Population Dynamics of *Bacillus megaterium* Strain B153-2-2 in the Rhizosphere of Soybean

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ABSTRACT

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Populations of *Bacillus megaterium* strain B153-2-2 with a rifampicin-resistant marker were studied in the rhizosphere and surrounding soil of soybean (*Glycine max*) plants for 2 yr in a silt loam soil in the field. B153-2-2 populations were established soon after introduction into the soil; a significantly greater population density (6 cfu/g of soil) was observed at 2 or 3 wk than at 1 or 4 wk after emergence in a root zone containing a taproot, its main branches, and adjacent soil. B153-2-2 populations increased after introduction, remained constant during the growing season, and then decreased toward the end of the year at a 10-cm depth in the root zone of a soil cylinder 0–10 cm out from the stem and 30 cm deep. Populations of the bacterium at 20- and 30-cm depths remained relatively constant throughout the season. Residual populations of B153-2-2 survived the winter at no less than 4 log cfu/g of soil and were recoverable during the second growing season without further introduction of the bacterium. Introduced bacterial populations in the rhizosphere and root zones were dispersed further vertically than horizontally from the application site and were recoverable to at least 50 cm deep and 30 cm laterally from a taproot 39 days after seedling emergence. B153-2-2 populations reached 6 log cfu/g of soil with minimum recovery of 2 log cfu/g of soil in the extended rhizosphere and root zones. The compatibility with indigenous soil microflora, the close association with soybeans, and the ability to colonize soybean roots and rhizosphere make *B. megaterium* B153-2-2 a potential candidate for management of microflora in the rhizosphere.

Additional keywords: ecology, rhizobacterium.

Colonization of plant roots or rhizosphere by bacteria has been widely studied, although the two categories often are not clearly distinguished (21). Populations of a bacterium introduced into a plant rhizosphere can be estimated by assaying the soil surrounding the roots. The assay involves the washing of roots in a buffered solution; the bacterial population is then determined by colony counts with serial dilutions (10,19,21). Sonication also may be used to remove bacteria from roots for such assays (22). However, high levels of variation occur because of soil moisture, soil type, plant species, and root age. In addition, those bacterial cells that adhere firmly to the root surfaces are not likely to be removed by washing (6,8).

The rhizosphere is a narrow zone of soil around the root and is influenced by the root. This sphere has been adapted for the investigation of populations of introduced bacteria associated with roots. Because the root system of a soybean plant in the field consists of a network of root branches, it is difficult to obtain a complete root system or rhizosphere. Many bacterial cells in close proximity to plant roots or adhering to root surfaces are dispersed and redistributed by water filtration in soil (5,11,23). Such bacterial redistribution through the soil can enhance root colonization (5,11). Introduced bacterial populations also have been recovered from bulk soil distal from a plant root (1). Therefore, bacterial populations in root zones are not necessarily restricted to the rhizosphere. For a rhizobacterium to be considered a potential biocontrol agent, information concerning its distribution in an extended rhizosphere is required (21). However, because of the extensive efforts required for such studies, little information is available (3,7,26).

Bacillus megaterium de Bary strain B153-2-2 reduced root decay symptoms of soybean (*Glycine max* (L.) Merr.) when co-infested with *Rhizoctonia solani* Kühn in soil in the greenhouse and field (15). B153-2-2 also improved root growth and was considered a potential biocontrol agent of soybeans (13,14). To better understand the fate of B153-2-2 as an introduced bacterium in the field, we characterized its colonization on the soybean rhizoplane (12). In this study, we investigated the population dynamics of *B. megaterium* B153-2-2 in the field over two seasons, and we evaluated the spatial and temporal development of this introduced bacterium in three parts of the soybean rhizosphere and the surrounding soils. A portion of this work has been reported (16).

