Control of Leaf Spot Diseases of Alfalfa and Tomato with Applications of the Bacterial Predator *Pseudomonas* Strain 679-2

L. E. CASIDA, JR., Professor, Department of Molecular and Cell Biology, and F. L. LUKEZIC, Professor, Department of Plant Pathology, The Pennsylvania State University, University Park 16802

ABSTRACT

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Pseudomonas strain 679-2 is a unique, highly competitive, nonobligate bacterial predator originally isolated from soil. Application of its cells to tomato and alfalfa provided a reduction in the severity of the leaf spot diseases caused by *Alternaria solani, Pseudopeziza medicaginis, Phoma medicaginis*, and *Stemphylium botryosum*. The treatment produced no adverse effects on the plants. Strain 679-2 produces a water-soluble compound that inhibited these fungi, but the role of the compound in the interaction was not determined. Application of a copper fungicide to the leaves with the bacteria did not interfere with activity of strain 679-2. Although strain 679-2 survived well on the leaves and in soil, it could be programmed by culture techniques for early mortality in order to reduce unwanted environmental exposure.

Soil contains several kinds of nonobligate bacterial predators (3–5) that attack other bacteria in soil. They do so, however, only if they are unable to obtain enough soluble nutrients from their environment (2,3). Although they can attack various prey species, they also can attack each other, which creates a hierarchy of bacterial predation in soil (4,5). At the top of this hierarchy is a unique *Pseudomonas* species, strain 679-2, ATCC 55089 (5). Although it is resistant to copper (0.01% $CuCl_2 \cdot 2H_2O$ added to the medium), 679-2 requires a small amount of copper to initiate growth. It obtains this (3,5,8) by synthesizing small amounts of growth initiation factor (Cu-GIF) to scavenge copper from the environment. Certain other nonobligate bacterial predators, such as *Cupriavidus nector* Makkar and Casida (2,3,8), also produce Cu-GIF.

Strain 679-2 destroys many species of bacteria and fungi, including many other prey bacteria, by producing a toxic compound (inhibitor) (5). The predation capability of strain 679-2 is such that the strain is highly competitive and will rapidly colonize soil (5). However, after maximum growth has occurred, it is able to maintain its numbers in the soil for prolonged periods of time (5). Strain 679-2 is also able to survive on leaf surfaces in the field (5). This combination of characteristics points to the potential of *Pseudomonas* strain 679-2 as a biocontrol agent for plant pathogens. This potential increased when initial trials showed no evidence of direct adverse effects of the bacterium on plants.

This study reports the results of field and greenhouse trials using *Pseudomonas* strain 679-2 cultures for biological control of selected leaf spot diseases of alfalfa and tomato.

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MATERIALS AND METHODS

Variant production. A stable variant of strain 679-2 is produced in the wild-type cultures (5). The variant, readily detectable because of colonial morphology, does not make the inhibitor but is resistant to it. Unless otherwise stated, the trials reported here use only the wild type of strain 679-2. Stock cultures for both the wild type and the variant were maintained on 0.1-strength heart infusion 1.5% agar (Difco Laboratories, Detroit, MI).

Cell suspensions for plant application. The cells of 679-2 were grown either in a synthetic medium broth or on heart infusion agar slants for application to plants. For the broth growth, strain 679-2 was first grown for 20 hr at 28 C on six slants of 0.1-strength heart infusion 1.5% agar. The cells from each slant were suspended in 4-ml aliquots of sterile distilled water, which were combined in a sterile flask. A portion (1 ml) of this suspension was used to inoculate each growth flask. Each 500-ml Erlenmeyer growth flask contained 100 ml of L-glutamic acid synthetic broth medium, composed of 0.1% L-glutamic acid, 0.1% NH_4NO_3, 0.1% KH_2PO_4, 0.02% Na_2SO_4, and 0.02% NaCl, adjusted to pH 7.2 with KOH. These flasks were incubated for 2 days on a reciprocal shaker (68 12-cm strokes per minute) at 28 C. At the end of this time, the cells had just reached the maximum stationary growth phase (1.1×10^7 colony-forming units [cfu] per milliliter), and autolysis had not yet started. The broth culture was sprayed as a mist on the plants, so that microdroplets became visible on the leaf surfaces. An in vitro assay of a cell-free filtrate made from this culture showed no inhibition or other activity against the inhibitor-sensitive test organisms *Micrococcus luteus* Schroeter and *Aspergillus niger* Tiegh. Paper chromatography of the filtrate usually showed no residual glutamic acid and only trace amounts of the Cu-GIF.

