

# The Influence of *Trichoderma harzianum* on the Root-knot *Fusarium* Wilt Complex in Cotton<sup>1</sup>

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**Abstract:** Wilt-susceptible cultivar 'Rowden' cotton was inoculated with *Meloidogyne incognita* (N), *Trichoderma harzianum* (T), and *Fusarium oxysporum* f. sp. *vasinfectum* (F) alone and in all combinations in various time sequences. Plants inoculated with F alone or in combination with T did not develop wilt. Simultaneous inoculation of 7-day-old seedlings with all three organisms (NTF) produced earliest wilt. However, plants receiving nematodes at 7 days and *Fusarium* and *Trichoderma* at 2 or 4 weeks later (N-T-F, N-TF) developed the greatest wilt between 49-84 days after initial nematode inoculation. During the same period, *Fusarium* added 4 weeks after initial nematode inoculation (N-F) and *Fusarium* added 4 weeks after initial simultaneous inoculation of nematode and *Trichoderma* (NT-F) produced the least wilt. The addition of *Fusarium* inhibited nematode reproduction. Simultaneous inoculation with nematodes and *Trichoderma* (NT-) resulted in the greatest root gall development, whereas nematodes alone produced the greatest number of larvae. In comparison with noninoculated controls (CK), treatments involving all three organisms inhibited plant growth. Plants inoculated with the nematode alone (N-) or with nematodes and *Trichoderma* (NT-) simultaneously had greatest root weight. Any treatment involving the nematode resulted in fewer bolls per plant and greater necrosis on roots than the noninoculated checks. **Key Words:** *Meloidogyne incognita*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Gossypium hirsutum*, nematode-fungus interactions.

Certain nematode-fungus disease complexes have been intensively investigated, and literature on this subject has been reviewed in detail (10, 13, 14, 16, 19). Recently, attention has been focused on the role of secondary organisms in disease complexes (4, 9, 15). For example, Powell et al. (15) reported the dominant role of *Meloidogyne* as that of a predisposing agent. But more importantly, they showed that combining root-knot nematodes with soil-inhabiting fungi, such as *Pythium*, *Curvularia*, *Botrytis*, *Aspergillus*, *Penicillium* or *Trichoderma* on tobacco, *Nicotiana tabacum* L., caused more root breakdown than each organism by itself. Mayol and Bergeson (9) investigated the effects of soil-borne bacteria in conjunction with *Meloidogyne incognita* and reached a similar conclusion. Because of these types of interactions, major difficulties are encountered in applying the doctrine of specific etiology to disease complexes. Furthermore, failure of disease control in certain situations can be due to incomplete diagnosis of

the disease agents present (13, 14).

Research on disease complexes has contributed to an understanding of increased disease severity resulting from situations involving different nematodes, and pathogens or saprophytes. There are also reports of the interactions of pathogens and associated antagonistic secondary organisms (5, 7, 17, 22). Such antagonism is a potential means of biological control, but to date this is unreliable as a control measure (6, 11, 21). Under natural conditions, disease development involving nematodes with other pathogens and secondary microorganisms undoubtedly is very common. This involvement is acknowledged by the routine procedures used to isolate pathogens from roots which require surface sterilization or selective media to exclude the presence of secondary organisms. The influence of secondary organisms in disease development involving nematodes and other pathogens, therefore, merits intensive study.

This research was designed to determine: (i) the potential interaction of *Trichoderma harzianum* Rifai, which is rarely considered a pathogenic organism, with *Meloidogyne incognita* (Kofoid & White) Chitwood and *Fusarium oxysporum* f. sp. *vasinfectum* (ATK) Synd. & Hans. on cotton, *Gossypium hirsutum* L., and (ii) the effects of sequence and timing of inoculations on wilt development.

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## MATERIALS AND METHODS

*Culture of test plants, nematode and fungus inoculum:* Acid-treated cotton seeds of cultivar 'Rowden,' susceptible to *M. incognita* and *F. oxysporum* f. sp. *vasinfectum*, were germinated in 7-cm diam pots containing a 2:1 (v/v) sandy loam soil-sand mixture and lightly covered with vermiculite. One-week-old seedlings were transplanted to observation boxes (ca. 52 x 42 x 20 cm) equipped with one glass side covered by black plastic which permitted periodic observation of root growth. The boxes contained a 2:1 (v/v) sandy loam-sand mixture. Three open-end glass inoculation tubes were placed equidistantly 3 cm from each plant at transplant time.

