Ecology and Epidemiology

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

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**ABSTRACT**


Overwintering of *Pseudomonas syringae* pv. *glycinea* (PSG) associated with soybean leaves in the field was evaluated with a streptomycin-rilampicin 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* (Cooper) 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 Paraná 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 hampers 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 MM1 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
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 kept at room temperature for 2 days to equilibrate 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 × 10⁶, 3.4 × 10⁶, 4.1 × 10⁶, and 3.6 × 10⁶ cfu/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 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 I). 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 I). There were no significant (P < 0.05) differences between numbers of colonies recovered from the soil

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**Fig. I.** Colonies formed by isolate PRS-81 of Pseudomonas syringae pv. glycinea on King’s B agar medium containing streptomycin sulfate (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 X15.

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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.

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 x 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 PSF from soil enabled detection of a population as low as

![Fig. 2. Survival of Pseudomonas syringae pv. glycinea under soil moisture of 15% (— - — ), 25% (O— O), 35% (— — — ), and 45% (Δ— Δ) at -12, 4, and 12°C.](image-url)

**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**

<table>
<thead>
<tr>
<th>Date</th>
<th>Days after burial</th>
<th>Recovery of the bacterium at indicated depths below the soil surface (cm):b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Surface</td>
</tr>
<tr>
<td>13 Nov 1981</td>
<td>3</td>
<td>1.86 x 10⁸</td>
</tr>
<tr>
<td>11 Dec 1981</td>
<td>11</td>
<td>6.17 x 10⁸</td>
</tr>
<tr>
<td>5 Jan 1982</td>
<td>56</td>
<td>2.63 x 10⁸</td>
</tr>
<tr>
<td>1 Feb 1982</td>
<td>83</td>
<td>7.94 x 10⁸</td>
</tr>
<tr>
<td>10 Mar 1982</td>
<td>120</td>
<td>1.26 x 10⁶</td>
</tr>
<tr>
<td>15 Apr 1982</td>
<td>156</td>
<td>ND</td>
</tr>
<tr>
<td>23 Nov 1982</td>
<td>6</td>
<td>1.00 x 10⁸</td>
</tr>
<tr>
<td>23 Dec 1982</td>
<td>36</td>
<td>3.39 x 10⁸</td>
</tr>
<tr>
<td>26 Jan 1983</td>
<td>64</td>
<td>5.75 x 10⁷</td>
</tr>
<tr>
<td>28 Feb 1983</td>
<td>97</td>
<td>9.33 x 10⁷</td>
</tr>
<tr>
<td>24 Mar 1983</td>
<td>121</td>
<td>5.89 x 10⁷</td>
</tr>
<tr>
<td>2 May 1983</td>
<td>162</td>
<td>ND</td>
</tr>
</tbody>
</table>

aInfect leaf tissue mixed with soil was placed on the soil surface and at 10, 20, and 30 cm below the surface.
bNumber of colony-forming units (cfu) per 100 cc of the leaf-soil mixture.
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**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Depths (cm)</th>
<th>Maximum</th>
<th>Minimum</th>
<th>Average</th>
<th>Average daily range²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1981–1982</td>
<td>Surface</td>
<td>19.4</td>
<td>-20.6</td>
<td>-1.0</td>
<td>5.6 (0–19)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>12.2</td>
<td>-17.2</td>
<td>-1.7</td>
<td>2.6 (0–13)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>9.4</td>
<td>-7.8</td>
<td>0.0</td>
<td>0.7 (0–4)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>8.9</td>
<td>-6.1</td>
<td>0.1</td>
<td>0.7 (0–3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trial</th>
<th>Depths (cm)</th>
<th>Maximum</th>
<th>Minimum</th>
<th>Average</th>
<th>Average daily range²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982–1983</td>
<td>Surface</td>
<td>20.6</td>
<td>-7.2</td>
<td>6.9</td>
<td>5.8 (0–16)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>18.3</td>
<td>-2.8</td>
<td>4.4</td>
<td>2.1 (0–14)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>13.9</td>
<td>-3.9</td>
<td>2.7</td>
<td>1.2 (0–7)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>15.6</td>
<td>-4.4</td>
<td>2.1</td>
<td>1.6 (0–7)</td>
</tr>
</tbody>
</table>

²Temperatures 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.

²Average daily temperature range, and (in parentheses) the range of daily temperature ranges.
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 8.0 x 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 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 these fluctuations 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 depth. 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 soil surface. 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 PSE 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. PSE, 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 PSE 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 PSE.

The dilution plate method, using a streptomycin-rifampicin resistant mutant, gave reliable estimates of soil population levels of PSE when the number of bacteria was greater than 10^3-10^4 CFU/g of soil. For PSE 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 PSE 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 PSE 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 PSE 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 PSE in nature. The effect of resistance to antibiotics on survival of PSE in soil needs to be further studied. Considering technical limitations in detecting soil populations of PSE and the great influence of soil temperature and moisture on its survival, we conclude that PSE associated with soybean leaf tissue could overwinter on the soil surface in central Illinois if weather is cold and dry.

**LITERATURE CITED**


