Interaction of Macrophomina phaseolina and Meloidogyne javanica on Ligustrum japonicum

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ABSTRACT

The combined pathogenic effects of *Macrophomina phaseolina* and *Meloidogyne javanica,* two previously unreported pathogens of *Ligustrum japonicum,* were greater than the independent effects of either. Leaf chlorosis and abscission, twig dieback, stunting, reduction and necrosis of roots, and eventual plant loss were greater on plants infected with both pathogens. Phytopathology 61:1297-1298.

Additional key words: Root rot complex, charcoal rot, histological pathology, dwarfing.

During the summer of 1969, dwarfed and unthrifty plants of *Ligustrum japonicum* L. were observed. Examination of the root system revealed the presence of the charcoal rot fungus *Macrophomina phaseolina* (Tassi) Goid. (= *Sclerotium bataticola,* [Taub., *M. phaseoli* (Maubl.) Ashby] and the root-knot nematode *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, in the absence of well-defined galls. In three widely separated localities, one or both organisms was associated with a foliar creasing on the host. Thus, it appeared necessary to determine the effect of individual and combined inoculations of these pathogens on *L. japonicum.*

MATERIALS AND METHODS.—Plants of *Ligustrum* were established from cuttings grown in 6-inch clay pots containing steam-sterilized greenhouse potting soil (1 part sandy loam to 1 part peat). All test plants were ca. 10 inches tall, with two to three branches above the central stem.

Inoculation treatments utilized three groups of plants, eight plants/group: the fungus alone; the nematode alone; and the fungus plus nematode. Twenty-four noninoculated plants served as controls. The fungus inoculum consisted of 7-day-old cultures grown on 2% potato-dextrose agar (PDA). The cultures were comminuted with a blender for 2 min in sterile tap water. Soil was infested by pouring the equivalent of one plate culture in 200 ml of water onto the surface of the soil in each of eight pots, then covering with ca. 0.5 inch steam-sterilized soil. Nematode inoculum consisted of progeny from a single egg mass on roots of *L. japonicum* which was propagated on roots of three successive seedling generations of squash, *Cucurbita pepo* L., var. *melopepo* Laef. 'Yellow Crookneck'. Severely galled roots of 14 squash plants were fractionated in distilled water with a blender for 3 min, and made up to a total volume of 1,600 ml. Each of eight pots in the nematode treatment received 100 ml of the root and nematode suspension, then were covered with a thin layer of soil followed by application of 100 ml distilled water. Similarly, the fungus inoculum plus 100 ml of the root and nematode suspension were poured into each of eight pots and treated as above. Of the control plants, 12 received PDA plus water, and 12 received water only.

Plants were spaced on a bench in a slat house where the average minimum and maximum temperatures were 15 and 27 C, respectively, during the 1-year test period. Plants were measured to assess treatment effect on over-all growth and disease expressions.

Roots from inoculated plants were studied to determine the nature of histological pathogenesis. Nematode-infected roots were killed and fixed in FAA, sectioned 10 μ thick, and stained with safranin-fast green.

RESULTS.—A general decline in plant vigor and a reduction in new growth were first noted 5 weeks after inoculation. This was particularly evident among plants grown in soil infested with the fungus. Moreover, the younger leaves of these plants exhibited a prominent creasing. The leaf folding or creasing occurred in a line parallel or oblique to the midvein, and was sharp and distinct with no evidence of any break or injury to the leaf tissue. The foliar creasing persisted, once initiated; and developed on succeeding, newly emerged leaves. In some cases, two creases occurred on a single leaf. Except for being creased, such leaves developed and matured in a normal manner. After 6 months' growth in infested soil, some plants in all three treatments showed yellowing and abscission of the terminal leaves. All plants in these treatments remained stunted, with little additional growth.

Plants grown in soil infested with the fungus plus nematode displayed the severest dieback symptoms, defoliation, and root rot 7 months after inoculation. This was apparent through the entire test period. Many nematodes and sclerotial bodies were present in the roots, the latter being scattered in the cortical tissue. After 12 months, three plants were dead in the fungus plus nematode treatment. These plants were completely defoliated, with severely rotted root systems. Four plants exhibited varying amounts of dieback, defoliation, and root necrosis. One plant was stunted, with no dieback.

Of the nematode-infected plants, three showed varying degrees of stunting, defoliation, and dieback. Five were stunted, with no defoliation and dieback. Roots of plants in this treatment were slightly galled and necrotic. The fungus-inoculated plants included two exhibiting varying degrees of defoliation and dieback, and six that were stunted. Roots of these plants were necrotic, and ca. half the size of contrasting control plants. Foliar creasing was present on plants grown in soil infested with any of the organisms. A comparison of the treatment effect on root systems and top growth is shown in Fig. 1. The degree of stunting exhibited by all plants in all treatments was marked, reflected by a 50-70% reduction in size.

