Survival of *Pseudomonas solanacearum* in Selected North Carolina Soils

W. C. Nesmith and S. F. Jenkins, Jr.

Department of Plant Pathology, University of Kentucky, Lexington 40546; and Department of Plant Pathology, North Carolina State University, Raleigh 27650, respectively.

Portion of a Ph.D. thesis submitted by the senior author to the Graduate School, North Carolina State University, Raleigh.
Journal Series Paper 6125 of North Carolina Agricultural Research Service, Raleigh.
Use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the product named, or criticism of similar ones not mentioned.
Accepted for publication 25 April 1983.

ABSTRACT

Nesmith, W. C., and Jenkins, S. F., Jr. 1983. Survival of *Pseudomonas solanacearum* in selected North Carolina soils. Phytopathology 73:1300-1304.

Survival of *Pseudomonas solanacearum* was monitored from one growing season to the next in microplots containing soils from four North Carolina sites that varied greatly in the occurrence of bacterial wilt of tomato from year to year. The microplots were infested with 2.5×10^9 colony-forming units of *P. solanacearum* (race 1, biotype 1) per gram of soil in September after soil in one-half of the plots was fumigated with methyl bromide. Survival was monitored by using a selective medium and susceptible tomato plants. The bacterial population declined more rapidly in the soils collected from nonpersistent sites than in soils from persistent sites. Fumigation of soils from nonpersistent sites was only partially effective in improving persistence and slowing the rate of decline of *P. solanacearum*. Populations of *P. solanacearum* declined rapidly upon addition to soil. Six months after infestation, tomato seedlings were planted directly into the microplots, because the bacterium was no longer detectable by testing it with the selective medium. Bacterial wilt on tomatoes did not develop in the nonfumigated soils collected from nonpersistent sites, but was common within 30 days after transplanting in fumigated soils and in both fumigated and nonfumigated soils collected from persistent sites. In greenhouse experiments, disease development was similar in steamed and nonsteamed soils regardless of the collection site, if tomato plants and inoculum of *P. solanacearum* were added simultaneously. However, when soils were infested with the bacteria and held for 60 days before the tomato seedlings were transplanted, significantly less disease developed in nonsteamed soils collected from nonpersistent sites, compared to steamed soils from the same site or steamed and nonsteamed soils from the persistent site. This indicates that suppressive soil factors (possibly of biological origin) exist at sites where *P. solanacearum* does not persist from season to season.

Additional key words: *Lycopersicum esculentum*.

Pseudomonas solanacearum E. F. Smith, causes a wilt of many plant species around the world. Three races are recognized based on host range: race 1 with a wide host range among mostly the solanaceous and composite families, race 2 restricted to banana and related plants, and race 3 attacking potato, tomato, and a few solanaceous weeds (5). Four biotypes are recognized based on cultural and physiological properties (12). Until 1962, when Sequeira (22) demonstrated that race 2 did not survive for long in soil, it was believed that *P. solanacearum* persisted for a long time at a site due to a significant soilborne phase or perennial hosts (5,13,15). In 1965, Dukes et al (7) reported that *P. solanacearum* produced the most severe disease in Georgia the first year following clearing of forest, and declined in subsequent years; they concluded that perennial weeds were important in long-term persistence at a site. Reports from Kenya (11) and Australia (10,23) indicated that race 3 has a limited soilborne phase, persisting less than 2 yr, apparently deep in the soil.

Race 1 is common in the southeastern United States; it occurs on a wide variety of soils and is believed to have a substantial soilborne phase. However, host range is considered important in the pathogen's survival (5,13,15,24). It persists in only certain regions of the coastal plain, piedmont, and delta and is totally absent in others or fails to persist long when introduced (15). Generally, bacterial wilt is not distributed uniformly within a field or from field to field (15,24), although secondary spread sometimes results in uniform distribution of the bacterial wilt pathogen over a wide area. Within a field, however, initial yearly distribution is relatively constant, indicating that survival may be limited to smaller areas and probably is influenced by permanent factors like soil type and topography rather than temporary factors like plant cover (24) and agents of spread. Smith (24) demonstrated that the bacterium survived bare fallow, but was reduced by crop rotation. McCarter (19) demonstrated that survival was better in Marlboro sandy loam than in Appling sandy loam.

