Effect of Bacillus spp. on Increased Growth of Seedlings in Steamed and in Nontreated Soil

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ABSTRACT

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A steamed soil mix in a commercial nursery was infested with *Bacillus* spp. and sown with seeds of 10 plant genera representing eight families. The plant response varied from increased germination and top weight, or both, to no effect, to reduction of either or both of these characters. There was marked plant specificity in response to the bacteria introduced into the soil mixture. In all cases, inoculating with mixed bacterial cultures produced less response than inoculating with a single bacterial isolate. The increase in seedling growth usually was greater under low than under high nutrient levels. Tomato and celosia seedlings, which showed growth response to bacterial inoculation, did not differ from the controls in nutrient analyses of the foliage. Little phosphate was solubilized in vitro by the *Bacillus* spp. isolates studied, and they did not fix nitrogen under aerobic conditions. Seedlings of most genera grew better in a peat-sand mix steamed at 60 C for 30 min than in one steamed at 100 C for 30 min. The phytotoxic effect of the latter treatment was overcome in some cases by subsequent infestation of the mix with bacteria. Field responses to pelleting of cabbage and grain sorghum seed with various bacterial isolates also were obtained.

Additional key words: bacterization, growth substances, biological control, nonparasitic pathogens.

Bedding-plant nurseries usually grow seedlings in steamed or chemically treated soil to reduce losses from soilborne *Pythium*, *Rhizoctonia*, and *Phytophthora* spp. Broadbent et al. (8) found that addition to steamed soil of selected antagonistic bacteria checked development of pathogens introduced subsequently. In the absence of known pathogens, some *Bacillus* spp. increased growth of pepper, snapdragon, and tomato seedlings in nutrient-deficient soils, but not in those adequately supplied with nutrients. However, some isolates of *Bacillus*, *Streptomyces*, and *Pseudomonas* spp. decreased seed germination or seedling growth. Still others produced no noticeable effect. Thus, there was a continuum from increased growth of the host, through no effect, to inhibition of seedling growth, produced by the soil microflora.

Isolate A13 of *Bacillus subtilis* Cohn emend. Prazm., which was isolated by Broadbent et al. (8) and found to increase plant growth, has been used extensively in field trials in nontreated soil in Victoria, Australia. Dipping oat (*Avena sativa* L.) seed in a suspension of this bacterium gave a significant ($P = 0.01$) increase of 40% in grain yield, and 28% in tiller number. Pelleting carrot (*Daucus carota* L. var. *sativa* DC.) seed with A13 gave a significant ($P = 0.01$) increase of 48% in marketable roots; dipping seed in a water suspension of A13 gave a significant ($P = 0.05$) increase of 18% in marketable roots (21). Sweet corn (*Zea mays* L.) pelleted with A13 showed increases of 17.2% in ear weight and 19.5% in number of ears (P. R. Merriman, *personal communication*).

This paper reports tests in an Australian bedding-plant nursery of the feasibility of applying bacteria to a soil mix treated with aerated steam, and of field trials on the pelleting of bacteria on seeds of grain sorghum (*Sorghum vulgare* Pers.) and cabbage (*Brassica oleracea* L. var. *capitata* L.). Studies on the mechanism of the effect on plant growth also are reported.

MATERIALS AND METHODS

Bacillus subtilis A13 was isolated from lysed mycelium of *Sclerotium rolfsii* in vegetable-garden soil, Penrith, N. S. W.; *Bacillus* sp. isolate WW27 was from wheat-field soil, Glen Osmond, South Australia; *Bacillus* sp. isolate Tx1 was from lupine (*Lupinus angustifolius* L.) rhizosphere, Tamborine Mt., Queensland; *Bacillus* sp. isolate DD32 was from wheat-field soil, Ceduna, South Australia; *Bacillus* sp. isolate AA43 was from wheat-field soil, Port Vincent, South Australia. Isolates were

streaked on yeast mannitol agar containing congo red, to determine homogeneity of the cultures. The bacteria were tested for antibiosis to *Phytophthora citrophthora*, *P. cinnamomi*, *P. nicotianae* var. *parasitica*, *Pythium ultimum*, *P. debaryanum*, *Fusarium oxysporum* f. sp. *lycopersici*, *Sclerotium rolfsii*, and *Rhizoctonia solani* on potato-dextrose, Czapek-Dox, and soil-extract agar media (8). All bacteria used in these studies were inhibitory to several of these plant pathogens in agar culture.

