Factors Relating to Peanut Yield Increases After Seed Treatment with *Bacillus subtilis*

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ABSTRACT

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Bacillus subtilis, when added as a seed treatment, consistently colonized the roots of peanut plants at rates exceeding 10^4 colony-forming units (cfu) per gram of root tissue when evaluated 120 days after planting. Yield increases from 1982 to 1985 ranged from −3.5 to 37%, with only two incidences of negative responses in 24 tests. Peanuts responded most favorably to the bacterial seed treatment when subjected to stresses, such as limited water availability, poor rotational practices, or cool soils, caused by early plantings. Treatment of peanut seed with *B. subtilis* was associated with improved germination and emergence, increased nodulation by *Rhizobium* spp., enhanced plant nutrition, reduced levels of root cankers caused by *Rhizoctonia solani* AG-4, and increased root growth. The means by which *B. subtilis* may affect yield in peanuts are multiple and are not operative at the same time, making predictions of degree of yield responsiveness difficult.

Additional keywords: bacterization, root rot

Bacillus subtilis (Ehrenberg) Cohn has been used for many years in attempts to control plant pathogens and increase plant growth. Seed treatments with *B. subtilis* have since been shown to control various diseases in a variety of crops, including diseases caused by *Rhizoctonia solani* Kühn in wheat, brown spot of rice, and damping-off in tomato and sugar beet (5,12–14).

Isolate A-13 of *B. subtilis*, obtained from lysed mycelium of *Sclerotium rolfsii* Sacc. (2), has been particularly useful in increasing yields and stimulating plant growth. Yield increases of up to 40% in oats and 48% in carrots were reported (13). Plant growth responses to seed treatments with *B. subtilis* A-13 have been reported in both unsteamed (2) and steamed soils (1).

In 1980, Backman (*unpublished data*) found that *B. subtilis* A-13 increased emergence and vigor in Florunner peanuts. Subsequent work showed a 13.6% increase in yield after seed treatment with *B. subtilis* in 1981 and a 24% increase in 1982 (4). In a 3-yr study conducted in Texas, the average yield increase attributable to seed treatment of peanuts with *B. subtilis* was 16.0% for cv. Florunner and 5.9% for Spanish types (7).

Production and harvest of *B. subtilis* in quantities necessary for agricultural use is relatively easy (8). Because it forms endospores, it can be formulated in dusts, wettable powders, and flowables while retaining viability. The bacterium is fully compatible with available chemical seed treatments for peanuts (4). The special suitability of the product, along with the favorable responses in peanuts, prompted commercialization of *B. subtilis* as a seed treatment for peanuts. It is currently produced by Gustafson, Inc. (Dallas, TX). It was first available on a limited basis in 1983 and has since been made fully available as Quantum-4000 for use on peanuts, cotton, and common beans.

Little information is available on the frequency and magnitude of peanut yield responses to *B. subtilis* in the southeast or on the interactions of *B. subtilis* with the peanut plant and with various components of the rhizosphere that affect plant growth. Because this is one of the first cases where an inoculant has reached successful commercialization, a series of studies was designed to elucidate the factors related to yield response of peanuts to *B. subtilis* with emphasis to those operable under field conditions.

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MATERIALS AND METHODS

Seed treatment. Seeds used in these experiments were treated in the laboratory with a fungicide at a rate of 7.8 ml/kg of seed. Pro-Ized II is a trade name for a flowable product consisting of 10% Botran (DCNA) and 16% Thiram (thiuram), each by weight (Gustafson, Inc., Dallas, TX). A wettable formulation of *B. subtilis* strain A-13 was added as Quantum-4000 (Gustafson, Inc.) to the flowable fungicide before its application to the seeds. The Quantum-4000 powder contained 1.13×10^8 spores per milligram, which was applied at a rate of 660 mg/kg of seed to achieve a final spore count of 7.5×10^{10} spores per kilogram of seed. The fungicide-bacterial spore mixture was then sprayed on the seeds with an air-gun sprayer while the peanut seeds were rolled for 1 min in a cement-mixer type drum. Seeds used in the 1983 regional field trials were treated by several commercial seed processors that market the products of Gustafson, Inc.; Pro-Ized II and a wettable powder of *B. subtilis* were used at the same rates as listed previously.

