2004 BELTWIDE COTTON CONFERENCES, SAN ANTONIO, TX – JANUARY 5-9

INCREASED PROTEIN, INSECT MORTALITY AND YIELD WITH CHAPERONE™

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Abstract

The plant growth regulator Chaperone™ has been reported to increase plant nitrogen levels, promote protein constituent transport, and increase overall yields. Field and growth chamber studies were conducted in 2002 and 2003 to quantify the effect of foliar applications of Chaperone on protein and endotoxin levels of cotton (*Gossypium hirsutum* L.) leaves and squares, and the subsequent effect on bollworm mortality and yield. In a growth chamber study in 2002, using cotton cultivar DP33B, Chaperone treatments were applied at the seventh true leaf as a foliar spray at 5, 10 and 20 oz/acre. The upper expanded main-stem leaves were sampled 10 days later for protein and endotoxin determinations, and insect mortality. Results indicated that insect mortality was increased 10, 14 and 23% on Chaperone-treated leaves and 16, 11, and 13% on squares at 5, 10, and 20 oz/acre, respectively, 10 days after application. In field studies in 2002, Chaperone at 5 oz/acre applied at matchhead square (MHS) and first flower (FF) increased yield 13.4% compared to the untreated control with an accompanying +7.7% increase in protein levels in leaves. In 2003, Chaperone at 5 oz/acre at MHS and FF increased protein in the leaves (+2.1 to 5.6%) and in the squares (+5.1 to 53.5%), with better expression of Bt endotoxin in leaves (-1.7%to +17.4%) and squares (+0.7 to 22.2%), and higher mortality of bollworms feeding (+9.5%) on treated plants compared to untreated leaves 10 days after application. Data from the growth chamber and field studies in 2002 and 2003 show that foliar applications of Chaperone may be a viable means for enhancing lint yields in cotton through the enhancement of plant protein levels. Furthermore, the enhanced protein status contributes to improved late season endotoxin levels, paricularly in the squares, that contributes to increase mortality of neonate bollworms feeding on the treated plants.

Introduction

Chaperone™ is a new protein transport enhancer registered by the Environmental Protection Agency in 2000 and the patent is pending. 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, (4) act as a part of lignin bio-synthesis, and (5) increase fruit retention (Robinson and Trevor, 1980). 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. Furthermore, 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. The concentration of Cry1Ac protein, as a proportion of total protein, also declines during the season (Holt 1998). The phenolic properties of Chaperone may aid in transgenic cotton by 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), the genes utilized for expression of the endotoxin protein *B. thuringiensis*.

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 into plant tissues in a greater concentration than non-treated plants. It was hypothesized that the unique phenolic properties of the plant growth regulator Chaperone increases plant total protein content and also enhances Bt endotoxin protein levels in transgenic cottons. These properties are conducive to increased bollworm mortality and also yield increases. The objectives of the current field and growth chamber research was to quantify the effect of foliar applications of Chaperone on leaf and square protein and endotoxin levels, bollworm mortality, and yield.

Methods and Materials

Field and growth chamber studies were conducted in 2001, 2002 and 2003 to quantify the effect of foliar applications of Chaperone on protein and endotoxin levels of cotton leaves and squares, and the subsequent effect on bollworm mortality and yield.

Growth Chamber Study

Cotton (*Gossypium hirsutum* L.) cv. DP 33B 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 a 12-h photoperiod, with day/night temperatures of 30/25 dg. 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 adequate 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 at 0.05% v/v was used. Chaperone treatments were sprayed at the seventh true leaf and the upper expanded main-stem leaf was sampled 10 days later. Treatments were sprayed again at the seventh true leaf +10 days and leaves and squares sampled 5 days later. Sampled tissues were placed in ziploc bags and immediately taken to the University of Arkansas Entomology Department for bollworm mortality testing. Bollworm mortality was assessed by placing single one-day old neonate bollworms on leaf sections in individual plastic cups with agarose in an incubator at 26 C. Mortality rates were assessed at 24, 48, 72, and 96 hours from the initiation of feeding for samples collected following the first spray application and assessed at 72 and 96 hours following the start of feeding for sampling taken after the second spray application. In 2001 and 2002, main-stem leaves along with the accompanying petioles and first position squares were sampled four main-stem nodes from the terminal at 5 and 10 days after Chaperone application, placed immediately on dry ice, and shipped to the Agdia testing facility to be analyzed for Bt endotoxin levels.

