Interaction of Corynebacterium michiganense and Meloidogyne incognita on Tomato

R. M. de Moura, E. Echandi, and N. T. Powell

Graduate Student and Professors, respectively, Department of Plant Pathology, North Carolina State University, Raleigh 27607. Present address of senior author: Escola Superior de Agricultura da Universidade Federal Rural de Pernambuco, Dois Irmaos, Recife, Pernambuco, Brazil.

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ABSTRACT

An interaction involving *Corynebacterium michiganense* and root-knot nematode, *Meloidogyne incognita*, was observed under greenhouse conditions when *C. michiganense* was inoculated in the stem of tomato plants. *M. incognita* increased severity of bacterial canker on tomato cultivars Manapal and MR-4, canker-susceptible and resistant cultivars, respectively. No significant difference in bacterial canker severity was observed in Manapal and MR-4 when inoculated with *M. incognita* simultaneously or 10 days prior to inoculation with *C. michiganense*. No increase in disease severity was observed on bacterial-canker-resistant *Lycopersicon hirsutum* (P.I. 251305) when inoculated with both pathogens. *C. michiganense* had no effect on reproduction of *M. incognita*.

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Additional key words: bacterial canker, root-knot nematode.

Bacterial canker of tomato caused by *Corynebacterium michiganense* (E.F. Sm.) H. L. Jens. and southern root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, are frequently found in close association on trellised tomatoes in western North Carolina. Although it is well-known that both pathogens can be very destructive when acting alone, their combined pathological potential may be far greater than their individual effects.

Nematode-bacteria relationships increase the incidence and severity of certain diseases caused by phytopathogenic bacteria (5, 6), but few reports on these relationships have been published.

The objectives of this study were to: (i) determine the effects of *M. incognita* on the development of bacterial canker in canker-susceptible and resistant tomato accessions, when inoculated simultaneously with and prior to *C. michiganense*, and (ii) to investigate the effect of *M. incognita* on a canker-susceptible tomato accession when inoculated with three isolates of *C. michiganense* with different virulence.

MATERIALS AND METHODS.—A population of *M. incognita* originally from tomato was maintained on tomato roots in the greenhouse at 28 C. Stock cultures of

