Overwintering of *Pseudomonas syringae* pv. *glycinea* in the Field

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ABSTRACT

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Overwintering of *Pseudomonas syringae* pv. *glycinea* (PSG) associated with soybean leaves in the field was evaluated with a streptomycin-rifampicin resistant mutant (PRS-81) of the bacterium. In 1981–1982, PRS-81 was recovered from buried samples (10, 20, and 30 cm below the surface) until February 1982 and from soil surface samples until March 1982. It was not detected after April. In 1982–1983, survival of the bacterium was not affected by depths at which samples were placed. Viable cells were detected from all samples until March. Soil temperature and moisture greatly influenced survival of the bacterium associated with soybean leaves. PRS-81 survived better under dry, cold conditions than under wet, warm conditions. Effects of soil temperature and moisture on survival of PRS-81 appeared to interact. Effects of soil moisture were more apparent at 4 C than at 12 or −12 C. Considering technical limitations in detecting PSG in soil and influences of soil temperature and moisture on its survival, it is concluded that PSG associated with soybean leaf tissue could overwinter on the soil surface in central Illinois if the weather is cold and dry.

Additional key words: bacterial blight, dilution-plate technique, *Glycine max*.

Bacterial blight, caused by *Pseudomonas syringae* pv. *glycinea* (Coerper) Young, Dye, and Wilke (PSG), is prevalent in soybean (*Glycine max* (L.) Merrill) fields in Illinois. Disease symptoms are commonly found on leaves and occasionally on stems, petioles, and pods (13,18). Approximately 18–22% soybean yield loss due to bacterial blight has been reported under favorable conditions for the disease (7,19).

Bacterial blight is usually more prevalent in the early growing season than later in the season. For early season diseases, in general, control measures emphasize reducing the amount of overwintered inoculum. Several studies (10,11,14) on survival of PSG demonstrated that infected seeds were sources of initial inoculum of the bacterium. Evidence for successful overwintering of the bacterium in association with diseased plant residues in the field is inconclusive. Two main reasons for the lack of conclusive evidence are: PSG, a soil invader, has poor competitive saprophytic ability (3,17), and methods employed to determine its persistence in soil are not sensitive enough to detect small numbers of viable cells (6).

Graham (10) found in a laboratory study that PSG survived at least 6–9 mo in sterile soil but less than 1 mo in unsterile soil at 25 C. He also noticed that the bacterium in soil survived longer under low temperature and dry conditions than under warm and wet conditions. In field studies, Daft and Leben (5) and Kennedy (12) concluded that PSG associated with diseased plant parts could survive between growing seasons on the soil surface in Ohio and Minnesota, respectively. However, in a study conducted in Parana State, Brazil, leaf debris, whether placed on the soil surface or buried below ground, apparently was not a site for between-season survival of the bacterium (8). These different results are probably due to interactions among environmental, biotic, and abiotic variables that influence survival of PSG in soil (6,8). Temperatures during the winter months in Brazil are higher than in Ohio and Minnesota. The increased activities of soil microorganisms in warm environments would induce rapid decay of plant residues in the field.

Technical difficulties in detecting low populations of bacteria in soil hamper studies of their survival (6). Two approaches to detect plant pathogenic bacteria in soil involve use of susceptible assay plants and selective media. Graham (10), Daft and Leben (5), Kennedy (12), and Fett (8) used susceptible assay plants to detect viable PSG populations in soil. Although this method is fairly sensitive (12), it only enables qualitative determination of bacterial survival in soil, and its ability to detect low populations decreases when saprophytic microorganisms are present (6,12).

Two selective media have been reported for PSG (9,16). Although Leben (16) reported that M71 agar medium was useful to detect PSG from soybean buds and leaves, results of a preliminary study (*unpublished*) indicated that it was not selective enough to detect PSG in soil. Fieldhouse and Sasser (9) developed an agar medium (BANQ) that could be used for quantitative recovery of PSG from soil. However, it was not available at the inception of our study. In the present study, an antibiotic resistant mutant of the bacterium was used to study persistence in soil. This technique enhanced detection of the bacterium in soil and enabled quantitative measurements of soil populations.

