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CHAPERONE™ RESULTS FROM THE TRI-STATE DELTA

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Abstract

Variability of endotoxin protein expression and/or concentration within transgenic cotton (Bt) varieties has been and continues to be a concern of cotton growers, researchers and many breeders. While not a consistent problem, it is one which can cause major economic problems. Transgenic cotton varieties, to have effective insecticidal properties, must produce additional levels of proteins that conventional cottons do not. Cotton needs high levels of protein to maintain high yields, whereas transgenic cottons require additional protein to sustain insecticidal endotoxin proteins. Chaperone, a protein transport enhancer, containing nitrophenolates has been shown to increase the efficacy of endotoxin protein levels within transgenic cottons, resulting in higher worm mortality and yield. Chaperone has increased endotoxin protein levels in leaves and squares, and feeding trials have shown an increase in the percentage of worm mortality. In addition, Chaperone, through its protein binding and translocation capabilities, has produced yield increases even at low worm threshold levels.

Introduction

Chaperone is a new protein transport enhancer for transgenic plants, registered by EPA in 2000 with the pending patent. Chaperone is a combination of nitrophenols , namely sodium 5-nitroguaiacolate, sodium o-nitrophenolate and sodium p-nitrophenolate. Phenolics play a central role in plant metabolism and growth. They are known to (1) increase photosynthetic electron transport (2) improve/protect membrane integrity (3) increase enzyme/protein production (eg. IAA oxidase and glucose 6-phosphate dehydrogenase) (Robinson and Trevor 1980), (4) act as a part of lignin bio-synthesis, and (5) increase fruit retention. Observances in transgenic cottons have shown that endotoxin levels have occasionally failed to be fully expressed under various conditions, including environmental factors and varietal differences, thus occasionally leading to less efficient insect control and subsequent yield losses. Cotton plants engineered to express the endotoxin protein, Cry1Ac, from (Bt) have shown significant declines in efficacy against Helicoverpa spp. during the season, particularly from flowering onwards (Fitt et al., 1998). Thompson et. al. (1976) reported that there was less total protein in the leaves of older plants as a result of a three- to five-fold reduction in protein synthesis over the season. Therefore, Olsen and Daly (2000) concluded that not only is there less Bt protein in older plants, it appears that the protein is either less available or less toxic to neonates. Furthermore, the concentration of Cry1Ac protein, as a proportion of total protein, also declines during the season (Holt 1998).

Our hypothesis was that utilization of the phenolic properties of Chaperone in transgenic cotton would aid in alleviating non-expression or under expression of Cry I Ac (BOLLGARD ™ by Monsanto) or a combination of Cry I Ac with Cry 2 Ab (BOLLGARD II ™ by Monsanto), which are currently the genes utilized for expression of the endotoxin protein *B. thuringiensis*. It has been observed that a reduction in the amount of expressed endotoxin protein occurs as plants mature leading to a loss of efficacy in the latter stages of the growing season thus increasing the probability of surviving pests which may develop immunity to the endotoxin protein (Greenplate,1999 & Benbrook and Hansen, 1997). In Bt cotton, it is theorized that expression of the Cry I Ac gene drops due to a decline in the concentration of the CAMV35S promoter causing the gene to be "silenced" or be affected by other post transcription events (Kennedy and Turner, 1999). The Cry I Ac protein may also be reduced due to increased turnover, sequestration within the plant, or dilution due to growth and aging (Greenplate, 1999). It is understood that Cry I Ac transcription levels are occasionally unstable in both immature and mature Bt cotton plants (Daly and Fitt, 1998). Testing for the past three years has indicated an increase in Bt expression, improved worm mortality and an increase in overall yield where Chaperone has been applied.

It is now thought that Chaperone acts in one or more of several ways: (1) as a form of protective water substitute for cellular membranes during times of water deprivation, and (2) as a protein stabilizer for the desired pesticidal protein and/or as a binder for protein constituents thus facilitating movement via intraplant transport mechanisms. The end result being that transgenic crops treated with Chaperone have been shown to express and move proteins, active in pest control, into plant tissues in a greater concentration than non-treated plants.

