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Penetration of Susceptible and Resistant Tobacco Cultivars by *Meloidogyne* Juveniles[1]

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Abstract: Rates of penetration of *Meloidogyne incognita, M. arenaria,* and *M. javanica* into tobacco cultivars NC2326 (susceptible to all three species) and K399 (resistant to *M. incognita*) and a breeding line that had been selected for resistance to *M. incognita* were compared. *Meloidogyne incognita* penetrated NC2326 rapidly during the first 24 hours after inoculation. Numbers of *M. incognita* continued to increase gradually through the 14-day experiment. Higher numbers of *M. incognita* were observed in the roots of K399 during the first 24 hours than were observed in NC2326. The number of *M. incognita* in K399 peaked 4 days after inoculation, then declined rapidly as the nematodes that were unable to establish a feeding site left the root or died. Numbers of *M. incognita* in the breeding line followed the same pattern as with K399, but in lower numbers. Numbers of *M. arenaria* showed little difference between cultivars until 7 days after inoculation, then numbers increased in NC2326. Numbers of *M. javanica* fluctuated in all cultivars, resulting in patterns of root population different from those observed for *M. incognita* or *M. arenaria.* Resistance to *M. incognita* appears to be expressed primarily as an inability to establish a feeding site rather than as a barrier to penetration. Some resistance to *M. arenaria* may also be present in K399 and the breeding line.

Key words: Meloidogyne arenaria, Meloidogyne incognita, Meloidogyne javanica, model, *Nicotiana tabacum,* penetration, regression, resistance, tobacco.

Mechanistic crop production models are based on our understanding of the physiology of the plant and economically important pests. In order to link plant and pest models in a realistic manner, the plant–pest interactions must be quantified. Determining an adequate mathematical representation of these dynamic interactions is a crucial component in the construction of crop production models.

Penetration by *Meloidogyne* spp. does not differ in susceptible and resistant cultivars of some crops (7–9; C. Opperman, pers. comm.). Although the nematodes penetrate resistant cultivars, the majority are unable to establish feeding sites and may leave the root (4,6,8,13). Of those nematodes that do establish a feeding site, development of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 and *M. arenaria* (Neal, 1889) Chitwood, 1949 does not differ in susceptible and resistant varieties, but development of *M. javanica* (Treub, 1885) Chitwood, 1949 does (5,9; C. Opperman, pers. comm.). Resistant tobacco cultivars are considered to be resistant only to *M. incognita,* although some resistance to *M. arenaria* may also be present (1,12). *Meloidogyne incognita* and *M. javanica* have been found to penetrate susceptible and resistant crop cultivars in higher numbers than *M. arenaria* (12; C. Opperman, pers. comm.). The synthesis of nicotine in tobacco roots may also be involved in resistance to root knot as the increase in nicotine content after infection by root-knot nematodes is greater in resistant cultivars than in susceptible (4).

The objective of this work was to compare the penetration of *M. incognita, M. arenaria,* and *M. javanica* into the roots of two *Nicotiana tabacum* L. cultivars—'NC2326' (susceptible to all three root-knot species) and 'K399' (resistant to *M. incognita*)—and a breeding line. The breeding line, the product of an interspecific hybridization between *N. tabacum* cv. NC2326 and *N. tomentosa* Ruiz & Pavon acc. 58, was selected for resistance to *M. incognita. Nicotiana tomentosa* has been suggested as the source of resistance to *M. incognita* in tobacco; however, the exact relationship is still in question (11).

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Materials and Methods

Seeds of tobacco cultivars NC2326 and K399 and a breeding line, RKN (BC_3) F_2 W/82-1, were germinated in sterile vermiculite. Six weeks after sowing, seedlings were transplanted to plastic tubes (5 cm d × 15 cm long) covered at one end with fine mesh cloth to confine the roots and filled with a steamed loamy sand (89% sand, 1% silt, 10% clay).

Populations of *M. incognita* (race 1), *M. arenaria* (nonpeanut race), and *M. javanica* were cultured in the greenhouse on tomato, *Lycopersicon esculentum* Mill. cv. Rutgers, plants. Heavily galled roots were placed on Baermann funnels in a mist chamber. Juveniles collected in the first 24 hours were discarded. Freshly hatched juveniles were collected and inoculum was calibrated.

