Effects of Snap Bean Cultivar, Seed Treatment Chemicals, and *Bacillus subtilis* on Snap Bean Seedling Diseases, Growth, and Yield

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Interpretative Summary

Populations of *Bacillus subtilis* on snap bean roots were generally proportional to the rate of *B. subtilis* applied. Seed treatment chemistry rarely affected root colonization by *B. subtilis*. Both seed treatment chemicals and snap bean cultivar significantly affected the disease control efficacy of *B. subtilis* supplements. Supplementing the chemical seed treatment combinations of Captan 400 + AS-50 (agricultural streptomycin) or Chloroneb 65W + Allegiance FL (metalaxyl) + AS-50 with Kodiak Concentrate at 1.0 oz/100 lb seed (an unregistered rate) increased yields by 38% and 60%, respectively.

Introduction

Previous studies indicated that the use of a commercial *Bacillus subtilis* preparation (Kodiak Concentrate, Gustafson Inc.) at 1.0 oz product per 100 lb seed could cause significant reductions in healthy stand and yield. However, when this product was used in combination with captan or captan plus streptomycin, no significant stand reductions occurred and snap bean yields were significantly increased. It was theorized that high levels of *B. subtilis* on roots were detrimental to plant growth but that these levels could be reduced with the addition of chemical seed treatments. The major objectives of this study were (1) to evaluate seed treatment chemicals, with and without *B. subtilis*, for control of snap bean seedling diseases, (2) to determine the effects of chemical seed treatments plus *B. subtilis* on plant growth and snap bean yield, and (3) to determine the effects of commonly used seed treatment chemicals and chemical combinations on root colonization by *B. subtilis* isolates GB03 (in Kodiak Concentrate) and MBI 600 (in Subtilex, MicroBio Ltd.), and (4) to determine whether choice of snap bean cultivar might affect the results.

Materials and Methods

The experiment was conducted at The University of Tennessee's West Tennessee Experiment Station on a Memphis-Calloway silt loam intergrade, 0.5% O.M., pH 6.4 (water), with high soil test levels of available P (46 lb/A) and K (184 lb/A). Available Ca, Mg, Zn, Fe, and Mn were >1280, >64, 5, 17, and 50 lbs/A, respectively. Total soluble salts were 490 ppm. The test was conducted in a field that had been planted annually to snap bean since 1989 with moderate to severe seedling disease losses. A split-plot design was used consisting of two snap bean cultivars as main plots (cv. Strike and Hialeah) and 16 different seed treatments as subplots. Seed treatments consisted of three combinations of seed treatment chemicals plus a water only control. The chemicals included three fungicides (Captan 400, Chloroneb 65W, and Allegiance FL),

one insecticide (Lorsban 50-SL), and one antibiotic (AS-50). Main plot treatments were replicated four times. Experimental units (subplots) were replicated eight times (four times per cultivar) and consisted of 20-foot-long two-row plots.

Seed treatment slurries were created by adding the test materials to distilled water. Treatments were applied 22-23 May to untreated seed at ca. 11 fl oz slurry/100 lb seed, then mixed with the seed for 2 min. in a rotating glass cylinder. The test area was cut with a field harrow on 24 May, then Treflan E.C. at 0.75 pint/A and D.Z.N. Ag 500 at 2.0 quart/A were broadcast over the field and incorporated. The test was planted 25 May into moist soil using a John Deere 71 Flexi-planter equipped with cone-seeders. The seeding rate was 5 seed/ft with an average planting depth of 1.6 in. (range: 1 - 2 in.; n = 24). Dual Magnum at 1.5 pint/A was applied as a pre-emergence herbicide on 26 May. The test was irrigated on 29 May with a lateral boom system (ca. 0.5 in. water). The lateral boom was used five more times during the growing season (on 19, 25, and 27 June plus 6 and 9 July; ca. 0.5 in. water/irrigation).

