Interaction of *Meloidogyne javanica* and *Macrophomina phaseoli* in Kenaf Root Rot[1]

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Abstract: Incidence and severity of root-rot caused by the fungus *Macrophomina phaseoli* was increased in screenhouse-grown kenaf (*Hibiscus cannabinus* L.) seedlings simultaneously infected by the nematode *Meloidogyne javanica*. In seedlings inoculated at 5, 10 and 15 days of age, root rot lesions increased 70.3, 44.1 and 21.8%, and nematode penetration increased 49.0, 36.7, and 12.3% when both fungus and nematode were present. *Key Words:* Interaction, *Meloidogyne javanica*, Root-knot, *Macrophomina phaseoli*, Root-rot, Kenaf.

The root-knot nematode *Meloidogyne javanica* (Treub.) Chitwood and the fungus *Macrophomina phaseoli* (Maubl.) Ashby (= *Sclerotium bataticola* Taub., the incitant of root and stem rot of kenaf (*Hibiscus cannabinus* L.) have been found associated on that crop throughout the Chianan Plain of Taiwan. Pre-plant fumigation of kenaf fields with 18–28 liter/ha Fumazone (70% 1,2-dibromo-3-chloropropane) for root-knot nematode control usually reduces the incidence of kenaf root-rot. This indicates a possible interrelation between the root-knot nematode and *M. phaseoli*.

The role of *Meloidogyne* spp. in disease complexes has received major attention. In 1892, Atkinson (1) noted that infection by root-knot nematodes appeared to increase the incidence of *Fusarium* wilt of cotton. Since then numerous observations of interactions between root-knot nematodes and other pathogens have been reported. Thomason (8) found *M. javanica* increased the severity of *Fusarium* wilt of black-eyed bean. Sasser *et al.* (7) showed that *M. javanica* greatly reduced the resistance of two tobacco varieties to black shank infection. This paper presents a study of the interrelationship between root-rot and root-knot of kenaf.

Materials and Methods

All pots (12-cm clay) received 700 g sterilized (boiling water) soil, were seeded with kenaf variety 'BG-7', and then placed in a screenhouse having temperature range of 24 to 32 C. Immediately after germination, seedlings were thinned to 15/pot. Hoagland's nutrient solution was applied twice a day throughout the experiment.

Kenaf seedlings 5, 10, and 15 days old were grown in sterilized soil and infected with *M. javanica* or *M. phaseoli* or both. Growth of seedlings, development of root-rot initial symptoms and root penetration by the nematode among the different treatments were compared. Each pot was considered a replicate and each treatment consisted of 6 replications.

M. phaseoli was originally isolated from diseased kenaf. A stock culture of the fungus was maintained in the laboratory by frequent transfers to fresh potato-sucrose agar (PSA) slants. To infest the soil with *M. phaseoli*, the fungus was grown for 5 days on 30 ml Czapek's solution in a 50-ml flask at 30 ± 2 C. The colony of the fungus was then filtered and macerated in a Waring blender with 30 ml distilled water for 30 seconds. The sterilized soil in each pot received 30 ml of mycelial suspension, and then sufficient

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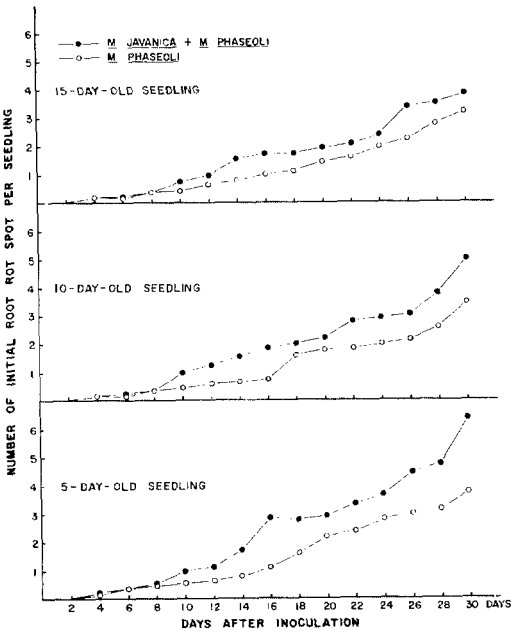

FIG. 1. Influence of *Meloidogyne javanica* on the infection of *Macrophomina phaseoli* on roots of kenaf seedlings.

