INCREASED PLANT PROTEIN, INSECT MORTALITY, AND YIELD WITH CHAPERONE™

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RESEARCH PROBLEM

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 1) the effect of foliar applications of Chaperone on protein and endotoxin levels of cotton (Gossypium hirsutum L.) leaves and squares, and 2) the subsequent effect on bollworm mortality and yield.

BACKGROUND INFORMATION

Chaperone™ is a new protein enhancer that was 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, e.g., increased photosynthetic electron transport, improved membrane integrity, increased enzyme/protein production, increased lignin bio-synthesis, and increased fruit retention and growth (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 Bacillus thurengiensis (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 Cry1Ac or a combination of Cry1Ac with Cry2Ab, the genes utilized for expression of the endotoxin protein, B. thuringiensis.

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RESEARCH DESCRIPTION

Field and growth-chamber studies were conducted in 2001, 2002, and 2003 to quantify 1) the effect of foliar applications of Chaperone on protein and endotoxin levels of cotton leaves and squares, and 2) 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°F/25°C and relative humidities of 60 to 80%. Plants were arranged in a completely randomized design with three 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 CO₂ backpack sprayer calibrated to deliver 10 gallons H₂O/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 samples 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 215*Bt*/RR was planted in early May 2002 and 2003 in a Captina silt loam at Clarkedale in northeast 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 first flower (FF) applied at 2.5, 5, 10, and 20 oz/acre, and two applications of Chaperone at mathead square (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-chamber studies. Measurements were made at select times each season and included leaf and square protein concentrations analyzed at the University of Arkansas (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 (Fig. 1). This yield increase was associated with increased plant protein levels (Fig. 2). Increased yields have also 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 (47.7%) in leaf protein content (Fig. 2). Similarly, in 2003, there was a numerical but not significant (P < 0.05) increase in leaf protein from Chaperone applications in Fayetteville (+2.1%) and Clarkedale (+7.7%) compared to the untreated control.

Effect of Chaperone on Endotoxin Levels in Leaves and Squares

In the 2001 field study at Clarkedale, Chaperone caused a significant increase in endotoxin levels in leaves, petioles, and squares, particularly at the higher concentrations of Chaperone (Fig.2). Similarly, in the 2003 field study in Clarkedale there was a trend for Chaperone to increase endotoxin levels of the squares (data not shown). The increase in endotoxin was associated with the enhanced protein levels observed in growth-chamber 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 (Fig. 4). 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 (data not shown).

PRACTICAL 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, in transgenic (Bt) cultivars the enhanced protein status contributes to improved late-season endotoxin levels, particularly in the squares, resulting in increased mortality of neonate bollworms feeding on the treated plants.

LITERATURE CITED


Greenplate, J.T. 1999. Quantification of Bacillus thuringiensis Insect Control Protein CryIAc Over Time in BOLLGARD™ Cotton Fruit and Terminals.
**Fig. 1.** Effect of Chaperone on lint yield averaged across locations in Arkansas, 2001-2003.

**Fig. 2.** Effects of Chaperone on total soluble protein in leaves at two locations in Ark., 2002-2003.
Fig. 3. Percentage increase in endotoxin level, above the control, following Chaperone applications at 5, 10 and 20 oz/acre.

Fig. 4. Effects of Chaperone on neonate mortality in leves and squares. Field study, Clarkedale, Ark., 2002.