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INCREASED EFFICACY OF BT COTTON WITH ARYSTA EXP-NP321

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Abstract

Variability of endotoxin expression and/or concentration within transgenic cotton (Bt) varieties has been and continues to be a concern of cotton growers, researchers and breeders. While not a consistent problem, it is one which can cause major economic problems. Transgenic cotton varieties, to be effective, must produce additional levels of proteins which conventional cottons do not. Arysta Experimental NP 321, a protein transport enhancer, containing nitrophenolates has shown that it increases the efficacy of the endotoxin levels within transgenic cottons, resulting in higher worm mortality and yield. By binding to proteins and enhancing their movement throughout the plant, with the ultimate destination being the fruiting structure, NP 321 has increased endotoxin levels in leaves, petioles and squares as determined by Elisa testing and by actual feeding trials which produced an increase in the percentage of worm mortality. In addition, NP 321, through its protein binding and translocation capabilities, has produced yield increases even without heavy worm pressures.

Introduction

ARYSTA EXP – NP 321 is a new Protein Transport Enhancer for Transgenic Plants, registered by EPA in 2000 and with patent pending. NP 321 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 yield losses.

Our hypothesis was that utilization of the phenolic properties of NP – 321 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 occurs as plants mature leading to a loss of efficacy in the latter stages of the growing season (the last 1/3) thus increasing the probability of surviving pests which may develop immunity to the endotoxin (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,G.G. and Turner, B.S.,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,J.C. and Fitt,G.P., 1998). Testing to date has indicated an increase in Bt expression, improved worm mortality and an increase in overall yield where NP – 321 has been applied.

It is now thought that NP – 321 acts in one or more of several ways: (a) as a form of protective water substitute for cellular membranes during times of water deprivation, and (b) as a protein stabilizer for the desired pesticidal protein and/or as a binder for protein thus facilitating movement via intraplant transport mechanisms. The end result being that transgenic crops treated with NP – 321 have been shown to express and move effective proteins into plant tissues in a greater concentration than non-treated plants.

Materials and Methods

Experiments Conducted

2001: Yield trials conducted at 18 locations.
2001: Plant samples taken for Endotoxin levels from 9 locations.
2001: Elisa testing done on plant samples by an independent laboratory.
2001: Growth chamber studies done for Endotoxin levels.
2002: Yield trials conducted at 46 locations.

2002: Plant samples taken for Endotoxin levels from 10 locations.
2002: Feeding trials for worm mortality at the University of Arkansas.

Treatments: Randomized Complete Blocks
2001: Application made Mid-Bloom
2001: Applications made at MHS and Mid-Bloom
2002: Applications made at Mid-Bloom.
2002: Applications made at MHS and/or Mid-Bloom.
2002: Applications made at MHS, EB, EB + 2wks and 3 weeks

Sampling for Endotoxin Levels
2001: Took samples of subtending leaves, petioles and fruiting structures at 5 and 10 days after application by horizon within the plant structure, placed immediately on dry ice and shipped to testing facility.
2002: Took samples of subtending leaves, petioles and fruiting structures at 5 and 10 days after application by horizon within the plant structure, placed immediately on dry ice and shipped to testing facility.

Growth Chamber Study Protocol 2002
Cotton (*Gossypium hirsutum L.*) cultivar Paymaster 1218 BtRR was planted in March 2002 at the Altheimer Laboratory, University of Arkansas into 2L pots containing sunshine mix. The growth chamber was set for 12-h photoperiod, with day/night temperatures of 30/25 degrees 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. NP 321 treatments were applied as a foliar spray with a CO_2 backpack sprayer calibrated to deliver 10 gallons H_2O/acre. An adjuvant, Penetrator Plus at 0.05% v/v was used.

The NP 321 treatments were sprayed at the 7th true leaf and sampled 10 days later for the upper expanded main-stem leaf. Treatments were sprayed again at the 7th true leaf +10 days and sampled 5 days later for the upper expanded main-stem leaf and 10 days later for the leaf and squares. 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 first spray application and assessed at 72 and 96 hours following start of feeding for sampling taken after the second spray application.

