Biological Control of Bacterial Wilt of Potatoes: Attempts to Induce Resistance by Treating Tubers with Bacteria

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ABSTRACT


Induced resistance and microbial antagonism were investigated as possible methods for control of bacterial wilt of potato caused by *Pseudomonas solanacearum*. Tuber seed pieces (containing buds) of the tolerant cultivar Ontario were treated by dipping them in suspensions of different bacteria or by introducing the bacteria directly into the bases of the emerging buds. The bacteria tested included avirulent (B82) and incompatible (70) strains of *P. solanacearum*, proteolytic (W163) and pectolytic (WP95) strains of *P. fluorescens*, and strains of *P. syringae pv. glycinea* (S-9-4) and *pv. lachrymans* (P1W214-6). Plants grown from treated tubers were challenged when 20–30 cm tall by stem or root inoculation with the highly virulent strain 276 of *P. solanacearum*. Treatment with the *P. solanacearum* and *P. fluorescens* strains caused a significant reduction in disease severity, particularly when plants were challenged by stem inoculation at relatively low inoculum levels and/or disease progress was slow in the control plants. When plants were challenged by root inoculation, there was a significant decrease in disease severity in treated plants when the roots were left intact but not when roots were injured. There was no correlation between effectiveness of tuber treatment with different bacterial strains and their ability to inhibit growth of *P. solanacearum* in vitro.

Additional key words: acquired resistance, potato brown rot, *Solanium tuberosum*

The wilt disease of many solanaceous crop plants caused by the soilborne bacterial plant pathogen *Pseudomonas solanacearum* has been controlled primarily by development of disease-resistant cultivars (18). It has been difficult, however, to find resistance to *P. solanacearum* in *Solanum tuberosum* L. Nearly 9,000 potato clones were tested in the greenhouse from 1947 to 1960 but only moderate resistance was found in a few clones (15). Of 22 *S. tuberosum* cultivars evaluated under field conditions in Georgia, Ontario was the only cultivar with relatively high resistance to bacterial wilt (5). Resistance in certain clones of *S. phureja* Juz. and Buk., a cultivated diploid potato, was reported by Thurston and Lozano (19) and introduced into *S. tuberosum* by Sequeira and Rowe (17). The genetics of resistance is complex and no hybrid clones have resistance to more than a few strains of the pathogen. Thus, breeding with *S. phureja* as a source of resistance is long-term, expensive, and difficult (18). In addition, resistance is not expressed under certain environmental conditions. For instance, resistance breaks down under high (above 30 C) ambient temperatures (17).

To resolve some of these difficulties, use of resistant varieties might potentially be coupled with biological control. The control of soilborne plant pathogens by biological methods usually involves reducing disease-producing activity of the pathogen by introducing an antagonist (1). Application of antagonistic bacteria to root or seed surfaces as a control mechanism is sometimes ineffective because the introduced antagonist is in turn subject to inhibition by adverse environmental conditions including the presence of other microorganisms. This problem might be reduced if the antagonist is placed in, rather than on, the plant. Biological control of *P. solanacearum* therefore might be effected by introducing an antagonist directly into the vascular system of tubers, provided the plant remains healthy.

High numbers of microorganisms within plant tissue, rather than directly affecting the pathogen, might influence the host's response to the pathogen. There are numerous reports of induced resistance brought about by prior inoculation of the host by a pathogen, avirulent or incompatible forms of a pathogen, heat-killed pathogens, etc. (3,11,12,16). These various agents presumably induce a physiological response by the host that confers resistance against subsequent inoculation by the virulent pathogen.

The primary objective of this research, therefore, was to explore the possibility of increasing the level of resistance in potato to bacterial wilt by means of antagonistic bacterial strains and/or strains capable of inducing disease resistance. The plan was to determine whether inoculation of tubers with avirulent variants of *P. solanacearum*, pathogenic strains of *P. solanacearum* not compatible with potato, and saprophytic cephalosporin pseudomonad species could reduce wilt caused by a highly virulent strain of *P. solanacearum*.