Procedures and techniques for obtaining and dispensing the inocula, except for the following exceptions, were previously described by Powell et al. (15). The North Carolina population of *M. incognita* (# 186) was maintained on 'Rowden' cotton plants. To obtain uniform egg masses for inoculation, nematodes from cotton were reared on cultivar 'Manapal' tomato, *Lycopersicon esculentum* Mill., for one generation. Egg masses were removed from roots, and four masses were added through each of three inoculation tubes, the additions thus giving a total of 12 egg masses per plant.

*F. oxysporum* f. sp. *vasinfectum* was isolated from infected cotton stems and cultured on potato-dextrose agar (PDA). The stock culture was kept in a sterilized mixture of 50% soil, 25% peat, and 25% coarse horticultural grade perlite as described by Toussoun and Nelson (20). The isolate of *T. harzianum* was obtained from galled roots of root-knot-infected tobacco plants grown in the greenhouse. For inoculum, fungi were grown separately for 7 days in petri dishes containing PDA. Fungal inocula were prepared separately by blending the contents of one petri dish in 100 ml distilled water in a food blender for 5 sec. For each fungal treatment, 60 ml of the mycelium-spore suspension were added to each plant by pouring 20 ml through each of the three inoculation tubes.

*Treatments and experimental design:* In each box were three plants which received a common treatment. There were

two boxes per treatment in two randomized blocks (Table 1). The experiment was terminated 105 days after nematode inoculation. Plant height, fresh top weight, fresh root weight, number of bolls and flowers were recorded. Roots were separated from the soil by screening. Soil from nematode treatments was saved for larval extraction. Roots were washed and indexed for galls and necrosis according to the classification system and the conversion formula reported by Powell et al. (15). This system consisted of: class 0 = no necrosis or gall on roots; class 1 = less than 10% necrotic or galled; class 2 = 11-25% necrotic or galled; class 3 = 26-50% necrotic or galled; class 4 = 51-75% necrotic or galled; class 5 = 76-100% necrotic or galled.

Wilt indices were recorded on all plants after initial wilt symptoms appeared. The classes for wilt development were: class 0 = no wilt symptoms; class 1 = trace with 1 or 2 leaves showing epinasty; class 2 = half of the leaves showing epinasty and a portion of leaves beginning to yellow; class 3 = 3/4 of the leaves showing epinasty and yellowing portion of the leaf beginning to dry; class 4 = all leaves showing epinasty and lower leaves beginning to fall off; class 5 = plant dead.

After plants and plant roots were classified, wilt, necrosis, or gall indices for each

TABLE 1. Descriptive key to the time and sequence of inoculations of cotton roots with *M. incognita* (N), *T. harzianum* (T) and *F. oxysporum* f. sp. *vasinfectum* (F).

Designation	Description of treatment
CK	= control, no inoculation
T-	= T inoculated 7 days after transplanting
T-F	= T then F 4 weeks later
-TF	= T + F 5 weeks after transplanting
-F	= F 5 weeks after transplanting
N-	= N inoculated 7 days after transplanting
NT-	= N + T 7 days after transplanting
NT-F	= N + T then F 4 weeks later
N-TF	= N then T + F 4 weeks later
N-T-F	= N then T 2 weeks later and F 4 weeks later
NTF	= N + T + F 7 days after transplanting
N-F	= N then F 4 weeks later

treatment were calculated. The following formula was used:

$$\text{Index} = \frac{(\text{No plants in class 1} \times 1 + \text{No plants in class 2} \times 2 + \dots + \text{No plants in class 5} \times 5)}{\text{Total no. plants in treatment} \times 5} \times 100$$

Before nematode assays were made, each soil sample was run through a sample divider four times. The nematodes in a 50-cm<sup>3</sup> soil sample were extracted by the sugar-flotation-sieving method (3). Egg counts were obtained by the method of Byrd et al. (2), except that 10-g chopped root samples were used. Numbers of eggs per plant and larvae per 500 cm<sup>3</sup> of soil were recorded.

