Influence of Meloidogyne incognita on the content of Amino Acids and Nicotine in Tobacco Grown under Gnotobiotic Conditions[1]

S. B. HANOUNIK and W. W. OSBORNE[2]

Abstract: Seedlings of *Meloidogyne incognita*-resistant (N.C. 95) and -susceptible (McNair 30) tobacco cultivars were grown aseptically for 55 days inside isolator chambers in autoclaved soil infested with 0 or 3,000 axenized eggs of *M. incognita* per 500 cc of soil. Healthy and infected plants were compared. Dry root weights of infected plants of resistant and susceptible cultivars were 16% and 84%, respectively, less than the controls. Sixteen amino acids, including those precursors for nicotine, and nicotine, increased significantly in infected roots of both cultivars. Increases in amino acids in infected roots ranged from 28% for valine to 103% for tyrosine in the resistant N.C. 95, and from 30% for leucine to 148% for tyrosine in the susceptible McNair 30. Nicotine content (dry weight basis) increased 42% and 62% in infected roots of resistant and susceptible cultivars, respectively. Nematode infection increased nicotine by 112% in leaves of N.C. 95, and decreased it by 56% in leaves of McNair 30. Root damage by *M. incognita* probably decreased nicotine movement into leaves of McNair 30. In N.C. 95, nicotine movement into leaves apparently was not adversely affected due to lack of significant root damage. *Key Words:* root-knot nematode, *Nicotiana tabacum.*

Tumorigenesis is one of the earliest plant responses to root-knot infections. Many experiments have been conducted to study the physiology and biochemistry of these local tumors, yet data on the metabolic changes of galls induced by root-knot nematodes on tobacco, *Nicotiana tabacum* L., are very limited. Owens and Specht (19) reported that root-knot galls induced by *Meloidogyne incognita* in tomato (*Lycopersicon esculentum* Mill.) contained higher levels of free amino acids, proteins, and nucleic acids. Hunter (7), Shafiee and Jenkins (24), and Shafiee (23) showed that higher concentrations of nitrogen, phosphorus, and potassium occurred in root-knot infected than in healthy tomato roots. Otiefa and El-Gindi (18) showed that galled tissues absorb and accumulate nutrients, but that heavily galled roots cannot translocate adequate amounts of nutrients to vegetative organs. Foliage of tomato plants infected with *M. incognita* have been shown to contain lower concentrations of nitrogen, phosphorus, potassium, sodium, calcium, and magnesium than healthy plants (14).

Nicotine, the principle tobacco alkaloid, plays a central role in tobacco quality (1, 6, 27). In most commercial tobacco cultivars, nicotine is generally recognized as the most important alkaloid which provides physiological stimulation to consumers. Tracer experiments have shown radioactivity in nicotine molecules from glutamic acid (9) and from proline (10). However, Lovkova and Il'in (12) demonstrated that the rate of incorporation of ^{14}C labeled amino acids into nicotine follows the order: glutamic acid, aspartic acid, arginine, proline, leucine, valine, serine, phenylalanine, alanine, histidine, lysine, and threonine.

Nicotine synthesis is almost wholly localized in tissue near the root tip, and the largest root system accordingly produces the largest total amount of nicotine (27). Larvae of root-knot nematodes penetrate tobacco roots mainly behind the root tips where they induce gall formation and develop into mature females (13). It is possible, therefore, that root-knot nematodes may influence nicotine synthesis and translocation because injury is involved at the site of nicotine synthesis.

The objective of this investigation was to study the influence of infection by *M. incognita* on total amino acids and nicotine content of tobacco grown under gnotobiotic conditions.

MATERIALS AND METHODS

Seedlings of *M. incognita*-resistant (N.C. 95) and -susceptible (McNair 30) tobacco cultivars were grown aseptically (Fig. 1) for 55 days inside an isolator chamber (Standard Safety Equipment Company, Palatina, Illinois 60067) in autoclaved soil infested with 0 or 3,000 axenized eggs of *M. incognita* per 500 cc soil. A split plot design with four replications and three plants per treatment was employed. Three isolator chambers were used. One chamber was used to grow tobacco

Received for publication 22 August 1974.

[1]Contribution No. 290 from the Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, Blacksburg.

[2]Plant Pathologist and Director of the Tobacco Research Station, Syrian Tobacco Monopoly, SYRIA–Lattakia; and Professor of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, Blacksburg 24061, respectively.