Root colonization by *B. subtilis*. Peanut roots were collected to a depth of 14 cm at harvest. Excess soil was removed by vigorous shaking, after which the roots were air-dried at room temperature for several days. Samples of each root contained a representative sample of secondary roots and several sections of the taproot. One gram (dry weight) of each sample was placed in 100 ml of water in a Waring blender and chopped at high speed for 1 min to produce a slurry. Large fragments were

removed by a single filtering through one layer of cheesecloth. The root suspension was diluted in a 10-fold series, after which 0.1-ml aliquots were spread onto a semiselective medium (400 ml of V-8 juice, 40 g of NaCl, 1 g of dextrose, 20 g of agar, and 600 ml of tap water) in petri dishes. The pH was adjusted to approximately 5.2 before autoclaving, resulting in a final pH of 4.8–5.1. Colonies of *B. subtilis* were enumerated after incubation at 30 C for 48 hr and are reported as colony-forming units (cfu) per gram of dry root weight.

Colonization of various regions of the peanut root system. Various regions of the root were analyzed separately by the above technique to determine the area(s) of heaviest colonization. Roots were cut so that the following areas were represented: 1) the crown (which consisted of the area of branching just above and below the soil line); 2) upper taproot, from the soil line to 3 cm below the soil line; 3) lower taproot, at depths greater than 6 cm from the soil line; 4) secondary roots, from the taproot outward to 3 cm; and 5) the secondary roots, at greater than 3 cm from the taproot. The study was repeated three times in 1983 on peanut roots from three field tests—two in Headland, AL, and one in Auburn, AL. Both treatments were present in six replicate blocks at each field test site.

Field tests. Field tests were conducted from 1983 to 1985 at the Wiregrass Substation in Headland. Each plot was 6.1 m long and consisted of two rows spaced 91 cm apart. Each row contained 70 seeds, which were planted with a cone planter (Kincaid Equipment Manufacturing Corp., Haven, KS). All soils were a Dothan sandy loam that had been previous seasons. Common to all field tests were the seed treatments of Pro-Ized II fungicide and Pro-Ized II fungicide + *B. subtilis*. Tests in 1983 included a test for emergence and vigor in which 24 seed lots were used to determine the extent to which these growth responses were dependent on seed lot. In 1984, tests included trials comparing colonization of the cultivars Pronto, Valencia, Florigiant, and Florunner and a test to determine the effects of planting time (19 April, 1 May, and 24 May) on Florunner seedling emergence and vigor. All field trials were randomized complete blocks with four to six replications.

In the regional field trial of 1983, 24 farms were chosen randomly by cooperating seed processors in Georgia, Alabama, and Florida to evaluate peanut responses to seed treatment with *B. subtilis*. Cooperating farmers were asked to return a questionnaire on the fields where the tests were performed. Information sought included planting date, cropping history, and other information on cultural practices. Seeds of Florunner treated with Pro-Ized II fungicide alone and seed treated with Pro-Ized II + *B. subtilis* were planted as paired plots at each location so that each location constituted a replication. Peanuts were evaluated for root disease at midseason (approximately 70 days after planting) at 15 locations and just before harvest at 14 locations. These same areas were harvested at the end of the season for yield by growers. Peanut yields were determined from equal-sized adjacent areas (0.4 ha) from each treatment and were weighed by the cooperating processors.

Plant nutrition. Experiments were performed to determine the effect of *B. subtilis* on various aspects of plant nutrition. This involved leaf and stem tissue analysis and analysis of rhizosphere soil from field experiments at the Wiregrass Substation at Headland and leaf and stem analysis from plants grown at the rhizotron plots at Auburn. For foliar analysis, three duplicates of each sample were analyzed. For the soil analysis, only two duplicates were used because of the small amounts of rhizosphere soil available. The treatment comparison was fungicide (see seed treatment) used alone and fungicide + *B. subtilis*. The descriptions of the general protocols follow and specific details are available (6).

Leaf and stem tissues were analyzed for nitrogen (N) with a Leco 600 CHN analyzer. Leaf samples were collected from plants grown in the field and at the USDA rhizotron (Auburn, AL) just before harvest (approximately 130 days postplanting). Fully expanded leaves, removed systematically from the upper (young leaves) and lower (old leaves) portions of 130- to 140-day-old peanut plants, were used. Stem sections comprising the most acropetal 15 cm of stem minus the meristematic region (upper 3 cm) were also analyzed. Tissues were dried in an oven at 60 C for 48 hr, then ground in a plant mill with 0.1 g of each sample used for N determination.

Levels of other nutrients (P, K, Mg, Ca, Cu, Fe, Mn, Zn, and B) in leaves were determined by the Auburn University Soil Testing Laboratory by means of inductively coupled argon plasma (ICAP) spectroscopy with a Jarrell-Ash 9000 analyzer. For this analysis, the first fully expanded leaves were taken from field grown plants in early August. These leaves were dried and ground as before. A sample size of 0.5 g was used for analysis.