MATERIALS AND METHODS

Host plants. Tubers of the potato cultivars Ontario and Russet Burbank were obtained from Dr. S. Slack, Department of Plant Pathology, University of Wisconsin, Madison (U.W.-Madison). They were stored at 4 C until 10–20 days before use, when they were placed at room temperature to stimulate germination. The cultivar Ontario was chosen because it showed some resistance to bacterial wilt in the field in Georgia (5).

Bacterial strains. All strains of *P. solanacearum* were obtained from the culture collection at the Department of Plant Pathology, U.W.-Madison. Strain 82 was from Colombia and strain 276 was from Mexico; both were isolated from potato. Highly virulent strain 276 was chosen for the challenge inoculation of potato plants. Strain 70 was from Colombia and was isolated from plantain; it is incompatible with potato. The strains were grown on TZC agar (7) at 28 C and maintained by transferring every 2–4 days.

Avirulent forms (designated with the letter B in front of the parental strain number) were obtained by growing virulent strains in still culture in test tubes (15 x 1.8 cm) containing 10 ml CPG broth (1 g caseinum acids, 10 g peptone, 5 g glucose per liter) for 2–3 wk at 28 C (4). A loopful from the surface of the broth was transferred to TZC agar and after 48 hr at 28 C, individual small red butyrous colonies typical of the avirulent mutants were selected.

Fluorescent pseudomonad strains (W163 and WP95) were obtained from J. S. Wang (U.S.-Madison), who had isolated them from soil in 1980. Strain W163 belongs to biotype G of *P. fluorescens* and is nonpectolytic, proteolytic, and lipase-positive. Strain WP95 belongs to biotype A of *P. fluorescens* and is pectolytic (J. S. Wang, personal communication). These were maintained either on King's B (8) or TZC medium at

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room temperature.

*P. syringae* pv. *lachrymans* (*P. s. lachrymans*) strain PHW214-6 (214) was isolated from cucumber by Dr. P. H. Williams (U.W.-Madison) and *P. syringae* pv. *glycinea* (*P. s. glycinea*) strain S-9-4 race 4 was isolated from soybean in 1975 by W. Fett (U.W.-

Bacterial inoculum preparation. Bacterial strains were transferred to CPG agar and incubated for 48 hr at either 28 C (*P. solanacearum*) or room temperature (all other strains). For stem inoculations, inoculum was prepared by transferring a loopful of bacteria from selected colonies on the plates to tubes containing sterile 0.01 M phosphate buffer, pH 7.2. The bacterial concentration was adjusted by diluting with buffer to the appropriate absorption at 600 nm. For root inoculation, suspensions were made in sterile buffer contained in 1- or 2-L autoclavable plastic jars. For root inoculation, suspensions were made in the same way, except bacteria were transferred from the plates to sterile distilled water. Serial dilutions were made for each inoculum suspension and plated onto TZC agar to determine actual colony-forming units per milliliter (cfu/ml).

Tuber treatments. For each treatment with a bacterial strain, 20–30 tuber pieces containing developing buds (*"eyepieces"*) were cut out from washed tubers with a melon-ball scoop. In most experiments, the cut eyepieces were placed immediately in a bacterial suspension of known concentration for 3–6 hr. Control eyepieces were placed in sterilized buffer. In an alternative treatment, the emerging bud of each eye was inoculated by placing a 20-μL drop of bacterial suspension at the base of the bud and then puncturing the drop with a sterile dissecting needle. All treated eye pieces were placed in a dew chamber at 20 C overnight to allow suberization before they were planted in soil in the greenhouse.

Plant growth conditions. Eye pieces were planted one to a pot in 10-cm clay pots containing 1:1:1 peat-soil-sand mixture that had been steamed for 1 hr. Plants were grown in an air-conditioned greenhouse at 20–24 C under a 14-hr photoperiod provided by cool-white and Gro-Lux fluorescent tubes. After the challenge inoculation, the plants were placed at 26–30 C under a 14-hr photoperiod provided by cool-white fluorescent tubes.

