# Effects of Individual and Multiple Infections with Three Bacterial Pathogens on Disease Severity and Yield of Soybeans

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**ABSTRACT**

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Bacterial blight, bacterial pustule, and wildfire resulting from multiple inoculations with individual or mixed cultures had no effect on yield of three soybean (*Glycine max*) cultivars (Pella, Williams, and Cumberland or Wells) in 1986 and 1987. In both years, disease severity at each rating and area under the disease progress curve (AUDPC) of Pella, which is susceptible to *Xanthomonas campestris* pv. *glycines*, causal agent of bacterial pustule, were highest in the plots inoculated with either *X. c. glycines* alone or combination with the other pathogens, *Pseudomonas syringae* pv. *glycinea* (bacterial blight) and *P. syringae* pv. *tabaci* (wildfire). The severity of individual diseases was not affected by mixed inoculations with the other pathogens; the pathogens did not act synergistically. Effects of individual and multiple diseases on yield were not statistically significant.

Bacterial pustule, bacterial blight, and wildfire are the most common bacterial diseases of soybeans (*Glycine max* (L.) Merr.) (6). Bacterial pustule, caused by *Xanthomonas campestris* pv. *glycines* (Nakano) Dye, is characterized by formation on abaxial surfaces of leaves of small pustules surrounded by a chlorotic halo. Bacterial blight, caused by *Pseudomonas syringae* pv. *glycinea* (Coerper) Young et al, develops on leaves as small, angular, translucent, water-soaked, yellow to light brown spots that turn reddish brown and become surrounded by a water-soaked margin bordered by a chlorotic halo. Centers of older leaves frequently drop out or tear away. In the earlier stages of symptom development, bacterial blight may resemble bacterial pustule. However, pustules are not associated with water-soaking and have a raised corky appearance. Lesions of wildfire, caused by *P. s. tabaci* (Wolf and Foster) Young et al, are necrotic spots of variable size and shape that are surrounded by a wide and sharply delineated yellow halo (1). In severely diseased leaves, lesions coalesce and tend to form large necrotic areas that eventually break apart to produce a tattered effect. Bacterial pustule and bacterial blight lesions are reported to be natural infection courts for the wildfire bacterium; the incidence of wildfire is associated with the development of the two diseases (2).

Bacterial pustule has been reported to affect soybean yield (5,8,16). Losses attributable to bacterial pustule were 4.3% in susceptible soybean lines compared with controls (16). Susceptible soybean lines yielded 11% less than resistant lines when plants were inoculated with *X. c. glycines* (5). In contrast, yield appeared to be stimulated with severe disease of bacterial pustule (8). Yield reductions of 17.9% attributable to bacterial blight, 17% attributable to brown spot, and 14.1% attributable to both diseases combined were observed (17). Yield reductions of 5–15% from bacterial blight have been observed in cv. Wells II (11). However, Daft and Leben (3) reported that bacterial blight did not cause yield losses in Ohio. The effect of wildfire on soybean yield has not been reported. Information on the effects of multiple bacterial diseases on yield has not been reported. The objective of this study was to evaluate the effect of individual and multiple infections by three bacterial pathogens on disease severity and yield of soybeans.

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## MATERIALS AND METHODS

**Experimental plots and soybean cultivars.** Experiments were done in 1986 and 1987 at the Agronomy/Plant Pathology Farm, Urbana, IL, on a Drummer silty clay loam soil that had been planted to corn in the previous year. Three cultivars were chosen, based on their reactions to the bacterial pathogens used in this study. In 1986, Williams, Pella, and Cumberland were used. Williams is resistant to *X. c. glycines*, and Pella is susceptible to *X. c. glycines* and *P. s. glycinea*. Cumberland is susceptible to *P. s. glycinea* but resistant to *X. c. glycines*. Because Cumberland was not highly susceptible to *P. s. glycinea* in the field in 1986, it was replaced by Wells in 1987. Reactions of the three cultivars to *P. s. tabaci* were not known, but in the greenhouse, wildfire symptoms were observed on inoculated Pella.