The objectives of this study were to evaluate overwintering of PSG associated with diseased soybean leaf tissue in the field in central Illinois and to determine the effect of soil moisture on survival of the bacterium.

MATERIALS AND METHODS

Isolation and inoculation. An isolate of PSG was obtained from a naturally infected soybean (cultivar Gnome) plant in Urbana, Illinois in 1980. The isolate was motile, rod-shaped, Gram-negative, obligate aerobic, and oxidase negative, and produced fluorescent pigment on King's B medium (15). When inoculated on soybean plants in a greenhouse, typical bacterial blight symptoms

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(18) with water-soaked lesions developed within 7 days after inoculation. Rifampicin-resistant mutants of the isolate were selected by using the gradient-plate method (4). By subculturing the mutants repeatedly on King's B medium with successively higher concentrations of rifampicin (3-[4-methyl-piperazinylimino-methyl] rifamycin SV, Sigma Chemical Co., St. Louis, MO), a mutant (RfPg-11) that exhibited resistance to rifampicin at 500 μg/ml was selected. From RfPg-11, a streptomycin-resistant mutant (PRS-81) was obtained by using the gradient plate method with streptomycin sulfate (Sigma Chemical Co., St. Louis, MO). PRS-81 showed resistance to both rifampicin at 500 μg/ml and streptomycin sulfate at 1,000 μg/ml.

Soybean cultivar Wells II seedlings grown on sandbenches in the greenhouse were inoculated with PRS-81 three times at weekly intervals beginning at the third trifoliate stage. The inoculum was made from a 2-day-old culture of PRS-81 on King's B agar plates and diluted to approximately 10^7 colony-forming units (cfu) per milliliter of distilled water. The bacterial suspension was sprayed onto soybean seedlings at 4.2 kg/cm^2 pressure.

Preparation of the leaf-soil mixture. All leaves, including both infected and healthy-looking leaves of Wells II plants, were collected three days after the third inoculation and air-dried at 20–25 C for 4 days. The dry leaves were broken into small pieces and mixed with soil (Drummer silt clay loam) from a soybean field at the ratio of 2 g of leaf tissue to 70 g of soil. The soil was dried at 20–25 C for 2 days and broken into fragments less than 0.5 cm in diameter before mixing with the leaf tissue.

Overwintering in the field. Survival of PSG associated with soybean leaf tissue in the field was studied during the winters of 1981–1982 and 1982–1983 at Urbana, IL. Individual glass fiber mesh (1.5 mm) bags (10 × 10 cm) containing 200 g of the leaf-soil mixture were placed at four different positions (soil surface and 10, 20, and 30 cm below the surface) in the soil. Treatments were arranged in a randomized complete block design with four replications. The experiments were initiated on 10 November 1981 and 17 November 1982. Samples were collected six times at 4- to 6-wk intervals from 13 November 1981 until 15 April 1982 and from 23 November 1982 until 2 May 1983. During the experiment, temperatures at the soil surface and at 10, 20, and 30 cm below the soil surface were monitored with automatic 7-day recording thermometers (Taylor Instrument Co., Arden, NC) to which sensing bulbs at the four depths were connected.