Field Study
Studies were performed in the field at Clarkdale, Arkansas, where cotton (*Gossypium hirsutum L.*) cultivar 1218 BtRR was planted on May 6, 2002. The experimental design was randomized complete block with six replications. Fertilizer, weed and insect control measures were carried out according to Extension Service recommendations for Arkansas. Plots were furrow irrigated as needed throughout the season. NP 321 treatments were applied as a foliar spray with CO_2 backpack sprayer calibrated to deliver 10 gallons H_2O/acre. An adjuvant, Penetrator Plus at 0.05% v/v was used. Samples were taken for testing at 72 and 96 hours after application of NP 321, placed in small ziploc bags, placed on dry ice and immediately taken to the University of Arkansas Entomology Department for bollworm mortality tests.

Results

Yield Studies
According to results from yield data reported and averaged from 24 Mid-South consultant trials, NP 321 increased yield from a single mid-bloom application at both 5 and 10 once per acre rates when compared to the untreated control (Figure 1). However, there was not a rate response as indicated by the similar yield results between the 5 oz/acre and 10 oz/acre treatments.

According to results from yield data reported from 11 university trials, NP 321 increased yield from a single mid-bloom application at both 5 and 10 once per acre rates when compared to the untreated Bt control (Figure 2). Also, the 5 oz/acre rate of NP 321 had a greater yield advantage than the higher rate of NP321 at 10 oz/acre.

According to results from yield data reported from 11 university trials and 24 consultant trials, NP 321 increased yield from a single mid-bloom application at both 5 and 10 once per acre rates when compared to the untreated Bt control (Figure 3). Also, the 5 oz/acre rate of NP 321 had a greater yield advantage than the higher rate of NP321 at 10 oz/acre.

Endotoxin Level Studies
The increase of endotoxin levels, compared to the non-treated Bt control, following a single mid-bloom application of NP 321 is shown in Figure 4 (Testing done by Agdia). All three rates of NP 321 showed significant increases in the level of tissue endotoxin levels compared to the untreated Bt control. As you increased the foliar-applied rate of NP 321, the level of expressed endotoxin level was also increased. The greatest increase in endotoxin levels was observed in the leaf tissue. Lower levels of endotoxin were observed in the petioles and squares, however, the application of NP 321 still made for improvements in endotoxin concentrations levels.

The percentage increase in worm mortality from imposed neonates feeding on cotton squares at first bloom is shown in Figure 5. Bollworm mortality was evaluated at 72 and 96 hours after the initiation of feeding on small squares from plants previously treated with NP 321. All three rates of foliar-applied NP 321 showed increased mortality levels of neonate bollworms at both the 72 and 96 hour ratings compared to a Bt control. The 5 oz/acre rate of NP 321 showed the highest mortality of neonate worms feeding on young squares. This result may help to explain the yield advantage that was achieved across test locations when NP 321 was applied at the lower rate of 5 oz/acre. A non-Bt treatment was also included in the test which showed less than five percent mortality of neonates.

The percentage increase in worm mortality from imposed neonates feeding on cotton leaves at the seventh true leaf stage is shown in Figure 6. Compared to the nontreated Bt-control plants, all NP 321 treatment rates showed increased levels of neonate mortality from feeding on young leaves at all rating times. The level of neonate mortality increased with each successive increase in NP 321 foliar rate. The non-Bt check showed less than five percent mortality levels of neonate bollworms at all three observation timings.

The percentage increase in worm mortality from imposed neonates feeding on cotton squares sprayed at MHS is shown in Figure 7. At 72 hours after neonate feeding on small squares, there was very little difference among treatments for increasing mortality. However, by 96 hours after feeding there were noticeable increases in mortality where squares were treated with NP 321. This increase in mortality also appeared to be rate dependent with the higher NP 321 rates showing the greatest increases in bollworm mortality.

The percentage increase in worm mortality from imposed neonates feeding on cotton leaves sprayed at MHS is shown in Figure 8. At both 72 and 96 hours after feeding there were noticeable increases in mortality where leaves were treated with NP 321. This increase in mortality also appeared to be rate dependent with the higher NP 321 rates showing the greatest increases in bollworm mortality.