**Inoculations.** A strain of *P. s. glycinea* (Pg61) was isolated from the naturally

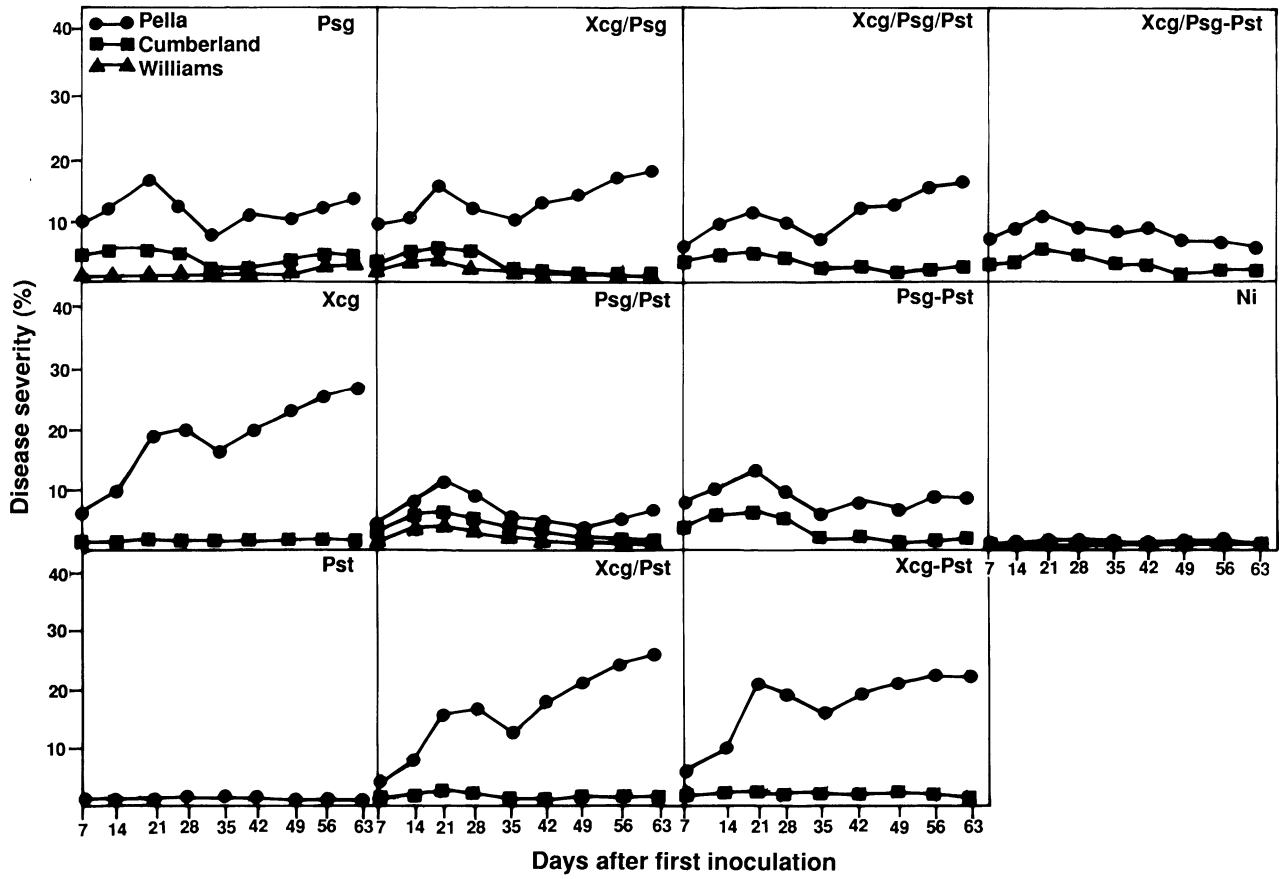


**Fig. 1.** Disease development on soybean cultivars Pella, Williams, and Cumberland inoculated with *Pseudomonas syringae* pv. *glycinea* (Psg), *Xanthomonas campestris* pv. *glycines* (Xcg), or *P. s. tabaci* (Pst) alone; *X. c. glycines* and *P. s. glycinea*; *P. s. glycinea* and *P. s. tabaci*; *X. c. glycines* and *P. s. tabaci*; *X. c. glycines*, *P. s. glycinea*, and *P. s. tabaci*; *P. s. glycinea* followed by *P. s. tabaci*; *X. c. glycines* followed by *P. s. tabaci*; *X. c. glycines* and *P. s. glycinea* followed by *P. s. tabaci*; and natural infection (Ni) in 1986.

