AGRONOMY AND SOILS

6-Benzyladenine Enhancement of Cotton

John J. Burke*

ABSTRACT

The influence of applied plant growth regulators (PGR) on growth, development and yield in cotton (Gossypium hirsutum L. and Gossypium barbadense L.) has been studied for over half a century. Studies of PGR containing cytokinin alone or in combination with gibberellins applied at the pinhead square developmental stage have reported both positive and negative effects; however, a majority of the studies report no significant effects on cotton. The objective of this study was to evaluate the effects of a foliar application of a commercial formulation of cytokinin (6-benzyladenine) during the early stages of seedling development, long before the pinhead square stage. Greenhouse studies in 2004 to 2006 compared untreated controls with 6-benzyladenine treated seedlings at the two to four-leaf stage. Initial studies determined that 25 µmol/mol 6-benzyladenine was optimal at this developmental stage. Concentrations of 30, 40 and 50 µmol/mol resulted in phytotoxic lesions on the leaf surfaces. Seedlings treated with 25 µmol/mol 6-benzyladenine approximately two-weeks after planting exhibited increased hypocotyl diameters, increased lateral root proliferation, and a breaking of apical meristem dormancy within one-week of treatment. Samples taken later in development exhibited increased boll weights and total root lengths in treated plants compared with untreated controls. Studies of plant water usage and water stress responses showed less water usage and stress avoidance in the 6-benzyladenine treated plants. This study showed that application of 6-benzyladenine to cotton early in development has the potential to increase yields and reduce water stress in cotton.

The class of plant growth regulators known as cytokinins have been reported to influence many aspects of plant growth and development including germination, cell division, cell enlargement, cell and organ differentiation, apical dominance, photosynthesis, nutrient translocation, flowering, fruit set, fruit growth, and plant senescence (Weaver, 1972; Elliott, 1982). Early studies of cytokinins in cotton evaluated the identification of the types of cytokinins synthesized by the plant (Sandstedt, 1971; Sandstedt, 1974; Shindy et al., 1973; Taylor et al., 1974) because native cytokinins probably played a practical role in abscission and its prevention in developing fruit.

Cothren and Cotterman (1980) reported that a two-year study in Arkansas with the commercial cytokinin mixture Cytozyme Crop+ showed numerical trends toward increased yields, but these changes were not significant. Mayeux and Illum (1985) described cotton yield enhancement from foliar application of Burst Yield Booster (BYB, Burst Agritech, Overland Park, KS), a cytokinin product. Products that were successfully used evolved from pesticide screening programs, and were shown to be effective primarily in altering the stature of the plant, and thereby assisting in protecting crop yields or promoting ease in harvest for a higher percentage of harvestable yield. Reviews of years of research on cytokinin application to cotton have reported mixed results on yield enhancement with an overall assessment that the results are inconclusive on the effectiveness of this hormonal application (Guinn, 1986; Cothren and Oosterhuis, 2010). Mayeux et al. (1987) reported that BYB provided an increase in yield at some locations each year tested; however, the product had not shown yield increases at all locations in any one year. They felt that the lack of yield increases at some locations could be explained by weather or other factors that masked or overrode the benefit of the product to the cotton plant.

Hofmann and Else (1989) investigated the response of cotton to cytokinin containing products. Burst Yield Booster was applied in single and multiple applications at various growth stages. Evaluations were conducted at the University of Arizona’s Maricopa Agricultural Center. There were no measurable differences in plant heights or flower and boll production during the two years these measurements were taken. There were also no significant differences, or consistent trends in yield for any growing season the material was evaluated.
Hedin and McCarty (1994a) reported on a series of tests conducted from 1986 to 1992 where kinetin was evaluated using foliar sprays applied to cotton plants in an attempt to improve yield, agronomic traits, and content of allelochemicals in the bud. Tests were conducted using kinetin riboside, indoleacetic acid (IAA), and gibberellic acid (GA). The effects of these plant growth hormones were near zero over the six-year period, although, in some individual tests, significant differences in yield were obtained. No multi-year trends were evident. They concluded that overall, these growth hormones appeared to be marginally effective at best for increasing yields.

