Histopathology of Selected cultivars of Tobacco infected with *Meloidogyne* species[1]

C. Sosa-Moss, K. R. Barker, and M. E. Daykin[2]

Abstract: Rates of nematode penetration and the histopathology of root infections in flue-cured tobacco cultivars 'McNair-944,' 'Speight G-28,' and 'NC-89' with either *Meloidogyne arenaria, M. incognita, M. hapla,* or *M. javanica* were investigated. Penetration of root tips by juveniles of all species into the *M. incognita*-resistant NC-89 and G-28 was much less than that on the susceptible McNair-944. Few juveniles of *M. incognita* were detected in resistant cultivars 7 and 14 days after inoculation. Infection sites exhibited some cavities and extensive necrotic tissue at 14 days; less necrotic tissue and no intact nematodes were observed 35 days after inoculation. Although some females of *M. arenaria* reached maturity and produced eggs, considerable necrosis was induced in the resistant cultivars. *Meloidogyne hapla* and *M. javanica* developed on all cultivars, but there was necrotic tissue at some infection sites in the resistant cultivars. The occurrence of single multistructured nuclei in the syncytia of most *M. hapla* infections differed from the numerous small nuclei found in syncytia caused by the other three species. *Key words: Nicotiana tabacum,* root knot, resistance, *M. arenaria, M. incognita, M. hapla, M. javanica.*

Journal of Nematology 15(3):392-397. 1983.

Root knot caused by *Meloidogyne* species is a major disease of tobacco (2,5,9,20). The development of vertical resistance (5) to the major species attacking tobacco, *Meloidogyne incognita* (Kofoid & White) Chitwood, has reduced losses to this disease in the past 25 years (2,3,9). Recent evidence (1) indicates that these cultivars may also have quantitative resistance to some populations of *M. arenaria* (Neal) Chitwood and *M. javanica* (Treub) Chitwood. In addition, these resistant cultivars may lessen the effects of root knot in predisposing the roots to invasion by other soilborne pathogens (19).

Root-knot resistant cultivars of tobacco are not problem free. Resistance may break down at temperatures of 30–35 C (6,17, 18,21). Races 2 and 4 of *M. incognita* and a newly described species, *M. grahami* Golden and Slana, reproduce on these resistant cultivars (9,10). High population densities of *M. incognita* may retard plant growth and limit yield (3). Continuous use of resistant cultivars in given fields results in populations of *M. incognita* and *M. arenaria* that readily reproduce and cause serious yield losses (K. R. Barker, unpublished).

Although the histopathology of root-knot infections in resistant and susceptible cultivars of other crops, such as tomato, have received much attention, little information of this type has been published for tobacco. For example, infection of resistant tomato by *M. incognita* causes a necrotic reaction which may be reversible by temperature (6) or by exogenous cytokinins (7). A similar hypersensitive reaction is known to occur in *M. incognita*-resistant tobacco (17; C. J. Nusbaum, unpublished).

The relative susceptibility to nematode penetration and root-tissue responses of selected tobacco cultivars to infection by *M. incognita, M. arenaria, M. hapla* Chitwood, and *M. javanica* are described herein. The comparative resistance of these cultivars to *Meloidogyne* spp. has been reported (1).

MATERIALS AND METHODS

The four nematode species used in these experiments were maintained on 'Floradel' tomato, *Lycopersicon esculentum* Mill. Eggs of given species were extracted from 50–70-day-old galled tomato roots with 1% sodium hypochlorite (14) and used as inocula at the rates indicated. Second-stage juveniles obtained from single egg-mass populations were used as inocula in a repeat of the basic experiment.

Seeds of tobacco cultivars (*M. incognita*-susceptible 'McNair-944' and *M. incognita*-resistant 'Speight G-28' and 'NC-89') were germinated in vermiculite. Seedlings of 5–

Received for publication 3 October 1982.

[1]Paper No. 8537 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh.

[2]Department of Plant Pathology, North Carolina State University, Raleigh, NC 27650. Permanent address of senior author: Centro de Fitopatologia, Colegio de Postgraduados, Chapingo, Mexico.

The technical assistance of D. W. Byrd, Jr., D. W. Corbett, Mrs. M. G. Gouge, and W. M. Williams is gratefully acknowledged.