Inoculum was prepared from cells grown on agar slants as follows. Six full-strength heart infusion agar slants were inoculated with strain 679-2 cells and incubated at 28 C. The incubation times were 2, 4, and 8 days, respectively, for cells to be tested against *Alternaria solani* Sorauer, *Phoma medicaginis* Malbr. & Roum. in Roum., and *Stemphylium botryosum* Wallr. The cells on each slant were then suspended by loop into 4 ml of sterile distilled water. The cell suspensions for each incubation time were combined and centrifuged. The cell pellet then was washed once by centrifugation in sterile distilled water and resuspended in 100 ml of sterile distilled water. These suspensions contained approximately 1.0×10^8 cfu/ml. These suspensions were sprayed to runoff onto the leaves of green snap bean, tomato, and alfalfa plants grown under greenhouse conditions. In addition, they were infused into tobacco and tomato leaves and streaked onto the surfaces of freshly cut potato tuber disks to determine possible phytopathogenicity or toxicity.

Production of inhibitor compound. The inhibitor compound was extracted after growth in either synthetic medium broth or heart infusion broth. In the synthetic medium broth, strain 679-2 was first grown for 24 hr on a shaker at 28 C in 100 ml of full-strength heart infusion broth. The cells were then separated from the broth and washed once by centrifugation in sterile distilled water. They were then resuspended in 100 ml of the L-glutamic acid synthetic broth in a 500-ml Erlenmeyer flask. The culture was shaken for 27 hr at 28 C, then frozen and thawed. The cells were removed by centrifugation, followed by passage of the supernatant through a 0.3-μm-pore membrane filter. Assay against *M. luteus* showed that this filtrate contained 3.5 units per milliliter of the inhibitor activity.

Higher yields of inhibitor could be obtained by inoculation of strain 679-2 cells into 50 ml of full-strength heart infusion broth in a 500-ml Erlenmeyer flask. This flask was shaken for 64 hr at 28 C. It was then frozen and thawed, followed by centrifugation and filtration as above. The filtrate contained 41 units per milliliter of the inhibitor activity, which was based on the *M. luteus* assay.

Inhibitor assay. Plates of 0.1-strength heart infusion agar were poured and allowed to dry at room temperature overnight. *M. luteus* was then streaked across the agar surface in various directions until the surface became covered. If needed, *A. niger* spores could be applied in a similar manner on separate plates. A sterile paper assay disk, 13 mm in diameter (Schleicher & Schuell, Inc., Keene, NH), was dipped in a sterile culture filtrate containing the inhibitor compound (or dilutions thereof) and drained against the edge of the vessel. It was then placed in the center of the plate, and the plate was incubated at 28 C. The diameter of the zone of inhibition, including the diameter of the disk, was measured after 2 days. For preparation of a standard curve, the *M. luteus* inhibition zone diameters corresponding to the various dilutions were plotted against the logs of units of activity. To obtain units of activity, a dilution that gave a 17-mm inhibition was arbitrarily designated as containing an activity of one unit per milliliter.