**Table 1.** The area under the disease progress curve (AUDPC)[a] of disease development and yield of soybean cultivars Pella, Williams, and Cumberland in 1986

| | Pella | | Williams | | Cumberland | |
|---|---|---|---|---|---|---|
| Treatment | Yield (kg/ha) | AUDPC | Yield (kg/ha) | AUDPC | Yield (kg/ha) | AUDPC |
| BB[b] | 192.1 | 636.2 | 148.2 | 103.8 | 175.0 | 190.2 |
| BP[c] | 197.4 | 912.1 | 147.3 | 0.0 | 174.5 | 64.5 |
| WF[d] | 188.3 | 62.5 | 152.3 | 0.0 | 179.2 | 0.0 |
| BB/BP[e] | 192.1 | 717.5 | 144.3 | 149.8 | 169.6 | 163.6 |
| BB/WF | 174.2 | 383.0 | 131.1 | 141.9 | 164.2 | 204.5 |
| BP/WF | 180.9 | 1,002.0 | 149.9 | 0.0 | 174.1 | 49.4 |
| BB/BP/WF | 183.3 | 657.6 | 142.6 | 119.2 | 164.0 | 166.8 |
| BB-WF[f] | 190.8 | 583.6 | 147.8 | 198.3 | 176.4 | 175.8 |
| BP-WF | 187.4 | 1,080.9 | 133.9 | 0.0 | 184.0 | 72.8 |
| BB/BP-WF | 191.1 | 526.3 | 135.7 | 149.0 | 179.0 | 160.6 |
| NI[g] | 197.8 | 25.4 | 131.7 | 0.0 | 168.0 | 26.4 |
| FLSD[h] ($P = 0.05$) | | 738.5 | | 161.8 | | 164.4 |

[a] AUDPC = $\Sigma[(Y_{i+1} + Y_i)/2][X_{i+1} - X_i]$, $Y_i$ = the proportion of diseased leaf area at the $i$th observation, $X_i$ = days of the $i$th observation, $n$ = total number of observations, $i$ = the day of rating.
[b] Inoculated with *Pseudomonas syringae* pv. *glycinea*.
[c] Inoculated with *Xanthomonas campestris* pv. *glycines*.
[d] Inoculated with *Pseudomonas syringae* pv. *tabaci*.
[e] / = Inoculated together.
[f] - = Inoculated with *P. s. tabaci* 10 days after.
[g] Natural infection.
[h] Fisher's protected least significant difference between two different means.

diseased Gnome soybean at Urbana, IL, in 1985. Strain 8ra of *X. c. glycines* was obtained from E. J. Braun, Department of Plant Pathology, Iowa State University, Ames, IA. Strain PT15 of *P. s. tabaci* was obtained from P. D. Shaw, Department of Plant Pathology, University of Illinois, Urbana. All bacteria were stored in 15% glycerol at −70 C during 1986–1987. Inoculum was prepared from 2-day-old cultures of the three pathogens grown on King's medium B (7) or yeast extract-dextrose-calcium carbonate medium (YDC) (13). Bacterial cells were suspended in distilled water, and the concentration was adjusted turbidimetrically to $10^8$ cells per milliliter. Soybean plants were sprayed with the bacterial suspension at a pressure of 5 kg/cm$^2$ until runoff. Inoculation dates and growth stages (4) in 1986 were 11 June (V3 growth stage), 23 June (V5 growth stage), and 22 July (R3 growth stage). In 1987, the inoculation dates were 11 June (V4 growth stage), 26 June (V6 growth stage), and 20 July (R3 growth stage). Postinoculation of *P. s. tabaci* was done 10 days after inoculation with the other pathogens, and plants that were not inoculated with the pathogen were sprayed with water.