Flats (33 × 29 cm) containing a mixture of equal parts of German peat and fine sand plus inorganic fertilizer, were treated with steam (100 C for 30 min) or aerated steam (60 C for 30 min) by the vault method (5). The soil surface was leveled, and seed that had been treated with aerated steam (54 C for 10 min) was sown by vacuum planting plates so that a known number of seeds were planted per flat. The flats were then conveyed by a moving belt under a device that automatically covered the seed with sterilized vermiculite. Two pipes, each fitted with four mist nozzles, were positioned to spray the surface of the soil in the flats with 100 ml of bacterial suspension (10^5 bacteria/ml water) or water (control) immediately before and after the vermiculite topping was applied (Fig. 1). The 500 flats were then transferred to the glasshouse and fertilized with $CaNO_3$, $(NH_4)_2SO_4$, KNO_3, $MgSO_4$, and $Na_2B_4O_7$ in each daily watering, as in commercial practice.

The plants used in the nursery flat tests were portulaca (*Portulaca grandiflora* Hook.), delphinium (*Delphinium cultorum* Voss.), eggplant (*Solanum melongena* L. var. *esculentum* Nees), snapdragon (*Antirrhinum majus* L.), celosia (*Celosia argentea* L.), dahlia (*Dahlia* sp.), zinnia (*Zinnia* sp.), cabbage (*Brassica oleracea* L. var. *capitata*), carnation (*Dianthus caryophyllus* L.), and alyssum (*Lobularia maritima* Desv.).

TABLE 1. Effect of two *Bacillus* isolates upon seed germination and the fresh top weight (g) of seedlings in soil steamed at two temperatures

Test plant and measurement[a]	*Bacillus* spp. isolates in soil treated:					
	60 C for 30 min			100 C for 30 min		
	A13	WW27	Control	A13	WW27	Control
Portulaca (*Portulaca grandiflora* 'Double Mixed')						
Mean wt/100 seedlings	18.2	18.2	19.0	18.3	24.7	12.0
Mean no. plants/flat	294	367	215	194	426	183
Delphinium (*Delphinium cultorum* 'Gold Medal')						
Mean wt/100 seedlings	7.3	4.3	5.3	5.6	3.3	4.0
Mean no. plants/flat	188	200	154	182	223	167
Eggplant (*Solanum melongena L.* var. *esculentum* 'Market Supreme')						
Mean wt/100 seedlings	30.6	35.3	30.3	27.7	32.8	33.3
Mean no. plants/flat	103	164	113	118	166	104
Snapdragon (*Antirrhinum majus* 'Tetraploid Mixed')						
Mean wt/100 seedlings	4.7	3.2	4.8	3.2	3.2	3.0
Mean no. plants/flat	739	284	484	484	317	343
Celosia (*Celosia argentea* 'Forest Triumph')						
Mean wt/100 seedlings	51.2	50.8	56.7	37.7	49.0	22.0
Mean no. plants/flat	155	181	146	175	188	174
Dahlia (*Dahlia* sp. 'Hi-Dolly')						
Mean wt/100 seedlings	172.5	134.0	146.2	164.2	129.3	153.9
Mean no. plants/flat	122	150	117	116	135	116
Zinnia (*Zinnia* sp. 'Thumbelina Mixed')						
Mean wt/100 seedlings	87.9	87.6	98.9	72.8	94.9	76.2
Mean no. plants/flat	212	199	178	188	182	208
Cabbage (*Brassica oleracea* var. *capitata* 'F_1 Superette')						
Mean wt/100 seedlings	214.3	176.9	252.2	174.1	120.4	177.9
Mean no. plants/flat	106	120	102	111	160	110
Carnation (*Dianthus caryophyllus* 'Chabaud Giant Mixed')						
Mean wt/100 seedlings	36.2	30.7	28.3	28.9	25.3	27.2
Mean no. plants/flat	223	164	185	149	152	148
Alyssum (*Lobularia maritima* 'Carpet of Snow')						
Mean wt/100 seedlings	48.6	31.1	54.5	24.5	25.0	41.4
Mean no. plants/flat	314	429	313	324	478	283

[a]Six flats in each series.