Use of trade names in this publication does not imply endorsement of the products named or criticism of similar ones not mentioned.

10-cm heights were transplanted to 5-cm containers and grown for 2–4 weeks. After the plants were 10–15 cm high, they were transplanted to 15-cm pots containing a 1:1 mixture of sand and a loamy soil. Nutrients were provided by periodic watering with a modified commercial material "VHPF" (Miller Chemical and Fertilizer Corporation, Baltimore, Maryland). The stock solution consisted of 700 g VHPF, 123 g KNO_3, and 227 g $MgSO_4$ per 133 liters of water.

Penetration of plant roots by nematodes: The four nematode species and noninoculated controls were used to determine relative rates of penetration of the susceptible McNair-944 and resistant G-28. Each plant growing in an 8-cm pot was inoculated with 2,000 juveniles from single-egg mass populations of a given nematode species. Roots from four plants per treatment were harvested 7 and 14 days after inoculation, stained with acid fuschin, and cleared with lactophenol or glycerin. Plants were maintained in the 8-cm pots for this short-term experiment.

Histopathology: Tobacco seedlings were inoculated with 20,000 eggs of a given nematode species. Three single-plant replicates per treatment were harvested 14 and 35 days after inoculation. Representative root fractions from the penetration study (with single egg-mass populations) also were utilized in this histological study (harvest dates were 7 and 14 days).

Root segments with galls and noninoculated roots were fixed in formalin-alcohol-acetic acid, dehydrated with T-butyl alcohol series, and embedded in tissue prep (Fisher Scientific Co., Pittsburgh, Pennsylvania). Sections (12–16 um thick) were cut with a rotary microtome, mounted with Haupt's adhesive and 4% formalin, and stained with Triarch's quadruple stain (Triarch, Inc., Ripon, Wisconsin). This stain is composed of safranin, orange G, fast green, and crystal violet.

RESULTS

Penetration of roots by nematodes: All four species of nematodes penetrated each of the cultivars, but fewer entered the resistant cultivars (Table 1). Most individuals of *M. incognita* that penetrated the resistant cultivars apparently died and deteriorated by day 14.

Table 1. Numbers of juveniles of *Meloidogyne* spp. detected in roots of selected tobacco cultivars.

Nematode species	7 Days†		14 Days†	
	M-944	G-28	M-944	G-28
M. arenaria	305	12*	470	92
M. hapla	172	181	699	361
M. incognita	799	148*	594	32*
M. javanica	405	394	522	121

†M-944 (McNair-944) is *M. incognita-susceptible* cultivar, whereas G-28 (Speight's G-28) is resistant to this pest.

*Asterisk indicates number for G-28 is less ($P = 0.05$) than that for M-944 for data indicated, as based on Waller-Duncan K-ratio t-test with $Log_{10}(X+1)$ transformation. (Data are means of four replicates.)

Histopathology—Meloidogyne incognita: Juveniles had penetrated the roots of the susceptible McNair-944 cultivar by day 7 (Fig. 1 A). By day 14, this nematode induced the development of typical syncytia with many small groups of nuclei (Fig. 1 C). Occasionally, some necrosis was observed in the cortex (Fig. 1 B) and the vascular cylinder was greatly modified.

In contrast to the extensive galls induced on the susceptible McNair-944, no root galls were evident on the resistant G-28 and NC-89. Nematodes caused an extensive necrotic reaction with little or no development of syncytia (Fig. 1 B). There was more necrotic tissue observed at 14 than at 35 days after inoculation. Cavities observed in the 14-day-old root tissue from nematode infection were rarely evident at 35 days. A few nematodes were detected in the necrotic tissue at 35 days (Fig. 1 D), but they were in poor condition or were dead.

Meloidogyne arenaria: This nematode species reproduced in all three tobacco cultivars tested but was most aggressive in the susceptible cultivar McNair-944 under greenhouse conditions. Giant cells were quite large, round or oval in shape, and some were lobed (Fig. 1 E). Many small groups of dispersed nuclei were generally present in a given syncytium. Plasmodesmata between giant cells were very conspicuous, and extensive hyperplastic tissue also was evident.

