

Survival of *Pseudomonas solanacearum* Race 3 in Plant Debris and in Latently Infected Potato Tubers

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ABSTRACT

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By the use of indicator plants, *Pseudomonas solanacearum* race 3 was detected in soil and debris collected from a heavily infested potato field 33 wk after the field was abandoned. Infested debris that was mixed into noninfested soil remained infested for 26 wk, and in one sample for 32 wk. It

Additional key words: bacterial wilt of potatoes.

Pseudomonas solanacearum E.F. Sm. is a bacterial plant pathogen of several economically important food crops, particularly potato, tomato, and banana in the tropical, subtropical, and warm temperate regions of the world (10). A number of different races or pathovars are recognized, based on their host range (1). Race 3 (biovar II) is a low temperature pathovar that poses a threat to potato cultivation in cool temperate climates (6,9,13,19).

Bacterial wilt of potatoes (which is caused by *P. solanacearum* race 3) is endemic in the Dorrigo area of the Northern Tablelands of New South Wales (N.S.W.) (13). Since the first report of the disease in that area in the 1956-57 season (2), it has caused seasonal crop losses. Thus, in the 1965-66 season an estimated 80 ha of potatoes was infested. The disease was more widespread in the following season, but in the 1967-68 season only 40 ha became infested, which represented an estimated loss of 9,000 bags of potatoes (15).

Long-term survival of *Pseudomonas solanacearum* in soil has been reported (12). Smith (17) showed that race 1 of *P. solanacearum* survived in bare-fallowed field plots for at least 4 yr and field trials conducted on the Northern Tablelands of N.S.W. over a 3 yr period have shown that race 3 could survive in similar bare-fallowed plots for 1-2 yr (16).

Notwithstanding the above evidence, *P. solanacearum* is reported to have poor survival ability in soil (18). It seems likely, therefore, that the bacterium survives mainly in sheltered sites such as the roots of alternative hosts, infested plant debris, volunteer tubers from earlier crops and perhaps, in some cases, in the deeper soil layers where microbial activity is likely to be lower due to the paucity of indigenous soil microorganisms.

Pseudomonas solanacearum race 3 has a limited alternative host range. Only two solanaceous weed hosts (*Solanum nigrum* L. sens. lat., [8] and *Solanum cinereum* R. Br. [5] have so far been reported in Australia. These species do not overwinter vegetatively under the severe climatic conditions of the Northern Tablelands, except, perhaps, for small pockets in the escarpment area where somewhat milder winter conditions prevail. The bacterium, therefore, either must overwinter in one or more of the other sheltered sites, or be dependent on reintroduction in latently infected seed tubers imported from warmer areas.

The purpose of the research reported here was to examine the role of potato plant debris and latently infected tubers as sheltered sites for the survival of *P. solanacearum* race 3 in soil.

is suggested that in a cool temperate climate, such as that of the Northern Tablelands of New South Wales, infested debris and latently infected self-sown tubers can serve, as short-term and long-term sheltered sites, respectively, for survival of *P. solanacearum* race 3 in the field.

MATERIALS AND METHODS

Infested soil, plant debris, and latently infected potato tubers were obtained from an abandoned potato field in the Dorrigo area of the Northern Tablelands of N.S.W. The field had been planted to potatoes in September 1976, and was abandoned in February 1977 after a severe outbreak of bacterial wilt. The potato grower allowed us to have unrestricted access to this abandoned field until the latter part of the year when his lease expired and the land was sown to pasture.

The soil was a fertile, well drained krasnozem derived from basaltic parent material and classified as part of the Fernbrook association (14). The area has a mean annual rainfall of about 160 cm with the highest rainfall in the late summer, and a mean seasonal temperature range of 8-20 C (14).

Survival of *P. solanacearum* in infested soil. Soil was collected from three sites occupied by diseased potato plants in the abandoned potato field. Approximately 8 kg of infested soil at the 10-30 cm level was removed from each site early in February 1977 and again at five successive sampling times up to October 1977. Each soil sample was sieved (5 mm × 5 mm sieve opening size); the portion retained by the sieve was termed "debris" and that which passed through was called "free soil".

Free soil from each sampling site was mixed by hand and then placed in eight separate 15-cm diameter plastic pots and a single "miniature" potato seedling (*Solanum tuberosum* 'Sequoia') was root-wounded by removal of approximately one-eighth of the root system, and transplanted into each pot (4). Debris from the sampling site was placed in similar pots and mixed with noninfested sandy soil prior to the transplanting of miniature potato seedlings.