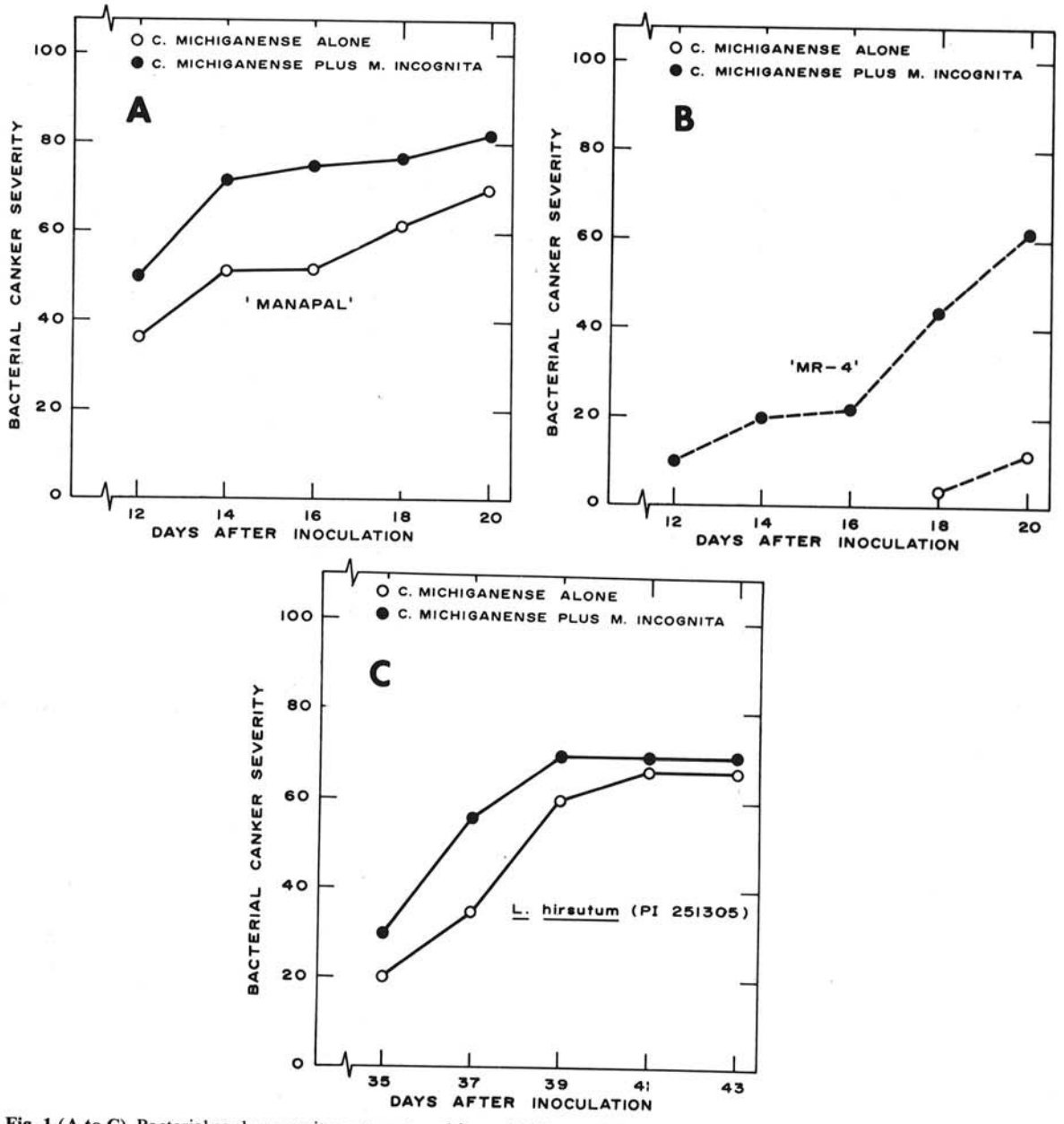

Fig. 1-(A to C). Bacterial canker severity on tomato cultivars **A)** Manapal, **B)** MR-4, and **C)** *Lycopersicon hirsutum* (P.I. 251305) inoculated with *Corynebacterium michiganense* alone and simultaneously with *Meloidogyne incognita*. Bacterial canker severity was based upon a 0-100 scale (see text). Each accession was inoculated separately and considered a separate experiment. Means represent the average of 10 replications. Disease evaluations for Manapal, MR-4, and *L. hirsutum* made 20, 20, and 43 days, respectively, after bacterial inoculations, were analyzed and found significantly different ($P = 0.05$) for Manapal and MR-4.

C. michiganense were maintained on nutrient agar at 4 C. *C. michiganense* for inocula was grown on yeast dextrose carbonate agar slants at 28 C for 24 hours. The same highly virulent isolate was utilized in all experiments and two others with intermediate and slight virulence were utilized in one experiment.

Canker-susceptible cultivar Manapal, resistant cultivar MR-4, and *Lycopersicon hirsutum* (P.I. 251305) (1) were grown from seed in vermiculite and transplanted when 12 days old into 5-cm diameter pots containing a mixture (3:1, v/v) of methyl bromide-fumigated soil and sand. After 30 days, plants that were 20 to 25 cm tall were transferred with soil and roots intact into the same type of soil mixture contained in 15-cm diameter clay pots. Just before transplanting, twelve freshly picked egg masses of *M. incognita* were suspended in sterilized water and dispensed into each hole made to receive the intact root system. Plants were then placed in the hole, and soil pressed around the roots. Control plants were similarly treated, but received no nematodes. Plants were inoculated with *C. michiganense* by puncturing the stem twice above cotyledonary leaves with a dental root-canal

file (Style D, No. 1, Kerry Manufacturing Co., Detroit, Mich.) previously dipped in a nutrient broth suspension containing 10^7 cells/ml. Bacterial inoculum was standardized with a Spectronic 20 colorimeter (1).

Ten plants were utilized per treatment in all experiments. Treatments consisted of: (i) *C. michiganense* alone; (ii) *C. michiganense* plus *M. incognita* inoculated simultaneously; (iii) *M. incognita* inoculated 10 days prior to *C. michiganense*; (iv) *M. incognita* alone; and (v) noninoculated control. Treatments were randomized in a complete block design in a greenhouse with air temperatures ranging 26-36 C during the day and 16-26 C at night. Plants were irrigated as required with tap water and fertilized once a week with a commercial general purpose 20-20-20 fertilizer. All experiments lasted about 45 days and were repeated twice.

