Effectiveness of *Bacillus uniflagellatus* in Controlling Plant Diseases

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ABSTRACT

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Under varied laboratory conditions, dry spore powder of *Bacillus uniflagellatus* failed to control *Meloidogyne incognita, Alternaria solani, Erysiphe cichoracearum, Fusarium solani, Pythium ultimum, Rhizoctonia solani,* and *Verticillium albo-atrum* when the spores were incorporated in the soil in which plants were grown. The bacterial powder did not markedly improve foliar development of corn or wheat grown under stress.

A readily produced, storable, easily applied microbial for controlling organisms that produce plant disease would have obvious commercial and biologic benefits. In recent years, bacteria and active substances from bacterial cultures have been used successfully to control several crop pathogens, most notably *Fusarium, Rhizoctonia,* and *Pythium* (1,2,10–13,17,18). Mann (14) reported on the isolation and identification of the soil bacterium *Bacillus uniflagellatus* and subsequently (15) on the effectiveness of control of tobacco mosaic virus through soil amendments with this bacterium. An active substance produced by this spore-former was reported to have nematicidal and fungicidal properties, and we have confirmed the latter in our laboratories. A dry spore preparation of *B. uniflagellatus* added to soil or seeds can effectively control certain plant diseases in suitable hosts (corn, wheat, cotton) grown in amended soil, especially when the plants are subjected to moisture and possibly fertilizer stress (E. W. Mann, *personal communication*).

Because of the apparent utility of this bacterial spore dust and the ease with which it can be produced, stored, and applied, we did a series of greenhouse experiments to evaluate its potential in control of several plant diseases.

MATERIALS AND METHODS

B. uniflagellatus spore dust (34 million spores per gram), a spore-free positive cocoa dust control, and the standard chemicals benomyl, fenaminosulf, carboxin, and prophos were used. Systemic tests were done to evaluate control of early blight caused by *Alternaria solani* (Ell. & G. Martin) Sor., powdery mildew caused by *Erysiphe cichoracearum* DC., and wilt caused by *Verticillium albo-atrum* Reinke & Berth. Soil incorporation tests were done to assess control of seedling blights caused by *Pythium ultimum* Trow, *Rhizoctonia solani* Kuehn, and *Fusarium solani* (Mart.) Appel & Wr. f. *phaseoli,* (Burk.) Snyd. & Hans., and the southern root-knot nematode, *Meloidogyne incognita.* In addition, *B. uniflagellatus* was evaluated for its potential to increase the growth of wheat and field corn grown under stress. All systemic, seedling blight, and nematicide tests were replicated five times per treatment.

Systemic tests. Seedlings of eggplant (*Solanum melongena* 'Black Beauty'), tomato (*Lycopersicon esculentum* 'Rutgers'), and squash (*Cucurbita moschata* 'Butternut') were transplanted to 7.5-cm pots in pasteurized soil (1:1:4, peat, muck, and sand) into which test material had been incorporated. The seedlings were inoculated with *Verticillium, Alternaria,* and *Erysiphe* 19 days after they were transplanted, and the percentage of healthy plants was determined 1 wk after inoculation.

Seedling blight tests. Pasteurized soil was inoculated with either *Pythium, Fusarium,* or *Rhizoctonia* 1 day before treatment. After test materials were incorporated, the infested soil was placed

Table 1. Systemic control of fungal pathogens

Pathogen	Percentage of healthy plants after treatment[x]			
	BU[y] (100 mg/pot)	CD[z] (100 mg/pot)	Benomyl 50W (20 mg/pot)	Untreated
Alternaria	0 a	0 a	0 a	0 a
Erysiphe	0 a	0 a	100 b	0 a
Verticillium	0 a	0 a	100 b	0 a

[x] Means followed by same letter in a test are not significantly different ($P = 0.05$).
[y] BU = *Bacillus uniflagellatus.*
[z] CD = cocoa dust.

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Table 2. Control of seedling blight pathogens incubated with seedling corn

Plant[w] Pathogen	Treatment (mg/pot)[v] BU[x] (100)	CD[y] (100)	Benomyl 50W (10)	Carboxin 75W (6.7)	Fenaminosulf 35W (28.6)	Untreated
Method 1						
Bean						
Fusarium						
Germinated	22 a	21 a	25 a			23 a
Healthy	0 a	0 a	25 b			0 a
Pea						
Pythium						
Germinated	2 a	0 a			21 b[z]	0 a
Healthy	2 a	0 a			21 b[z]	0 a
Method 2						
Cotton						
Rhizoctonia						
Germinated	6 a	6 a		22 b		7 a
Healthy	1 a	0 a		22 b		2 a
Pythium						
Germinated	1 a	1 a			13 b	1 a
Healthy	1 a	0 a			13 b	0 a
Method 3						
Cotton						
Rhizoctonia						
Germinated	11 a	10 a		24 b		13 a
Healthy	3 a	3 a		22 b		3 a
Pythium						
Germinated	7 a	3 a			22 b	3 a
Healthy	1 a	1 a			20 b	1 a

[v] Means followed by same letter in a row are not significantly different ($P = 0.05$).
[w] Number of germinated and healthy plants based on initial population of 25.
[x] BU = *Bacillus uniflagellatus.*
[y] CD = cocoa dust.
[z] 14.3 mg/pot.

in 7.5-cm pots and planted immediately with field corn (*Zea mays* 'Asgrow RX-43A') seedlings (method 1) or cotton (*Gossypium hirsutum* 'Delta Pine 16') seeds (method 2) or was held 1 wk before seeding with cotton (method 3). The corn seedlings were permitted to grow for 1 wk before they were removed, and the pots were immediately seeded with peas (*Pisum sativum* 'Little Marvel') or beans (*Phaseolus vulgaris* 'Pinto'). The number of germinated and healthy plants was recorded 2 wk after seeding.