Recovery of PRS-81. In order to reisolate PRS-81, 100 cc of the leaf-soil mixture from each sample was homogenized for 4 min in 200 ml of distilled water with a Virtis "45" homogenizer (The Virtis Co., Inc., Gardiner, NY) at speed scale 60. Serial dilutions of suspensions were spread on King's B agar medium containing streptomycin sulfate (100 μg/ml), rifampicin (50 μg/ml), cycloheximide (3-[2(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide, Sigma Chemical Co., St. Louis, MO) (60 μg/ml), and Botran (2,6-dichloro-4-nitroaniline, TUCO, Div. of the Upjohn Co., Kalamazoo, MI) (5 μg/ml). Cycloheximide and Botran were included to inhibit fungal growth. After 3–5 days of incubation at 25 C, PRS-81 colonies were counted at ×12 magnification under a stereomicroscope (Wild Heerbrugg Ltd., CH-9435 Heerbrugg, Switzerland). The characteristic rough colony type (Fig. 1) of PRS-81 was easily identified at this magnification, especially during early growth of colonies. Pathogenicity of the reisolates was confirmed by inoculating seedlings of cultivar Wells II.

Soil moisture study. The effect of soil moisture on survival of PSG was studied in incubation chambers at −12, 4, and 12 C. Seventy-five grams of dry, infested leaf tissue was mixed with 2,400 g of dry soybean field soil (Drummer silt clay loam). The moisture content of the leaf-soil mixture was approximately 5% based on the oven-dry weight. Seven grams of the leaf-soil mixture was placed in a petri plate 3.5 cm in diameter.

Treatments included four different moisture levels (15, 25, 35, and 45% based on the oven-dry weight) of the leaf-soil mixture. Treatments were arranged in a completely randomized design with three replications at each temperature. Samples were collected six times at 4- to 6-wk intervals for 153 days. The four moisture levels were achieved by adding appropriate amounts of distilled water to each sample plate. In order to maintain constant moisture levels, each sample plate was placed in a 5-cm-diameter plate. Six milliliters of distilled water were poured in each outside plate and it was then sealed tightly with Parafilm. All plates were left at room temperature for 2 days to equilibriate moisture in the sample plates. Extreme care was taken to prevent water in the outside plate from entering the sample plate during the experiment. To check moisture levels at every sampling time, approximately 0.5 g of the leaf-soil mixture was removed from each sample plate before homogenization and its moisture content was measured based on the oven-dry weight. PRS-81 was reisolated and colonies were counted as in the field overwintering study except that samples were homogenized in 30 ml instead of 200 ml of distilled water.

Efficiency of PRS-81 recovery. PRS-81 recovery efficiency was determined based on the recovery of known numbers of cells added to soil under laboratory conditions. Two ml of bacterial suspensions with five concentrations of PRS-81 cells (32, 3.2×10^2, 3.4×10^3, 4.1×10^4, and 3.6×10^5 cfu per ml) were added to 9 g of soybean field soil. By using the same technique as for the soil moisture study, PRS-81 colonies were recovered from the soil and compared with the estimated number of cells added to the soil.

Since we were interested in PSG populations associated with soybean leaf tissue in soil rather than free in soil, efficiency of PRS-81 recovery after mixing infected leaf tissue with soil was also checked. PRS-81 colonies were recovered from infected leaf and soil mixture which contained 7 g of soil and 0.2 g of infected soybean leaf tissue. The numbers of colonies recovered from the leaf-soil mixture were compared with those from 0.2 g of infected leaf tissue alone. This experiment was replicated 10 times in a completely randomized design. The *F*-test with single degree of freedom was performed to compare treatment effects throughout the experiment.

RESULTS

Overwintering in the field. Survival of PSG associated with leaf tissue was greater on the surface than below the soil surface in 1981–1982 (Table 1). PRS-81 populations in buried samples declined at about the same rate at all depths during the winter. However, the population on the soil surface declined more slowly. Recovery of PRS-81 colonies from the soil surface samples was significantly ($P < 0.01$) higher than that from the buried ones throughout the winter. Viable cells were detected until 10 March 1982 from the soil surface but not from buried samples. In 1982–1983, PRS-81 populations from all samples declined at about the same rate (Table 1). There were no significant ($P < 0.05$) differences between numbers of colonies recovered from the soil

