Persistence of *Pseudomonas solanacearum* (Race 1) in a Naturally Infested Soil in Costa Rica

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ABSTRACT

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The persistence of *Pseudomonas solanacearum* in an inceptisol was studied at Turrialba (600 m above sea level) in Costa Rica. The relative inoculum level in the soil after different crop rotations and weed management practices was determined indirectly by an assay based on the percentage of wilted plants of bacterial wilt-susceptible and tolerant potato clones growing in this soil. Cultivar Atzimba was highly susceptible, whereas clone MS 35-22 had a high level of tolerance in trials in which maize, sweet potatoes, and kudzu were used in the rotations. No crop significantly reduced the relative inoculum potential. No effect could be demonstrated in other crop rotations in which maize, cowpeas, wilt-resistant tomatoes, and sweet potatoes were used. The severity of the disease on potato was significantly reduced only in plots in which weeds had been eliminated by a contact herbicide. In this case, the surface of the soil was exposed for 4 mo in contrast to those plots in which weeds either were controlled through tillage or allowed to grow in a weed fallow treatment.

Additional key words: bacterial wilt, *Solanum tuberosum*, soilborne bacteria, weed hosts, weed management.

Bacterial wilt, which is caused by *Pseudomonas solanacearum* E. F. Smith, is among the most serious plant diseases in the tropics and subtropics. The host range of the pathogen is wide and it includes nonsolanaceous crop plants as well as those of weed species from other taxonomic groups (8). The disease is widespread on potatoes (*Solanum tuberosum*) throughout Central America, where race 3 is the most commonly found variant (2), particularly in the moderately cool highlands.

In Costa Rica, the farming system in the main potato-growing area includes rotations with pasture and vegetable crops and, even in marginal areas, farmers managed to control the disease by using these rotations and planting selected healthy seed tubers. In fact, outbreaks of bacterial wilt often are traced to the use of contaminated seed (3).

At altitudes lower than 1,000 m above sea level, many soils appear to be infested naturally with race 1 of the bacterium (L. Sequeira and E. R. French, *personal communications*), even where susceptible crops have not been cultivated recently. When potatoes are grown in the hot, humid lowland tropics, race 1 of *P. solanacearum* becomes a limiting factor. Even though sources of resistance to *P. solanacearum* have been identified (12,13), relatively few evaluations of resistance against race 1 have been made under field conditions (7).

In this study, the persistence of race 1 bacterial inoculum in a naturally infested soil was evaluated during a 3.5-yr period after different crop rotations. Studies were initiated to investigate the role of weeds in maintaining the bacterial population; the importance of several local weeds, especially *Melampodium perfoliatum* (Cavanilles) H. B. K., in Turrialba was reported (7). Preliminary reports of this study were included in a planning conference at the International Potato Center (5) and in an abstract (6).

MATERIALS AND METHODS

All experimental work was carried out in the La Montaña experimental field of the Tropical Agricultural Research and Training Center (CATIE), Turrialba, Costa Rica, which is at an altitude of approximately 600 m. The average maximum temperature is 27 C and the average minimum is 17 C, with little variation from month to month. Annual precipitation is in excess of 2,500 mm, and there is an ill-defined short dry season during February and March. The soil is an inceptisol (pH 4.9) with stony and normal phases (1).

The relative inoculum potential was determined indirectly by observing the percentage of wilted potato plants of wilt-susceptible and tolerant cultivars in the various treatments. The number of wilted plants was counted weekly from plant emergence until maturity, and from all these readings average percentage wilt (APW) was calculated. A severity index at 9 or 11 wk (SI_9 or SI_{11}, respectively) based on a scale of 1 (no wilt) to 6 (plant dead) (7) also was calculated to differentiate levels of resistance or susceptibility among different genotypes and severity of wilt among treatments. Analyses of variance were carried out using angular transformed percentage data.

Experiments I and II were designed to study the effects of crop rotations on the persistence of the bacterium; experiment III was designed to study the effect of weed management. Site I was 150 m from sites II and III, which were contiguous.

For Experiment I, which extended over four growing seasons, seed tubers of cultivar Atzimba were planted (55,500 plants per hectare) in December 1976 in a randomized complete block design with three replicates. Subsequently these were divided into six main plots, 8.10 × 5 m, with raised grass borders left between plots to prevent the spread of soil. Maize (*Zea mays* 'Tuxpeño Planta Baja C-7'), 20,000 plants per hectare, and beans (*Phaseolus vulgaris* 'Guaria'), 200,000 plants per hectare, were planted in 1977, and maize and sweet potato (*Ipomoea batatas* 'C-15'), 50,000 plants per hectare, in 1978, as rotation crops (Fig. 1). In subsequent potato plantings in December 1978 after crop rotations, main plots were subdivided equally and the relative disease susceptibility of the potato cultivars Atzimba and Rosita, and clone MS 35-22 (50,500 plants per hectare) was compared in 1977–1978, and Atzimba and MS 35-22 in 1978–1979, both in a split-plot design with three replicates, with rotation crops as main plots and potato genotypes as subplots. From 1977 to 1979, fallow periods between potatoes and rotation crops lasted up to 3 mo. After the 1978–1979 planting of potatoes, all plots were left in a weed fallow (11) until September 1979. At that time they were planted with maize in those plots that previously contained sweet potatoes, while tropical kudzu (*Pueraria phaseoloides*) was planted in plots that had maize in

