INFLUENCE OF ROOT-KNOT NEMATODE ON BACTERIAL WILT SEVERITY IN TOMATO

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ABSTRACT

Wilt susceptible tomato plants grown in both soils infested with *Pseudomonas solanacearum* and *P. solanacearum - Meloidogyne incognita* combination started to die of wilt one week after transplanting; all plants died of wilt one week earlier in the latter than in the former. With resistant cultivars, wilted (dead) plants in soils infested with both the bacterium and the nematode occurred one to four weeks earlier than those plants grown in the bacterium-infested soil alone. Yields of wilt resistant cultivars grown in soils infested with the bacterium-nematode combination were lower (13%) than those plants grown in soils infested with the bacterium alone (23%). None of the plants inoculated or grown in naturally or artificially nematode-infested soil alone died of wilt after the experiment.

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INTRODUCTION

Bacterial wilt caused by *Pseudomonas solanacearum* E. F. Smith is one of the most serious diseases of solanaceous crops in both tropical and sub-tropical regions of the world. Young plants are known to develop bacterial wilt symptoms more readily than older plants (Kelman, 1953). Natural infection is usually observed in the field at flowering stage or later. Tomato cultivars believed to be wilt resistant succumb to bacterial wilt when planted in different localities at different times. This phenomenon has been attributed to a number of factors, notable among which are differences in aggressiveness of strain, plant age, and presence of other soil pathogens (Buddenhagen and Kelman, 1964; Mew and Ho, 1976; Powell, 1971).

Among the root-invading pathogens which predispose plants to *P.*

solanacearum, the root-knot nematode appears to be the most notorious (Steward and Schindler, 1956). This study aimed to determine whether addition of *M. incognita* affects the yield and influence the development and severity of bacterial wilt on locally-developed wilt-resistant tomato cultivars.

MATERIALS AND METHODS

Preparation of Test Plants. — Six resistant tomato cultivars (Marikit, VC 48-1, Pope, 1169, Venus and VC 11-1) and two susceptible cultivars (Yellow plum and 2029) were used in the study. The seeds were treated with Arasan 75 and were sown in seedboxes containing heat sterilized soil. The seedlings were transplanted into 15 cm pots with decontaminated soil.

Test Organisms and Preparation of Inocula for Artificial Inoculation. — The organisms used were *Pseudomonas solanacearum* E. F. Smith (Race I, Biovar, IV, isolate LE 100) and *Meloidogyne incognita* Chitwood. Fresh inoculum of bacteria was prepared by taking a loopful of the pure culture in sterile distilled water and streaking it onto plates of Kelman's medium (Kelman, 1954). After 36 hr incubation at room temperature, a pure, single and fluidal colony was picked with sterile wireloop and streaked on a freshly prepared Kelman's medium without the tetrazolium salt. The bacteria from this culture were multiplied on plates with the same medium for 36-48 hr and then suspended in tap water. The bacterial density of the required inoculum was standardized by using a Bausch and Lomb "Spectronic 20" spectrophotometer.

The egg inoculum of *M. incognita* was prepared using Sasser's technique (1976). The concentration of eggs per ml was determined by removing three-1ml samples, counting the eggs per sample and using the average to represent the number of eggs per ml. Finally, the volume of water was adjusted to obtain the desired number of eggs per ml.

Preparation of Soils Naturally Infested with Bacteria or Nematode. — Preparation of soil naturally infested with bacteria was carried out as follows: Wilt susceptible tomato cv 2029 was planted in sterilized soil. Three-week old tomato plants were inoculated with *P. solanacearum* using the root injury technique by cutting the lateral roots with a scalpel approximately 3 cm away from the stem and 6 cm deep. One hundred ml of the bacterial suspension was then poured over the injured roots. The infested soils were then mixed thoroughly to obtain a uniformly infested soil for natural inoculation experiments.