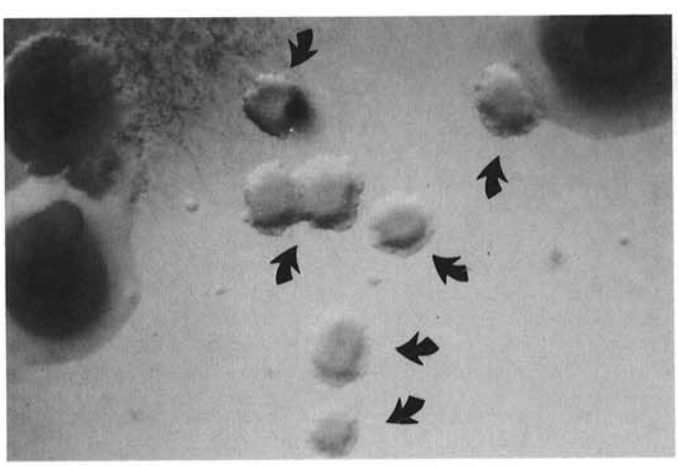

Fig. 1. Colonies formed by isolate PRS-81 of *Pseudomonas syringae* pv. *glycinea* on King's B agar medium with streptomycin (100 μg/ml), rifampicin (50 μg/ml), cycloheximide (60 μg/ml), and Botran (5 μg/ml) 5 days after incubation at 24 ± 1 C. Magnification ×15.

surface and at different depths throughout the winter. Viable cells were detected from all samples until 24 March 1983.

Surface soil was colder than soil at depths of 20 and 30 cm during the first winter, but it remained warmer than soil at 20 and 30 cm below the soil surface the following winter (Table 2). Diurnal fluctuations of temperature were greater on the soil surface than below during both winters (Table 2). Differences in moisture were also noticed between surface samples and buried samples; surface samples were drier than buried ones throughout the experiment.

