Identification of Bacteria for Biological Control of *Botrytis cinerea* on Petunia Using a Petal Disk Assay

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ABSTRACT

Gould, A. B., Kobayashi, D. Y., and Bergen, M. S. 1996. Identification of bacteria for biological control of *Botrytis cinerea* on petunia using a petal disk assay. Plant Dis. 80:1029-1033.

A total of 172 bacterial strains recovered from the surface of petunia leaves and flowers was evaluated for the ability to suppress Botrytis blight of petunia using a rapid, small-scale petal disk assay. Twenty-four strains were identified that consistently suppressed *Botrytis cinerea* sporulation by 100% on petal disks. Twenty-three of these strains were identified as fluorescent pseudomonads. One strain, *Pseudomonas fluorescens* PB92B10E, was tested for the suppression of Botrytis blight at the whole plant level. This bacterium reduced disease incidence by an average of 77% on whole flowers inoculated with *B. cinerea* conidia in seven different trials. Populations of a rifampicin-resistant mutant of PB92B10E increased by 10^4 g^{-1} of tissue (fresh weight) over a 7-day period on whole, nonsterile flowers. The isolation of *P. fluorescens* PB92B10E demonstrates the utility of the petal disk assay for the development of potential antagonists for management of greenhouse crop diseases.

Production of the common garden petunia (*Petunia* × *hybrida* Hort. Vilm.-Andr.) accounts for two-thirds of the value of all bedding plants sold in the United States (21). *Botrytis cinerea* Pers. is a common opportunistic plant pathogen on petunia and a number of other greenhouse crops; symptoms of this disease on petunia include blossom and leaf blight (7).

Botrytis spores are disseminated throughout greenhouses by air currents and splashing water. In addition, plant debris from flower and leaf tissues provides an excellent substrate for infection and growth of this fungus (15). Environmental conditions conducive to the development of Botrytis blight on herbaceous plants in the greenhouse include cool to moderate temperatures and high humidity (15). As a result, this disease is more prevalent in greenhouses with poor air circulation, improper plant spacing, and poor sanitation (16).

Strategies used to control Botrytis blight in the greenhouse include proper cultural practices combined with the use of fungicides. Unfortunately, strains of *B. cinerea* resistant to the benzimidazole and dicarboximide fungicides commonly used to control this disease have been reported (19). It is essential, therefore, that additional methods of control be identified to supplement or replace the use of fungicides on greenhouse crops. One such option is the use of antagonistic microorganisms for the biological control of Botrytis blight.

A number of laboratory and greenhouse studies have demonstrated that certain bacteria and fungi can suppress *B. cinerea* on a variety of plants (8,14,20,22). Mechanisms of antagonism include competition for nutrient substrates, preemptive colonization of plant surfaces, antibiosis, mycoparasitism, and fungistasis (5). Preliminary in vitro testing of potential microbial suppressants, however, is often cumbersome and labor intensive. We have developed a petal tissue assay for petunias that simplifies and speeds the screening of large numbers of bacterial strains for their ability to suppress *B. cinerea* in vitro.

MATERIALS AND METHODS

Isolation of *B. cinerea*. Between March 1992 and October 1993, 15 isolates of *B. cinerea* were obtained from common garden petunias by harvesting conidia with sterile forceps from diseased flowers or leaves and plating the spores on malt extract agar (MEA; Difco). Single-spore isolates were then maintained on MEA and stored at 4°C until needed. Four fungal isolates that sporulated vigorously on MEA were chosen for use in biological control assays. To maintain pathogenicity, conidia of certain isolates were inoculated onto surface-sterilized petunia leaf or petal tissue and then reisolated by harvesting freshly produced spores from diseased tissue.

Isolation of bacteria. Petunias used for the isolation of bacteria were obtained from several commercial nurseries in northern and central New Jersey. All plants were free of obvious symptoms of disease. Four to five grams of entire leaves or flowers from each of 103 plants were rinsed in distilled water, placed in 100 ml of 10 mM potassium phosphate buffer (pH 7), and shaken at 250 rpm on a rotary shaker for 1 h at room temperature (approximately 21°C). Samples were sonicated for 30 s and then shaken for one additional hour. Tenfold dilutions of the resulting suspensions were prepared immediately, and 100-μl aliquots of the dilutions were plated onto five plates of each of three media: nutrient yeast agar (NYA) (6), yeast dextrose calcium carbonate agar (YDC) (6), and King's B medium (KB) (6) agar. After 5 days, arbitrarily selected colonies were isolated and streaked on NYA to obtain pure cultures. All 1,529 strains were stored in 25% glycerol at –80°C until further use. Gram reaction and colony morphology were recorded for all bacteria used in petal disk and plate assays (see below).

