# Bacterial Blight of Soybeans as Influenced by Populations of Yellow Bacteria on Leaves and Buds

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ABSTRACT

A bacterium characteristic of a group of yellow bacteria was isolated from soybean leaves infected with *Pseudomonas glycinea* and designated as YB-3. It is a small, gram-negative rod that has a polar flagella, is highly motile, oxidase-positive, and produces a light yellow, water insoluble pigment. When *P. glycinea* was mixed with YB-3 at a 1:9 ratio and inoculated onto soybean leaves, lesion development of bacterial blight was inhibited; at a 1:4 ratio, there was ca. 50% reduction in symptoms, and at a 1:1 ratio there was no reduction in lesion development. By incubating the 1:4 mixture of *P. glycinea*:YB-3 24-48 hr before inoculating the leaves, complete inhibition of bacterial blight symptoms resulted.

Population levels of *P. glycinea* and YB-3 were assayed 1, 2, 3, 7, and 14 days after atomizing mixtures of them onto uninjured leaf surfaces. Seven days after application of *P. glycinea*:YB-3 at a 1:9 ratio there was a sharp reduction in the number of *P. glycinea* cells; whereas when a 1:1 mixture of *P. glycinea* and YB-3 or *P. glycinea* alone was applied, the number of *P. glycinea* cells increased. Numbers of YB-3 increased slightly through day 3 but dropped sharply thereafter.

When mixtures of *P. glycinea*: YB-3 at a 1:9 ratio were placed on soybean buds, YB-3 multiplied more rapidly than did *P. glycinea*. From day 3 throughout the remainder of the test period, the number of YB-3 cells remained at the same level but *P. glycinea* cell numbers decreased.

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*Additional key words: Pseudomonas glycinea*, biological control.

The importance of the resident phase in which bacteria colonize a plant but do not produce disease symptoms has been investigated by many workers (2, 3, 4, 5). This paper reports a study of the effect of a group of yellow bacteria, isolated from soybean [*Glycine max* (L.) Merr.] leaves infected with *Pseudomonas glycinea* Coerper, on the development of bacterial blight. Objectives of the study were to determine: (i) the degree of inhibition of bacterial blight by the yellow bacteria; (ii) the population changes of yellow bacteria and *P. glycinea*; and (iii) the interactions of the resident phases of these bacteria.

MATERIALS AND METHODS.—Plants of the soybean cultivar 'Clark 63' used for inoculations were grown in the greenhouse in steam-sterilized soil. The two youngest trifoliolate leaves on ca. 21-day-old plants were used for inoculation. *Pseudomonas glycinea* cultures were obtained through the courtesy of B. W. Kennedy. Yellow bacteria used in this study were isolated from leaves of field-grown soybeans infected with *P. glycinea*. All isolates of the yellow bacteria had similar characteristics; i.e., small, gram-negative rods that passed through a 0.45-$\mu$m Millipore filter, polar flagella, light yellow, water insoluble pigment, highly motile, and oxidase-positive. Isolate YB-3, which is representative of the group, was isolated from leaves of field-grown Clark 63 infected with *P. glycinea*. Bacteria used for inoculations were obtained from 48-hr cultures grown on TYPA (0.1 M Tris buffer, pH 7.2; Bacto yeast extract, 0.3%; Bacto peptone, 0.06%; glucose, 0.3%; and Bacto-agar, 2.0%). Bacteria were suspended in 0.85% saline solution and adjusted to $10^8$ cells/ml initial concentration for all plant inoculation studies.

*Bacterial blight inhibition studies.—Pseudomonas glycinea* isolate R-6 was mixed with yellow bacteria isolate YB-3 at cell:cell ratios of 1:1, 1:4, and 1:9 and immediately applied by means of a sterile cheesecloth pad to Carborundum-dusted soybean leaves. *Pseudomonas putida* (Trevisan) Migula obtained as ATCC No. 12633 was mixed with *P. glycinea* in the same ratios and *P. glycinea* tested alone was diluted with 0.85% saline to obtain approximate cell concentrations. The *P. putida* – *P. glycinea* mixtures and *P. glycinea* alone inoculated onto soybean leaves served as controls. Each treatment was replicated four times with three plants per replication in two experiments. Bacterial blight severity ratings were taken 7 days after the plants were inoculated. Ratings were made according to a scale for which the values 0, 1, 2, 3, and 4, indicated, respectively, no symptoms, 1-25%, 26-50%, 51-75%, and 76-100% of the inoculated leaf area with lesions.