Nematicide tests. Seedling tomatoes were set directly into nematode-infested soil with test materials incorporated (method 4) or were grown for 2 wk in amended pasteurized soil before soil was inoculated with nematodes (method 5). The degree of galling on roots was evaluated 3–4 wk after planting.

Corn and wheat stress test. Test material was incorporated into unpasteurized soil (1:1, Fox sandy loam and sand). The soil was placed in 10-cm pots and immediately seeded with either three field corn or four wheat (*Triticum aestivum* 'Ionia') seeds. The pots were placed at random in the greenhouse and subirrigated to field capacity. Only one seedling was permitted to grow in each pot. Twenty pots per treatment rate per plant type were divided into two equal groups. The unstressed group was watered regularly and fertilized twice during the holding period with 20-20-20 plus trace elements (Greenfield Instant Plant Food); the stressed group was not fertilized and was watered only when definite signs of wilting appeared. Four weeks after planting, the aboveground portion of each plant was measured and the wet weight recorded.

Table 3. Control of nematodes

Method	Root-knot index[x] after treatment: BU[y] 100 mg/pot	BU[y] Dip[z]	Cocoa dust 100 mg/pot	Cocoa dust Dip[z]	Prophos tech. 1.25 mg/pot	Untreated
4	3.8 a	3.8 a	4.0 a	4.0 a	1.0 b	4.0 a
5	3.4 a	3.2 a	3.6 a	3.4 a	···	3.2 a

[x] Root-knot index: 1 = no galling to 5 = all roots severely galled. Means followed by same letter in a row are not significantly different ($P = 0.05$).
[y] BU = *Bacillus uniflagellatus.*
[z] Roots dipped directly into the dry dust and planted.

RESULTS AND DISCUSSION

In all trials, *B. uniflagellatus* spore powder was applied at 1 and 10 mg per 7.5-cm pot, but only the results of the high (100 mg) rates are included because no differences were observed at lower rates.

As shown in Table 1, *B. uniflagellatus* was ineffective in providing systemic control of *Alternaria, Erysiphe,* or *Verticillium* when the spore dust was applied to the soil. Benomyl was completely effective at the lowest rate (5 mg a.i. per pot) against the latter two organisms.

B. uniflagellatus did not provide significant control of *Pythium* or *Fusarium* even though the spores were placed in combination with a preferred host, corn, for 1 wk before the test (Table 2). Benomyl and fenaminosulf provided significant control of *Fusarium* and *Pythium,* respectively. *B. uniflagellatus* treatment failed to control Pythium or Rhizoctonia seedling blights of cotton, but fenaminosulf and carboxin were markedly effective.

Neither soil incorporation nor root dip treatments with the spore powder effectively controlled the southern root-knot nematode, despite preincubation with the host plant for 2 wk before infestation. Prophos provided complete control even at 1.25 mg a.i. per pot

Table 4. Influence of *Bacillus uniflagellatus* (BU) on growth of corn and wheat

Treatment (mg/pot)	Plant[z]			
	Height (cm)		Weight (g)	
	Stressed	Not stressed	Stressed	Not stressed
	Corn			
BU				
2	41.7 a	85.7 a	1.61 ab	10.70 a
20	41.1 a	90.6 a	1.51 a	11.93 a
200	46.7 b	90.1 a	1.97 c	11.57 a
Cocoa dust				
200	41.7 a	86.7 a	1.59 ab	11.42 a
Untreated	43.2 ab	84.5 a	1.88 bc	11.05 a
	Wheat			
BU				
2	30.7 a	42.8 a	0.31 ab	1.36 a
20	29.0 a	43.7 a	0.31 ab	1.31 a
200	30.7 a	42.5 a	0.29 a	1.45 a
Cocoa dust				
200	31.0 a	43.5 a	0.31 ab	1.40 a
Untreated	31.5 a	40.7 a	0.34 ab	1.45 a

[z]Means followed by same letter in a test are not significantly different ($P = 0.05$).

(Table 3).

Results of growing wheat and corn under stressed conditions combined with *B. uniflagellatus* treatment were not encouraging (Table 4). A rate of 200 mg of spore dust per 10-cm pot significantly increased the height and weight of corn, compared with the cocoa dust control treatment, but the differences were not significant from the untreated controls. At the high rate, the spore dust produced significant weight reduction in wheat grown under stress conditions, but the results may be due more to chance than to significant interaction between the treatment and the crop.

In laboratory and field studies of *B. uniflagellatus* for disease control (3–9), results have varied greatly, with significant positive responses in some, negative responses in others, and, most commonly, no differences between treatments. It is apparent from our tests and those of Cole and co-workers (3–9) that *B. uniflagellatus* and typical fungicides and nematicides cannot be expected to perform similarly. More clarification is required concerning the growth and development of the bacterium, its production of antibiotic substances, and their relation to soil type, temperature, pH, and moisture, as well as soil microorganism and host plant interactions.

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