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# POPULATION BIOLOGY OF *BACILLUS CEREUS* UW85 IN THE RHIZOSPHERE OF FIELD-GROWN SOYBEANS

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**Summary**—We studied the spatial and temporal distribution and abundance of the biocontrol agent *Bacillus cereus* UW85 in the rhizosphere of soybeans during two growing seasons in the field. For this study, we used a neomycin-resistant mutant, UW85n1, derived from UW85. UW85n1 spores were applied to seeds and by 24 h after planting 72% of the spores had germinated. UW85n1 colonized emerging radicles, but populations were 10–50-fold smaller on radicles than they were on seeds at planting. UW85n1 population sizes in the rhizosphere of 10–35-day old plants were largest near the crown of the plant and smaller further from the crown. For example, in a 1989 experiment 21 days after planting, UW85n1 populations were 158-fold larger 0–2 cm below the crown than they were 7–8 cm below the crown. UW85n1 population sizes were largest right after planting, and by seed harvest they decreased to levels comparable to indigenous *B. cereus* populations on untreated roots; however, at harvest the entire *B. cereus* population on treated roots was neomycin resistant, whereas on untreated roots <3% was neomycin resistant. The proportion of the heterotrophic bacterial population that was represented by UW85n1 was generally largest right after planting and decreased at subsequent sampling times. Our results indicate that UW85n1 grew and spread on soybean roots, and it persisted in the rhizosphere until seed harvest.

## INTRODUCTION

*Bacillus cereus* UW85 reduces damping-off diseases of alfalfa (Handelsman *et al.*, 1990) tobacco (Handelsman *et al.*, 1991), and soybean (L. J. Halverson, unpubl. Ph. D. thesis, University of Wisconsin, 1991) caused by zoosporic Oomycete fungi, and it increases soybean nodulation (Halverson and Handelsman, 1991). Successful biocontrol and plant growth promotion by UW85 varies with location and year of the field trials (J. Handelsman and R. S. Smith, unpubl. data). Knowledge of its rhizosphere population biology will contribute to an understanding of the mechanisms of biocontrol and nodulation enhancement by UW85 and may suggest strategies to improve its efficacy.

In this paper, rhizosphere colonization is defined as the ability of an organism to spread from the source of inoculum (the seed) onto the root. Growth is defined as an increase in population size over time. Most studies of bacterial colonization and growth in the rhizosphere have focused on fluorescent pseudomonads and rhizobia under various field, greenhouse, and growth chamber conditions (Kloepper *et al.*, 1980; Weller, 1983; Mowad *et al.*, 1984; Loper *et al.*, 1985; Bahme and Schroth, 1987; Howie *et al.*, 1987; Scher *et al.*, 1988; Liddell and Parke 1989; Osburn *et al.*, 1989; Abaido *et al.*, 1990). Fewer studies have focused on rhizosphere colonization and growth of Gram-positive bacteria under controlled conditions (Dikstra *et al.*, 1987; Juhnke *et al.*, 1987; Maplestone and Campbell 1989; Reddy and Rahe 1989a, b), and little attention has been directed towards describing the spatial and temporal rhizosphere population biology of Gram-positive bacteria in the field.

Our objective was to describe the population biology of UW85 in the rhizosphere of soybeans grown in the field. The data show that UW85n1 spores germinated on seeds, and that UW85n1 colonized roots, persisted until seed harvest, and increased the abundance of heterotrophic bacteria in the rhizosphere.

## MATERIALS AND METHODS

### *Bacterial strains and bacterial seed treatments*

Strains used in this study included the wild-type *B. cereus* UW85 (ATCC 53522) and its spontaneous neomycin-resistant (UW85n1) and streptomycin-resistant (UW85s1) mutants. Soybean seeds (*Glycine max* L. Merr), cultivar AP-200 (Agripro Seeds, Ames, Iowa), were coated with a paste of UW85, UW85n1 or UW85s1 spores. To produce a spore paste, Trypticase soy agar (TSA, BBL Cockeysville, Md) plates were inoculated with 1.0 ml per plate of a mid-log-phase culture of *B. cereus* grown in half-strength Trypticase soy broth (TSB). After 4 days at 28°C, the lawn of spores was scraped off the plates; 25 soybean seeds were mixed with the resulting paste of spores from one plate of spores in a 50 ml conical centrifuge tube (VWR, Chicago, Ill.). Seeds were spread in a

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single layer and air-dried in a laminar flow hood. *B. cereus* populations on coated seeds varied from $9.7 \times 10^7$ to $7.4 \times 10^8$ cfu. For simplicity, we will refer to roots derived from treated seeds as "treated roots".