Effects of *B. subtilis* on *Rhizobium*. Levels of root nodulation by *Bradyrhizobium* spp. were determined by a subjective rating of the taproots and secondary roots of untreated peanut plants and peanut plants treated with *B. subtilis* before harvest in 1984. Plants from field plots in Headland were rated approximately 110 days after planting. Ten roots from plants within each of six replicate plots were collected randomly from both bacterized and nonbacterized plants by removing the plants and cutting off the root systems (upper 20 cm of taproot with attached secondary and feeder roots). The roots were then rated on a scale from 1 to 5, in which 1 = no nodules, 2 = 25%, 3 = 50%, 4 = 75%, and 5 = 100% of the root surface nodulated.

In addition to comparisons of nodulation of treated and control plants, *Rhizobium* spp. were isolated from peanut nodules and evaluated for sensitivity to antibiotics produced by *B. subtilis*. Excised nodules were surface-sterilized in 1.0% NaOCl for 5 min, cut aseptically in half, and placed on yeast extract-mannitol agar. Four isolates recovered in this manner were examined for sensitivity to antibiotics produced by *B. subtilis*. A loop of *B. subtilis* was streaked across the agar so that a single solid band of bacterial growth could form. Three days later, the isolates of *Rhizobium* spp. were streaked onto the same plate perpendicular to the direction of the growth of *B. subtilis*, by starting at the edge of the plate and pulling the inoculation loop into contact with the *B. subtilis*. Plates were observed for clear zones adjacent to the growth of *B. subtilis* after 3 days.

Rhizotron studies. The USDA rhizotron facility at Auburn, a facility designed for plant root research, was used to compare growth of peanut plants that received *B. subtilis* as a seed treatment with those that did not. The plots were 112 × 61 cm in area and 1.8 m deep. The entire volume of soil used was topsoil of a Dothan sandy loam from a field in Headland in which peanuts had been grown for seven successive years. One of the walls of each compartment was glass, allowing root observations and measurements. Growth comparisons were made under two water regimes, one in which plants received natural rainfall all season, and one in which the plots were covered in order to impose moisture stress on the plants. A total of four compartments were used, allowing comparisons between seeds treated with *B. subtilis* and untreated seeds under both moisture regimes. To each of the dry plots, 2.4 cm of water was supplied on each of three occasions as the plants neared the point of permanent wilting as determined visually by a lack of recovery from wilt at night. Soil moisture was determined by electrical conductivity through ceramic soil moisture blocks imbedded in the soil at a depth of 30.5 cm.

Root growth measurements were made twice weekly during the early part of the season (0–60 days) and once weekly during the latter part (60–132 days). The measurements were recorded as centimeters of new roots measured against the glass. Different colored wax

markers were used each week so that root age could be determined at later dates. Root decline or death was based on visual observations twice during the growing season with a subjective rating scale where 1 = newly formed healthy roots and 5 = roots that appeared brown and necrotic. Intermediate values corresponding to the various degrees of discoloration were also recorded. Roots of similar and different age classes were evaluated with this method by erasing the colored marks representing the appropriate age classes and observing the condition of the underlying roots.

Studies on emergence, vigor, and plant phenologies. Peanuts grown at Headland were assessed for peanut emergence twice after planting and for vigor several times during the growing season. Vigor was assessed on a subjective rating scale from 1 to 5, with 1 being the least vigorous and 5 being the most vigorous in terms of height, width, and general appearance of the plant. Vigor was assessed on the basis of an entire plot, irrespective of emergence in the plot. (A plot with poor emergence could receive a high vigor rating.) Plants grown in microplots at the rhizotron were evaluated with respect to emergence, vigor, numbers of nodes, lengths of vertical and horizontal runners, numbers of pods, numbers of pegs, and pod weights. Peanut plants growing on farms that were part of the regional field trials of 1983 were similarly evaluated for vigor at pod set (60 days after planting) and again near harvest.

Taproot disease assessments. A subjective root disease rating scale (18), ranging from 1 to 5 where 1 = no taproot cankers, 2 = 25% of taproot surface area diseased, 3 = 50% of taproot diseased, 4 = 75% of taproot diseased, and 5 = taproot entirely diseased, was used. These taproot cankers were previously reported to be primarily caused by *R. solani* AG-4 (18). Assessments were performed for the regional field trials in 1983 and in all Headland field trials from 1983 to 1985. Taproots were collected throughout the season and from inverted plants at harvest. Five to 10 taproots (extending from the soil line to 20 cm deep) were removed from randomly chosen plants in each plot to assess disease severity. Each root was thoroughly washed and rated while wet to enhance visualization of the dark, sunken cankers.