Conclusions

Applications of NP 321 in 2001-2002 exhibited

- Yield increases in both University and Consultant trials.
- An increase of Endotoxin levels for Bt cotton in leaves, petioles and squares as evidenced in Elisa testing performed by Agdia.
- An increase in bollworm mortality as displayed in feeding trials from growth chamber and field studies conducted by the University of Arkansas.

Acknowledgements

We would like to acknowledge the help and abilities of Dr. Randolph Luttrell and Dr. Ibriham Ali, University of Arkansas, for their work in the 2002 feeding trials.

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Plate 1. Freshly laid bollworm egg on cotton square.

Plate 2. Bollworm damage to square showing flared bracts and brownish excrement.

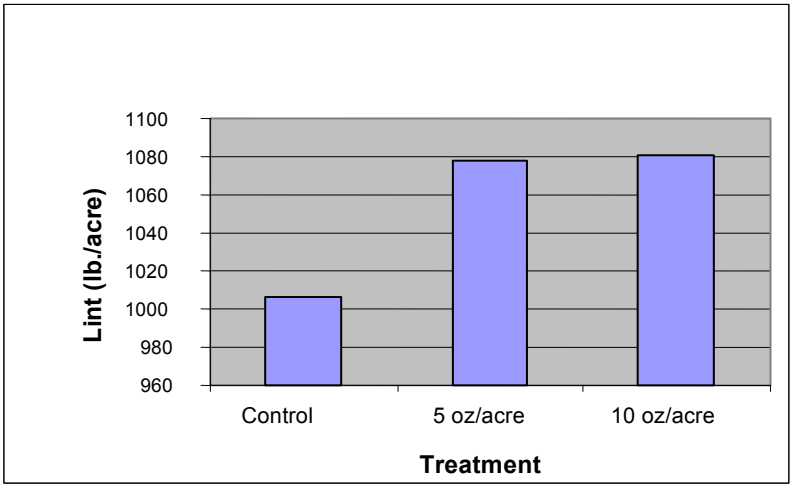

Figure 1. Mid-South Consultants yield data following NP 321 treatment for 2001-2002 crop years.

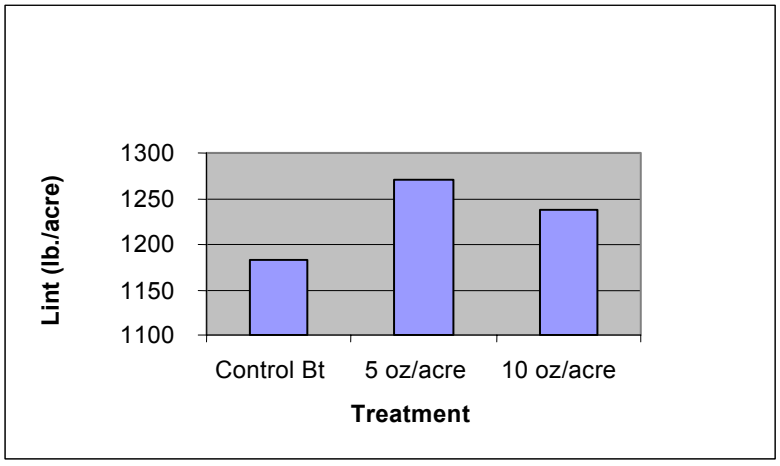

Figure 2. University yield data following NP 321 treatment for 2001-2002 crop years.

