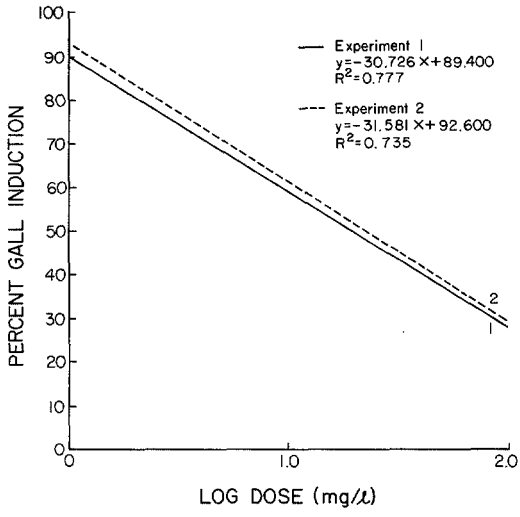

FIG. 2. Dosage-response relationship between nicotine and gall induction by juveniles of *Meloidogyne incognita* in two experiments.

cised at the crown and dried at 50 C to a constant weight, and a 200-mg sample was analyzed manually for nicotine by the procedure of Harvey et al. (9). Since chloroplasts were absent, treatment of extracted material with activated carbon was omitted. A conversion factor from dry weight to fresh weight of 9.56 was determined from 30 separate dried root systems. Nicotine percentage was converted to micrograms per gram of fresh root tissue. Nicotine concentration in fresh roots, therefore, was estimated as percentage of nicotine (dry weight) (1,046.25) = μg nicotine/g root (fresh weight).

Roots of five plants from each treatment were stained with acid fuchsin and cleared in lactophenol. One gram of stained root tissue was excised from each root system. Roots were arranged in a single layer and pressed between glass microscope slides. The number of *M. incognita* within the roots was counted at 40× magnification. After 8 days, the remaining plants were analyzed for nematode numbers and nicotine content as described above.

In the second test, 10-week-old NC 89 and NC 2326 tobacco seedlings were transplanted into 150-cm³ Conetainers (Leach Nursery, Canby, Oregon) containing steam-sterilized soil. Eighty plants of each cultivar were grown in a greenhouse at 27–29 C. Two weeks later, 40 plants of each cultivar were each inoculated with 7,500 *M. incognita* J2. Plants were arranged in a completely randomized design.

Four days after inoculation, 10 inoculated and 10 noninoculated plants of each cultivar were randomly selected, five for analysis of root nicotine content and five for determination of nematode penetration. The same procedure was followed at 8, 12, and 16 days after inoculation. The test was a 2 × 2 × 4 factorial with two cultivars, two inoculation treatments, and four durations of exposure to *M. incognita*.

RESULTS

Exposure of *M. incognita* to nicotine significantly reduced J2 motility, with the number of motile nematodes decreasing as nicotine concentration increased. Nicotine at 100 μg/ml reduced nematode motility by 73 and 71% compared with deionized water in the two experiments. Dosage-response plots of motility percentage (control = 100%) versus the log of the nicotine concentration exhibited a significant linear relationship (Fig. 1). The concentrations

TABLE 1. Numbers of *Meloidogyne incognita* in roots of resistant and susceptible tobacco 4 and 8 days after inoculation, Experiment 1.

Cultivar	Plant age (weeks)	Nematodes/g root (fresh wt.) 4 days	8 days
NC 89	8	35 ± 7	40 ± 9
(resistant)	10	62 ± 8	51 ± 9
	12	56 ± 11	29 ± 6
	14	69 ± 13	58 ± 10
NC 2326	8	233 ± 19	268 ± 17
(susceptible)	10	211 ± 19	320 ± 25
	12	129 ± 21	147 ± 21
	14	171 ± 28	286 ± 16

Mean of five replications ± standard error.

of nicotine that reduced the motility of 50% of the test organisms (EC_{50}) were 14.5 and 16.5 μg/ml in the two experiments.