A separate statistical analysis was performed for each parameter. LSD values were calculated for comparisons of means of all characteristics except wilt indices. Duncan's new multiple range test values were calculated for each date of wilt development for individual comparison of means.

## RESULTS

Treatments involving *M. incognita* (N), *T. harzianum* (T), and *F. oxysporum* f. sp. *vasinfectum* (F) in combination significantly inhibited plant growth, except that treatment NT-F did not affect plant height (Fig. 1 A-C). However, roots of plants inoculated with *M. incognita* alone (N-) and simultaneously with *T. harzianum* (NT-) weighed more than those of the noninoculated controls. All treatments involving *M. incognita* alone or in combination with either or both fungi caused more root necrosis (Fig. 1-D) and fewer bolls than treatments lacking nematodes. There were no significant differences among treatments for number of flowers per plant. In comparisons between *M. incognita* with *Fusarium* and *M. incognita* with various combinations of *Fusarium* plus *T. harzianum*, treatment N-F resulted in greater top weight and induced less root necrosis than treatment N-TF and induced greater height than treatment NTF.

Plants inoculated simultaneously with *M. incognita* and *T. harzianum* (NT-) produced more root galls than plants inoculated with *M. incognita* alone (N-); however, the latter contained more nematode

larvae per 500 cm<sup>3</sup> of soil than any other treatment (Fig. 2-A). Plants inoculated with *M. incognita* and *Fusarium* (N-F) supported more nematodes per 500 cm<sup>3</sup> of soil than plants initially inoculated with *M. incognita* followed by *T. harzianum* and *Fusarium* 2 and 4 weeks later (N-TF, N-T-F). Egg production per plant was less in treatments containing *Fusarium* than in other treatments (Fig. 2-B).

Wilt initially appeared 14 days after simultaneous inoculation of *M. incognita*, *F. oxysporum* f. sp. *vasinfectum*, and *T. harzianum* (NTF) on 1-week-old seedlings (Fig. 3). The same incubation period was noted for plants receiving *Fusarium* 4 weeks and *T. harzianum* 2 and 4 weeks after initial *M. incognita* inoculation (N-TF, N-T-F). However, in plants in treatments NT-F and N-F, the initial wilt symptoms appeared 21 days and 35 days after *Fusarium* inoculation, respectively. Wilt never developed on plants inoculated with *Fusarium* alone (-F) or *Fusarium* and *T. harzianum* combinations (T-F, -TF). Between 63 and 84 days, plants in treatments N-T-F, N-TF, and plants in NTF showed greater wilt development than plants inoculated with *M. incognita* and *Fusarium* (N-F). After 84 days, no significant difference occurred among treatments involving all three organisms or *M. incognita* in combination with *Fusarium*. However, at 105 days when the experiment was terminated, all combination treatments involving *M. incognita* were significantly different from the various treatments involving only fungi.

## DISCUSSION

These results confirm the importance of *M. incognita* in a disease complex with *Fusarium* and indicate the potential role of the saprophytic fungus, *T. harzianum*. The phenomenon of increasing the incidence of wilt in the presence of root-knot nematode is not new (13, 14). However, the addition of *T. harzianum* in this disease complex resulted in greater wilt development than occurred with the nematode and *Fusarium*.

The finding that simultaneous inoculations with all three organisms on cotton seedlings produced the greatest wilt is in