MATERIALS AND METHODS

Bacterial inoculum. *B. megaterium* strain B153-2-2 (ATCC 55000) is a rifampicin-resistant derivative from the wild type originally isolated from the crown of a field-grown soybean plant. The bacterium was identified by biochemical, morphological, and physiological characteristics (4,9) and was verified by L. K. Nakamora (Microbial Properties Research, USDA, Peoria, IL). Rifampicin-resistant mutants of the wild type were selected on nutrient agar (NA) (Difco Labs, Detroit, MI) amended with serial concentrations from 50 to 300 μg/ml of rifampicin. We prepared this selective medium, NA^{rif}, by adding rifampicin dissolved in a small amount of methanol after the NA was autoclaved for 15 min at 120 C and cooled to 50 C. We avoided exposure to light during medium preparation, and we incubated all plates in the dark. Colonies smaller than those grown on NA under the same incubation conditions were avoided; colonies that appeared similar to the wild type were selected from NA^{rif} at 100 μg/ml. Selected colonies were incubated in nutrient broth (NB) (Difco) and then plated on NA. Thousands of individual colonies were picked separately with sterilized toothpicks and inoculated on NA. All the selected colonies were compared with the wild type for their antagonism to *R. solani* isolate 65L-2 on potato-dextrose agar (Difco). Like the wild type, several strains inhibited *R. solani*. After comparing growth rate, morphological, biochemical, and physiological characteristics with the wild type, we selected B153-2-2 as the representative strain from the rifampicin-resistant mutants that appeared at a frequency of 0.3%. Stock cultures of B153-2-2 were routinely maintained on NA and stored in NB at −80 C. The rifampicin resistance of B153-2-2 remained stable during our studies for more than 5 yr.

Inoculum of B153-2-2 was prepared as a dry mix. A cell suspension was prepared by transferring a loop of cells from a fresh NA culture to a 250-ml Erlenmeyer flask containing 50 ml of NB and incubating it on a rotary water bath shaker at 120 rpm for 12 h at 30 C. The final concentration ranged from 5 to 8 $\times$ 10^7 cfu/ml of NB. The cell suspension was mixed with ground autoclaved soybean stems at 1:15 (v/w). Inoculum density ranged from 6 to 8 $\times$ 10^7 cfu/g of the dry mix.

Fungal inoculum. Inoculum of *R. solani* isolate 65L-2 (ATCC 66489) (intraspecific group 2B, anastomosis group 2-2) (17,18) was prepared by culturing mycelia in potato-dextrose broth (Difco) on a rotary shaker at 120 rpm for 6 days at 25 C. Mycelial mats were separated from the broth by filtration, pressed dried, ground into a mycelium suspension, diluted, and mixed with an autoclaved soil mix (sand/soil/pea gravel/vermiculite, 1:1:1:1, v/v) to give an inoculum density of 100 μg of fresh mycelium per gram of soil mix.

Soil conditions. The field soil was Catlin silt loam (pH 6.0, 20 μg/ml of P and 164 μg/ml of K) located in an experimental field plot on the Agronomy-Plant Pathology South Farm, Urbana, Illinois, which had a history of continuous soybean cultivation. The field was plowed in fall 1986, after harvest, as routine management and was fertilized with 47.0 kg of P_2O_5 and 57.4 kg/ha of K_2 before being plowed in the spring 1987. Treflan herbicide (2,6-dinitro-*N*,*N*-dipropyl-4-[trifluoromethyl]benzenamine) was applied at 0.373 kg a.i./ha. In 1987, the field was not tilled after harvest. In spring 1988, the same quantity of fertilizer used in 1987 was surface-applied and disk-plowed. Precipitation was 4,879 mm from June to September 1987 and 2,258 mm from June to September 1988. Because of the drought, overhead irrigation was applied in 1988, but the amount of water applied was not recorded.

Sampling procedure. We sampled three defined root zones to estimate populations of the introduced B153-2-2 in the soybean field. Root zone 1 was a cylinder of soil 10 cm in diameter centered by a soybean plant and 10 cm deep from the soil surface; soil was associated with the soybean taproot and its branches (Fig. 1). Root zone 2 was a cylinder of soil 20 cm in diameter and 30 cm deep containing soil associated with the taproot, its branches, and secondary branches. When the soil in root zone 2 was sampled, soil in root zone 1 was not, because the former included the latter. Root zone 3 was a cylinder of soil 60 cm in diameter and 50 cm deep containing soil associated with zone 2 and the extended root branches and some bulk soil.

The total volume of root zone 1 was collected as one sample and used to study B153-2-2 populations early in the growing season. Zone 2 was used to investigate the population dynamics of B153-2-2 with time for the entire growing season. We collected soil samples in zone 2 by digging the entire soil cylinder with a shovel and then sampling with a garden trowel. One sample was taken from each 10-cm-depth level. Each sample was a mixture of four subsamples of 10^3 cm of soil from four sites equidistant from the taproot. A combined soil sample weighed no less than 2 kg and was marked by its original location. For example, the sample taken at 10 cm down from the soil surface and 10 cm laterally from the taproot was marked 10/10; at 20 cm down and 10 cm laterally from the taproot, 20/10; at 30 cm down and 10 cm laterally, 30/10. Samples from each of four replications were taken for zones 1 and 2, and two replications were taken for zone 3. To obtain samples from zone 3, we dug a hole with a shovel and a garden trowel, and we took 14 samples in sequence (each came from four subsamples as described): 10/10, 10/20, 10/30, 20/10, 20/20, 20/30, 30/10, 30/20, 30/30, 40/10, 40/20, 40/30, 50/10, and 50/20 cm. No sample was taken at 50/30 cm. The site for sampling was chosen at random. The plant at each site was cut off at the crown, and the soil surface of the sampling area was cleared of plant debris. Soil samples were placed into polyethylene bags and processed within 12 h.