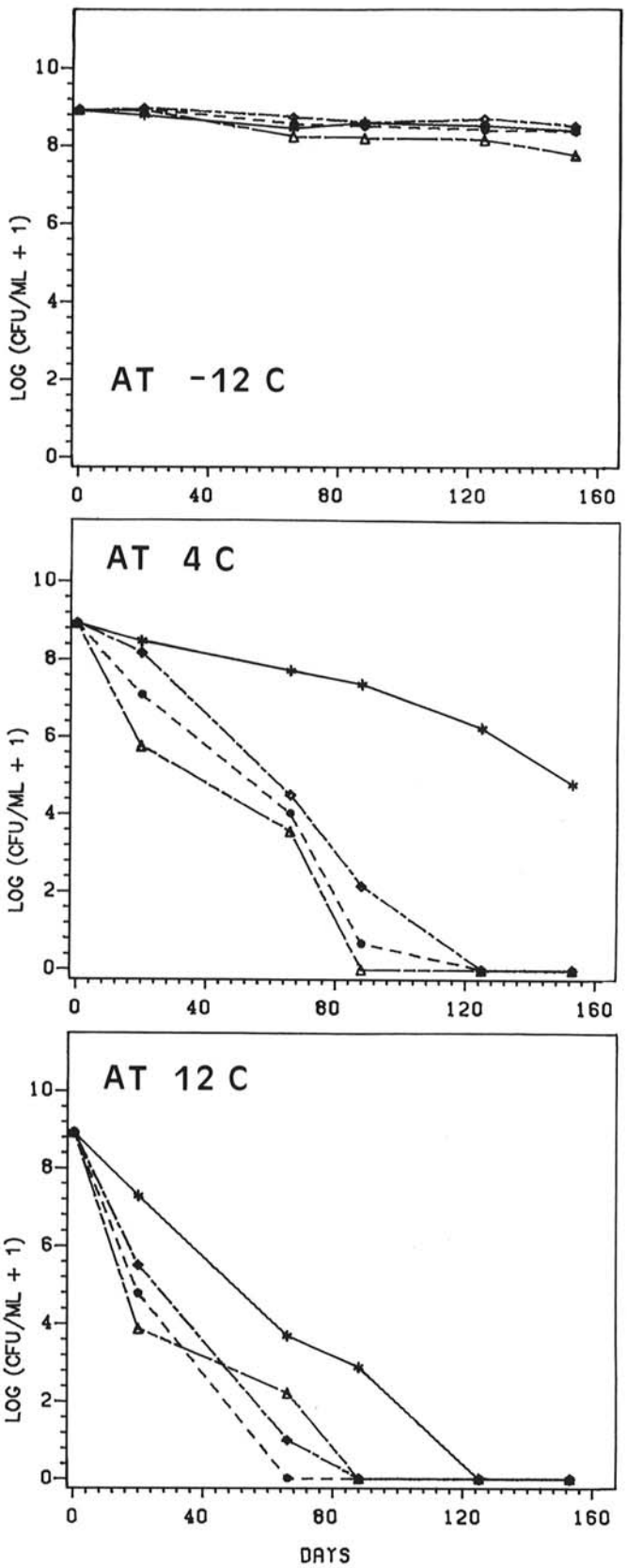

Fig. 2. Survival of *Pseudomonas syringae* pv. *glycinea* under soil moistures of 15% (*——*), 25% (◇ —·— ◇), 35% (•——•), and 45% (Δ– – –Δ) at −12, 4, and 12 C.

Effects of soil moisture. Actual moisture levels observed throughout the experiment were 14.8 ± 4.2, 24.7 ± 3.9, 33.9 ± 3.2, and 46.2 ± 5.7% for the intended 15, 25, 35, and 45% levels, respectively. Soil moisture affected survival of PRS-81 differently at −12, 4, and 12 C. At −12 C, PRS-81 populations under all moisture regimes remained almost unchanged (Fig. 2). Large numbers of colonies were consistently recovered at every sampling time throughout the experiment. PRS-81 populations at 15% moisture at 4 C decreased more slowly than those at 25, 35, and 45% at the same temperature (Fig. 2). Viable cells were detected from 15%-moisture samples after 153 days, whereas none were recovered from 25-, 35-, and 45%-moisture samples after 125, 125, and 88 days, respectively. PRS-81 populations declined more rapidly at 12 C than at 4 C (Fig. 2). After 88 days of incubation at 12 C, the bacterium was detected only from 15%-moisture samples. Viable cells were no longer detected thereafter. Soil moisture significantly ($P<0.01$) affected survival of PRS-81 in soybean leaf tissue at all three temperatures. Time × moisture interactions were significant at 4 C ($P<0.01$) and 12 C ($P<0.05$) but not at −12 C.

Efficiency of recovery of PRS-81. The technique employed to recover PSG from soil enabled detection of a population as low as

TABLE 1. Recovery of *Pseudomonas syringae* pv. *glycinea* mutant PRS-81 from four different depths in the field during the winters of 1981–1982 and 1982–1983

Date	Days after burial	Recovery of the bacterium at indicated depths below the soil surface (cm)[a,b]			
		Surface	10	20	30
13 Nov 1981	3	1.86×10^9	1.62×10^9	1.82×10^9	2.40×10^9
11 Dec 1981	31	6.17×10^8	2.95×10^7	1.38×10^7	1.02×10^7
5 Jan 1982	56	2.63×10^7	7.59×10^5	8.91×10^5	4.47×10^5
1 Feb 1982	83	7.94×10^3	1.02×10^2	1.86×10^2	1.66×10^2
10 Mar 1982	120	1.26×10^3	ND[c]	ND	ND
15 Apr 1982	156	ND	ND	ND	ND
23 Nov 1982	6	1.00×10^{10}	1.38×10^{10}	1.48×10^{10}	1.55×10^{10}
23 Dec 1982	36	3.39×10^7	2.29×10^7	1.55×10^7	1.10×10^7
26 Jan 1983	64	5.75×10^5	6.46×10^5	4.68×10^5	4.90×10^5
28 Feb 1983	97	9.33×10^4	5.13×10^4	1.55×10^4	3.16×10^4
24 Mar 1983	121	5.89×10^3	1.07×10^3	4.07×10^3	2.75×10^3
2 May 1983	162	ND	ND	ND	ND