Preparation of soil naturally infested with nematodes was carried out as follows: Mature egg masses of *M. incognita* were taken from galled tomato roots; the eggs were freed by tearing off the culticle of the egg mass. An egg suspension in tap water was prepared and inoculated into the root zone of tomato cv

2029. Forty-five days after inoculation, the roots of the inoculated plants were carefully removed and the nematode eggs were collected using Sasser's technique. These egg inocula (650 egg/plant) were used to inoculate several potted tomato plants. The plant were allowed to mature to enable the nematodes to multiply. At maturity, the plants were cut close to the soil, leaving the root system. The nematode infested soils and the galled tomato roots were chopped finely and then mixed thoroughly to obtain a uniformly infested soil for the natural inoculation experiments.

Effects of Bacteria-Nematodes Combination on Bacterial Wilt Severity. —

A. *Artificial Inoculation*

Effects of Bacteria-Nematodes Combination on Bacterial Wilt Severity. —

A. *Artificial Inoculation*

Wilt resistant and wilt susceptible cultivars received the following treatments:

(1) Uninoculated control
(2) *P. solanacearum*
(3) *M. incognita*
(4) *P. solanacearum* and *M. incognita* simultaneously
(5) *P. solanacearum* first plus *M. incognita* after one week
(6) *M. incognita* first plus *P. solanacearum* after one week

A 5 x 6 factorial experiment in randomized complete block design was used. Inoculations were made on three week-old plants grown in pots with decontaminated soil. Inoculation with *P. solanacearum* was made by pouring 100 ml of bacterial suspension (0.3 OD at 425 nm) around the base of each plant. *M. incognita,* on the other hand, was introduced into a depression in the soil in the root zone using 600 eggs per plant. Control plants were treated with distilled water.

B. *Natural Inoculation*

Natural inoculations using soils naturally infested with bacteria and/or nematodes were carried out using four wilt-resistant cultivars, namely, Marikit, VC 48-1, Pope, 1169, and one wilt-susceptible cultivar, 2029. The treatments were: (1) uninoculated control, and, (2) soils infested with *P. solanacearum* and *M. incognita.* A 5 x 4 factorial experiment in RCBD was used.

Preparation of the soil for the different treatments was carried out as follows: Soil infested with *P. solanacearum* was mixed with an equal volume of *M. incognita*-infested soil to have soils infested with the bacterium and the nematode. Soil infested with *M. incognita* (or *P. solanacearum)* was mixed with an equal volume of sterilized soil to have soil infested with the nematode (or bacterium) alone. Sterilized soil was used in the control.

The seedlings of the varieties were transplanted in pots containing

the necessary soils for the different treatments two weeks after pricking.

RESULTS AND DISCUSSION

Natural Inoculation.

Percentage mortality. — Plants grown in *M. incognita*-infested soil and the control plants remained relatively free of wilt throughout the experimental period in all the varieties used (Fig. 1). Plants grown in soils infested with *P. solanacearum* alone and in *P. solanacearum-M. incognita* combination developed the disease one week after transplanting in wilt susceptible tomato cv 2029. However, mortality increased with time such that all plants died of wilt five and four weeks after transplanting for those grown in soils infested with bacteria and bacteria-nematode combination, respectively.

In wilt-resistant tomato cv Marikit (Fig. 2), wilting occurred four weeks after transplanting in bacteria-nematodes combination treatment, while that of *P. solanacearum* alone, six weeks after transplanting. In cv VC 48-1 and Pope, the disease started to develop two weeks after transplanting in bacterium-nematode treatment, which was earlier by one and four weeks, respectively, than plants grown in soil infested with *P. solanacearum* alone. The disease developed much later among the wilt resistant cultivars in cv 1169 which started six weeks after transplanting for the bacterium-nematode treatment, while that of the bacterium alone treatment was one week later.