Soil samples were mixed and crushed in a bag until each had a uniform appearance; any visible plant debris was removed by hand. A 1-g sample was removed from each bag and stored at 4 C. Each soil sample was then suspended in deionized distilled water, and serial dilutions were made. For each dilution, an aqueous suspension of 100 μl was spread on either NA or NA^{rif}. Three plates were used for each dilution. Plates were incubated for 12 h at 30 C, and the number of colonies was recorded. Plates

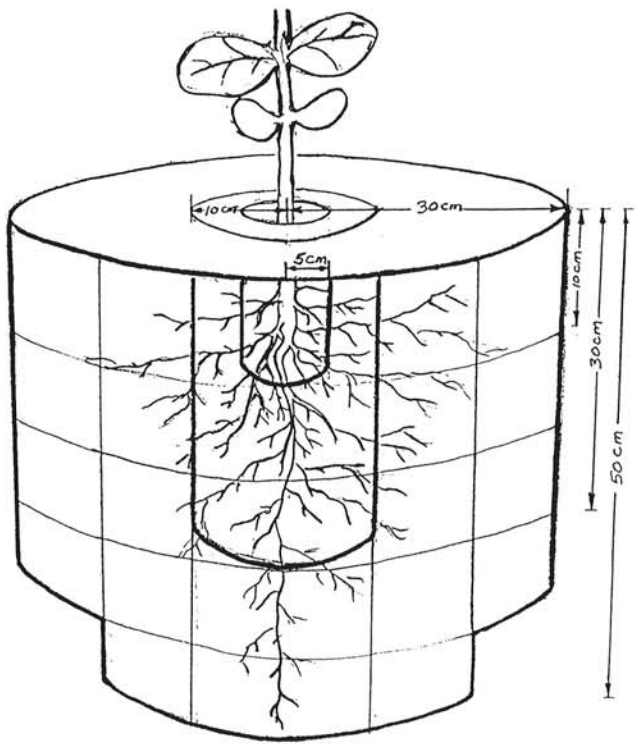

Fig. 1. Schematic diagram of root zones centered around a soybean plant showing three defined sampling zones, with 10-cm divisions, from the taproot to 30 cm laterally and down from the soil surface to 50 cm vertically. Root zone 1 represents a soil cylinder 10 cm in diameter and 10 cm deep centered on a soybean plant; root zone 2, 20 cm in diameter and 30 cm deep; and root zone 3, 60 cm in diameter and 50 cm deep. Each zone was sampled independently without reference to the others.

with more than 300 colonies did not provide accurate estimates, and were not counted.

Experimental design and data analysis. The field experiment consisted of four treatments: B153-2-2 alone, *R. solani* 65L-2 alone, B153-2-2 plus 65L-2, and a control without either organism. The treatments were arranged in a randomized block design with four replications. The plot treated with B153-2-2 alone received the bacterial inoculum at 1 g/m^2 (0.67 g/m of row) plus the sterile soil mix at 50 g/m^2 (34 g/m of row). The plot treated with *R. solani* alone received the fungal inoculum at 50 g/m^2 plus the sterile dry mix at 1 g/m^2. The treatment of B153-2-2 plus 65L-2 was applied with the bacterial inoculum at 1 g/m^2, and the fungal inoculum was applied at 50 g/m^2. The control was applied with the sterile dry mix at 1 g/m^2 plus the sterile soil mix at 50 g/m^2. All inocula and additives were distributed by hand into furrows opened with a hoe at planting time. Soybeans were planted on 28 May 1987. Each plot consisted of four 3-m rows. Rows were spaced on 76-cm centers, and each plot was separated with a 1-m-wide border. Four hills per row were planted with seeds of cv. Williams 82. There were 16 plants per plot after the seedlings were thinned.

Two duplicated field experiments with the same design were planned in 1987. Only plots in field 1 were sampled, whereas those in field 2 remained undisturbed. In 1988, neither field plot was tilled. Each treatment in the field plot 1 received the same quantity of each amendment and was applied to the same rows as in 1987. In field plot 2, no additional treatment was made, and this plot was used to estimate residual populations of B153-2-2, which was applied in 1987. Seeds were planted by hand with vinyl gloves that were changed between treatments to avoid cross contamination.

