Persistence of Pseudomonas solanacearum in Artificially Infested Soils

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ABSTRACT

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Pseudomonas solanacearum persisted 4 years in two field soils artificially infested by clipping beds of young tomato plants with a contaminated mower followed by soil incorporation of the resulting diseased plants. The infestation remained high in one soil as indicated by 99.0, 91.0, 95.2, and 98.1% infection of Marion tomato plants during the 1972-75 test period. In the second soil, 93.0% of Marion plants were infected the second year after the soil infestation, but infection percent dropped to 65.9, 60.8, and 63.1 during the following 3 years. More than 90% of Venus and Saturn plants, used as resistant checks, remained free of wilt symptoms although as high as 29.5% of symptomless plants had *P. solanacearum* in their vascular systems.

Additional key words: bacterial wilt, *Lycopersicon esculentum*, soil-borne bacteria.

I reported earlier (5) that high infestations of *Pseudomonas solanacearum* E. F. Sm. were established in two field soils by clipping beds of young tomato plants with a contaminated rotary mower followed by soil incorporation of the diseased plants with a tractor-mounted rotary tiller. Both soils were uniformly infested the year following the incorporation of the diseased material as indicated by 93 and 99% kill of Marion tomato plants at two locations (Athens and Midville, Georgia, respectively). It was suggested that the described procedure may be useful for establishing or increasing infestations of *P. solanacearum* in field soils to be used for experimental purposes. To be most useful, however, newly established infestations should remain high permanently or at least for several years. The present paper reports the persistence of *P. solanacearum* during the three years following the initial determination in 1972, the year following the infestation.

MATERIALS AND METHODS

Studies were conducted each year from 1973 through 1975 at Athens and Midville, Georgia, in field plots established and infested as described earlier (5). The plot area at each location was approximately 30 × 50 meters. A different experimental design was used each year in that rows of plants of susceptible and resistant cultivars were assigned at random within the infested plots at each location. Soils were an Appling sandy loam (pH 6.2, when infested in 1971) at Athens in the piedmont and a Marlboro loamy sand (pH 5.9, when infested) at Midville in the middle coastal plain. After land preparation, the plots were fertilized annually with 60, 26, 48, and 67, 29, 85 kg/hectare (ha) of N, P, and K at Athens and Midville, respectively, applied broadcast as complete fertilizer. These application rates were based on soil test results and the recommended rates for tomato production. Plants at both locations were side-dressed 3 weeks after transplanting with an additional 40 kg/ha of N applied as ammonium nitrate (1973 and 1974) or as calcium nitrate (1975). Trifluralin (*a,a,a*-trifluoro-2,6-dinitro-N,N-dipropyl-*p*-toluidine, Treflan, 1.75 liters/ha) was applied for weed control. Plots at both locations were also hand-weeded and cultivated during the growing season to control major infestations of nutsedge (*Cyperus* sp.) and common purslane (*Portulaca oleracea*) that occurred. Plant debris and remaining weeds were plowed under or disked in at the end of each test. Plots were maintained in a fallow condition between tests except for the growth of a few winter weeds, mainly henbit (*Lamium amplexicaule*) and common chickweed (*Stellaria media*).

Tomato (*Lycopersicon esculentum* Mill.) transplants were grown on a greenhouse bench in individual 266-ml paper cups filled with methyl bromide-fumigated soil. Plants of the Marion cultivar (susceptible to *P. solanacearum*) were used at both locations to determine the level and distribution of the infestation from year to year. Plants of a resistant cultivar (Venus or Saturn) were used in most experiments as checks (2). Plants, 20 to 30 cm tall, were hand-planted during April or May of each year (except in 1975 when excessive rainfall caused delay of transplanting until 20 June) and were given 850 ml of Peters water-soluble fertilizer solution (20-20-20, 2.5 g/liter). Plants were spaced 0.70 to 0.85 m in the row with rows 0.97 m apart. At Athens, 585, 900, and 960 Marion plants were transplanted in 1973, 1974, and 1975, respectively. At Midville, 900, 1,530, and 1,500 Marion plants were transplanted during the same period. Numbers of resistant plants (Venus or Saturn) were 90, 90, 0, and 720, 180, and 60 at Athens and Midville in the years 1973, 1974, and 1975, respectively. The resistant plants were transplanted either in alternate rows with Marion (Midville, 1973) or in one to three rows of 60 to 90

plants assigned at random within each experimental plot. Irrigation was used as needed at both locations to maintain vigorous growth. Carbaryl (1-napthyl methylcarbamate, Sevin 80%, 2.2 kg/ha) and chlorothalonil (Bravo 6F, 2.3 liters/ha) or maneb (manganese ethylenebisdithiocarbamate, Manzate D or Dithane M-22 special 80%, 2.2 kg/ha) were applied with a back-mounted motorized Solo Mistblower at 7- to 10-day intervals to control insects and foliar pathogens. Data were taken from June through August each year except in 1975 when the plants transplanted in June at Athens were maintained in the field until 7 October. As plants showed symptoms of bacterial wilt, they were cut at soil level and a section of the stem from the base of each plant was suspended in a test tube of tap water to determine whether bacteria could be observed streaming from the vascular system (3). At the end of the growing season, living plants were cut and checked for the presence of the wilt bacterium in the vascular system. Throughout the growing season, suspensions from representative tubes were streaked on tetrazolium chloride medium (4) and incubated at 35 C to determine the presence of the wilt bacterium. Additionally, representative bacterial cultures obtained from both susceptible and resistant plants, and picked from streaked tetrazolium chloride plates, were tested for pathogenicity by inoculating Marion tomato plants (15-20 cm tall) in the greenhouse.