Challenge inoculation. Three to four weeks after planting, plants of similar size (20–30 cm tall) were challenged with strain 276 either by stem inoculation (drop or micropipette methods) or by root inoculation. For stem inoculation, 1) the bud was removed from the axil of the third fully expanded leaf from the top, a 20-μL drop of the inoculum was placed at the axil, and a dissecting needle was thrust through the drop and into the stem (6), or 2) a micropipette containing 20 μL of the inoculum was inserted diagonally into the stem at the leaf axil (K.-Y Lum, unpublished). Before insertion of the micropipette, the stem at the leaf axil site was punctured with a wire of diameter slightly greater than that of the micropipette. The micropipette was removed 1 day later. For root inoculation, 100 ml of bacterial suspension (10^4 cfu/ml) was poured onto the soil around the plant and the roots were either left undisturbed or wounded by inserting a knife into the soil near the eye piece.

Disease ratings. Disease ratings for individual plants were recorded at 1- to 5-day intervals up to 21-48 days after the challenge inoculation. The fusing scale was used: 0 = no symptoms, 1 = up to 25% of the foliage wilted, 2 = 25–50% of the foliage wilted, 3 = 50–75% of the foliage wilted, and 4 = 75–100% of the foliage wilted. Plants with ratings of 3 or 4 after 21 days always died, whereas those with ratings of only 1 or 2 sometimes recovered.

Means, standard errors, analysis of variance, and least significant difference tests were calculated with a computer programed with the Statistical Package for the Social Sciences, which takes into account unequal sample size (14).

Test for bacterial antagonism. Strains of *Pseudomonas* used in tuber inoculations were tested for their ability to inhibit growth of strain 276 in vitro. The growth from a 2-day-old culture of strain 276 on CPG agar was suspended in water and added to 300 ml of cooled melted CPG agar. This agar suspension then was dispensed into petri plates (15 ml/plate) and allowed to solidify. Sterile toothpicks were used to transfer the test strains from 2-day-old cultures onto the surface of the solidified bacterial lawn. Two plates were used for each strain tested and three test spots were placed on each plate. The plates were incubated at 28 C and observed for inhibition zones after 2 days.

Test for tissue necrosis. The bacterial strains used as potential inducers of disease resistance were tested for their ability to induce localized necrosis in leaves of potato plants (cultivar Ontario). Strains containing 10^1–10^7 cfu/ml in buffer were infiltrated into the intercellular spaces of fully expanded potato leaves with a hypodermic syringe fitted with a 30-gauge needle according to the method of Klement (9). The inoculated plants were placed at 28 C and observed for tissue necrosis 2 days later or for wilt symptoms up to 21 days after infiltration.

RESULTS

Inhibition of bacterial growth in vitro. The bacterial strains chosen for tuber treatments were tested for their ability to inhibit the growth of strain 276 in vitro. Strain 70 of *P. solanacearum* produced diffusible substances that inhibited the growth of strain 276, but strain B82 and the strains of *P. s. lachrymans* and *P. s. glycinea* did not. The two *P. fluorescens* strains (W163 and WP95) were inhibitory to strain 276. Strain 276, on the other hand, did not inhibit the growth of any of the other strains used.

Infiltration of potato leaves. When infiltrated into potato leaves, the strains of *P. s. lachrymans* and *P. s. glycinea*, strain B82 of *P. solanacearum*, and strains W163 and WP95 of *P. fluorescens* caused localized necrosis of the infiltrated tissue within 2 days. This rapid necrosis was interpreted as a hypersensitive response. Strains 82 and 276 gave a compatible response characterized by slow, spreading necrosis followed by wilting of the plant. Strain 70 of *P. solanacearum* caused wilting of the infiltrated leaf only.