Materials and Methods

Protocol for Growth Chamber Studies

Cotton (*Gossypium hirsutum* L.) cultivar Paymaster 1218 BtRR was planted in March 2002 at the Altheimer Laboratory, University of Arkansas into 2 L pots containing a soilless horticultural mix. The growth chamber was set for 12-h photoperiod, with day/night temperatures of 30/25 C° and relative humidities of 60 to 80%. Plants were arranged in a completely randomized design with 3 replications. All pots received half-strength Hoagland's nutrient solution daily to maintain ade-

quate nutrients and water. Chaperone treatments were applied as a foliar spray with a CO_2 backpack sprayer calibrated to deliver 10 gallons H_2O/acre. The adjuvant, Penetrator Plus 0.05% v/v was used. The Chaperone treatments were applied as foliar sprays at the seventh true leaf stage and the upper expanded main-stem leaf was sampled 10 days later for bollworm mortality feeding. Treatments were sprayed again at the seventh true leaf +10 days. Five days after the second application of Chaperone, the upper expanded main-stem leaf was collected and 10 days after the second application, the upper expanded and main-stem leaf and subsequent first position square were collected. After each sampling, tissue samples were placed in small ziploc bags and immediately taken to the University of Arkansas Entomology Department for bollworm mortality testing. Bollworm mortality rates were assessed at 24, 48, 72, and 96 hours from the initiation of feeding for samples following the first spray application and assessed at 72 and 96 hours following start of feeding for sampling taken after the second spray application.

Protocol for Field Studies

Yield studies were performed at numerous locations in the Tri-state Delta from 2001-2003 using commercially available transgenic (*Gossypium hirsutum* L.) cultivars recommended for each location. The experimental designs were randomized complete blocks with 4 to 6 replications depending on the test site. Fertilizer, weed and insect control measures were initiated according to state recommendations. Irrigation was applied as needed throughout the season. Chaperone treatments (5 or 10 oz./acre) were applied at mid-bloom as a foliar spray with a CO_2 backpack sprayer calibrated to deliver 10 gallons H_2O/acre. In addition to yield, studies were conducted from 2001-2003 at the University of Arkansas to determine protein and endotoxin concentrations and percent bollworm mortality following applications of Chaperone. These studies evaluated Chaperone sprayed at MHS and early bloom under different spray rates (5, 10 or 20 oz/acre). The field experiments in Arkansas were planted in early May in Clarkedale, northeast Arkansas. Leaf and square samples were collected one week after application of Chaperone, placed in small ziploc bags, placed on dry ice and immediately taken to the University of Arkansas Entomology Department for bollworm mortality tests. Leaf and square samples were also taken for protein and endotoxin analyses (University of Arkansas Altheimer Laboratory and Agdia, respectively).

Results and Discussion

Lint Yields

The effect that foliar applications of Chaperone had on lint yields in cotton was evaluated from 2001-2003 in numerous field trials by both mid-South consultants and University researchers. Pooled results from mid-South consultant trials from 2001-2003 showed that a single application of Chaperone mid-bloom at both 5 and 10 oz/acre rates increased lint yields by 80 lb/acre compared to the untreated Bt check (Fig. 1). Likewise, lint yield results from 11 University trials conducted from 2001-2003 showed similar increases in lint yield following a single application of Chaperone at mid-bloom (Fig. 2). Results from University trials also suggested that lint yields were higher when Chaperone was applied at the lower 5oz/acre rate compared to the higher 10 oz/acre rate (Fig. 2). Yield results, combined from all University and Consultant trials from 2001-2003, indicated that a single Chaperone application at mid-bloom increased lint yield by 80 lb/acre (Fig. 3).

Leaf Total Soluble Protein

Total soluble protein levels in leaves was determined by collecting samples from two experiment sites in Arkansas at various time intervals following foliar applications of Chaperone. Leaf samples collected in 2003 from both Clarkedale and Fayetteville, Arkansas locations were collected 10 days after the last application at two weeks after first flower (FF2). In 2002, leaf samples from a growth chamber trial were collected five days after the last Chaperone application occurring 10 days after the initiation of the seventh true-leaf. Results from these studies indicated that leaf protein concentrations from Chaperone treated plants was higher compared to untreated Bt cotton plants (Fig. 4).