Table 1. Colonization by *Bacillus subtilis* of roots of 140-day-old peanut plants of various cultivars grown at Headland, AL

Cultivar	Root colonization (log cfu/g root ± standard error)
Florunner	4.32 ± 0.09
Pronto	4.72 ± 0.19
Valencia	4.58 ± 0.37
Florigiant	4.25 ± 0.11

RESULTS

A mean population of *B. subtilis* on roots of field-grown Florunner peanut plants from all tests in Headland was found to be 3.21×10^4 cfu/g of dry root tissue. In tests conducted from 1983 through 1985, peanut taproots were consistently colonized at populations greater than 10^4 cfu/g of dry root in Headland. Peanut roots sampled from the four tests of the 1983 regional field trials were always found to be colonized at populations exceeding 10^4 cfu/g of root tissue. Recovery from treated, unplanted seed averaged 1.91×10^5 cfu per seed, whereas the population for total root systems and associated rhizosphere soil was 9.84×10^6 cfu per root system, representing an increase of nearly 100-fold.

B. subtilis colonized roots of all cultivars tested (Table 1) at levels similar to those for Florunner, a cultivar previously shown to be responsive to seed treatment with *B. subtilis* (4). These populations were based on samples representing all areas of the root.

Certain regions of the roots of Florunner were more heavily colonized by *B. subtilis* than others (Table 2). In general, colonization was greatest in the upper taproot region, and populations decreased toward the tip of the taproot. Relatively few bacteria were found on the secondary roots.

Field experiments. The greatest yield increase attributable to *B. subtilis* in the five Headland field trials conducted from 1983 to 1985 was 17% in 1984. The only other significant ($P = 0.05$) yield increase during this period was 11.7% in 1983. Yield improvements averaged 5.7% in Headland trials. Statistically significant differences in levels of root cankers induced by *Rhizoctonia* spp. were not observed, although root rot severity was almost always negatively correlated ($P = 0.05$) to yield.

The most extensive field study on yield response to *B. subtilis* was conducted in 1983 when responses were measured at 24 locations throughout Georgia and Alabama. The average yield of fields receiving seeds treated with fungicides alone was 3,918 kg/ha, whereas those with seeds treated with fungicide + *B. subtilis* averaged 4,219 kg/ha, an increase of 7.6%. Yield responses to bacterization ranged from −3.5 to 37%, with only two locations producing negative responses. Based on information received for 16 of the 24 sites, two cultural practices (rotational history and planting date) appeared to be related to yield response (Table 3). The average yield response among fields planted early (before 7 May) and having poor rotational histories (legumes in either of the previous 2 yr) was 14.5%. Significant differences ($P \leq 0.05$) in root disease severity were not observed in any of 15 locations evaluated during the growing

Table 2. Colonization of various regions of the peanut root system by *Bacillus subtilis* determined by dilution plating of comminuted root sections of 120-day-old peanuts grown in Auburn and Headland, AL, in 1985

Root area	Colonization (log cfu/g root tissue)			
	Auburn field	Headland test 1	Headland test 2	Mean
Secondary roots, < 3 cm from taproot	ND	0.75	1.05	0.90
Secondary roots, > 3 cm from taproot	0.75	1.15	ND	0.95
Taproot, lower, > 9 cm below crown	ND	2.46	2.15	2.30
Taproot, middle, 3–9 cm below crown	4.50	3.74	4.15	4.13
Taproot, upper, crown to 3 cm	4.93	4.42	4.44	4.59
Crown area[a]	4.21	4.06	3.92	4.06
LSD ($P = 0.05$)	1.28	0.96	1.68	

[a] Crown area was defined as the area of branching near the soil line.

Table 3. Effects of crop rotation and planting date on yield response to seed treatment with *Bacillus subtilis* in 16 regional field trials done in Georgia, Alabama, and Florida in 1983

Planting time	Average yield increase (%)[a]		Means[d]
	Legumes[b]	No legumes[c]	
Early (before 7 May)	14.5 [6]	2.0 [2]	11.4 ± 3.6 [8]
Late (after 7 May)	8.9 [5]	3.6 [3]	6.8 ± 4.4 [8]
Mean	12.0 ± 3.8 [11]	2.96 ± 1.18 [5]	

[a] Numbers in brackets are the number of fields in each mean.
[b] Legumes in either of previous 2 yr.
[c] No legumes in either of previous 2 yr.
[d] Factorial means are followed by a standard error for that mean.

season and at only one of the 14 sites sampled near harvest time. At this location, roots of treated plants received a root rot rating of 2.02 as opposed to 2.31 for the controls. The field had been continuously planted to legumes.