RESULTS

Growth response of bedding plants in steamed soil.—Six replications of each of the species listed in Table 1 were inoculated with *Bacillus* isolates A13 or WW27, or with water as a control. Twenty days after seeding, the number of seedlings and fresh shoot weights were determined (Table 1). Seed germination was increased for portulaca, delphinium, eggplant, celosia, dahlia, cabbage, and alyssum by *Bacillus* WW27, and for portulaca, delphinium, and snapdragon by *Bacillus* A13. Germination frequently was increased 25%. Germination for portulaca was increased an average of 132.8% by *Bacillus* WW27 in soil steamed at 100 C for 30 min (Table 1). Seedling top weight was increased by isolate A13 for delphinium, dahlia, and carnation in both 60 C- and 100 C-treated soil, and by WW27 in soil steamed at 100 C for portulaca, celosia, and zinnia. The weight increase for most plants was 25%, and for celosia it was 122.7%. Marked specificity was shown in plant response to the bacteria, and top weight, seed germination, or both sometimes were reduced.

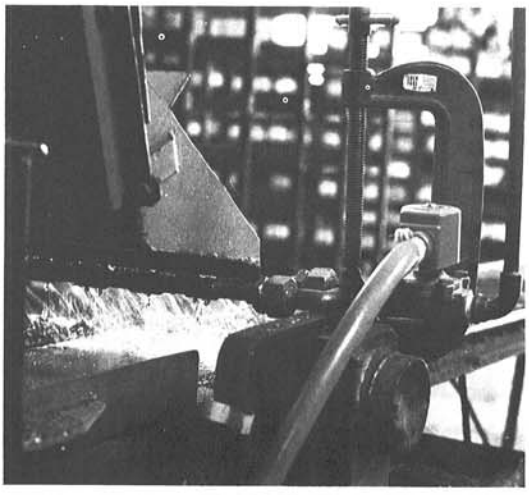

Fig. 1. Close-up of equipment used to apply topping and bacterial suspensions to seeded flats in commercial tests of bacterization.

The germination of seeds of the 10 test plants was increased an average of 9.3% (range, −16.1 to +41.1%), and the top weight an average of 26.4% (range, −9.0 to +157.7%), in soil steamed at 60 C over that steamed at 100 C, which confirms previous results (3). Seed germination was increased in 60 C-treated soil for six species, and top weight for eight of the 10 species over that in soil steamed at 100 C. The addition of selected bacteria to the soil mix steamed at 100 C for 30 min sometimes overcame these inhibitory effects (see portulaca, celosia, and zinnia, Table 1).

Growth response of cabbage in field soil.—*Bacillus* isolates WW27, A13, Tx1, AA43, and DD32 were grown in shake flasks of nutrient broth for 24 hr, centrifuged at 5 *g* for 30 min, and resuspended in sterile deionized water. Cabbage seed (cultivar Ballhead Yates Hybrid) was soaked in the bacterial suspension overnight, and sown in flats of the steamed (60 C for 30 min) peat-sand potting mix. At time of transplanting, seedlings from DD32-inoculated seed were clearly larger and more vigorous than the checks. Seedlings were transplanted to a clay-loam field soil in randomized blocks. The heads were harvested and weighed at maturity (Table 2). Inoculation with *Bacillus* isolate DD32 significantly ($P = 0.01$) increased yield by 22.1%, and AA43 by 18.1%, but the other *Bacillus* isolates reduced yield significantly ($P = 0.05$).