A study of stained, serially sectioned roots para-

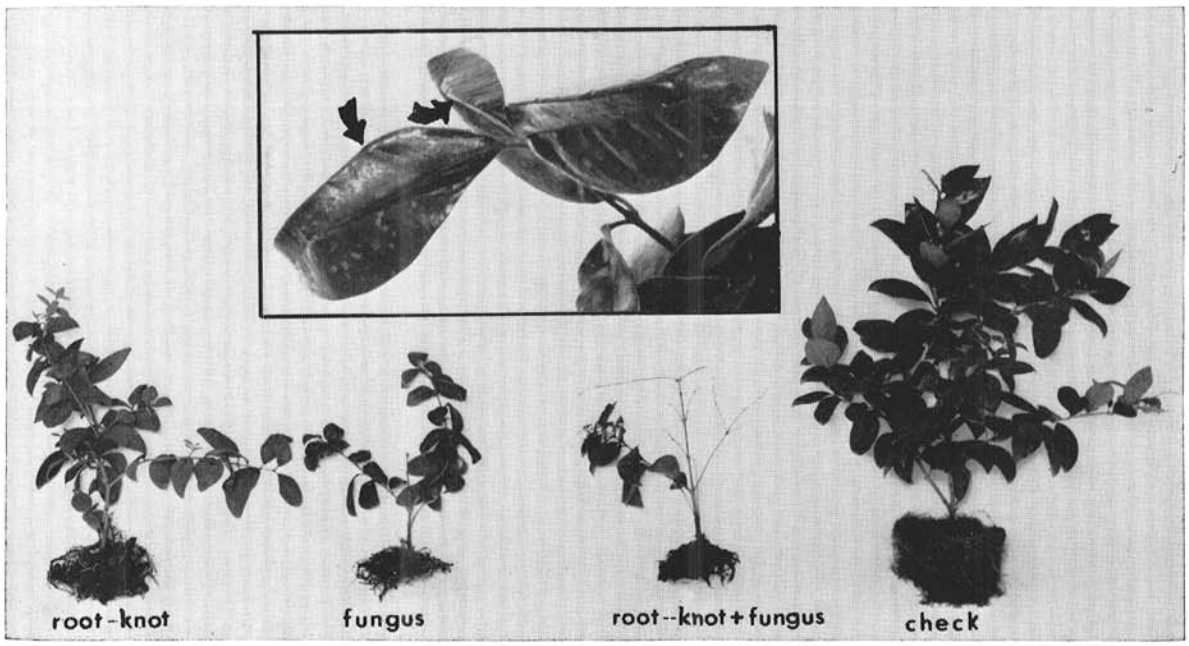

Fig. 1. Comparative effects of roots and top growth of *Ligustrum japonicum* inoculated with *Macrophomina phaseolina* (fungus) and *Meloidogyne javanica* (nematode), singly or in combination; insert, foliar creasing (arrows).

sitized by *M. javanica* revealed that the nematodes fed only in the vascular cylinder with the head against or between multinucleate giant cells of altered xylem and phloem parenchyma. Impairment of the vascular system was produced by hypertrophied vascular cells and bodies of nematodes.

DISCUSSION.—That interactions between nematodes and other plant-pathogenic fungi cause greater disease manifestations in certain hosts is well recognized. In earlier reports (3, 4), the charcoal rot fungus, *M. phaseolina,* did not appear to appreciably influence disease severity in the presence of certain nematodes, whereas the role of *M. javanica* significantly influenced the wilt severity among other hosts (1, 2, 5, 6, 7, 8). More recently, Tu & Cheng (9) demonstrated an increase of incidence and severity of root rot in kenaf seedlings due to combined infection by *M. javanica* and *M. phaseolina.*

From our studies, it is evident that the combined effect of *Macrophomina phaseolina* and *Meloidogyne javanica* to *L. japonicum* plants was greater to the root system of *L. japonicum,* leading to greater defoliation and dieback with eventual plant loss, than with either pathogen alone. The symptom of foliar creasing was produced and clearly evident when any of the pathogens were present singly or in combination; thus, foliar creasing appears to be a manifestation of a root disturbance of *L. japonicum.* Finally, a study in histological pathogenesis reveals that *Meloidogyne javanica* in the absence of well-defined root galls is present in the vascular system of the root, and characteristically induces the formation of giant cells.

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