Partial purification of the inhibitor was obtained by ascending paper chromatography of strain 679-2 culture filtrates or concentrated preparations containing the inhibitor. This was performed with a solvent system composed of ethanol, NH_4OH, and water (80:5:15 v/v). After ascent of the solvent, the sheets were air-dried overnight, then observed by short wavelength UV illumination (Mineralight models UVS11 or SL2537, UVP, San Gabriel, CA.). A lengthwise portion taken from the chromatogram was dipped in a solution of ninhydrin in acetone (straw-colored). After the acetone had evaporated, the sheet was subjected momentarily to flowing steam, then heated for 5 min at 105 C in an oven. Glutamic acid appeared at R_f 0.43. The Cu-GIF (2,3) (R_f 0.14) reacted (became purple) with the ninhydrin, but the inhibitor compound did not. The position of the inhibitor (R_f 0.84) on the chromatogram was found by cutting lengths of approximately 1.5 cm from a lengthwise strip taken from the chromatogram. Each piece was placed on an *M. luteus* plate as described above. After incubation, the plates were examined for possible zones of inhibition, indicating the presence of active material that had diffused from the pieces.

Solutions of the inhibitor were evaluated in the absence of the strain 679-2 cells. Preparations containing 3.5 or 41 units per milliliter were sprayed to runoff on the leaves of green snap bean, tomato (cv. Rutgers), and alfalfa (cv. Sarnac) plants grown under greenhouse conditions. In addition, the 3.5 units per milliliter preparation was infused into 2-wk-old tobacco (cv. Samsun) and 2-wk-old tomato (cv. Rutgers) leaf tissue.

Enumeration of strain 679-2 cells. Soil dilutions were made in 10-fold increments in tubes containing 9 ml of sterile distilled water. The initial 1-g soil sample was blended for 1 min in 100 ml of distilled water in a sterile Waring blender. Tubes for other dilutions were mixed with a vortex Genie mixer (American Hospital Supply Corporation, Evanston, IL) before 1 ml was removed for preparing the next dilution of the sequence. The dilutions were plated on "copper" agar (0.1-strength heart infusion 1.5% agar containing 0.01% $CuCl_2 \cdot 2H_2O$ [pH 6.5]). Strain 679-2 colonies were easily recognized on this medium. They were beehive-shaped, 1.5- to 2-mm wide and 1.0- to 1.5-mm high, opaque, off-white, and shiny, with entire margins.

Counts of strain 679-2 cells on leaf surfaces were obtained by blending 1 g of leaf tissue in 100 ml of sterile distilled water in a sterile Waring blender for 1 min. Serial dilutions were then plated on "copper" agar.

Trifoliolate plates. Sterile sand (about 1 cm deep) in a 226.8-g (8-oz) clear, capped deli bowl (Microlite DO8Cl bowl and L409Cl lid, Amoco Foam Products, Atlanta, GA) was dampened with sterile 0.5-strength Hoagland's solution (1) supplemented with 10 ml per liter of 0.01% kinetin (Sigma Chemical Co., St. Louis, MO). The petioles of three trifoliolate leaflets of alfalfa were placed into the sand in each plate (six plates per treatment). Strain 679-2 cells (5.5×10^8 cfu/ml), either wild-type or variant, were sprayed on the leaflets until just damp. The lids were then removed for 10 min so that the leaflet surfaces could dry. Afterward, some plates received an additional spraying with *P. medicaginis* spores (1.4×10^6 spores per milliliter) or *S. botryosum* spores (1.4×10^6 spores per milliliter) suspended in 0.01 M phosphate buffer, pH 7.0. The leaflets were allowed to dry again if copper fungicide (Champion WP, 77% $Cu(OH)_2$, equal to 50% metallic copper) (0.05% active ingredient in 0.01 M phosphate buffer, pH 7.0) was to be sprayed on them. The covered plates were incubated for 1 wk at 22 C, in a 16-hr photoperiod, in a controlled-environment chamber (Model RI 23-555 A, Revco Scientific Inc., Asheville, NC). Light intensity was at 0.474×10^3 $\mu W/cm^2$ at the leaf level and 0.357×10^3 $\mu W/cm^2$ at leaf level measured through the dish cover. Light was provided by 20W cool-white fluorescent lights.