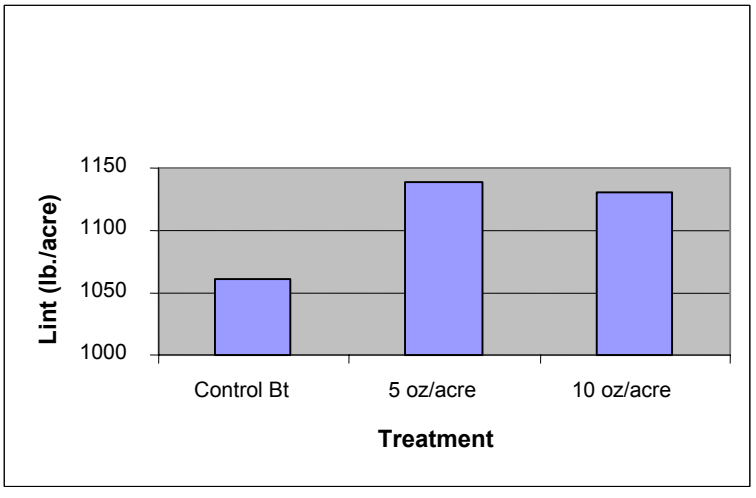

Figure 3. Yield summary for all 35 university and consultant trials following NP 321 treatment for 2001-2002 crop years.

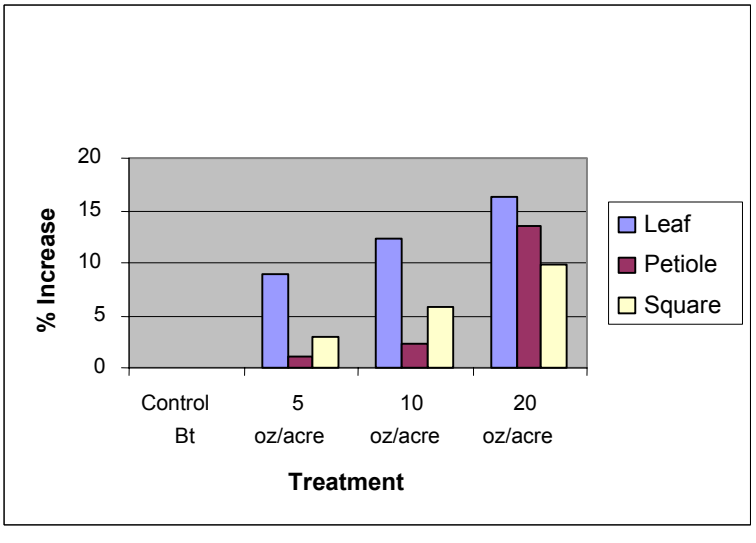

Figure 4. Percentage increase in plant endotoxin levels following foliar treatment with NP 321 in Bt cotton.

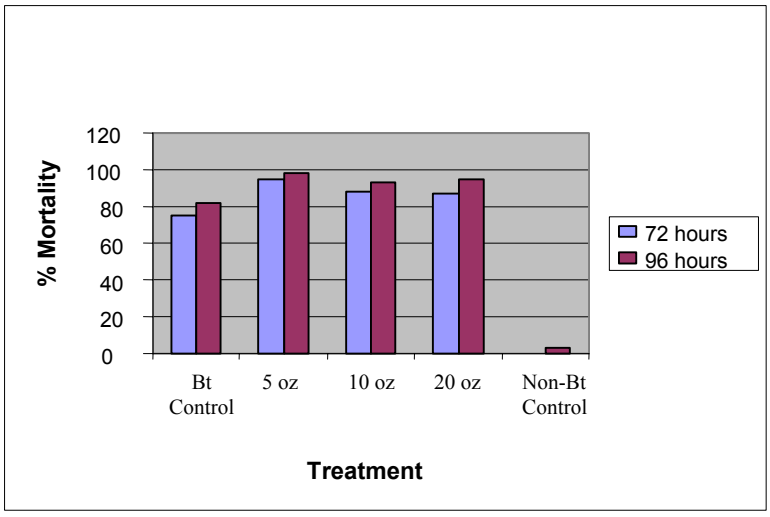

Figure 5. Mortality levels of neonate bollworms feeding on squares following NP 321 applications. Study was conducted in 2002 in Fayetteville, AR in a controlled growth chamber environment.

