Cotton Response to High Temperature Stress During Reproductive Development

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COTTON RESPONSE TO HIGH TEMPERATURE STRESS DURING REPRODUCTIVE DEVELOPMENT
COTTON RESPONSE TO HIGH TEMPERATURE STRESS DURING REPRODUCTIVE DEVELOPMENT

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Crop Soil and Environmental Science

By

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ABSTRACT

Temperature is a primary controller of the rate of plant growth, developmental events, and fruit maturation. Increased temperatures from global climate change are projected to cause substantial losses in crop productivity by the end of the twenty-first century. Elevated temperatures affect all stages of cotton development, but the crop seems to be particularly sensitive to adverse temperatures during reproductive development. In Arkansas, temperature stress is considered to be one of the main factors affecting cotton yield. Environmental stress during floral development is a major reason for the disparity between actual and potential yields. Field and growth chamber studies were conducted with the objectives of investigating the effects (1) of high temperature stress during flowering and early boll development on early seed growth, (2) of foliar-applied 1-Methylcyclopropene (1-MCP) on the growth and yield of field grown cotton, and (3) investigate the amelioration of high temperature stress in cotton flowers and young cotton fruit using 1-MCP. In growth room studies high day temperature (38°C) compared to the control temperature (32°C) resulted in increased glutathione reductase (GR) activity and decreased ovary carbohydrate concentrations. In field studies GR activity, calcium and carbohydrate concentrations of ovaries and leaves were not significantly affected by applications of 1-MCP. Yield parameters of lint, seed, and seedcotton were also not affected by 1-MCP applications in Marianna, whereas in Fayetteville yield was significantly increased. The increases in yield in Fayetteville were attributed to higher temperatures during the reproductive period when the 1-MCP applications were made. Overall the studies show that foliar applied 1-MCP may potentially help to ameliorate the effects of high temperature on cotton, but may also exhibit no effect or a negative effect on non-stressed cotton.
This thesis is approved for recommendation to the Graduate Council.

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DEDICATION

CHAPTER II. Effects of 1-MCP on the Physiology and Yield of Field-Grown Cotton

Abstract

Introduction

Materials and Methods

Results

Discussion

References

INTERPRETATIVE SUMMARY

APPENDIX I – CHAPTER I

APPENDIX II – CHAPTER II
INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is a major world crop grown for the production of fiber, fuel, and feed. Cotton is reputed to be the most complicated row crop due to its perennial nature, indeterminate growth habit and sympodial fruiting pattern (Mauney, 1986). Cotton cultivars used in today’s agriculture have become more dependent on the grower to provide the water and nutrients needed for growth and development. This dependency has created sensitivity to adverse environmental conditions. The U.S. cotton crop has shown extreme and unpredictable year-to-year variability in yields, which has been attributed to genetics, management practices, and unfavorable weather conditions (Lewis et al., 2000; Robertson, 2001), with high temperatures considered to be the main environmental factor contributing to variable yields (Oosterhuis, 1994). This is especially true for cotton in the Mississippi River Delta. These crops show great yield potential during mid-season, but as environmental constraints become more prevalent during flowering and boll development, the yield potential decreases.

Although cotton originated in hot climates, it does not yield best at excessively high temperatures (Oosterhuis, 2002). The optimum temperature for cotton growth is reported to be between 20 to 30°C (Reddy et al., 1991). In the Mississippi River Delta, these optimum temperatures are usually exceeded daily during the flowering and boll development, thus reducing reproductive efficiency (Bibi et al., 2008). Higher temperatures affect all stages of growth and development of cotton, but the crop sensitivity to adverse temperatures seems to increase during reproductive development. Excessively high temperatures can decrease seed size, fibers per seed, and fiber length (Oosterhuis, 1999). High temperatures can also lead to decreased pollen viability and reduced fertilization efficiency (Snider et al., 2009). A major reason for the disparity between potential and actual yields is attributed to environmental stress during floral
development, yet there is a lack of information on the physiological effects of high temperatures during the flowering process.

Growers have become accustomed to using chemicals to ameliorate stresses caused by plant diseases, insects, and weeds. There are also chemicals that may be effective at alleviating high temperature stress, specifically 1-Methylcyclopropene (1-MCP). This chemical is already widely used in horticulture to successfully prolong the shelf life of climacteric fruits, and there is some evidence for preventing boll-shedding in cotton (Kawakami et al., 2006). The synthetic plant growth regulator 1-MCP works by inhibiting the plant stress hormone ethylene, the levels of which increase during plant stress and can cause fruit shed, pollen sterility, or poor fertilization. Preliminary work has indicated that 1-MCP may be able to decrease the severity of high temperature stress on cotton (Storch, 2010). However, this has not been positively demonstrated.