*Experimental design*

Experiments were performed at the University of Wisconsin Experimental Farms in Arlington, Wis. The Arlington site is situated on a Joy silt-loam soil (fine-silty, mixed, mesic aquic Hapludolls, pH 6.8). The 1987 and 1989 experiments were planted on 19 and 24 May, respectively. The design for the 1987 experiment was a randomized complete block with three seed treatments and three blocks. Seed treatments were UW85n1, UW85s1 and an untreated control. Seeds were spaced 15 cm apart in rows that were 7.6 m long and 76 cm apart. Seeds were planted at a depth of 2.5–4 cm. Sampling times were 7, 14, 21, 28, 35, 49, 63, 91 and 126 days after planting, and within each block, three plants were subsampled from every treatment plot, and subsample values were averaged prior to analysis. All analyses were based on the averages of three plants.

The design for the 1989 experiment was a randomized complete block with two treatment factors: sampling time and seed treatment. Each combination of seed treatment and sampling time was randomly assigned a different plot within each of five blocks, and each plot consisted of four seeds spaced 15 cm apart, and planted at a depth of 2.5–4 cm. Seed treatments were UW85, UW85n1, and an untreated control. Sampling times were 1, 2, 3, 5, 7, 10, 14, 21, 28 and 116 days after planting, and within each block we sampled one plant from every treatment plot.

*Sample preparation and dilution plating*

Populations of aerobic, heterotrophic bacteria, total *B. cereus* and neomycin-resistant *B. cereus* were estimated by dilution plating sonicated material from seeds, cotyledons, radicles or 1–2 cm root segments. Root segments were taken 0–1, 0–2, 2–3, 3–5, 4–5, 5–7, 7–8 or 9–10 cm below the crown, or from the last centimeter of the root, which was 5–6, 6–7, 8–9, 10–11, 11–12, 12–13 or 18–19 cm below the crown. We will refer to the last centimeter of the root as the root tip (RT). When roots could no longer be removed intact from the soil, we measured bacterial population sizes on a root segment 9–10 cm below the crown, since at all samplings we were able to obtain at least 10 cm of root. The crown is defined as the part of the plant at the soil–air interface, which was marked on each plant as it was removed from the soil. Samples were sonicated in 5 or 10 ml of sterile distilled water for 15 s at 20% output with a 250 W Vibra-cell sonicator (Sonics and Materials, Danbury, Conn.), serially diluted in sterile distilled water, and then 0.1 ml aliquots of the dilutions were spread on the appropriate media.

*Semi-selective media*

In the 1987 experiment, the medium for enumerating *B. cereus* was half-strength TSA. In the 1989 experiment, the medium for enumerating *B. cereus* was changed to Min IC because fewer non-bacilli were able to grow on this medium, thereby facilitating the detection of *B. cereus*. Furthermore, when root samples were plated onto both media, up to 20% more *B. cereus* grew on Min IC medium than on half-strength TSA medium. Min IC media contained ($l^{-1}$): 2.0 g L-glutamic acid; 1.0 g trisodium citrate $2H_2O$; 10 mg thiamine hydrochloride; 2.0 g $(NH_4)_2SO_4$; 6.0 g $KH_2PO_4$; 0.2 g $MgSO_47H_2O$; 0.25 mg $MnSO_4$; 5.0 g acid-hydrolized casein (Sigma Chemical Co., St Louis, Mo). After autoclaving, 10 ml of a sterile 50% (w/v) dextrose solution and 10 ml of $FeCl_36H_2O$ (4.0 mg $ml^{-1}$) were added per liter. All media used to enumerate *B. cereus* contained 12.5 $\mu$g polymyxin $\beta$-sulfate $ml^{-1}$, 50 $\mu$g ampicillin $ml^{-1}$, and 100 $\mu$g cycloheximide $ml^{-1}$. The antibiotics were included to reduce growth of other microorganisms; all *B. cereus* isolates we screened grew on the semi-selective medium.

The medium for enumerating heterotrophic bacteria was half-strength TSA in 1987. This was changed to 10% strength TSA in 1989 since it supported growth of a greater diversity of colony types than 50% strength TSA. All media used to enumerate heterotrophic bacteria contained 100 $\mu$g cycloheximide $ml^{-1}$. Antibiotics were obtained from Sigma Chemical Co., St Louis, Mo.

*Enumeration of bacteria*

*B. cereus* colonies were identified by their broad, flat, cream-colored colony morphology (Claus and Berkeley, 1986) on the semi-selective media. We tested 600 colonies selected by this method and all except one were hemolytic (Halverson, Stabb and Handelsman, data not shown), which is diagnostic of *B. cereus*. Total and neomycin-resistant *B. cereus* colonies were counted after 2 and 3 days at 28°C, respectively, and populations of heterotrophic bacteria were determined after 3 days at 28°C. The number of cfu per sample was $\log_{10}$ transformed, and expressed as log cfu $cm^{-1}$ root. For purposes of statistical analysis, in samples that had no colonies at our detection limit we assigned a value that was 10-fold less than the detection limits.

*Enumeration of B. cereus spores*

The proportion of spores and vegetative cells was estimated by plating aliquots of diluted samples before and after exposing them in a water bath at 85°C for 15 min, which kills vegetative cells but not spores. Controls for this experiment included heat treatment of vegetative and fully sporulated cultures of UW85n1 grown in 10 ml Min IC medium. There were no detectable survivors in vegetative cultures after heat treatment, and there were no significant

differences between populations in the sporulated cultures before and after heat treatment.