In North Carolina, bacterial wilt nurseries established on a Worsham sandy loam at the Tobacco Research Station at Oxford required annual infestation to maintain bacterial wilt, while in the nursery on a Norfolk loamy sand on the Horticultural Crops Research Station at Clinton, the bacterium survived and was highly pathogenic from season to season. Similarly, on commercial potato farms in eastern North Carolina potato production was abandoned in some fields (Torhaunta fine loamy sands) due to bacterial wilt, while adjacent fields (Arapahoe coarse sands) under the same farming operation remained relatively free of the disease during 20–30 yr of potato production (L. W. Nielsen, *personal communication*). These experiments were conducted to evaluate survival of *P. solanacearum* in soils from these sites.

MATERIALS AND METHODS

Soils. Two soils, a Norfolk loamy sand (8,18) from Clinton, NC and a Torhaunta fine loamy sand from Beaufort County, NC, were selected because bacterial wilt occurred from season to season at these sites (Table 1). Another two soils, a Worsham sandy loam (8,18) from Oxford, NC and an Arapahoe coarse loamy sand from Pamlico County, NC (Table 1) were selected because bacterial wilt did not develop season after season at these sites, despite frequent introduction of the pathogen. Soil samples were from the plow layer (upper 15–20 cm) and were screened (5-mm mesh) to remove large particles.

Inoculum. *P. solanacearum*, isolate K-60 (race 1, biotype 1) (12) was inoculated into cultivar Rutgers tomato plants and reisolated on triphenyltetrazolium chloride medium (TTC) (16). Ten virulent fluidal-type colonies developed from single-cell origin (6) were placed in sterile tap water in screw-top test tubes to comprise the stock cultures. Inoculum was increased by streaking a loop of the stock suspension onto TTC, and three, single, virulent, fluidal-type

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

colonies from each isolate were transferred to a synthetic liquid medium (minus the calcium carbonate) (14). After 72 hr, the bacterial suspension was centrifuged at 5,000 *g* for 3 min, washed twice in sterile tap water, and suspended in a few milliliters of sterile tap water. After 1 hr, all 10 stock culture suspensions were combined, diluted with sterile deionized water, and used to infest soils.

Microplots. In September 1974, sixty-four microplots were established at Raleigh, NC, in plastic buckets (20 L capacity) with bottom drainage holes which were filled with 15 L of soil. Each bucket was embedded in field soil with the experimental soil inside the bucket, level with the field soil outside, and the top edge of the bucket extending ~10 cm above ground level. To aid drainage, a 6-cm layer of experimental soil (same soil as inside the bucket) was added under each bucket to ensure that uniform soil pores developed at the drainage holes (17). The microplots were confined to an area 7 × 20 m with 1.2 m between microplots and 1.8 m between blocks. The area around each microplot was established sod of mixed grasses and legumes. The experimental soil inside each microplot was maintained weed free by twice-weekly hand cultivation to minimize pathogen survival on weed hosts.

Test I. The following treatments were established in September in microplots for each of the four soils and arranged in a randomized complete block design with four replications (25): field soil (Fd), field soil infested with 2.5×10^9 colony-forming units (cfu) per gram of dry soil (Fd + I); fumigated control soil (F); and fumigated soil reinfested with 2.5×10^9 cfu per gram of dry soil (F + I). Fumigated soils were treated three times at 4-day intervals with methyl bromide (6.8 g per liter of soil) while enclosed under a 100 μm-thick polyethylene tarp. A 30-day-old cultivar Rutgers tomato plant was planted in a subsample from each soil and placed in the greenhouse to determine inoculum viability following infestation.

Monitoring survival. Populations of *P. solanacearum* were monitored with a selective medium (FSM) (20). A 10-g soil sample was taken at random from two of the four replicate microplots, diluted serially in sterile deionized water, and plated on FSM in three petri dishes. The number of fluidal colonies of *P. solanacearum* was counted on those dilution plates having approximately 30–100 colonies. The mean number of cfu per gram of oven dry soil averaged from six plates was used in regression analysis, and slope values of survival curves in log I/I_0 (cfu at later dates divided by initial cfu) units per unit of time were computed after semilogarithmic transformation of the data (3,25). Populations of the bacterium were monitored from September through the fall and winter. When the population dropped below a level detectable by culturing on FSM, susceptible tomatoes were planted in each microplot to determine if the bacterium was present.

Test II. During the second year of the study, the plots were reinfested such that survival of *P. solanacearum* produced in association with a host could be monitored. A cultivar Homestead 24 tomato plant was planted in each of the 64 plots and 50 ml of a laboratory-produced inoculum (2.5×10^7 cfu per milliliter) was mixed into the Fd + I and F + I plots to inoculate the tomato plants. About 30 days after transplanting, the plants were pulled, the soil was shaken from the roots into each plot, and the plants were discarded. The soil was mixed well with a small garden trowel to uniformly disperse the pathogen. Populations of *P. solanacearum* were monitored and evaluated as in Test I.