Petal disk assays. Bacterial inocula were prepared by growing bacterial strains in 30 ml of Luria-Bertani (LB; Difco) broth for 48 h at room temperature. Bacterial suspensions were centrifuged at 3,000 × *g* for 5 min, the supernatant was decanted, and the pellet was resuspended in a 0.03% solution of Tween 20 in water to attain an optical density of 0.3 (at 595 nm), which approximates a concentration of 10^8 CFU of bacteria ml^{-1} (as determined by standard curve).

Fungal inoculum was prepared from *B. cinerea* grown on MEA for 5 days at approximately 21°C. Conidia were harvested from fungal colonies with sterile forceps and dispensed in 1 ml of sterile water in a microcentrifuge tube. The tube was vortexed for 30 s to disperse conidia, and the spores were counted using a hemacytometer. Spore concentration was then adjusted to 1,000 conidia ml^{-1}.

Ultra-white hybrid petunias (Goldsmith Seeds, Gilroy, CA) grown from seed in a greenhouse for 3 to 4 months were used as sources of petal disks. The plants received standard cultural care, and no fungicides were applied. For petal disk assays, the third, fourth, or fifth fully opened flowers from the floral apex were surface-sterilized by dipping entire flowers three times in 0.05% sodium hypochlorite and rinsing in five changes of sterile water. Care was taken to select plant tissue that appeared free of insects or disease.

Petal disks (14 mm diameter) were cut from surface-sterilized plant tissue using a

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Accepted for publication 31 May 1996.

Publication no. D-1996-0715-04R
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sterile cork borer and were fully submerged in bacterial inoculum. Four disks were tested for each bacterial strain. Check disks were prepared by dipping tissue in sterile 0.03% Tween 20 solution only. Once treated, individual disks were placed in a single well of a sterile Falcon 3047 multiwell plate with 24 15-mm-diameter wells (six lanes of four wells each). Treatments were placed in the multiwells in the following manner (Fig. 1): molten MEA was placed in the first well of lane 1 and allowed to solidify. In the remaining three wells of lane 1 and in all wells of lane 2, check petal disks dipped only in sterile 0.03% Tween 20 solution were placed singly at the bottom of each well. Tissue disks inoculated with bacteria were placed at the bottom of all wells in each of the four remaining lanes; a different bacterium was tested in each lane.

Loaded plates were covered with sterile lids, placed in closed plastic boxes lined with wet paper towels, and incubated in a lighted, continuous-mist incubation chamber at 15 ± 2°C with a relative humidity of near 90% and a photoperiod set to 12 h. After 24 h, 100-µl aliquots of fungal inoculum were dispensed to the center of all tissue disks in lanes 2 to 6 and to the well in lane 1 containing MEA. The remaining three wells in lane 1 were not inoculated with the fungus. After 6 days of further incubation in the incubation chamber, petal disks were visually examined for fungal sporulation. Disease suppression by each bacterial strain was assessed on a scale of 0 to 4, which represented the number of petal disks in a given lane colonized by *B. cinerea*. An assay was repeated if the fungus failed to sporulate in the well with MEA or if the well became contaminated. The test was also repeated if any of the noninoculated check disks in lane 1 became infected with the fungus (which rarely occurred), or if inoculated check disks in lane 2 were not colonized.

Bacteria were screened in three trials. In the first trial, 172 arbitrarily chosen bacterial strains were assayed using *B. cinerea* isolates BC9204 and BC9301. Of the 96 bacterial strains that inhibited fungal colonization in all four tissue disks in any given lane (disease rating = 0), 76 strains with the most vigorous growth on agar media were selected and evaluated for a second time. Twenty-four of the 47 bacteria that completely inhibited fungal development in this latest assay were tested a third time.

The antifungal activity of each of the 24 strains that completely inhibited sporulation of *B. cinerea* after three successive petal disk assays was evaluated in plate assays. Assays consisted of 1-cm plugs of *B. cinerea* BC9204 grown for 1 week on potato-dextrose agar (PDA; Difco) and placed in the center of PDA plates. Four different bacterial strains were screened per plate by placing single loopfuls of each strain, grown for 3 days on NYA, in one of four locations around the periphery of each plate. After incubation for 1 week at room temperature, the plates were examined for zones of fungal growth inhibition ≥5 mm around the margin of each bacterial colony. Each strain was tested in this manner three times.

