ATR Notes:

VARYING INOCULUM LEVELS OF BACTERIA-NEMATODES AND THE SEVERITY OF TOMATO BACTERIAL WILT

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ABSTRACT

Development of bacterial wilt was consistently earlier on plants inoculated with both bacterium *(Pseudomonas solanacearum* **E.F. Smith) and nematode** *Meloidogyne incognita* **Chitwood) compared to those inoculated with the bacterium alone. The percentage mortality also increased with increasing nematode population in the bacterium-infested soil. The presence of** *M. incognita* **enhanced the development and severity of bacterial wilt and lowered the yield of wilt-resistant tomato cultivars.**

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Bacterial wilt of solanaceous plants caused by *Pseudomonas solanacearum* E.F. Smith continues to reappear in renewed crops under diverse climates and crop handling practices. In the Philippines, it is destructive to tomato, eggplant, pepper, white potato, ginger, castor bean, banana and abaca (Zehr, 1969; Kam, 1977; Rillo, 1978). It does not only cause death of plants but also reduces their potential yield. Nematodes, along with other disease-causing agents, are of tremendous importance as components of disease complexes (Powell, 1971). Interactions may be the major economic hazard posed by these pathogens. Some of the damage normally ascribed to nematodes may result from a complex in which nematodes are only a part.

This study determined the level of inocula that would influence the

development and severity of tomato bacterial wilt.

Artificial inoculation using varying inoculum levels of *P. solanacearum* (Race I, Biovar IV, Isolate LE 100) and *M. incognita* on wilt-susceptible tomato CV Yellow Plum was carried out as follows: Three-week-old seedlings, previously pricked in seed boxes, were planted in 15 cm pots containing decontaminated soil one week after transplanting. Inoculation was done simultaneously with the two pathogens using different combinations of inoculum levels (Table 1). The bacterial inoculum was prepared following the technique of Napiere and Quimio (1980). The nematode egg suspension, on the other hand, was poured into a depression made in the soil on the root zone of the plant. One hundred ml of the bacterial inoculum was poured around the base of the plant. The nematode inoculum was prepared following Sasser's technique (1976).

Soils infested with *P. solanacearum* or *M. incognita* which were taken from the previous experiment were used for the natural inoculation tests. Sterilized soil was mixed with infested soil to attain various concentrations of the pathogens in the soils.

The following treatments were used to determine the effects of *M. incognita* on bacterial wilt severity:

T_1 = Soil infested with *P. solanacearum* only

T_2 = Soil infested with *M. incognita* only

T_3 = Equal volume of soil infested with *P. solanacearum* and *M. incognita*

T_4 = Equal volume of soil infested with *P. solanacearum* and *M. incognita* plus one volume of sterilized soil;

T_5 = Equal volume of soil infested with *P. solanacearum* and *M. incognita* plus 9 volumes of sterilized soil;

T_6 = Equal volume of soil infested with *P. solanacearum* and *M. incognita* plus 99 volumes of sterilized soil;

T_7 = Control (sterilized soil only)

Three-week-old seedlings of CV

Table 1. Combinations of the different inoculum levels used to inoculate Yellow Plum tomato with bacterial wilt.

Pseudomonas solanacearum concentration (cells/ml)	**Meloidogyne incognita concentration (eggs/plant)**			
	500	**250**	**50**	**Control**
2.9×10^6	**A**	**B**	**C**	**D**
2.9×10^3	**E**	**F**	**G**	**H**
2.9×10^2	**I**	**J**	**K**	**L**
Control	**M**	**N**	**O**	**P**

2029 were planted in 15 cm pots containing infested soils. A randomized complete block design was used to layout the various treatments.

The disease started to develop during the first week after transplanting in all soils naturally infested with *P. solanacearum,* be it alone or in combination with *M. incognita* (Table 2). Treatments with higher concentrations of *P. solanacearum* and *M. incognita,* however, showed more severe wilt than treatments with lesser concentrations of the pathogens (Fig. 1).