TABLE 1. Texture analysis, pH, and percent organic matter of four North Carolina soils

Soil	Texture analysis: sand (%)	silt (%)	clay (%)	pH	Organic matter (%)
Norfolk loamy sand	81	17	2	6.0	1.9
Torhaunta fine loamy sand	71	23	6	5.4	6.0
Worsham sandy loam	67	24	9	6.4	1.2
Arapahoe coarse loamy sand	85	12	3	5.8	3.0

TABLE 2. Population changes of *Pseudomonas solanacearum* during the winter in four North Carolina soils, either nonfumigated or fumigated, following fall infestation

Soil type	Treatment[y]	cfu/g oven dry soil (days after infestation): 3	7	14	30	60	90	120	150
Test I[w]									
Norfolk loamy sand	Fd	3×10^5	5×10^4	5×10^4	5×10^3	5×10^3	–[z]	–	–
Norfolk loamy sand	Fd + I	4×10^8	9×10^7	8×10^7	2×10^7	5×10^5	9×10^4	4×10^3	2×10^3
Norfolk loamy sand	F + I	3×10^8	5×10^7	4×10^7	3×10^7	8×10^5	5×10^4	6×10^4	4×10^3
Torhaunta fine loamy sand	Fd	9×10^4	7×10^4	3×10^4	–	–	–	–	–
Torhaunta fine loamy sand	Fd + I	3×10^8	8×10^7	7×10^7	8×10^7	5×10^5	3×10^4	–	–
Torhaunta fine loamy sand	F + I	9×10^7	8×10^7	8×10^7	2×10^7	4×10^4	4×10^4	4×10^3	–
Arapahoe coarse loamy sand	Fd	–	–	–	–	–	–	–	–
Arapahoe coarse loamy sand	Fd + I	2×10^8	9×10^7	7×10^7	2×10^7	4×10^5	–	–	–
Arapahoe coarse loamy sand	F + I	2×10^8	9×10^7	6×10^7	6×10^6	4×10^5	3×10^4	4×10^3	–
Worsham sandy loam	Fd	1×10^4	3×10^4	3×10^4	–	–	–	–	–
Worsham sandy loam	Fd + I	2×10^8	9×10^7	6×10^7	5×10^6	2×10^5	–	–	–
Worsham sandy loam	F + I	2×10^8	2×10^8	5×10^7	6×10^6	4×10^5	–	–	–
Test II[x]									
Norfolk loamy sand	Fd	2×10^4	3×10^4	2×10^4	4×10^3	2×10^3	–	–	–
Norfolk loamy sand	Fd + I	5×10^5	4×10^5	5×10^5	4×10^5	4×10^5	9×10^4	3×10^4	3×10^3
Norfolk loamy sand	F + I	4×10^5	3×10^5	4×10^5	4×10^5	5×10^5	7×10^4	8×10^3	3×10^3
Torhaunta fine loamy sand	Fd	–	–	–	–	–	–	–	–
Torhaunta fine loamy sand	Fd + I	4×10^5	4×10^5	3×10^5	8×10^4	9×10^4	2×10^3	–	–
Torhaunta fine loamy sand	F + I	5×10^5	4×10^5	2×10^5	9×10^4	3×10^3	–	–	–
Arapahoe coarse loamy sand	Fd	–	–	–	–	–	–	–	–
Arapahoe coarse loamy sand	Fd + I	2×10^5	9×10^4	3×10^4	9×10^3	8×10^3	–	–	–
Arapahoe coarse loamy sand	F + I	2×10^5	8×10^4	2×10^4	4×10^4	9×10^3	–	–	–
Worsham sandy loam	Fd	–	–	–	–	–	–	–	–
Worsham sandy loam	Fd + I	1×10^5	9×10^4	4×10^4	3×10^4	4×10^3	–	–	–
Worsham sandy loam	F + I	1×10^5	1×10^5	4×10^4	2×10^6	6×10^3	–	–	–

[w] Test I: Soils were infested with 2.5×10^9 cfu/g soil in September 1974.
[x] Test II: Plots of treatments Fd + I and F + I were reinfested by placing 50 ml of 2.5×10^7 cfu per milliliter of solution of *P. solanacearum* onto the soil around a young tomato transplant in May 1975 to insure some inoculum was produced in association with a host. Each Fd plot also had a tomato transplant.
[y] Treatments: Fd = field soil control, Fd + I = artificially infested field soil, and F + I = fumigated then infested.
[z] – = not detected.