The development of the disease in the bacterium-nematode treatment was consistently earlier than those treated with the bacterium alone in all the varieties used. This indicates that the presence of *M. incognita* hastened the development of bacterial wilt of tomato. In general, mortality of plants grown in bacterium-nematode infested soil was significantly higher by 47.6% than those plants grown in soils infested with the bacterium alone. Plants grown in soils infested with the two pathogens combined had significantly higher mortality than those plants grown in soils infested with the bacterium alone. Data also showed that the two pathogens could interact to cause early development and severe damage to wilt-resistant tomato.

Although the plants succumbed to bacterial wilt in bacterium alone treatment, development and severity of the disease was consistently earlier in plants grown in soils infested with the combination of bacterium and nematode. Severe infection of resistant plants grown in soils infested with the bacterium alone could be attributed to the high concentration of inoculum that could have increased per unit area due to continued multiplication of the bacterium. Kelman and Sequira (1962) and Libman (1964) stated that infection may take place in uninjured roots when the concentration of the inoculum was sufficiently heavy.

The results corroborated the

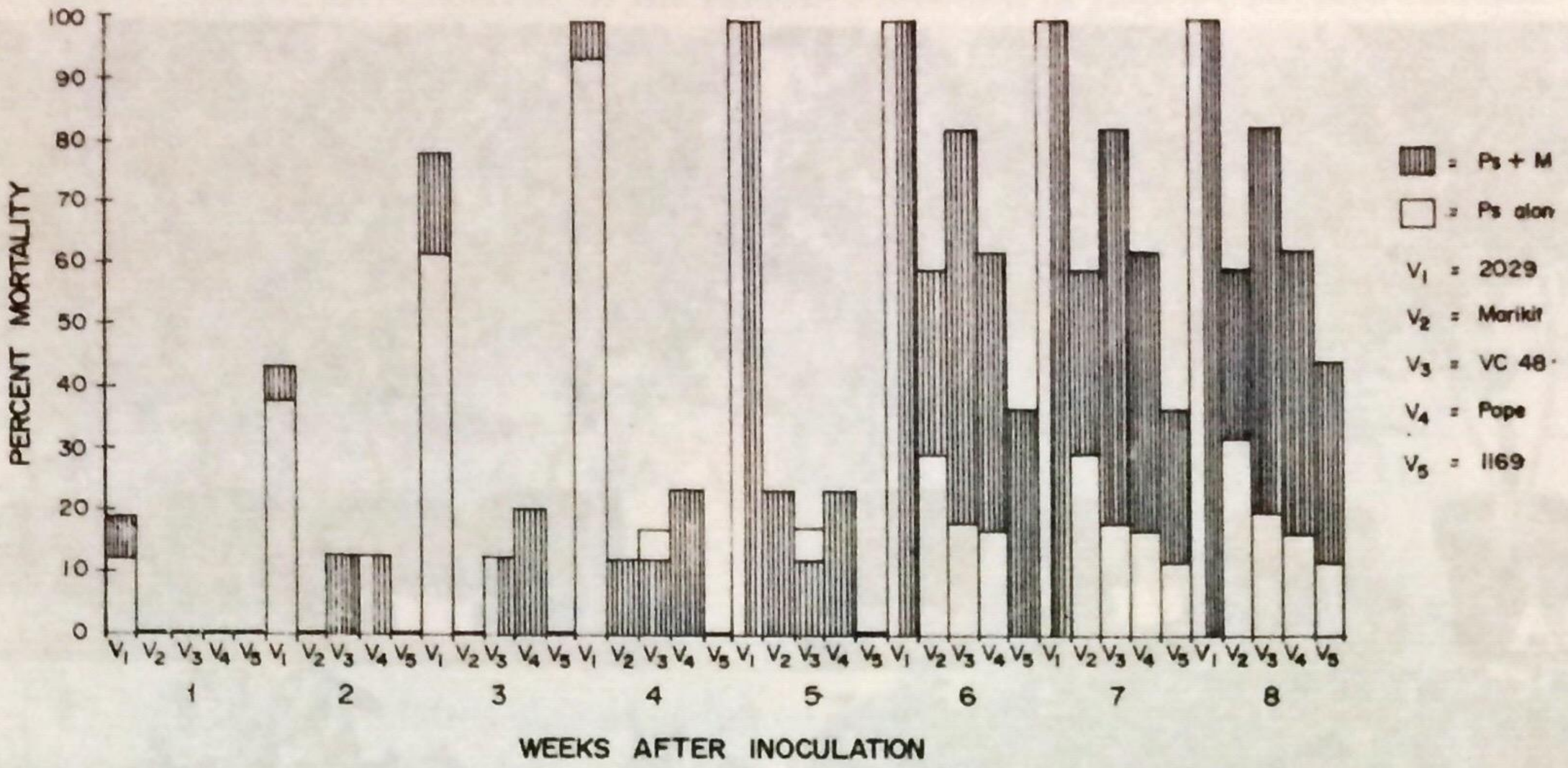

Fig. 1. Weekly percentage mortality of five tomato cultivars grown in soil naturally infested with *Pseudomonas solanacearum* and *Meloidogyne incognita* alone or in combination.

reports of other workers on tobacco (Lucas *et al.*, 1955; Johnson and Powell, 1969) and white potato (Feldmesser and Goth, 1970) that the presence of root-knot nematodes increases the incidence and severity of bacterial wilt caused by *P. solanacearum.* These authors concluded that the role of nematodes in the complex appeared principally that of providing wound through which the bacteria may enter and also useful metabolites for bacterial growth.

Number of fruits per plant. — Instead of fruit weight, the number of marketable fruits per plant was used as the criterion for determining the effect of the pathogens on the yield of the test varieties.