Field Studies

Cotton (*Gossypium hirsutum* L.) cultivar Suregrow 215Bt/RR was planted in early May 2002 and 2003 in a Captina silt loam at Clarkedale in NE Arkansas, and in Fayetteville in northwest Arkansas. The design was a randomized complete block with three replications. Fertilizer, pesticides and irrigation practices were according to current extension recommendations. Treatments consisted of an untreated control and single foliar applications of Chaperone at FF applied at 2.5, 5, 10, and 20 oz/ /acre, and two applications of Chaperone at MHS and FF at 2.5, 5, and 10 oz/acre in 2002. In 2003, Chaperone was applied at a rate of 5 oz/acre at MHS and FF. Yields were determined by mechanically harvesting the middle two rows of each four row plot and components of yield and fiber quality were determined from a two-meter sample from each plot. The methods for testing for neonate mortality and the times for testing for mortality were the same as in the growth room studies.

Measurements were made at select times each season and included leaf and square protein concentrations analyzed at the University of Arkansas utilizing the Bradford method (Bradford, 1976), leaf and square endotoxin concentration (conducted by Agdia), insect mortality (University of Arkansas Department of Entomology), and yield and yield components.

Results and Discussion

Effect of Chaperone on Cotton Yield

Foliar application of Chaperone in field trials at two locations in Arkansas for three years increased lint yields by an average of 61 lb/acre (Figure 1). This yield increase was associated with increased plant protein levels (Figure 2). Increased yields have been reported for three years from numerous consultant field trials across the US Cotton Belt (Lackey et al., 2004).

Effect of Chaperone on Protein Content of Leaves and Squares

In 2002, in the field study at Clarkedale, foliar application of Chaperone at first flower caused an increase in leaf protein content (Figure 2). Similarly, in 2003, there was a numerical but not significant ($P \leq 0.05$) increase in leaf protein from Chaperone applications in Fayetteville (+5.6%) and Clarkedale (+7.7%) compared to the untreated control.

Effect of Chaperone on Endotoxin Levels in Leaves and Squares

In the 2002 field study at Clarkedale, Chaperone caused a significant increase in endotoxin levels in leaves, petioles and squares, particularly at the higher concentrations of Chaperone (Figure 3). Similarly, in the 2003 field study in Clarkedale there was a trend for Chaperone to increase endotoxin levels of the squares (Figure 4). The increase in endotoxin was associated with the enhanced protein levels observed in growth room and field studies. 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 and thus increasing the probability of surviving pests which may develop immunity to the endotoxin protein (Greenplate, 1999; Benbrook and Hansen, 1997). Chaperone appears to be a viable means for enhancing endotoxin levels and thereby improving insect mortality.

Effect of Chaperone on Bollworm Mortality on Leaves and Squares (2002-2003 Field Studies)

Increases in bollworm mortality were recorded in the growth chamber study in Fayetteville in 2002 (Figure 5). These results indicated that all Chaperone treatments resulted in higher bollworm mortality compared to the untreated control, and also that mortality increased with increasing rates of Chaperone. Likewise, field studies in Arkansas have confirmed increases in bollworm mortality following applications of Chaperone, particularly worms feeding on squares (Figures 6 & 7).

Effect of Increasing Temperature and Chaperone on Leaf Protein Content. (2003 Growth room Study)

A preliminary study in the growth chamber showed a decrease in plant proteins with elevated temperatures (data not shown). In a subsequent growth room study, a foliar application of Chaperone applied at 5 oz/acre at MHS resulted in a numerical increase in protein levels at higher temperatures (36C and 39C) compared to the untreated controls (Figure 8). These data suggests a beneficial effect of Chaperone for enhancing protein levels under the high temperatures experienced in mid-summer. This research is currently being repeated.

