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# Penetration and Development of *Meloidogyne incognita* in Roots of Resistant and Susceptible Corn Genotypes[1]

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*Abstract:* Rates of penetration and development of *Meloidogyne incognita* race 4 in roots of resistant (inbred Mp307, and S4 lines derived from the open-pollinated varieties Tebeau and Old Raccoon) and susceptible (Pioneer 3110) corn genotypes were determined. Seedlings grown in styrofoam containers were inoculated with 5,000 eggs of *M. incognita*. Roots were harvested at 3-day intervals starting at 3 days after inoculation (DAI) to 27 DAI and stained with acid fuchsin. Penetration of roots by second-stage juveniles (J2) at 3 DAI was similar for the four corn genotypes. *Meloidogyne incognita* numbers in Tebeau, Old Raccoon, Mp307, and Pioneer 3110 peaked at 12, 12, 15, and 27 DAI, respectively. Nematode development in the resistant genotypes was greatly suppressed compared to Pioneer 3110. Resistance to *M. incognita* in these genotypes appears to be expressed primarily as slower nematode development rather than differences in J2 penetration.

*Key words:* corn, *Meloidogyne incognita*, nematode development, resistance, root penetration, southern root-knot nematode, *Zea mays*.

*Meloidogyne incognita* (Kofoid & White) Chitwood is commonly found associated with corn (*Zea mays* L.) in the southeastern United States (7,8). Although corn is considered tolerant to *M. incognita*, yield losses in heavily infested fields may be 30% or greater (8). Corn included in cropping systems in fields infested with *M. incognita* can result in 10- to 20-fold greater nematode populations (7,16,17). This increase in *M. incognita* numbers may adversely affect yields of root-knot susceptible crops following corn. Crop rotations would be more effective in managing *M. incognita* populations if commercial corn hybrids were available with resistance to root-knot nematodes.

Mass screenings of commercial corn hybrids and corn inbreds have been conducted in recent years in an effort to find plants resistant to *M. incognita* (26,27). All of the commercial hybrids tested were excellent hosts for *M. incognita* (26). However, considerable variation in the host suitability of inbred lines of corn to *M. incognita* was observed (27). One inbred line, Mp307, had a high degree of resistance to *M. incognita*. Hybrids with Mp307 as a parent have supported significantly less *M. incognita* reproduction than other hybrids in greenhouse (24) and field (25) tests. Open-pollinated varieties have also been evaluated as sources of resistance to *M. incognita* (1). The open-pollinated varieties, Tebeau and Old Raccoon, were found to be highly resistant to *M. incognita* when compared with susceptible genotypes.

The mechanisms of resistance in the corn inbred line, Mp307, and the lines from the open-pollinated varieties, Tebeau and Old Raccoon, are not known. The objectives of this research were to compare penetration and development of *M. incognita* on these resistant corn genotypes with a susceptible corn hybrid.

## MATERIALS AND METHODS

A race 4 population of *M. incognita* was increased on tomato (*Lycopersicon esculentum* Mill. cv. Floradel) in the greenhouse. After 8–10 weeks, eggs were collected from tomato roots with NaOCl (12).

The resistant corn genotypes used in this study included the inbred, Mp307, and two S4 lines derived from the open-pollinated varieties Tebeau and Old Raccoon. A susceptible (26) corn hybrid, Pioneer 3110, was also included in the tests as

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a check. Corn seeds were planted singly into 7-cm-d styrofoam containers filled with 215 g of a mixture of methyl bromide-treated sandy loam soil and river sand (80% sand, 14% silt, 6% clay). Ten-day-old seedlings were inoculated with 5,000 eggs by applying 1 ml water–egg suspension into the soil of each cell. Plants were grown in a greenhouse maintained at ca. 26 C.