Plants were inoculated 3 weeks after transplanting to allow injuries due to transplanting to heal before inoculation. One milliliter of inoculum was introduced into each of two holes, 4 cm deep, one on either side of the plant. Inoculum contained 1,481 ± 120 *M. arenaria*, 1,053 ± 172 *M. incognita*, or 943 ± 127 *M. javanica* second-stage juveniles per tube. Tubes were watered gently and embedded in a 0.75-cm-thick layer of sand on a heating mat in a completely randomized design. A heating mat sensor was embedded in a tube at the average root depth and the thermostat was set to 26 C. The soil temperature averaged 25.5 ± 2.3 C over the course of the experiment. Plants were watered and fertilized as needed. At 1, 4, 7, and 14 days after inoculation, five tubes of each cultivar–nematode species combination were randomly chosen for sampling. Roots were carefully rinsed free of soil, weighed, and stained with acid fuchsin. Numbers of stained nematodes in each root system were determined (3).

The effect of the host on penetration and the ability of *Meloidogyne* species to penetrate and remain in the roots of a given cultivar were compared by plotting actual numbers of nematodes in the roots of the three cultivars against time. Where appropriate, data were subjected to regression analysis.

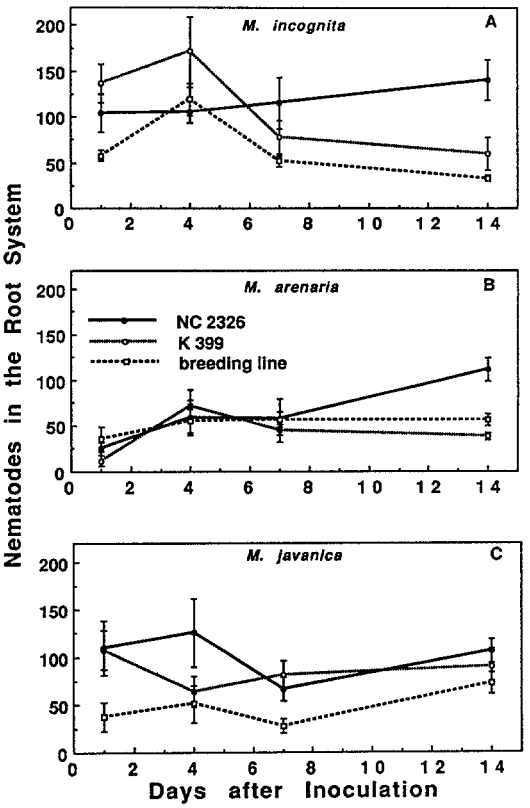

FIG. 1. Numbers of *Meloidogyne* juveniles in the root systems of two tobacco cultivars and a breeding line over time. Points are the means of five replicates. Vertical bars represent the standard error of the means. In some cases the error bars are smaller than the symbols.

Results and Discussion

The interaction between *M. incognita* and NC2326 illustrated a classical compatible plant and nematode combination (Fig. 1A). The rapid increase in the first 24 hours after inoculation represented invasion of the root by the bulk of the initial J2 population followed by a slow but continued population increase as the few individuals left in the soil penetrated the roots.

The higher number of *M. incognita* in K399, relative to NC2326, at 1 and 4 days after inoculation suggested that the roots of the resistant variety may be more easily penetrated by young, vigorous J2 (Fig. 1A).

This agreed with other studies which have shown similar penetration patterns by *Meloidogyne* spp. in susceptible and resistant cultivars (7,8; C. Opperman, pers. comm.). Older, weaker J2 may still be able to penetrate or repenetrate NC2326, but they are not able to penetrate or repenetrate K399 or the breeding line. The drastic drop between 4 and 7 days might be the result of nematodes leaving the roots, presumably after unsuccessful attempts to establish feeding sites. Several previous studies of root-knot nematode species on various hosts have indicated that juveniles unable to establish a feeding site may leave the root (4,6–8,10). It is unlikely the root population would ever decline to zero. Some small proportion of the population is able to establish feeding sites and continue development even in resistant cultivars (5,9,13; C. Opperman, pers. comm.).

The breeding line showed a similar response to *M. incognita* as K399 but allowed less penetration (Fig. 1A). Although K399 ultimately allows fewer nematodes to remain in the roots than NC2326, there undoubtedly is a physiological cost of resistance or damage due to the penetration process (2,12). Cultivars that show similar resistance, but allow less initial penetration, as observed in the breeding line, may offer greater protection against yield loss.