Severity of infection. The percent area of leaf surface infected was estimated by using the keys of James (6). Results were analyzed by the paired *t* test, with uninoculated leaves as controls.

Laboratory experiments (disease interaction). Trifoliolate plates were used to determine if the strain 679-2 wild-type variant cells were effective in reducing the incidence of *P. medicaginis* (alfalfa spring leaf spot). Strain 679-2 wild-type variant cells were prepared separately as broth cultures. These were sprayed on trifoliolate plates that, in selected cases, were also sprayed with *P. medicaginis* spores. Other plates received only the *P. medicaginis* spores.

Field experiments (disease interaction). The effect of strain 679-2 on the incidence of early blight disease of tomatoes (caused by *A. solani*) was studied, using field-grown plants where disease was endemic. The plants, the cultivar Merit, were grown according to standard horticulture recommendations for commercially produced green-pack tomatoes. Duplicate trials were conducted in separate fields of tomato plants. The plots consisted of three well-separated sets of 12 plants in each field that were sprayed with strain 679-2 cells grown in synthetic broth medium. In the same row as the treated plants, three separate sets of 12 plants sprayed with H_2O were used an controls. About 3.7×10^9 cfu were sprayed toward each plant, but a portion of these cells drifted onto the ground. Each plant received three of these sprayings, with intervals of 4 and 3 days, respectively, between the sprayings.

Strain 679-2 grown in synthetic medium broth culture was evaluated on an alfalfa field where common leaf spot (*P. medicaginis*) was endemic. The plants (cv. Centurion) were grown following the standard agronomic recommendations for fertilizer and pesticide applications. A total of 12 well-separated, 1-m^2 plots were randomly marked off. Six of these were retained, sprayed with the culture medium, to serve as controls. The rest were sprayed with the culture. Each plot received 333 ml sprayed onto the leaves, with some of the culture deposited on the soil. Two additional applications were made at 5 and 7 days after the initial applications. The evaluations of percent incidence of disease and survival of the strain 679-2 cells were made 12 days after the last application.

The effect of applying strain 679-2 as a preplant treatment and as a postplant soil treatment was investigated with tomato transplants and established alfalfa plants, respectively. A Fumigun model 470-2A (Neil A. Maclean Co., Belmont, CA) was used to inject 2.0×10^8 cfu at a depth of 7.5 cm (with a 15-cm diameter of soil exposed) into soil prepared to receive tomato transplants 2 days before planting. The alfalfa was an established stand in its second year. A similar amount of bacterial cells was mixed with potting soil at a 7.5-cm depth 1 day be-

fore tomato plants were transplanted into it. These plants were grown under greenhouse conditions.

Field survival of strain 679-2. Samples were periodically collected from the treated field-grown tomato plants. The samples consisted of an aseptically removed third leaf from the stem apex. The collected leaf was placed in a sterile plastic bag and handled as above within .5–1 hr after removal. The effect of leaf canopy was determined by a separate trial, using plants that were sprayed only once with strain 679-2 synthetic medium broth culture. They grew closer together (approximately 8 cm apart in each direction). The plants were maintained on a cart outside of a greenhouse and received daily watering of the leaves and soil.

Preliminary laboratory experiments had shown that strain 679-2 cells grown on full-strength heart infusion agar were unaffected by incubation (storage) at either 4 or 28 C for periods of 9–25 days. Thereafter, they suddenly lost their copper resistance. A few days later (when they were about 13–39 days old), the cells died. The loss of copper resistance meant that the cells could no longer maintain their dominance in nature (2). Cells grown on a more dilute medium (e.g, 0.1-strength heart infusion broth or agar) do not demonstrate this "programmed" death. To determine if this factor affected survival, a field trial with well-separated tomato plants was conducted, but the strain 679-2 cells that were to be used were grown in a different manner. Each of three tomato plants with endemic early blight was sprayed with 33 ml of an aqueous cell suspension prepared by suspending strain 679-2 growth from the surfaces of full strength heart infusion agar slants (see above). A total of 3.3 $\times 10^9$ cfu was sprayed toward each plant, although some of the cells went onto the ground. The plants received only this spray.