Test III. A range of ten-fold dilutions of inoculum densities of *P. solanacearum* from 25 to 2.5 × 10^9 cfu per gram of soil was established in the greenhouse in each of the four soils. One half of a soil sample of each of the four soils was steamed for 1 hr at 82 C, then both steamed and nonsteamed soils were leached with three volumes of sterile, deionized water. The experimental unit consisted of a 12-cm-diameter clay pot containing soil with a known inoculum density replicated six times. Uniform 17-day-old cultivar Homestead 24 tomato seedlings were transplanted into three of the pots on the day of infestation and into the other three pots 60 days later. Noninfested steamed and nonsteamed soils were used as controls. This experiment was repeated three times, and disease incidence (DI) was assessed as: DI 100 = plants wilted by day 7, DI 60 = plants wilted by day 20, and DI 30 = plants wilted by day 30. Plants wilted on days between these points were given appropriate DI values read from a graph constructed from those three points. The presence of *P. solanacearum* in each wilted plant was confirmed by isolation on the FSM.

TABLE 3. Variation in bacterial wilt incidence on tomatoes among four soil types (fumigated versus nonfumigated) after holding the soils in microplots overwinter following a fall infestation[w]

Soil type	Treatment[x]	Disease incidence[y] June 1975	Disease incidence[y] June 1976
Norfolk	Fd	28 c[z]	14 b
	Fd + I	52 d	29 c
	F + I	53 d	31 c
	F	0 a	0 a
Torhaunta	Fd	11 b	0 a
	Fd + I	29 c	7 ab
	F + I	48 d	13 b
	F	0 a	0 a
Worsham	Fd	0 a	0 a
	Fd + I	0 a	0 a
	F + I	26 c	0 a
	F	0 a	0 a
Arapahoe	Fd	0 a	0 a
	Fd + I	0 a	0 a
	F + I	33 cd	0 a
	F	0 a	0 a

[w] Microplots were infested September 1974 and August 1975 with laboratory grown inoculum of *P. solanacearum* and in May 1975 a susceptible tomato was introduced to ensure some of the population was produced in association with a host.

[x] Treatments: Fd = field soil control, Fd + I = artificially infested soil, F + I = fumigated then infested, and F = fumigated.

[y] Plants wilting within 7 days were assigned a disease incidence (DI) of 100, by day 20 a DI of 60, and on day 30 a DI of 30.

[z] Values followed by different letters are significantly different at $P = 0.05$ as determined by the Duncan's multiple range test. Average of four replications.

TABLE 4. Slopes for the survival curves of *Pseudomonas solanacearum* in four North Carolina soils during the winters of 1974–1975 and 1975–1976, based on semilogarithmic transformation of I/I_0 (cfu at later dates/initial cfu)

Soil type	Treatment[y]	Slope value 1974–1975	Slope value 1975–1976
Norfolk loamy sand	Fd + I	−0.0376 a[z]	−0.0111 a
Norfolk loamy sand	F + I	−0.0332 a	−0.0128 a
Torhaunta fine loamy sand	Fd + I	−0.0508 a	−0.0646 b
Torhaunta fine loamy sand	F + I	−0.0424 a	−0.0638 b
Worsham sandy loam	Fd + I	−0.0908 b	−0.0875 c
Worsham sandy loam	F + I	−0.0905 b	−0.0866 c
Arapahoe coarse loamy sand	Fd + I	−0.0905 b	−0.0862 c
Arapahoe coarse loamy sand	F + I	−0.0377 a	−0.0867 c

[y] Treatments: F + I = soil fumigated then infested, and Fd + I = soil infested without fumigation.

[z] Values within columns followed by different letters are not homogenous at $P = 0.05$ as determined by an analysis of covariance.

RESULTS

Survival of *P. solanacearum* in microplots. *P. solanacearum* was not detected at any time during the experiments in soils that were fumigated and not reinfested; this indicated that spread among plots either did not occur or was minimal. The bacterium was known to be present in the Norfolk, Torhaunta, and Worsham control field soils (Fd) because bacterial wilt had been present at the collection sites. *P. solanacearum* was absent in the Arapahoe Fd, and bacterial wilt had not been observed at this collection site for several years.