Yield response of grain sorghum in field soil.—An experiment was conducted during the 1974-75 season on red-brown earth at the Agricultural Research Center, Tamworth, to determine the effect of phosphate fertilization and inoculation of seed of grain sorghum Hybrid E57 with *Bacillus subtilis* A13. The experimental design was a 2 × 2 factorial with six replications. Treatments were: (i) nontreated seed, no phosphate fertilizer; (ii) nontreated seed, 22 kg/ha P as double superphosphate; (iii) seed pelleted with *B. subtilis* A13 (4×10^5 *B. subtilis*/seed), no phosphate fertilizer; (iv) seed pelleted (4×10^5 *B. subtilis*/seed) with 22 kg/ha P as double superphosphate. The seed was sown by a cone-seeder at the rate of 10 kg/ha. Plots were thinned to give a final plant stand of approximately 95,000 plants/ha.

TABLE 2. Effect of seed inoculation with *Bacillus* isolates on yield of cabbage transplanted to the field, and on fresh shoot weight of tomato seedlings grown in steamed soil

	Cabbage, field test		Tomato seedlings, steamed soil[a]	
Bacillus spp. isolates	Heads, number	Mean weight (g/head)	Mean seedling number/flat	Mean plant weight (g)
DD32	37	1,822.2	217.0	0.4506
AA43	42	1,764.3	206.0	0.3660
A13	46	1,466.4	230.7	0.3489
Tx1	52	1,445.9	217.7	0.3208
WW27	37	1,344.9	210.7	0.4607
Mixture of above	...	...	195.0	0.2369
Control	58	1,493.4	249.3	0.3603
S.E.	...	...	6.6	0.0172
LSD	...	...	20.2	0.0531
F ($P = 0.01$)	...	139.37	...	...

[a]Means of six replicates.

Heads were hand harvested with a sickle, and subsequently threshed.

Grain yields for each plot (kg/ha) at 3% grain moisture were as follows: nontreated seed, no phosphate fertilization, 1,341; nontreated seed, with phosphate fertilizer, 1,987; seed pelleted with A13, 1,748; seed pelleted with A13, with phosphate fertilization, 2,291 (C.V. = 19.9%; LSD = 452 kg/ha). Observations on flowering indicated that both phosphate fertilizer and pelleting with *B. subtilis* A13 had slightly hastened development. Seed pelleting with *Bacillus subtilis* A13 resulted in a 30.4% grain yield increase over the control. There was a 48.2% yield increase from the application of 125 kg/ha double superphosphate, but analysis of variance showed no significant interaction between the seed inoculation and phosphate application. This indicates that improved phosphate nutrition probably was not responsible for the yield response to seed treatment.

Fig. 2. Growth of wheat in a nutrient-deficient steamed U.C. mix; seed was pelleted with gum arabic-lime-irradiated peat with *Bacillus subtilis* A13 (left) or water control (right). No additional nutrients were added.

Pelleting with *B. subtilis* A13 did not significantly affect the number of fertile tillers per hectare, or individual grain weight, indicating that the yield increase came mainly from an increased grain set.

Comparative growth response of wheat in field and laboratory tests.—The results of glasshouse or growth-cabinet experiments generally are reproducible, but field trials often are not. For example, wheat (*Triticum aestivum* L. 'Eagle') seed pelleted with a mixture of gum arabic, lime, and gamma-irradiated peat inoculated with *Bacillus subtilis* A13 (265,500 cells/seed), grown in nutrient-deficient peat-sand mixture in a growth chamber (27 C, 16-hr day, and 19 C, 8-hr night) gave striking response, compared to pelleted control seed (Fig. 2). Considerable leaching of nutrients occurred in these pots. However, some of the same seed lot sown in a field test in a dry season at the N. S. W. Agricultural Research Station at Tamworth gave no response.

Possible modes of action of bacteria inoculated on seed in increasing plant growth.—*Protection against nonparasitic root pathogens.*—The bacteria used in these tests, and in those of Merriman et al. (20), were screened for antibiosis against several pathogens of plant roots (8). By contrast, bacteria used by other investigators in bacterization were not screened for antibiotic production (6, 19).