Roots were harvested at 3-day intervals starting at 3 days after inoculation (DAI) to 21 DAI; harvests at 24 and 27 DAI were added when the experiment was repeated. Roots were thoroughly washed, and whole root systems were stained with acid fuchsin (3). Root systems were placed in 10-cm-d inverted plastic petri dishes and flattened with slight pressure (9). As the experiment progressed, root systems were divided in half or thirds to facilitate counting. Total numbers of nematodes in each root system were recorded at each observation date, and individuals were assigned to one of three growth stages described by Noe (20). The growth stages were "vermiform," which included second-stage juveniles (J2) with no swelling, "midstage," which included individuals from early swelling to partially globose with conical tails, and "swollen," which included individuals that were fully globose preadults or adults. Nematode development as the percentage of the population in each growth stage at each harvest date was calculated as (individuals in each growth stage/total number of nematodes in root) × 100.

The experiment was conducted using a randomized complete block design with five replicates per treatment. Data from both experiments were combined and subjected to analysis of variance, and means were compared by least significant differences (LSD) at $P = 0.05$. Regression equations were calculated to express changes over time in numbers of nematodes in the root systems of the four corn genotypes.

## Results

*Meloidogyne incognita* penetration of resistant and susceptible corn genotypes was similar at the first harvest date (3 DAI) (Table 1). After the first harvest, differences ($P = 0.05$) in numbers of nematodes in the vermiform stage were seen among genotypes. Numbers of nematodes in Tebeau, Old Raccoon, Mp307, and Pioneer 3110 root systems peaked at 12, 12, 15, and 27 DAI, respectively. By 27 DAI, Pioneer 3110 roots had five times as many nematodes as the resistant genotypes.

Nematode development on susceptible and resistant genotypes differed ($P = 0.05$) greatly (Table 1, Fig. 1). Nematodes in the midstage were first found on Pioneer 3110 at 6 DAI but were not observed on the resistant genotypes until 9 DAI. A higher percentage of the *M. incognita* population in roots of Pioneer 3110 were midstage than for populations in the resistant genotypes at 6, 12, and 15 DAI. Nematodes in the swollen stage was first found on Pioneer 3110 and Old Raccoon on 15 DAI, although more were observed on Pioneer 3110 than on Old Raccoon. Numbers of nematodes in the swollen stage increased greatly over time on Pioneer 3110, peaking on the last harvest (27 DAI). From 15 DAI until the last harvest (27 DAI), Pioneer 3110 had a higher percentage ($P = 0.05$) of the *M. incognita* population in the swollen stage than did the resistant corn genotypes.

There was a quadratic relationship between DAI and numbers of nematodes in roots for Pioneer 3110 ($Y = -145.21 + 56.59\ [DAI] - 1.16\ [DAI]^2$, $R^2 = 0.93$, $P = 0.01$), Mp307 ($Y = -183.98 + 63.4\ [DAI] - 1.94\ [DAI]^2$, $R^2 = 0.63$, $P = 0.02$), and Tebeau ($Y = -75.64 + 42.78\ [DAI] - 1.39\ [DAI]^2$, $R^2 = 0.56$, $P = 0.03$). Numbers of nematodes in Pioneer 3110 increased rapidly and then levelled off toward the end of the tests (Fig. 1A). Nematode numbers in Mp307 and Tebeau peaked midway through the test and then declined until the test ended (Fig. 1B). A cubic model ($Y = -334.96 + 135.57\ [DAI] - 9.11\ [DAI]^2 + 0.17\ [DAI]^3$, $R^2 = 0.93$, $P = 0.002$) best described the relationship between DAI and numbers of nematodes in roots for Old Raccoon. Nematode numbers increased until 12

TABLE 1. Percentage of the *Meloidogyne incognita* population in three different developmental stages and total number of nematodes per root system on a corn hybrid and three corn lines at 3-day intervals from 3 to 27 days after inoculation (DAI).