In 1987, soil samples of zone 1 were collected on a weekly basis for 4 wk after emergence. Samples from zone 2 were collected separately on a weekly basis for the first month and then monthly for the remainder of the year. Samples of zone 3 were taken at 32 and 39 days after emergence. In 1988, zone 1 was sampled at weeks 1 and 3, zone 2 was sampled from May to October, but zone 3 was not sampled.

Population data from all the plate counts were converted to common logarithms (base 10) of colony-forming units per gram of soil before statistical analysis. We performed analyses of variance and comparison of the general linear model by using SAS (25). Means were separated on the basis of least significant difference (LSD) at $P = 0.05$.

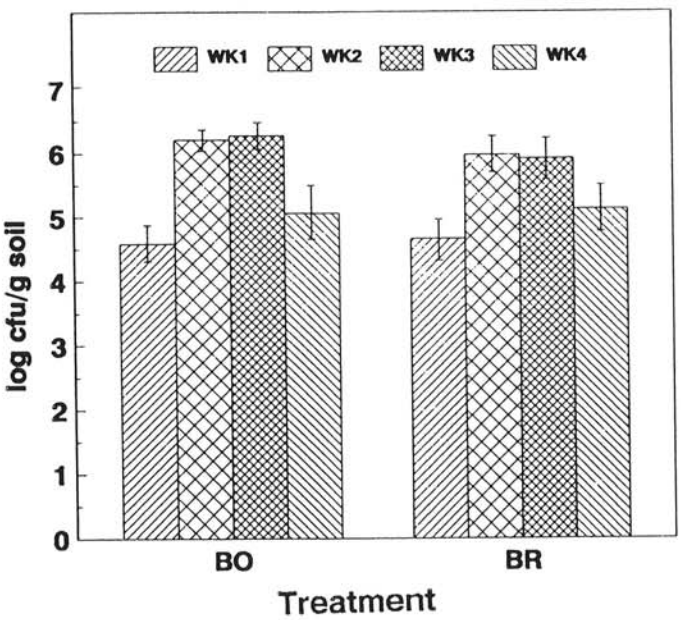

Fig. 2. Mean populations (log colony-forming units [cfu] per gram of soil) of *Bacillus megaterium* B153-2-2 in the soybean root zone 1 at 1, 2, 3, and 4 wk after emergence in plots in which the bacterium was introduced without (BO) or with (BR) *Rhizoctonia solani* 65L-2. Vertical lines indicate standard deviations for the mean populations.

RESULTS

Colonies of B153-2-2 from soil samples in root zone 1 were recovered on NA^{rif} only from the plots in which it was introduced for 2 yr. No colonies were recovered on NA^{rif} from soil samples from plots treated with *R. solani* alone or the control in each year. Populations of B153-2-2 showed no significant difference between plots treated with the bacterium alone compared to those co-infested with *R. solani* (data not shown). At week 1, B153-2-2 populations were recovered at 4.6 log cfu/g of soil for both treatments. The highest populations of 6.3 and 6.0 log cfu/g of soil were recorded at week 3 for treatment with the bacterium alone, and at week 2 for the treatment with *R. solani* plus B153-2-2, respectively. The population size of B153-2-2 was significantly greater at weeks 2 and 3 than at weeks 1 and 4 for both treatments (Fig. 2). Similar populations were recovered in the second year after the second application of the bacterium (data not shown).

Colonies of *Bacillus* spp. were identified by their distinct morphological characteristics on NA compared with other soil microflora from the soil dilutions. The gram-positive reaction was confirmed for pre-identified colonies. We counted the colonies of *Bacillus* spp. at 12 h to avoid colonies of slow-growing bacteria. Total populations of naturally occurring *Bacillus* spp., including B153-2-2, were similar for all treatments at the end of the growing season (Table 1). However, B153-2-2 populations were significantly higher in the treatment with B153-2-2 alone compared to those co-infested with *R. solani* for both years.