Post-emergence damping-off losses were recorded 11 and 18 June. Representative dead and dying seedlings were collected for pathogen identification. Differences in seedling vigor were recorded 12 June by measuring to the nearest 0.5 cm the length of the blade of the center leaflet of the first trifoliolate leaf on five representative plants per row. Healthy stands were recorded 3 ½ and 6 weeks after planting (on 18-19 June and 6 July, respectively). To compensate for possible nitrogen loss from the heavy rainfall that fell during the first two weeks of the test, all rows were sidedressed at early flowering (26 June) with calcium nitrate at ca. 19 lb N/A. The heights of five representative plants per row were recorded 11 July. The snap beans were mechanically harvested with a one-row Pix-All, the Hialeah on 18 July and the Strike on 19 July. All data were subjected to analysis of variance for a split-plot design. Mean separation tests were performed where significant differences were indicated.

Samples for determining the effects of seed treatment chemistry on root colonization by *B. subtilis* were collected from subplots when the first trifoliolate leaves were ½ - 2½ in. long (on 11, 12, 13, and 14 June for replications 1, 2, 3, and 4, respectively). Each sample consisted of the roots of six plants per subplot (three/row) recovered to a depth of ca. 4.5 in. Roots were excised with scissors near the cotyledonary node and gently shaken to remove most of the adhering soil. Collection tools were surface disinfected with 70% isopropyl alcohol between subplots. Samples were kept cool until shipment to Knoxville, TN, via overnight express mail where they were processed as outlined below.

All six roots collected from a single subplot were combined into one sample for processing. Roots with adhering soil were excised and weighed, then placed in sterile phosphate buffered saline (PBS). Each sample was agitated on a rotary shaker for 5 min., placed in an ultrasonic water bath one min., then placed in a vortex blender for 30 sec. Serial dilutions of the agitated PBS were prepared for each sample. These were then plated onto duplicate plates of a modified V-8 juice medium that was semi-selective for

B. subtilis, GB03. Plates were incubated on the lab bench at room temperature. After 7-14 days, colonies on the agar plates with the characteristic morphologies of the *B. subtilis* isolates were counted. Results were expressed as cfu/gram root based on the fresh weight of the root samples. Counts included rhizosphere (soil remaining on root), rhizoplane (root surface), and some endorhizosphere (internal colonization) bacteria.

Results and Discussion

Rain showers (some very intense) 30 May - 1 June added 3.6 in. of water to the test area, flooding the margins of the test area for several hours and creating conditions very favorable for seedling diseases. Rainfall 2 - 7 June totaled another 2.2 in. The weather then turned warm and moderately dry with <1.4 in. rainfall during the remainder of the test. These heavy rains early in the test followed by the very warm, dry weather created conditions conducive for seedling diseases caused by *Rhizoctonia solani* (Rhizoctonia damping-off and stem rot) and by *Macrophomina phaseolina* (ashy stem blight). Over 60 damped-off seedlings were collected 11-20 June. *M. phaseolina* was identified as the causal pathogen for seedling death in ca. 90% of the cases. Stem lesions typical of those due to infection with *R. solani* were apparent on <10% of the collected plants.

Significant differences due to snap bean cultivar were noted in seedling emergence, seedling vigor, plant stand 3 ½ weeks after planting, and plant height at early flowering (Table 1). These cultivar differences in disease and plant growth did not translate into differences in snap bean yield (Table 1), presumably because the primary pathogen in this study (*M. phaseolina*) is not a "root-pruning" pathogen.

Snap bean cultivar had no significant effect on root colonization by either of the *B. subtilis* isolates ($P > 0.51$). There were no significant interactions between the seed treatments and cultivars that affected root colonization by the bacteria ($P > 0.79$). Significant differences in root colonization by *B. subtilis* due to seed treatment were noted. These were due, in part, to the application rate of the biological (Table 2). There were no significant differences in root colonization by GB03 when applied at the high application rate (1.0 oz). Seed treatment #10 (Captan 400 2.5 fl oz + Lorsban 50-SL 1.5 oz + Allegiance FL 0.75 fl oz + AS-50 0.89 oz) significantly reduced root colonization by GB03 at the low application rate (0.125 oz) compared to other seed treatments receiving the same rate (Table 2). Root colonization by *B. subtilis* isolate MBI 600 appeared unaffected by application rate.