Perhaps the introduced bacteria inhibit nonparasitic but pathogenic microorganisms in the rhizosphere that decrease plant growth without producing other recognized symptoms, or they may destroy the phytotoxins produced by these microorganisms. Brown (9, 10) showed that bacteria (mostly *Pseudomonas* and *Achromobacter* spp.) abundant in the rhizosphere of 6-day-old wheat seedlings produced materials inhibitory to extension of pea internodes and lettuce hypocotyls. This is consistent with the statement of Rovira (29) that "at least 20% of the production potential [of plants] is lost simply to satisfy an unfavourable microbiological situation."

No pathogenic root fungi were isolated from the soil mix used in our glasshouse experiments. Treating soil with aerated steam at 60 C for 30 min kills known plant-pathogenic fungi, bacteria, and nematodes, but spore-

TABLE 3. Effect of *Bacillus* spp. on fresh shoot weights (g) of celosia under two nutrient regimes in soil steamed at 60 C for 30 min

Plant measurement[a]	Fresh shoot weights of celosia grown with:							
	Nutrients applied in daily watering				No nutrients applied			
	A13 (g)	WW27 (g)	A13 + WW27 (g)	Control (g)	A13 (g)	WW27 (g)	A13 + WW27 (g)	Control (g)
Mean no. of plants/flat	137.6	147.0	139.0	137.6	152.3	151.6	141.1	146.6
Mean plant wt	1.02	1.16	1.04	0.98	0.17	0.42	0.41	0.24
Percentage increase in plant wt	4.08	18.3	6.1	0	0	75.0	70.8	0

Standard deviation of plant numbers: 9.0
Standard deviation of mean plant weight: 0.08

[a]Six flats in each series.

forming bacteria, some actinomycetes, and *Penicillium* and *Aspergillus* spp. survive (3, 4, 8). The increased plant growth found in our studies of steamed soil is not inconsistent with the hypothesis that the action of inoculated bacteria is to inhibit nonparasitic pathogenic microorganisms, in view of the demonstrated rapidity with which contamination of treated soil may occur (30), and the known production of phytotoxins in the rhizosphere by *Aspergillus* and *Penicillium* spp. (7, 14, 17).

Altman (1) suggested that exoenzymes of high molecular weight (MW) were responsible for antagonistic activity of *B. subtilis* A13, which focused attention on laminarinase, chitinase, and cellulase. Subsequent isolation of the exoenzymes of isolate Tx1 revealed only limited chitinase activity. A sensitive biological assay was developed which showed that the proteins involved in the lytic process required no cofactors for activity, had maximum activity at pH 8.0, and could be stored in a freeze-dried state. By using a freeze-dried exoenzyme protein sample and gel filtration, the exoenzymes produced by Tx1 could be divided into two groups, those of low MW, which were devoid of lytic activity, and those of high MW which possed lytic activity. The high-MW exoenzymes were separated by disc electrophoresis, which revealed the presence of at least four proteins. Lastly, it was found that the high-MW exoenzymes could be separated on a preparative scale using preparative disc electrophoresis.

Plant growth substances produced by Bacillus subtilis A13.—Plant hormones have been shown (9, 10, 18) to be produced by rhizosphere bacteria. M. E. Brown (*personal communication*) found that *B. subtilis* A13 produced moderate quantities of gibberellin, and increased the growth of tomato, radish, pea, and lettuce. Indoleacetic acid (IAA) was not detected.

Transformation of unavailable mineral and organic compounds into forms available to plants.—Bacillus megaterium var. *phosphaticum*, for example, is thought to affect growth by increasing phosphate availability (2, 25). *Bacillus* isolates A13 and WW27 were applied singly and in combination to seeds of celosia and portulaca in flats of steamed (60 C for 30 min) peat-sand potting mix. Of the 12 flats per treatment, six were supplied in daily watering with the nutrients previously listed, and six were given no additional nutrients. The test was concluded 21 days after sowing, and fresh weight of shoots determined (Tables 3 and 4). Isolate WW27 again caused an increased fresh weight of celosia and portulaca plants. The increased growth of celosia from the bacteria was greater in soil with low nutrients than with adequate nutrition (Table 3). Portulaca seedlings in flats inoculated with WW27 were twice the size of those in control flats, and were green and healthy (Fig. 3). A mixture of the two