RESULTS

Plant pathogenicity, toxicity, and hypersensitivity. There was no evidence of plant pathogenicity, toxicity, or hypersensitivity in any trial when synthetic medium broth or heart infusion broth cultures of strain 679-2 were evaluated. In addition, strain 679-2 did not rot potato tuber disks or elicit a hypersensitive response in tobacco. Finally, the injection of 2.0×10^8 cfu into soil having an established alfalfa stand and the preplant treatment of tomato transplants in the field had no discernible effect on the plants during their subsequent growth. The same was true when the cells were mixed with soil 1 day before young tomato plants were transplanted into it in a greenhouse experiment.

The inhibitor compound also did not show adverse effects on plants in the absence of the strain 679-2 cells. Preparations containing 3.5 or 41 units per milliliter were sprayed onto the leaves of bean, tomato, and alfalfa plants grown under greenhouse conditions. No phytotoxicity was observed up to 6 days after application. In addition, the 3.5 unit per milliliter preparation was not toxic when it was infused into tobacco and tomato leaf tissue. However, infusion of the 41 unit per milliliter preparation produced necrotic lesions on the leaves of both plants. Control plants infiltrated with sterile, full-strength heart infusion broth (the control) were symptom-free.

For the first tomato field plot (Table 1), the mean values for incidence of infection and standard deviations were, respectively, for treated and control plots, 7.9 ± 7.7 and 37.0 ± 23.7%. For the second plot, the respective values were 8.8 ± 7.4 and 28.7 ± 21.8%. The differences between treated and control plants were highly significant ($P = 0.01$). The soil under the control plants did not contain any strain 679-2 cells, either initially or later on. At the time of performing the disease incidence evaluations, the soil under the treated plants contained 3.2×10^5 of strain 679-2 cfu per gram of soil. A test of this soil 44 days later (i.e., well after normal tomato harvest time) showed 1.6×10^5 of strain 679-2 cfu per gram of soil. The following spring (in March) 1.6×10^3 cfu per gram of soil were still present. Survival of strain 679-2 cells on the field-grown leaves was much lower than in soil. At the time of evaluation of the severity of *A. solani* infection, no living cells of strain 679-2 were detected. However, on the plants grown to produce a dense canopy, the strain 679-2 cells survived well. Initially after the spraying, the cells were present at 5.4×10^4 cfu per gram of leaf tissue. This approximate number was found at sequential samplings. After 54 days, 9.2×10^3 cfu per gram of leaf were present.

The diseased plants receiving the cells of strain 679-2 grown on full-strength heart infusion agar slants did not show toxicity or any other alteration in the plants attributable to the bacteria. At 15 days after application, the mean and standard deviation for percent disease severity of treated and control plants was 27.7 ± 12.9 and 58 ± 2.8, respectively (Table 1). The differences were highly significant ($P = 0.01$). Strain 679-2 cells could not be recovered from the leaves at the time of the disease incidence evaluation, or from the fruits or soil.

The disease severity of common leaf spot of alfalfa was reduced by the application of strain 679-2. Values for treated and control plants were 3.4 ± 1.0 and 5.5 ± 1.3% diseased foliage, respectively, ($P = 0.01$) (Table 1). Strain 679-2 cells were not present initially in the soil, nor were they present on the leaves of the control plants or in their respective soils at the end of the experiment. At 12 days after the last spraying, the treated plants had 1.6×10^5 strain 679-2 cfu per gram of leaf tissue, and the soil beneath the plants had 1.6×10^6 cfu/g. At 45 days after the last spraying, the soil still contained 4.2×10^5 strain 679-2 cfu/g. The foliage had been removed 25 days earlier. Nevertheless, the alfalfa regrowth contained a few epiphytic strain 679-2 cells, but the colony numbers on the plates were too low for enumeration. In the spring, approximately 6 mo later, the soil still contained 5.8×10^4 strain 679-2 cfu per gram of soil. However, the bacterium was not present on the new foliage.