The overall effects (i.e., on both cultivars) of snap bean seed treatment on seedling emergence, post-emergence damping-off, and healthy stand 3 ½ weeks after planting are given in Table 3. Supplementing the seed treatments with *Bacillus subtilis* generally did not significantly change the effects of the seed treatments. Where used, seed treatment #10 significantly increased damping-off losses and reduced plant stands compared to the water control (Table 3). The supplemental biologicals could not reverse these trends and may have actually exacerbated them. There was a significant

interaction (P = 0.02) between cultivar and seed treatment on damping-off losses. The main difference between cultivars appeared to be the effect of supplementing seed treatment #10 with a biological (Table 4). For Hialeah, combining seed treatment #10 with either the 0.125 oz rate of Kodiak Concentrate or the 1.0 oz rate of Subtilex significantly reduced post-emergence damping-off. With Strike, combining seed treatment #10 with the 1.0 oz rate of Subtilex significantly increased disease losses (Table 4). The effects of these interactions on healthy stand 3 ½ weeks after planting are also provided in Table 4.

Disease losses due to ashy stem blight continued during the period 4 - 6 weeks after planting. Rows in areas that were flooded during the heavy rains in early June lost many plants. By the end of the test, healthy stands in these rows were often reduced by >80%, and yields were near zero. Fortunately, only one row out of each two-row plot was severely affected. Results recorded >4 weeks after planting and reported here are therefore based on the observations recorded for the "best-yielding" row of each two-row plot.

The effects of snap bean seed treatment on plant stand 6 weeks after planting, plant height during early flowering, and snap bean yield are given in Table 5. Five treatments significantly reduced healthy stand six weeks after planting compared to the water-only control:1.0 oz Kodiak Concentrate, treatment #10 with 0.125 oz Kodiak Concentrate or with either rate of Subtilex, and treatment #4 (Captan 400 2.5 fl oz + AS-50 0.89) with 0.125 oz Kodiak Concentrate. There were no significant effects due to seed treatment on plant height or yield. Highest snap bean yields were observed with the combination of treatment #4 plus 1.0 oz Kodiak Concentrate (Table 5). Though not significant at the P = 0.05 level in the current test, the trends support the observations of earlier studies.

Table 1. Snap Bean Seed Treatment Test, Jackson, TN, 2001: Effects of snap bean cultivar on seedling emergence, seedling vigor, stand 3 ½ weeks after planting, plant height, and yield [1].

Cultivar	Seedling emergence [2] (%)	Seedling vigor [3] (cm)	Healthy stand 18-19 June (%)	Plant height 11 July (cm)	Snap yie (lb/
1) Hialeah	70 b	4.7 a	65 b	36 a	35
2) Strike	84 a	3.3 b	76 a	32 b	34
ANOVA F value	28.49	40.38	50.86	12.58	0.(

Probability > F	0.01	0.008	0.006	0.003	0.77

[1]Values are the means of 64 observations (16 seed treatments X four replications). Means in the same column followed by the same letter do not differ significantly by Fisher's (protected) LSD (P = 0.05).

[2]Estimated total seedling emergence is based on the sum of post-emergence damping-off losses 1-19 June plus healthy stands 3 ½ weeks after planting.

[3] Values are the mean length of the blade of the center leaflet of the first trifoliolate on ten representative plants per plot on 12 June.

Table 2. Snap Bean Seed Treatment Test, Jackson, TN, 2001: Effects of snap bean seed treatment on root colonization by *B. subtilis* isolates. [1]

Seed treatment materials and rate(s) per 100 lb seed	Log10 cfu/g root [2]		
	GB03		MBI(
1) water 10.0 fl oz	0.0	e [3]	0.0
2) Kodiak Concentrate 0.125 oz	2.62	b	0.0
3) Kodiak Concentrate 1.0 oz	3.06	ab	0.0
4) Captan 400 2.5 fl oz + AS-50 0.89 oz	0.0	e	0.0
5) treatment #4 + Kodiak Concentrate 0.125 oz	2.88	ab	0.0
6) treatment #4 + Kodiak Concentrate 1.0 oz	3.43	a	0.0
7) Chloroneb 65W 4.0 oz + Allegiance FL 0.75 fl oz + AS-50 0.89 oz	0.0	e	0.0
8) treatment #7 + Kodiak Concentrate 0.125 oz	2.45	b	0.0