[a] Infected leaf tissue mixed with soil was placed on the soil surface and at 10, 20, and 30 cm below the surface.
[b] Number of colony-forming units (cfu) per 100 cc of the leaf-soil mixture.
[c] ND = not detected.

TABLE 2. Temperatures (C) at the soil surface and at 10, 20, and 30 cm below the surface during the 1981–1982 and the 1982–1983 trials[a]

Trial	Depths (cm)	Maximum	Minimum	Average	Average daily range[b]
1981–1982	Surface	19.4	−20.6	−1.0	5.6 (0–19)
	10	12.2	−17.2	−1.7	2.6 (0–13)
	20	9.4	−7.8	0.0	0.7 (0–4)
	30	8.9	−6.1	0.1	0.7 (0–3)
1982–1983	Surface	20.6	−7.2	6.9	5.8 (0–16)
	10	18.3	−2.8	4.4	2.1 (0–14)
	20	13.9	−3.9	2.7	1.2 (0–7)
	30	15.6	−4.4	2.1	1.6 (0–7)

[a] Temperatures were monitored during the experiment using automatic 7-day recording thermometers (Taylor Instrument Co., Arden, NC) to which sensing bulbs at the four depths were connected.
[b] Average daily temperature range, and (in parentheses) the range of daily temperature ranges.

TABLE 3. Numbers of colony-forming units of *Pseudomonas syringae* pv. *glycinea* mutant PRS-81 detected in soil to which known numbers of cells were added under laboratory conditions[a]

Colonies added to soil (cfu/g soil)			Colonies recovered from soil (cfu/g soil)		
Mean	Standard deviation	Coefficient of variation	Mean	Standard deviation	Coefficient of variation
7	2.8	40	0	0	-
70	18	26	103	179	73
7.6×10^2	1.7×10^2	22	4.4×10^2	3.8×10^2	87
9.0×10^3	1.9×10^3	21	1.0×10^3	7.0×10^3	7
8.0×10^4	2.5×10^4	31	1.2×10^4	1.5×10^4	13

[a] Two milliliters of bacterial suspension with five concentrations of cells of PRS-81 were added to 9 g of soybean field soil. Numbers of colonies of PRS-81 in 2 ml of the suspension were estimated before and after adding to soil. This experiment was replicated three times.

approximately 100 cfu/g of soil (Table 3). The coefficient of variation (C.V.) indicated that the sensitivity of the technique decreased when the bacterial population was less than 10^3–10^4 cfu/g of soil.

Recovery of PRS-81 was reduced when infected leaf tissue was mixed with soybean field soil (Table 4). Compared to the number of colonies detected from infected leaf tissue without soil, approximately 60% of the colonies were recovered from the leaf-soil mixture. The C.V. was higher for the leaf-soil mixture (C.V. = 63%) than for leaf tissue alone (C.V. = 49%), indicating that estimates of the number of viable cells obtained by the technique varied greatly when soil factors were involved.

DISCUSSION

These results indicate that soil temperature and moisture may greatly influence survival of PSG associated with soybean leaf tissue in the field. Higher survival of PRS-81 on the soil surface than below the surface in 1981–1982 was probably due to abnormally cold and dry weather, and considerable diurnal fluctuation of temperature at the surface as compared to below the surface. Diurnal fluctuation of temperature reduces microbial activity in soil and degradation of plant tissue (1). Although the average temperature at 10 cm was slightly lower than at the soil surface, survival of PRS-81 at 10 cm was significantly ($P < 0.01$) lower than on the surface. Apparently higher soil moisture coupled with less temperature fluctuation at 10 cm than at the surface may have accelerated leaf decomposition.