In root zone 2, mean populations of B153-2-2 for the bacterium treatment alone at a 10-cm depth showed an increase with time, reaching its highest level of 6.8 log cfu/g of soil on day 210 of the calendar year (end of July) and then decreased to 4 log cfu/g of soil on day 338 (early December) in 1987 (Fig. 3A). Without an additional application, B153-2-2 was recovered in the second growing season at 5–6 log cfu/g of soil from May to October. At the 20-cm depth, B153-2-2 populations were stable during the growing season, but decreased toward the end of the year (Fig. 3B). In 1988, the residual populations at this level were recovered between 4 and 6 log cfu/g of soil in June and July. Mean populations of B153-2-2 recovered at the 30-cm depth showed overall low levels, about 5 log cfu/g of soil, with a recovery range differing between replications (Fig. 3C). In 1988, these populations were recovered at 3–5 log cfu/g of soil in June and July. No linear or quadratic relationship fit these populations at any of the three depths. However, B153-2-2 populations at depths of 10 and 20 cm in zone 2 in 1987 showed a relationship close to a cubic model with time, although the fitness was low. Similar results were obtained for B153-2-2 populations from the *R. solani* and B153-2-2 treatment in zone 2 (data not shown).

In root zone 3, B153-2-2 was recovered from all locations in plots treated with the bacterium, except at locations of 10/30 and 50/20 cm at 32 days after emergence (Fig. 4). The lowest

TABLE 1. Mean populations of *Bacillus megaterium* B153-2-2 (Bm) and the indigenous populations of *Bacillus* spp. from soil samples in soybean root zone 1 in December 1987 and October 1988 in the plots co-infested or not infested with *Rhizoctonia solani* 65L-2 (Rs)

	Population (log cfu/g of soil)			
	B153-2-2		*Bacillus* spp.	
Treatment	Dec. 1987	Oct. 1988	Dec. 1987	Oct. 1988
Bm	4.97 a[x]	4.43 a	5.13 a	7.03 a
Bm + Rs	4.07 b	3.40 b	4.97 a	7.43 a
Rs	ND[y]	ND	...[z]	6.87 a
Control	ND	ND	...	6.63 a

[x]Numbers followed by the same letter in a column are not significantly different by LSD at $P = 0.05$.
[y]Not detected.
[z]Missing data.

mean populations (between 2 and 3 log cfu/g of soil) were recovered at 10/20, 20/20, 20/30, 30/30, 40/20, 40/30, and 50/10 cm, and the highest (greater than or equal to 5 log cfu/g of soil) at 20/10 and 30/10 cm. The remaining sites had intermediate populations of greater than or equal to 4 log cfu/g of soil. However, at 39 days, the lowest populations of B153-2-2 recovered were at 10/20, 10/30, 20/20, 20/30, and 50/20; the highest were at 10/10, 20/10, and 30/10; and intermediate populations were found for the remaining locations.

DISCUSSION

Information regarding the fate of a bacterium introduced into soil is essential before such a bacterium can be used for management of rhizosphere microflora; this information assists in the understanding of the relationship between the bacterium and the indigenous microflora. The spatial and temporal distribution and population dynamics of *B. megaterium* B153-2-2 at various levels of soybean root zones provided a comprehensive view of the development of an introduced bacterium in relationship to the soybean rhizosphere and the adjacent soil with time. This information is useful in understanding the ecology of the bacterium, its rhizosphere competence, and potential as a biological control agent of soilborne pathogens. The methods used in this study could be adapted for studies of other introduced bacteria.

The high populations of B153-2-2 in the soybean root zone soil at 2 or 3 wk compared to those at 1 wk after seedling emergence showed that this bacterium was established soon after introduction and increased rapidly early in the season. Similar results were reported from other studies (2,20). A population of 6 log cfu/g of soil is considered an adequate effective concentration for suppression of *R. solani* (13,16). The ability of B153-2-2 to reach this population level early in the season is a desirable characteristic for an introduced bacterium used to suppress soilborne pathogens.

B153-2-2 populations increased with root development throughout the season and then decreased with root senescence at the end of the season; the highest populations were found in the areas of root zone 2. This suggested a close association of these populations with intensive root development. High levels of B153-2-2 populations were recovered from soybean roots (12). Crude soybean root exudates were shown to attract B153-2-2 cells (X. Y. Zheng, Z. L. Liu, and J. B. Sinclair, *unpublished data*), which suggested a role of soybean root exudates in the distribution and growth of B153-2-2 in soybean root zones. Cells of B153-2-2 were found attached to specific sites on the seedling hypocotyl after it was vigorously washed (Z. L. Liu and J. B. Sinclair, *unpublished data*), which could also be associated with root exudates.