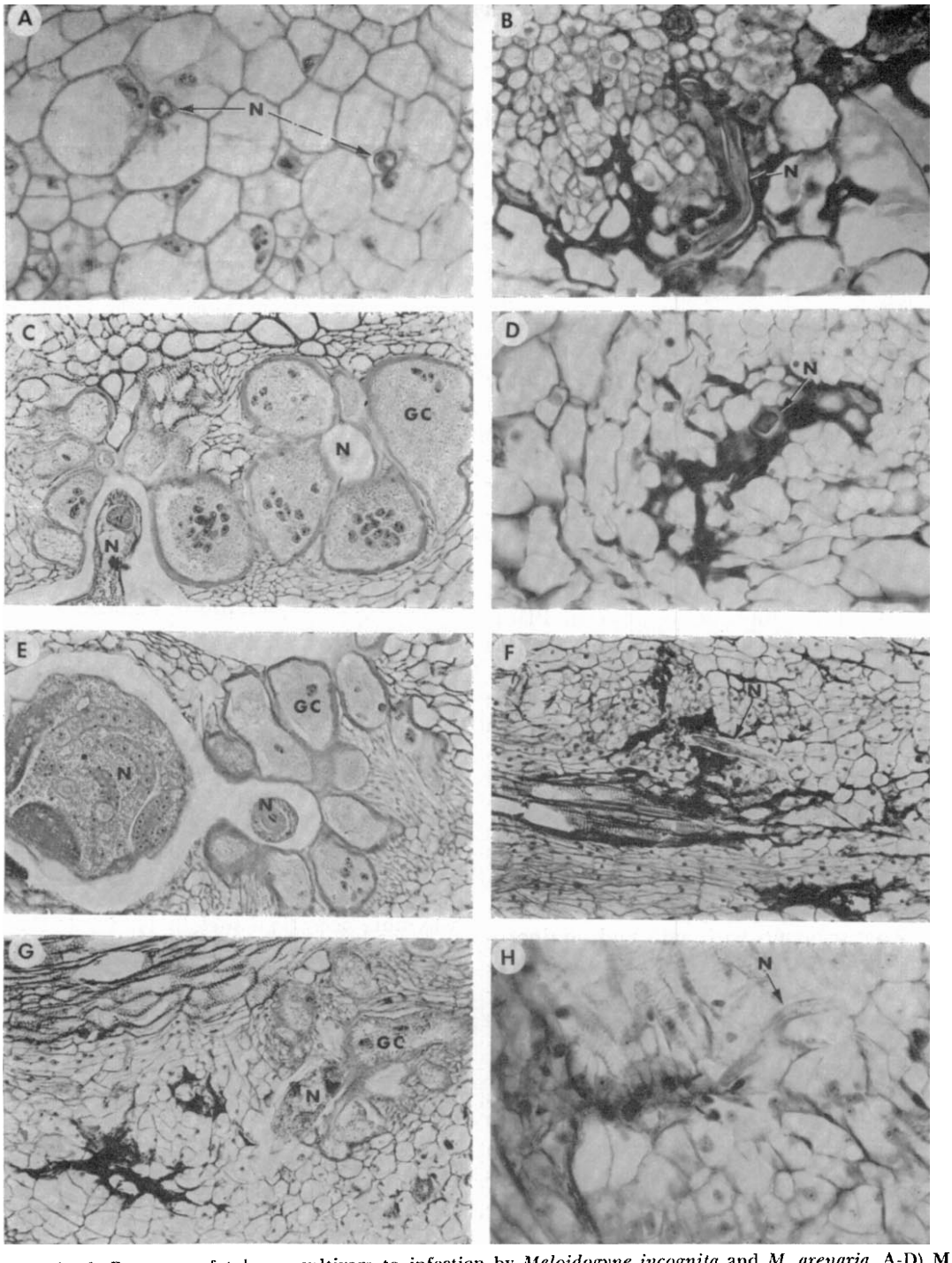

Fig. 1. Response of tobacco cultivars to infection by *Meloidogyne incognita* and *M. arenaria*. A-D) M. *incognita:* A) McNair-944, 7 days after inoculation with juveniles (J-2). B) Speight G-28, 14 days after inoculation (J-2). C) McNair-944, 14 days after inoculation D) Speight G-28, 35 days after inoculation. E-H) *M. arenaria:* E) McNair-944, 14 days after inoculation. F, G) Speight G-28, 14 days after inoculation. H) NC-89, 14 days after inoculation (J-2). Inocula for A, B, D, and H were juveniles from single egg-mass cultures and those for others were eggs from mass cultures). GC = giant cell; N = nematode.