In a second study, Hedin and McCarty (1994b) reported the results of a study at Mississippi State during 1986-1992, in which five commercial plant growth regulators and urea were evaluated as foliar sprays on growing cotton plants for their effects on yield, agronomic traits, and pest allelochemicals. Of the five tested, the activities of three (Burst, Foliar Triggrr [Westbridge Chemical Co., San Diego, CA], and Maxon [Terra International, Blytheville, AR]) were attributed by their providers to cytokinins. FPG-5 Foliar (Baldridge Bio-Research, Inc., Cherry Fork, OH) contained cytokinins, IAA, GA, and several inorganic micronutrients, and PG-IV (Microflo, Lakeland, FL.) contained indolebutyric acid (IBA), GA, and micronutrients, but no cytokinins. They reported that FPG-5 and Foliar Triggrr caused significant increases in yield, but only in 1992. Burke (2009) saw similar yield increases in field studies of the commercial cytokinin, MaxCel (Valent BioSciences, Chicago, IL). Hedin and McCarty (1997) concluded that while they observed positive effects with various plant growth hormones and bioregulators in individual years, multi-year results were not significant. That same year Oosterhuis et al. (1997) found that Maxon (cytokinin) application during the 1996 season produced a significantly greater number of open bolls 7 weeks after first flower.

Finally, Bednarz and van Iersel (1998) showed that Stimulate and Early Harvest, products containing cytokinin, failed to show significant influence on photosynthesis or any associated parameters. The lack of a significant increase in leaf area, leaf area ratio, or shoot dry weight compared to the untreated control support this conclusion.

The majority of studies performed to date evaluated cytokinin foliar applications at the pinhead square stage or later. We hypothesized that the beneficial effects of cytokinin application would best be realized early in plant development before reproductive-induced changes in plant hormonal concentrations occurred. The objective of the present study was to evaluate the effects of a foliar application of a commercial formulation of cytokinin (6-benzyladenine) during the early stages of seedling development, long before the pinhead square stage, potentially enhancing the beneficial effects of cotton’s hormonal response.

**METHODS AND MATERIALS**

**Greenhouse Cultural Practices.** Sure-Grow 215 RR/BG (Delta Pine and Land Co.; Scott, MS) cotton seeds were planted into 16 cm diameter pots containing 900 g of Sunshine Mix #1 soil (Sun Gro Horticulture Distributors Inc., Bellevue, WA). Three seeds were planted per pot and pots were placed on benches in a greenhouse set to provide a 31/27°C day/night cycle. Plants were thinned to one plant per pot and grown throughout the year. 430 W high-pressure sodium lights (P. L. Light Systems, Beamsville. ON Canada) were used to maintain a 16/8 h photoperiod. Nutrients were maintained by daily application with Peters Excel fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH) through the automated watering system.

**Cytokinin Concentration Determination for Cotton Seedlings at the Two-Leaf Stage.** The experiment was arranged in a completely randomized design with five replications of six concentrations of 6-benzyladenine. The experiment was repeated three times to determine optimum concentration of cytokinin. When the Sure-Grow 215 RR/BG plants reached the two-leaf stage (Fig. 1, approximately 14 days after planting) they were sprayed with 0 (water), 10, 20, 30, 40, or 50 µmol/mol 6-benzyladenine (MaxCel, Valent BioSciences, Chicago, IL). A single pass sprayer equipped with an 8003EV fan-tipped spray nozzle located in a safety hood was used for the hormone application at 20 PSI. Plants were placed 30 cm beneath the spray nozzle and allowed to sit at room temperature for 1 hour following the hormone application prior to being returned to the glasshouse. Phenotypic responses of the plants were evaluated eight days after treatment. Based upon the findings of this initial study, all subsequent treatment groups received a single spray application of 25 µmol/mol 6-benzyladenine at the two-leaf stage.
Evaluation of Cytokinin-Induced Changes in Hypocotyl Diameter.