**HYPOTHESIS AND OBJECTIVES**

It is hypothesized that high temperature stress will detrimentally affect fertilization and early seed development in cotton, and secondly, that the application of 1-MCP will partially ameliorate the detrimental effect of high temperature stress on reproductive growth.

The general objective is to document the response of cotton reproductive structures (fertilization and early seed development) to high temperature stress, and to investigate possible methods of amelioration of the stress condition so as to sustain yield potential. The specific objectives are:
1. To quantify the effect of high temperature stress during flowering and early boll development on early seed growth.

2. To study the effect of foliar-applied 1-Methylcyclopropene (1-MCP) on the growth and yield of field grown cotton.

3. To investigate the amelioration of high temperature stress in cotton flowers and young cotton fruit using 1-MCP.

These studies will involve both field and growth chamber environments. It is hoped that from this project we will be able to better explain how environmental high temperature stress during the critical flowering period affects yield, and also formulate strategies to ameliorate the stress and protect potential yield.

LITERATURE REVIEW

History of Cotton

Cotton (Gossypium hirsutum L.) is a major industrial crop. It is not known exactly how long cotton has been cultivated, but scientists have found bits of cotton bolls and pieces of cotton cloth in caves in Mexico that proved to be at least 7,000 years old (Anonymous, 2010). The industrial revolution in England and the invention of the cotton gin in the U.S. paved the way for the important place cotton holds in the world today (Anonymous, 2010). Cotton is used more than any other textile fiber produced. All parts of the cotton plant are considered to be useful; the fiber is used to make cloth, cottonseed is crushed in order to make oil, meal, and feed and the remainder of the plants such as stalks, cotton burrs and leaves are plowed under to enrich the soil.
There are four main cultivated species of cotton in the world of which two: *Gossypium barbadense* L. (known as Pima) and *Gossypium hirsutum* L. (known as Upland cotton) are grown commercially in the USA. Pima cotton has longer fibers and is referred to as extra-long staple, while Upland cultivars have shorter fiber and are known as short staple. Upland cotton cultivars are grown in the Southeast (Georgia, North Carolina, South Carolina, and Virginia), the Mississippi Delta (Arkansas, Louisiana, Mississippi, Missouri, and Tennessee), the Southwest (Kansas, Oklahoma, and Texas), and the West (Arizona, California, and New Mexico). Pima cotton cultivars are grown in Arizona, California, New Mexico, and West Texas.

**Overview of Stress**

There are many different types of stress that can affect crop growth and yield. Stresses may be biotic or abiotic. Common abiotic stresses include soil acidity, mineral deficiency, drought, and heat stress. Any single stress can affect crop growth and yield depending on the duration and severity of the stress. However, stresses rarely occur alone and are often interconnected. The major stresses affecting row crop agriculture in the US Cotton Belt are nutrient stress, drought and extreme temperatures.

Nutrients are essential for plants to function and grow normally. However, deficiencies do occur, which decrease growth and yield. Nutrient availability is affected by soil pH. Cotton prefers a pH of 6.0 to 6.5, but soils are often too acidic. There are four major causes for soil becoming acidic; rainfall and leaching, acidic parent material, organic matter decay, and harvest of high yielding crops (Johnson, 1992). Acidic soils have a low pH that causes elements such as aluminum and manganese to become toxic which leads to poor crop growth (Johnson, 1992). Higher pHs of 5.5 to 6.5 allow for more nutrient availability to the crops. If a soil is acidic, an
application of lime will help to raise the pH to a desired level. Soil nutrient status can be determined using a simple soil test before planting, and fertilizer applied accordingly. If a deficiency is detected during the growing season, a foliar application can usually be applied to sustain the plants throughout the rest of the growing season. The longer it takes to detect a deficiency the more detrimental it can be to crop growth and yield.