RESULTS

A few plants were killed by *Rhizoctonia solani* and *Sclerotium rolfsii* soon after transplanting in all tests, and these plants were eliminated in the calculation of results. *Pseudomonas solanacearum* persisted at both locations during the 3-year test period as indicated by the infection of Marion plants grown in the test plots (Fig. 1). Plants died rapidly at Midville during all 3 years and 81.6 to 99.0% were killed by the end of the growing season. A high percentage of the few surviving plants had the wilt bacterium in the vascular system as determined by streaming of bacteria into water, isolation in the laboratory, and greenhouse pathogenicity tests. Percent infection (plants killed by bacterial wilt plus living plants infected) in 1975 was 98.1 compared with 99.0 in 1972, the year following the infestation. The infestation was distributed uniformly throughout the plot area at Midville. Plants died less rapidly at Athens than at Midville each year, despite the occurrence of environmental conditions conducive to disease development. Approximately 30-40% of the plants remained alive at the end of each growing season during 1973-75. Percentage of Marion plants infected decreased from 93.0% in 1972 to 65.9, 60.8, and 63.1% during the 1973-75 period. The infestation in the field plot at Athens became less uniformly distributed than at Midville during the 3-year test period.

More than 90% of resistant plants (Venus or Saturn) remained free of bacterial wilt symptoms at both locations during the 3-year test period. Furthermore, there was no evidence of a change in the resistance of the resistant cultivars during the tests. However, the wilt bacterium was present in low populations in the vascular system in as high as 29.5% of resistant plants (Midville, 1974) at the end of the growing season as determined by laboratory studies and greenhouse pathogenicity tests. Resistant plants with the wilt bacterium in the vascular system appeared (no actual measurements were taken) to grow as vigorously and to yield as well as the noninfected plants of the same cultivar.

The soils in the two plots were not assayed for plant parasitic nematodes. However, the root systems of randomly selected plants were examined at the end of each growing season for galling caused by root-knot nematodes (*Meloidogyne* spp.). There was no evidence that root-knot nematodes occurred in either plot in significant numbers.

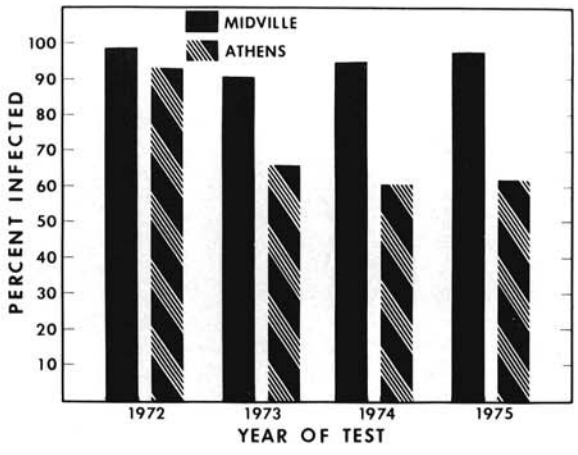

Fig. 1. Percentage of Marion tomato plants infected with *Pseudomonas solanacearum* when grown in field plots of Marlboro loamy sand at Midville and Appling sandy loam at Athens during 1972-75. Both soils were artificially infested in 1971 by clipping beds of tomatoes of transplant size with a *P. solanacearum*-contaminated mower followed by soil incorporation of the resulting diseased plants.

DISCUSSION

Pseudomonas solanacearum is known for its erratic distribution and unpredictable persistence in field soils under natural conditions (1, 3, 6). Kelman (3) noted that the organism appears to survive in some soils for long periods in the absence of susceptible crops, whereas in other soils it declines rapidly even with cultivation of susceptible hosts. Furthermore, strains of the wilt bacterium may vary in their soil survival capacity (9). Maintenance of a high and uniform natural infestation of *P. solanacearum* is one of the most perplexing problems that we have encountered in conducting field tests in Georgia. Several inoculation procedures have been evaluated by others (3, 7) to insure uniform disease development in the field. The artificial infestation procedure that I described earlier (5), and further evaluated in long-term tests reported here, apparently provides an acceptable method for overcoming field infestation irregularities. Our results suggest that high populations of *P. solanacearum* can be maintained in the presence of a susceptible host for at least 4 years, provided a suitable soil type is selected. Although bacterial wilt occurs to some extent in soils of many types (3) and the causal organism has overwintered as far north as New

Jersey (10), in the United States it occurs naturally only in southern areas and, in Georgia, is especially adapted to the light, sandy soils of the lower coastal plain. The persistence of a high population at Midville probably occurred because the light, sandy soil at that location was more conducive to survival of *P. solanacearum* than soils with a higher clay content at Athens (3). The Venus and Saturn cultivars released in 1972 by the North Carolina Agricultural Experiment Station (2) continue to survive well in our heavily infested plots despite their apparent susceptibility to strains of *P. solanacearum* in some other areas (8).

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