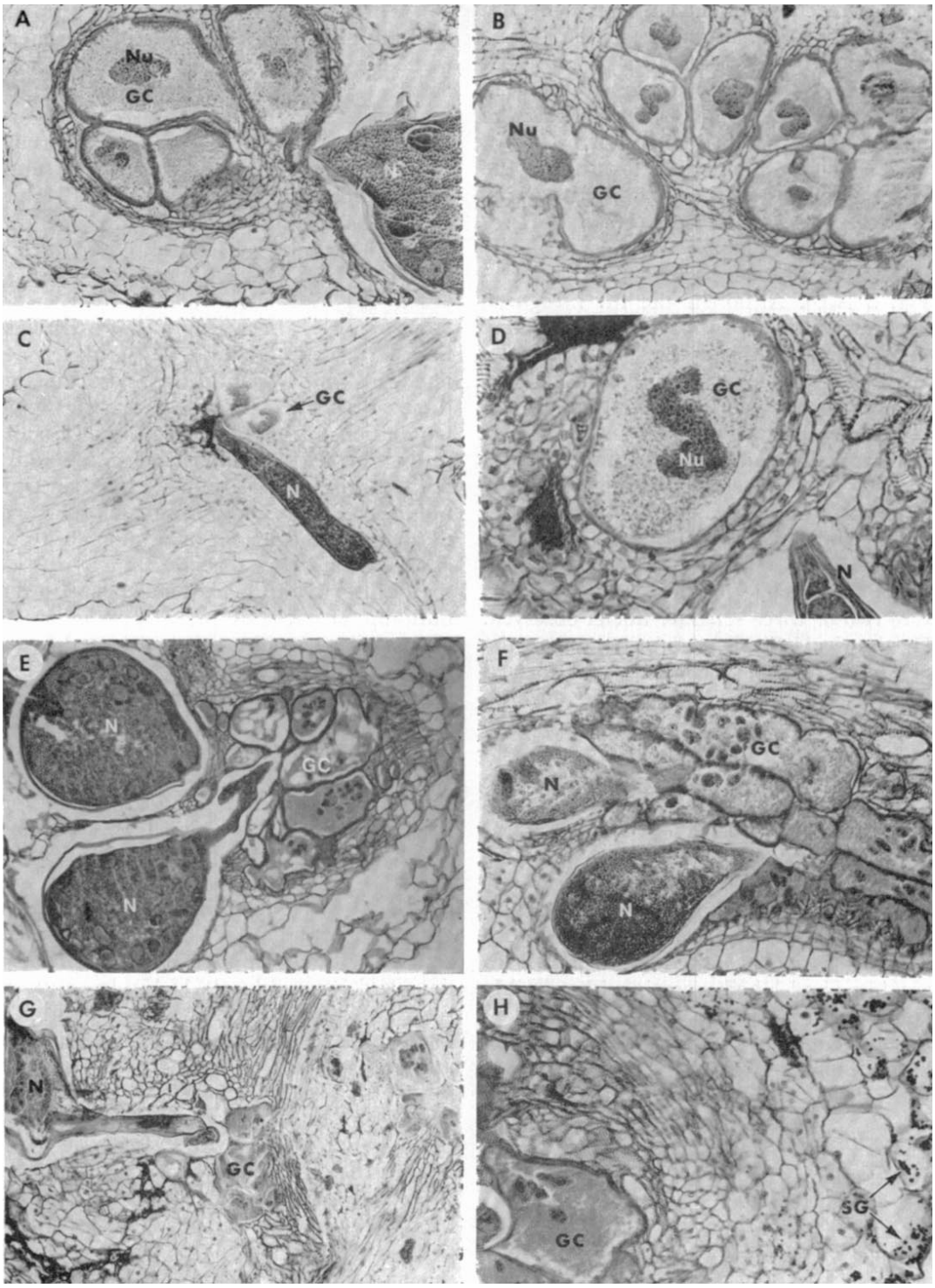

Fig. 2. Reaction of tobacco cultivars to *Meloidogyne hapla* and *M. javanica*. A-D) *M. hapla:* A, B) McNair-944, 35 days. C, D) NC-89, 14 days (note the multi-structured single nucleus per syncytium). E-H) *M. javanica:* E) McNair-944, 35 days after inoculation. F) Speight G-28, 14 days after inoculation. G) Speight G-28, 35 days after inoculation. H) NC-89, 35 days after inoculation. GC = giant cell; N = nematode; Nu = nucleus. (Note slight necrosis in G and H and apparent accumulation of starch granules [SG] in H.)