Seven days after treatment, cotton hypocotyl thicknesses were measured 2.54 cm above the soil surface using a 0-25 mm micrometer having 0.001 mm accuracy (Mitutoyo Corporation, Aurora, IL). Two experiments were arranged in a completely randomized design with five untreated (water) and five treated (25 µmol/mol 6-benzyladenine) seedlings evaluated. Mean hypocotyl diameters and standard deviations were determined. Significant differences between hypocotyl diameters at the 0.05 level were determined using a Least Square Mean Differences Student’s t test using JMP Version 5.0 statistical discovery software (SAS Inst., Cary, NC).

Cytokinin-Induced Breakage of Apical Dominance. Control cotton seedlings (water treatment) and treated cotton seedlings (25 µmol/mol 6-benzyladenine) were evaluated visually for breakage of apical dominance on the axillary meristems. Breakage of apical dominance was rated qualitatively by visually comparing leaf development in the axillary meristems of the cotyledons with that of the water treated seedlings. Seedlings were evaluated seven and twenty five days after treatment. Three replicate experiments were performed.

Evaluation of Cytokinin-Induced Changes in Root and Boll Development.

Control (water treatment) and treated (25 µmol/mol 6-benzyladenine) cotton plants were evaluated for the affect of hormone treatment on root and boll development. Two experiments were arranged in a completely randomized design with four control (water) and four 6-benzyladenine treated plants. One set of four plants were grown for 31 days in the greenhouse, terminated, and roots were washed. Roots were photographed and root length densities were determined according to the method of Tennant (1975). A second set of plants were grown for 70 days after planting and boll fresh weights were measured. Significant differences between root lengths were determined using a Least Square Mean Differences Student’s t test using JMP Version 5.0 statistical discovery software (SAS Inst., Cary, NC).

Method for Determining Whole Plant Transpiration: Three Sure-Grow 215 cotton seeds were planted in ten 1-gallon pots containing equal amounts of Sunshine® 3-Mix soil (Sun Gro Horticulture Canada Ltd, Bellevue, WA) based upon soil weight prior to watering. Each pot was well watered and placed in a greenhouse (Rainbow Plus, Stuppy Greenhouse, Kansas City, MO) set to maintain air temperatures at 32C/28C day/night temperatures. Seven days after planting, seedlings were thinned to one plant per pot, and pots were drenched with water and allowed to drain overnight. Seedlings in five pots selected at random were sprayed with 25 µmol/mol 6-benzyladenine eight days after planting. A single pass sprayer equipped with a Spraying Systems 8003 EVS fan-tipped spray nozzle located in a safety hood was used in the application of the hormone at 20 PSI. Seedlings were placed 30 cm beneath the spray nozzle. After hormone treatment, the pots were covered from both ends with 2 Mil poly bags (S-3478, Uline, Waukegan, IL), which are permeable to air but impermeable to water vapor (Xin et al., 2009). A slit was cut in the top bag to permit seedling growth. The slit was further sealed with a piece of clear adhesive tape and covered with a layer of dry potting mix to minimize water loss through the slit. Pots were weighed immediately, randomized in the greenhouse, and then weighed again 13 days after treatment when the first sign of leaf wilting was observed in one of the pots. The experiment was replicated twice and whole plant level transpiration was determined gravimetrically according to the procedure of Xin et al. (2009). Significant differences between treatments were determined using a Least Square Mean Differences Student’s t test using JMP Version 5.0 statistical discovery software (SAS Inst., Cary, NC).

