Effects of 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 roots were proportional to the rate of *B. subtilis* applied. Neither variety nor seed treatment chemistry significantly affected root colonization by *B. subtilis*. Supplementing common seed treatment chemicals with Kodiak Concentrate (*B. subtilis*) at 1.0 oz/100 lb seed significantly increased mean yields. The greatest yield increase with this biological fungicide was with the least effective chemical seed treatment. Highest stands and yield were obtained with the seed treatment combination of Chloroneb 65W + Allegiance FL (metalaxyl) + AS-50 (agricultural streptomycin) without the Kodiak Concentrate supplement.

Introduction

Previous studies had indicated that the use of a commercial *Bacillus subtilis* preparation (isolate GB03 in Kodiak Concentrate, Gustafson Inc.) at 1.0 oz product per 100 lb seed would 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 GB03 on roots were detrimental to plant growth, but these levels were reduced with the addition of chemical seed treatments. The major objectives of this study were (1) to determine the effect of commonly used snap bean seed treatment chemicals on root colonization by GB03, (2) to determine the effects on snap bean stand, growth, and yield of supplementing these seed treatment chemicals with GB03, and (3) to determine the effects of different snap bean varieties on these interactions.

Materials and Methods

The experiment was conducted at The University of Tennessee's West Tennessee Experiment Station in a field that had been planted annually to snap bean since 1989 with moderate to severe seedling disease losses. The soil was a Memphis-Calloway silt loam intergrade, 0.7% O.M., pH 6.9 (water), with high soil test levels of available P and K. A split-plot design was used with three snap bean varieties (cv. Strike, Hialeah, and Hystyle) as main plots. Main plot treatments were replicated four times. Subplots consisted of twelve combinations of seed treatment chemicals and GB03. Each subplot consisted of two rows of beans, 20 ft long, planted 30 in. apart.

Seed treatment slurries were created by adding distilled water to the test materials. Treatments were applied 20-26 Apr to untreated seed at ca. 10 fl oz slurry/100 lb seed,

then mixed with the seed for 2 min in a rotating glass cylinder. Ammonium nitrate at 44 lb N/A, triple super phosphate at 53 lb P_2O_5/A, Treflan E.C. at 0.75 pint/A, and D.Z.N. Ag 500 at 2.0 quart/A were broadcast over the test area 15-16 May and incorporated. The test was planted 16 May into slightly moist soil using a John Deere 71 Flexi-planter equipped with with cone-seeders, a seeding rate of 5 seed/ft, and an average planting depth of 1.4 in. Dual II at 1.5 pint/A was then applied as a pre-emergence herbicide. The test was irrigated 17 May with a lateral boom system (ca. 0.5 in. water).

Snap bean emergence began 23 May, and differences in seedling emergence were recorded 24 May. Differences in seedling vigor were recorded 6 June by measuring the length of the blade of center leaflet of the first trifoliolate leaf on five representative plants per row. Post-emergence damping-off losses were recorded 6 June. Healthy stands were recorded 14 June and again 28-29 June (at full bloom). The center 10 - 15 feet of each row was harvested 8 July (Hialeah) and 10 July (Strike and Hystyle) with a one-row mechanical snap bean harvester. The number of plants harvested was recorded immediately after each row was picked. Yield data were corrected for differences in row length harvested by multiplying yields by the ratio of (healthy stands 28-29 June)/(number plants harvested). All data were subjected to analysis of variance. Mean separation tests were performed where significant differences were indicated. Arsine transformations were used for analyses of percentage data. Values in tables are the untransformed percentages.

Samples for determining the effects of chemical seed treatments on root colonization by GB03 were collected from each subplot on 1, 5, 6, and 7 June (replications 1, 2, 3, and 4, respectively) when the first trifoliolate leaves were ½ - 2½ in. long. 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 near the cotyledonary node with scissors and gently shaken to remove most the adhering soil. Collection tools were surface disinfected with 70% isopropyl alcohol between subplots. Samples were kept cool until shipment via overnight express mail to Knoxville, TN, 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 were prepared for each sample in PBS, and then plated onto duplicate plates of a modified V-8 juice medium that was semi-selective for *Bacillus subtilis*, GB03. Plates were incubated on the lab bench at room temperatur. After 7-10 days, colonies on the agar plates with the morphology of GB03 were counted. Results expressed as cfu/gram root are based on the fresh weight of the root sample. Actual counts included rhizosphere (soil remaining on root), rhizoplane (root surface), and some endorhizosphere (internal colonization) bacteria.