Abnormally warm weather during the winter of 1982–1983 may have resulted in the same survival trend of PRS-81 regardless of depths. Drier conditions and larger fluctuations of temperature at the soil surface than at 20- and 30-cm depths may have counteracted deleterious effects of warm temperatures at the surface soil. The results from 1981–1982 and 1982–1983 cannot be compared with each other because the infected soybean leaf tissue used for the two trials probably had different degrees of colonization by the bacterium.

In general, the results of the soil moisture study support the field observations and agree with a previous report (10). Low survival of PRS-81 at 4 and 12 C as compared to −12 C were probably caused by rapid decomposition of leaf tissue by saprophytic microorganisms at warmer temperatures. Survival of soil invaders like PSG in soil depends on the rate of decomposition of plant debris (17). Under high moisture conditions, populations of PRS-81 declined more rapidly than under low moisture conditions. This could be accounted for by the influence of moisture on oxygen levels in soil and subsequently on soil microbial activity. PSG, an obligate aerobic bacterium (2), cannot survive for long periods under anaerobic conditions. The effects of temperature and moisture on survival appeared to interact. Less apparent effects of soil moisture at 12 and −12 C than at 4 C suggest that the effect of soil moisture on survival of PSG could be masked at certain temperatures. Further study is needed to elucidate the interactions between soil moisture and temperature and their effects on the survival of PSG.

TABLE 4. Numbers of colony-forming units of *Pseudomonas syringae* pv. *glycinea* mutant PRS-81 recovered from dry infected soybean leaf tissue assayed alone and after mixing with soil[a]

Source of colonies	Mean	Standard deviation	Coefficient of variation
Leaf tissue	2.05×10^5	9.96×10^4	49
Soil + leaf tissue mixture	1.18×10^5	7.48×10^4	63

[a] This experiment was replicated 10 times and leaf tissue (0.2 g), or soil (7 g) + leaf tissue (0.2 g), were used for each replication. Means of two treatments are significantly different at $P < 0.05$ (F-test with single degree of freedom).

The dilution plate method, using a streptomycin-rifampicin resistant mutant, gave reliable estimates of soil population levels of PSG when the number of bacteria was higher than 10^3–10^4 cfu/g of soil. For PSG populations of less than 10^3–10^4 cfu/g of soil, the technique did not provide a reliable quantitative estimation of bacterial populations in soil. This technique appears similar to the susceptible-assay-plant technique in sensitivity. Kennedy (12) reported that the susceptible-assay-plant technique could detect PSG when the concentration of the bacterium in a pure culture suspension was greater than 100 cfu/ml. However, he was not able to obtain consistent results when a saprophyte was mixed in the bacterial suspension. Although recovery of PRS-81 in the present study was as low as approximately 100 cfu/g of soil in the laboratory, the sensitivity of detecting PSG from field soil may not be as high. Differences between laboratory and field conditions with respect to bacterial survival were summarized by De Boer (6).

The rather low recovery rate from the leaf-soil mixture may have been caused by soil particles which prevented leaf tissue from being homogenized as completely as when soil was absent. Therefore, the bacterial cells in leaf tissue may not have been released readily into the suspending medium. If this is true, then the dilution plate technique could underestimate the size of populations of PSG associated with soybean leaf tissue in soil.

It is possible that PRS-81, a spontaneous streptomycin-rifampicin-resistant mutant, may have lost some ecologically advantageous characteristics that affect the ability to survive in soil. However, because of rifampicin and streptomycin resistance, PRS-81 may be less affected by antibiotic-producing microorganisms such as *Streptomyces* sp. than the wild-type PSG in nature. The effect of resistance to antibiotics on survival of PSG in soil needs to be further studied. Considering technical limitations in detecting soil populations of PSG and the great influence of soil temperature and moisture on its survival, we conclude that PSG associated with soybean leaf tissue could overwinter on the soil surface in central Illinois if weather is cold and dry.

