Symptomless Resistance of Alfalfa to *Meloidogyne incognita acrita*[1]

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Abstract: Penetration, development and migration of the cotton root-knot nematode, *Meloidogyne incognita acrita*, in resistant and susceptible alfalfa varieties was compared. Larvae entered both resistant and susceptible plants in approximately the same numbers. After 3 to 4 days, the number of larvae in resistant roots decreased sharply until at 7 days fewer than 5 larvae/seedling and no nematode development could be found. In susceptible roots, larvae became sedentary and developed normally; egg production began as early as 18 days after penetration of the host. *Key Words: Meloidogyne incognita acrita, Medicago sativa*, Alfalfa, Resistance, Susceptibility, Larva migration.

Invasion of a susceptible root by larvae of *Meloidogyne* spp. Goeldi, usually causes major changes in plant cells surrounding the nematode. Near the head of the parasite, normal vascular tissue is replaced by giant cells, and hypertrophy and cell proliferation occur (2, 5). Once feeding begins, larvae become sedentary and develop to maturity.

In resistant roots larvae may either fail to enter, enter in reduced numbers with little or no development, or enter in large numbers with varying degrees of development (1, 3). Hypersensitivity is a common type of response of resistant plants (8, 9). The larvae may enter roots of resistant plants in large numbers, but hypersensitive cells quickly die and wall off the pathogen so that injury to the host is confined to a few cells (8). This results in the subsequent death of the nematode. The hypersensitive reaction in some cases may be delayed until after the parasitic relationship becomes well established (6).

'African' alfalfa (*Medicago sativa* L.) is highly resistant to *M. incognita acrita* Chitwood and *M. javanica* (Treub) Chitwood (7), but susceptible to *M. hapla* Chitwood (10). While studying the reaction of 'African' to invasion by *M. incognita acrita,* we observed large numbers of larvae in the roots 1 to 4 days after inoculation. During the 6th or 7th day after inoculation, however, staining revealed only a few larvae remaining which were morphologically unchanged and migrating within the roots. We observed no evidence of hypersensitive plant tissue reaction to the invading larvae and dissection of the roots revealed no larval cuticles.

This study was undertaken to compare the penetration, subsequent development, and migration of *M. incognita acrita* larvae in roots of resistant and susceptible alfalfa varieties.

MATERIALS AND METHODS

Three varieties of alfalfa, 'African,' 'Moapa' and 'Sonora,' resistant to *M. incognita acrita*, and one susceptible variety, 'Lahontan,' were used in these studies. 'Moapa' and 'Sonora' are selections from 'African' and have retained most of its resistance. Seeds of each alfalfa variety were germinated in Supreme Perlite®. After 48 hr, a germinated seedling was transplanted singly into 12 × 100 mm cylindrical polyethylene-film containers filled with sterilized

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loamy sand and embedded in a plastic tray of moist sand. The tray was placed in a constant temperature water tank at 27 ± 1 C. After 4 days, each alfalfa seedling was inoculated with 100 viable *M. incognita acrita* larvae counted and transferred as follows: Freshly-hatched larvae were concentrated in a small Baermann funnel and then distributed in droplets in a counting dish. Nematodes per droplets were counted and the droplets consolidated until 100 larvae were in one large drop which was drawn into an eye dropper and placed in the soil next to the seedling.

PENETRATION OF THE ROOTS OF RESISTANT AND SUSCEPTIBLE PLANTS BY *MELOIDOGYNE INCOGNITA ACRITA*: Four replicate plants of each variety were harvested at 1, 2, 3, 4, 5, 6, 7, 10, 14, 18, and 21 days following inoculation. Each seedling was processed according to the following schedule: (i) gentle washing in tap water and fixation in FAA for 48 hr; (ii) staining with a weak Fleming's solution for 50 min; (iii) serial dehydration in ethyl alcohol at 35, 50, 70, 85, 95 and 100% concentrations at 2 hr intervals; and (iv) preservation and examination in clove oil. The number of larvae that had penetrated the roots, changes in host tissue, and developmental stages of the nematodes were recorded.

MIGRATION OF LARVAE FROM RESISTANT ROOTS: In this experiment we devised a method to compare the number of larvae leaving the resistant 'African' roots and the susceptible 'Lahontan' roots. Two plants each of the resistant and the susceptible varieties were harvested from the polyethylene containers at 3, 4, 5, 6 and 7 days after inoculation. Each seedling was carefully washed to remove nematodes on the surface of the roots, then placed in a counting dish of sterile distilled water at 25 C. The roots were examined microscopically five times daily for 72 hr; water in the dishes was changed daily. Plant responses to the nematode infection were observed and recorded. All emerged larvae were counted and removed from the counting dish. Larvae that had migrated from the resistant roots were transferred to susceptible alfalfa plants to determine their ability to infect a susceptible host after passage through a resistant root.

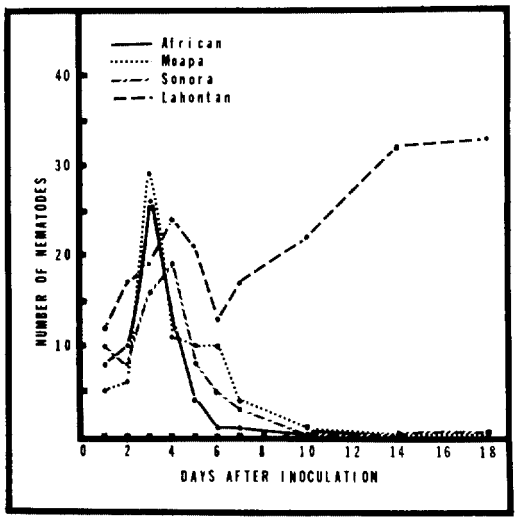

FIG. 1. The number of *M. incognita acrita* observed within the roots of the resistant alfalfas, 'African,' 'Moapa' and 'Sonora,' and the susceptible alfalfa, 'Lahontan.'