Potato plants were kept in a greenhouse maintained at 26-34 C during the day and 22-28 C during the night; the actual temperatures depended to some extent on the ambient temperature. Plants were considered to be diseased if they showed typical symptoms of bacterial wilt, that is, inrolling of the lateral leaflets, wilting, and often yellowing of the leaves. Potato tubers from wilted plants, when cut transversely and squeezed, exuded bacterial ooze from the vascular ring, a test which is strongly diagnostic for bacterial wilt (4). A few stem sections from diseased plants were collected, individually placed in sterile water and examined for streaming of bacterial ooze from the cut ends. In a few cases, the bacterium was grown and isolated on tetrazolium chloride agar medium (11) and identified as *P. solanacearum* biovar II (7) which can be equated with race 3 (1,9). Typical symptoms of bacterial wilt also were obtained when both ooze from

infected stem sections and cell suspensions prepared from the growth on tetrazolium chloride medium were inoculated into young tomato plants (*Lycopersicon esculentum* 'Grosse Lisse').

Each diseased plant was given a disease rating which was converted to a disease index, according to the system of Winstead and Kelman (20), but modified so that irreversibly wilted plants were given the same numerical grade as dead ones. This enabled sacrifice of those plants to check for streaming of bacterial ooze from cut stems before the plant died.

Survival of *P. solanacearum* in tubers and root segments. About 40 diseased, self-sown potato plants in the bud or early bloom stage were collected from the Dorrigo field in March 1977. From these plants, 18 tubers and 18 bundles of root segments were selected. Each bundle of root segments was loosely wrapped in nylon gauze, tagged, and three bundles of root segments or one tuber were buried in each 18-cm diameter, porous, earthenware pot containing noninfested soil. The pots were buried in the ground so that the tubers and root segments were at about the same depth that tubers are normally formed on potato plants in the field.

At intervals after burial, three each of tubers and bundles of root segments were excavated, homogenized as two separate lots, and the homogenate then was mixed with a quantity of noninfested sandy soil in 15-cm diameter plastic pots. A root-wounded potato seedling was transplanted into each pot, the pots were kept in a greenhouse, and disease symptoms were recorded.

Tuber indexing. Externally symptomless tubers were collected in February from the Dorrigo field, washed in tap-water, dipped in

ethanol, and then flamed to sterilize the outside of the tubers. A section of the stolon end of each tuber was cut away, a second cut was made at right angles to the first and the exposed surfaces were examined closely for signs of vascular discoloration. Tubers with visible disease symptoms were discarded. Exposed surfaces were allowed to heal in the sun and then 90 tubers were planted separately in 15-cm diameter plastic pots containing noninfested sandy soil. Pots were kept in an unheated greenhouse and, when plants had attained a height of 20–30 cm, the temperature was increased to 26–34 C during the day and 22–28 C during the night to promote disease expression. As soon as disease symptoms appeared, plants were cut at ground level and stem sections were checked for streaming of bacterial ooze. Several suspensions obtained in this way were streaked on plates of tetrazolium chloride agar medium (11) to verify the presence of *P. solanacearum*.

Eleven externally symptomless tubers also were kept in large sealed desiccators containing free water, incubated at 30 C, and examined daily for release of bacterial ooze from the "eyes".

RESULTS

Debris remained infested with *P. solanacearum* 233 days after the crop was abandoned in the field in February 1977 (Fig. 1). Severe bacterial wilt symptoms generally developed 15–30 days after transplanting of indicator plants into noninfested soil amended with infested debris. Only in the debris sampled at 39 days did the time required for disease expression in the indicator plants