A bacterial canker severity index was used to quantitate on a scale of 0 to 100 the host population reaction to bacterial canker. At a given time all plants were rated into six categories as follows: 0 = no wilt; 1 = one lower leaf wilted; 2 = two to four lower leaves or fewer than half of the lower leaves wilted; 3 = half to three-quarters of the leaves wilted; 4 = two of the top five leaves or more than three-quarters of the leaves wilted, but the terminal leaves of the main shoot not wilted; and 5 = terminal leaves of the main shoot and most leaves wilted or dead (1). Then the following formula was applied to compute the bacterial canker severity index (BCSI):

$$\text{BCSI} = \frac{(0n_0 + 1n_1 + \ldots\ldots + 5n_5)\ 100}{n_t\ (n_c - 1)}$$

where n_0 = the number of plants in disease category 0 etc., n_t = total number of plants, and n_c = total number of disease categories (8).

Nematode reproduction was determined using the method described by Loewenberg et al. (4).

RESULTS AND DISCUSSION.—Bacterial canker-susceptible and resistant tomato accessions inoculated simultaneously at transplanting with *C. michiganense* and *M. incognita*, developed more severe bacterial canker symptoms than those inoculated with *C. michiganense*. Bacterial canker symptoms were observed first in plants of the canker-susceptible Manapal (Fig. 1). In the canker-resistant MR-4, severe symptoms were observed in plants inoculated with both pathogens 12 days after inoculation, whereas 18 days were required for expression in plants inoculated with only bacteria (Fig. 1). Symptoms appeared about 30 days after inoculation in plants of *L. hirsutum* inoculated with both pathogens and with bacteria alone, but differences in disease severity were not statistically significant (Fig. 1).

In this study, *C. michiganense* did not affect the reproduction of *M. incognita* on Manapal or MR-4. Similar study-observations were not made on *L. hirsutum*. Schuster and Wagner (7) observed no relationship between *C. michiganense* and *M. incognita* when both pathogens were simultaneously added to the

TABLE 1. Bacterial canker index on tomato cultivars Manapal, MR-4, and *Lycopersicon hirsutum* (P.I. 251305) inoculated with *Corynebacterium michiganense* and *Meloidogyne incognita*

Inoculation	Bacterial canker index[z]		
	Manapal	MR-4	*L. hirsutum*
C. michiganense plus *M. incognita* simultaneously	88 a	76 a	70 a
M. incognita 10 days prior to *C. michiganense*	82 a	38 a	26 b

[z]Each accession was inoculated separately and considered a separate experiment. Means represent the average of 10 replications. Values with the same letters are not significant, $P = 0.05$. Disease evaluations for Manapal, MR-4, and *L. hirsutum* were made 19, 38, and 43 days (respectively) after inoculation with *C. michiganense*.

A bacterial canker severity index (BCSI) was based upon scale of 0 to 100. At a given time all plants were rated into six categories as follows: 0 = no wilt; 1 = one lower leaf wilted; 2 = two to four lower leaves or fewer than half of the lower leaves wilted; 3 = one-half to three-quarters of the leaves wilted; 4 = two of the top five leaves or more than three-quarters of the leaves wilted, but the terminal leaves of the main shoot not wilted; and 5 = terminal leaves of the main shoot and most leaves wilted or dead (1). Then the following formula was applied to compute the bacterial canker severity index:

$$\text{BCSI} = \frac{(0n_0 + 1n_1 + \ldots\ldots + 5n_5)\ 100}{n_t\ (n_c - 1)}$$

where n_0 = the number of plants in disease category 0 etc., n_t = total number of plants, and n_c = total number of disease categories (8).

TABLE 2. Bacterial canker index on tomato cultivar Manapal inoculated with three isolates of *Corynebacterium michiganense* differing in virulence in the presence and absence of *Meloidogyne incognita*

Isolates and virulence	Bacterial canker index[z]	
	C. michiganense alone	*C. michiganense* plus *M. incognita*
High	76 b	86 a
Intermediate	22 d	38 c
Slight	8 e	18 d

[z]Bacterial canker severity was based upon a 0-100 scale (see text). Means represent the average of 10 replications. Differences between means with common letters are not significant, $P = 0.05$. Disease evaluation was made 38 days after bacterial inoculation.