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previous rotations. Kudzu covered these plots for 7 mo, although its establishment was slow. Maize was harvested in December 1979 and maize stalks were left in a weed fallow in the plots until they were prepared in April 1980 for a potato planting. In May, plots were planted with cultivar Atzimba, and wilt readings were taken weekly.

In Experiment II, started in December 1977, the effect of continuous cropping between two plantings of potatoes 1 yr apart was studied. There were five treatments, replicated four times in plots 3 × 3 m: continuous plantings of wilt-susceptible potatoes, cultivar Atzimba (50,500 plants per hectare); maize followed by cowpea (*Vigna unguiculata* 'Vigna-44') 100,000 plants per hectare; wilt-resistant tomato (*Lycopersicon esculentum* 'Kewalo') 20,000 plants per hectare, followed by the same; cowpea followed by sweet potato; a maize with potato association at 22,250 and 33,700 plants per hectare, respectively, followed by the same.

In Experiment III, the amount of wilt was compared in two plantings of potato cultivar Atzimba 1 yr apart, starting in December 1977, in plots with a 4-mo weed fallow (treatment 1) and those where weeds were controlled either by tillage (treatment 2) or through the use of the contact herbicide, paraquat (0.4 kg a.i./ha), 10 applications over a 4-mo period (treatment 3). These treatments all followed a cowpea rotation that lasted 3 mo. Treatments 4 and 5 were continuous plantings of potatoes and bacterial wilt-resistant tomatoes. The experimental design was identical to that of Experiment II.

In Experiments II and III there was a 2-mo fallow in all plots from mid-March to mid-May 1978 after the first planting of potatoes was harvested. In all experiments, fertilizer was applied at the recommended rates for each crop.

RESULTS

In all plantings of potatoes the first wilted plants appeared 3–5 wk after planting. Time to maturity varied among potato genotypes, but never exceeded 100 days.

The amount of bacterial wilt in the potato plots was not reduced significantly after any crop rotation. In Experiment I, however, different potato cultivars were compared and there were slight changes from year to year. For example, the amount of wilt in Atzimba in 1978–1979 was lower than in the previous season, although APW values in both years were considerably higher than those observed in the first planting of potatoes in 1976. The amount of wilt in MS 35-22, however, increased from 1978 to 1979, despite the rotation crops planted between potato crops (Fig. 1).

In the 1977–1978 planting when three potato genotypes were evaluated, cultivar Atzimba was the most susceptible and clone MS 35-22 the most tolerant; cultivar Posita was intermediate. In the 1978-1979 planting, although 50–60% of MS 35-22 plants were infected with *P. solanacearum*, with APW_{12} values of 12.48 and 16.20 after maize and sweet potato rotations, respectively, SI_{11} values were only 1.73 and 1.97, and less than 1% of tubers had visible symptoms of the disease at harvest; yields were the equivalent of 30 t/ha. On the other hand, cultivar Atzimba had APW_{11} values of 41.96 and 38.71 after the same rotations, and SI_{11} values of 4.44 and 4.36, respectively, and yielded only 100 g of healthy tubers per plant, of which a further 15% rotted after storage for 5 wk.

In 1980, potatoes were planted during wetter months than in the previous three plantings, and consequently a direct comparison could not be made. Nevertheless, the increase of bacterial wilt in all plots was rapid. At 4 wk approximately 25% of the potato plants had wilted, and this had increased to over 80% by 5 wk after planting. APW values were slightly higher in plots with kudzu (APW_8 = 55.95) than in plots with maize in rotation (APW_8 = 49.48), even though weed suppression in the former was greater.

In Experiment II, none of the five rotation treatments reduced the level of bacterial inoculum. The disease developed much more rapidly in the 1979 planting than in the 1977–1978 planting. There were no significant differences between the amount of wilt in cultivar Atzimba in plots with continuous cropping of potatoes and those that had two rotation crops, although APW values were slightly higher for the former (Table 1).