*Statistical analysis*

The SAS computer statistics program (SAS Institute, 1985) was used for statistical analyses. In the 1987 experiment, a split-plot analysis was used for total *B. cereus*, neomycin-resistant *B. cereus*, and heterotrophic bacterial populations. In these analyses, seed treatments and sampling times within a block were whole-plot treatment factors, and locations on the root were sub-plot treatment factors. For each sampling time, for each of the total *B. cereus*, neomycin-resistant *B. cereus*, and heterotrophic bacterial populations, a separate ANOVA was performed. For each sampling time, a separate least significant difference (LSD) was calculated to compare populations among treatments (a comparison between whole-plots) and at various locations on the root (a comparison between sub-plots; Milliken and Johnson, 1984).

In the 1989 experiment, at 0, 1, 2, 3, 5 and 7 days after planting, measurements of the total *B. cereus*, neomycin-resistant *B. cereus*, and heterotrophic bacterial populations were each analyzed as randomized complete block experiments. An LSD was calculated to compare populations among treatments (Snedecor and Cochran, 1980).

In the 1989 experiment after 7 days after planting, total *B. cereus*, neomycin-resistant *B. cereus*, and heterotrophic bacterial populations were analyzed as split-plot experiments. In these analyses, seed treatments and sampling times (10, 14, 21, 28 and 116 days after planting) within a block were whole-plot treatment factors, locations on the root were the first sub-plot treatment factors. A separate ANOVA was performed on each of the total *B. cereus*, neomycin-resistant *B. cereus*, and heterotrophic bacterial populations. A separate LSD was calculated to compare populations among treatments (a comparison between whole-plots) and at various locations on the root (a comparison between sub-plots; Milliken and Johnson, 1984).

Populations of *B. cereus* before and after heat treatment were analyzed as a split-plot experiment. In this analysis, seed treatments and sampling times (0, 1, 2, 3, 7 and 10 days after planting) were whole-plot treatment factors, and *B. cereus* populations before and after heat treatment were the sub-plot treatment factors. An LSD was calculated to compare populations before the heat treatment with populations after the heat treatment (a comparison between sub-plots; Milliken and Johnson, 1984).

## RESULTS

Neomycin-resistant (Neo$^r$) *B. cereus*, total *B. cereus*, and heterotrophic bacterial populations were measured on untreated, UW85n1-, UW85-, and UW85s1-treated roots in 1987 and 1989 in the field. We present data only for bacterial populations on untreated and UW85n1-treated roots, because populations on UW85s1- and UW85-treated roots were similar to those on UW85n1-treated roots (L. J. Halverson, *loc. cit.*).

Three lines of evidence led us to conclude that most members of the *B. cereus* populations on UW85n1-treated roots were derived from our inoculum, and were not from the indigenous population. First, the total *B. cereus* populations on UW85n1-treated roots were usually significantly larger ($P = 0.05$) than those on untreated roots (Tables 1 and 2; Figs 1 and 2). Second, the *B. cereus* populations on UW85n1-treated roots were primarily neomycin-resistant whereas little neomycin resistance was found in the populations on untreated roots, (Tables 1 and 2; Figs 1 and 2), and third, the *B. cereus* populations on UW85n1-treated roots maintained the biocontrol phenotype, as indicated by testing representative isolates in an assay for suppression of alfalfa damping-off (Handelsman *et al.*, 1990). Biocontrol activity was associated with UW85 derivatives and not associated with indigenous populations. We are cautious about basing conclusions solely on the neomycin-resistant populations because we have found that maintenance of neomycin-resistance in rhizosphere populations can be variable (L. J. Halverson, *loc. cit.*). Therefore we base our conclusions about population dynamics on total *B. cereus* populations, but we present data on neomycin-resistant *B. cereus* populations as well. For simplicity, we will refer to the *B. cereus* populations on UW85n1-treated roots as populations of UW85n1.

*UW85n1 colonization of radicles*

At planting, 100% of the UW85n1 population on seeds consisted of spores, compared with 28% within 24 h after planting. The size of populations of vegetative cells and spores did not change during the first 3 days after planting (Table 2). On radicles, 100% of the UW85n1 population consisted of spores, suggesting that either the radicles were colonized only by spores or that vegetative cells of UW85n1 had sporulated (Tables 1 and 2).

*Growth of UW85n1 on seeds and roots*

Two lines of evidence suggest that UW85n1 grew on seeds and roots. In the 1987 experiment, UW85n1 population sizes on seed coats 7 days after planting were 10-fold larger than at planting (Table 1). In the 1987 experiment, between 35 and 49 days after planting, UW85n1 populations increased 6–79-fold [Fig. 1(D) and (E)], which suggests that UW85n1 grew in the rhizosphere. In addition, in the 1989 experiment >70% of the UW85n1 spores applied to seeds germinated.