The control plants, in all the varieties tested, had significantly higher yield than those treated with the pathogens. The combined effects of the two pathogens, however, further reduced the yield by 51% (Table 1). Among wilt-resistant cultivars, plants grown in soils infested with bacterium-nematode combination had lower yield compared to those plants grown in soils infested with *P. solanacearum* alone by 23, 20, 13 and 13% for Marikit, VC 48, Pope and 1169, respectively. However, there was no significant difference among the treated plants though interactions were observed. Comparison among variety means showed that yield of 1169 differed significantly from that of Marikit, VC 48-1 and Pope; differences among the last three varieties, however, were insignificant.

Artificial Inoculation.

Percentage mortality. — Development of the disease started during the first week after inoculation in wilt-susceptible tomato cv Yellow plum (Fig. 3) inoculated simultaneously with *P. solanacearum* and *M. incognita.* Those inoculated with *P. solanacearum* alone,


2A
B
C
D
4A
V1To
B
V1T2
C
V1T1
D
V1T3

Table 1. Comparative analysis of the percent reduction in yield of different tomato varieties grown in soils naturally infested with *Pseudomonas solanacearum* and/or *Meloidogyne incognita*.

Treatment[1]	Reduction in yield (%)					Treatment
	2029	Marikit	VC 48-1	Pope	1169	Mean
P. solanacearum	100	13.3	44.4	30.3	16.6	40.9
M. incognita	28.6	46.6	44.4	30.3	16.6	33.3
P. solanacearum + *M. incognita*	100	33.3	55.5	39.4	27.7	51.18

[1] Soils naturally infested with the indicated pathogens. Control had sterilized soil.

P. solanacearum first plus *P. solanacearum* one week after, developed the disease in the second week after inoculation. However, those plants treated with the two pathogens combined had higher mortality and died of wilt on the fifth week compared to those plants inoculated with the bacterium alone. Those inoculated with the bacterium alone died of wilt on the seventh week (Fig. 4).

In a wilt-resistant cultivar, like VC 48-1, wilt developed after one week when the two pathogens were inoculated simultaneously. Five weeks after, wilt developed in *P. solanacearum* alone and *P. solanacearum* first plus *M. incognita* one week later or vice-versa. The same pattern of development was observed in the other resistant varieties.