The numbers of *M. arenaria* observed from 1 to 7 days were similar for all three cultivars tested (Fig. 1B). From 7 to 14 days populations in NC2326 continued to increase, whereas populations in K399 and the breeding line slowly decreased. This suggests some degree of resistance to *M. arenaria* may be present in K399 and the breeding line, resulting in a decrease or lack of continued increase in root populations.

Populations of *M. javanica* fluctuated on all three cultivars (Fig. 1C). The plotted interaction between *M. javanica* and the breeding line approximately paralleled that observed for NC2326, but at lower numbers. Although it is difficult to predict from these data, the breeding line may be somewhat less susceptible than NC2326 to *M. javanica.*

Comparison of the three nematode species on the same host illustrated the relative ability of each species to penetrate and remain in the root. Although the *M. arenaria* population showed the same general trends as *M. incognita* on each cultivar, it was at a much lower level (Fig. 1A, B). Whether this is due to a characteristic of the nematode (less aggressive) or of the plant (less susceptible to penetration) cannot be determined from these data, but it is in agreement with other work on tobacco and soybean in which *M. arenaria* penetrated at lower levels than did *M. incognita* or *M. javanica* (12; C. Opperman, pers. comm.). The pattern for *M. javanica* was somewhat different than that for *M. incognita* and *M. arenaria,* suggesting a different interaction with the host (Fig. 1C). *Meloidogyne incognita* and *M. arenaria* have been shown to respond similarly to susceptible and resistant soybeans, but the response of *M. javanica* was different (C. Opperman, pers. comm.).

The responses of *M. arenaria* and *M. incognita* to K399 were similar, suggesting some measure of resistance to *M. arenaria* (Fig. 1A, B). The decline in population of *M. arenaria* following the peak at 4 days was slight, relative to *M. incognita,* indicating relatively fewer nematodes leaving the roots. Most *M. arenaria* that penetrate appear to remain in the root, as contrasted with the *M. incognita* population which penetrates in high numbers but may also leave in high numbers. This might indicate that the initial ability to penetrate is the primary determinant of root populations of *M. arenaria,* whereas the ability to establish a feeding site is the primary determinant of *M. incognita* populations.

The breeding line still contains substantial natural genetic variability. In general, the three *Meloidogyne* species responded similarly to the breeding line and K399. *Meloidogyne incognita* had little trouble penetrating but did not remain in the root. *Meloidogyne arenaria* entered the root in much lower numbers, but the majority that

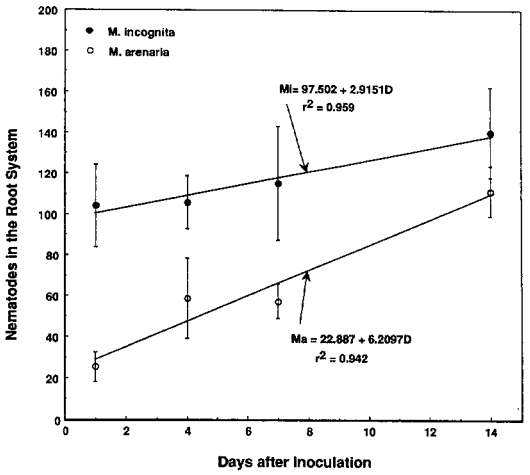

FIG. 2. Regression lines describing penetration and establishment of a feeding site in tobacco cultivar NC2326 by *Meloidogyne incognita* and *M. arenaria*. Points are the means of five replicates.

entered remained. The *M. javanica* population fluctuated, suggesting the nematode penetrates, leaves, and possibly re-enters in relatively high numbers. This line shows promise against *M. incognita* and *M. arenaria* and should be continued in a breeding program.

The regression equations developed for penetration of NC2326 by *M. incognita* and *M. arenaria* (Fig. 2) can be used as one of the linkages between the plant and nematode models that are under development. These equations are based only on data between 1 and 14 days after inoculation; whether they are valid beyond 14 days cannot be determined by the data presented here. Separation of the processes of penetration and establishment of feeding site would facilitate the inclusion in the model of damage due to penetration (such as simple physical damage or predisposition to other threats) even in the resistant case where penetration does not necessarily lead to a parasitic root population. Additional research to quantify the interaction between root-knot nematode and tobacco will be necessary to accomplish the dynamic linking of plant and nematode models.

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