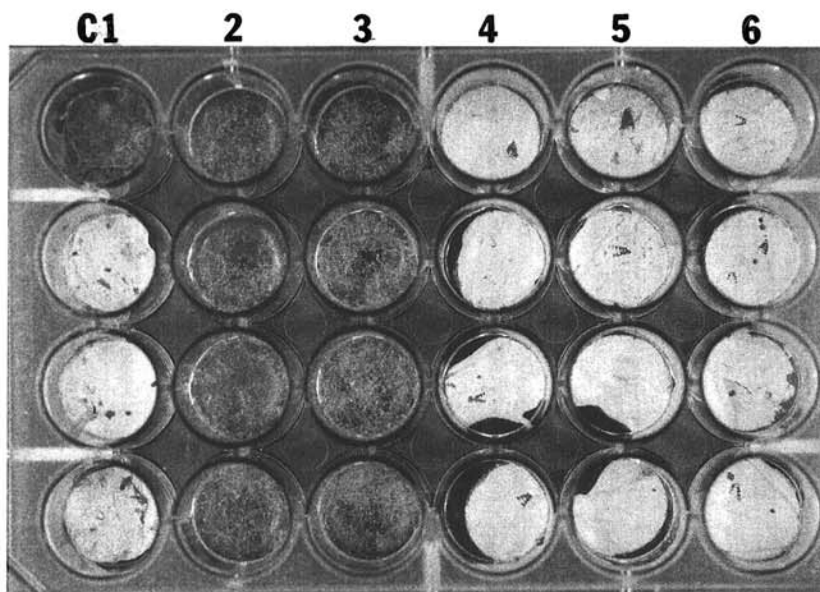

Fig. 1. Petal disk assay for control of *Botrytis cinerea* sporulation by bacterial strains in Falcon 3047 multiwell plates. Except for top well of lane C1, all wells contain surface-sterilized petal disks. The various treatments are (from left to right): top well of lane C1, malt extract agar + *B. cinerea*; remaining wells of lane C1, negative check (no *B. cinerea*, no bacterium); lane 2, positive check (*B. cinerea* only); lanes 3 to 6, *B. cinerea* + bacterium (a different bacterial strain per lane). Note fuzzy, gray sporulation of the fungus in all disks of lanes 2 (*Botrytis* check) and 3 (disease rating = 4), and no sporulation (disease rating = 0) in all disks of lanes 4 to 6. Caret-shaped marks on petal disks are small wounds made by forceps when the disks were placed in wells.

Whole flower assays. Of the 24 bacterial strains that inhibited the growth of *B. cinerea* in three successive petal disk assays, a single representative bacterium, PB92B10E, was selected for further characterization. This isolate, which produced a large zone of inhibition in plate assays, was originally obtained from petunia leaves. Control of *B. cinerea* sporulation on whole flowers using PB92B10E was tested on plants in the lighted incubation chamber previously described. Using a hand-held spray bottle in the greenhouse, all flowers (total of six to eight) of 6-month-old Ultra-white petunia plants grown from seed were sprayed to runoff with suspensions of bacteria at a concentration of 10^8 CFU ml^{-1} in 0.03% Tween 20. Flowers on check plants were sprayed in a similar manner with 0.03% Tween 20 only, and all plants were allowed to dry overnight in the greenhouse.

The following day, 100-µl aliquots of *B. cinerea* BC9204 conidia in water (1,000 conidia ml^{-1}), prepared as described for petal disk assays, were dispensed in the interior base of the corolla tube of each open flower on a plant. Using a modification of the technique described by Wood (25), flowers were wounded to enhance infection by applying a fragment of dry ice for 3 s near the base of the corolla tube just prior to inoculation. In each of seven trials, all open flowers on 4 (trial 1 only) or 15 (trials 2 to 7) plants were inoculated with both *B. cinerea* BC9204 and bacterial strain PB92B10E or a rifampicin mutant of this strain (PB92B10Er+) (see below). Nine plants served as checks: all flowers on three plants were inoculated with *B. cinerea* conidia only, three more plants were treated with the bacterium, and the three remaining plants were sprayed with 0.03% Tween 20. The petunias were then placed in the lighted incubation chamber in a completely randomized pattern. After 6 days, each flower was examined for colonization by *B. cinerea*, and disease incidence was expressed as percent infected flowers per treatment. The incubation chamber assay was repeated six times using PB92B10E (trials 1 to 4) or PB92B10Er+ (trials 5 to 7). Statistical differences between treatments were determined using a nested analysis of variance with treatment and plant nested within treatment as main effects. The least squares means procedure was used to separate treatment means. All statistical analyses were performed using Statistical Analysis Software (SAS Institute, Inc., Cary, NC.).