Endotoxin Levels

In 2001, a field study was conducted in northeast Arkansas to determine the endotoxin level of cotton leaf, petiole and square material following application of Chaperone at 5, 10, and 20 oz/acre. Samples for endotoxin analysis were submitted to Agdia for testing. Endotoxin results were expressed as the percent increase in endotoxin levels above the control. Results indicated that endotoxin levels were increased in all plant component parts as a result of foliar Chaperone applications (Fig. 5). Furthermore, endotoxin levels of plant component parts increased with each increasing rate of Chaperone (Fig. 5).

Bollworm Mortality

The effect of foliar Chaperone applications on the mortality of neonate bollworms was evaluated in 2002 and 2003 at two test sites in Arkansas. Only the results from the 2002 season are presented in this paper. Mortality results are from the growth chamber study in Fayetteville (Fig. 6) from leaves collected 10 days after Chaperone applications at main-stem node seven, and leaves and squares collected from Clarkedale (Fig. 7) 10 days after application at FF2. Results from the growth chamber study in Fayetteville indicated an increase in neonate bollworm mortality from all Chaperone treatments compared to the untreated Bt check (Fig. 6). Also, there was an increase in bollworm mortality with increasing application rate of Chaperone

(Fig. 6). Results from the field study at Clarkedale indicated that Chaperone application at both 5 and 10 oz/acre rates increased mortality of neonate bollworms feeding on leaf and square material for a period of 96 hours (Fig. 7).

References

Benbrook, M. and Hansen, M. 1997. Return to the Stone Age of Pest Management. Proceedings of Plant Pesticide Resistance Management, A Public Meeting, March 21 1997, Washington DC

Kennedy, G.G., Turner B.S., 1999. Emerging Technologies for Integrated Pest Management Proceedings , March 8-10, 1999 Raleigh, N.C., APS Press

Daly, J.C. and Fitt, G.P., 1998 Efficacy of Bt Cotton Plants in Australia – "What is Going On?" pp 675-678 In New Frontiers in Cotton Research-Proceedings World Cotton Research Conference-2 Athens, Greece, Sept. 6-12, 1998. Editor: F. Gilham, Published P. Petridis, Thessaloniki, Greece.

Fitt, G.P. Daly, J.C., Mares, C.L. and Olsen, K. 1998. Changing efficacy of transgenic cotton plant patterns and consequences. Sixth Australian Entomological Research Conference, Brisbane, Australia.

Greenplate, J.T., 1999. Quantification of Bacillus thuringiensis Insect Control Protein CryIAc Over Time in BOLLGARD™ Cotton Fruit and Terminals. J. Econ. Entom. 92:1377-1383

Holt, H. 1998. Season-long monitoring of transgenic cotton plants development of an assay for the quantification of *Bacillus thuringiensis* insecticidal protein. pp. 331-335, In Proc. 9th Australian Cotton Grower's Research Association, Queensland, Wee Waa, Australia.

Olsen, K.M. and Daly, J.C. 2000. Plant-toxin interations in transgenic Bt cotton and their effects on mortality of helicoverpa armigera (lepidoptera: Noctuidae). Entomol. Soc. Amer. 93:1293-1299.

Robinson, Trevor, 1980. The Organic Constituents of Higher Plants, 4th edition. Cordus Press, Amherst, Mass.

Thompson, A.C., Lane, H.C., Jones, J.W. and Hesketh, J.D. 1976. Nitrogen concentration of cotton leaves, buds, and bolls in relation to age and nitrogen fertilization. Agron. J. 68:617-621.

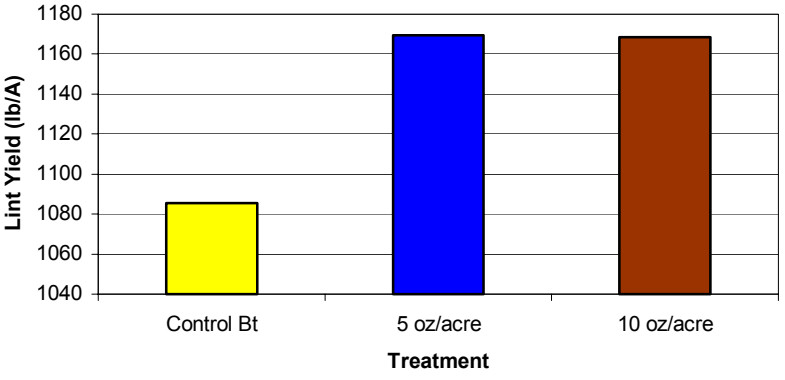

Figure 1. Chaperone Lint Yield Results for Mid-South Consultant Trials (2001-2003).