In a second experiment, the effect on development of bacterial blight of incubating *P. glycinea* and YB-3 mixtures prior to inoculating the soybean plants was tested. The same inoculation procedures, number of replicated experiments, and bacterial cell ratios were used except that the mixtures were incubated for 4, 24, and 48 hr at 24 C prior to inoculating the leaves. Immediately before each mixture was rubbed onto the leaves a 1.0-ml sample was removed and live cell counts of *P.*

*glycinea* and YB-3 were made by the dilution plate method.

*Assay of bacterial growth on leaf surfaces.*—To monitor the population changes of *P. glycinea* and yellow bacteria on the leaf surface, an inoculation and assay procedure developed by Mew & Kennedy (7) was used. *Pseudomonas glycinea* and YB-3 were mixed at ratios of 1:1 and 1:9 for the inoculations; comparable concentrations for *P. glycinea* alone also were prepared. The leaf surfaces were gently sprayed with the bacteria by means of an atomizer that produces a fine spray; care was taken to avoid any injury to the leaves. Disease symptoms developed on less than 1% of the leaves and assays were made only on symptomless leaves. A random sample of 10 leaves for each treatment was assayed 0, 1, 2, 3, 7, and 14 days after the spray was applied and this experiment was conducted three times. Leaves were washed for 1 hr by shaking them in 100 ml of sterile water containing 0.001% Tween 80. Bacterial numbers were determined by the dilution plate method on Kado & Heskett's medium D selective for *Pseudomonas* spp. (1) as modified by Mew & Kennedy (7) in which 3% sucrose and 0.0001% crystal violet were added.

*Assay of bacterial growth on buds.*—Buds of soybean plants were inoculated by placing a 0.05-ml drop of the bacterial suspension on the bud shortly after the primary leaves had opened. Extreme care was taken in placing the droplet on the bud to avoid injury to the plant surface. The inoculation procedure used was similar to one developed by Leben et al. (6). Seedlings used for inoculations were grown from a seed lot of Clark 63 that was found to be free of *P. glycinea*; few of the usual resident bacteria were present. The same cell ratios of *P. glycinea*:YB-3, assay procedure, and number of replicated experiments were used except that the initial concentration of bacteria was $10^7$ cells/ml for each isolate. All buds assayed were symptomless. Fourteen days after inoculation, buds from the different treatments were ground with a mortar and pestle and assayed for virulent *P. glycinea* on soybean leaves. Inoculations were made by rubbing Carborundum-dusted leaves with a sterile cheesecloth pad that had been dipped in the inoculum. Ten buds from each treatment were assayed. None of the buds assayed showed any bacterial blight symptoms.

RESULTS.—*Inhibition of bacterial blight by YB-3.*—Isolate YB-3 mixed with *P. glycinea* and inoculated onto soybean leaves resulted in a reduction of bacterial blight symptoms as compared to inoculation with *P. glycinea* alone (Table 1). No inhibition of bacterial blight lesions occurred when the commonly occurring saprophyte *P. putida* was mixed with *P. glycinea* at the same ratios as those used in testing YB-3, indicating that *P. putida*, unlike YB-3, did not inhibit disease development.

When the mixtures of *P. glycinea* and YB-3 were incubated prior to leaf inoculation, the longer the incubation period the greater the effectiveness of YB-3 in inhibiting blight for *P. glycinea*:YB-3 ratios of 1:4 and 1:9; at a 1:1 ratio, however, YB-3 was ineffective (Table 2). Assay of bacteria at the time of inoculation showed a 10-28% decrease in the number of viable cells of *P. glycinea* and no change in the cell numbers of YB-3 in the *P. glycinea*:YB-3 ratios of 1:4 and 1:9 for 4, 24, and 48 hr. No change occurred in cell numbers of either *P. glycinea* or YB-3 at a 1:1 ratio.

