**COMPARISONS OF GLIOCLADIUM VIRENS** AND A COMBINATION OF TRICHODERMA SPECIES (BINAB T) IN POLYMER COATINGS OF COTTON SEED FOR **CONTROLLING SEEDLING DISEASES** J. E. DeVay<sup>1</sup>, R. H. Garber<sup>2</sup>, C. R. Howell<sup>3</sup>, R. J. Wakeman<sup>1</sup>, T. Ricard<sup>1</sup>, B. L. Weir<sup>4</sup>, and R. N. Vargas<sup>5</sup> <sup>1</sup>Department of Plant Pathology, University of California, Davis, CA; <sup>2</sup>California Planting Cotton Seed Distributors, Shafter, CA; <sup>3</sup>USDA-ARS, Southern Crops Research Laboratory, College Station, TX; <sup>4</sup>CE-DANR University of California, Merced, CA; <sup>5</sup>CE-DANR, University of California, Madera, CA.

### **Abstract**

Polymer coatings of both acid delinted and machine delinted cotton seed have been effective carriers for chemical and biological seed treatments. In field comparisons of three polymer coatings, Incotec, Colorcon, and Seedbiotics, applied to both acid delinted and machine delinted Acala Maxxa seed, all were similar based on percentage seedling survival. Combinations of fungicides were mixed with the polymer coatings before application to the seed. When seed treatments were made using acid delinted seed, the preparations of biocontrol fungi mixed with the Colorcon or Seedbiotics polymers, were as effective as chemical seed treatments based on percentage seedling survival and lint yields. In greenhouse tests, Binab T (*Trichoderma harzianum* + *T. polysporum*), was effective both as a seed treatment or with in-furrow applications for controlling black root rot caused by Thielaviopsis basicola.

# **Introduction**

Among the difficulties in using fungi and bacteria as biocontrol agents for seed and seedling diseases is their application to seed and effective shelf-life. The use of polymer coatings, similar to those used for coating pharmaceuticals and foodstuffs, provided an excellent medium as a carrier for biocontrol organisms and for chemical seed treatments (DeVay et al., 1991, 1994). Combining chemical pesticides with polymer coatings eliminates the irritating dust associated with acid delinted seed and pesticides when seed are dumped into hopper boxes. Moreover, combining biocontrol organisms with polymer materials provided a carrier in which the density of live propagules on each seed could be maintained at a high level and also give an effective shelf-life of approximately 5 months.

Another possible use of polymer seed coatings involved the use of machine delinted seed which was spray-coated, a process which resulted in a smooth coat and allowed the seed to slip and be planted singly the same as acid delinted seed. Combining the pesticides and biocontrol organisms in the polymer materials prior to application to the seed, eliminated the steps involved with acid delinting, neutralization of the seed, and additional steps in treating the seed with pesticides. It also eliminated the hazards of acid delinting with sulfuric acid or hydrogen chloride gas and the problems associated with disposing of these substances (DeVay et al., 1995).

The objectives of the present work were to compare several polymer coatings and their effectiveness as carriers for biocontrol organisms.

#### **Materials Methods**

# Cotton seed

Acala Maxxa cultivar, both acid and machine delinted, was obtained from the Anderson Clayton Gin, Fresno, CA.

# **Biocontrol fungi**

Strains of *Gliocladium virens* were provided by Dr. Charles Howell. The cultures were grown on millet seed and the colonized seed was ground to fine granules (#100 mesh sieve). The granules were mixed with Colorcon Opadry Red polymer (Colorcon, Inc., West Point, PA) or Seedbiotics polymer (Seedbiotics, St. Joseph, MO) and applied in a water slurry to the seed in a rotating drum. Approximately 54 oz of the fungal preparation were mixed with 32 oz of the polymer coatings and applied per cwt. of seed.

A preparation (Binab T wettable powder) consisting of a mixture of *Trichoderma polysporum* and *T. harzianum* was obtained from Binab Bio-Innovation AB (Algaras, Sweden). Approximately 16 or 32 oz of the fungal preparation were mixed with 32 oz of the Colorcon or Seedbiotics polymers and applied per cwt. of seed.

### Polymer seed coatings

Acid and machine delinted Acala Maxxa seed were coated with the Colorcon Opadry Red polymer, the Seedbiotics limestone-polymer combination, or a polymer of Incotec Co. (Salinas, CA). The polymers were prepared as suspensions, mixed with appropriate fungicides, and sprayed on the seed in rotating drums. The seed were dry within minutes after treatment. The amount of polymer applied was 1.5 to 2.0% of seed weight.

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All the seed treatments, including the biocontrol fungi, involving the Seedbiotics polymer were applied by Dr. W. R. Ellis of Seedbiotics Co. The Incotec polymer was applied by Ms. Cher Brandt of Incotec Co., and the Colorcon polymer by Mr. R. Long, C.M.S, Ames, IA.

# **Experimental plots**

Plots at the California Planting Cotton Seed Distributors Research and Development Station, Shafter, CA were randomized complete block design with eight replications. Treatment rows in each block were 30 feet long and 120 seed were planted per treatment row using a four-row cone planter.

# Data collection and analysis

Seedling emergence, stand count or percentage seedling survival during the first month after planting were recorded. Seed cotton in the treatment rows was handpicked. Analysis of variance of the data were done using the type III sums of squares method of the general linear models procedure of SAS.

