A Procedure for Infesting Field Soils with Pseudomonas solanacearum

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ABSTRACT

Field soils became uniformly infested with *Pseudomonas solanacearum* when beds of tomato of transplant size were inoculated by clipping with a contaminated rotary mower and the resulting diseased plants incorporated into the soil. Mortality among H-1350 tomato plants transplanted immediately after soil incorporation of the diseased plant material was 93 and 98% at two locations in Georgia. Mortality among plants transplanted the year following infestation ranged from 0% in plants of a resistant breeding line to 99% in a susceptible cultivar. The clipping procedure appears to be a useful tool for increasing the level and uniformity of infestation by *P. solanacearum* in fields to be used for experimental purposes.

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Field studies on *Pseudomonas solanacearum* E. F. Sm. are often complicated by erratic infestations (1, 5) and by the unpredictable persistence of the organism in different fields (3). Earlier, we reported (6) that more than 90% of field-grown tomato (*Lycopersicon esculentum* Mill.) transplants were killed when plant beds were clipped with a rotary mower contaminated with *P. solanacearum* and suggested that this technique might be used to produce uniform soil infestations for experimental work. The present paper reports the results of field tests to determine soil infestations of *P. solanacearum* after clipping to produce disease and to evaluate resistance of various tomato cultivars grown in the same soils.

Field plots (30 × 50 m) were established on an Appling sandy loam soil (pH 6.2) near Athens in the piedmont and on a Marlboro loamy sand (pH 5.9) at Midville in the upper coastal plain of Georgia. Both soils were free of *P. solanacearum* based on the absence of wilt among tomato crops grown in the fields previously. Plots were fertilized for tomato production according to soil test results and irrigation was used as needed to promote vigorous growth. In 1971 plots were hand seeded with a model 300A Planet Jr. at the rate of 60 seed of the cultivar 'Urbana' per m of row in rows spaced 20 cm apart. Plants were grown until 20 cm tall and then were clipped to ca. 16 cm with a modified rotary mower (6) with blades sprayed with a suspension of *P. solanacearum*. Inoculum was prepared by suspending growth from petri dish cultures (grown at 35 C for 3 days on potato-dextrose agar) in distilled water. During the clipping operation the resulting suspension (10^8 cells/ml) was applied to the mower blade until runoff at 30-m intervals with a hand-operated Hudson compression sprayer. Initial disease symptoms appeared within 5 to 7 days after clipping and were similar to those described previously (6). Two additional clippings were made at 7- to 10-day intervals after the first. More than 95% of plants were dead within 2 weeks after the final clipping. The dead plants were cut to soil level with a tractor-mounted rotary mower and incorporated into the soil. After land preparation, 500 H-1350 tomato plants, grown on a greenhouse bench singly in 266-ml paper cups filled with methyl bromide-fumigated soil, were transplanted into the plots at both Athens and Midville to determine the degree and uniformity of the infestation by *P. solanacearum*. Plants were transplanted on 15 July, spaced 0.85 m in the row with rows 1.3 m apart. Counts of plants with bacterial wilt were made from July through September. As plants wilted, they were cut at soil level, and a section of the stem was suspended in a test tube containing water to check for streaming of the wilt bacterium from the vascular system (3, 5). Suspensions from representative tubes were streaked on tetrazolium chloride medium (4) and cultured to verify the presence of *P. solanacearum*. At Midville many of the plants were killed by *Rhizoctonia solani* within 2 weeks after transplanting. However, 93% of the 253 remaining plants died of bacterial wilt. Approximately 98% of the 428 plants which survived transplanting at Athens died of bacterial wilt.