9) treatment #7 + Kodiak Concentrate 1.0 oz	3.16	ab	0.0	b
10) Captan 400 2.5 fl oz + Lorsban 50-SL 1.5 oz + Allegiance FL 0.75 fl oz + AS-50 0.89 oz	0.31	e	0.0	b
11) treatment #10 + Kodiak Concentrate 0.125 oz	1.66	c	0.0	b
12) treatment #10 + Kodiak Concentrate 1.0 oz	3.22	ab	0.0	b
13) Subtilex 0.25 oz	0.29	e	2.83	a
14) Subtilex 1.0 oz	0.31	e	2.74	a
15) treatment #10 + Subtilex 0.25 oz	0.59	de	2.96	a
16) treatment #10 + Subtilex 1.0 oz	1.13	cd	2.83	a
ANOVA F value	23.98		253.48	
Probability > F	< 0.0001		< 0.0001	
LSD (P = 0.05)	0.78		0.22	

[1] Values are the means of eight replications (four per cultivar).

[2] cfu = colony-forming units; GB03 and MBI 600 are the *B. subtilis* isolates in Kodiak Concentrate and Subtilex, respectively.

[3] Means in the same column followed by the same letter do not differ significantly by Fisher's (protected) LSD (P = 0.05). DAP =days after planting.

Table 3. Snap Bean Seed Treatment Test, Jackson, TN, 2001: Effects of snap bean seed treatment on seedling emergence, post-emergence damping-off, and healthy stand 25 DAP after planting [1].

Seed treatment materials and rate(s) per 100 lb seed	Seedling emergence [2] (%)	Damping-off losses (post) (%)	He 1

1) water 10.0 fl oz	77	4	c	74	abc
2) Kodiak Concentrate 0.125 oz	75	4	c	71	abcd
3) Kodiak Concentrate 1.0 oz	76	4	c	73	abc
4) Captan 400 2.5 fl oz + AS-50 0.89 oz	80	4	c	76	a
5) treatment #4 + Kodiak Concentrate 0.125 oz	74	5	c	69	abcd
6) treatment #4 + Kodiak Concentrate 1.0 oz	80	3	c	77	a
7) Chloroneb 65W 4.0 oz + Allegiance FL 0.75 fl oz + AS-50 0.89 oz	78	3	c	75	ab
8) treatment #7 + Kodiak Concentrate 0.125 oz	78	4	c	74	abc
9) treatment #7 + Kodiak Concentrate 1.0 oz	77	2	c	75	ab
10) Captan 400 2.5 fl oz + Lorsban 50-SL 1.5 oz + Allegiance FL 0.75 fl oz + AS-50 0.89 oz	77	12	ab	65	cde
11) treatment #10 + Kodiak Concentrate 0.125 oz	73	9	b	63	de
12) treatment #10 + Kodiak Concentrate 1.0 oz	73	10	b	63	de
13) Subtilex 0.25 oz	78	3	c	75	ab
14) Subtilex 1.0 oz	77	3	c	74	abc
15) treatment #10 + Subtilex 0.25 oz	80	14	a	66	bcde
16) treatment #10 + Subtilex 1.0 oz	74	15	a	59	e
ANOVA F value	0.76	11.44		2.99	
Probability > F	0.71	< 0.0001		0.001	
LSD (P = 0.05)	7.6	3.6		9.0	

[1] Values are the means of eight replications (four per cultivar). Means in the same column followed by the same letter do not differ significantly by Fisher's (protected) LSD (P = 0.05). DAP =days after planting.

[2] Estimated seedling emergence based on the sum of post-emergence damping-off losses 1-19 June plus healthy stands 3 ½ weeks after planting.

Table 4. Snap Bean Seed Treatment Test, Jackson, TN, 2001: Effects of snap bean seed treatment on post-emergence damping-off and healthy stand 25 DAP for each cultivar [1].

