Barry J. Jacobsen[1], S. Kiewnick[1], J. Bergman[2] and Joyce Eckhoff[2]. [1]Department of Plant Sciences, Montana State University, P.O. Box 173140, Bozeman, MT 59717-3140 and [2] Eastern Agricultural Research Center, Sidney, MT 59270. **Integrated Control of Soilborne Diseases of Sugar Beet with Antagonistic Bacteria and Fungicide Seed Treatments**

ABSTRACT

In two years of field studies, 4 Bacillus sp. used in combination with seeds treated either with Apron-Thiram or with Apron-Thiram-Tachigaren provided increased stands and sucrose yields by controlling Pythium damping-off or Aphanomyces black root when compared to the fungicides applied alone. In 1998, 3 field trials were done in Montana using commercial formulations on seed pelleted by Seed Systems Inc. Stands where bacteria were combined with the seed treatment fungicides were increased over either Apron-Thiram or Apron-Thiram-Tachigaren in all locations at 4 and 8 weeks after planting and at harvest. In 1998, yield of untreated plots averaged 8067, Apron-Thiram 8250, Apron-Thiram-Tachigaren 8616, bacteria plus Apron-Thiram 8994 and bacteria plus Apron-Thiram-Tachigaren 9089 lbs of sucrose/A. Percentage sucrose content was not influenced by seed treatment.

INTRODUCTION and OBJECTIVES:

Damping-off caused by several species of *Pythium*, *Rhizoctonia solani*, *Phoma betae* and *Aphanomyces cochlioides* is the primary pathological cause of poor stands wherever sugar beets are grown. Establishment of uniform vigorous stands is critical to achieving maximum yields and controlling weeds. In Montana, damping-off from Aphanomyces is a common disease with stand losses of 10-50% being common throughout the Yellowstone River valley. Most severe losses to Aphanomyces damping-off occur with late planting where soil temperatures are in the 20-30 $^{0}$C range. It is interesting that unlike in the nonirrigated Minnesota or eastern North Dakota production areas, the late season root rot phase is less common in Montana or western North Dakota. Currently, damping-off diseases addressed in these studies are controlled by application of the fungicides Apron, Thiram and Tachigaren applied as seed treatments to either pelleted or nonpelleted seeds. Tachigaren is applied only to pelleted seeds for control of Aphanomyces. These fungicides provide protection for 1-3 weeks. The objectives of these studies were to further test MSU *Bacillus* sp. under field conditions where Pythium and Aphanomyces damping-off had historically been problems. These isolates all provided damping-off control equal to or better than standard fungicide treatments in inoculated greenhouse tests with *Pythium ultimum*, *Rhizoctonia solani*, and *Aphanomyces cochlioides* and in 1996 field trials. Four isolates were chosen for field tests in 1997 and 1998 based on earlier performance and their ability to be commercially fermented. Combinations of *Bacillus* isolates with fungicides was done to examine improved fungicide performance and to achieve the short term benefits of fungicide seed treatments plus the long term benefits of root colonization by root colonizing bacteria. *Bacillus* sp. were chosen because of their production of endospores and capacity to be formulated into commercial seed treatments.

PROCEDURES:

In 1997, a six replicate randomized complete block design was planted on June 2 ( soil temperature @10 cm was 15 $^{0}$C) using commercial planting equipment in a field near Forsyth, MT. This field had a history of severe Aphanomyces damping-off. Seeds (Beta 8754) were hand treated and pelleted using 24 hr. cultures grown on Tryptic soy broth (Difco) at 28 $^{0}$ C.