Tuber treatments. In the first experiment, Ontario eye pieces were treated by dipping them in a suspension of strains 70 or B82 of *P. solanacearum* or of strain W163 of *P. fluorescens*. The mean inoculum concentration was 1 × 10^7 cfu/ml (range 5.1 × 10^6 to 2.1 × 10^7 cfu/ml). The plants grown from these eye pieces (six to nine in each treatment) were challenged when they were about 33 cm tall (27 days after planting) by stem inoculation (drop method) with 1 × 10^6 cfu/ml of strain 276. Immediately thereafter, the plants were transferred to the 28 C greenhouse. Because temperature controls were not working correctly, temperatures in the greenhouse dipped as much as 10 degrees below 28 C at night. As a result, disease progress in the control plants was slow and disease severity (mean disease rating) was low until 37 days after inoculation (Fig. 1A). Even before 37 days, however, the disease severity in the treated plants was significantly lower (*P = 0.1*) than that in the control plants.

The experiment was repeated under conditions more favorable for disease progress. The plants were challenge-inoculated by the micropipette method (1.9 × 10^7 cfu of strain 276) when they were slightly younger (about 27 cm tall) and therefore more susceptible than in the previous experiment. There were 15 plants per treatment. The plants were maintained at 28 ± 2 C and a 14-hr photoperiod. Disease progress was more rapid than in the previous experiment, with control plants reaching a mean disease rating of 2.7 at 25 days after challenge (Fig. 1B). Although disease severity in plants treated with strain 70 was lower than in control plants, the difference was not significant at *P = 0.1*. Treatment with strains W163 or B82 had no effect on disease severity in this experiment.

To determine whether other bacterial
strains might be effective in inducing resistance, eye pieces were tested with 2.7 \times 10^5 \text{ cfu/ml} of virulent strains of \textit{P. s. glycinea} and \textit{P. s. lachrymans} and strain WP95 of \textit{P. fluorescens}. Plants were challenge-inoculated with 4.1 \times 10^4 \text{ cfu/ml} by the micropipette method and kept at 28 C. Treatment with the bean or cucumber pathogens did not affect disease severity, but treatment with strain WP95 resulted in a significant decrease (P = 0.1) in disease severity after 2 wk (Fig. 1C). The latter was the only treatment that reduced disease severity.

To determine whether the concentration of bacteria in the challenge inoculum affects the degree of resistance, plants grown from eye pieces treated with 7.2 \times 10^4 \text{ cfu/ml} of strains W163 or B82 were inoculated with 2 \times 10^4, 6 \times 10^4, or 6 \times 10^5 \text{ cfu/ml} of strain 276 by the micropipette method. There were 12 plants per treatment and their average height was 25 cm. Results indicated that the lower the challenge inoculum concentration, the greater the difference in disease severity between treated and control plants. Disease severity was always lower in the treated plants (Fig. 1D, E) but was statistically significant (P = 0.1) from the control only for plants inoculated with 6 \times 10^5 \text{ cfu/ml}.

In another experiment, eye pieces were treated by thrusting a dissecting needle into the base of the developing bud through a drop of 6.5 \times 10^5 \text{ cfu/ml} of either strains W163 or B82. There were 11-14 plants in each treatment. The plants were challenged 25 days later, when their average height was 27 cm, by stem inoculation (drop method) with 1 \times 10^5 \text{ cfu/ml} of strain 276. Both treatments reduced disease severity significantly (P = 0.1), compared with controls, by 9 days after challenge (Fig. 1F).