The variant cells of strain 679-2 did not protect the leaves against *P. medicaginis*. In contrast, a high level of protection of the leaves by the wild-type cells (Table 1) was recorded. The mean percent value and standard deviation for *P. medicaginis*–induced disease severity in the absence of strain 679-2 cells was 24.2 ± 13.7. When the wild-type cells had been added with the spores, the values were 10.0 and ±7.5% ($P = 0.01$). Evaluations determined that neither the variant nor the wild-type cells were phytotoxic to the leaves.

The broth cultures used in the preceding trials were not altered in any way. For example, the cells were not separated from the spent medium, and the pH was not changed. This was possible because culture filtrates (0.3-μm membrane filtration) prepared from wild-type and variant cultures grown in this way were not inhibitory in the *M. luteus* assay. The filtrates also did not inhibit *P. medicaginis* when they were tested as above on alfalfa trifoliolates.

A trial similar to those above was

Table 1. Inhibition of leaf spot diseases of alfalfa and tomato by *Pseudomonas* strain 679-2[a] in field plot experiments

Crop	Plot	Fungus controlled	Strain 679-2 preparation[a]	Percent leaf area infected[b]	
				Treated	Control[c]
Tomato	1	*Alternaria solani*	Broth-grown	7.9 ± 7.7	37.0 ± 23.7
Tomato	2	*A. solani*	Broth-grown	8.8 ± 7.4	28.7 ± 21.8
Tomato	...	*A. solani*	Agar-grown	27.7 ± 12.9	58.0 ± 2.8
Alfalfa	...	*Pseudopeziza medicaginis*	Broth-grown	3.4 ± 1.0	5.5 ± 1.3

[a] Broth-grown used synthetic medium broth; agar-grown used full-strength heart infusion agar slants.
[b] Values are mean ± SD.
[c] For control SD, all differences were highly significant ($P = 0.01$) by the paired *t* test.

Table 2. Inhibition of leaf spot diseases of alfalfa by *Pseudomonas* strain 679-2 in laboratory experiments

Fungus controlled	Strain 679-2 preparation[a]	Percent leaf area infected[b]	
		Treated	Control[c]
Phoma medicaginis	Broth-grown	10.0 ± 7.5	24.2 ± 13.7
P. medicaginis	Agar-grown	1.4 ± 2.2	38.8 ± 28.5
Stemphylium botryosum	Agar-grown	6.0 ± 14.0	60.0 ± 28.9

[a] Broth-grown used synthetic medium broth; agar-grown used full-strength heart infusion agar slants.
[b] Values are mean ± SD.
[c] For control SD, all differences were highly significant ($P = 0.01$) by the paired t test.

conducted, but the *P. medicaginis* preparation that was used contained both mycelium and spores. With this preparation, 49% of the leaf surface area of the trifoliolates became infected. Application of strain 679-2 synthetic medium broth culture decreased this value to 35%, copper fungicide instead of the culture decreased it to 30%, and the culture plus the fungicide decreased it to 14% (SD = ±12.5%). This was highly significant ($P = 0.01$).

***Phoma* and *Stemphylium*.** Most of the preceding trials used whole cultures of young strain 679-2 cells grown in synthetic medium broth. The inhibitor compound was not produced under these conditions. In other trials, such as with *A. solani* on tomatoes, strain 679-2 was grown on full-strength heart infusion agar, and then the cells were washed. This meant they were actively producing the inhibitor compound when they were ready for testing, although the inhibitor was not added for the trial. This type of preparation was also evaluated for inhibition of *P. medicaginis* and *S. botryosum* on alfalfa trifoliolates (Table 2). In the absence of strain 679-2 cells, *P. medicaginis* infected 38.8% of the leaf surface (SD = ±28.5%). In the presence of the strain 679-2 cells, *P. medicaginis* infection was only 1.4% (SD = ±2.2%) ($P = 0.01$). Control plants showed 60% of the leaf surface area infected by *S. botryosum* (SD = ±28.9%) (Table 2). In the presence of the strain 679-2 cells, *S. botryosum* infection was only 6% of the surface area (SD = ±14%) ($P = 0.01$).