Plant nutrition studies. In 1985, the leaf and stem N analysis revealed significantly higher levels of N in leaves of plants grown from seeds treated with *Bacillus* in field plots at Headland (Table 4). This was also true for plants grown in the rhizotron (*data not presented*). The range of N increase in these two tests was 2.3–24.0%. For the rhizotron-grown plants, N was higher for all plants that were not water stressed, regardless of bacterial treatment. The soil analysis performed in 1985 revealed no differences in levels of available nutrients caused by bacterization in either the rhizosphere soil of field grown plants or of plants grown at the rhizotron.

In 1986, when leaves of field grown plants were subjected to a complete nutrient analysis, nitrogen was again higher by 12.3% ($P = 0.05$) among plants treated with *Bacillus*; boron and potassium were also increased by 16.4 and 10.3% ($P = 0.1$), respectively (Table 5).

Nodulation by *Rhizobium* was found to be greater on roots of plants treated with *B. subtilis* than on those of control plants in a 1984 Headland field test but not in a 1985 test. The average nodulation rating for peanuts receiving the Pro-Ized II fungicide seed treatment was 3.5 compared with 4.3 for the peanuts treated with the same fungicide + *B. subtilis* ($LSD_{0.05} = 0.52$) in 1984. All isolates of *Rhizobium* tested were sensitive to the antibiotics produced by *B. subtilis* in vitro.

In studies not reported here, there was no effect of *B. subtilis* on vesicular-arbuscular mycorrhizae colonization when assessments were made 40–60 days after planting.

Rhizotron studies. Responses of root growth to inoculation by *B. subtilis* were observed when water stress was imposed. Figure 1 shows the soil moisture content for each of the treatments. For plots receiving normal rainfall, root growth occurred for only the first 80 days (early pod development stage), after which growth abruptly ceased (Fig. 2). Under this water regime, there was little difference in root populations for plants treated with *B. subtilis* compared with peanut plants not treated with the bacterium. Divergence of the curves (Fig. 2) in water-stressed peanut plots occurred principally during the middle of the season (50–80 days, corresponding to the pegging growth stage) when growth rates were near maximal. At the end of the season, the bacterial treatment had 28.5% more total root length.

No differences in rates of root senescence or death were observed among any of the four treatments. For all treatments, roots started to decline quickly 2 wk after they were produced, with most having root ratings between 4 and 5 after 5 wk. Root browning, indicative of root death, was gradual with very few observations of acute decline.

Studies on emergence and plant phenologies. Only plants from one of the 16 location/seed lots included in the 1983 regional field trials exhibited a significant increase in seedling emergence after treatment with *B. subtilis* (*data not presented*). In the five other tests, established in 1983, there was only one significant emergence response among the seed lots and locations evaluated. In 1984, an increase in emergence attributable to *B. subtilis* was observed ($P = 0.05$) only at the earliest planting date (19 April). At this time, emergence was 43% for the *B. subtilis* treatment and 31% for the control. Emergence from seed treated with *B. subtilis* increased to more than 60% for the second planting, but no differences from the control were observed at either this or the final planting. Corresponding mean soil temperatures (9 cm) for the three dates were 21.1, 26.7, and 27.8 C. In 1985, no significant emergence responses were detected. However, soil temperatures were found to be 27 C or greater for all 1985 experiments.

Between 1983 and 1985, visible differences in vigor occurred twice in five tests at the field plots in Headland. In both cases, the increased vigor was most obvious approximately 50 days after planting. Corresponding yield increases for treatments with improved vigor over their control associated with the use of *B. subtilis* were 12 and 17%, respectively. Of the 16 other sites used for the regional field tests that same year, plants in three sites were observed to have a vigor increase in response to the bacterial seed treatment, with appreciable yield increases in response to *B. subtilis* occurring at these three and numerous other locations.

Plant phenology measurements made in two fields in 1984 revealed only small differences in height, width, or numbers of nodes on the vertical stem in the first field. The easily discernible visible responses that had been recorded in 1983 in this field were not apparent. For the second field, one that did display a visible vigor response in 1984, significant differences ($P = 0.05$) in height were found after treatment with *B. subtilis*. Average height of plants treated with *B. subtilis* was 45 cm, compared with 41 cm for the controls. Numbers of nodes on the vertical stem averaged 23 for treated plants, compared with 21 for the untreated plants ($P = 0.1$).

Phenological measurements were also made on plants grown in the rhizotron. Plants treated with *Bacillus* had 16% more pods and more than 100% more pegs than untreated plants when grown under the water-stress conditions. Plants treated with *Bacillus* also had 16% more pods than untreated plants when plants were not grown under water-stress conditions. No differences were detected between treated and untreated plants with respect to yield, vertical height, or number of nodes on the central stem under either of the moisture regimes.