**Experimental design.** The experiment was a split-plot design arranged in randomized complete blocks with four replicates. Cultivars were whole plots with inoculations (treatments) as subplots. Treatments consisted of 11 inoculations: *X. c. glycines*, *P. s. glycinea*, or *P. s. tabaci* alone; *X. c. glycines* and *P. s. glycinea*; *P. s. glycinea* and *P. s. tabaci*; *X. c. glycines* and *P. s. tabaci*; *X. c. glycines*, *P. s. glycinea*, and *P. s. tabaci*; *P. s. glycinea* followed by

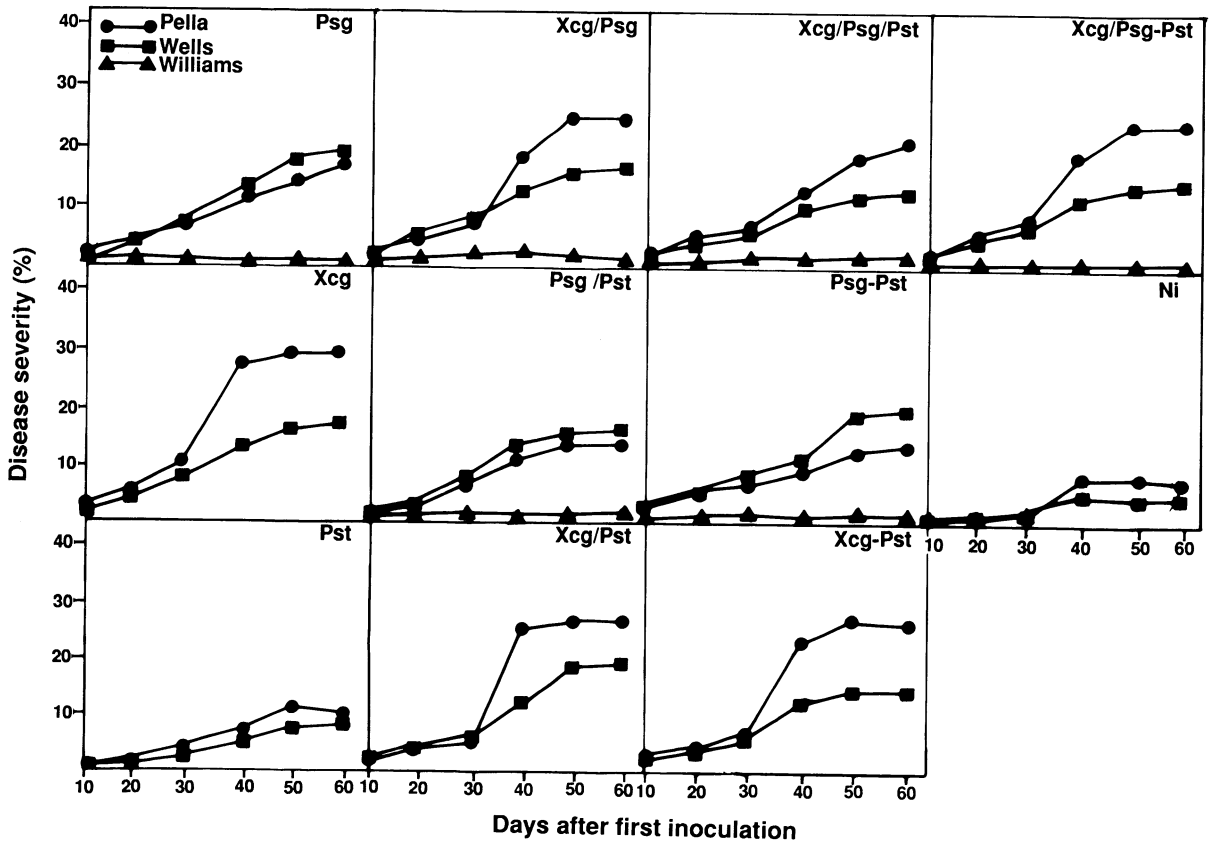


**Fig. 2.** Disease development on soybean cultivars Pella, Williams, and Cumberland inoculated with *Pseudomonas syringae* pv. *glycinea* (Psg), *Xanthomonas campestris* pv. *glycines* (Xcg), or *P. s. tabaci* (Pst) alone; *X. c. glycines* and *P. s. glycinea*; *P. s. glycinea* and *P. s. tabaci*; *X. c. glycines* and *P. s. tabaci*; *X. c. glycines*, *P. s. glycinea*, and *P. s. tabaci*; *P. s. glycinea* followed by *P. s. tabaci*; *X. c. glycines* followed by *P. s. tabaci*; *X. c. glycines* and *P. s. glycinea* followed by *P. s. tabaci*; and natural infection (Ni) in 1987.