Test I. Populations of *P. solanacearum* declined rapidly upon addition to all soils regardless of soil type or treatment (Table 2). Major differences among treatments were not evident for 60 days after infestation using the FSM. However, beyond 60 days marked differences were noted: the bacterium was not detected in the Worsham soils regardless of treatment, the Arapahoe Fd + I, or Fd plots of any soil; populations remained detectable longest (150 days) in the Norfolk Fd + I and F + I; and populations in the Arapahoe and Torhaunta soils were detected longer in F + I soils than in Fd + I soils.

Beyond 180 days, the bacterium was not detected regularly in any treatment using the FSM, but occasionally the pathogen was detected in some samples from the Norfolk Fd, F + I, and Fd + I treatments. By April (210 days after infestation) the bacterium was not detected in any treatment by the FSM. Preliminary bioassays conducted in the greenhouse using tomato seedlings planted into soil subsamples removed from these microplots indicated the bacterium was still present in some plots. However, the results of this bioassay was inconsistent. Thus, in May a susceptible tomato plant (cultivar Homestead 24) was transplanted directly into each microplot in a final effort to detect differences in survival of *P. solanacearum* among the plots.

The bacterium was detected by using the bioassay in the F + I treatments of all soils and the Fd + I and Fd treatments of the Norfolk and Torhaunta soils (Table 3). In the Torhaunta soil, disease incidence was significantly higher in F + I than in other treatments. No disease was observed in the Worsham or Arapahoe plots unless the soils were F + I. A second planting into these same microplots showed similar results except that some limited vascular browning was observed among some plants in the Worsham and Arapahoe Fd + I, which had not been seen in the earlier assay.

There were significant differences ($P = 0.05$) among the covariance slope values for population counts on FSM (Table 4). The rate of population decline fell into two distinct groups with the faster decline associated with both Worsham soil treatments and Arapahoe Fd + I (range of slope values = −0.0905 to −0.0908) compared with the slower decline associated with Norfolk and Torhaunta soils (range of slope values (−0.0332 to −0.0508).

Test II. Plants in all Fd + I and F + I plots, plus those in Norfolk Fd wilted within 30 days of reestablishment (infestation and transplanting). Populations of *P. solanacearum* in the soil reached 10^7 cfu per gram of soil while plants were wilting, but the population dropped to about 10^5 cfu per gram of soil by the time most plants were dead. Regular assays with the FSM were initiated at that time (Table 2). As in Test I, in which laboratory-produced inoculum was used, the pathogen population in Test II, in which host-produced inoculum was used, declined rapidly. Again, the bacterium was detected longer with the FSM in Norfolk than all other soils. The bacterium was not detected beyond 60 days in the Worsham or Arapahoe soils. Generally, unlike Test I, the population level did not differ among soil treatments within the same soil type. By 150 days after infestation the bacterium was not detected regularly with the FSM, but soil subsamples removed from the microplots and bioassayed in the greenhouse indicated the bacterium was still present in Norfolk and Torhaunta soils and absent in all Worsham and Arapahoe plots. In spring (about 210 days after infestation) tomato plants were again planted directly

into the microplots, since the pathogen was not being detected with other means. Typical bacterial wilt symptoms developed in the plants transplanted into Norfolk Fd, Fd + I, and F + I and the Torhaunta Fd + I and F + I, but bacterial wilt symptoms were not detected in plants in other plots (Table 3). The disease developed more slowly in Test II than it had in Test I. The rate of population decline as indicated by covariance analysis differed significantly by soil type, but not by treatment (Table 3). The slowest decline was associated with Norfolk soils, Torhaunta soils were intermediate, and the Worsham and Arapahoe soils showed the fastest decline.

Test III. Tomato plants died within 7–10 days when planted immediately into soils infested with $\geq 2.5 \times 10^7$ cfu per gram of soil. Transplants in soil infested with $< 2.5 \times 10^3$ cfu per gram of soil seldom developed symptoms within 14 days. Thus, only data from the plots with inoculum densities of 2.5×10^4, 2.5×10^5, and 2.5×10^6 were analyzed statistically. The correlation between inoculum density and disease incidence was $r = 0.88$, with all soils and treatments, if plants were planted at the time of soil infestation. A delay of 60 days between soil infestation and transplanting reduced the correlation to $r = 0.39$. There was a significant interaction between soil, time of transplanting, and steaming of soil. There was no difference in the incidence of disease between the steamed and nonsteamed soils if transplanting occurred on the date of infestation (Fig. 1). Less disease was observed on plants in Arapahoe and Worsham than in other soils. Differences between steamed and nonsteamed soils were evident when a 60-day waiting period was imposed between infestation and transplanting (Fig. 1). In all soil-treatment combinations with a 60-day waiting period, less disease was detected in nonsteamed than in steamed soil. Greatest differences occurred with Arapahoe and Worsham soils, where only vascular browning was observed unless soil was steamed. The DI was lower following the waiting period in all treatments, although the difference was not significant for steamed Arapahoe or Worsham soils.