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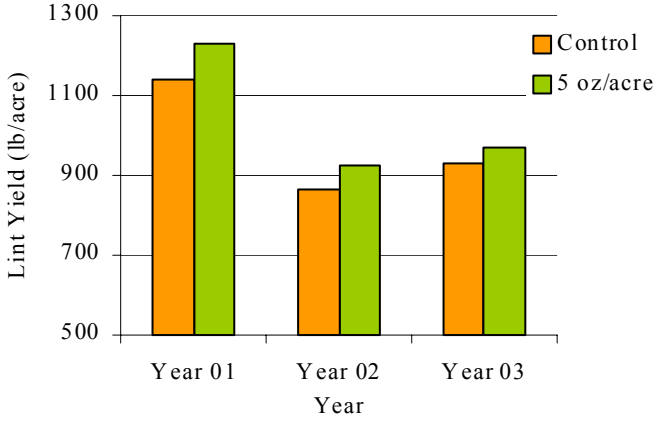

Figure 1. Effect of Chaperone on lint yield averaged across locations in Arkansas, 2001-2003.

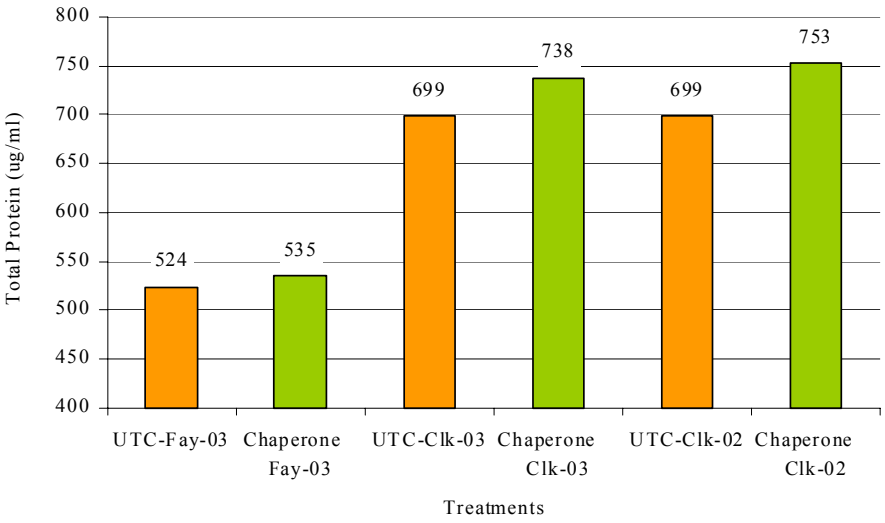

Figure 2. Effects of Chaperone on total soluble protein in leaves at two locations in Arkansas, 2002-2003.

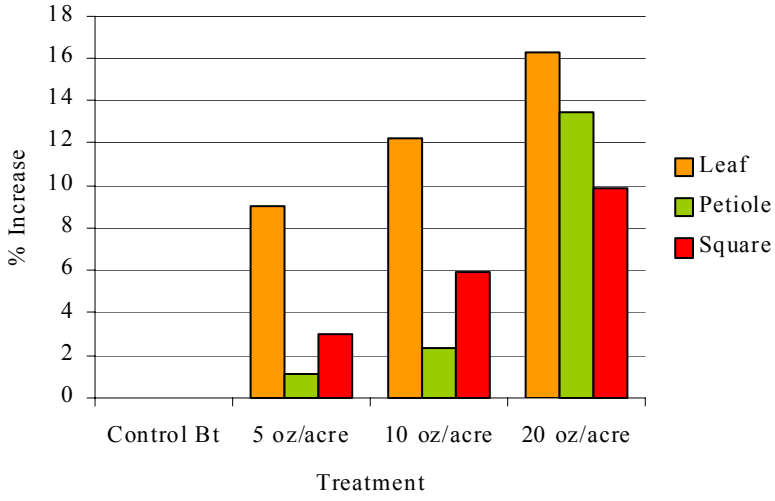

Figure 3. Percentage increase in endotoxin level, above the control, following Chaperone applications at 5, 10 and 20 oz/acre. Clarkedale, Arkansas 2002.