DISCUSSION

The ability of *B. subtilis* to consistently colonize the roots of peanuts is of considerable importance. This consistency indicates that *B. subtilis* can remain viable and successfully inoculate the host under a wide range of conditions. The ability of *B. subtilis* to endure environmental stresses is an advantage over some of the root-colonizing bacteria that do not form endospores, which reportedly

Table 4. Leaf and stem nitrogen content just before harvest of field-grown peanuts treated with *Bacillus subtilis* and fungicide-treated (Pro-Ized II) controls from Headland, AL, in 1985

Treatment	Percentage of nitrogen[a]		
	Upper leaves	Lower leaves	Stems
Pro-Ized II	2.41	2.25	1.19
Pro-Ized II + *B. subtilis*	2.77	2.49	1.33
LSD ($P = 0.05$)	0.22	0.23	0.08

[a] Percent dry weight of nitrogen. Means of three samples removed from each of six replications per treatment.

Table 5. Nutrient content of leaf tissues of field plants grown from seeds treated with Pro-Ized II (fungicide) and those treated with Pro-Ized II + *Bacillus subtilis* in Headland, AL, in 1986

Element	Nutrient content[a]	
	Pro-Ized II + *B. subtilis*	Pro-Ized II
N (%)	2.72**[b]	2.42
P (%)	0.19	0.19
K (%)	0.96*	0.87
Mg (%)	0.92	0.92
Ca (%)	1.60	1.59
Cu (ppm)	11.70	12.29
Fe (ppm)	212.56	213.96
Mn (ppm)	70.43	76.55
Zn (ppm)	27.40	26.23
B (ppm)	93.85*	80.61

[a] Expressed as percentage of dry weight or μg/g of dry weight.

[b] ** = Significantly different from control at 0.05 level; * = significantly different at 0.1, according to Least Significant Difference test.

can drop to population levels insufficient to promote plant growth, particularly under dry conditions (16). The increase of populations of *B. subtilis* attributable to replication on the peanut root may be much greater than 100-fold because bacterial cells can become nonviable, they may be ingested by bacteriaphagous organisms, or they may be moved away from the root and rhizosphere because of physical movement by agents such as rain water.

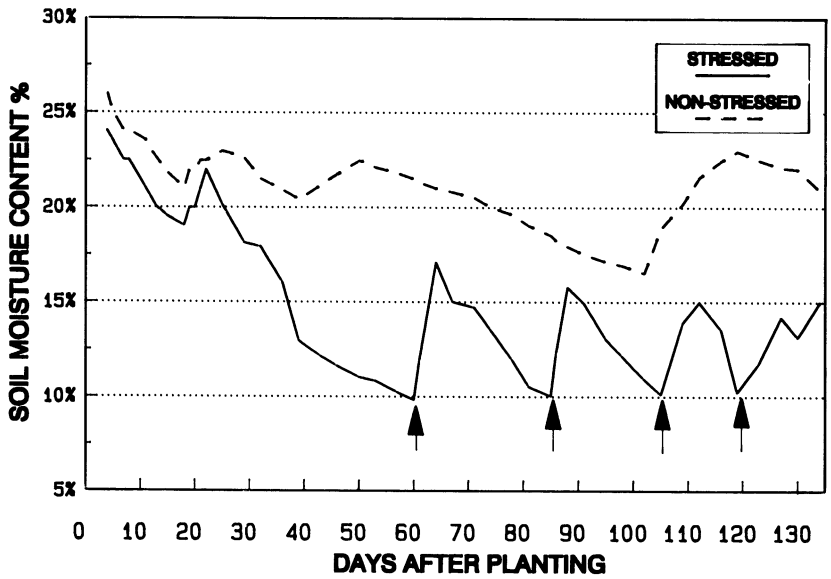

Fig. 1. Average water content at 30.5-cm depth of soils in compartments at the USDA rhizotron, Auburn, AL, as determined by electrical conductivity through ceramic blocks. An arrow indicates the time of application of 2.4 cm of water to the stressed peanut treatments.