The amount of wilt in cultivar Atzimba in plots of Experiment III where weeds were controlled by spraying with paraquat was significantly lower than that of the other treatments (Table 2), except where resistant tomato had been grown. In these, however, APW_9 was not significantly different from plots with a weed fallow,

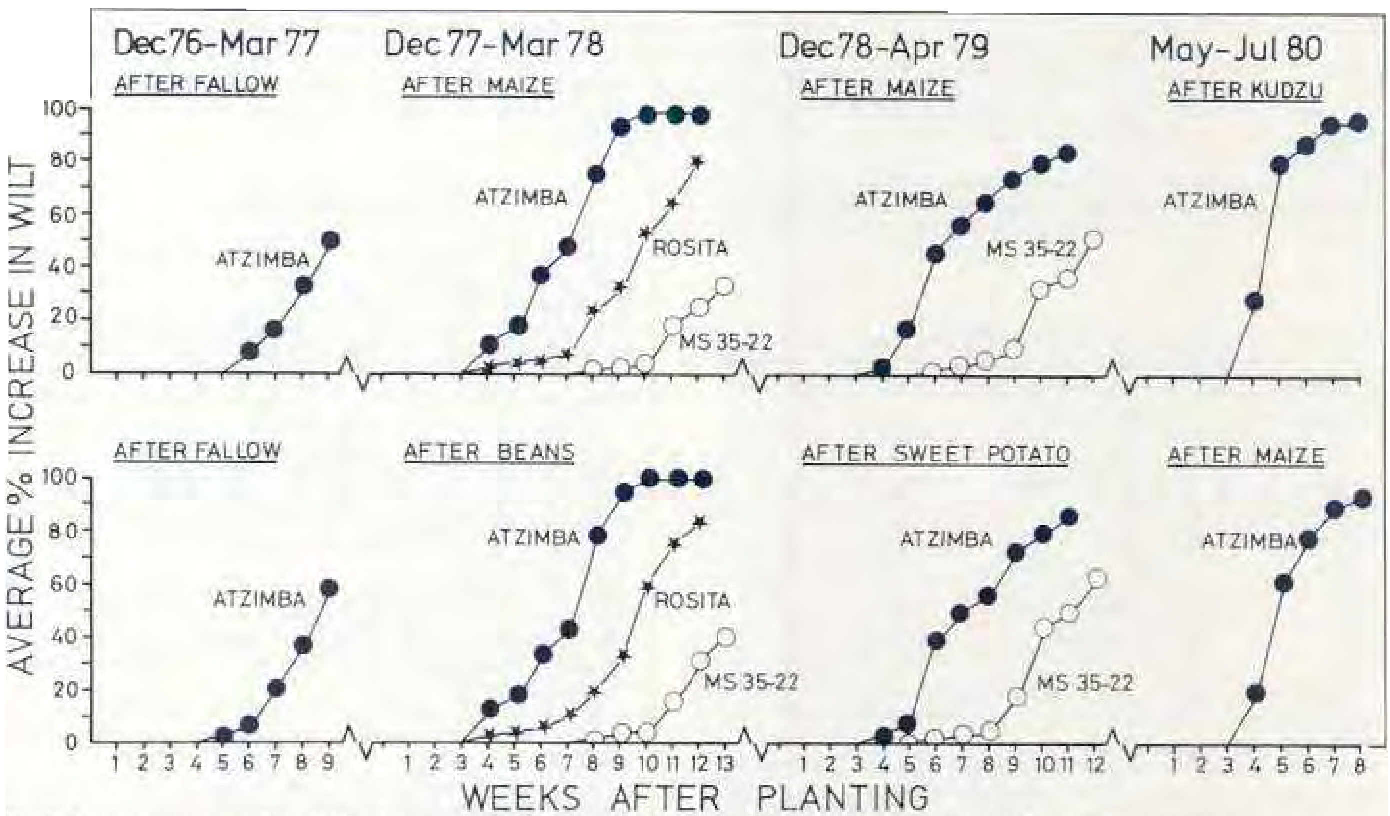

Fig. 1. Average percentage of bacterial wilt in potatoes after different crop rotations at Turrialba, Costa Rica, 1976–1980 (Experiment I).

weed control through tillage, or where potatoes had been cropped continuously. The wilt observed at the end of the season in plots having previous herbicide applications reached 60%, but the disease developed more slowly; SIs were almost half those of the other treatments. In plots with the weed fallow, the disease developed as rapidly as in plots continuously planted with potatoes.

In both Experiments II and III there was a high correlation ($r = 0.95$) between APW and SI values at 9 wk ($P = 0.01$).

DISCUSSION

Once *P. solanacearum* became established in this inceptisol at Turrialba the inoculum level was maintained even in the absence of susceptible crops. After the inoculum potential increased during potato cultivation, it remained at a high level despite several different crop rotations.

In Turrialba the soil rarely dries completely and conditions are favorable for the persistence of the bacterium. Although rotations have proven successful against race 3 isolates at higher elevations in Costa Rica and in other countries (4), this was not the case with race 1 in this study.