The apparent delayed severity of the disease, especially in resistant varieties, was possibly due to the low concentration of *M. incognita*. Davide (1967) reported that on tomato and bitter gourd under field condition when the temperature

Fig. 2. (TOP) Wilt-resistant tomato cv Marikit growing in soil naturally infested with *P. solanacearum* and *M. incognita* alone or in combination . A) Uninoculated control, B) *M. incognita* alone, C) *P. solanacearum* alone, D) *P. solanacearum* and *M. incognita*.

Photographs taken 56 days after inoculation. Note healthy plants in treatments A and B. One plant died of wilt in treatment C and several in treatment D.

Fig. 4. (BOTTOM) Wilt susceptible tomato cv Yellow plum artificially inoculated with *P. solanacearum* and *M. incognita* alone or in combination. A) Uninoculated control, B) *M. incognita* alone, C) *P. solanacearum* alone, D) *P. solanacearum* plus *M. incognita* simultaneously.

Photographs taken 3 weeks after inoculation. Note severe wilting of plants inoculated with *P. solanacearum* and *M. incognita* (treatment D). All plants in treatments D and C died of wilt within 4 and 7 weeks, respectively, after inoculation.

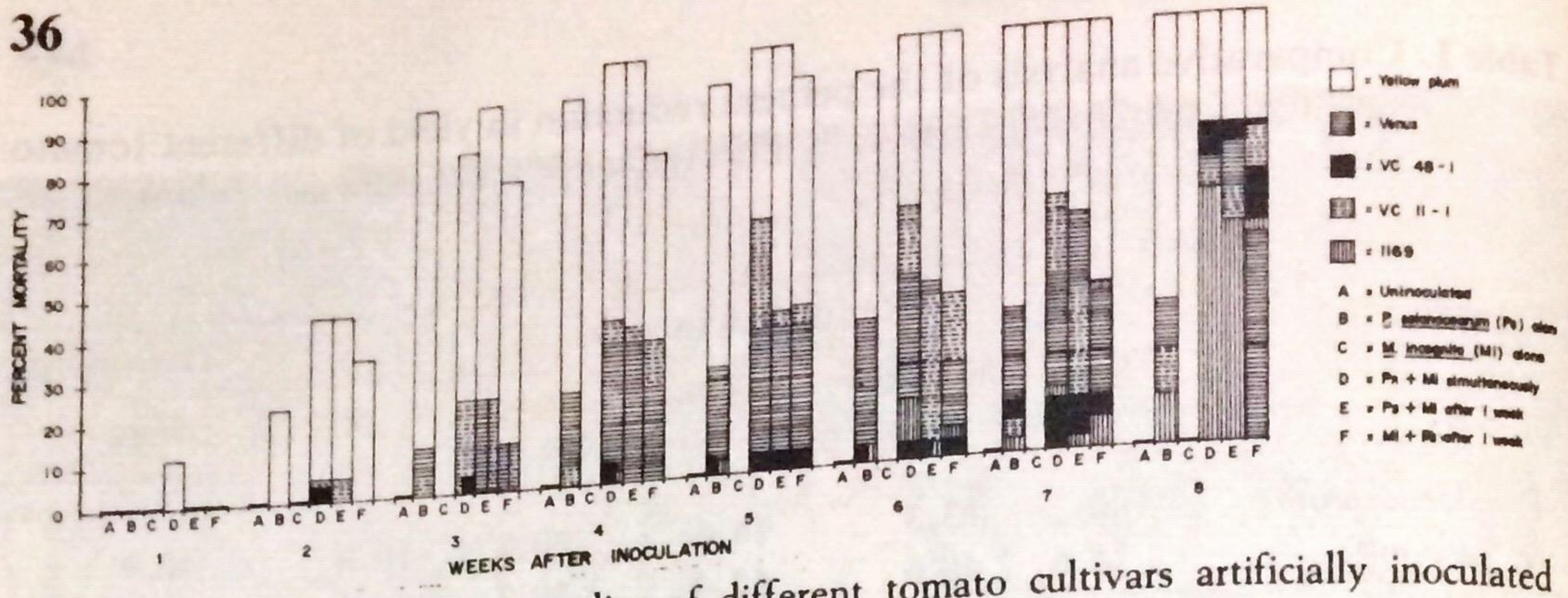

Fig. 3. Weekly percentage mortality of different tomato cultivars artificially inoculated with *Pseudomonas solanacearum* and *Meloidogyne incognita* alone or in combination.