Bacterial population studies. Bacterial persistence on inoculated whole petunia flowers was evaluated using a rifampicin-resistant mutant of bacterial strain PB92B10E. To select the mutant, tubes containing 25 ml of LB medium amended

with rifampicin (100 μg/ml) were inoculated with bacteria grown for 48 h on NYA medium. Tubes were wrapped in aluminum foil and shaken at 250 rpm for 7 days at approximately 21°C. Each culture was centrifuged, the supernatant was decanted, and the pellet was resuspended in 0.03% Tween 20 to a concentration of 10^8 CFU ml^{-1}. Aliquots were streaked on KB amended with rifampicin at 50 μg/ml, and the plates were incubated at room temperature. After 2 days, arbitrarily selected colonies were again added to separate centrifuge tubes containing 30 ml of LB amended with antibiotic at 50 μg/ml. Cultures were shaken for an additional 3 days, centrifuged, and resuspended in 0.03% Tween 20.

Bacterial inoculum for population studies was prepared by growing an arbitrarily selected rifampicin-resistant strain (PB92B10Er+) in 30 ml of LB medium + 50 μg of rifampicin per ml for 3 days at room temperature. Bacterial suspensions were centrifuged at 3,000 × *g* for 5 min, the supernatant was decanted, and the pellet was resuspended in a 10 mM solution of potassium phosphate buffer (pH 7) to yield a bacterial concentration of 10^8 CFU ml^{-1}.

In whole flower tests, PB92B10Er+ in 0.03% Tween 20 was sprayed to runoff on all flowers of three nonsterile Ultra-white petunia plants (6 months old). The plants were allowed to dry in the greenhouse for 24 h and were then placed in the incubation chamber as described for petal disk assays. On a daily basis for 7 days, one flower from each plant was removed, weighed, placed individually in 100 ml of potassium phosphate buffer, and shaken for 1 h. The suspensions were then sonicated for 30 s, shaken for one more hour, and serially diluted in a 10-fold series. The drop plate method (11) was used to enumerate bacteria, in which 10-μl aliquots of each dilution were placed on KB plates containing rifampicin. The plates were wrapped in aluminum foil and incubated for 2 days at room temperature, and bacterial colonies were counted. Bacterial population studies on whole flowers were repeated twice, and population changes with time were analyzed using linear regression analysis. Population data were subjected to log transformation to achieve a normal distribution prior to analysis.

RESULTS

Petal disk assays. Visual rating of the petal disk assay was facilitated by abundant sporulation that accompanied infection of petal disk tissue by *B. cinerea.* In all assays, petal disks inoculated only with fungal conidia (fungal checks) were consistently infected, and surface-sterilization of petal tissue in 0.5% sodium hypochlorite was sufficient so that negative checks were rarely contaminated.

In three successive trials, 24 strains from the original 172 strains screened consistently suppressed the development of *B. cinerea* in all petal disks tested (Table 1). Of these 24 strains, 10 inhibited the growth of *B. cinerea* in plate assays, and all but one were gram negative and produced pale yellow to yellow, mucoid, and fluorescent colonies on KB medium. The remaining strain stained positive in gram reaction and produced off-white, rapidly growing colonies on NYA. No attempt was made to identify the majority of these bacteria to species. The original source of 19 of these 24 strains was leaf tissue; the remainder were recovered from petunia flowers.

Whole flower assays. The bacterial strain used in whole flower assays, PB92B10E, was identified as *Pseudomonas fluorescens* by fatty acid analysis (0.912 similarity index; Microbial ID Inc., Newark, DE). Under controlled, incubation chamber conditions, *P. fluorescens* PB92B10E reduced fungal infection of flowers inoculated with *B. cinerea* from 61 to 100% compared to the fungal check (Table 2). It was apparent during these assays that blossoms on check plants not intentionally inoculated with *B. cinerea* became infected with this fungus, although the percentage varied considerably from trial to trial (6 to 87%). Nonetheless, PB92B10E or its rifampicin mutant significantly reduced this incidental infection by 37 to 100% in six of seven trials (Table 2).