FIG. 1. Tobacco plants inside the isolator chamber. Legend: inlet air filter (A); entry port (B); cylinder (C) attached to the entry port of the chamber through sleeve (S).

seedlings, and two for the actual experiments. Each isolator chamber was made of 0.305-mm (12-mil) clear vinyl, and measured 120 × 118 × 60 cm. Glove ports were attached to the sides for manipulation of the materials inside the chamber. Air-filter ports were fitted at each end and an entry port at one end. The inlet air was forced through a filter and into the chamber by a blower, and was exhausted through a second filter at the opposite end of the chamber. Before use, the openings of the air filters were sealed with mylar tape and sterilized with dry heat at 150 C for 4 h. Sterile filters were attached to the filter ports of the chamber and the inside of the chamber was sprayed with 600 ml of 2% aqueous solution of peracetic acid (3, 11, 15, 25). After 12 h the mylar seals on the filters were broken and the blower was run for 96 h to exhaust the peracetic acid.

All materials to be introduced into the chambers, except living plants and nematode eggs, were sterilized by heat (2, 3, 4). The chambers were checked for possible contamination at 15-day intervals by making cotton swab cultures on four different culture media.

Tobacco seeds were obtained from closed pods of greenhouse-grown plants to avoid seed contamination which may occur under field conditions. Seeds were soaked for 20 min in a 1:2 (v/v) dilution of 5.25% commercial sodium hypochlorite, and transferred with sterile 1-ml pipettes to test tube slants of agar dextrose medium (15 g agar and 10 g of dextrose to one liter of water). After 10 days, caps of contamination-free tubes were tightened and placed inside the entry port of the chamber. The entry port of the chamber was closed and the inside sprayed with peracetic acid. Twelve h later, the tubes were moved into the chamber, unscrewed, filled with water, shaken till the germinated tobacco seeds were brought into suspension. The suspension was sprayed on the surface of Weblite® (expanded shale, Weblite Corporation, Roanoke, Virginia 24065) contained in aluminum pans and moistened with half-strength Hoagland solution. The germinated seeds grew inside this chamber for 65 days in continuous light from cool white fluorescent lamps at an intensity, 20 cm above the plants, of 10,545 lux (980 ft-c) at a temperature of 28 ± 1 C. This chamber was designated as the seedling chamber. At the end of 65 days, when seedlings averaged about 10 cm high, they were transferred to the connected plant chamber, which was sterilized by methods mentioned previously.

Seedlings of resistant and susceptible cultivars were selected for uniformity, removed with as little root damage as possible, and transplanted (one per beaker) into 600-ml beakers. The beakers had a 1-cm

hole in the bottom for aeration, and contained 500 cc of a sandy clay loam/Weblite (1:1, v/v) mixture. The mixture has been washed in tap water for 48 h and autoclaved 12 h at 1.0 atmosphere pressure (15 psi) and 121 C. After 20 days, eggs of *M. incognita* were obtained from 45-day-old tomato plants using the Hussey and Barker method (8). These eggs were obtained from the progeny of a single female removed from a tobacco plant and propagated on 'Rutgers' tomato in a greenhouse. They were identified as *M. incognita* by the perineal pattern characteristics (20, 26) and the differential host reaction (20, 21, 22). These eggs were washed with 12 liters of sterile distilled water, rinsed into two sterile bottles, and introduced into the plant chambers as described previously. Each plant was watered, pulled up gently, 3,000 eggs added and mechanically mixed with the soil and the plant replanted. Plants were grown in continuous light from cool-white fluorescent lamps at an intensity (20 cm above the plants) of 9,684 lux (900 ft-c) at a temperature of 27 ± 1 C. Each beaker received 100 ml of water every other day. Half-strength Hoagland's solution was added to the soil at the rate of 100 ml per beaker (replacing one watering) per week.

Fifty-five days after inoculation, the entire root system of inoculated and uninoculated tobacco plants were collected from each cultivar per replicate per treatment. These roots were dried at 75 C to constant weight, ground, and analyzed for total amino acids (16, 17) and nicotine content (5). Leaves number 7, 8, and 9 (from bottom) were harvested from each cultivar per replicate per treatment, dried and ground in the same manner, and analyzed for nicotine (5).

Data were subjected to analysis of variance and Duncan's multiple range test.

TABLE 1. Influence of *Meloidogyne incognita* on dry weight of roots and nicotine content of leaves number 7, 8 and 9 (from bottom) of resistant (N.C. 95) and susceptible (McNair 30) tobacco cultivars under gnotobiotic conditions.

	Roots			Leaves: Nicotine content		
	Dry weight (g)		Weight decrease	(%, dry weight basis)		More (+) than or less (−) than healthy
Cultivar	Healthy	Infected	(%)	Healthy	Infected	(%)
McNair 30	6.5	1.0**[a]	84	0.245	0.107**	−56
N.C. 95	6.0	5.0 ns	16	0.220	0.467**	+112

[a]** = Significant, $P = 0.01$, and ns = nonsignificant.