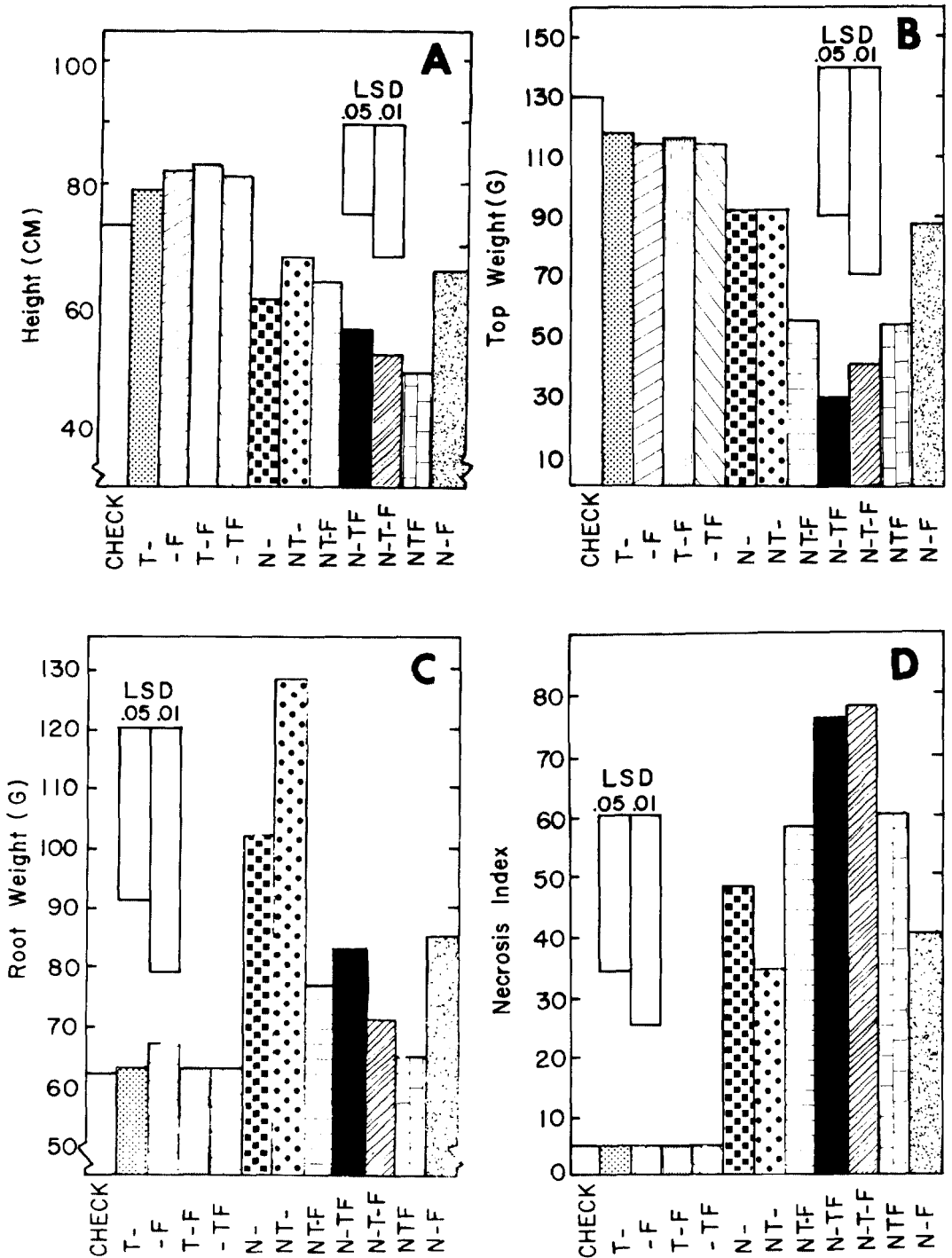


FIG. 1 (A-D). Effects of a sequence of inoculations with *Meloidogyne incognita* (N), *Fusarium oxysporum* f. sp. *vasinfectum* (F), and *Trichoderma harzianum* (T), alone and in combination on growth and root necrosis of cotton. (A) Plant height. (B) Top weights. (C) Root weights. (D) Necrosis developing on roots (refer to Table 1 for key to treatment symbols and to time and sequence of inoculations).