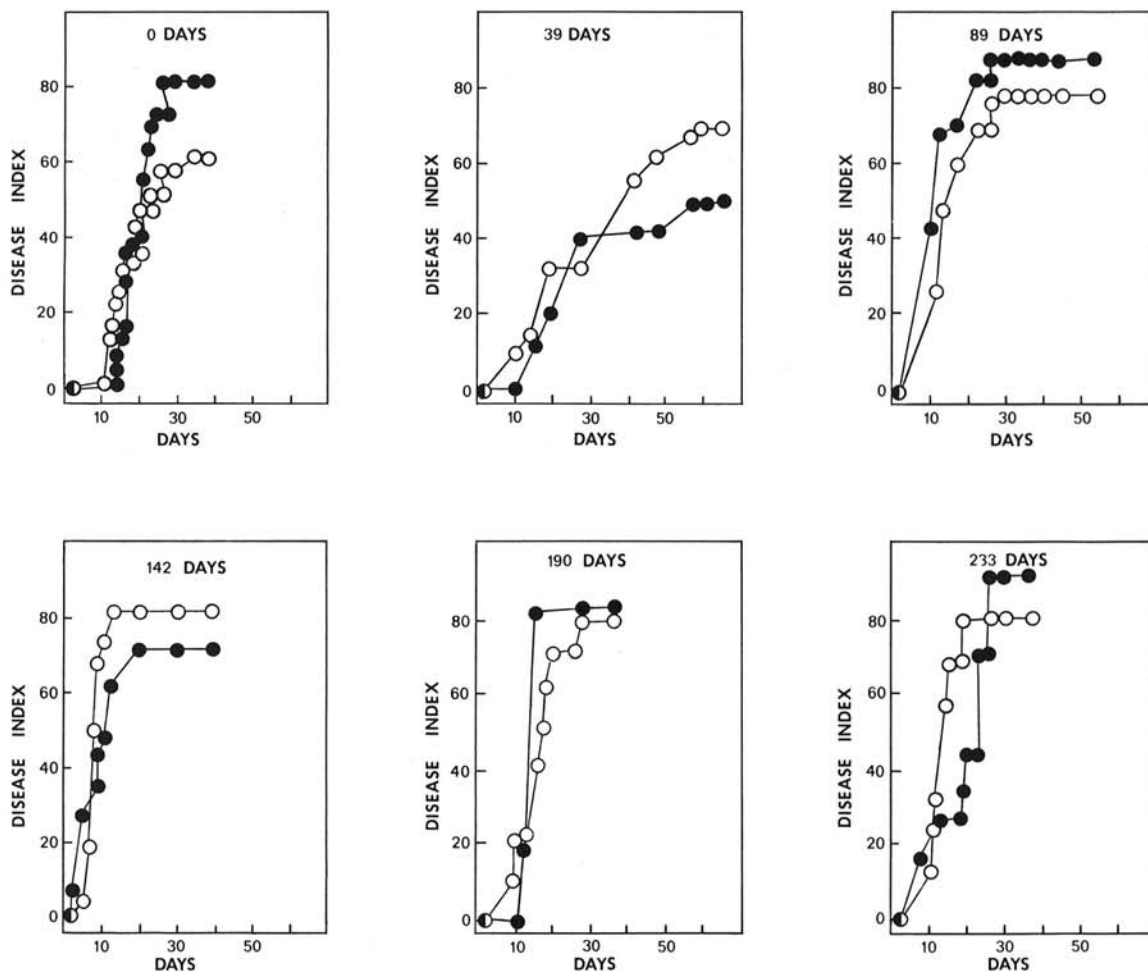


Fig. 1. Disease index rating of potato seedlings grown for different lengths of time in *Pseudomonas solanacearum*-infested field soil collected at 0, 39, 89, 142, 190, and 233 days after the potato field was abandoned because of bacterial wilt. Each diseased plant was given a disease rating based on a scale of 0–5, in which 5 represented irreversibly wilted plants. The separate ratings were totalled and expressed as a percentage of the maximum possible numerical rating. The solid circles are disease ratings for potato seedlings grown in noninfested soil amended with debris sieved from the infested potato field, and the light circles are those for infested field soil "free" from debris.

exceed 30 days, and this difference was probably due to a low initial level of infestation in the soil sampled.

Contrary to expectation, free soil remained uniformly infested over the entire sampling period, except for soil sampled at 39 days, which gave results similar to the debris samples, perhaps again due to a low initial level of infestation in the soil sample.

Tubers and root segments, taken from diseased plants and buried in earthenware pots, remained infested throughout the winter of 1977 (Table 1). However, at the last sampling (223 days), only one indicator plant grown in soil amended with infested debris developed disease symptoms, suggesting that bacterial populations had declined in both tubers and root sections near the end of the sampling period.

Tuber indexing. Of the 81 externally symptomless tubers which sprouted and produced plants, *P. solanacearum* was isolated from the cut stem ends of eight plants.

When 11 externally symptomless tubers were incubated at 30 C in desiccators containing free water, one showed oozing from the eyes at 7 days and two others showed the same symptoms by the end of the 3rd wk. Continued incubation beyond this time was not practical because of extensive tuber breakdown caused by microorganisms other than *P. solanacearum*.

DISCUSSION

Pseudomonas solanacearum can survive in infested debris for at least 33 wk under the climatic and soil conditions of the Northern Tablelands of N.S.W. The debris remained infested from January, the month when the summer crop usually is harvested in the Dorriggo area, until the following September when the next season's potato crop is planted. In addition, when infested debris was mixed with noninfested soil and kept in earthenware pots buried in the ground, the debris remained infested for 26 wk, and in one sample for 32 wk. It is apparent that partially decomposed debris of the previous season's potato crop is important in the carryover of *P. solanacearum* between successive crop plantings.