TABLE 2. Influence of *Meloidogyne incognita* on amino acids and nicotine content in roots of the resistant (N.C. 95) and susceptible (McNair 30) tobacco cultivars.

	Amino acid and nicotine analysis (%, dry weight basis)					
	N.C. 95			McNair 30		
Component	Healthy	Infected	Increase over healthy[a] (%)	Healthy	Infected	Increase over healthy (%)
Glutamic acid	0.522	0.783	50**	0.448	0.750	67**
Aspartic acid	0.580	0.750	29**	0.512	0.687	34**
Arginine	0.180	0.300	67**	0.142	0.260	83**
Proline	0.273	0.409	50**	0.248	0.517	108**
Leucine	0.369	0.515	40**	0.376	0.489	30**
Valine	0.299	0.383	28**	0.265	0.361	36**
Serine	0.287	0.379	32**	0.266	0.350	32**
Phenylalanine	0.252	0.341	35*	0.231	0.327	42*
Alanine	0.300	0.435	45**	0.262	0.387	48**
Histidine	0.078	0.119	53**	0.077	0.116	51**
Lysine	0.364	0.487	34**	0.316	0.694	120**
Threonine	0.257	0.360	40**	0.221	0.356	61**
Glycine	0.269	0.374	39**	0.228	0.384	68**
Metheonine	0.076	0.146	92**	0.087	0.155	78**
Isoleucine	0.213	0.294	38**	0.220	0.302	37**
Tyrosine	0.104	0.212	104**	0.090	0.224	149**
Nicotine	0.350	0.500	42**	0.230	0.372	62**

[a]** = Significant, $P = 0.01$; and * = significant, $P = 0.05$.

RESULTS

Healthy and infected plants of both cultivars were compared. Dry root weights of infected N.C. 95 and McNair 30 tobacco plants were 16% and 84%, respectively, less than the controls (Table 1). However, amounts of 16 amino acids, including those precursors for nicotine, and nicotine, were significantly greater in infected than in healthy roots of N.C. 95 and McNair 30 (Table 2). Individual amino acids ranged from 29% more for aspartic acid to 104% more for tyrosine in N.C. 95, and from 30% more for leucine to 149% more for tyrosine in McNair 30. Nematode infections increased nicotine content of leaves by 112% in N.C. 95 and decreased it by 56% in McNair 30 (Table 1). These effects were significant in both cultivars.

DISCUSSION

This investigation has established that *M. incognita* influenced the amino acid and nicotine content of the resistant (N.C. 95) and susceptible (McNair 30) tobacco cultivars under gnotobiotic conditions. Sixteen amino acids, including the precursors for nicotine, and nicotine, were greater in infected than in healthy roots of the resistant N.C. 95 tobacco. This was also evident in the susceptible McNair 30 where roots were more severely galled and damaged than those of N.C. 95. Nematode infections apparently increased amino acid precursors for nicotine, which in turn seemed to have stimulated more nicotine formation through an unknown mechanism. Nicotine is formed in tobacco roots and then translocated to the leaves (27). Therefore, a higher nicotine content in the roots should result in a higher nicotine level in the leaves. This was the case if the roots were not damaged and the translocation was not impaired by nematode infections. In N.C. 95, roots were not damaged significantly and higher nicotine content in infected roots resulted in higher leaf nicotine levels, because nicotine translocation apparently was not restricted. In McNair 30, severe root damage occurred, and the nicotine content of leaves was decreased, apparently due to inadequate translocation from roots to vegetative organs.

This is the first report of the nicotine content of tobacco plants being directly related to infection by plant parasitic nematodes.