Table 1. Bacterial populations on seeds and 7-day old seedlings during the 1987 experiment

| Days after planting | Treatment | Mean $\log_{10}$ cfu ± SEM: Neo$^r$ *B. cereus* | Total *B. cereus* | Heterotrophic bacteria | *B. cereus* (%)† |
|---|---|---|---|---|---|
| | Seed | | | | |
| 0 | UW85n1 | 8.4 ± 0.2 | 8.1 ± 0.3 A* | —‡ | — |
| | Untreated | ND§ | 1.9 ± 0.2 B | — | — |
| | Seed coat | | | | |
| 7 | UW85n1 | 8.3 ± 0.5 | 9.2 ± 0.4 | — | — |
| | Untreated | — | — | — | — |
| | Cotyledon | | | | |
| 7 | UW85n1 | 7.1 ± 0.4 A | 8.1 ± 0.2 A | 8.7 ± 0.1 A | 33.8 |
| | Untreated | 1.7 ± 0.3 B | 4.2 ± 0.2 B | 8.2 ± 0.4 A | 0.01 |
| | Radicle | | | | |
| 7 | UW85n1 | 5.8 ± 0.5 A | 6.4 ± 0.2 A | 7.3 ± 0.4 A | 24.5 |
| | Untreated | 2.0 ± 0.1 B | 3.9 ± 0.3 A | 6.9 ± 0.2 A | 0.0013 |

*Values at the same sampling time in the same column followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's LSD. The neomycin-resistant and total *B. cereus* populations on UW85n1-treated seeds, cotyledons and radicles were significantly larger than those on untreated seeds, cotyledons, and radicles.

†$B.\ cereus\ (\%) = \dfrac{\text{total } B.\ cereus \text{ population per sample}}{\text{heterotrophic bacterial population per sample}} \times 100.$

‡Not determined.

§None detected at detection limits (100 cfu per sample).

*Spatial distribution of UW85n1 in the rhizosphere*

Between 10 and 35 days after planting, UW85n1 population sizes generally decreased with increasing distance from the crown (Figs 1 and 2; Tables 1 and 2), and they averaged 120- and 213-fold larger on root segments 2–3 cm below the crown than those on the last centimeter of roots sampled in the 1987 and 1989 experiments, respectively. At 35 and 49 days after planting, there was some evidence that

Table 2. Bacterial populations on seeds and seedlings during the first week after planting in the 1989 experiment

| Days after planting | Treatment | Mean $\log_{10}$ cfu ± SEM: Neo$^r$ *B. cereus* | Total *B. cereus* | Heterotrophic bacteria | *B. cereus* (%)† |
|---|---|---|---|---|---|
| | Seed | | | | |
| 0 | UW85n1 | 8.4 ± 0.2 | 8.5 ± 0.2 A* | —§ | — |
| | Untreated | ND‡ | 2.0 ± 0.3 B | — | — |
| | Seedling¶ | | | | |
| 1 | UW85n1 | 8.3 ± 0.1 A | 8.6 ± 0.2 A | 8.6 ± 0.1 A | 100.0 |
| | Untreated | 1.4 ± 0.3 B | 3.0 ± 0.2 B | 5.7 ± 0.2 B | 0.2 |
| 2 | UW85n1 | 8.8 ± 0.2 A | 8.8 ± 0.1 A | 8.7 ± 0.1 A | 100.0 |
| | Untreated | 1.6 ± 0.2 B | 3.4 ± 0.1 B | 6.6 ± 0.2 B | 0.07 |
| 3 | UW85n1 | 7.7 ± 0.3 A | 8.4 ± 0.2 A | 8.2 ± 0.1 A | 100.0 |
| | Untreated | 2.4 ± 0.2 B | 3.6 ± 0.2 B | 6.6 ± 0.1 B | 0.09 |
| | Seed coat | | | | |
| | UW85n1 | 7.9 ± 0.3 | 8.2 ± 0.3 | — | — |
| | Untreated | — | — | — | — |
| | Cotyledon | | | | |
| 5 | UW85n1 | 5.2 ± 0.3 A | 6.3 ± 0.3 A | 7.6 ± 0.3 A | 5.0 |
| | Untreated | 1.4 ± 0.3 B | 3.2 ± 0.2 B | 6.7 ± 0.2 B | 0.03 |
| | Radicle | | | | |
| 5 | UW85n1 | 5.5 ± 0.3 A | 5.6 ± 0.3 A | 7.2 ± 0.3 A | 2.5 |
| | Untreated | 2.6 ± 0.1 B | 3.9 ± 0.1 B | 6.8 ± 0.1 A | 0.1 |
| 7 | UW85n1 | 4.4 ± 0.3 A | 4.5 ± 0.3 A | 7.0 ± 0.2 A | 0.3 |
| | Untreated | 2.1 ± 0.2 B | 3.0 ± 0.3 B | 6.5 ± 0.2 B | 0.04 |

*Values at the same time in the same column followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's LSD.

†See Table 1.

‡None detected at detection limits (50 cfu per sample).

§Not determined.

¶The proportion of the UW85n1 population that was spores on seedlings 1, 2, and 3 days after planting was 28, 27, and 12%, respectively.