# **Results and Discussion**

Comparisons of several polymer coatings of both acid and machine delinted seed, based on seedling emergence and percentage seedling survival, indicated that were no significant differences among these treatments affecting seedling performance (Table 1). Seedling emergence and survival were not delayed or enhanced by the polymers or by the chemical seed treatments mixed with the polymers. However, all chemical seed treatment/polymer combinations were significantly better than the untreated acid delinted seed in percentage seedling survival (Table 1).

When Colorcon seed coating of Acala Maxxa acid delinted seed were mixed with biocontrol fungi, these treatments were significantly better than the untreated seed based on percentage seedling survival (Table 2). Two of the treatments consisting of Gliocladium virens strain G-6 or G. virens strain G-6 + Apron FL were also significantly better than the chemical control seed treatment. Although the Acala Maxxa cultivar has excellent tolerance or resistance to Pythium seed and root rot, the biocontrol treatments with G virens strain G-6 were effective against Rhizoctonia solani and probably other seedling pathogens. The importance of these results is their confirmation of earlier findings (DeVay et al., 1994) that G. virens strain G-6 is a practical and useful seed treatment for controlling seedling diseases of cotton. The mixture of the Colorcon polymer with the Trichoderma species in Binab T was also an effective seed treatment and similar to the chemical seed treatment based on seedling survival (Table 2). In regard to affects on lint yields, none of the seed treatments were significantly different from the untreated seed. This is not surprising since cotton plants have unusual ability to compensate in growth and yield for missing or dead plants. In other field tests, the Seedbiotics limestone-polymer coating when mixed with preparations of *G. virens* strains G-4 + G-6 was similar in effectiveness to the untreated seed and seed treated with chemical fungicides. However, in combination with Binab T at 32 oz/cwt seed, the seed coating was significantly better than any other treatment, including the untreated seed and seed treated with chemical fungicides (Table 3). Among the seed treatments, lint yields were similar to the untreated seed.

In greenhouse tests, Binab T also has been effective against *R. solani, Pythium ultimum, and Thielaviopsis basicola* both as a seed treatment and as in furrow treatments (data not shown).

# References

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Table 1. Field comparisons of acid an machine delinted Acala Maxxa cotton
seed with different polymer seed coatings - Shafter, CA

Seed Treatments	Percentage	Percentage Seedling Survival	
(oz/cwt seed)	acid delinted	machine delinted	
1. Untreated seed	80 e <sup>1</sup>		
2. Uncoated seed with			
Apron TL (1 oz)			
+ Nu-Flow ND (14.5 oz)	91 a		
3. Incotec coating			
+ Apron TL $(1 \text{ oz})$ +			
Nu-Flow ND (14.5 oz)	90 ab	90 ab	
4. Seedbiotics coating			
+ Apron TL $(1 \text{ oz})$ +			
Nu-Flow ND(14.5 oz)	88 abcd	88 abcd	
5. Colorcon coating			
+ Apron FL (1 oz)			
+ PCNB-Vitavax (6.4 oz)			
+ Baytan 30 (1.5 oz)	89 abc	85 bcde	

<sup>1</sup>Percentage seedling survival values followed by the same letter are not significantly different (P = 0.05).

Table 2. Field comparisons of fungal preparations for the biocontrol of seedling diseases of Acala Maxxa cotton - Shafter, CA

Treatments (oz/cwt seed) <sup>1</sup>	Percentage Seedling Survival	Lint Yields (lbs/ac)
1. Untreated seed	73 e <sup>2</sup>	906 a
2. Apron FL (1 oz)		
+ Baytan 30 (1.5 oz)	78 d	925 a
3. Gliocladium virens		
Strain G-6 (54 oz)	86 ab	926 a
4. G. virens Strain G-6		
(54 oz) + Apron FL (1 oz)	88 a	1003 a
5. Binab T (Trichoderma		
harzianum +		
T. polysporum - 16 oz)	81 cd	957 a
6. Binab T (16 oz) +		
Apron FL $(1 \text{ oz}) +$		
Baytan 30 (1.5 oz)	83 bcd	859 a

<sup>1</sup>Spore preparations of *Gliocladium* and *Trichoderma* species and chemical fungicides were applied to seed in Colorcon Opadry polymer (Red, YA-38-15059) at 32 oz/cwt seed.

 $^2\text{Values}$  in columns followed by the same letter are not significantly different (P = 0.05).

Table 3. Field comparisons of fungal preparations for the biocontrol of seed and seedling diseases of Acala Maxxa cotton - Shafter, CA

Seed Treatments (oz/cwt seed) <sup>1</sup>	Percentage Seedling Survival	Lint Yields (lbs/acre)
1. Untreated seed	91 $bc^2$	1053 a
2. Seedbiotics coating +		
Apron TL $(1 \text{ oz}) +$		
Nu-Flow ND (14.5 oz)	93 ab	1036 a
3. Seedbiotics coating +		
Binab T (Trichoderma		
polysporum +		
T. harzianum - 32 oz)	98 a	1256 a
4. Seedbiotics coating +		
Gliocladium virens Strains		
G-4 + G-6 - 32 oz)	91 bc	1111 a

<sup>1</sup>Fungal preparations and chemical fungicides were premixed with a standard coating of the Seedbiotics limestone-polymer mixture and applied to the seed as a spray. Seed weight was increased approximately 2%.

 $^2Values$  in columns followed by the same letter are not significantly different (P = 0.05).