Similarly, 33 wk after the potato field was abandoned, soil from which debris had been removed by sieving was shown to be infested. This suggests either that the population size of *P. solanacearum* in free soil remained above the inoculum level necessary for infection, or the decline of the bacterial population in free soil was offset by the release of bacterial cells from infested debris during the course of the sampling period.

Inspection of debris and tubers in the abandoned potato field, at different times throughout 1977, suggested that the buildup and release of bacterial ooze was at a maximum in the late summer months when warm, moist soil conditions were most conducive to rapid tuber breakdown. With the onset of cooler, drier soil conditions in late autumn (April-May), tuber breakdown was arrested and release of bacterial ooze into the free soil declined.

Infested debris is an important short-term shelter for the bacterium in soil. However, *P. solanacearum* race 3 can survive for 1-2 yr in bare-fallowed or weed-fallowed ground on the Northern

TABLE 1. Bacterial wilt disease in potato seedlings transplanted into triplicate pots containing either infested potato tubers or root segments, previously buried in soil for varying lengths of time

Period of burial (days)	Disease rating ^a	
	Tubers	Root segments
0	+++	+++
34	+++	-+
70	+--	++
138	++-	+-
185	+++	++-
223	---	+-

^aSymbols: + = Bacterial wilt symptoms in indicator plants within 8 wk of transplanting into infested soil, and - = no disease symptoms in indicator plants within 8 wk.

Tablelands (16), and information from potato growers suggested that longer periods of field infestation are not exceptional. For example, one infested field, which had been abandoned to potato growing in the spring of 1972 because of bacterial wilt, produced diseased plants when sown to potatoes in the spring of 1976 (3). A detailed inspection of the latter area failed to show the presence of alternative weed hosts.

Inspections of particulate debris buried in earthenware pots for 32 wk, and observations made in infested potato fields, indicated that debris gradually decomposes, especially in the warm, moist soil conditions experienced in the late summer period. It therefore seems unlikely that debris would serve as a sheltered site for periods as long as 1-2 yr.

An important sheltered site for long-term survival of the bacterium in the field therefore appears to be symptomless potato tubers. Approximately 10% of such tubers, when grown in noninfested soil gave rise to diseased plants. In addition, three of the 11 externally symptomless tubers, stored under conditions of high humidity, exuded bacterial ooze from the eyes.

Probably tubers that are latent carriers of *P. solanacearum* can regenerate in a way similar to healthy tubers. If environmental conditions are unfavorable for disease expression, as can often occur in cold temperate regions, then the infected, but externally symptomless plants, survive and produce latently infected tubers. With the onset of a warm, wet growing season these tubers produce heavily infected plants that may become infection centres for the secondary spread of bacterial wilt throughout the crop.

Pseudomonas solanacearum race 3 is a low-temperature adapted pathovar which tends to occupy the altitudinal and latitudinal extremes of *P. solanacearum* distribution (7,9,19). Indeed, it would seem that the unique distribution of this race in cool temperate areas is due mainly to its close association with the potato, and the ability to survive in soil under temperature conditions prevailing in the areas most suitable for potato cultivation. Very likely *P. solanacearum* race 3 has limited distribution in warm temperate regions because of its restricted host range, its close affinity with potatoes which often are not grown in warm climates, and the probable rapid decline of pathogen populations in warm, moist soils where host debris is degraded rapidly.

By contrast, *P. solanacearum* race 1 (also a pathogen of potatoes) is found in the warmer regions of the world where it infects a number of susceptible solanaceous crops and weeds, which often are present during the extended growing season. In these tropical and sub-tropical regions, debris is rapidly decomposed by microorganisms in the warm moist soils, and therefore would provide only temporary sheltered sites for the bacterium. Thus, races 1 and 3, although both are pathogens of potato, clearly have different natural host ranges and distribution patterns, and control measures for one may be inappropriate for the other.

In summary, the wide distribution of *P. solanacearum* race 3 in cool temperate regions of the world is probably due to close association with the potato plant, (including debris and latently infected tubers) and the undesirable cultural practices attending the growing of potatoes, such as the transport of latently infected seed tubers over long distances.

In the cool soil conditions that occur on the Northern Tablelands of N.S.W., survival between growing seasons is dependent on the presence in soil of pieces of infested host debris. In addition, the bacterium may survive in buried volunteer tubers from the previous crop, and under appropriate environmental conditions, could provide focal points for secondary spread of the bacterium within the subsequent potato crop. Thus, any agricultural practice which tends to enhance breakdown or removal of infested debris and volunteer tubers, such as sowing to pasture or deep plowing, should result in accelerated destruction of sheltered sites by exposing the bacterium to increased competition from indigenous soil microorganisms.

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