*P. s. tabaci*; *X. c. glycines* followed by *P. s. tabaci*; *X. c. glycines* and *P. s. glycinea* followed by *P. s. tabaci*; and natural infection. For mixed inoculation, final bacterial cells of each pathogen were adjusted to $10^8$ cells per milliliter. Each subplot consisted of six rows 6.1 m long and 76 cm apart. Seeds were planted at 27 per meter of row on 13 May in both years. The center two rows were inoculated, and two remaining rows on each side were not inoculated to reduce interplot interference.

**Disease and yield assessment.** Disease severity was rated nine times in 1986 and six times in 1987 about 7–12 days apart. Disease severity, portion of diseased area from total leaf area, was rated by a modified Horsfall-Barratt scale, then converted into percent severity (11). The area under the disease progress curve (AUDPC) (14) was calculated from the combined severity data of the individual diseases. Two center rows of each plot were trimmed to 4.6 m long, and soybeans were harvested from the trimmed rows. The yield and 300-seed weight were adjusted to the basis of 1.3 g/kg of moisture content. Results from 1986 and 1987 were analyzed separately because different soybean cultivars were used. Analysis of variance was performed to determine effects of the diseases on soybean yield.

**Table 2.** The area under the disease progress curve (AUDPC)[a] of disease development and yield of soybean cultivars Pella, Williams, and Cumberland in 1987

| | Pella | | Williams | | Cumberland | |
|---|---|---|---|---|---|---|
| Treatment | Yield (kg/ha) | AUDPC | Yield (kg/ha) | AUDPC | Yield (kg/ha) | AUDPC |
| BB[b] | 187.5 | 341.3 | 176.2 | 16.1 | 213.0 | 458.1 |
| BP[c] | 186.8 | 906.5 | 178.9 | 0.0 | 215.4 | 446.6 |
| WF[d] | 181.4 | 308.1 | 173.6 | 0.0 | 217.1 | 201.8 |
| BB/BP[e] | 197.1 | 650.9 | 176.1 | 44.3 | 203.6 | 415.3 |
| BB/WF | 195.3 | 328.3 | 178.6 | 18.1 | 262.0 | 356.0 |
| BP/WF | 190.9 | 750.0 | 174.0 | 0.0 | 207.9 | 418.0 |
| BB/BP/WF | 188.5 | 503.1 | 160.0 | 13.6 | 213.9 | 426.6 |
| BB-WF[f] | 200.0 | 346.4 | 173.2 | 8.3 | 206.0 | 508.6 |
| BP-WF | 190.1 | 852.2 | 172.2 | 0.0 | 208.9 | 424.6 |
| BB/BP-WF | 184.0 | 759.7 | 184.6 | 2.8 | 209.5 | 446.9 |
| NI[g] | 199.2 | 302.1 | 176.9 | 0.0 | 201.2 | 215.2 |
| FLSD[h] ($P = 0.05$) | | 475.8 | | 28.0 | | 215.3 |

[a] AUDPC = $\Sigma[(Y_{i+1} + Y_i)/2][X_{i+1} - X_i]$, $Y_i$ = the proportion of diseased leaf area at the $i$th observation, $X_i$ = days of the $i$th observation, $n$ = total number of observations, $i$ = the day of rating.
[b] Inoculated with *Pseudomonas syringae* pv. *glycinea*.
[c] Inoculated with *Xanthomonas campestris* pv. *glycines*.
[d] Inoculated with *Pseudomonas syringae* pv. *tabaci*.
[e] / = Inoculated together.
[f] - = Inoculated with *P. s. tabaci* 10 days after.
[g] Natural infection.
[h] Fisher's protected least significant difference between two different means.