Seed treatment materials and rate(s) per 100 lb seed	Damping-off losses (post) (%)				Health 18-19 (%	
	Hialeah		Strike		Hialeah	
1) water 10.0 fl oz	3	ef	4	de	67	abc
2) Kodiak Concentrate 0.125 oz	4	ef	4	e	66	abc
3) Kodiak Concentrate 1.0 oz	4	def	3	e	69	abc
4) Captan 400 2.5 fl oz + AS-50 0.89 oz	5	def	3	e	65	abc
5) treatment #4 + Kodiak Concentrate 0.125 oz	5	de	5	de	63	bc
6) treatment #4 + Kodiak Concentrate 1.0 oz	3	ef	3	e	66	abc
7) Chloroneb 65W 4.0 oz + Allegiance FL 0.75 fl oz + AS-50 0.89 oz	3	ef	3	e	68	abc
8) treatment #7 + Kodiak Concentrate 0.125 oz	3	ef	5	de	70	ab
9) treatment #7 + Kodiak Concentrate 1.0 oz	3	ef	2	e	66	abc
10) Captan 400 2.5 fl oz + Lorsban 50-SL 1.5 oz + Allegiance FL 0.75 fl oz + AS-50 0.89 oz	12	a	12	bc	60	cd

11) treatment #10 + Kodiak Concentrate 0.125 oz	7	cd	12	bc	60	cd	67	cd
12) treatment #10 + Kodiak Concentrate 1.0 oz	10	abc	11	cd	52	d	74	abc
13) Subtilex 0.25 oz	2	f	5	de	72	a	78	abc
14) Subtilex 1.0 oz	2	ef	4	e	68	abc	79	abc
15) treatment #10 + Subtilex 0.25 oz	10	ab	18	ab	65	abc	68	cd
16) treatment #10 + Subtilex 1.0 oz	9	bc	22	a	61	bc	57	d
ANOVA F value	8.75		6.40		2.25		2.10	
Probability > F	< 0.0001		0.0001		0.02		0.03	
LSD (P = 0.05)	3.1		6.7		9.1		15.7	

[1]Values are the means of four replications. Means in the same column followed by the same letter do not differ significantly by Fisher's (protected) LSD (P = 0.05). DAP = days after planting.

Table 5. Snap Bean Seed Treatment Test, Jackson, TN, 2001: Effects of snap bean seed treatment on healthy stand 42 DAP, plant height, and yield [1].

Seed treatment materials and rate(s) per 100 lb seed	Healthy stand 6 July (%)		Plant height 11 July (cm)
1) water 10.0 fl oz	53	ab	37
2) Kodiak Concentrate 0.125 oz	48	abcde	34
3) Kodiak Concentrate 1.0 oz	42	cdef	31
4) Captan 400 2.5 fl oz + AS-50 0.89 oz	52	abc	33
5) treatment #4 + Kodiak Concentrate 0.125 oz	37	f	30

6) treatment #4 + Kodiak Concentrate 1.0 oz	57	a	35	5282
7) Chloroneb 65W 4.0 oz + Allegiance FL 0.75 fl oz + AS-50 0.89 oz	47	abcdef	34	2950
8) treatment #7 + Kodiak Concentrate 0.125 oz	51	abc	33	3800
9) treatment #7 + Kodiak Concentrate 1.0 oz	57	a	37	4738
10) Captan 400 2.5 fl oz + Lorsban 50-SL 1.5 oz + Allegiance FL 0.75 fl oz + AS-50 0.89 oz	47	abcdef	36	3425
11) treatment #10 + Kodiak Concentrate 0.125 oz	39	ef	34	3017
12) treatment #10 + Kodiak Concentrate 1.0 oz	45	bcdef	34	3658
13) Subtilex 0.25 oz	48	abcdef	35	3372
14) Subtilex 1.0 oz	50	abcd	34	3991
15) treatment #10 + Subtilex 0.25 oz	40	def	34	2917
16) treatment #10 + Subtilex 1.0 oz	38	ef	34	3036
ANOVA F value	2.28		0.69	1.31
Probability > F	0.01		0.79	0.22
LSD (P = 0.05)	10.9		4.7	1707

[1] Values are the means of eight replications (four per cultivar)and reflect observations from the "best-yielding row" of each two-row plot. Means in the same column followed by the same letter do not differ significantly by Fisher's (protected) LSD (P = 0.05). DAP = days after planting.

[2]The test was mechanically harvested with a one-row Pix-All on 18 July (cv. Hialeah) and 19 July (cv. Strike).

This research represents one season's data and does not constitute recommendations. After sufficient data is collected over the appropriate number of seasons, final recommendations will be made through research and extension publications.