RESULTS

Cytokinin Concentration Determination for Cotton Seedlings at the Two-Leaf Stage. Greenhouse studies in 2004 to 2006 compared untreated controls with 6-benzyladenine treated seedlings at the two to four-leaf stage (Fig. 1). Initial studies evaluated phenotypic responses of seedlings to foliar applied 0 (water), 10, 20, 30, 40, or 50 µmol/mol 6-benzyladenine approximately one week after treatment. Concentrations of 30, 40 and 50 µmol/mol resulted in phytotoxic lesions on the leaf surfaces (Fig. 2) that became more apparent as the applied concentration increased. Lower concentrations of 6-benzyladenine (10 and 20 µmol/mol) showed no apparent injury to leaf surfaces when compared with the water treated control.
Evaluation of Cytokinin-Induced Changes in Hypocotyl Diameter. The first observation of a response to the 25 µmol/mol 6-benzyladenine was an apparent thickening of the hypocotyl diameters (Fig. 3). The graph shows the measured hypocotyl diameter of the control and treated plants. The average hypocotyl diameter 2.54 cm above the soil surface of the control seedlings was 3.27 mm, while the average hypocotyl diameter of the treated seedlings was 4.38 mm diameter.

Cytokinin-Induced Breakage of Apical Dominance. The cytokinin-induced breaking of apical dominance was visible within 7 days after treatment (Fig. 3). The development of leaves in axillary buds of the cotyledons was enhanced in the treated plants. The rapid breaking of apical dominance in the treated plants provided an advantage in the rate of fruiting branch development. The cytokinin-treated plant shown in the right side of Fig. 4A exhibited greater branch development compared to the water control on the left.
Evaluation of Cytokinin-Induced Changes in Root and Boll Development. Root size of the control and cytokinin-treated cotton plants was evaluated approximately one month after planting. There was an increase in root development in cytokinin-treated cotton compared with controls (Fig. 5). A two-fold increase in average root length was measured in the cytokinin-treated cotton. This increase was significant at the 0.02-level using the Least Squares Means Differences Student’s t test. Boll weights from plants harvested for root evaluations showed average boll weights per plant of 196 g for controls and 264 g for cytokinin-treated plants (Fig. 6).

Cytokinin-Induced Changes in Whole Plant Transpiration. The increase in root length and hypocotyl thickness suggested an improved water harvesting and transport system in the cytokinin-treated cotton. The impact of the morphological changes on plant performance was evaluated under controlled conditions in greenhouse studies. The amount of water lost by each plant was determined according to the procedure of Xin et al. (2009) at the first sign of any plant wilting (Fig. 7). Relative stress levels were evaluated according to the procedure of Burke (2007) before and at the first sign
Foliar application of plant cytokinins to enhance cotton yield and development has provided sometimes promising, yet inconsistent results (Guinn, 1986; Cothren and Oosterhuis, 2010). The vast majority of these studies applied the hormone at the pinhead square stage or later under field conditions. The consensus in the literature is that the lack of yield increases at some locations could be explained by weather or other factors that masked or overrode the benefit of the product to the cotton plant. Our investigation of the cytokinin enhancement of cotton was conducted under controlled environmental conditions and treatments were applied early in plant development before reproductive-induced changes in plant hormonal concentrations could occur.