Cells were separated from beer by centrifugation @10,000 rpm for 5 minutes and washing with phosphate buffer. This procedure was repeated 3 times. Cells were resuspended in 1% methyl cellulose and 1ml was used to treat 110 seeds. This provided 1 x $10^{6-7}$ cfu/seed. Seeds were overtreated with Apron FL @ 0.98ml/kg seed, Thiram 42S @ 5.2ml/kg seed and Tachigaren @ 45gm/kg seed. For lots treated with Tachigaren, treatment was done following pelleting with Seed Systems Custom Coating @ 2.2 gm/110 seeds. Pelleting was done after the Apron-Thiram-Bacterial treatments. Where bacteria were applied they were applied in the Apron-Thiram mixture. A total of 60 row ft per plot. was harvested by hand on 10/17/97. In 1998, research trials with the same six replicate randomized complete block design were established in Sidney, MT and in two growers fields with a history of Aphanomyces black root rot one in Forsyth , MT and the other in Fallon, MT. The Sidney location has a history of Pythium damping-off but not Aphanomyces. A single seed lot of Beta 8757 was treated by Seed Systems using commercially formulated bacteria prepared by Gustafson Inc. and were applied to achieve $1x10^{6-7}$ cfu/seed for field trials. These bacteria provided control of Aphanomyces black root rot in 1997 field trials. Apron FL was applied @ 0.98 ml/kg seed, Thiram 42S @ 5.2 ml/kg seed and Tachigaren @ 45g/kg seed. Trials were planted in the first 10 days of May using commercial planting equipment and 30 row ft. per plot was harvested in the last two weeks of September. Percent sucrose, tare and quality factors were determined by the Western Sugar lab in Billings MT for Forsyth and Fallon trials. The Holly Sugar lab in Sidney handled the Sidney samples. Stand counts were determined 30 days after planting and at harvest.

RESULTS:

Results of the 1997 Forsyth trial is given in Table 1. Isolates LS 199, 01 and 202 significantly increased stands compared to the Apron-Thiram control and treatments including Tachigaren reduced stands although the bacterial co-treatments partially compensated for this effect. The reduced stands with Tachigaren treatments are thought to be due to phytotoxicity and poor pellet formation compared to commercially pelleted seed. Sucrose yield were increased at the LSD =0.05 level by the LS 199 and 201 treatments on Apron-Thiram treated seed and the LS 199 on Apron-Thiram-Tachigaren treated seed. No significant differences were observed in % sugar. Yield differences were due to differences in tons/A, tare and sugar quality.

Results for 1998 are given in Table 2. 1998 Sugarbeet Seed Treatment Trials for Control of Pythium and Aphanomyces using Apron-Thiram (AT), Apron-Thiram-Tachigarin (ATT) alone or in combination with MSU Biologicals LS 199,200, 201 and 202. Only Pythium damping-off and root rot was evident at the Sidney location and both Aphanomyces black root rot and Pythium were evident in the two grower field locations as evidenced by the response to Tachigaren. At the Sidney location there was no benefit to Apron-Thiram-Tachigaren treatment while all MSU biologicals increased yields over the Apron-Thiram treatment and the Apron-Thiram-Tachigaren plus LS 200 increased harvested stand. At the Forsyth location, all treatments increased yield over the standard Apron-Thiram treatment and Apron-Thiram plus LS 199, 201 and 202 out yielded the Apron-Thiram-Tachigaren treatment. Apron-Thiram-Tachigaren plus LS 200 had higher final stands than the Apron-Thiram treatment. At Fallon, Apron-Thiram-Tachigaren, Apron-Thiram-Tachigaren plus LS 201 and 202 and Apron-Thiram plus LS 200 and 201 increased yield over the standard Apron-Thiram treatment. At the 5% level of statistical difference there were no final stand differences other than Apron-Thiram-

Tachigaren plus LS 202, although at the 10% level Apron-Thiram plus LS 201 or Apron-Thiram-Tachigaren plus LS 201 increased stands. Differences are statistically significant for all three trials but should not be discussed because of differences in diseases present. Sucrose yield did not differ due to % sugar.