In all experiments described, the plants were challenged by stem inoculation. To determine whether challenge by root inoculation might be less severe, a 100-ml suspension of 10^8 \text{ cfu/ml} of strain 276 was poured onto the soil at the base of each plant grown from eye pieces treated with a suspension of 1.1 \times 10^5 \text{ cfu/ml} of strains 70, W163, or B82. There were 20 plants per treatment. Disease progress was less rapid than in stem-inoculated plants and only 40% of the control plants had a disease rating of 3 or 4 by 5 wk after inoculation of intact roots (Fig. 2). By 26 days after challenge, however, disease severity in the treated plants was significantly lower (P = 0.1) than that in the controls. When the roots were

Fig. 1. Effect of treating potato eye pieces (cultivar Ontario) with different bacterial strains on disease severity in plants challenged by stem inoculation with the virulent strain 276 of \textit{Pseudomonas solanacearum}: (A) Eye pieces dipped in 1 \times 10^5 \text{ cfu/ml} of strains 70 (incompatible) and B82 (avirulent) of \textit{P. solanacearum} and strain W163 of \textit{P. fluorescens}. Plants challenged with 1 \times 10^5 \text{ cfu/ml} of strain 276. (B) Eye pieces dipped in 1 \times 10^5 \text{ cfu/ml} of strains 70, B82, and W163. Plants challenged with 1.9 \times 10^5 \text{ cfu/ml} of strain 276. (C) Eye pieces dipped in 2.7 \times 10^5 \text{ cfu/ml} of \textit{P. syringae pv. glycinea}, \textit{P. s. pv. lachrymans}, and \textit{P. fluorescens} (WP95). Plants challenged with 4.1 \times 10^4 \text{ cfu/ml} of strain 276. (D) Eye pieces dipped in 7.2 \times 10^5 \text{ cfu/ml} of strain W163. Plants challenged with 6 \times 10^5, 6 \times 10^4, and 2 \times 10^5 \text{ cfu/ml} of strain 276. (E) Eye pieces dipped in 7.2 \times 10^5 \text{ cfu/ml} of strain B82. Plants challenged as in (D). (F) Emerging buds treated with 6.5 \times 10^5 \text{ cfu/ml} of strains B82 and W163. Plants challenged with 1 \times 10^5 \text{ cfu/ml} of strain 276.
**P. fluorescens** is pectolytic and will rot tuber slices in vitro but there was no obvious effect on the treated eyepieces. Both of these strains, therefore, may have caused more injury than the other strains, eliciting a greater response by the plant and leading to a higher level of induced resistance. This is consistent with the finding that the magnitude of resistance in cucumber induced by **P. pisi**, *P. s. phaseolicola*, or *P. s. angulara*, which cause localized necrosis, was significantly lower than that elicited by *P. s. lachrymans*, which causes a spreading necrosis (2). In tobacco plants hypersensitive to TMV, the degree of systemic protection against *Phytophthora parasitica* var. *nicotianae* by prior inoculation with the virus was directly related to the average number of TMV lesions per leaf (13).

Reduction in disease severity also occurred after direct inoculation of strains W163 and B82 into the bud even though disease progress in the controls was rapid (Fig. 1F). These strains do not cause observable damage to the tuber but cause localized necrosis when infiltrated into potato leaves. The presence of these bacteria in the vascular system of the stem, while not affecting plant growth, may have elicited a high level of induced resistance.

When plants were challenged by root inoculation, there was a significant decrease in disease severity in treated plants when the roots were left intact (Fig. 2) but not when they were wounded, even though the rate of disease progress and the disease severity in the controls were similar in both cases. It is possible that wounding the roots allowed so many bacteria to enter the vascular system that any induced resistance in the stem was overwhelmed.

In experiments where treatments reduce disease severity, strain B82, which does not inhibit growth of strain 276 in culture, was as effective as strains W163 or W163, which do, suggesting that production of an antibacterial agent probably is not requisite for a protective response. This response, therefore, may be the result of induced systemic resistance.

Although the application of fluorescent pseudomonads antagonistic to *Erwinia carotovora* to potato seed pieces has been found to enhance growth and yield of potato plants in the field, the principal effect on the pathogen is via antagonism rather than via induced resistance (10).

Our results indicate that even under conditions highly favorable to disease progress (high challenge concentrations, direct introduction of the challenge inoculum into the vascular system, and high temperatures), treatment of potato tubers with avirulent, incompatible, or antagonistic bacteria may result in a significant reduction in severity of bacterial wilt.

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**LITERATURE CITED**