| Genotype | Percentage† | | | Nematodes per root system |
|---|---|---|---|---|
| | Vermiform‡ | Midstage‡ | Swollen‡ | |
| | | 3 DAI | | |
| Pioneer 3110 | 100 | 0 | 0 | 12 |
| Mp307 | 100 | 0 | 0 | 18 |
| Old Raccoon | 100 | 0 | 0 | 12 |
| Tebeau | 100 | 0 | 0 | 15 |
| LSD ($P$ = 0.05) | NS | NS | NS | NS |
| | | 6 DAI | | |
| Pioneer 3110 | 97.8 | 2.2 | 0 | 140 |
| Mp307 | 100.0 | 0 | 0 | 67 |
| Old Raccoon | 100.0 | 0 | 0 | 155 |
| Tebeau | 100.0 | 0 | 0 | 151 |
| LSD ($P$ = 0.05) | 1.6 | 1.6 | NS | 68 |
| | | 9 DAI | | |
| Pioneer 3110 | 68.7 | 31.3 | 0 | 231 |
| Mp307 | 93.5 | 6.5 | 0 | 337 |
| Old Raccoon | 86.0 | 14.0 | 0 | 260 |
| Tebeau | 75.6 | 24.4 | 0 | 129 |
| LSD ($P$ = 0.05) | 10.9 | 10.9 | NS | 100 |
| | | 12 DAI | | |
| Pioneer 3110 | 50.0 | 50.0 | 0 | 463 |
| Mp307 | 81.5 | 18.5 | 0 | 120 |
| Old Raccoon | 72.8 | 27.2 | 0 | 341 |
| Tebeau | 73.5 | 26.5 | 0 | 375 |
| LSD ($P$ = 0.05) | 14.5 | 14.5 | NS | 148 |
| | | 15 DAI | | |
| Pioneer 3110 | 19.8 | 73.3 | 6.7 | 430 |
| Mp307 | 58.0 | 42.0 | 0 | 439 |
| Old Raccoon | 57.3 | 42.2 | 0.5 | 234 |
| Tebeau | 45.3 | 54.7 | 0 | 282 |
| LSD ($P$ = 0.05) | 15.7 | 14.0 | 2.9 | 128 |
| | | 18 DAI | | |
| Pioneer 3110 | 6.3 | 60.0 | 33.7 | 521 |
| Mp307 | 25.1 | 74.1 | 0.8 | 311 |
| Old Raccoon | 39.5 | 60.4 | 0.1 | 161 |
| Tebeau | 19.5 | 78.6 | 1.9 | 187 |
| LSD ($P$ = 0.05) | 12.2 | 12.1 | 4.4 | 86 |
| | | 21 DAI | | |
| Pioneer 3110 | 0.4 | 66.6 | 33.3 | 461 |
| Mp307 | 8.2 | 88.7 | 3.1 | 296 |
| Old Raccoon | 6.9 | 88.7 | 4.4 | 125 |
| Tebeau | 0.9 | 90.0 | 9.1 | 160 |
| LSD ($P$ = 0.05) | 5.6 | 10.1 | 9.7 | 147 |
| | | 24 DAI | | |
| Pioneer 3110 | 0 | 35.2 | 64.8 | 507 |
| Mp307 | 2.2 | 89.4 | 8.4 | 262 |
| Old Raccoon | 2.5 | 82.1 | 15.4 | 110 |
| Tebeau | 1.2 | 69.4 | 29.4 | 103 |
| LSD ($P$ = 0.05) | NS | 13.4 | 14.3 | 143 |
| | | 27 DAI | | |
| Pioneer 3110 | 0 | 23.3 | 76.7 | 583 |
| Mp307 | 3.4 | 81.2 | 15.3 | 80 |
| Old Raccoon | 0 | 65.0 | 35.0 | 152 |
| Tebeau | 0 | 55.4 | 44.6 | 134 |
| LSD ($P$ = 0.05) | 2.3 | 9.3 | 9.5 | 188 |

Data are means of five replications and two repetitions, except that 24 and 27 DAI were means of one repetition.

† Percentage = number of nematodes in growth stage/number of nematodes in root system.