TABLE 4. Effect of two *Bacillus* isolates on fresh shoot weights of portulaca in steamed soil to which nutrients had been applied in daily watering

Plant measurement[a]	Seedling emergence and fresh shoot wt of portulaca grown in steamed soil			
	A13 (g)	WW27 (g)	A13 + WW27 (g)	Control (g)
Mean no. plants/flat	205	195	219	212
Mean seedling wt	.177	.521	.351	.197
Standard deviation of plant numbers: 76.				
Standard deviation of mean seedling wt.: .076				

[a]Six flats in each series.

TABLE 5. Tissue analysis of tomato and celosia grown from seed pelleted with *Bacillus* isolates in steamed soil to which nutrients had been added[a]

Host plant and *Bacillus* spp. isolates	Cl (%)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)	Mn (μg/g)	Cu (μg/g)	Zn (μg/g)
Tomato										
Control	0.79	3.05	0.60	5.04	2.14	0.64	0.12	98	13	66
DD32	0.75	3.19	0.57	5.56	1.96	0.80	0.12	109	14	86
WW27	0.74	3.09	0.59	4.31	2.10	0.75	0.13	98	14	101
AA43	0.64	3.16	0.80	4.99	1.84	0.71	0.11	90	11	68
Tx1	0.81	2.94	0.64	5.14	1.98	0.74	0.14	101	12	82
A13	0.75	3.02	0.67	5.14	1.96	0.71	0.14	96	15	101
Celosia										
Control	1.20	4.55	1.08	7.07	2.06	1.26	0.03	241	13	72
A13	1.02	4.31	1.15	6.93	1.96	1.20	0.03	249	15	96
WW27	1.17	4.79	1.10	7.43	2.22	1.33	0.03	243	15	80

[a]Six replicates per treatment were combined to provide sufficient plant tissue for analysis.

Fig. 3. Growth of portulaca seedlings following inoculation at time of seeding with a bacterial suspension of *Bacillus* isolate WW27 (left) or water control (right).

TABLE 6. Solubilization of phosphates in modified Pikovskaya broth by *Bacillus* and *Streptomyces* isolates

Isolates	Available phosphate (μg/ml) after inoculation[a]		
	3 wk	4 wk	8 wk
Control	131.0	131.0	170.0
Bacillus spp.			
A13	165.6	159.0	310.0
AA43	155.4	192.0	277.0
DD32	166.0	168.0	179.2
Tx1	126.4	124.0	270.0
WW27	226.0	212.4	257.0
Streptomyces sp.			
2-24	...	152.0	222.0
LSD = 2.81; C.V. = 1.52%			

[a]Two replicates in the 3-wk series, and five replicates in 4- and 8-wk series, combined for analyses.

Bacillus isolates decreased the plant response from that obtained with WW27 alone. Isolate A13 is inhibitory to growth of WW27 on yeast mannitol agar, and might also affect its growth in soil. Plant tissue analyses of celosia with added bacteria, and growing in soil adequately supplied with nutrients, did not show increased nutrient levels over the controls (Table 5).

The effect of individual *Bacillus* isolates and a mixture of them (grown individually and mixed after centrifuging and washing) was tested on seedlings of tomato *(Lycopersicon esculentum* Mill. 'Grosse Lisse'). Six flats of steamed (60 C for 30 min) peat-sand mixture per treatment were sown with seeds, and sprayed with bacterial suspensions (100 ml/flat, 10^5 bacteria/ml). The nutrients previously listed were applied in daily watering. Plants were harvested 14 days after sowing, and fresh top weight determined (Table 2). Isolates WW27 and DD32 significantly increased mean plant weight by 27.8 and 25.1% respectively; Tx1 and A13 decreased growth, but the amount was nonsignificant. All isolates except A13 significantly decreased germination. The mixture of the five bacteria significantly decreased top weight by 34.2%, and germination by 21.8%. Plant tissue analyses did not show any increase in nutrient levels over the control plants (Table 5). Plants were analyzed for N, P, K, Cl, Ca, Mg, Na, Mn, Cu, and Zn. Repetition of the experiment gave similar results, suggesting that the bacteria were not transforming unavailable mineral and organic compounds into forms available to the plants.