RESULTS

Figure 1 summarizes the penetration of resistant and susceptible alfalfa varieties by larvae of *M. incognita acrita*. Approximately the same number of larvae entered the roots of the resistant alfalfas as the susceptible variety. After 3 to 4 days, an abrupt decrease in the number of larvae in resistant roots was observed. After 7 days, resistant roots contained less than 5 larvae per seedling with no nematode development observed (Fig. 2A) and ten days after inoculation, no larvae were detected in any of the resistant roots. After entering the sus-

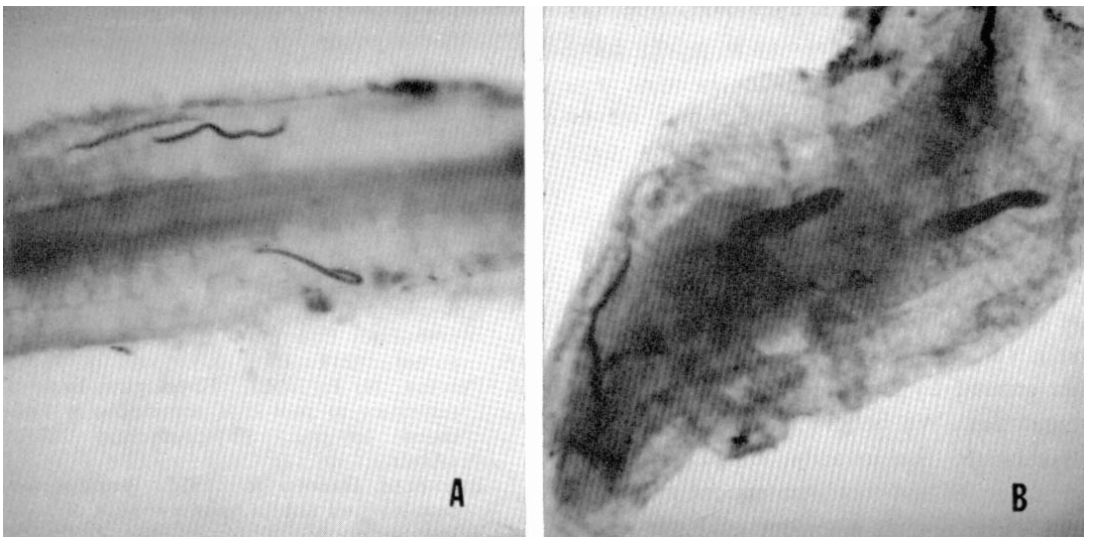

FIG. 2. **A–B.** Development of *M. incognita acrita* in 'African' and 'Lahontan' alfalfa varieties (×50): **A.** 'African'; and **B.** 'Lahontan', both 7 days after inoculation.

ceptible 'Lahontan' roots, the larvae became sedentary and development proceeded normally (Fig. 2B). Egg production began as early as 18 days after host penetration.

Figure 3 summarizes data comparing the migration of *M. incognita acrita* larvae from the resistant 'African' and the susceptible 'Lahontan' alfalfas. Greatest migration of larvae from 'African' roots occurred 4 days after inoculation. Figure 1 (the reciprocal of Fig. 3) shows maximum decrease in numbers of larvae within 'African' roots during the third and fourth day after inoculation.

Although a few larvae migrated from the 'Lahontan,' the number was small compared with the larvae recovered from 'African.' Larvae that had migrated from 'African' roots showed no morphological changes that indicated development or feeding. Larvae that had migrated from the resistant roots and were transferred to susceptible alfalfa plants penetrated the roots, matured and produced eggs within 20 days.

DISCUSSION

Wallace (11) suggested that nematodes may invade and then leave a resistant root; whereas they tend to remain in a susceptible root. De Guiran (4) found larvae of *M.*

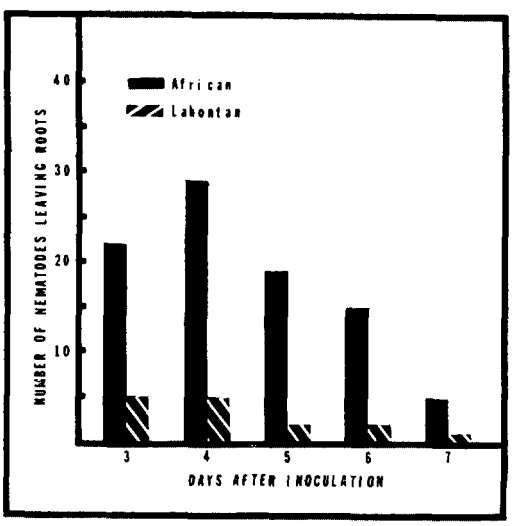

FIG. 3. The number of *M. incognita acrita* larvae that emerged from 'African' and 'Lahontan' roots.

javanica which had invaded *Crotalaria astragatina* and then migrated from the roots although many remained in the root and failed to develop. In our studies most larvae left the resistant roots within a few days after penetration.

Some symptomatic response is usually evident when a nematode invades a resistant plant. Christie (2), however, observed that the destruction of cells by *Meloidogyne* larvae is surprisingly light when compared with that caused by the passage of many other nematodes. We believe the lack of visible response to the invasion of *M. incognita acrita* larvae and their subsequent migration out of the root is a unique and previously unreported type of resistance to *Meloidogyne* spp. Apparently the larvae do not elicit a pronounced response by the plant since neither syncytia nor necrotic cells were observed.

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