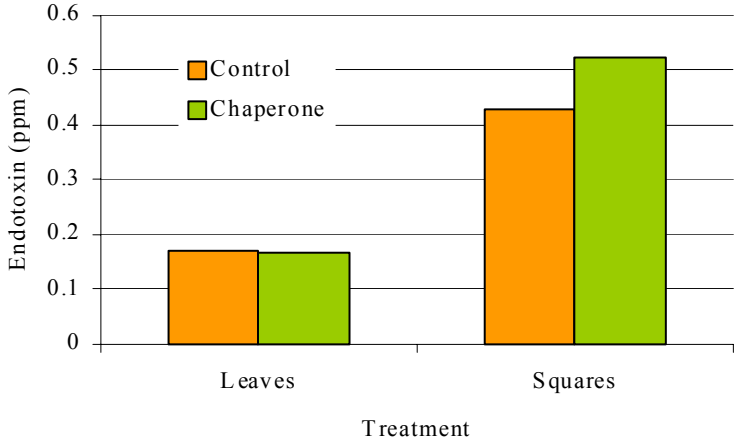

Figure 4. Effect of Chaperone on endotoxin levels in leaves and squares 10 days after application at first flower. Clarkedale, Arkansas 2003.

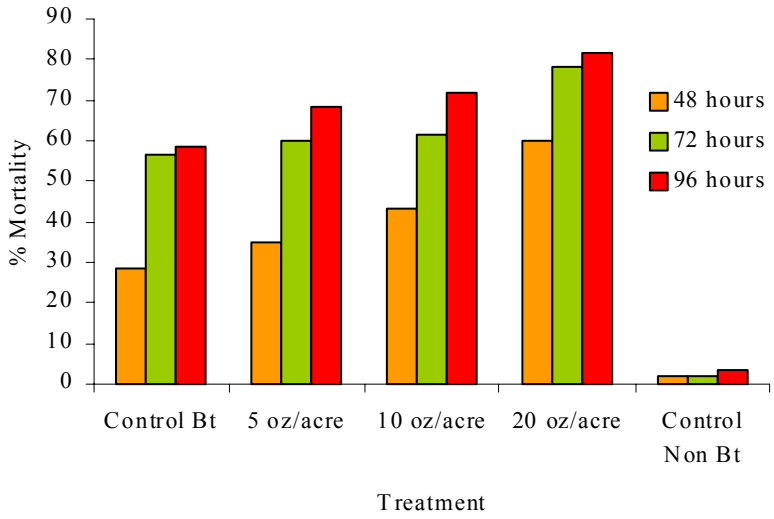

Figure 5. Effect of Chaperone applications on neonate bollworm mortality. Growth chamber study 2002, University of Arkansas.

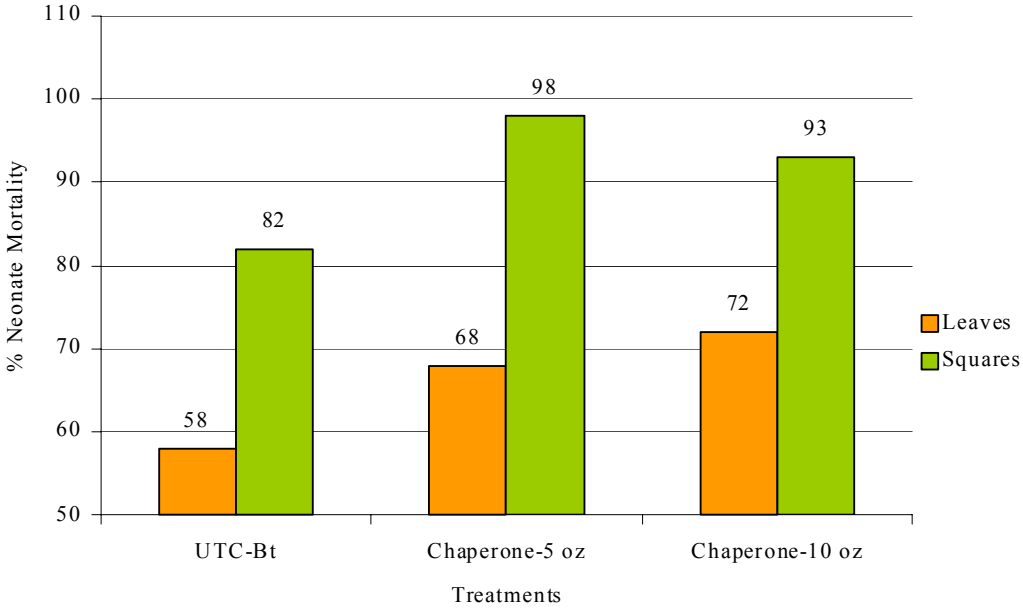

Figure 6. Effects of Chaperone on neonate mortality in leaves and squares. Field study, Clarkedale Arkansas 2002.