‡ Growth stage: vermiform = no swelling; midstage = slightly swollen to partially globose, conical tail; swollen = fully globose preadult or adult.

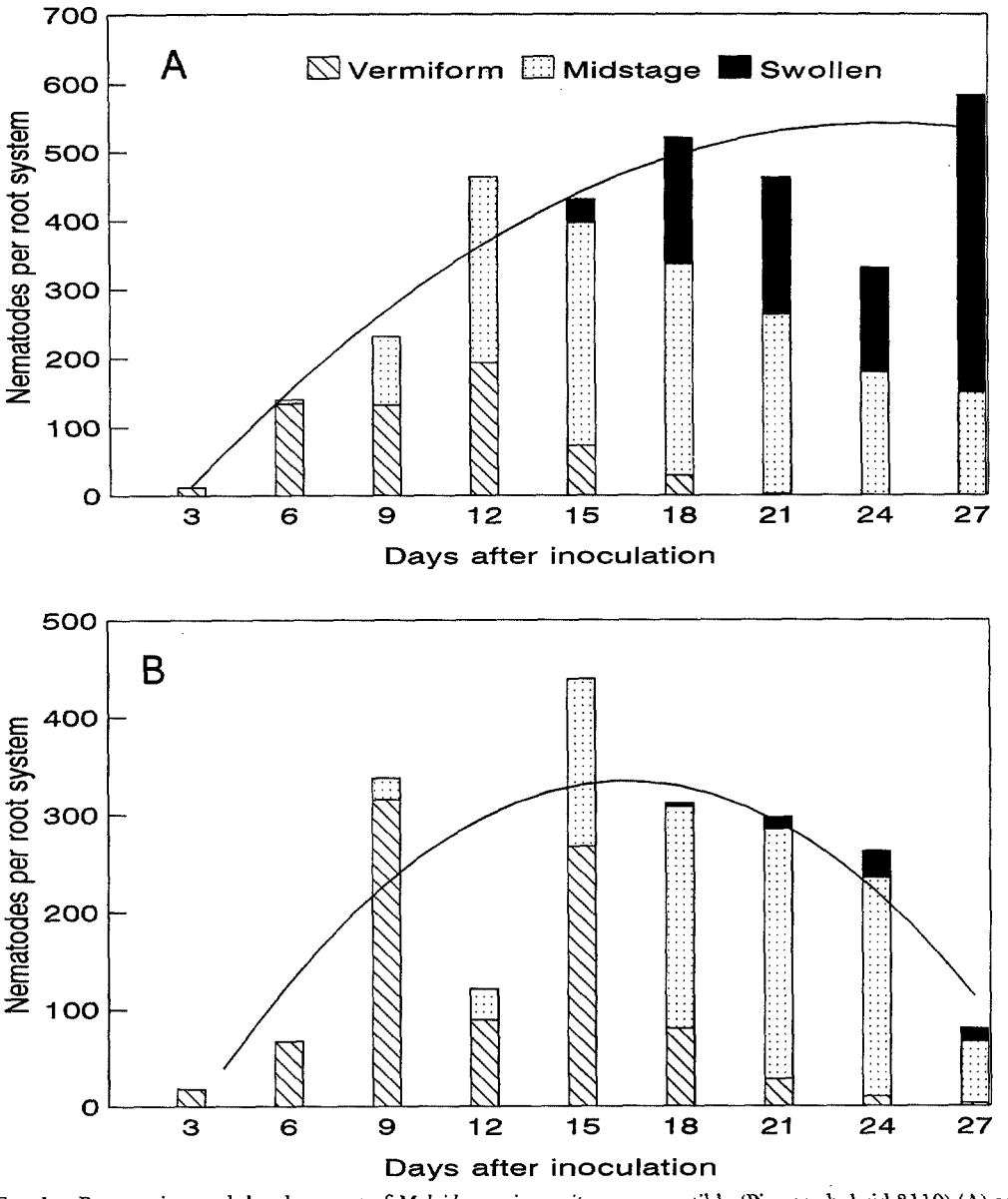


FIG. 1. Penetration and development of *Meloidogyne incognita* on susceptible (Pioneer hybrid 3110) (A) and resistant (Mp307) (B) corn at 3-day intervals from 3 to 27 days after inoculation. Nematode growth stage: vermiform = no swelling; midstage = slightly swollen to partially globose, conical tail; swollen = fully globose preadult or adult. Regression curves describing the relationship between days after inoculation (DAI) and number of nematodes (all stages) in roots for Pioneer 3110 ($Y = -145.21 + 56.59$ [DAI] $- 1.16$ [DAI]$^2$, $R^2 = 0.93$) and Mp307 ($Y = -183.98 + 63.4$ [DAI] $- 1.94$ [DAI]$^2$, $R^2 = 0.63$) are shown.

DAI and decreased until 24 DAI, and there was a slight increase in nematode numbers at 27 DAI.

## DISCUSSION

Resistance to *M. incognita* in the inbred Mp307 and the S4 lines from the open-pollinated hybrids Tebeau and Old Raccoon was manifested most noticeably by slower nematode development as opposed to differential rates of penetration. Juveniles entered the susceptible check and the resistant genotypes in equal numbers. In some cases, greater numbers of *M. incog-*

*nita* juveniles penetrated the resistant plants than the susceptible plants. *Meloidogyne incognita* usually penetrate resistant and susceptible plants in equal numbers (6, 10,14,19,23), but differences have been observed (4,15,21). Baldwin and Barker (2) found that *M. incognita* J2 penetration of resistant and susceptible corn hybrids differed at 4 and 8 days after inoculation. Apparently the mechanisms of resistance in the corn hybrids they used differ from those of the resistant genotypes included in this study.

Root-knot resistance may also be characterized by slow nematode development when compared with susceptible hosts (4, 6,15,18,21). Although nematodes may enter resistant and susceptible plants in equal numbers, juveniles may never become adults or are greatly slowed in their development on resistant hosts. This was the type of response we observed in the resistant genotypes, in contrast to the *M. incognita* development observed in Pioneer 3110. The midstage and the swollen stage were either observed first in the susceptible plants or in greater numbers than in resistant plants. The number of adult nematodes and the percentage of nematodes developing to adults in resistant genotypes were much less when compared to the susceptible hybrid.