Severity of the disease increased with time so that on the 5th week, all plants grown in equal volume of soil infested with bacteria and nematodes were all dead. On the 6th week, 95.83% and 83.33% of plants grown in equal volume of soils infested with bacteria and nematode plus 99 parts sterilized soil, and soils infested with the bacterium alone, respectively, were also dead. All plants grown in soils infested with the bacterium regardless of their concentration died of wilt on the 7th week.

Although the actual population of the pathogens was not determined, it was presumed that the lesser the volume of infested soil added, the lower the population of the pathogens. The results indicate that bacterial wilt developed earlier

Table 2. Weekly percentage mortality of tomato cv 2029 grown in soils naturally infested with *Pseudomonas solanacearum* and *Meloidogyne incognita*.

Treatments[1]	Percent Mortality (weeks after transplanting)						
	1	2	3	4	5	6	7
Ps + Mi + 1 ST	16.67	29.17	54.17	79.17	95.83	100	100
Ps + Mi + 9 ST	8.33	25.00	45.83	62.50	91.67	100	100
Ps + Mi + 99 ST	4.17	20.83	54.17	62.50	91.67	95.83	100
Ps + Mi	29.17	58.33	66.67	87.50	100	100	100
Ps	4.17	12.90	20.83	33.33	62.50	83.33	100
Mi	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0

[1] Naturally infested soils were diluted with sterilized soil (ST) to obtain varying concentrations of the pathogen inoculum.

Ps + Mi + 1 ST = 1 part soil with *P. solanacearum*, 1 part sterilized soil, and 1 part soil with *M. incognita*.

Ps + Mi + 9 ST = 1 part soil with *P. solanacearum*, 9 parts sterilized soil, and 1 part soil with *M. incognita*.

Ps + Mi + 99 ST = 1 part soil with *P. solanacearum*, 99 parts sterilized soil, and 1 part soil with *M. incognita*.

Ps + Mi = 1 part soil with *P. solanacearum* and 1 part soil with *M. incognita*.