A reduced yield associated with drought stress is a major problem in the world as many agriculture areas do not receive, adequate or timely rainfall. Many producers in the US have some type of irrigation provides the needed water requirements, but there are still large agriculture areas that depend solely on rainfall for the water needed for their crops. Drought stress has resulted in total yield losses on millions of hectares in the world each growing season. Producers who have irrigation are still affected by drought stress because they frequently cannot keep up with the plant’s water requirements either physically or economically without supplementary rainfall. Plants develop water deficits when demand exceeds the supply of water. Water deficit causes stomata to close and reduce transpiration, which also reduces CO₂ intake and photosynthesis. In addition, leaf temperatures rise as evaporative cooling ceases, which can lead to leaf damage and to an increase in leaf senescence (Gardner et al., 1985). As a response to desiccation the growth hormone abscisic acid (ABA) is produced, which can cause arrested growth and reproductive failure (Gardner et al., 1985).

Heat stress occurs when temperatures are high enough to detrimentally affect growth and may cause irreversible damage to plant functions and development. High temperatures can lead to plant water-deficit stress because the evaporation rate tends to increase with high temperatures. The reproductive development of many crops can be damaged because they may not produce flowers or the flowers that are produced may not set seed or fruit (Hall, 2004). This
is a serious problem as the seeds or fruit are the harvested components. Heat stress should be a big concern in agriculture with global warming causing climate changes to warmer environments and shifts in rainfall patterns. Of all the stresses, heat stress appears to impose the greatest risk to successful crop production because of global warming and climate change (Parry, 1992).

**Heat Stress**

Global warming trends over the last 50 years show a 0.13°C increase per decade (Craufurd and Wheeler, 2009). The current projection of global temperature shows an increase of 4.0°C by the end of this century (Craufurd and Wheeler, 2009). Currently high temperatures limit growth and development processes in much of the cotton producing areas (Reddy et al., 2002). Change to warmer climates in the future can shorten all development stages and change crop suitability areas (Craufurd and Wheeler, 2009). Projections of the future climate changes show a nine percent decrease in cotton yield by the middle or latter part of the 21st century (Reddy et al., 2002). While crop production practices will adapt with global warming, such as earlier planting dates, practices may be limited by availability of radiation in non-summer periods (Reddy et al., 2002).

**Vegetative and Reproductive Growth**

Cotton has a predictable development pattern which can be affected by temperature (Oosterhuis et al., 2002). Cotton’s main-stem apex continuously initiates axillary buds and leaves, where lower axillary buds usually develop vegetative branches and main-stem nodes five and higher develops fruiting branches (Reddy et al., 1997; Oosterhuis and Jernstedt, 1999). One of the most important variables to the growth and development processes of cotton is
temperature (Hodges et al., 1993). Fruiting branches increase rapidly with an increase in temperatures while vegetative branches increase in cooler temperatures (Reddy et al., 1992). An explanation for more vegetative branches developing under cooler temperatures is that an accumulation of metabolites occur when growth and development of the plants is slowed allowing for more vegetative branches to develop (Reddy et al., 1992).

Growth of plants accelerates as temperatures increase, thus allowing plants to reach maturity earlier (Reddy et al., 1996). This will give less time for the bolls to develop and reach their genetic potential size (Reddy et al., 1996). High temperatures also result in insufficient carbohydrate production which causes boll shedding, malformed bolls, smaller bolls, decreased lint, and lower yields (Oosterhuis, 1999). If the temperature increase is distributed equally throughout the growing season, it could shorten cotton development from emergence to maturity by as much as 24 days (Reddy et al., 1996). Every 1°C average rise in air temperature during the growing season could potentially lead to a 17% decrease in yields of crops (Lobell et al., 2003).

Reproductive growth is visible at about four weeks after planting in the form of floral buds (pinhead squares) in the apex of the plant, but microscopic squares are actually present just a few weeks after planting (Oosterhuis et al., 2002; Mauney, 1986). Although cotton starts reproductive growth at this time, it still continues vegetative growth throughout the season, but too much vegetative growth can cause excess shading and excessive fruit shedding (Oosterhuis et al., 2002). Since vegetative growth is favored by cool temperatures, and temperatures tend to increase during the growing season, excessive vegetative growth is not a main cause of yield reduction. Furthermore, the use of a growth retardant (mepiquat chloride) controls excessive vegetative growth. About three weeks after visible squares are evident, flowers will start to
appear (Oosterhuis and Jernstedt, 1999). During the critical period for the plants, pollination and fertilization occur in this stage and is necessary for successful seed set and subsequent boll development (Stewart et al., 1993). This stage of development is particularly sensitive to high temperature stress (Snider et al., 2009; 2010; 2011) which can lead to decreased components of yield, boll numbers and boll weight (due to lower seed number) and poor fiber quality.