Discussion and Conclusion

The LS 201 and LS 199 isolates used with Apron-Thiram or Apron-Thiram-Tachigaren seed treatments appear to be a promising additions to the arsenal for Aphanomyces and Pythium control. Research planned for 1999 will repeat these experiments plus investigate the use of these biologicals as a planter box treatments or as an additions to Apron-Thiram on non-pelleted seeds. These later treatments are important since Aphanomyces damping-off is often a serious disease in replant situations where pelleted seed is not available.

**Table 1. Effect of combinations of Bacillus sp. and fungicide seed treatments on sugar beet stand and yield at Forsyth, MT in 1997.**

| Treatment | % Stand 7/6/97 | | % Stand at harvest | | Sucrose lb/A | |
|---|---|---|---|---|---|---|
| | AT [1] | ATT [2] | AT | ATT | AT | ATT |
| none | 46.3 | 31.1 | 46.0 | 29.4 | 3820 | 2660 |
| LS 201 | 70.1 | 45.5 | 57.0 | 49.0 | 5700 | 4060 |
| LS 199 | 72.9 | 51.2 | 80.4 | 52.0 | 5860 | 4980 |
| LS 200 | 53.7 | 34.8 | 61.4 | 35.4 | 4320 | 3120 |
| LS 202 | 38.9 | 36.9 | 44.4 | 39.4 | 4400 | 3040 |
| LSD 0.05 | 12.5 | | 21.3 | | 1760 | |
| LSD 0.10 | 10.4 | | 17.6 | | 1480 | |

1. Apron-Thiram 0.98 ml + 5.2 ml/kg seed
2. Apron-Thiram-Tachigaren 0.98 ml + 5.2 ml + 45 gm/kg seed

**Table 2. 1998 Sugarbeet Seed Treatment Trials for Control of Pythium and Aphanomyces using Apron-Thiram (AT), Apron-Thiram-Tachigarin (ATT) alone or in combination with MSU Biologicals LS 199,200, 201 and 202**

| Treatment | Sidney (Pythium) | | Forsyth (Pyth.+ Aphan.)[1] | | Fallon (Pyth. + Aphan.)[1] | | Average | |
|---|---|---|---|---|---|---|---|---|
| | *Yield* | *Stand* | *Yield* | *Stand* | *Yield* | *Stand* | *Yield* | *Stand* |
| none | 8409 | 45.5 | 6756 | 41.1 | 9037 | 45.0 | 8067 | 43.8 |
| AT | 8921 | 51.2 | 6494 | 42.9 | 9365 | 47.6 | 8250 | 47.2 |
| ATT | 8420 | 52.1 | 7270 | 42.4 | 10158 | 51.8 | 8616 | 48.8 |
| AT-199 | 9439 | 52.8 | 7814 | 44.1 | 8904 | 47.1 | 8719 | 48.0 |
| AT-200 | 9211 | 51.7 | 7619 | 43.3 | 9809 | 47.2 | 8907 | 47.4 |
| AT-201 | 9226 | 52.1 | 8013 | 44.4 | 11002 | 52.1 | 9414 | 49.5 |
| AT-202 | 9728 | 56.0 | 7866 | 45.4 | 9220 | 47.3 | 8938 | 49.6 |
| ATT-199 | 9406 | 53.8 | 7296 | 45.5 | 9704 | 47.0 | 8802 | 48.8 |
| ATT-200 | 9973 | 57.3 | 7281 | 46.5 | 9045 | 50.1 | 8766 | 51.3 |
| ATT-201 | 9715 | 52.8 | 7197 | 46.0 | 10918 | 53.7 | 9277 | 50.8 |
| ATT-202 | 9477 | 51.7 | 7726 | 44.7 | 11332 | 55.7 | 9512 | 50.7 |
| LSD 0.05 | 176 | 8.7 | 532 | 3.3 | 457 | 8.9 | 369 | 6.6 |

Yield=Lbs. Of sugar/A, Stand = per 30 ft. of row, Variety Beta 8757, seed pelleted and treated by seed systems
1. Diseases present Pythium and Aphanomyces