Annual weeds are abundant under the hot, humid conditions of the lowland tropics. At Turrialba, weeds grew more slowly under dead maize stalks than in plots from which beans had been recently harvested, even though the beans had previously suppressed weed growth. Where sweet potatoes were grown and the soil was quickly covered with foliage, wilt-susceptible weeds, such as *M. perfoliatum* were uncommon, but the bacterial populations still remained high. Sequeira (11) showed that a weed fallow was as effective as rotations with kudzu for the control of bacterial wilt of bananas, caused by *P. solanacearum* race 2; he concluded that the elimination of plant hosts was the factor largely responsible for the control of the disease, because the banana strain of *P. solanacearum* has a limited host range. A similar situation appears to hold for race 3 in view of the efficiency of pasture rotations. This is not the case with race 1, which has a wider host range.

With race 1 under our conditions, a weed fallow promoted the survival of the bacterium while continual tillage of the soil constantly brought weed seeds to the surface where they could germinate. We have observed wilting young weed plants in soil plowed a few weeks before (7); however, in a related research project in the same area, no wilted plants were seen in plots with a weed fallow and it was not possible to isolate the bacterium from healthy-appearing, wilt-susceptible weeds even though a high inoculum level had been detected in previous potato plantings (C. Rodríguez, *personal communication*). These findings are similar to those reported by Moffett and Hayward (9).

A direct reduction of bacterial inoculum of race 3 was attributed by Navarro (10) to weed control through the application of an herbicide (DNBP), which eliminated weeds for up to 6 mo, even though plots with weed fallow or tillage were not included for comparison in the study. For paraquat, which is inactivated upon contact with the soil, a direct effect of the chemical upon the bacterium can probably be discounted. This contact herbicide killed annual weeds before they completed their reproductive cycle. In this case the soil remained bare for a period of 4 mo, and may have contributed to the apparent reduction of the bacterium in the plots thus maintained devoid of live vegetation cover. This occurred during the wet season, when the bacterium can survive longer in the soil. Nevertheless, the use of contact herbicides on the scale used in this study may not be a commercial feasibility and other weed control alternatives should be studied.

If the potato becomes a more important crop in the hot, humid tropics, integrated control, including disease resistance and inoculum reduction or eradication, will be necessary. The level of wilt resistance maintained by the clone MS 35-22 even when grown in heavily infested soil is reported here for the first time. Genetic resistance such as this may provide an increment of control. But even as this or better wilt-resistant potatoes against race 1 are selected, it will be necessary to provide an environment in which the risk of wilt infection is reduced. The rotation crops used in this study proved ineffective, but minimum tillage management involving herbicides might help by lowering the inoculum level in the environment.

TABLE 1. Average percentage wilt (APW_9)[a] and severity index (SI_9)[b] caused by *Pseudomonas solanacearum* in potato plants of cultivar Atzimba before and after different crop rotations at Turrialba, Costa Rica (Experiment II)

Potatoes Dec'77–March'78 APW_9	Intermediate cropping sequence May '78–Jan '79	Potatoes Jan–April '79 APW_9	SI_9
26.70[c]	Potato-potato	54.60	
25.67	Tomato-tomato	51.36	
25.93	Maize-cowpea	47.30	
31.36	Cowpea-sweet potato	49.92	
30.52	Maize with potato-Maize with potato	55.05	

[a]The number of wilted plants in each treatment was counted weekly from plant emergence until maturity at 9 wk; from all these readings APW_9 was calculated.

[b]SI_9 is based on a scale of 1 (no wilt) to 6 (plant dead) at 9 wk after planting.

[c]Values represent the mean of four replicates. Means in the same column are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 2. Average percentage wilt (APW_9)[a] and severity index (SI_9)[b] caused by *Pseudomonas solanacearum* in plants of potato cultivar Atzimba before and after rotation with cowpeas and weed management at Turrialba, Costa Rica (Experiment III)

Potatoes Dec '77–March '78 APW_9	Intermediate crop or weed management sequence May '78–Jan '79	Potatoes Jan–April '79 APW_9	SI_9
27.06 x[c]	1. Cowpea-weed fallow	53.79 y	4.97 y
28.52 x	2. Cowpea-tillage	43.50 y	4.13 y
23.93 x	3. Cowpea-herbicide	23.81 x	2.65 x
29.56 x	4. Potato-potato	49.69 y	5.00 y
26.13 x	5. Tomato-tomato	38.27 xy	3.96 xy

[a]The number of wilted plants in each treatment was counted weekly from plant emergence until maturity at 9 wk; from all these readings APW_9 was calculated.

[b]SI_9 is based on a scale of 1 (no wilt) to 6 (plant dead) at 9 wk after planting.

[c]Values represent the mean of four replicates. Mean in the same column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

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