Bacterial populations on whole flowers. From populations of PB92B10Er+ determined in three separate trials on nonsterile, whole flowers, a linear relationship between bacterial populations and time following inoculation was evident (Fig. 2). Populations were established at approximately 10^4 CFU g^{-1} flower tissue and appeared to increase by greater than 10^4 over the 7-day period.

DISCUSSION

Promising results have been attained with several biological control agents developed for diseases caused by *Botrytis* (2,8,14,20,22). Few studies, however, have attempted biological control on ornamental bedding plants such as petunias. In this

Table 1. Number of bacterial strains that inhibited colonization of *Botrytis cinerea* in petal disks inoculated with both fungal conidia and bacteria in tissue disk assays

	Number of bacterial isolates[z]		
Rating[y]	**Trial 1**	**Trial 2**	**Trial 3**
0	96	47	24
1	32	9	0
2	19	11	0
3	11	4	0
4	14	5	0
Total strains	172	76	24

[y] Measured on a scale of 0 to 4, which represents the number of petal disks in petal disk assays exhibiting any degree of *B. cinerea* sporulation after 6 days at 15°C.

[z] The bacteria screened in trial 2 were those strains that grew most vigorously from the pool of strains that completely inhibited fungal development (disease rating = 0) in trial 1. Similarly, the 24 bacteria screened in trial 3 were selected from those bacteria that completely inhibited fungal development in trial 2.

Table 2. Control of Botrytis flower blight with *Pseudomonas fluorescens* PB92B10E (trials 1 to 4) and PB92B10Er+ (trials 5 to 7) in whole flower assays

	Disease incidence (%)[x]						
Treatment	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**	**Trial 7**
Uninoculated check	7.7 a[y] (13)[z]	61.8 b (23)	6.8 a (29)	20.8 b (24)	70.0 b (23)	87.5 b (8)	25.0 a (4)
Pseudomonas check	0 a (11)	21.4 a (14)	9.1 a (22)	0 a (12)	43.8 ab (16)	23.0 a (13)	0 a (10)
Botrytis check	81.8 b (11)	68.7 b (16)	55.0 b (20)	57.1 c (21)	96.0 c (25)	55.6 b (9)	77.8 b (9)
Botrytis + *Pseudomonas*	0 a (12)	24.7 a (81)	6.2 a (81)	6.9 a (87)	37.2 a (94)	24.1 a (58)	26.1 a (46)

[x] Disease incidence is expressed as the percent flowers per treatment infected with *Botrytis cinerea.*

[y] Means in a column followed by the same letter are not significantly different (least squares means separation procedure) ($P < 0.05$). There were no significant differences ($P < 0.05$) between plants within treatment for each trial except for trial 6.

[z] Number of flowers in treatment.

study, *P. fluorescens* PB92B10E was identified as a potential biological control agent for Botrytis blight of petunia, reducing disease incidence by an average of 77% in whole flower assays. These results give promise to the use of biological agents for the control of Botrytis blight of petunia as well as other diseases of ornamental greenhouse crops.

Bacterial strain PB92B10E was identified using a petal disk assay developed for rapid preliminary screening of potential fungal antagonists on petunia. The protocol for the petal disk assay was developed using methods previously described for identifying epiphytes for biological control of *Botrytis* (14,20) combined with a protocol determining residual efficacy of fungicides used in *Botrytis* management (19). Using this petal disk assay, several candidate antagonists consistently suppressed petal tissue infection by 100% in repeated tests. The use of disk assays to isolate antagonists does not appear to be confined specifically to petals; leaf tissue has been used with promising success (A. Gould and M. Bergen, *unpublished*). Furthermore, the disk assay has worked in our laboratory on other hosts such as geranium and exacum, which highlights the utility of such an assay for screening biological control agents for these ornamental plants.

In the present study, infection of petal tissue by *B. cinerea* was facilitated by incubation under conditions of high humidity and by wounding tissue with dry ice. Numerous reports confirm the requirement of high humidity (1,3,4,10,13,23,24) and wounding (9,12,17,18,25) for infection by *Botrytis*. In addition, a number of studies have confirmed the importance of decaying or damaged tissue in the infection process (12,17,18,25). For example, Laemmlen and Sink (18) found that conidial concentrations on petunia significantly increased on senescent flowers and leaves and on fallen petunia debris.