LITERATURE CITED

1. Biederbeck, N. O., and Campbell, C. A. 1973. Soil microbial activity as influenced by temperature trends and fluctuations. Can. J. Soil Sci. 53:363-376.
2. Buchanan, R. E., and Gibbons, N. E. 1974. Bergey's Manual of Determinative Bacteriology. 8th ed. The Williams & Wilkins Co., Baltimore, MD.
3. Buddenhagen, I. W. 1965. The relation of plant pathogenic bacteria to the soil. Pages 269-284 in: Ecology of Soil-borne Plant Pathogens. Baker and Snyder, eds. U. of Calif. Press, Berkeley.
4. Carton, B. C., and Brown, B. J. 1981. Gene mutation. Pages 222-242 in: Manual of Methods for General Bacteriology. Gerhardt, ed. Amer. Soc. for Microbiology. Washington, DC.
5. Daft, G. C., and Leben, C. 1973. Bacterial blight of soybeans: Field-overwintered *Pseudomonas glycinea* as possible primary inoculum. Plant Dis. Rep. 57:156-157.
6. De Boer, S. H. 1982. Survival of phytopathogenic bacteria in soil. Pages 285-306 in: Phytopathogenic Prokaryotes. Vol. 1. M. S. Mount and M. L. Lacy, eds. Academic Press, New York.
7. Dunleavy, J. 1973. Soybeans. Pages 270-286 in: Breeding Plants of

Disease Resistance. R. R. Nelson, ed. The Penn State Univ. Press, University Park.
8. Fett, W. F. 1979. Survival of *Pseudomonas glycinea* and *Xanthomonas phaseoli* var. *sojensis* in leaf debris and soybean seed in Brazil. Plant Dis. Rep. 63:79-83.
9. Fieldhouse, D. J., and Sasser, M. 1982. A medium highly selective for *Pseudomonas syringae* pv. *glycinea*. (Abstr.) Phytopathology 72:706.
10. Graham, J. H. 1953. Overwintering of three bacterial pathogens of soybean. Phytopathology 43:189-192.
11. Kendrick, J. B., and Gardner, M. W. 1921. Seed transmission of soybean bacterial blight. Phytopathology 11:340-342.
12. Kennedy, B. W. 1959. Detection and distribution of *Pseudomonas glycinea* in soybean. Phytopathology 59:189-192.
13. Kennedy, B. W., and Tachibana, H. 1973. Bacterial diseases. Pages 491-504 in: Soybeans: Improvement, Production, and Uses. B. E. Caldwell, ed. American Society of Agronomy, Madison, WI.
14. Kent, G. C. 1945. A study of soybean diseases and their control. Pages 221-222 in: Rep. Agric. Res., Iowa Agric. Exp. Stn. 30 June 1945.
15. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Med. 44:301-307.
16. Leben, C. 1972. The development of a selective medium for *Pseudomonas glycinea*. Phytopathology 72:674-676.
17. Schroth, M. N., Thomson, S. V., and Weinhold, A. R. 1979. Behavior of plant pathogenic bacteria in rhizosphere and nonrhizosphere soils. Pages 105–156 in: Ecology of Root Pathogens. S. V. Krupa and Y. R. Dommergues, eds. Elsevier, Amsterdam.
18. Sinclair, J. B. 1982. Compendium of Soybean Diseases. 2nd ed. Pages 3-4, American Phytopathological Society, St. Paul, MN.
19. Williams, D. J., and Nyvall, R. F. 1980. Leaf infection and yield losses caused by brown spot and bacterial blight diseases of soybean. Phytopathology 70:900-902.