In 1972, tomato plants of 13 breeding lines, two cultivars, 'Venus' and 'Saturn' [recently released by the North Carolina Agricultural Experiment Station (2)] all selected for resistance to *P. solanacearum*, and 'Marion' (susceptible check) were transplanted on 22 June at Athens and on 29 June at Midville. All plants were seeded in the greenhouse and transplanted into the field as described above. The Marion plants were planted in alternate rows with the various resistant lines and cultivars to determine the uniformity of infestation and to allow direct comparison between susceptible and resistant types. Survival counts were made at 7- to 10-day intervals from transplanting until 12 September at Athens and 14 September at Midville. Dying plants were tested for the presence of *P. solanacearum* as previously described. Results from six representative breeding lines and cultivars are shown in Fig. 1. Plants of Marion died rapidly at both Athens and Midville. Plants of some breeding lines selected for resistance also died rapidly, whereas other lines and the Saturn and Venus cultivars survived well throughout the growing season. Final survival ranged from less than 1% in Marion plants to 94% in the most resistant breeding line (Ceylon 1960-8) at Midville, and from less than 7% in Marion plants to 100% in a resistant breeding line (NC 1965-54) at Athens. These results showed that both soils were still uniformly infested the year following soil incorporation of the diseased plants resulting from clipping.

The clipping procedure described earlier (6) and evaluated in these tests appears to be a useful method for increasing infestations of *P. solanacearum* in field soils that are to be used for experimental purposes such as studies to determine organism survival, effectiveness of control measures, and host resistance. The wilt organism survived equally well when introduced into two soils of markedly different types, one of which (Athens plot) was outside the range where *P. solanacearum* is found naturally in soils. The organism varies greatly in its

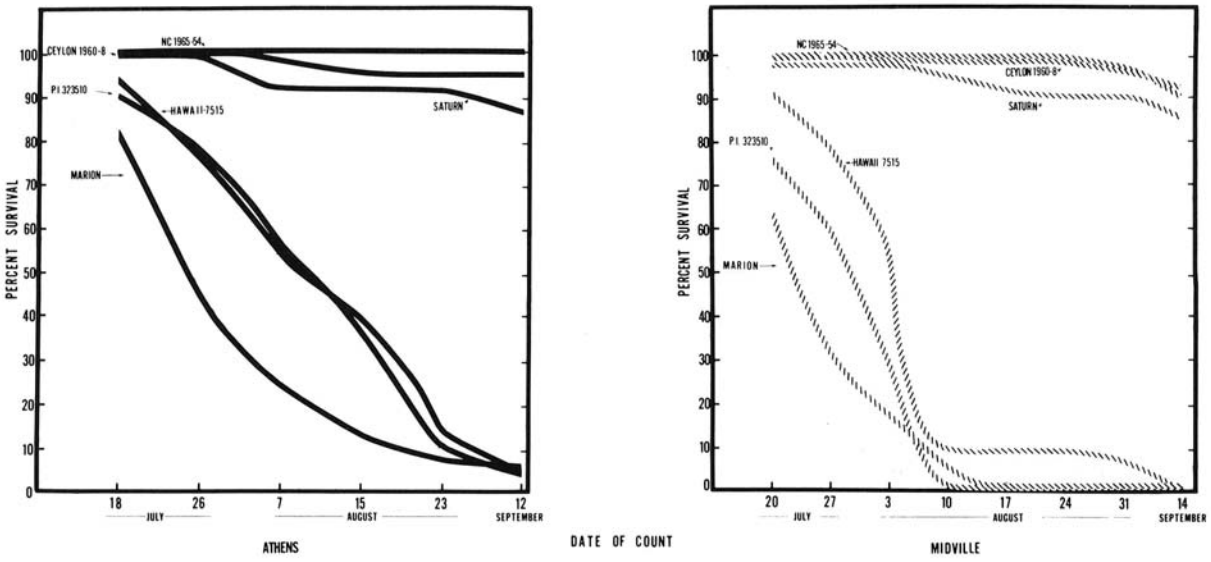

Fig. 1. Survival of six tomato cultivars or breeding lines grown at Athens and Midville, Georgia, in field soils infested with *Pseudomonas solanacearum*. Both soils were infested the previous year by clipping thickly seeded tomato plants with an artificially contaminated rotary mower followed by soil incorporation of the diseased plants.

persistence in different soils (3) and strains may differ in their survival capacity (7). Although in the USA the organism is found naturally only in the southern areas, it has overwintered as far north as New Jersey (8). In my tests susceptible plants grown in the soils infested by clipping were almost eliminated by the wilt organism, whereas survival within resistant lines varied with the line or cultivar concerned. Thus, soils infested by the clipping technique seem to be well suited for resistance screening studies although some earlier inoculation experiments with diseased plant material added to the soil often gave erratic results (3, 9).

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