*Changes in population of bacteria on the leaf surface.*—There was an initial increase in the cell numbers of both *P. glycinea* and YB-3 on the uninjured leaf surface (Fig. 1-A, C). Both *P. glycinea* and YB-3 increased at approximately the same rate; after 7 days, however, the population levels of both dropped (Fig. 1-C). Bacterial blight development was greatly inhibited when *P. glycinea*:YB-3 at a ratio of 1:9 was rubbed onto Carborundum-dusted leaves (Table 1). YB-3 showed the same pattern of increase for both ratios (Fig. 1-B, C) but the population level at 3 days was slightly higher for the 1:9 ratio of *P. glycinea*:YB-3 (Fig. 1-C).

TABLE 1. Disease severity of bacterial blight of soybean leaves (cultivar 'Clark 63') inoculated with different ratios of *Pseudomonas glycinea* and test bacteria

| Bacteria | Disease severity[a,b,c] | | |
|---|---|---|---|
| | Ratio of *P. glycinea* to test bacteria | | |
| | 1:1 | 1:4 | 1:9 |
| *P. glycinea* + YB-3 | 3.5 ab | 1.7 c | 0.8 d |
| *P. glycinea* + *P. putida* | 3.7 ab | 3.5 ab | 3.1 b |
| *P. glycinea* + saline | 3.8 a | 3.2 ab | 3.0 b |

[a] Rating scale: 0 = no symptoms; 1, 2, 3, and 4 = 1-25, 26-50, 51-75, and 76-100%, respectively, of the inoculated leaf area with lesions.

[b] Each value is the mean of the disease severity rating for 24 plants in each treatment.

[c] Values with different letters are statistically different at the 5% level (Duncan's Multiple Range Test).

TABLE 2. Effect on bacterial blight of soybean leaves (cultivar 'Clark 63') of incubating mixtures of *Pseudomonas glycinea* and YB-3 prior to inoculation of the plants

| Preinoculation incubation (hr) | Disease severity[a,b,c] | | | |
|---|---|---|---|---|
| | *P. glycinea* and saline | Ratio of *P. glycinea* to YB-3 | | |
| | 1:1 | 1:1 | 1:4 | 1:9 |
| 0 | 3.8 a | 3.7 a | 1.7 b | 0.7 c |
| 4 | 3.8 a | 3.6 a | 0.5 cd | 0.4 d |
| 24 | 3.5 a | 3.6 a | 0.4 d | 0.2 d |
| 48 | 3.6 a | 3.5 a | 0.2 d | 0.2 d |

[a] Rating scale: 0 = no symptoms; 1, 2, 3, and 4 = 1-25, 26-50, 51-75, and 76-100%, respectively, of the inoculated leaf area with lesions.

[b] Each value is the mean of the disease severity rating for 24 plants in each treatment.

[c] Values with different letters are statistically different at the 5% level (Duncan's Multiple Range Test).