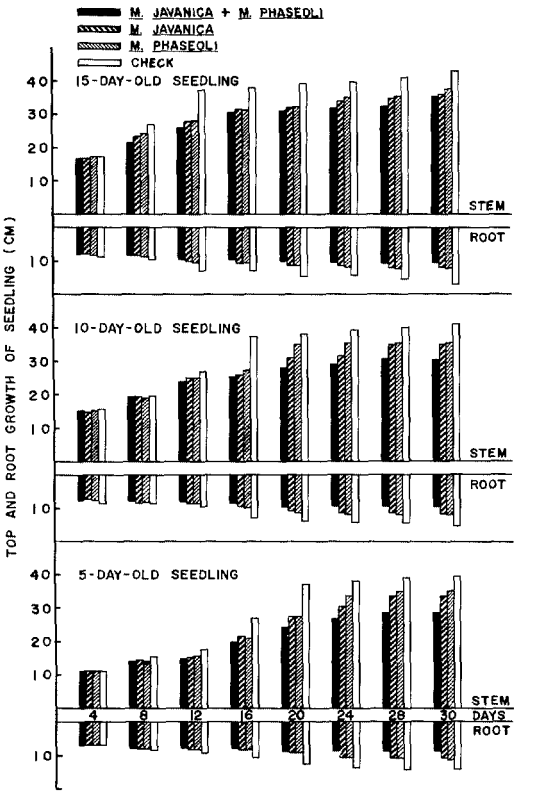

FIG. 2. Growth of kenaf seedlings after inoculation of *Meloidogyne javanica* and/or *Macrophomina phaseoli* at different ages.

water was applied to assure even distribution of the inoculum.

M. javanica collected from kenaf plants was multiplied on susceptible tomato plants in the greenhouse. In the nematode series, each pot received about 180 eggs in a small amount of distilled water. The pot was then flooded with additional water.

Examination of kenaf seedlings was made at intervals of 2 days after inoculation. The seedlings in each treatment (18 pots) were dug carefully and washed. The roots of seedlings were examined under a dissecting microscope after measuring the height of seedlings and root growth. For examination of nematode infection, Goodey's method (4) was adapted. The roots, after examination for growth, were immersed into boiling 0.05% acid fuchsin lactophenol solution for about 3 min, and allowed to cool in the stain. The roots were then washed in running water for 10 min and passed through 30, 50, 70, 85, 95, and 100% ethanol for 5–10 min, respectively. Finally, the roots were left in plain lactophenol overnight.

RESULTS

Distinct root-rot lesions appeared 4 days after inoculation of kenaf seedling roots with *M. phaseoli* mycelia in the sterilized soil (Fig. 1). Examination of roots at 2-day intervals revealed that the number of initial spots per seedling tended to increase more rapidly when both *M. phaseoli* and *M. javanica* were present. At the end of the experiment (30 days after inoculation), both pathogens together caused 6.3, 5.0, and 3.9 initial spots of root-rot/seedling in the 5, 10, and 15-day-old seedlings, respectively, vs. 3.7, 3.4, and 3.2 for *M. phaseoli* alone.

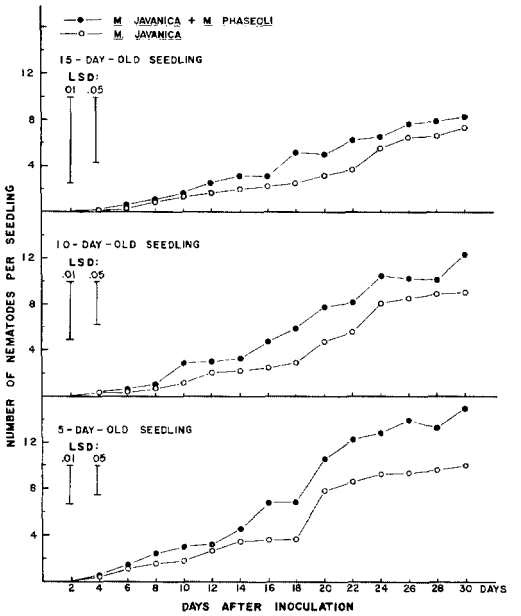

FIG. 3. Effect of *Macrophomina phaseoli* on penetration of *Meloidogyne javanica* into roots of simultaneously inoculated 5, 10, and 15-day-old kenaf seedlings [180 nematode eggs and 63 mg (wet wt) mycelia introduced per pot (6 reps) containing 15 seedlings].