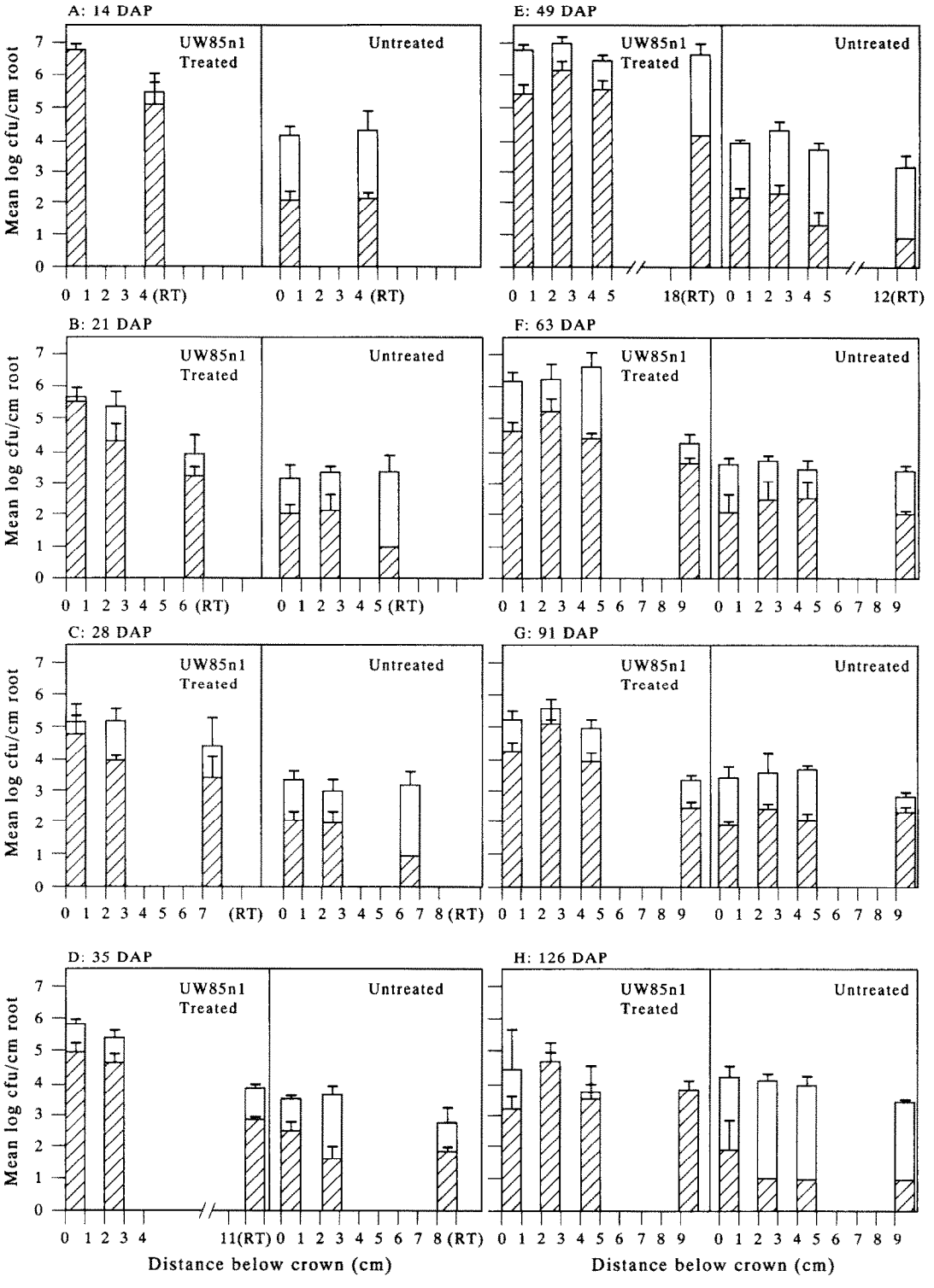


Fig. 1. Total and neomycin-resistant *B. cereus* populations on UW85n1-treated and untreated soybean roots during the 1987 experiment. DAP, days after planting; total *B. cereus* populations, open bars; Neo$^r$ *B. cereus* populations, solid bars; error bars indicate the SEM. The total *B. cereus* populations were significantly larger on UW85n1-treated roots than on untreated roots, except 91 DAP 9–10 cm below the crown, and 126 DAP at all locations on the roots. The Neo$^r$ *B. cereus* populations were significantly larger on UW85n1-treated roots than on untreated roots, except 91 DAP 9–10 cm below the crown. The LSDs to compare total *B. cereus* populations at different locations on the root within a treatment at 14, 21, 28, 35, 49, 63, 91 and 126 days after planting, are 1.4, 1.2, 0.9, 0.7, 0.8, 0.6, 0.7 and 1.8 $\log_{10}$ cfu, respectively. The LSDs to compare Neo$^r$ *B. cereus* populations at different locations on the root within a treatment at 14, 21, 28, 35, 49, 63, 91 and 126 days after planting, are 0.9, 1.3, 1.0, 0.7, 1.2, 1.0, 0.5 and 1.1 $\log_{10}$ cfu, respectively.