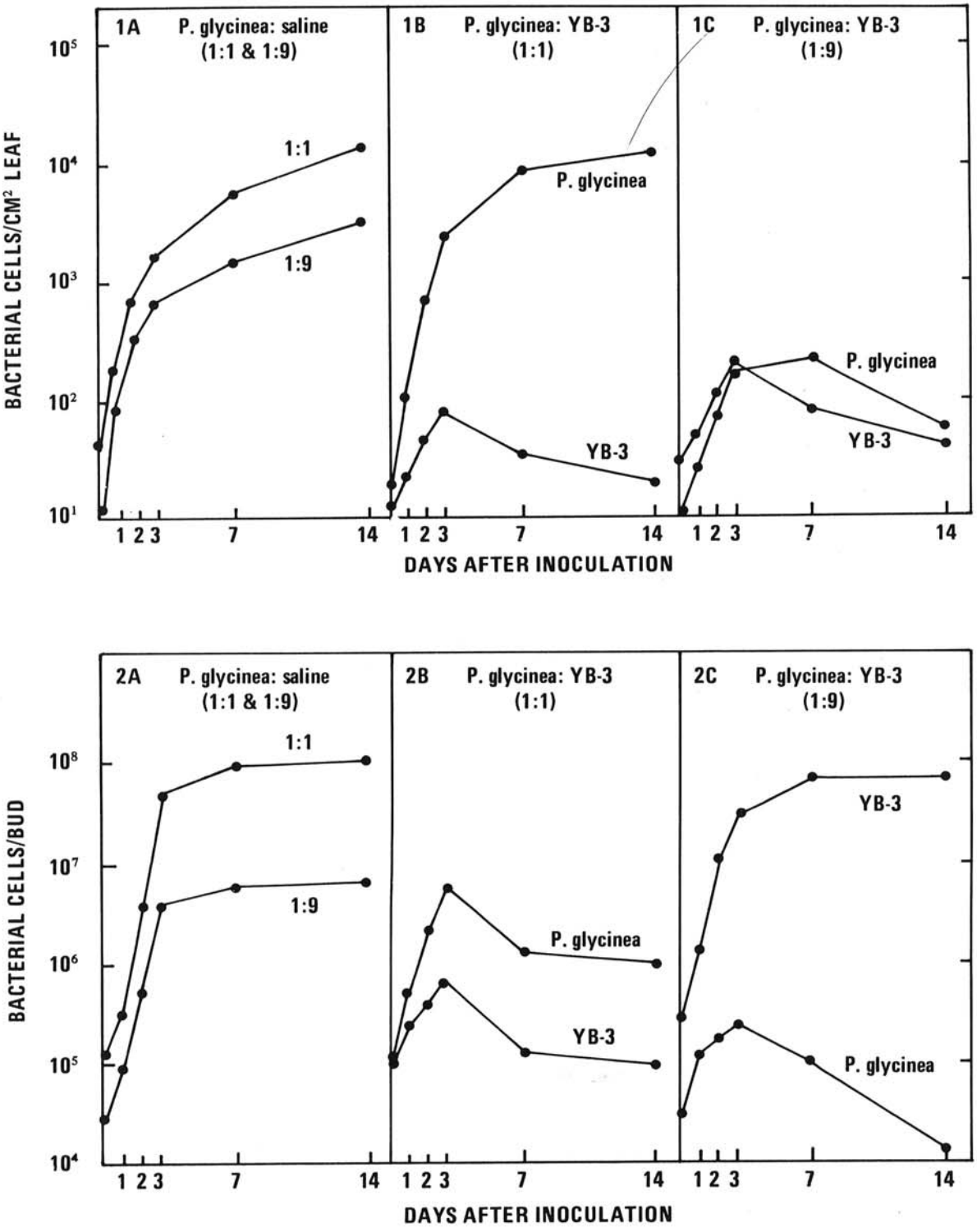


Fig. 1-2. 1) Growth on soybean (cultivar 'Clark 63') leaf surfaces of *Pseudomonas glycinea* and YB-3, a saprophytic yellow bacterium isolated from soybean leaf surfaces: (A) *Pseudomonas glycinea*:saline ratios of 1:1 and 1:9; (B) *Pseudomonas glycinea*:YB-3 ratio of 1:1; (C) *Pseudomonas glycinea*:YB-3 ratio of 1:9. **2)** Population changes of *Pseudomonas glycinea* and YB-3 on soybean (cultivar Clark 63) buds: (A) *Pseudomonas glycinea*:saline ratios of 1:1 and 1:9; (B) *Pseudomonas glycinea*:YB-3 ratio of 1:1; (C) *Pseudomonas glycinea*:YB-3 ratio of 1:9.

*Changes in population of bacteria on the bud.*—Population levels of *P. glycinea* and YB-3 on the buds changed at rates different than those for populations on the leaf surfaces. The number of *P. glycinea* cells remained constant after 3 days on buds (Fig. 2-A), whereas numbers continued to increase on the leaf surface (Fig. 1-A). *Pseudomonas glycinea* and YB-3 at a 1:1 ratio on buds reached population peaks at 3 days, and then dropped slightly (Fig. 2-B). This was in contrast to the development on the leaf surface where cell numbers of *P. glycinea*, whether in combination with YB-3 (Fig. 1-B) or alone (Fig. 1-A), continued to increase throughout the 14-day period. On buds inoculated with *P. glycinea*:YB-3 at a ratio of 1:9, cell numbers of YB-3 increased sharply to day 3 and then stabilized (Fig. 2-C), which is similar to the pattern exhibited for buds inoculated with *P. glycinea* alone (Fig. 2-A).