## RESULTS

**Disease development.** Bacterial pustule and bacterial blight symptoms appeared about 7 days after inoculation, but wildfire symptoms were not observed. Leaves of Pella and Wells inoculated with *X. c. glycines* and *P. s. glycinea* at a V3 growth stage (4) were defoliated. Spread to upper leaves was slow but occurred after rain.

In 1986, bacterial pustule and bacterial blight increased on Pella for 2–3 wk after

the initial inoculation and then decreased until after the second and third inoculations (Fig. 1). Bacterial blight severity on Cumberland ranged from 2 to 7%. Disease severity of bacterial blight and bacterial pustule from natural infections on Pella was about 2–4% and at trace levels on Williams and Cumberland (Fig. 1). The highest disease severity was observed when Pella was inoculated with *X. c. glycines*, and the highest AUDPC value was obtained from Pella inoculated with *X. c. glycines* and *P. s. tabaci* (Table 1). Direct effects of bacterial blight and bacterial pustule on wildfire symptom expression and development were not detected in the field. Bacterial blight and bacterial pustule development were greater in 1987 than in 1986. Disease severity on Pella and Wells increased significantly 2 wk after the first inoculation (Fig. 2). The highest disease severity and the highest AUDPC value occurred on Pella inoculated with *X. c. glycines* (Table 2 and Fig. 2). Disease severity of bacterial blight and bacterial pustule by natural infections was about 8% on Pella and at trace levels on Williams and Wells (Fig. 2). In both years, disease severity caused by infections of the two pathogens did not differ from those caused by individual pathogens.

**Yield reduction.** Mean yields of inoculated plots of Pella in 1986 ranged from 0 to 11.7% lower when compared with yield from the check plot (Table 1), but they were not statisticallysignificant ($P = 0.05$). In 1987, yields from Pella were not significantly different among all treatments (Table 2). Yield reductions of Williams, Wells, and Cumberland inoculated with individual or combined bacteria of *P. s. glycinea*, *X. c. glycines*, and *P. s. tabaci*, were not significant in either year (Tables 1 and 2).

## DISCUSSION

Weather conditions in Urbana from early June to late August were similar in 1986 and 1987. Disease severity in 1987 generally was higher than in 1986. Disease severity in the check plot ranged from 2 to 9% and from 2 to 3% in 1987 and 1986, respectively, indicating that natural infection of bacterial blight and bacterial pustule in 1987 was greater than in 1986. The development of wildfire is known to be associated with lesions of bacterial blight and bacterial pustule (2). However, in this study, wildfire symptoms were not observed in any of the cultivars used in the field.

Bacterial blight and bacterial pustule developed mainly on the top of the canopy and spread very slowly to newly formed leaves. This resulted in a "horizontal layer pattern" (3) of bacterial blight and bacterial pustule development. Disease severity decreased beginning about 3 wk after the first inoculation. Because disease severity is a proportion of diseased leaf area to the total leaf area, this might be attributable to defoliation of inoculated leaves, slow spreading to the new leaves, or rapid growth of plants (11). For this reason, disease development could not be characterized by fitting asymptotic growth functions (9).

Bacterial blight and bacterial pustule did not affect yields of Pella and Wells, which are known to be susceptible to *P. s. glycinea* and *X. c. glycines*. High disease severity in the early growing season may not reduce yields because soybean plants compensate for the loss of photosynthetic area by rapid vegetative growth. In a simulated defoliation study, the loss of photosynthetic area during the vegetative growth stage did not affect yield, whereas if the loss occurred at the R3 growth stage, yields were reduced significantly (15). The highest disease severity after the R3 stage was about 28 and 41% on Pella inoculated with *X. c. glycines* in 1986 and 1987, respectively, resulting in no yield difference. This suggests that Pella may possess a degree of tolerance to the disease. Brown spot and bacterial blight of soybeans caused yield loss by reducing seed weight (11,12). In this study, synergistic effects among the two bacterial diseases were not observed, and their effects on yields and seed weights of the four cultivars used were not significant.

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