The spatial and temporal distribution patterns of B153-2-2 showed that the bacterium moved further downward than it did laterally. Bacterial dispersal in the soil was at least 30 cm horizontally and 50 cm vertically from the application site. These results can be explained, in part, by the physical filtration and adsorptive forces that affect bacterial movement in soils (26,27),

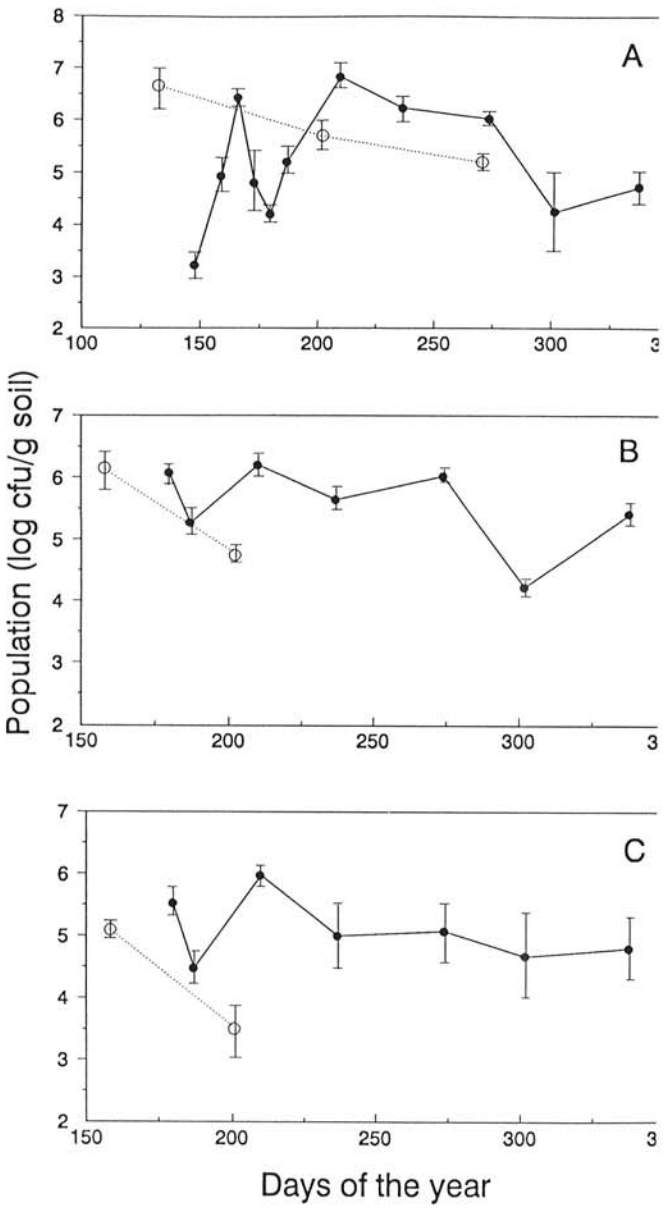

Fig. 3. Mean populations (log colony-forming units [cfu] per gram of soil) of *Bacillus megaterium* B153-2-2 recovered with time in soybean root zone 2 at **A**, 10-; **B**, 20-; and **C**, 30-cm depths within the first year (1987, ●–●) after application and the subsequent year (1988, ○–○) without the addition of the bacterium. Vertical lines indicate standard deviations for the mean populations.