Our observations of increased hypocotyl diameter within days of cytokinin treatment were not entirely surprising. Suttle (1986) reported the cytokinin-induced ethylene biosynthesis in non-senescent cotton leaves, and ethylene previously had been shown to promote an increase in cotton hypocotyl diameter (Goeschl and Kays, 1975). Suttle (1986) reported that treatment of intact cotton (cv LG 102) seedlings with both natural and synthetic cytokinins resulted in an increase in ethylene production by excised leaves. The effectiveness of the cytokinins tested was thidiazuron >> benzyladenine >> isopentyladenine 2 zeatin >> kinetin. Our study used 6-benzyl adenine as it was the second most effective cytokinin previously tested, and it was available in a commercial form for foliage applications.
Cytokinins have long been known to stimulate the growth of lateral buds and thus suppress apical dominance (Catalano and Hill, 1969; Sachs and Thimann, 1964). Our results showed a rapid response to the 6-benzyladenine treatments in cotton, with apical buds breaking and new leaves and branches appearing within a week of treatment. Cytokinins are now known to influence the production and localization of lateral roots on excised root segments. Using pea (Pisum sativum L.) root segments, Torrey (1962) showed that low kinetin concentrations (5 X 10^{-8} M) slightly stimulated lateral root initiation, but at higher concentrations, kinetin was inhibitory. We also found increased lateral root production in our investigation with a two-fold increase in overall root length within three week of cytokinin treatment. The increased root length promoted exploration of a greater soil volume and potentially, access to more available soil water. This may in part explain our finding of a lower level of water stress in the cytokinin treated plants than in the controls.

Another possible explanation for the observed results is that cytokinins are also known to influence genes associated with cuticular wax formation. Xia et al. (1997) showed that application of the cytokinin 6-benzylaminopurine induced ectopic expression of CER2-GUS in all cell types of leaves that emerge following treatment. The CER2 gene is required for normal accumulation of cuticular waxes. Although not measured directly in the current study, our cytokinin treatments may have increased cuticular wax on the leaves, as less water was lost from the pots of treated seedlings than from untreated controls. We also observed a slower desiccation of leaves from the cytokinin-treated plants when seedlings were removed from the soil (data not shown). Hedin et al. (1994b) reported that FPG-5 and Foliar Triggrr caused significant yield increases, but only in 1992. If you look at the average May rainfall for Mississippi State, Mississippi during the years of this study (http://weather-warehouse.com/WeatherHistory/PastWeatherData_StateUniversity_MississippiState_MS_April.html), the overall precipitation in May was 5.4 inches per year. In 1992, however, only 1.28 inches of precipitation occurred in May. It is interesting to speculate that they only saw a cytokinin benefit in 1992 because of the improved morphological attributes of a cytokinin-treated plant for water uptake and loss prevention from the leaves.

The benefits of water stress avoidance on cotton growth have been previously reported (Burke et al., 1985). The severity of the soil water deficit was analyzed by monitoring changes in growth parameters of photoperiodic cotton strains T185 and T25 at 106 DAP. All parameters exhibited dramatic declines under stress conditions, however, plant height decreased 65% between irrigated and dryland treatments of T25, while T185 showed an 80% reduction in plant height. The magnitude of the reduction in plant height, leaf area index, plant dry weight, and leaf number between irrigated and dryland was greater in T185 than T25 treatments. T25 previously was identified as a drought resistant cotton line exhibiting enhanced stress avoidance because of elevated lateral root production (Quisenberry et al., 1981). Significant correlations between root characteristics and dry-matter yield under dryland conditions suggested that overall root vigor allowed T25 to be a better competitor for limited soil water. They concluded that root morphology and root growth potentials appear to be important for the adaptation of cotton to conditions where limited soil-water availability is a major growth constraint. In the present study, cytokinin resulted in a two-fold enhancement of lateral root development, and the treated plants exhibited reduced water-deficit stress levels.

The present study evaluated the effects of a foliar application of a commercial formulation of cytokinin (6-benzyladenine) during the early stages of seedling development, prior to the pinhead square stage, on the growth response of the cotton plant. The increased hypocotyl diameter, breaking of apical dominance, and increased lateral root formation are classic cytokinin responses in plants. The increased speed of lateral branch formations in treated plants also resulted in enhanced boll development. The time to first flower was reduced in the treated plants (data not shown), and bolls were able to more fully develop compared to control plants. This is supported by increased boll weights per plant that we observed in this study. The findings of this study suggest that cytokinin treatment of young cotton seedlings may enhance overall performance and yield, especially in water-limited environments.

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REFERENCES