Recovery of PB92B10Er+ at high populations from nonsterile whole flowers indicates that this isolate effectively colonized and persisted on flower tissue of petunia. Populations of PB92B10Er+ (when expressed on a logarithmic scale) increased steadily in a linear manner to greater than 10^8 CFU g^{-1} flower tissue after 7 days; these increases may have been facilitated by the same environmental conditions that are conducive to disease development. These conditions include wounding, natural senescence, or incubation under conditions of high humidity.

Proposed mechanisms of biological control for agents effective against *Botrytis* include the inhibition of fungal growth by the production of antibiotic-like compounds (8,14). In a manner similar to other biological control agents, activity by PB92B10E may be due to antibiosis, resulting in inhibition of fungal growth (as indicated in plate assays), or to colonization and competition for nutrient substrates (as indicated by rapid increases in bacterial populations on petal tissue). Microscopic observation of inoculated petal disk tissue indicated that conidia failed to germinate in the presence of PB92B10E. This was further supported by observations that inoculation of petal disks with hyphal inoculum was not controlled by this bacterial isolate (unpublished observation). Although the exact mode of inhibition is unclear, the early establishment of bacteria and maintenance of high populations on petal tissue should prove useful for controlling Botrytis blight under greenhouse conditions.

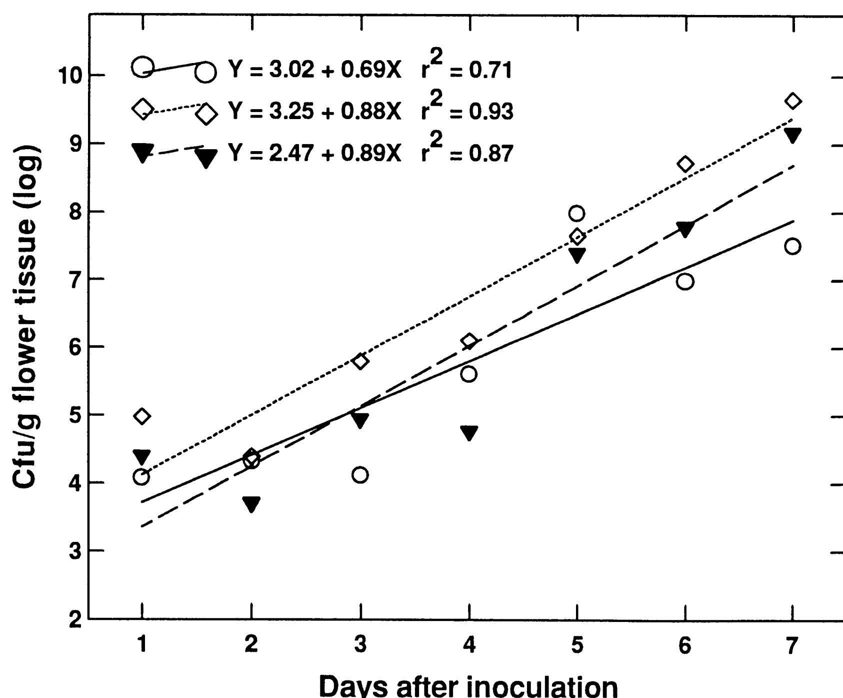

Fig. 2. Populations of bacteria (log CFU g^{-1} flower tissue fresh weight) 1 to 7 days following inoculation with bacterial strain PB92B10Er+ on nonsterile whole petunia flowers. Each regression line represents a different trial; all equations were significant at $P = 0.0001$.

ACKNOWLEDGMENTS

We thank M. Dubos and B. Hajdari for technical support and B. Clarke for thoughtful review of this manuscript. We also thank J. Bogard of Goldsmith Seeds, Inc., Gilroy, CA, for the generous gift of petunia seeds. Funding for this project was received from the Bedding Plants Foundation, Inc., Lansing, Michigan.