Because the number of *P. glycinea* cells decreased from that in the 1:9 mixture of *P. glycinea* and YB-3 placed on the bud initially (Fig. 2-C), buds from all treatments were assayed 14 days after inoculation for bacteria capable of producing bacterial blight lesions. None of the leaves inoculated with preparations from buds inoculated with *P. glycinea*:YB-3 at a ratio of 1:9 developed lesions. On the other hand, 100, 100, and 70% of the leaves inoculated with preparations from buds inoculated with *P. glycinea*:saline at a ratio of 1:1 and 1:9, and with *P. glycinea*:YB-3 ratio of 1:1 respectively, developed lesions.

DISCUSSION.—These studies indicate that within the parameters set up for this investigation, interaction of YB-3 with *P. glycinea* resulted in inhibition of development of bacterial blight. Increase of incubation time of the *P. glycinea*:YB-3 mixture prior to inoculation increased the effectiveness of blight inhibition when the ratio was 1:4 or 1:9 but had virtually no effect when the mixture was 1:1. When the *P. glycinea*:YB-3 ratio of 1:4 was used for inoculation immediately after mixing, reduced symptom development about 50%, but an incubation period of 48 hr after mixing almost totally inhibited symptom development. In contrast when the *P. glycinea*:YB-3 ratio of 1:1 was inoculated immediately after mixing, no symptom reduction was evident and a 48-hr incubation period resulted in no increased inhibition. Apparently a certain threshold ratio of YB-3 to *P. glycinea* is necessary to get inhibition of bacterial blight and if this threshold is not attained, increasing the incubation time does not effect blight inhibition.

YB-3 apparently is antagonistic toward *P. glycinea* since the number of viable cells of *P. glycinea* decreased 10-28% for the *P. glycinea*:YB-3 ratios of 1:4 and 1:9. No change was noted in the viability of YB-3 or *P. glycinea* cells for the 1:1 mixture.

Mew & Kennedy (7) have demonstrated that when *P. glycinea* was sprayed gently onto soybean leaves to avoid injury to the leaf surface, 1,000-fold increases in cell numbers occurred on susceptible leaves, whereas numbers were unchanged or declined on leaf surfaces of resistant cultivars. On plants intermediate in susceptibility *P. glycinea* first increased in numbers then declined. In my studies, in which *P. glycinea* was placed only on susceptible leaves, the populations of *P. glycinea* increases similarly when tested in combination with YB-3 or alone (Fig. 1-A, B). When the ratio of *P. glycinea*:YB-3 was 1:4 or 1:1 no inhibition occurred but at a ratio of 1:9 bacterial blight was inhibited (Table 1) and *P. glycinea* cell numbers decreased (Fig. 1-C) in much the same way as Mew & Kennedy determined for plants intermediate in susceptibility (7).

Leben et al. (6) have shown that *P. glycinea* can multiply on soybean buds but the buds do not become diseased. The data presented in this paper substantiate their findings. YB-3 apparently competes better with *P. glycinea* on buds than on the surface of expanded leaves as evidenced by the maintenance of higher population levels of YB-3 on buds than on leaves. Isolations from field-grown soybean plants indicated that the group of yellow bacteria represented by YB-3 is one of the most common epiphytic bacteria inhabiting soybean buds (*unpublished data*).

There may be many resident bacteria, which like YB-3 colonize both leaves and buds and under environmental conditions favorable for growth increase in population relative to other bacteria. It is possible that bacteria similar to YB-3 may determine whether pathogenic bacteria such as *P. glycinea* remain in the resident phase or cause disease. If conditions on the plant surface can be manipulated to shift the balance in favor of multiplication of nonpathogenic bacteria, we may be able to reduce the severity of a particular bacterial disease.

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