The strain 679-2 cell preparations used above did not contain inhibitor compound. A cell preparation containing 48 units per milliliter of inhibitor compound and 1.0×10^{10} cells per milliliter was obtained by growing strain 679-2 for 48 hr (at 28 C) in shaken flasks containing full-strength heart infusion broth. This culture, including the inhibitor compound, was tested against *S. botryosum* on alfalfa trifoliolates. In the absence of strain 679-2, the incidence of infection was 50.8% (SD = ±24.0%). With the culture present, the incidence of infections was 2.4% (SD = ±4.7%). The difference was highly significant ($P = 0.01$). Note that the values obtained with inhibitor compound present were similar to the values (above) where inhibitor was absent and the cell numbers (1.0×10^8 cfu/ml) were lower.

DISCUSSION

Strain 679-2 produced no detectable phytotoxic effects when applied to either plants or soil. Likewise, the inhibitor compound produced in vitro also was not phytotoxic when sprayed onto foliage. However, when a preparation demonstrating high antibacterial and antifungal activity was perfused into the intercellular spaces of leaves, phytotoxicity was detected. Use of the inhibitor compound as an antifungal agent on plants in the absence of strain 679-2 cells was not tested. It is known that the inhibitor compound is quite water-soluble. However, no attempts were made to formulate preparations that would have reduced water solubility.

The cells of strain 679-2 reduced the severity of several leaf spot diseases of alfalfa and tomato. The strain 679-2 cells survived at a high number on the tomato leaves grown under conditions of high cover and frequent watering. These conditions would reduce desiccation of the leaf surface, which according to Leben (7) is important in bacterial survival in the phyllosphere. They also survived and multiplied for prolonged periods in the soil. Concerns about long-term survival can be answered by growing the bacteria in such a manner to ensure rapid population decline. For example, when grown in a synthetic broth medium, the cells survived well in nature. In contrast, when grown on slants of a nutritionally rich heart infusion agar, cells died after a few days. This rapid death occurred on leaves and in soil in the field as well. In the field, however, the cells lived long enough to be effective as a biocontrol agent. Both types of cell preparations reduced the incidence of infection of leaf spot.

Strain 679-2 cells attach to prey cell walls (5). It is assumed that they deliver a tiny but lethal dose of the inhibitor compound to the prey. This is difficult to prove, particularly in nature, because of the apparent small size of the effective lethal dose. Nevertheless, the variant strain of strain 679-2, which was resistant to the inhibitor compound but was unable to synthesize it in the laboratory, did not protect plants in the field. The wild type strain, which could synthesize this compound, did protect plants in the field. The wild-type cells, even when present in relatively low numbers on mycelium, apparently synthesized and delivered an inhibitor compound dose directly to the mycelium that was sufficient to kill it. Cell suspensions at either 1.0×10^8 or 1.0×10^{10} cfu/ml sprayed on alfalfa trifoliolates were equally effective against *S. botryosum* in the presence or absence of 48 units per milliliter of extracellular inhibitor compound.

Strain 679-2 is quite resistant to copper, and copper-containing fungicides are routinely used in production of various crops, especially commercial tomato crops (9). The results presented here demonstrated that, at least with *P. medicaginis* infections of alfalfa leaves, the fungicide and strain 679-2 cells could be used simultaneously. They did not interfere with each other (i.e., the respective fungal inhibitors obtained appeared to be additive). It may be that the biocontrol associated with strain 679-2 can be effectively combined with copper fungicides in an integrated program.

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