Ps = Soils with *P. solanacearum* alone

Mi = Soils with *M. incognita* alone

C = Sterilized soils

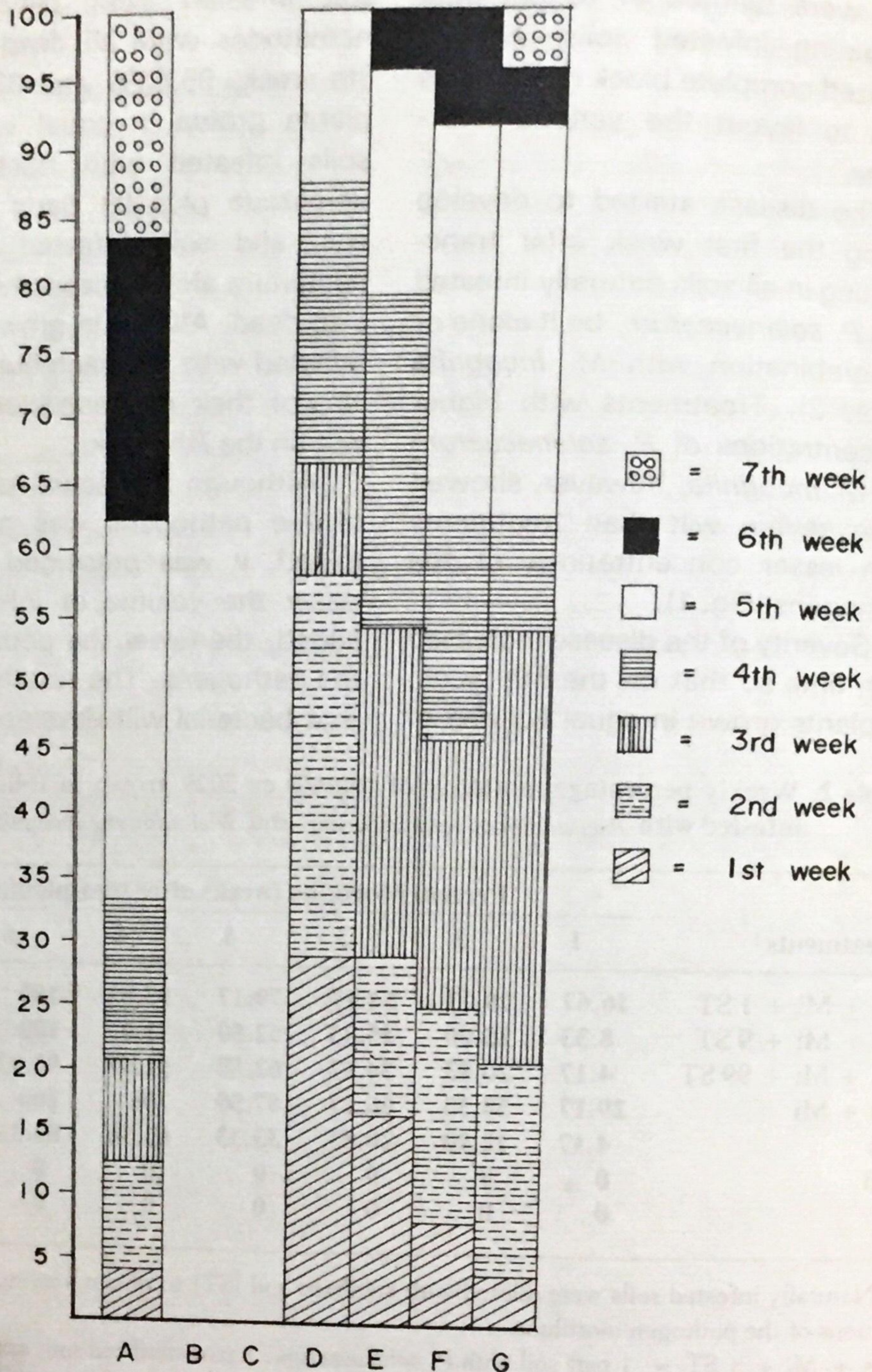

Fig. 1. Weekly percent mortality of wilt susceptible tomato cv 2029 grown in soils naturally infested with *Pseudomonas solanacearum* and *Meloidogyne incognita* alone or in combination. A) *P. solanacearum* alone); B) *M. incognita* alone; C) control; D) equal volume of *P. solanacearum* and *M. incognita;* E) equal volume of *P. solanacearum* and *M. incognita* plus 1 part sterilized soil; F) equal volume of *P. solanacearum* and *M. incognita* plus 9 parts sterilized soil; G) equal volume of *P. solanacearum* and *M. incognita* plus 99 parts sterilized soil.

and became more severe when the population of the two pathogens and their combinations were higher. With high population of *M. incognita,* bacterial wilt developed earlier and faster even at lower concentrations of *P. solanacearum,* which if alone, would not cause severe disease development. The two pathogens, therefore, interacted together to cause early development of the disease and inflict more damage to tomato seedlings. The plants in the control and in soil infested with *M. incognita* only remained relatively healthy throughout the experimental period.

With artificial inoculation, the disease developed earliest at the highest densities of the two pathogens used (Table 3). It was evident, however, that *M. incognita* enhanced the severity of bacterial wilt as shown by increased mortality with increasing number of *M. incognita* eggs in the inoculum. These data corroborate the findings in the natural inoculation study. All plants died of wilt at highest inoculum density of the two pathogens (2.9×10^6 bacterial cells/ml + 500 eggs nematode).

Highly significant differences were observed on percent mortality among treatment means within variety. The different inoculum levels of the pathogens, be they separately or in combination, had an effect on the development and severity of the disease. The results show that the two pathogens can interact to influence disease expression and cause more damage to the tomato plants and that bacterial wilt incidence is dependent on the inoculum levels of both pathogens.

Table 3. Weekly percentage mortality of tomato cv Yellow Plum inoculated with varying levels of inoculum of *Pseudomonas solanacearum* and/or *Meloidogyne incognita*.