ranged from 25-29°C, *M. incognita* reached adult stage in 10 days, started producing eggs with second stage larvae in 15 days after inoculation, and reached the peak of egg production within 18 to 20 days. The temperature from July to October, when the experiment was conducted, ranged from 23.1 to 31.7°C. Furthermore, it is possible that the second generation started at the third week after inoculation, hence severity to the disease started in that week.

None of the plants died of wilt in the control and nematode alone treatments. Plants inoculated with the bacterium-nematode combination had consistently higher mortality than plants inoculated with the bacterium alone in all the test varieties. For all wilt resistant cultivars, significant differences in percent mortality between plants inoculated with the bacterium-nematode combination and with the bacterium alone were exhibited on the seventh week after inoculation.

Among the test varieties, Yellow plum had the highest mortality (67%) followed by VC 48-1 (37%), Venus (36%), VC 11-1 (36%), and 1169 (29%). Highly significant differences among varieties, treatment and variety x treatment were observed. The results confirmed the findings of Johnson and Powell (1969), Katsura and Uemura (1963) and Davide (1972) that the presence of root-knot nematode greatly enhanced the severity of bacterial wilt.

Comparison among treatment means within variety (DMRT) showed that mortality did not differ significantly from each other in treatments where the bacterium and nematode were combined. However, these treatments had significantly higher percentage mortality than the treatment with the bacterium alone.

Number of fruits per plant. — Results showed that all plants of all test varieties inoculated with the pathogens, be it separately or in combination, had lower yields than the control (Table 2). In Yellow plum, no data were gathered in all treatments with *P. solanacearum* since all the plants died before the fruits matured. However, plants inoculated with the nematode alone treatment had lower yield (29%)

than the control plants.

In wilt resistant cultivars, i.e., Venus, VC 48-1, VC 11-1 and 1169, lowest yield was consistently observed when the two pathogens were combined compared to the bacterium alone treatment. Highly significant differences among treatment means were observed. Interaction between variety and treatment did not show significance.

Comparison among treatment means within variety (DMRT) revealed that all plants inoculated with the pathogens have significantly lower yield than the control. The combined effects of the two pathogens lowered the yield by 16 to 23% compared to those inoculated with the bacterium alone. The plants inoculated with the pathogens, be it separately or in combination, did not show significant difference from each other. This showed that the presence of bacteria and nematode in combination did not further reduce the potential yield of tomatoes although differences were observed.

Table 2. Comparative analysis of percent reduction in yield of five tomato varieties artificially inoculated with *Pseudomonas solanacearum* and/or *Meloidogyne incognita*.

	Reduction in yield (%)					Treatment
Treatment[1]	**Yellow plum**	**Venus**	**VC 48-1**	**VC 11-1**	**1169**	**Mean**
Ps	100	7.6	18.2	30.7	13.4	34.0
Mi	29.8	23.1	18.2	15.2	20.0	21.3
Ps + Mi	100	23.1	18.2	46.2	33.4	44.2
Ps + Mi after 1 week	100	38.3	9.3	30.7	33.4	42.3
Mi + Ps after 1 week	100	38.3	18.2	38.3	33.4	45.6
Variety mean	85.9	26.1	16.4	32.2	26.7	

[1] P_s = *P. solanacearum* alone
Mi = *M. incognita* alone
Ps + Mi = *P. solanacearum* + *M. incognita* simultaneously.
Ps + Mi after 1 week = *P. solanacearum* first + *M. incognita* after 1 week.
Mi + Ps after 1 week = *M. incognita* first + *P. solanacearum* after 1 week.

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