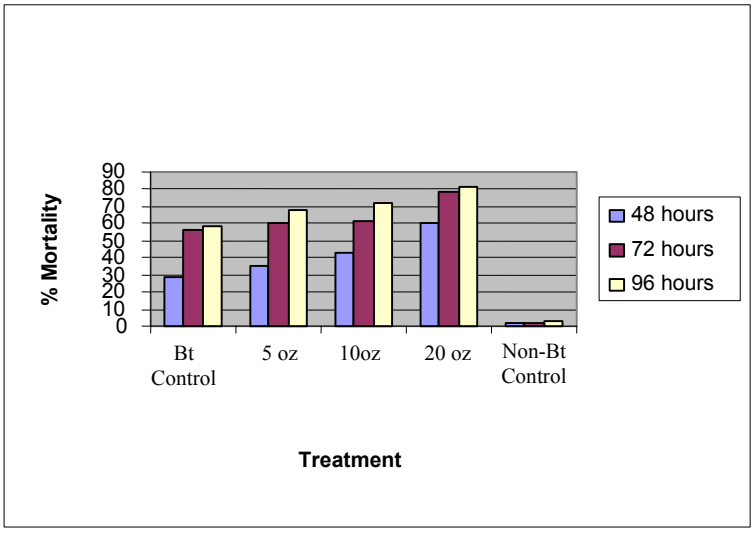

Figure 6. Mortality levels of neonate bollworms feeding on leaves following NP 321 applications. Study was conducted in 2002 in Fayetteville, AR in a controlled growth chamber environment.

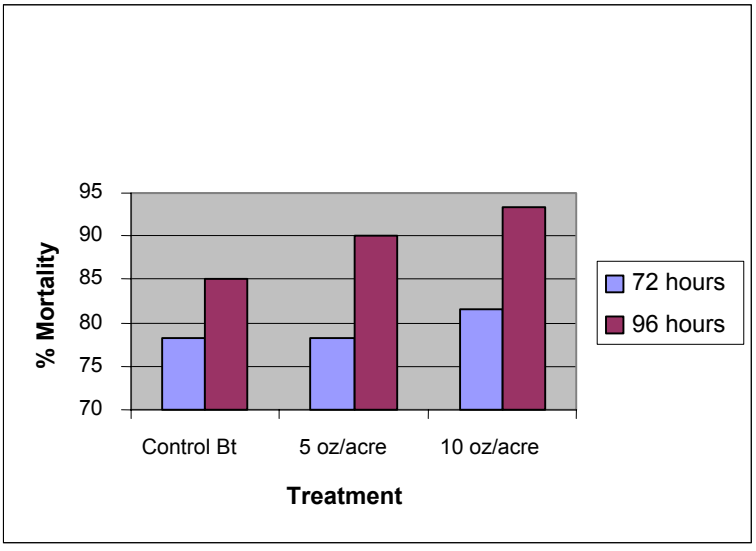

Figure 7. Mortality levels of neonate bollworms feeding on squares following NP 321 applications. Samples were obtained from the field trial at Clarkedale, Arkansas during the summer of 2002.

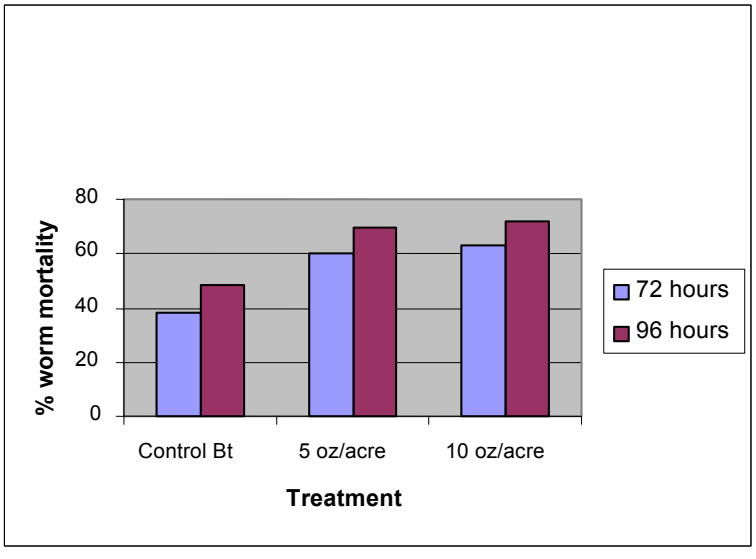

Figure 8. Mortality levels of neonate bollworms feeding on leaves following NP 321 applications. Samples were obtained from the field trial at Clarkedale, Arkansas during the summer of 2002.