To determine phosphate-solubilizing activity, *Bacillus* isolates WW27, Tx1, A13, AA43, DD32, and *Streptomyces* 2-24 (obtained from P. R. Merriman) were grown on a modified Pikovskaya medium (31). All isolates except WW27 grew well, but there were no cleared zones. *Bacillus* isolates A13, AA43, Tx1, DD32, and WW27 also were inoculated onto the phosphate-precipitated medium of Gerretson (15). The isolates grew on the medium, but again no cleared zones developed. Modified Pikovskaya broth was inoculated with the *Bacillus* isolates and analyzed for available phosphate after 3, 4, and 8 wk, using the ascorbate reduced molybdenum blue method. Some phosphate apparently was slowly solubilized by the *Bacillus* isolates (Table 6).

Nitrogen fixation.—Bacillus polymyxa, B. racemosus, and *B. circulans* fix free nitrogen under anaerobic conditions. Mulder (23) and Rovira (27) were able to establish a facultative nitrogen-fixing *Bacillus* in the rhizosphere.

The bacterial isolates used in glasshouse trials were tested for ability to grow on nitrogen-free M9 agar (27), and to fix nitrogen under aerobic conditions. Only

isolates AA43 and DD32 grew. None of the bacteria grew under anaerobic conditions.

Peppers and tomatoes were grown in sterile media with and without nitrogen, using the Gibson (16) technique of plant culture. No increase in plant growth could be demonstrated when *Bacillus* isolates WW27, A13, or DD32 were used.

The acetylene reduction test (24) for nitrogen fixation was performed six times, using *Bacillus subtilis* A13 grown in nutrient broth or in nitrogen-free medium (27). No acetylene reduction occurred under aerobic conditions.

DISCUSSION

Although the results reported for the use of *Azotobacter chroococcum* and *Bacillus megaterium* as bacterial fertilizers in the Soviet Union (13, 22) met with skepticism in the Western world, growth increase obtained with *Azotobacter* (11, 12, 32), with *Azotobacter*, *Clostridium*, and *Bacillus* spp. (26, 27, 28), and with *Bacillus* and *Streptomyces* spp. (20, 21), have confirmed many of the Soviet observations. Some bacteria that increase plant growth produce gibberellin or auxin, or solubilize phosphate, while others that do not have been shown to alter the rhizosphere microbial population.

The major difficulty in commercial application of seed or root bacterization is the variable results encountered in field trials, compared with glasshouse trials (Fig. 2). Field results vary from season to season and place to place (21) owing to uncontrolled physical or biological factors. For example, most soil bacteria become inactive when soil water potential falls below −15 bars (4). Soil in glasshouse and chamber tests, and in irrigated culture in the field, is more likely to remain wetter than −15 bars, and the bacteria more likely to remain actively growing, than would be the case in soil for nonirrigated or dryland crops.

Since it appears unlikely that this variability can be overcome by using mixtures of microorganisms, the cause must be studied and corrected in each situation. Information is needed, inter alia, on the influence of: (i) soil water potential and nutrition on the growth of the inoculant, (ii) the inoculant on root growth and metabolism, (iii) the interaction of seedborne microorganisms with the inoculant before and after sowing, (iv) the interaction of the inoculant with the rhizosphere microflora, (v) different methods of inoculating seed with bacteria on the success attained, and (vi) different seed-storage conditions on longevity of inoculum.

The benefits from seed and root bacterization seem to result from several effects that may operate singly or in concert: (i) protection against nonparasitic root pathogens; (ii) production of biologically active substances (e.g., auxins and gibberellins); (iii) transformation of unavailable mineral and organic compounds into forms available to plants; (iv) possibly, nitrogen fixation. The different conditions under which these effects may operate may also contribute to the variability in results of field experiments.

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