Nicotine significantly suppressed tomato root-galling by *M. incognita*, and the mean number of galls per root system decreased as nicotine concentrations increased. At 100 μg/ml of nicotine, the numbers of galls per root system were suppressed 69 and 66% in the two experiments. Dosage-response plots of gall induction percentage (control = 100%) versus the log of the nicotine concentration produced a significant linear relationship (Fig. 2). The EC_{50} of nicotine was 19.2 and 22.3 μg/ml in the two experiments.

M. incognita were present in roots of inoculated resistant and susceptible tobacco in both greenhouse experiments, but root swellings were apparent only on susceptible tobacco. Most nematodes within roots of both cultivars were near the root tips, but some were observed throughout the root. The number of nematodes per gram of root was greater in susceptible than resistant tobacco roots (Tables 1, 2). Interaction of cultivar with plant age and cultivar with duration of exposure to *M. incognita* also had a significant effect on the number of nematodes per gram of root. There were fewer per gram of root in 12-week-old tobacco in Experiment 1, particularly in susceptible tobacco. In susceptible tobacco, the number of *M. incognita* per gram of root increased as time of exposure to *M. incognita* increased from 8 to 16 days. In contrast, numbers of nematodes in roots of resistant tobacco decreased as exposure time increased from 8 to 16 days.

In both greenhouse experiments, the nicotine level was higher in roots of *M. incognita*-inoculated resistant and susceptible tobacco than in noninoculated controls (Tables 3, 4). Nicotine content was higher in inoculated resistant tobacco roots than in inoculated susceptible tobacco. Nicotine content of tobacco roots was positively correlated with plant age (0.379), but not with the duration of exposure to *M. incognita* nor to the number of nematodes per gram of root.

DISCUSSION

Nicotine mimics the action of acetylcholine in insects, and the activity is readily reversible (14). The general effect of nicotine may be similar to that of carbamate nematicides in that neuromuscular and chemotactic activity is impaired, resulting in reduction of nematode movement and root invasion (4). Motility of *M. incognita* and amount of root-gall induction were inversely related to nicotine concentration; however, a 100% reduction in mean nematode motility or gall induction did not occur at any concentration examined.

TABLE 2. Numbers of *Meloidogyne incognita* observed in roots of resistant and susceptible 12-week-old tobacco at 4, 8, 12, and 16 days after inoculation, Experiment 2.

Cultivar	Nematodes/g root (fresh wt.) 4 days	8 days	12 days	16 days
NC 89 (resistant)	94 ± 10	101 ± 7	42 ± 4	23 ± 6
NC 2326 (susceptible)	317 ± 13	296 ± 15	409 ± 17	440 ± 22

Mean of five replications ± standard error.

TABLE 3. Nicotine content of inoculated and noninoculated resistant NC 89 and susceptible NC 2326 tobacco roots 4 and 8 days after inoculation with *Meloidogyne incognita*, Experiment 1.

Treatment and cultivar	Plant age (weeks)	Nicotine % (dry wt.)		Nicotine μg/g (fresh wt.)*	
		4 days	8 days	4 days	8 days
Inoculated					
NC 89 (resistant)	8	0.72 ± 0.02	0.74 ± 0.09	754.2	777.2
	10	0.84 ± 0.03	0.76 ± 0.03	878.6	797.9
	12	0.77 ± 0.04	0.72 ± 0.02	800.2	754.2
	14	0.94 ± 0.04	0.83 ± 0.04	979.1	870.3
NC 2326 (susceptible)	8	0.65 ± 0.05	0.65 ± 0.01	675.7	675.7
	10	0.65 ± 0.05	0.67 ± 0.03	684.1	699.8
	12	0.68 ± 0.05	0.68 ± 0.02	715.5	715.5
	14	0.74 ± 0.01	0.76 ± 0.03	777.2	792.9
Noninoculated					
NC 89 (resistant)	8	0.64 ± 0.06	0.65 ± 0.20	673.6	674.7
	10	0.66 ± 0.10	0.66 ± 0.20	691.4	690.4
	12	0.69 ± 0.02	0.70 ± 0.71	729.1	731.2
	14	0.76 ± 0.03	0.76 ± 0.03	791.8	795.0
NC 2326 (susceptible)	8	0.63 ± 0.09	0.63 ± 0.03	661.1	662.1
	10	0.65 ± 0.01	0.65 ± 0.05	675.1	675.1
	12	0.68 ± 0.06	0.69 ± 0.01	715.5	716.5
	14	0.73 ± 0.03	0.74 ± 0.02	767.8	769.9

Mean of five replications ± standard error.
* Calculated as % nicotine (dry wt.) (1,046.25).