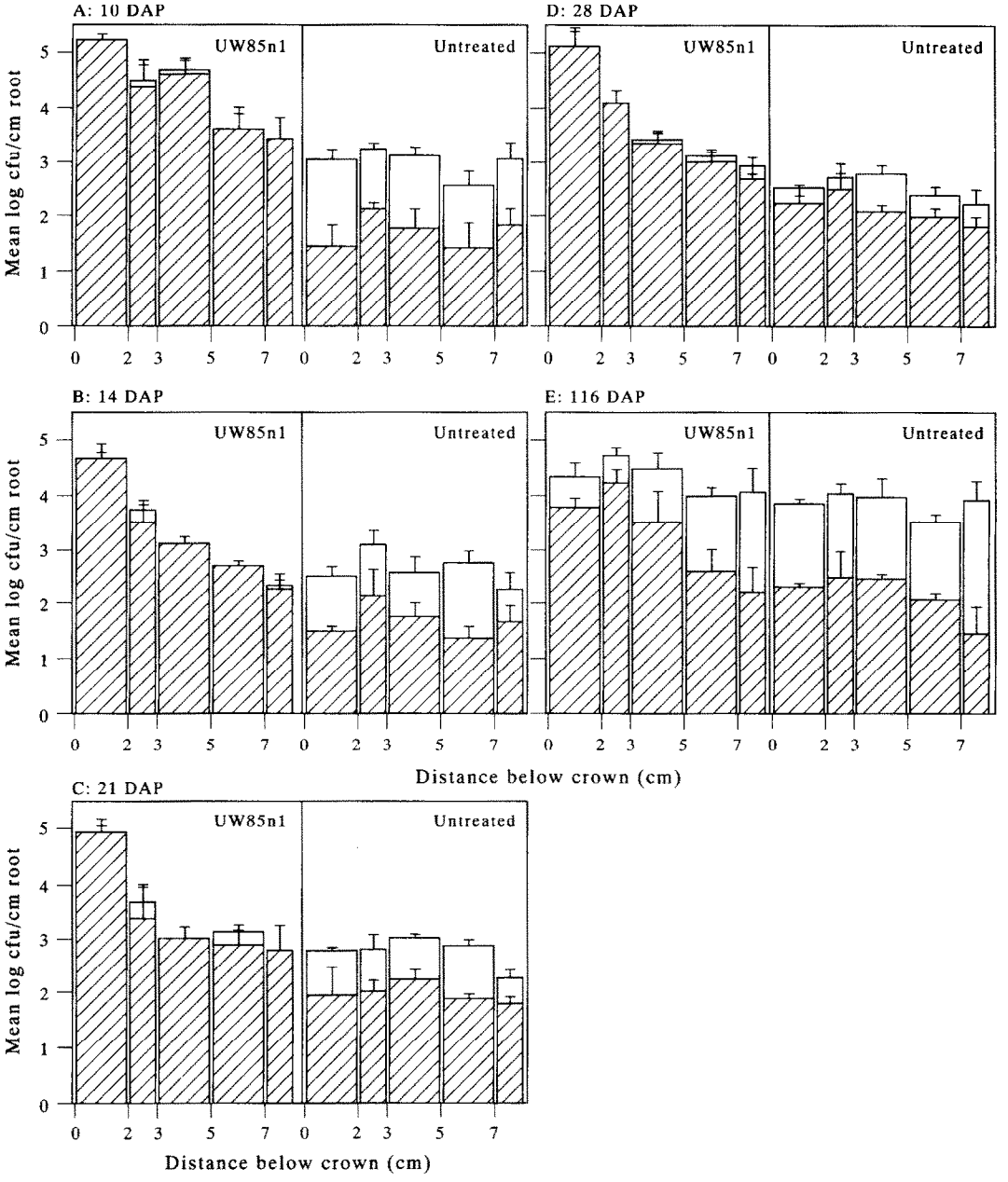


Fig. 2. Total and neomycin-resistant *B. cereus* populations on UW85n1-treated and untreated soybean roots during the 1989 experiment. DAP, days after planting; total *B. cereus* populations, open bars; Neo$^r$ *B. cereus* populations, solid bars; error bars indicate the SEM. The total *B. cereus* populations were significantly larger on UW85n1-treated roots than on untreated roots, except 10 DAP 7–8 cm below the crown, 14, 21 and 116 DAP 3–8 cm below the crown, 28 DAP 3–5 cm below the crown. The Neo$^r$ *B. cereus* populations were significantly larger on UW85n1-treated roots than on untreated roots, except 10 DAP 7–8 cm below the crown and 116 DAP 5–7 cm below the crown. The LSD to compare total *B. cereus* populations at different locations on the root within a treatment at all sampling times is 0.6 $\log_{10}$ cfu. The LSD to compare Neo$^r$ *B. cereus* populations at different locations on the root within a treatment at all sampling times the LSD is 0.8 $\log_{10}$ cfu.