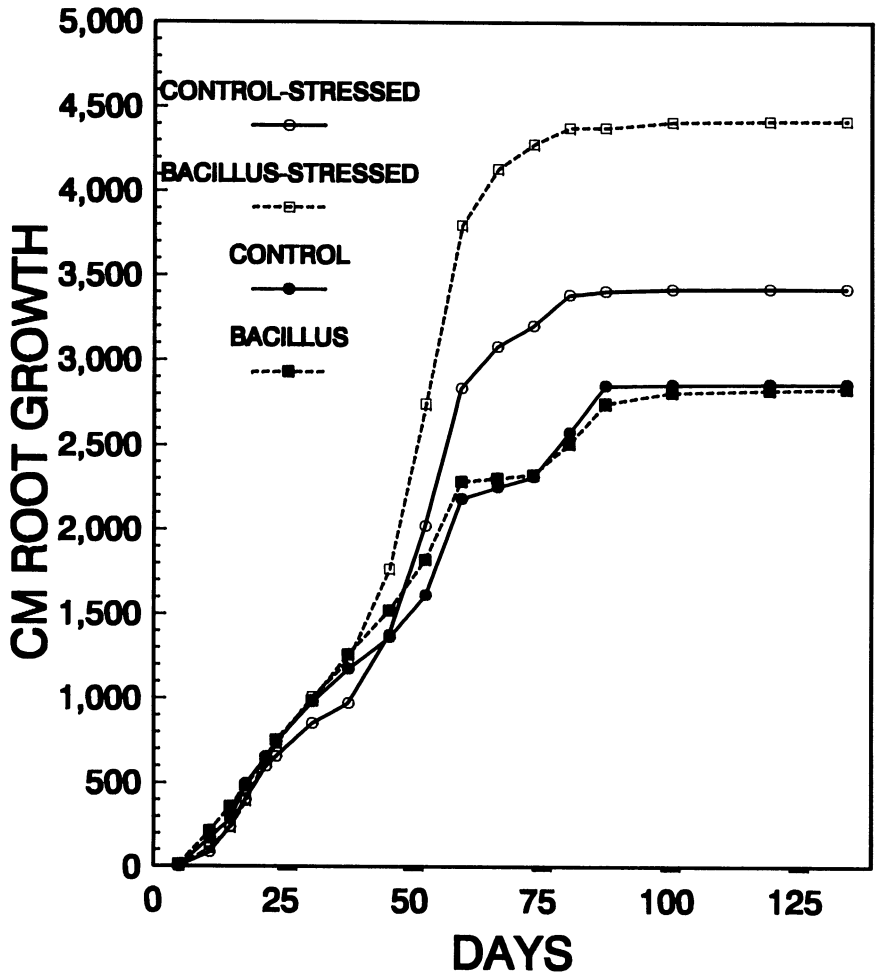

Fig. 2. Root growth curves of roots treated with *B. subtilis* and untreated subjected to water stress or receiving normal rainfall during the growing season at the USDA rhizotron, Auburn, AL. Cumulative root growth refers to the total length of root against the glass wall.

The pattern of inoculation of peanut roots by *B. subtilis* indicated that populations diminish as the distance from the source of the inoculum (the seed coat) increases. This would be expected where the applied organism is subjected to more and more competition as it moves away from the point of inoculation. Second, we found that lateral roots were poorly colonized, even when nearby areas of taproot were highly colonized. This disparity in colonization in the vertical plane as opposed to the horizontal plane indicates that *B. subtilis* does not colonize by maintaining itself on the growing point of roots, but more likely it is carried down the soil profile by the movement of soil water, where it colonizes available roots. This would be consistent with the fact that *B. subtilis* A-13 is nonmotile, and while it is an efficient colonizer, it is not highly competitive.

Taken as a whole, the regional field trials conducted at 24 sites indicated that the responses of peanut to colonization by *B. subtilis* were variable. Yields varied from no response to a 37% increase, whereas vigor responses and root rot control were not routinely correlated with yield increases. When locations were broken down by rotation and time of planting, reasons for the variability became more obvious. Locations that had legumes in their recent rotation and were planted early were most responsive. These results, although compatible with the control of deleterious organisms, could not be justified solely on the basis of control of root cankers caused by *R. solani*. There may be suppression of deleterious rhizobacteria causing chronic reductions in plant vigor (17). Second, there may be a modification of host physiology that accounts for some of the yield increases in the absence of any overt pathology.

The 1984 field test supported previous observations (4) that increased emergence in response to seed treatment with *B. subtilis* was dependent on temperature. A response was observed only at the earliest date when mean soil temperatures were lowest (21 C). It was previously shown that accelerated germination and increased root growth in a seed germination chamber occurred in peanuts at 20 C but not at 27 C (4). The causes for the temperature dependency of these responses was not determined, although fungal damage was ruled out because all seed were treated with effective fungicides. The temperatures that are conducive to enhanced germination and emergence responses for seed treated with *B. subtilis* are not conducive to maximal peanut germination for seed receiving only chemical fungicides. Further research is needed to determine whether the seed treatment with *B.*

subtilis is providing an escape from physiological or pathological stresses at low temperatures. No differences in levels of root disease were found in numerous samplings of field plants taken just after emergence (*data not reported*).