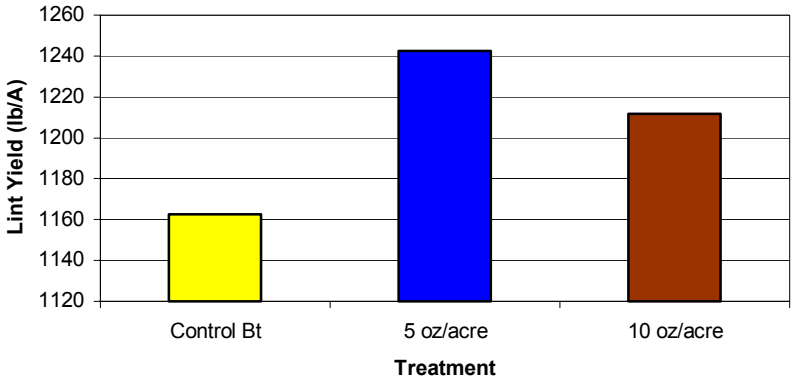

Figure 2. Chaperone Lint Yield Results for University Trials (2001-2003).

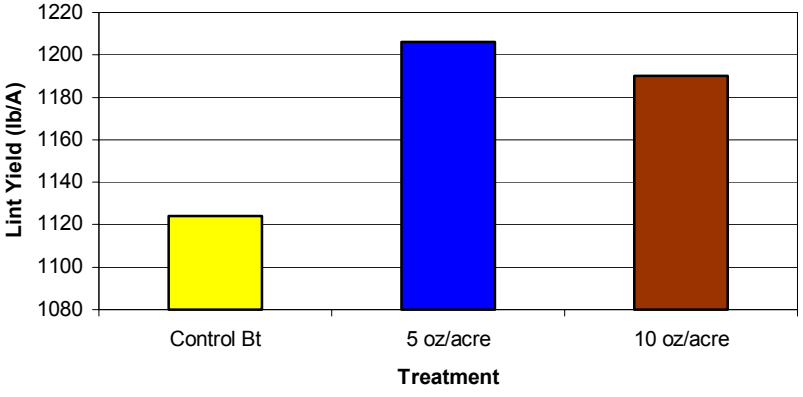

Figure 3. Chaperone Lint Yield Results from all Trials (2001-2003).

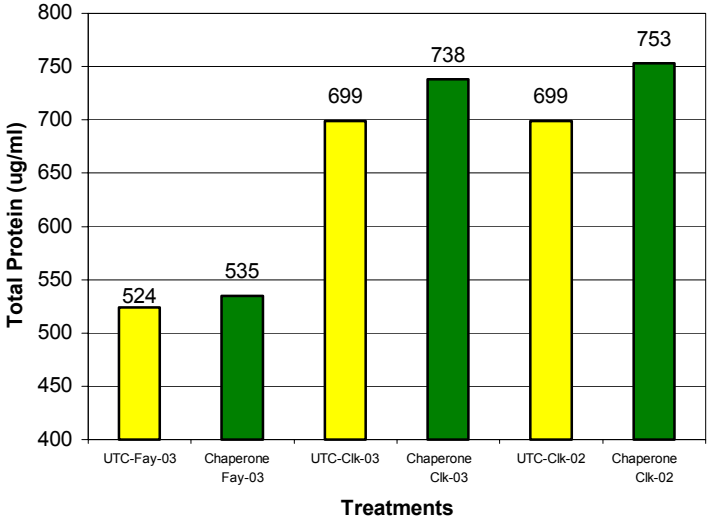

Figure 4. Effect of CHAPERONE on Total Protein in Leaves from two Arkansas Locations, Fayetteville and Clarkedale (2002-2003)