Bacteria/Nematodes[1] (cells/ml) (eggs/plant)	Percent Mortality (weeks after inoculation)								
	1	2	3	4	5	6	7	8	9
2.9×10^6 + 500	4.17	20.83	29.17	58.33	66.67	70.83	100	100	100
2.9×10^6 + 250	0	16.67	29.17	54.17	58.33	58.33	100	100	100
2.9×10^6 + 50	0	4.17	20.83	37.50	50.00	62.50	62.50	75.00	75.00
2.9×10^6 + 0	0	8.33	16.67	20.83	37.50	37.50	37.50	37.50	37.50
2.9×10^3 + 500	0	12.50	37.50	50.00	50.00	54.17	62.50	87.50	91.67
2.9×10^3 + 250	0	4.17	12.50	29.17	41.67	45.83	45.83	54.17	54.17
2.9×10^3 + 50	0	4.17	25.00	37.50	54.17	54.17	54.17	54.17	54.17
2.9×10^3 + 0	0	12.50	29.17	37.50	37.50	37.50	37.50	37.50	37.50
2.9×10^2 + 500	0	4.17	16.67	29.17	37.50	54.17	54.17	54.17	54.17
2.9×10^2 + 250	0	0	8.33	20.83	29.17	29.17	37.50	37.50	37.50
2.9×10^2 + 50	0	4.17	12.50	25.00	45.83	50.00	50.00	50.00	50.00
2.9×10^2 + 0	0	8.33	16.67	25.00	25.00	25.00	25.00	25.00	25.00
0 + 500	0	0	0	0	0	0	0	0	0
0 + 250	0	0	0	0	0	0	0	0	0
0 + 50	0	0	0	0	0	0	0	0	0
0 + 0	0	0	0	0	0	0	0	0	0

[1] Inoculum per plant: 100 ml bacterial suspension and/or nematode egg suspension with desired number.

The results confirmed the report of Harrison and Young (1941) that differences in the severity of bacterial wilt under field condition depends upon the nematode population. Areas having high nematode population had more severe tomato wilt than those having low nematode population.

Plants inoculated with the nematode alone had higher yields compared to those inoculated with the bacterium alone or with bacterium in combination with the nematode. The lowest yield observed was in plants inoculated with the highest concentration of bacterium and nematode.

Although there were differences noted among treatment means within variety, these differences were not significant. The different treatments did not affect the potential yield of tomatoes at the densities of the pathogens tested.

LITERATURE CITED

HARRISON, A.L., and YOUNG, P.A. 1941. Effect of root-knot nematode in tomato wilt. Phytopathology 31: 749-752.

KAM, E.Y. 1977. Characteristics of *Pseudomonas solanacearum* E.F. Smith infecting ginger *(Zingiber officinale* Roscoe) in the Philippines. MS thesis, U.P. at Los Baños.

NAPIERE, C.M., and QUIMIO, A.J. 1980. Influence of root-knot nematode on the severity of bacterial wilt. *Ann. Trop. Res.* 2: 29-39.

POWELL, N.T. 1971. Interaction of plant paristic nematodes with other disease-causing agents. *In* Plant parasitic nematodes. Vol. II. 119-135. *Ed. Zuckerman,* B.M., W.F. Mai, and R.A. Rhode, Academic Press. New York.

RILLO, A.R. 1978. Characteristics of *Pseudomonas solanacearum* E.F. Smith affecting banana in the Philippines. M.S. thesis, U.P. at Los Baños.

SASSER, J.N. 1976. Suggested procedure for obtaining and applying root-knot nematode inoculum and for determining the amount of reproduction on host plants. *In* Proc. Asia Regional Planning Conf. Root-knot Nematode Res. Prog. CEC, College, Laguna, Philippines.

ZEHR, E.I. 1969. Studies on the distribution and economic importance of *Pseudomonas solanacearum* E.F. Smith in certain crops in the Philippines. *Phil. Agric.* 53:218-233.