The reactions of the cultivars G-28 and NC-89 to *M. arenaria* were similar. Many nematodes in the root tissues had unusual shapes and were poorly developed (Fig. 1 F–H). Furthermore, some of the syncytia were small and in the cortical tissue instead of the vascular cylinder. Infection sites in the resistant cultivars often exhibited varying amounts of necrotic tissue (Fig. 1 F–H).

Meloidogyne hapla: M. hapla readily penetrated and developed in McNair-944 (Fig. 2 A,B). Although the *M. incognita*-resistant cultivars supported considerable reproduction of this species, some females developed very slowly (Fig. 2 C). Nevertheless, numerous egg masses were visible in all three cultivars.

Transverse and tangential root sections of each infected cultivar usually showed 2–5 syncytia that were oval or pear shaped. These syncytia had an unusual characteristic clumping of the nuclear material into one or two nuclei (Fig. 2 A,D). Only rarely were two or three separate nuclei observed. The hyperplastic tissue continguous to the giant cells was very compact and formed by many small cells each having one nucleus. Some necrosis was observed in the root tissue of all cultivars. The cortex and vascular tissue were very disorganized, especially where many females were observed at sites of root branching.

Meloidogyne javanica: Large numbers of egg-masses of this nematode were evident in the roots of all three cultivars at 35 days. Transverse and tangential sections of infected roots showed many small to very large and lobed syncytia with a highly altered vascular system (Fig. 2 E,F). The resistant cultivars G-28 and NC-89 usually showed some necrotic reaction in cortical tissues (Fig. 2 G,H) but not in all instances (Fig. 2 F). These necrotic areas were adjacent to the infection loci of the nematodes. Giant cells developed poorly in some roots of G-28 and NC-89.

DISCUSSION AND CONCLUSIONS

The hypersensitive reaction to *M. incognita* in the resistant cultivars was similar to that of other resistant crops (6). The lower rate of nematode penetration in the resistant cultivars also is in general agreement with those obtained for tomato (11, 16). The disappearance of juveniles in the resistant cultivar over time is in agreement with the findings of Hadisoeganda and Sasser (11) for *M. incognita* on resistant tomato.

A hypersensitive host reaction occurs after penetration by many nematodes (8) as well as other plant parasites (23). Nevertheless, a hypersensitive reaction of the host is not a general feature of vertical resistance when host-pathogen specificity is absent (24). The slight to moderate necrosis observed in *M. incognita*-resistant cultivars infected by *M. arenaria* apparently is related to some quantitative resistance in these cultivars to the latter nematode. Some populations of *M. arenaria* induce galls readily in the greenhouse but cause only limited root galls in the field (Barker, unpublished); this resistance may be sufficient to limit reproduction in a more natural environment. However, continuous use of these cultivars in the field may result in the build-up of races of *M. incognita* or *M. arenaria* that could cause severe damage (Barker, unpublished). G-28 and NC-89 apparently have insufficient resistance to *M. javanica* and *M. hapla* to give effective field resistance.

The different shapes and structures of the syncytia induced by the four *Meloidogyne* species in tobacco may provide some insight into the origin and development of syncytia. The compact, rounded syncytia associated with *M. incognita* and *M. hapla* appear to be the result of increased nuclear activity without subsequent cytokinesis as described previously (12,13,15). In contrast, the formation of very extensive, highly lobed giant cells associated with *M. arenaria* and *M. javanica* on tobacco may involve cell wall dissolution and coalescence of cytoplasm. Both types of development may be functioning as previously suggested (4). Multi-structured syncytial nuclei similar to those occurring in *M. hapla-infected* tobacco occur in onion parasitized by this nematode (22). This restricted number of nuclei could be related to the limited root-gall development associated with *M. hapla*.

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