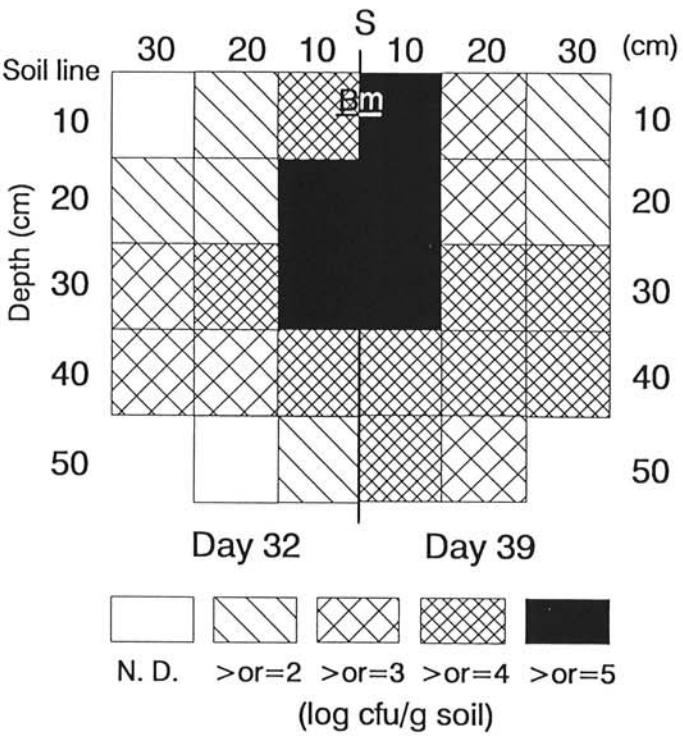

Fig. 4. Spatial and temporal distributions of populations (log colony-forming units [cfu] per gram of soil) of *Bacillus megaterium* B153-2-2 in an extended soybean root zone at 32 and 39 days after plant emergence in the field in which the bacterium was introduced. Site Bm indicates the relative location of B153-2-2 application, and site S indicates the location of a soybean plant. N.D. indicates not detectable, where the bacterial populations were below 2 log cfu/g of soil. The root zone was assayed every 10 cm laterally out from a taproot and vertically down from the soil surface.

particularly the percolation of water that enhances downward movement of bacteria (5,11,23). Therefore, rainfall and irrigation water could contribute to the downward movement of B153-2-2 in soil. However, populations of B153-2-2 were not distributed randomly in the soil but were closely associated with the root zones. These results agreed with previous studies (24).

Residual populations at 4 and 6 log cfu/g of soil were recovered from soil treated with the bacterium (May 1987) in December 1987 and May 1988, showing that B153-2-2 survived the winter in the field and increased in the next growing season when soil and other environmental conditions were favorable for its multiplication. Because the populations of B153-2-2 recovered at the end of 1987 were no less than 4 log cfu/g of soil for treatments with the bacterium alone or in combination with *R. solani*, 4 log cfu/g of soil could be considered the survival population threshold for B153-2-2 in a soybean field. B153-2-2 was recovered during the growing season and at the end of the year, showing that B153-2-2 was competitive with indigenous microflora in the soybean field.

Populations of B153-2-2 recovered from plots treated with the bacterium alone or in combination with *R. solani* did not show a significant difference during the growing seasons. However, the bacterial populations from the combined treatment were significantly lower compared to the bacterium treatment alone at the end of the season for 2 yr. The reason for this low recovery was not clear. Nonetheless, co-infestation with *R. solani* did not affect the colonization of root zone soil by B153-2-2. Indigenous soil microflora does not limit soybean root colonization by B153-2-2 (12) and its colonization in the rhizosphere and root zones, which suggested that B153-2-2 was competitive in soybean field soil.

The wild type of B153-2-2 was originally obtained from a soybean plant grown in the same soil type in which these field studies were conducted; thus, it would be expected that B153-2-2 should be compatible with the indigenous microflora of the site. The compatibility of B153-2-2 with the microflora in other soil types and ecosystems needs to be studied.

The close association of B153-2-2 with soybean, its compatibility with soil microflora, and the ability of the bacterium to colonize soybean roots, rhizosphere, and extended root zones make *B. megaterium* B153-2-2 a potential candidate for use in rhizosphere management. The bacterium could also serve as a vehicle for introducing genetically engineered characteristics into the rhizosphere.