LITERATURE CITED

1. AKEHURST, B. C. 1968. Tobacco. Longmans, Green, London and Harlow, England. 551 p.
2. HALE, M. G., D. L. LINDSEY, and K. M. HAMEED. 1973. Gnotobiotic culture of plants and related research. Bot. Rev. 39:261-273.
3. HAMEED, K. M. 1971. Influence of Pencillium simplicissimum (Oud.) Thom. and Penicillium citrinum Thom. on growth, chemical composition and root exudation of axenic marigold. Ph.D. Diss., Virginia Polytech. Inst. and State Univ., Blacksburg. 143 p.
4. HANOUNIK, S. B. 1974. Population dynamics and effects of Meloidogyne incognita on tobacco plants. Ph.D. Diss., Va. Polytech. Inst. and State Univ., Blacksburg, Va. 78 p.
5. HARVEY, W. R., H. M. STAHR, and W. C. SMITH. 1969. Automated determination of reducing sugars and nicotine alkaloids on the same extract of tobacco leaf. Tob. Sci. 13:13-15.
6. HAWKS, S. N., JR. 1970. Principles of flue-cured tobacco production. S. N. Hawks, Jr., N.C. State Univ., Box 5155, Raleigh, N.C. 239 p.
7. HUNTER, A. H. 1958. Nutrient absorption and translocation of phosphorus as influenced by the root-knot nematode Meloidogyne incognita acrita. Soil Sci. 86:245-250.
8. HUSSEY, R. S., and K. R. BARKER. 1973. A comparison of methods of collecting inocula of Meloidogyne spp., including a new technique. Plant Dis. Rep. 57:1025-1028.
9. LAMBERTS, B., and R. V. BYERRUM. 1958. Glutamate as a precursor for the pyrrolidine ring in nicotine. J. Biol. Chem. 223:939-943.
10. LEET, E. 1958. Biogenesis of nicotine. V. New precursors of the pyrrolidine ring. J. Am. Chem. Soc. 80:2162.
11. LINDSEY, D. L. 1967. Growth of beans, tomatoes and corn under gnotobiotic conditions. Phytopathology 57:960-964.
12. LOVKOVA, M. Y., and G. S. IL'IN. 1963. Participation of labeled amine acids in the biosynthesis of nicotine. Biochemistry 385:612-614.
13. LUCAS, G. B. 1965. Disease of tobacco. 2nd. ed. The Scarecrow Press, New York. 778 p.
14. MAUNG, B. A., and W. R. JENKINS. 1959. Effect of root-knot nematodes Meloidogyne incognita acrita Chitwood 1949, and stubby-root nematode Trichodorus christei Allen 1957, on the nutrient status of tomato Lycopersicon esculentum hort. var. Chesapeake. Plant Dis. Rep. 43:791-796.
15. MILLER, R. H., and T. J. CHAU. 1970. The influence of soil microorganisms on the growth and chemical composition of soybeans. Plant Soil 32:146-161.
16. MOORE, S., D. H. SPACKMAN, and W. H. STEIN. 1958. Chromatography of amino acids on sulfonated polystyrene resins. Anal. Chem. 30:1185.
17. MOORE, S., and W. H. STEIN. 1951. Chromatography of amino acids on sulfonated polystyrene resins. J. Biol. Chem. 192:663.
18. OTEIFA, B. A., and D. M. EL-GINDI. 1962. Influence of parasitic duration of Meloidogyne javanica (Treub) on host nutrient uptake. Nematologica 8:216-220.

19. OWENS, R. G., and H. N. SPECHT. 1966. Biochemical alterations induced in host tissues by root-knot nematodes. Contrib. Boyce Thompson Inst. 22:181-198.
20. SASSER, J. N. 1954. Identification and host-parasite relationships of certain root-knot nematodes (Meloidogyne spp.) Maryland Agric. Exp. Stn. Bull. No. A-77. 31 p.
21. SASSER, J. N. 1967. Identification of root-knot nematodes, pages 110-111 *in* A. Kelman, ed., Sourcebook of laboratory exercises in plant pathology. W. H. Freeman, San Francisco.
22. SASSER, J. N. 1972. Physiological variation in the genus Meloidogyne as determined by differential hosts. Eur. Mediterr. Plant Prot. Organ. Bull. 6:41-48.
23. SHAFIEE, M. F. 1962. Histo- and physiopathological studies of pepper, Capsicum frutescens, infected with Pratylenchus penetrans, Meloidogyne acrita and Meloidgyne hapla. Ph.D. Dis., Rutgers Univ., New Brunswick, N.J.
24. SHAFIEE, M. F., and W. R. JENKINS. 1963. Host-parasite relationships of Capsicum frutescens and Pratylenchus penetrans, Meloidgyne incognita acrita and M. hapla. Phytopathology 53:325-328.
25. SHAY, F. J. 1971. The effect of low levels of calcium on exudation of sugars from peanut roots under gnotobiotic conditions. Ph.D. Diss., Virginia Polytech. Inst. and State Univ., Blacksburg. 81 p.
26. TAYLOR, A. L., V. H. DROPKIN, and G. C. MARTIN. 1955. Perineal patterns of root-knot nematodes. Phytopathology 45:26-34.
27. TSO, T. C. 1972. Physiology and biochemistry of tobacco plants. Dowden, Hutchinson and Ross. Stroudsburg, Pennsylvania. 393 p.