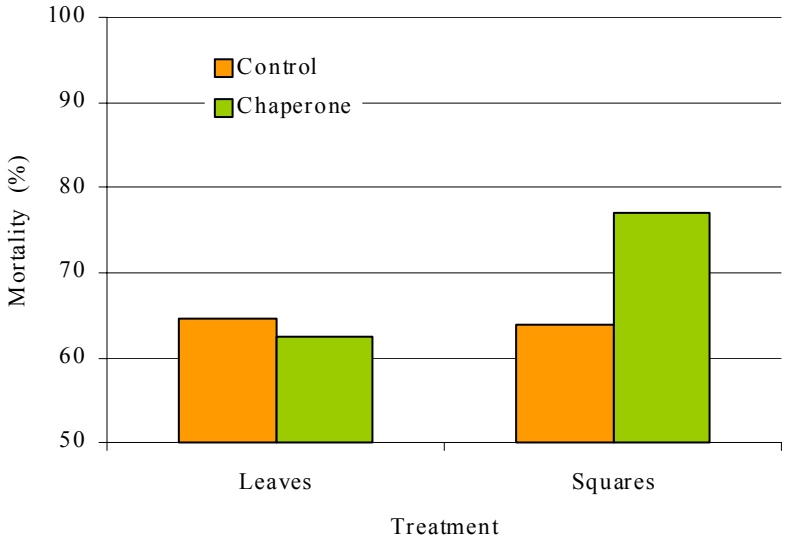

Figure 7. Effect of Chaperone on neonate mortality on leaves and squares 10 days after application at first flower. Fayetteville, Arkansas 2003.

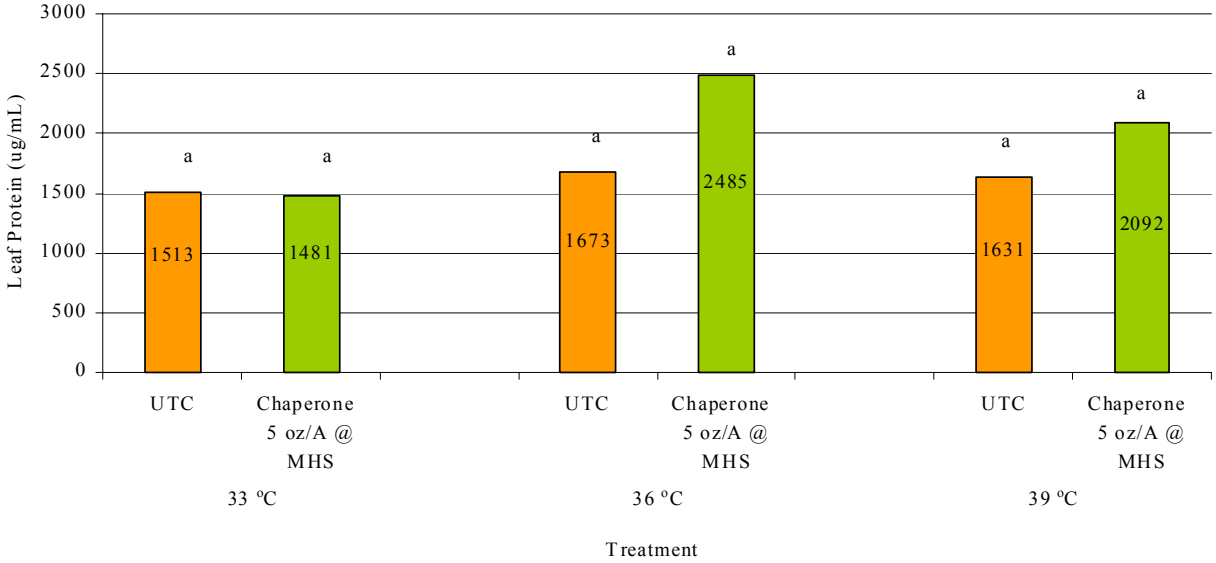

Figure 8. Effect of foliar applications of Chaperone applied at 5.0 oz/acre at matchhead square under elevated temperatures on total leaf protein concentrations. Growth chamber study, Fayetteville 2003. *Bars superseded by the same letter are not significantly different at $P \leq 0.05$.