DISCUSSION

These studies were initiated to investigate why there appeared to be adequate inoculum of *P. solanacearum* after overwintering for significant bacterial wilt infections of commercial crops planted at the Norfolk and Torhaunta sites, but an apparent lack of such inoculum at the Worsham and Arapahoe sites. Our first hypothesis was that suitable weed hosts were absent at the Worsham and Arapahoe sites, because weed hosts play a major role in the persistence of *P. solanacearum* elsewhere (5,11,15,22,26). In order to eliminate overwintering hosts, half of each soil was fumigated prior to reinfestation, and all microplots were cultivated twice weekly to eliminate weeds. Despite these treatments, bacterial wilt developed the next spring in all plots that had been fumigated and reinfested the previous fall, and in the Norfolk and Torhaunta Fd + I plots (Table 2). Also, all four collection sites were visited about every 60 days, and weeds reported to be hosts were found at all sites, but we were unable to isolate the pathogen from their roots or stems using the FSM (W. C. Nesmith, *unpublished*). These data indicate that, in North Carolina, *P. solanacearum* can overwinter in some soils in the absence of a suitable host, but do not eliminate the possibility that weed hosts were involved at the collection site.

Data obtained with the FSM from the microplots in Test I clearly indicate that survival of *P. solanacearum* was affected by the soil used (Table 3). McCarter (19) reported a similar phenomenon earlier. In our study, the pathogen was detected longer and at a higher level in the Norfolk and Torhaunta soils collected from sites where the disease reoccurs regularly, than in the Worsham and Arapahoe soils which were collected from sites where the disease does not persist (Table 2). The rate of population decline was much more rapid in the Worsham and Arapahoe soils than the Norfolk and Torhaunta, except where the Arapahoe soils had been fumigated prior to infestation. We concluded that the Norfolk loamy sand and Torhaunta fine loamy sand are conducive to *P. solanacearum* and that the Worsham sandy loam and the Arapahoe coarse loamy sand are suppressive. Similar reports have been made involving other soilborne pathogens (2,4).

Changes of the pathogen population in the conducive soils that we studied showed a rapid initial decline followed by a slower rate of decline with sufficient virulent population persisting through the winter to produce abundant disease in the spring crop (Table 3). Conversely, in the suppressive soils the population disappeared rapidly and was not detected with the host in the spring planting, which indicated that a virulent population did not persist, unless the soils were first fumigated, then infested, in which case wilt developed in the spring crop, indicating that a virulent population did persist (Table 3). We do not understand why a virulent population was detected with the bioassay in the Worsham F + I, when it was not detected longer with the FSM in the F + I than the Fd + I. Likewise, we do not understand the rapid rate of population decline by this pathogen in soils, because the literature suggests long-term survival of race 1 in soil (5,15,19,21,24,26). The rapid rates of decline observed in these tests suggest that *P. solanacearum* is not as well adapted to survival in soil as previously believed, especially in suppressive sites (1,2,9).

Fumigation of the suppressive soils nullified some of the suppressiveness, especially with the Arapahoe soils. Slope values (Table 4) for the Arapahoe F + I were similar to those for the Norfolk and Torhaunta soils, but the slope values for the Worsham F + I were not different from those of the Worsham and Arapahoe Fd + I. In Test III, the greenhouse inoculum density experiment (Fig. 1), most suppression appeared during the 60-day waiting period between infestation and introduction of a host, with major differences noted in the DI among nonsteamed soils, but not among steamed soils. These data suggest microbial antagonism