LITERATURE CITED

1. Bahme, J. B., and Schroth, M. N. 1987. Spacial-temporal colonization patterns of a rhizobacterium on underground organs of potato. Phytopathology 77:1093-1100.
2. Bowen, G. D. 1980. Misconceptions, concepts, and approaches in rhizosphere biology. Pages 283-304 in: Contemporary Microbial Ecology. D. C. Ellwood, J. N. Hedger, M. J. Lathan, J. M. Lynch, and J. H. Slater, eds. University of Warwick, Warwick, U.K.
3. Bowen, G. D. 1991. Microbial dynamics in the rhizosphere: Possible strategies in managing rhizosphere populations. Pages 25-32 in: The Rhizosphere and Plant Growth. D. L. Keister and P. B. Cregan, eds. Kluwer Academic Publishers, Dordrecht, Netherlands.
4. Buchanan, R. E., and Gibbons, N. E. 1974. Bergey's Manual of Determinative Bacteriology. 8th ed. Williams and Wilkens & Co., Baltimore, MD. 1246 pp.
5. Chao, W. L., Nelson, E. B., Harman, G. E., and Hoch, H. C. 1986. Colonization of the rhizosphere by biological control agents applied to seeds. Phytopathology 76:60-65.
6. Fletcher, M., Latham, M. J., Lynch, J. M., and Rutter, P. R. 1980. The characteristics of interface and their role in microbial attachment. Pages 67-78 in: Microbial Adhesion to Surfaces. R. C. W. Berkeley, J. M. Lynch, J. Melling, P. R. Rutter, and B. Vincent, eds. Ellis Horwood Lts., Chichester, W. Sussex, U.K.
7. Foster, R. C. 1985. The Biology of the Rhizosphere. Pages 75-79 in: Ecology and Management of Soilborne Plant Pathogens. C. A. Parke, A. D. Rovira, K. J. Moore, P. T. Wong, and J. F. Kollmorgen, eds. The American Phytopathological Society, St. Paul, MN.
8. Foster, R. C., and Bowen, G. D. 1982. Plant surface and bacterial growth: The rhizosphere and rhizoplane. Pages 159-195 in: Phytopathogenic Prokaryotes. I. M. S. Mount and G. H. Lacy, eds. Academic Press, New York.
9. Gordon, R. E., Hanyes, W. C., and Pang, C. H-N. 1973. The Genus *Bacillus*. USDA, Agric. Res. Ser. Washington, DC. 284 pp.
10. Katzenlson, H., Lochhead, A. G., and Timonin, M. I. 1984. Soil microorganisms and the rhizosphere. Bot. Rev. 14:543-587.
11. Liddell, C. M., and Parke, J. L. 1989. Enhanced colonization of pea taproots by a fluorescent pseudomonad biocontrol agent by water infiltration into soil. Phytopathology 79:1327-1332.
12. Liu, Z. L., and Sinclair, J. B. 1988. Colonization and population dynamics of *Bacillus* isolate B153-2-2 on soybean root and rhizosphere. (Abstr.) Phytopathology 78:1523.
13. Liu Z. L., and Sinclair, J. B. 1989. A primary study of biological control of Rhizoctonia damping-off, root and crown decay of soybeans. (Abstr.) J. Cell. Biochem. Suppl. 13A:177.
14. Liu, Z. L., and Sinclair, J. B. 1990. Enhanced soybean root growth and nodulation by *Bradyrhizobium* in the presence of strains of *Bacillus megaterium* B153-2-2 in soybean rhizosphere soil (Abstr.) Phytopathology 81:1179.
15. Liu, Z. L., and Sinclair, J. B. 1991. Effects of seed coating by *Bacillus* spp. on suppression of Rhizoctonia damping-off, root and stem rot on soybeans. Biol. Cult. Tests 6:62.
16. Liu, Z. L., and Sinclair, J. B. 1991. Population dynamics of *Bacillus megaterium* B153-2-2 in soybean rhizosphere soil. (Abstr.) Phytopathology 81:1179.
17. Liu, Z. L., and Sinclair, J. B. 1991. *Rhizoctonia solani* anastomosis group 2-2 pathogenic to soybean. Plant Dis. 75:682-687.
18. Liu, Z. L., and Sinclair, J. B. 1992. Genetic diversity of *Rhizoctonia solani* anastomosis group 2. Phytopathology 82:778-787.
19. Loper, J. E., Suslow, T. V., and Schroth, M. N. 1984. Lognormal distribution of bacterial populations in rhizosphere. Phytopathology 74:1454-1460.
20. Newman, E. I., and Watson, A. 1977. Microbial abundance in the rhizosphere: A computer model. Plant Soil 48:17-56.
21. Parke, J. L. 1991. Root colonization by indigenous and introduced microorganisms. Pages 33-42. in: The Rhizosphere and Plant Growth. D. L. Keister and P. B. Gregan, eds. Kluwer Academic Publishers, Dordrecht, Netherlands.
22. Parke, J. L., Liddell, C. M., and Clayton, M. K. 1990. Relationship between soil mass adhering to pea taproots and recovery of *Pseudomonas fluorescens* from the rhizosphere. Soil Biol. Biochem. 22:495-499.
23. Parke, J. L., Moen, R., Rovira, A. D., and Bowen, G. D. 1986. Soil water flow affects the rhizosphere distribution of a seed-borne biological control agent, *Pseudomonas fluorescens*. Soil Biol. Biochem. 18:583-588.
24. Rovira, A. D., Bowen, G. D., and Foster, R. C. 1983. Significance of rhizosphere microflora and mycorrhicas in plant nutrition. Pages 61-93 in: Encyclopedia of Plant Physiology. New Ser. Vol. 15. A. Lanchli and R. L. Beileski, eds. Springer-Verlag, Berlin.
25. SAS Institute, Inc. 1985. SAS User's Guide: Statistics. Version 5. SAS Institute, Inc., Raleigh. 956 pp.
26. Weller, D. M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopathol. 26:379-407.
27. Wong, P. T. W., and Griffin, D. M. 1976. Bacterial movement at high metric potentials. I. In artificial and natural soils. Soil Biol. Biochem. 8:215-218.