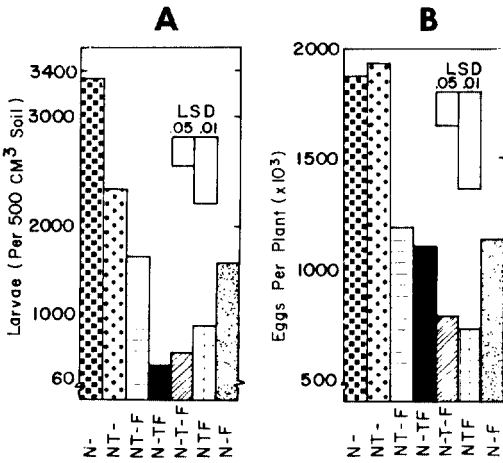


FIG. 2 (A-B). The effects of sequence of inoculation with *Meloidogyne incognita* (N), alone, and combined with *Fusarium oxysporum* f. sp. *vasinfectum* (F) and/or *Trichoderma harzianum* (T) on nematode population densities. (A) Final population of second-stage larvae. (B) Numbers of eggs (in 1000s)/plant (refer to Table 1 for key to treatment symbols).

agreement with Inagaki and Powell (8). This greater wilt production may not occur if the inoculations are made when plants are older (12). Several workers (1, 12, 15) have reported that the predisposing effects of *M. incognita* are most pronounced when the nematodes have been in the roots for a period of 3-4 weeks. This effect was reflected in the treatments N-T-F and N-TF, when *T. harzianum* was inoculated 2 and 4 weeks after initial nematode

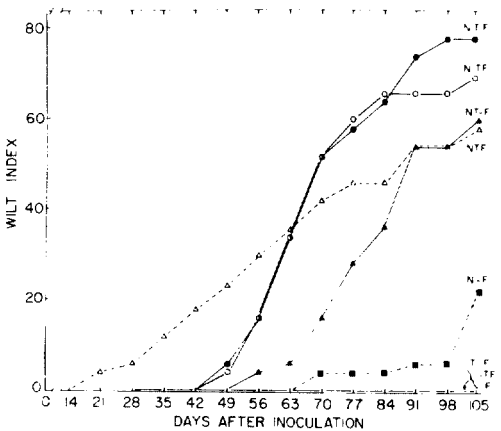


FIG. 3. Effects of the time of inoculation with *Meloidogyne incognita* (N), *Fusarium oxysporum* f. sp. *vasinfectum* (F), and *Trichoderma harzianum* (T) alone and in combinations on wilt development of 'Rowden' cotton (refer to Table 1 for key to treatment symbols).

inoculation, and greater wilt development occurred between 63-84 days after initial *M. incognita* inoculation. The more severe wilt was responsible for these two treatments having fewer larvae than NT-F. Further evidence for supporting this conclusion lies in the fact that the effect of *T. harzianum* in treatment NT- is not significantly different from treatment N- except for numbers of larvae per 500 cm<sup>3</sup> of soil and root-gall indices, whereas the N-T-F and N-TF treatments had a significantly greater necrosis index in comparison with N-F. The apparent ineffectiveness of *T. harzianum*, when it is inoculated at the same time as root-knot nematode, may be due to the difficulties in maintaining a high fungal population throughout the experiment. Prior modification of the rhizosphere by *M. incognita* in a manner favorable to the fungus may be necessary. This modification might be accomplished either by changes in the pH of the soil or by physiological changes in the root brought about by the root-knot nematode (6, 14).

The role that each of the three organisms plays in wilt development is not completely clear. The root-knot nematode is required for *Trichoderma* to accentuate wilt, and the predisposing effect of this nematode reaches a maximum 2-4 weeks after its addition. In this situation, a saprophyte, such as *T. harzianum*, is quite capable of causing a breakdown of root tissues. This fungus may further predispose the plant to wilt by making the vascular portion of the root more accessible to *Fusarium* (18). *T. viride*, which was not used in this study, is widely considered as an antagonist to other fungi occurring in the soil. In these tests, wherein *T. harzianum* functioned as an important component of the disease complex, this was not the case.

With the increased interest in ecology, pathologists are becoming more interested in biological control through antagonistic action among organisms such as saprophytes and parasitic fungi. Results may often be promising in the laboratory; yet failures occur under actual field conditions. It is possible that failure in the field may be due to a new environment produced by a third organism. Furthermore, "antagonists" such as *Trichoderma* may actually be increasing

plant disease by interacting with nematodes and certain soil microflora.

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