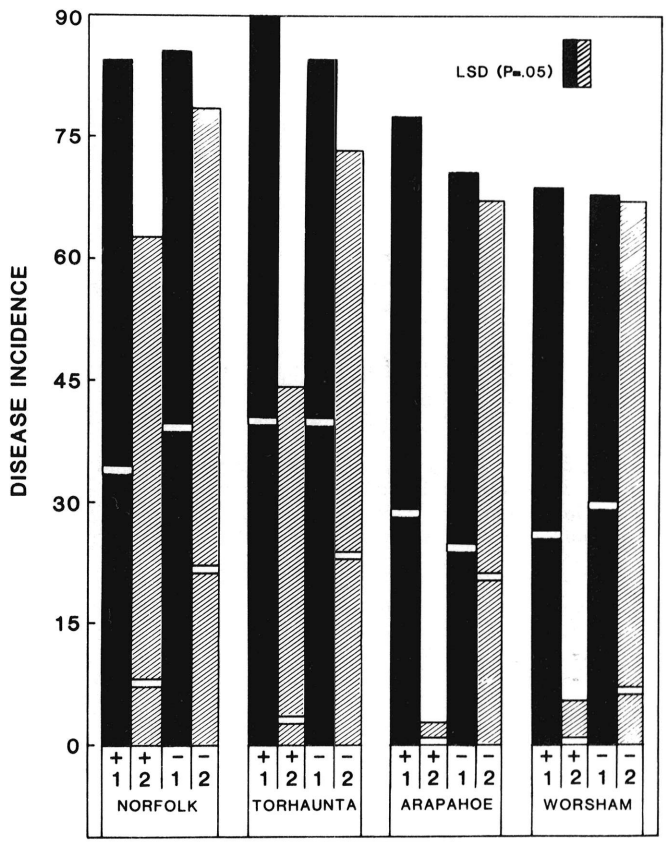

Fig. 1. Effect of the initial inoculum density of *Pseudomonas solanacearum* on the incidence of disease in Homestead 24 tomato plants transplanted on the same day as soil infestation, versus transplanting 60 days later in steamed or nonsteamed soils. Soils: Norfolk = Norfolk loamy sand, Torhaunta = Torhaunta fine loamy sand, Arapahoe = Arapahoe coarse loamy sand, Worsham = Worsham sandy loam. Soil treatments: (+) = nonsteamed, (−) = steamed. Time of transplanting: (1) = same date as infestation with *P. solanacearum*, (2) = 60 days after infestation. Full length of bar is incidence of disease produced with 2.5×10^6 cfu of initial inoculum per gram of soil and the height to the break represents the incidence produced with an initial population of 2.5×10^4 cfu per gram of soil.

may be a cause of the suppression. However, factors which may not be related to survival may be involved, because significantly less disease developed in suppressive than in compatible soils when infestation and transplanting occurred simultaneously (Fig. 1). These data indicate different inoculum potentials are needed for different soils. Reasons for these differences should be investigated.

Data obtained in Test II cannot be directly compared with that obtained in Test I because: the pathogen was produced in association with a living host compared to laboratory-produced inoculum in Test I, and fumigation was not repeated prior to reinfestation of the F + I plots as had been done in Test I. However, it should be noted that generally similar results were obtained between the tests in that the Worsham and Arapahoe plots were suppressive to *P. solanacearum* while the Norfolk and Torhaunta were more conducive to survival (Table 2).

The cause of these differences in apparent persistence of *P. solanacearum* at the four collection sites is not understood. More sensitive techniques for monitoring *P. solanacearum* in soil are needed. However, the results of this study provide some information about the four sites that were investigated and suggest other questions that need further investigation. Even though our microplots were located within a 7 × 20-m block so climatic factors were not directly involved, climatic differences are known to play a major role in the distribution of this pathogen (5,15). All plots were fallowed from September through April and half were fumigated prior to infestation, so unavailability of a suitable host during the noncrop period seems unlikely to account for the differences observed. The soil involved at the collection site appears to be associated with survival. The Norfolk loamy sand and Torhaunta fine loamy sand appeared conducive to *P. solanacearum* while the Worsham sandy loam and Arapahoe coarse loamy sand appeared to be suppressive. This last point may be more significant than would appear from these limited data, because it is not uncommon to find areas in eastern North Carolina differing widely in the yearly recurrence of bacterial wilt. Such areas may be only a short distance apart (3–100 m) and often differ in soil type. For example, near Cash Corner, NC, bacterial wilt is a problem year after year in one potato field, but 30 m away the disease is seldom seen. When it occasionally occurs, it does not persist, even though both fields are under the same farming operation. The soil type in which bacterial wilt persists is a Torhaunta while that in which bacterial wilt seldom occurs is an Arapahoe-Portsmouth association.