Emigration of *Meloidogyne* spp. juveniles has been observed from resistant plants (10,22). Differential emigration of J2 from resistant and susceptible corn genotypes may have occurred in our study. Nematode numbers in the resistant plants peaked at approximately 2 weeks, whereas nematode numbers peaked at the last harvest on 27 DAI in the susceptible plants. Juvenile emigration may be occurring, since juveniles occur at the same or higher numbers in resistant plants as in susceptible plants from 3 to 15 DAI, and then drop off drastically. Total nematode numbers in resistant roots had decreased significantly at the last harvest dates, possible because of juvenile egression.

The slowed nematode development and decrease in nematode numbers observed in the resistant corn genotypes could be the result of several factors. It has been suggested that some resistant plants lack nutrients, which may result in emigration, slowed development, or and altered sex ratio in root-knot nematodes (11). However, a more active response by the resistant plants may be occurring. It is possible that some nematotoxic substance may have accumulated in the roots after infection by J2. Phytoalexins have been associated with *M. incognita* resistance in soybean (14). It is difficult to speculate on the importance of phytoalexins in corn because little progress has been made in characterization of phytoalexins in the Gramineae. Further studies are warranted to determine the mechanisms of resistance in corn genotypes and whether they produce postinfection inhibitory chemicals.

We have confirmed that Mp307, Tebeau, and Old Raccoon are sources of resistance for *M. incognita.* Delayed nematode development on resistant genotypes accounted for differences seen in egg production in earlier studies (1,27). Material in this study will be used to develop *M. incognita*-resistant germplasm, which can be used for the development of nematode-resistant hybrids.

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