Juveniles treated with 100 μg/ml nicotine exhibited no symptoms of paralysis although nematode motility was reduced. Paralysis or death may be a function of the nicotine concentration permeating into the body cavity and exposure time. Even though J2 motility and orientation may have been impaired, random movement may have resulted in their passing through the nylon screen. A treatment that completely inhibited nematode motility would have been desirable. Likewise, high inoculum density could result in some J2 contacting and penetrating roots simply by chance. Root penetration may have protected the nematodes from external nicotine exposure and allowed alteration of nicotine effects.

The nicotine content of tobacco roots significantly increased within 4 days of initial exposure of roots to *M. incognita*. This increase was greater in resistant NC 89 than in susceptible NC 2326 tobacco. Hanounik and Osborne (7), however, reported a greater increase in nicotine in susceptible than in resistant tobacco roots when challenged by *M. incognita*. Since they analyzed nicotine content 55 days after inoculation, the greater increase in susceptible roots was attributed to reduced translocation of nicotine from roots to shoots within heavily galled root systems of the susceptible tobacco. In the experiments reported here, root damage may not have been extensive enough to significantly affect the translocation of nicotine.

Increase in exposure time to *M. incognita* had no effect on the nicotine content of tobacco roots. The increase in nicotine content with plant age is consistent with a previous report (18). Lack of a correlation between nematodes per gram of root and nicotine percentage suggests that the nicotine content of tobacco roots is independent of the number of nematodes in root tissue. Any initial nematode attack may work as a stimulus for increased overall nicotine production in roots, and the response occurs within 4 days.

The increase in nematode numbers per gram of root in susceptible tobacco and the decrease in resistant tobacco with longer exposure to *M. incognita* agrees with another report (16). Failure to detect de-

TABLE 4. Nicotine content of *Meloidogyne incognita*-inoculated and noninoculated 12-week-old resistant NC 89 and susceptible NC 2326 tobacco roots, Experiment 2.

Treatment and cultivar	Nematode exposure time (days)	Nicotine % (dry wt.)	Nicotine µg/g root (fr. wt.)*
Inoculated			
NC 89	4	0.81 ± 0.01	849.4
(resistant)	8	0.89 ± 0.04	929.9
	12	0.81 ± 0.06	833.7
	16	0.83 ± 0.06	867.1
NC 2326	4	0.69 ± 0.03	722.8
(susceptible)	8	0.69 ± 0.04	719.6
	12	0.70 ± 0.03	733.2
	16	0.71 ± 0.03	743.7
Noninoculated			
NC 89	4	0.68 ± 0.04	706.1
(resistant)	8	0.69 ± 0.08	725.9
	12	0.69 ± 0.06	717.6
	16	0.73 ± 0.03	765.7
NC 2326	4	0.67 ± 0.03	700.8
(susceptible)	8	0.67 ± 0.03	704.0
	12	0.68 ± 0.02	708.1
	16	0.69 ± 0.02	717.6

Mean of five replications ± standard error.
* Calculated as % nicotine (dry wt.) (1,046.25).

teriorated juveniles or the exit of nematodes from within resistant roots (6,16) may account for these results. The generally greater number of nematodes per gram of root in Experiment 2 is probably the result of a fivefold increase over Experiment 1 in initial inoculum density.

Nematodes grow and reproduce in susceptible tobacco roots even in the presence of nicotine at apparently much higher concentrations than those found to be toxic in laboratory tests. Nicotine was extracted from entire root systems, however, and localized concentrations of nicotine within roots were not determined. In resistant tobacco roots, a localized increase in nicotine at the site of infection may be sufficient to impair normal nematode feeding activity resulting in giant cell collapse and cessation of growth and development of *M. incognita.*

Our data indicate that nicotine may be involved in resistance of tobacco to *M. incognita,* but further studies are necessary to substantiate this suggestion. Other alkaloids such as nornicotine and anabasine may also be involved in tobacco resistance to *M. incognita* and should be investigated.

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