LITERATURE CITED

1. Alexander, M. 1971. Microbial Ecology. John Wiley & Sons, New York. 472 pp.
2. Baker, K. F., and Cook, R. J. 1974. Biological Control of Plant Pathogens. W. H. Freeman and Co., San Francisco. 433 pp.
3. Benson, D. M., and Baker, R. 1974. Epidemiology of *Rhizoctonia solani* preemergence damping-off of radish: Survival. Phytopathology 64:1163-1168.
4. Broadbent, P., and Baker, K. F. 1974. Behavior of *Phytophthora cinnamomi* in soils suppressive and conducive to root rot. Austr. J. Agric. Res. 25:121-137.
5. Buddenhagen, I. W., and Kelman, A. 1964. Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol. 2:204-230.
6. DeVay, J. E., and Schnathorst, W. C. 1963. Single cell isolation and preservation of bacterial cultures. Nature 119:775-776.
7. Dukes, P. D., Morton, D. J., and Jenkins, S. F., Jr. 1965. Infection of indigenous hosts by *Pseudomonas solanacearum* in south Georgia. (Abstr.) Phytopathology 55:1055.
8. Fiskell, G. A., and Perkins, H. F., eds. 1970. Selected coastal plain soil properties. Southern Co-op. Series Bull. 148:111.
9. Gray, T. R. G., and Parkinson, D. 1968. The Ecology of Soil Bacteria: An International Symposium. University of Toronto Press. 681 pp.
10. Graham, J., and Lloyd, A. B. 1979. Survival of potato strain (race 3) of *Pseudomonas solanacearum* in the deep soil foyers. Austr. Agric. 30:489-496.
11. Harris, D. C. 1976. Epidemiology and control of *Pseudomonas solanacearum* in Kenya. Pages 148-150 in: Proc. First Int. Planning Conf. and Workshop on the Ecology and Control of Bacterial Wilt Caused by *Pseudomonas solanacearum*. L. Sequeira and A. Kelman, eds. 18–23 July 1976, Raleigh, NC. University of California/AID, Pest Management and Related Environmental Protection Project: Berkeley. 166 pp.
12. Hayward, A. C. 1964. Characteristics of *Pseudomonas solanacearum*. J. Appl. Bact. 27:265-277.
13. Jackson, M. T., and Gonzales, L. C. 1981. Persistence of *Pseudomonas solanacearum* (race 1) in a naturally infested soil in Costa Rica. Phytopathology 71:690-693.
14. Jenkins, S. F., Jr., and Kelman, A. 1976. Techniques for the study of *Pseudomonas solanacearum*. Pages 143-147 in: Proc. First Int. Planning Conf. and Workshop on the Ecology and Control of Bacterial Wilt Caused by *Pseudomonas solanacearum*. L. Sequeira and A. Kelman, eds. 18–23 July 1976, Raleigh, NC. University of California/AID, Pest Management and Related Environmental Protection Project: Berkeley. 166 pp.
15. Kelman, A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum*. N.C. Agric. Exp. Stn. Tech. Bull. 99. 194 pp.
16. Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on tetrazolium medium. Phytopathology 44:693-695.
17. Konke, H. 1968. Soil Physics. McGraw-Hill, New York. 397 pp.
18. Lee, W. D. 1955. The soils of North Carolina. N.C. Agric. Exp. Stn. Tech. Bull. 115. 187 pp.
19. McCarter, S. M. 1976. Persistence of *Pseudomonas solanacearum* in artificially infested soils. Phytopathology 66:998-1000.
20. Nesmith, W. C., and Jenkins, S. F., Jr. 1979. A selective medium for the isolation and quantification of *Pseudomonas solanacearum* from soil. Phytopathology 69:182-186.
21. Okabe, N. 1971. Population changes of *Pseudomonas solanacearum* and soil microorganisms in artificially infested natural field soil. Rev. Plant Prt. Res. 4:105-108.
22. Sequeira, L. 1958. Bacterial wilt of bananas—dissemination of the pathogen and control of the disease. Phytopathology 48:64-69.
23. Shamsuddin, M., Lloyd, A. B., and Graham, J. 1979. Survival of the potato strain of *Pseudomonas solanacearum* in soil. Austr. Agric. 4:212-215.
24. Smith, T. E. 1943. Distribution of bacterial wilt, *Bacterium solanacearum*, in successive crops of tobacco grown on the same field. Phytopathology 33:1076-1080.
25. Steel, R. G. D., and Torrie, J. H. 1960. Principles and Procedures of Statistics, with Special Reference to the Biological Sciences. McGraw-Hill, New York. 481 pp.
26. Tanaka, Y., and Noda, N. 1973. Studies on the factors affecting survival of *Pseudomonas solanacearum* E. F. Smith, the causal agent of tobacco wilt disease. Bull. Okayama Tobacco Exp. Stn. 32:81-94.