LITERATURE CITED

1. Baker, K. F. 1946. Observations on some *Botrytis* diseases in California. Plant Dis. Rep. 30:145-155.
2. Blakeman, J. P. 1972. Effect of plant age on inhibition of *Botrytis cinerea* spores by bacteria on beetroot leaves. Physiol. Plant Pathol. 2:143-152.
3. Bolkan, H. A., Ogawa, J. M., and Teranishi, H. R. 1984. Shoot blight of pistachio caused by *Botrytis cinerea*. Plant Dis. 68:163-165.
4. Bulger, M. A., Ellis, M. A., and Madden, L. V. 1987. Influence of temperature and wetness duration on infection of strawberry flowers by *Botrytis cinerea* and disease incidence of fruit originating from infected flowers. Phytopathology 77:1225-1230.
5. Campbell, R. 1989. Biological Control of Microbial Plant Pathogens. Cambridge University Press, Cambridge.
6. Dhingra, O. D., and Sinclair, J. B. 1985. Basic Plant Pathology Methods. CRC Press, Boca Raton, FL.
7. Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossman, A. Y. 1989. Fungi on Plants and Plant Products in the United States. American Phytopathological Society, St. Paul, MN.
8. Hammer, P. E., Evensen, K. B., and Janisiewicz, W. J. 1993. Postharvest control of *Botrytis cinerea* on cut rose flowers with pyrrolnitrin. Plant Dis. 77:283-286.
9. Hausbeck, M. K., and Pennypacker, S. P. 1991. Influence of grower activity and disease incidence on concentrations of airborne conidia of *Botrytis cinerea* among geranium stock plants. Plant Dis. 75:798-803.
10. Hausbeck, M. K., and Pennypacker, S. P. 1991. Influence of time intervals among wounding, inoculation, and incubation on stem blight of geranium caused by *Botrytis cinerea*. Plant Dis. 75:1168-1172.
11. Hoben, H. J., and Somasegaran, P. 1982. Comparison of the pour, spread, and drop plate methods for enumeration of *Rhizobium* spp. in inoculants made from presterilized peat. Appl. Environ. Microbiol. 44:1246-1247.
12. Hunter, J. E., Rohrbach, K. G., and Kunimoto, R. K. 1972. Epidemiology of Botrytis blight of macadamia racemes. Phytopathology 62:316-319.
13. Hyre, R. A. 1972. Effect of temperature and light on colonization and sporulation of the *Botrytis* pathogen on geranium. Plant Dis. Rep. 56:126-130.
14. Janisiewicz, W. J., and Roitman, J. 1988. Biological control of blue mold and gray mold on apple and pear with *Pseudomonas cepacia*. Phytopathology 78:1697-1700.
15. Jarvis, W. R. 1980. Epidemiology. Pages 219-250 in: The Biology of *Botrytis*. J. R. Coley-Smith, K. Verhoeff, and W. R. Jarvis, eds. Academic Press, London.
16. Jarvis, W. R. 1992. Managing Diseases in Greenhouse Crops. American Phytopa-

thological Society, St. Paul, MN.
17. Johnson, K. B., and Powelson, M. L. 1983. Analysis of spore dispersal gradients of *Botrytis cinerea* and gray mold disease gradients in snap beans. Phytopathology 73:741-746.
18. Laemmlen, F. F., and Sink, K. C. 1978. Evaluation of petunia cultivars for *Botrytis* resistance. Plant Dis. Rep. 62:361-365.
19. Moorman, G. W., and Lease, R. J. 1992. Residual efficacy of fungicides used in the management of *Botrytis cinerea* on greenhouse-grown geraniums. Plant Dis. 76:374-376.
20. Redmond, J. C., Marois, J. J., and MacDonald, J. D. 1987. Biological control of *Botrytis cinerea* on roses with epiphytic microorganisms. Plant Dis. 71:799-802.
21. Sink, K. C, ed. 1984. Petunia. Springer-Verlag, Berlin.
22. Sutton, J. C., and Peng, G. 1993. Biocontrol of *Botrytis cinerea* in strawberry leaves. Phytopathology 83:615-621.
23. Thomas, C. S., Marois, J. J., and English, J. T. 1988. The effects of wind speed, temperature, and relative humidity on development of aerial mycelium and conidia of *Botrytis cinerea* on grape. Phytopathology 78:260-265.
24. Wilcox, W. F., and Seem, R. C. 1994. Relationship between strawberry gray mold incidence, environmental variables, and fungicide applications during different periods of the fruiting season. Phytopathology 84:264-270.
25. Wood, R. K. S. 1951. The control of diseases of lettuce by the use of antagonistic organisms. I. The control of *Botrytis cinerea* Pers. Ann. Appl. Biol. 38:203-216.