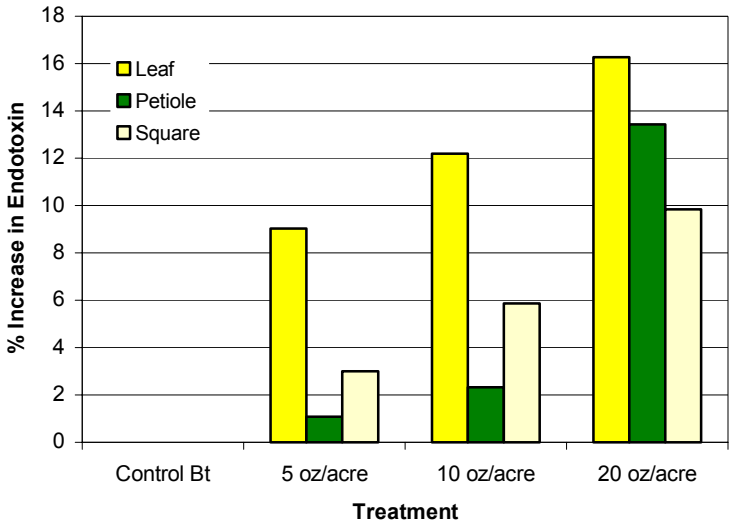

Figure 5. Percentage Increase of Endotoxin Level Above the Control (Clarkedale, AR-2001).

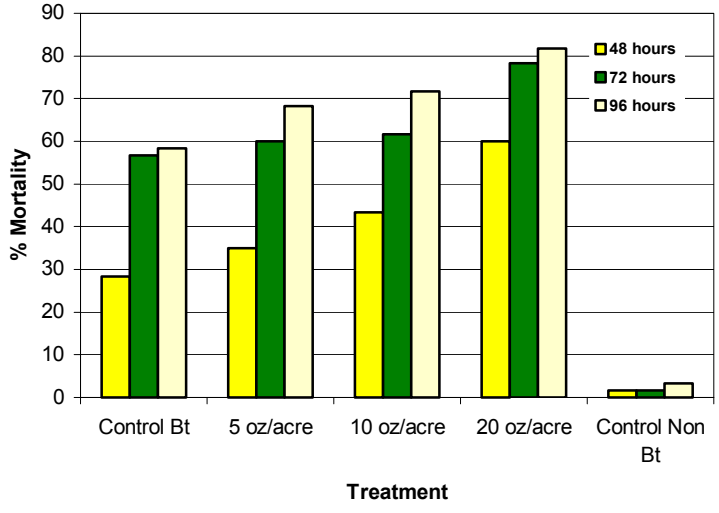

Figure 6. Percent Bollworms Mortality Following chaperone Applications. Growth Chamber, Arkansas, 2001.

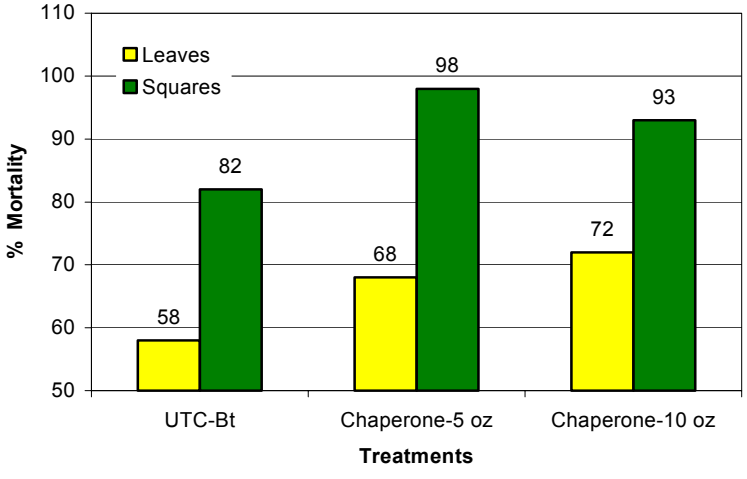

Figure 7. Effects of CHAPERONE on Neonate Mortality in Leaves and Squares. Field Study, Arkansas 2002.