**Fertilization**

High temperatures in the midsouth of the US Cotton Belt occur during the flowering period in the months of July to August (Oosterhuis, 2002). It is crucial to limit stress at this development stage in order to optimize yields. A major disparity between actual and potential yields in crops with valuable reproductive structures is due to environmental stresses during floral development (Boyer, 1982). The maximum daily temperature that cotton experiences during flowering often exceeds the optimal temperatures needed for successful pollen tube growth (Snider et al., 2009). There is a strong correlation between maximum pollen tube growth and boll retention (Liu et al., 2006). Pollen grains act as independent functional units once they are released from anthers making them more susceptible to damage from high temperatures (Kakani et al., 2005). Thus high temperature damage during anthesis can result in poor fertilization, which leads to decreased seed numbers and fewer bolls (Kakani et al., 2005). With high temperature damage to pollen and pollen tube growth there is a decrease in the amount of fertilized ovules, which leads to lower yield (Snider et al., 2009).
Square and Boll Shedding

The shedding of squares and bolls is a natural occurrence in cotton when adverse environmental conditions are experienced (Oosterhuis, 1990). However, the concern is when excess shedding occurs as can be caused by environmental stress, such as high temperatures, or drought, or insect damage in particular.

Boll shedding has been linked to the boll load of the cotton plant (Guinn, 1982). Some producers believe it is a good thing to have boll shedding so the plant can optimize its fruit load with available nutrients (Oosterhuis, 1990). While it is true that some boll shedding can be beneficial to crops yield, excessively high temperatures cause increased shedding (Reddy et al., 1992). In the Mississippi River Delta producers often experience great yield potential mid way through the season, but as the temperatures rise during flowering and boll development the producers experience decreased yield potential due to reduced boll numbers and boll size. This is due to temperatures reaching well above 35°C on a daily average (Reddy et al., 1992). Brown and Zeiher (1995) reported that high temperatures significantly decreased boll size and seed number, with fruit retention being the most severely affected.

Physiological Effects

Photosynthesis is defined as the process by which green plants, algae, diatoms, and certain forms of bacteria make carbohydrates from carbon dioxide and water in the presence of chlorophyll, using energy captured from sunlight by chlorophyll, and releasing excess oxygen as a byproduct (Gardner et al., 1985). The process of photosynthesis is considered to be central to plant survival, but extreme environmental conditions can disrupt the photosynthesis process. Stress conditions that can negatively affect photosynthesis include: high light intensity,
temperature extremes, low water availability, and low carbon dioxide conditions. One of the most important factors limiting photosynthesis is temperature extremes (Salvucci et al., 2004). Bibi et al. (2008) stated the upper threshold temperature for decreased photosynthesis in cotton was 35°C.

Photosynthesis can be completely restrained by high temperature before the detection of other stress symptoms (Berry et al., 1980). There are several components of the photosynthetic apparatus and associated metabolic processes that are sensitive to heat (Law, 1999). High temperatures inhibit photosynthetic CO₂ fixation and damage photosynthetic electron transport, particularly at the site of photosystem II (PSII) in the thylakoid membranes (Berry et al., 1980). Many reports show decreases in photosynthesis could develop from suppression of the PSII function, which has been shown to be the most thermally unstable component of the electron transport chain (Quinn et al., 1985; Havaux et al., 1996).

Inhibition of the PSII system has been shown to result in increased chlorophyll fluorescence (Krause et al., 1991). Thus, chlorophyll fluorescence can be used to detect and even quantify temperature induced changes in the photosynthesis mechanism (Krause et al., 1991; Govindjee, 1995; Strasser, 1997). Camejo et al. (2005) observed that high temperatures reduce the maximum fluorescence ratio and fluorescence quantum yield (φPSII) of tomatoes (Lycopersicon esculentum L.) indicating that the photosynthetic efficiency of PSII had been severely decreased. Decreased photosynthetic efficiency of plants due to high temperatures has been reported for St. John’s wort (Hypericum perforatum L.) (Zoybayed et al., 2005), cotton (Gossypium hirsutum L.) (Downtown et al., 1972; Reddy et al., 1991; Burke et al., 1998; Bibi et al., 2008), potato (Solanum tuberosum L.) (Havaux, 1993; Havaux et al., 1996), maize (Zea mays L.) (Crafts-Brandner et al., 2002) and several other plant species.
High temperatures during the vegetative stage can destroy components of leaf photosynthesis, reducing CO₂ assimilation rates (Hall, 2004). Jiao et al. (1996) reported that assimilate export from leaves is also inhibited by high temperatures. Weis (1981) reported that light-dependent activation of the enzyme Rubisco in spinach (Spinacia oleracea) chloroplasts was inhibited by moderately elevated temperatures and the inhibition was closely correlated with reversible inhibition of CO₂ fixation. The enzyme Rubisco activase regulates the activation of ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) in the light (Portis, 1992; Andrews et al., 1995; Salvucci et al., 1996). A similar effect of temperature on Rubisco activation and CO₂ fixation was reported for wheat leaves (Triticum aestivum L.) (Kobza et al., 1987).