UW85n1-treated roots were longer ($P = 0.08$) than untreated roots [Fig. 1(D) and (E)].

In the 1989 experiment, 14, 21 and 116 days after planting, 3–8 cm below the crown, total *B. cereus* population sizes on UW85n1-treated roots were not significantly different from indigenous *B. cereus* population sizes on untreated roots; however, the entire *B. cereus* population on UW85n1-treated roots was neomycin-resistant (Fig. 2), suggesting that UW85n1 replaced the indigenous *B. cereus* population. In contrast to UW85n1-treated roots, there were no gradients in the spatial distribution of the indigenous *B. cereus* on untreated roots (Figs 1 and 2).

*Temporal changes in UW85n1 populations in the rhizosphere*

Temporal population dynamics of UW85n1 varied considerably between the two growing seasons. In 1987, UW85n1 populations decreased 10–39-fold between 14 and 28 days after planting, increased 6–79-fold between 35 and 49 days after planting, the highest populations were measured at 49 days after planting, and the populations were lower at later samplings so that by 126 days after planting UW85n1 population sizes were not significantly different from those of indigenous *B. cereus* on untreated roots (Fig. 1). Between 35 and 49 days after planting, there was a 10–96-fold increase in UW85n1 populations on the last centimeter of root [Fig. 1(D) and (E)]. Increases in UW85n1 populations at the root tip could be due to growth. Alternatively, since there were 41 mm of precipitation 48 days after planting (S. Baker, pers. commun.), UW85n1 might have been carried to the root tip by water movement. In 1989, UW85n1 populations decreased 8–25-fold between 10 and 14 days after planting, did not change further by 28 days after planting, and after this sampling period, UW85n1 population sizes were similar to those of indigenous *B. cereus* on untreated roots.

*Proportion of the heterotrophic bacterial population that was UW85n1*

During the first 3 days after planting, heterotrophic bacterial populations on UW85n1-treated seedlings consisted entirely of UW85n1 (Table 2), but by 7 days after planting, the proportion that was UW85n1 on radicles was much smaller (Tables 1 and 2).

In 1987, between 35 and 49 days after planting at all locations on the root, UW85n1 increased 2–6-fold in proportion to the heterotrophic bacterial population (Table 3). This was due to increases in UW85n1 populations [Fig. 1(D) and (E)], since total heterotrophic bacterial populations did not decrease during this period. In 1989, by 14 days after planting at all locations on the root, the proportion of the heterotrophic bacterial population that was UW85n1 was smaller than in 1987 (Tables 3 and 4). At four sampling times in 1987 and at four sampling times in 1989, heterotrophic bacterial populations on UW85n1-treated roots were significantly greater ($P = 0.05$) on UW85-treated roots than on untreated roots (data not shown).

Table 3. Proportion of the heterotrophic bacterial population on UW85n1-treated roots that was *B. cereus* (1987)

| Distance below the crown (cm) | *B. cereus* (%)* Days after planting | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 14 | 21 | 28 | 35 | 49 | 63 | 91 | 126 |
| 0–1 | 28.2 | 5.2 | 1.5 | 4.6 | 10.7 | 7.9 | 0.3 | 0.06 |
| 2–3 | —† | 10.3 | 5.0 | 2.3 | 13.3 | 28.9 | 1.5 | 0.006 |
| 4–5 | 3.84 | — | — | — | 6.43 | 11.7 | 0.43 | 0.004 |
| 6–7 | — | 0.66 | — | — | — | — | — | — |
| 8–9 | — | — | 0.32 | — | — | — | — | — |
| 9–10 | — | — | — | — | — | 0.12 | 0.07 | 0.012 |
| 11–12 | — | — | — | 0.16 | — | — | — | — |
| 18–19 | — | — | — | — | 66.4 | — | — | — |

*See Table 1.
†Not determined.

Table 4. Proportion of the heterotrophic bacterial population on UW85n1-treated roots that was *B. cereus* (1989)

| Distance below the crown (cm) | *B. cereus* (%)* Days after planting | | | |
|---|---|---|---|---|
| | 10 | 14 | 21 | 28 |
| 0–2 | 26.3 | 0.31 | 1.10 | 0.132 |
| 2–3 | 4.07 | 0.0056 | 0.016 | 0.027 |
| 3–5 | 2.04 | 0.019 | 0.0054 | 0.0032 |
| 5–7 | 6.31 | 0.0078 | 0.0037 | 0.013 |
| 7–8 | 0.47 | 0.0062 | 0.013 | 0.0022 |

*See Table 1.

## DISCUSSION

Our results show that UW85n1 spores germinated on soybean seeds, and that UW85n1 colonized emerging radicles, spread on growing roots, and persisted until seed harvest (Figs 1 and 2). UW85n1 persisted in the rhizosphere throughout the growing season at population densities greater than $10^4$ cfu per root segment, although it usually represented only a small proportion of the heterotrophic bacterial population (Tables 3 and 4). UW85n1 populations increased between sampling periods [Fig. 1(D) and (E)], which could be due to long-term population trends or to short-term diel patterns for example (Hirano and Upper, 1989), that were not detected by our sampling procedure.