Results and Discussion

The irrigation on 17 May plus rainfall during the first 12 days after planting (1.7 in.) created disease-conducive conditions. Hypocotyl lesions characteristic of those due to *Rhizoctonia solani* were common on collected seedlings. Effects of seed treatments on root colonization by *B. subtilis* are shown in Figure 1. In general, root colonization by *B. subtilis* significantly increased with application rate but appeared unaffected by seed treatment chemistry (Fig. 1A). These increases, however, were not consistent across variety (Fig. 1B, 1C, and 1D). Populations of *B. subtilis* on roots were not correlated with seedling stands, plant growth, or yield (R $ 0.10; n = 144). Yield increases attributable to use of *B. subtilis* were determined by subtracting the yields without Kodiak Concentrate from those with Kodiak Concentrate for each seed chemistry - variety - replication combination. When the application rates of Kodiak Concentrate were subjected to ANOVA for a split-plot design, the average yield increase with the high rate of Kodiak Concentrate (563 lb/A) was significantly higher than the yield with no Kodiak Concentrate, while the average yield increase with the low rate of Kodiak Concentrate (110 lb/A) was not significantly different. The yield increase with *B. subtilis* was greatest (1078 lb/A) with the seed treatment that had the lowest yield without *B. subtilis* (Captan 400 + Lorsban 50-SL + Allegiance FL + AS-50).

Effects of treatments on seedling emergence, leaflet length, post-emergence damping-off, snap bean stands, and yield are summarized in Tables 1 and 2. The rate of seedling emergence, seedling growth, and healthy stands were significantly affected by variety. There was no significant effect of variety on yield. Seedling damping-off, healthy stands, and yield were significantly affected by seed treatment. Highest yields were obtained with the seed treatment combination of Chloroneb 65W + Allegiance FL + AS-50 without Kodiak Concentrate. There were no significant interactions between variety and seed treatment for seedling emergence, seedling growth, healthy stands, or yield.

Table 1. Snap Bean Seed Treatment Test, Jackson, TN, Spring 2000: Effects of variety and seed treatment on seedling emergence, seedling vigor (leaflet length), and post-emergence damping-off.

Seed treatment materials and rate per 100 lb seed	Seedling emergence on 5/24 (%)	Mean leaflet length on 6/06 (in.)	Dam c losse (%
VARIETY			

1) Strike	19	a	1.1	b	6	
2) Hialeah	16	a	1.3	a	4	
3) Hystyle	6	b	1.1	b	3	
SEED TREATMENT						
1) water 10.0 fl oz	15		1.2		2	cde
2) Kodiak Concentrate 0.125 oz	12		1.2		3	cde
3) Kodiak Concentrate 1.0 oz	17		1.2		2	cde
4) Captan 400 2.5 fl oz + AS-50 0.89 oz	14		1.2		2	c
5) treatment #4 + Kodiak Concentrate 0.125 oz	11		1.2		3	cd
6) treatment #4 + Kodiak Concentrate 1.0 oz	15		1.2		4	c
7) Chloroneb 65W 4.0 oz + AS-50 0.89 oz + Allegiance FL 0.75 fl oz	15		1.2		1	de
8) treatment #7 + Kodiak Concentrate 0.125 oz	13		1.2		1	e
9) treatment #7 + Kodiak Concentrate 1.0 oz	15		1.2		1	e
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		1.2		15	a
11) treatment #10+Kodiak Concentrate 0.125 oz	12		1.2		10	ab
12) treatment #10+Kodiak Concentrate 1.0 oz	11		1.2		10	b

Values are the means of either four replications (variety) or 12 replications (seed treatment). Means in the same column for the same factor (variety or seed treatment) followed by the same letter do not differ significantly by Fisher's (protected) LSD (P = 0.05).

Table 2. Snap Bean Seed Treatment Test, Jackson, TN, Spring 2000: Effects of variety and

seed treatment on healthy stand four weeks (6/14) or six weeks (6/28-29) after planting and on yield.

Seed treatment materials and rate per 100 lb seed	Healthy stand 6/14 (%)		Healthy stand 6/28-6/29 (%)		Yie (lb/a 7/08
VARIETY					
1) Strike	71	a	71	a	35
2) Hialeah	59	b	60	b	41
3) Hystyle	51	c	52	c	31
SEED TREATMENT					
1) water 10.0 fl oz	59	c	60	c	3150
2) Kodiak Concentrate 0.125 oz	61	bc	62	bc	3420
3) Kodiak Concentrate 1.0 oz	65	abc	65	abc	4032
4) Captan 400 2.5 fl oz + AS-50 0.89 oz	62	abc	65	abc	3692
5) treatment #4 + Kodiak Concentrate 0.125 oz	66	abc	65	abc	3454
6) treatment #4 + Kodiak Concentrate 1.0 oz	68	ab	68	a	4031
7) Chloroneb 65W 4.0 oz + Allegiance FL 0.75 fl oz + AS-50 0.89 oz	67	ab	70	a	4179
8) treatment #7 + Kodiak Concentrate 0.125 oz	68	ab	67	ab	3709
9) treatment #7 + Kodiak Concentrate 1.0 oz	69	a	67	ab	4132

10) Captan 400 2.5 fl oz + Lorsban 50-SL 1.5 oz + Allegiance FL 0.75 fl oz + AS-50 0.89 oz	46	d	44	d	2419	bc
11) treatment #10 + Kodiak Concentrate 0.125 oz	49	d	48	d	3298	abc
12) treatment #10 + Kodiak Concentrate 1.0 oz	50	d	50	d	3497	ab

Values are the means of either four replications (variety) or 12 replications (seed treatment). Means in the same column for the same factor (variety or seed treatment) followed by the same letter do not differ significantly by Fisher's (protected) LSD (P = 0.05).

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.