The ability of *B. subtilis* to stimulate root growth at the rhizotron was related to the water status of peanut plants in that root growth was enhanced to a much greater extent by *B. subtilis* when plants were under water stress. The nature of this interaction is not understood. Water stress is known to cause many changes in plants, including increased levels of abscissic acid, decreased levels of cytokinins (19), and shifts in peanut rhizosphere ecology. It is possible that the hormonal levels of the plant and/or the rhizosphere microflora present determine the ability of *B. subtilis* to induce growth increases. These observations are supported by the work of Okon (15), who found that the greatest yield increases in cereal and forage crops in response to inoculation with *Azospirillum* spp. occurred under nonirrigated conditions in semiarid climates.

The mechanism by which *B. subtilis* stimulates root growth is unknown. Gibberellins have been detected in some cultures of *B. subtilis*, although auxins have not (2). While auxins and gibberellins have been shown to be important as products of bacterial origin affecting plant growth (3), ethylene, cytokinins, and abscissic acid may also be important. Mechanisms other than production of growth stimulators by which *B. subtilis* might improve plant growth include: 1) production of a substance that interacts with the plant's own growth regulatory system, 2) enhancement of plant nutrition, or 3) competition with other organisms in the rhizosphere that reduce plant growth (9,11,17). Promotion of root growth in radish by various bacterial treatments has been shown to take place only when other deleterious rhizosphere bacteria are present (19).

Many of the growth responses associated with the use of *B. subtilis* may be merely the result of increased root growth. A more extensive root system would provide more contact surface for nitrogen-fixing bacteria as well as nutrient uptake. Further, the increased growth rate may allow the roots to escape certain root diseases in the rare instances where reductions in levels of root disease have been demonstrated. A combination of the above benefits would result in more vigorous growth. A similar response to *B. subtilis* has been reported in soybeans (10).

The implications of the increased plant vigor response to inoculation with *B. subtilis*, which occurred erratically, are not understood. Yield increases frequently occurred when there was no apparent aboveground vigor response. Vigor increases were not observed in Texas (7), even though yield benefits were routinely obtained. The additional growth in the shoots could be an aboveground response to the additional root growth that results from use of the bacterium under dry conditions. In Texas, where vigor response has not been observed, all experimental plots were irrigated, diminishing the need for additional root growth. The additional yields may also be the result of plants bacterized by *B. subtilis* setting more pods and pegs, escaping root disease, and/or taking up more nutrients.

Experiments conducted in 1985 revealed that nitrogen content was higher in peanut plants treated with *B. subtilis*. At that time, it was believed that this was related to increased nodulation observed previously in response to treatments with *B. subtilis* (4). However, in 1986, two other nutrients, boron and potassium, were also found to be slightly higher for treated plants. Because several nutrients have been shown to increase because of *B. subtilis*, this would suggest that enhanced nutritional status may be caused by increased root growth or improved root health, rather than by the direct interaction of *B. subtilis* with the nutrient ions themselves or with microorganisms that affect their availability.

The yield responses associated with *B. subtilis* cannot be attributed to any single factor. The relative importance (with respect to yield) of the various plant responses to the bacterial seed treatment varies with field and environmental parameters. It is probable that increased root growth, whether caused by competition with deleterious bacteria or by some direct interaction with the plant, is the primary effect of the use of *B. subtilis* and that many of the other responses are related to this response. Enhanced nodulation and increased nutrient uptake associated with use of *B. subtilis* may be the result of more root contact with *Rhizobium* spp. and available nutrient ions. Reductions in taproot cankers may be attributable to the escape phenomenon (the avoidance of disease which is often associated with faster growing plants). Aboveground vigor increases can be explained as response to the production of a more extensive root system and the resulting enhanced nutrition.

Any yield increase associated with the use of *B. subtilis* is, therefore, the additive result of those plant growth responses that occur and are beneficial under a particular set of environmental conditions. Yield increases in response to *B. subtilis* are generally predictable (Table 3). Fields subjected to poor rotational practices and those that are planted early (in cool soils) are likely to benefit from seed treatment with *B. subtilis*.

The consistency with which *B. subtilis* colonizes roots demonstrates environmental insensitivity with respect to survival. This feature will be of great importance should *B. subtilis* be modified genetically to deliver a pesticide or other compound to plant rhizospheres. Environmental insensitivity not only assures colonization under a variety of field conditions, but it ensures that viability will be retained during the time between production and planting, a period in which a variety of stresses may be encountered during transport and storage. The fact that only the upper taproot and nearby branch roots are effectively colonized indicates that yield responses shown here may be small in comparison to a similar organism that can colonize the entire root system.

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