A bacterial canker severity index (BCSI) was based upon scale of 0 to 100. At a given time all plants were rated into six categories as follows: 0 = no wilt; 1 = one lower leaf wilted; 2 = two to four lower leaves or fewer than half of the lower leaves wilted; 3 = one-half to three-quarters of the leaves wilted; 4 = two of the top five leaves or more than three-quarters of the leaves wilted, but the terminal leaves of the main shoot not wilted; and 5 = terminal leaves of the main shoot and most leaves wilted or dead (1). Then the following formula was applied to compute the bacterial canker severity index:

$$\text{BCSI} = \frac{(0n_0 + 1n_1 + \ldots\ldots + 5n_5)\ 100}{n_t\ (n_c - (n_c - 1)}$$

where n_0 = the number of plants in disease category 0 etc., n_t = total number of plants, and n_c = total number of disease categories (8).

nutrient solution of hydroponically-grown tomatoes of the cultivar Michigan-Ohio. It is possible that when tomato roots are inoculated with both pathogens, different results are obtained.

When Manapal and MR-4 were inoculated with *M. incognita* 10 days prior to inoculation with *C. michiganense*, no significant difference in canker severity was observed when compared to treatments receiving the two pathogens simultaneously (Table 1). *L. hirsutum* inoculated with both pathogens at the time of transplanting was more severely affected by canker than when inoculated with the nematode and 10 days later with the bacteria. *L. hirsutum* grows slowly requiring several days to become established. Plants inoculated with the bacteria 10 days after transplanting were well established and could be more resistant to canker than those inoculated immediately after transplanting.

Griffin and Hunt (2) found no significant difference in disease severity between simultaneously inoculated susceptible alfalfa cultivars and cultivars inoculated with *C. insidiosum* 30 days before and after inoculating with *M. hapla*. But Johnson and Powell (3) demonstrated that inoculating bacterial wilt-susceptible tobacco cultivars with *M. incognita* 3 or 4 weeks prior to inoculation with *Pseudomonas solanacearum* increased wilt symptoms to a greater degree than inoculating with the pathogens simultaneously. It appears that, in some bacterium-nematode combinations, the time of nematode inoculation has no influence on disease severity; however, with others, it is of major importance.

Regardless of the level of virulence of the bacterium, bacterial canker was more severe in Manapal plants inoculated simultaneously with *C. michiganense* and *M. incognita* than in those inoculated with the bacterium alone (Table 2).

LITERATURE CITED

1. FORSTER, R. L., and E. ECHANDI. 1973. Relation of age of plants, temperature, and inoculum concentration to bacterial canker development in resistant and susceptible Lycopersicon spp. Phytopathology 63:773-777.
2. GRIFFIN, G. D., and O. J. HUNT. 1972. Effects of temperature and inoculation timing on the Meloidogyne hapla/Corynebacterium insidiosum complex in alfalfa. J. Nematol. 4:70-71.
3. JOHNSON, H. A., and N. T. POWELL. 1969. Influence of root knot nematodes on bacterial wilt development in flue-cured tobacco. Phytopathology 59:486-491.
4. LOEWENBERG, J. R., T. SULLIVAN, and M. L. SCHUSTER. 1960. The effect of pH and minerals on the hatching and survival of Meloidogyne incognita incognita larvae. Phytopathology 50:215-217.
5. PITCHER, R. S. 1963. Role of plant-parasitic nematodes in bacterial diseases. Phytopathology 53:35-39.
6. POWELL, N. T. 1971. Interaction of plant parasitic nematodes with other disease-causing agents. Vol. II, p. 119-136 *in* B. M. Zuckerman, W. F. Mai, and R. A. Rohde. (ed.) Plant-parasitic nematodes. Academic Press, New York. 347 p.
7. SCHUSTER, M. L., and L. J. WAGNER. 1972. Control of bacterial canker and root knot of hydroponic tomatoes. Plant Dis. Rep. 56:139-140.
8. SILBERNAGEL, M. J., and A. M. JAFRI. 1974. Temperature effects on curly top resistance in Phaseolus vulgaris. Phytopathology 64:825-827.