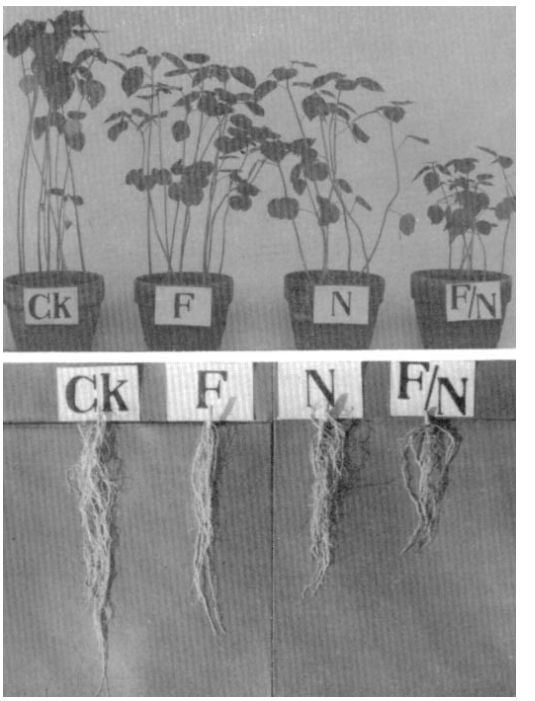

FIG. 4. The top and root growth of kenaf seedlings after 30 days in soils infested with *Macrophomina phaseoli* alone (F), *Meloidogyne javanica* alone (N), *M. phaseoli* plus *M. javanica* (F/N), and neither pathogen (CK).

Growth of kenaf seedlings and roots was also depressed by both root-knot nematode and root-rot fungus. In the nematode-infested soil, kenaf roots were much more slender than control plants and had numerous galls (Fig. 4). Roots infected by *M. phaseoli* only were short, had less laterals, and became darker because of coalescing lesions (Fig. 4). The growth of kenaf seedlings was retarded and severe injury was found on the roots in the presence of both parasitic agents (Fig. 2, 4).

The results summarized in Fig. 3 indicates that *M. phaseoli* infection was also favorable to the reproduction and infection of *M. javanica* in kenaf roots. Under a 24–32 C temperature range, *M. javanica* completed the first generation in 20 days and second stage larvae of the second generation appeared in the roots 22 days after inoculation. The nematode multiplied more rapidly in kenaf roots infected by *M. phaseoli*, causing an increase in nematodes in kenaf seedlings infected at the age of 5 days (Fig. 3).

Penetration by *M. javanica* tended to increase when associated with *M. phaseoli*. Twenty days after inoculation of both parasites, nematode penetration into the 5, 10, and 15-day-old seedlings was 10.7, 7.7, and 4.9 nematodes/seedling, respectively. This decreased to 7.9, 4.8, and 3.1 nematodes/seedling, respectively, in the absence of *M. phaseoli*.

Although severe injury of kenaf roots resulted from infection by *M. javanica* and *M. phaseoli*, a considerable degree of resistance to the nematode and to the root-rot fungus was found in the older seedlings,

particularly when introduction of the two organisms was delayed until 15 days after germination (Fig. 3).

Discussion

The effects of *M. phaseoli* and *M. javanica* on *Macrophomina* root-rot of kenaf seedlings were found to be synergistic in this experiment. Although the presence of *M. javanica* did not shorten the incubation period of *Macrophomina* root-rot, it was closely associated with the increase of the lesions. This effect might be due to the action of the nematode in providing readily available infection courts for the fungus. As suggested by Faulkner and Skotland (3), it is possible that liberation of plant materials through root wounds produced by the nematode may stimulate hyphal penetration.

Mountain and McKeen (5) reported an increase in reproduction of *Pratylenchus penetrans* when certain hosts were grown in soil infected with *Verticillium dahliae*. In our study, *M. phaseoli* also increased the population and infection of the root-knot nematode. Mountain and McKeen (5), and Faulkner and Skotland (3) concluded that the presence of *V. dahliae* could cause changes in host physiology favorable to *P. penetrans* and *P. minyus*. Many fungi, such as *V. albo-atrum* and others, are known to produce indole-3-acetic acid which stimulates roots (6). Christie (2) reported that invasion by root-knot nematode larvae is limited mainly to root tip regions with comparatively undifferentiated tissues. Based on these findings, it is presumed that a small amount of IAA-like substance produced by *M. phaseoli* would stimulate root growth during the early stage of infection and thus make the roots more favorable for root-knot nematode penetration. However, further research is needed to elucidate the population increase of *M. javanica* caused by *M. phaseoli* on kenaf roots.

Development of the initial symptoms of root-rot and the number of nematode penetration were found to be affected by the age of kenaf seedlings. These results indicate that adult resistance of the host may exist.

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