Our examination of populations has focused exclusively on mean population sizes to describe the distribution and abundance of rhizosphere bacteria; a study of other statistical variables such as population variance and probability distribution would also contribute to our understanding of these populations. It must also be recognized that because of limitations inherent in dilution plating and sampling rhizosphere populations, and because we did not measure colonization of lateral roots by UW85n1, our estimates of bacterial population sizes are presumably fractions of real populations.

There are several explanations for the samples in which there were no significant differences between the total *B. cereus* populations on UW85n1-treated and untreated roots, but the entire *B. cereus* population on UW85n1-treated roots was neomycin resistant [Fig. 2(B) and (C)]. First, UW85n1 could have completely displaced indigenous *B. cereus* from UW85n1-treated roots. However, this may not be the most reasonable explanation since UW85n1 colonized emerging radicles shortly after they emerged from the seed (Tables 1 and 2), suggesting that UW85n1 probably arrived before indigenous *B. cereus*. Second, previously established populations of UW85n1 in the rhizosphere could have competitively excluded (Lindow, 1987) indigenous *B. cereus* from colonizing the rhizosphere. Alternatively, UW85n1

seed treatments could have altered the composition of the rhizosphere microbial community (G. S. Gilbert *et al.*, unpubl. data) which might have prevented colonization by indigenous *B. cereus*.

The ability of UW85n1 spores to germinate on seedlings within 1 day after planting could be important in preventing disease and modifying rhizosphere microbial communities. If the antifungal antibiotic implicated in biocontrol of damping-off diseases by UW85n1 (Handelsman *et al.*, 1990, unpubl. data) is packaged within the spore, germination of UW85n1 spores on seeds could provide antifungal antibiotic around the seed, thereby protecting it from seed-colonizing pathogens (Handelsman and Parke, 1988). Alternatively, large UW85n1 populations on germinating seeds could consume nutrients that are consumed by the microbial flora that usually colonize germinating seeds. Competition for these nutrients could alter the composition of rhizosphere microbial communities, affect the density of heterotrophic bacteria in the rhizosphere, and contribute to disease prevention and nodulation enhancement (Halverson and Handelsman, 1991).

Extracellular factors produced by UW85n1 may contribute to its ability to colonize, persist, and grow in the rhizosphere. UW85 produces antifungal (Handelsman *et al.*, 1990) and antibacterial (L. S. Silo and J. Handelsman, unpubl. data) metabolites in culture media, and it secretes ammonia and removes calcium from culture media causing an ionic environment that lyses *Phytophthora* zoospores (Gilbert *et al.*, 1990). These metabolites could provide UW85n1 with a competitive advantage by inhibiting growth of microorganisms that compete with it for nutrients on soybean roots.

It is often suggested that bacteria must become established in the rhizosphere in high numbers in order to prevent disease or promote plant growth (Weller, 1988). However, in the 1987 experiments, UW85n1 promoted root growth (Fig. 1), increased nodulation (Halverson and Handelsman, 1991), and increased emergence (L. J. Halverson *loc. cit.*) without its establishment in the rhizosphere at high population densities or as a dominant member of the heterotrophic bacterial community. For example, in the 1989 experiment, 28 days after planting, there were 87% more nodules on UW85n1-treated roots than on untreated roots (Halverson and Handelsman, 1991), and since soybean nodules appear 10–12 days after initiation of nodulation, UW85n1 likely affected the nodulation process around 16–18 days after planting. However, by 14 days after planting, UW85n1 population sizes on three out of five locations were not significantly different ($P = 0.05$) from those of indigenous *B. cereus* on untreated roots [Fig. 2(B)], and UW85n1 represented only 0.31–0.0062% of the heterotrophic bacterial population (Table 4).

The spatial and temporal population biology of UW85n1 in the rhizosphere varied between the two growing seasons (Figs 1 and 2). In another experiment in the 1988 season, the plants were severely stressed by a drought and high temperatures, stands were poor, and yields were low. The 1988 field season and plant growth were so aberrant and the conditions so extreme, that no conclusions can be based on the data gathered. In the 1988 experiments, UW85n1 colonized roots, but its survival and ability to spread on roots was very poor and highly variable among plants (L. J. Halverson, *loc. cit.*). The results from our three field experiments demonstrate the importance of performing multiple-year field experiments when examining the population biology of biocontrol or plant growth-promoting agents.

We have presented preliminary results about the spatial and temporal population biology of a Gram-positive biocontrol agent in the field. It is interesting that a bacterium that is present in low populations in the rhizosphere can have a significant effect on nodulation (Halverson and Handelsman, 1991), disease control, and seed yield (L. J. Halverson, *loc. cit.*). Long-term, detailed studies are needed to identify the biotic and abiotic factors that contribute to the ability of UW85n1 to grow, spread, persist, and affect heterotrophic communities in the rhizosphere. These studies may contribute to our understanding of variability in disease control and nodulation enhancement.

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