An essential role for Rubisco activase in maintaining the active state of Rubisco in the light at levels that are adequate for photosynthesis have been reported in numerous studies (Portis et al., 1986; Salvucci et al., 1986; Mate et al., 1993; Eckhardt et al., 1997). Isolated Rubisco activase is particularly sensitive to inactivation by elevated temperatures (Robinson et al., 1989; Holbrook et al., 1991; Crafts-Brandner et al., 1997). Therefore, inactivation of Rubisco activase provides a potential biochemical explanation for the inactivation of Rubisco at elevated temperatures (Weis, 1981; Kobza et al., 1987).

High day and high night temperatures increase respiration and photorespiration with an additional loss in carbohydrates (Krieg, 1986; Ludwig et al., 1965; Guinn, 1974). When high temperatures persist, they are detrimental to plant growth because plants are induced to respire at an increased rate (Arevalo et al., 2004; Oosterhuis, 2002). Rapidly respiring plants use carbohydrates for respiratory energy instead of filling developing bolls (Loka, 2008). Increasing temperature adversely affects the plants ability to gain carbohydrates (Cothren, 1999). Overall, high temperatures result in an inability to produce enough carbohydrates to fulfill all the plants
needs. The limited amount of carbohydrates can be reflected by increased boll shedding, malformed bolls, smaller boll size, decreased lint percentage, and lower yields (Oosterhuis, 1999).

Although cotton is more heat-tolerant than many C3 plants, excessively high temperatures increase square and boll shedding and decrease yield (Oosterhuis, 1997). The most significant factors affecting boll retention or shedding, however, are the magnitude and the duration of exposure to high temperature (Reddy et al., 1992). This is particularly important as high temperatures normally occur during peak boll development in the Mississippi River Delta. Cotton fibers are composed primarily of carbohydrates (Constable and Oosterhuis, 2010). Under normal conditions, a cotton seed produces about 12,000-15,000 fibers (Oosterhuis, 1997). Therefore, when carbohydrate supplies are reduced, fiber weight per seed is reduced and ultimately yield is reduced (Arevalo et al., 2004).

Environmental stresses during floral development are thought to cause the disparity in actual and potential yields (Boyer, 1982). Weather conditions affect ovule development, pollen fertility, and pollen dispersal (Powell, 1969; Stewart, 1986). Pollen grains are more inclined to damage from high temperatures (Kakani et al., 2005). Thus high temperature damage during anthesis can result in lack of fertilization, which leads to decreased seed numbers and fewer bolls (Kakani et al., 2005). The number of seeds per boll is a major component of yield and fiber quality, and is a function of the number of locules (carpels) per boll and the number of ovules per locule (Stewart, 1986). Variation in seeds per boll is the result of either the lack of seed fertilization or post-fertilization termination of embryo growth, and both cultivar and environment contribute to the variation in the number of seed per boll (Stewart, 1986; Turner et al., 1977).
Overall, high temperatures affect many of the physiological functions necessary for survival and yield production. Without an understanding of these functions, it would not be possible to improve the yield and quality of row crops. With a clear understanding of how high temperatures affect row crops, researchers can begin to understand the best ways to ameliorate this heat stress.

**Amelioration of Heat Stress**

There are several options for possible to amelioration of heat stress on row crops. These options include selecting heat tolerant cultivars, irrigation, mulching, and agrochemicals. However, with all these options there is no perfect method for ameliorating heat stress completely.

Plant breeders are becoming more aware of the importance of heat tolerance in cotton. However improvements to cotton cultivars through plant breeding have been hindered by many traits, such as lint yield, fiber properties, and insect resistance, as these traits are quantively inherited (Bauer, 1994). However, it has been reported that public breeders have dramatically improved yields in Pima cotton (*G. barbadense* L.) by increasing high temperature tolerance (Kittock et al., 1988). Heat tolerant cultivars of cotton have been developed by screening important traits and physiological properties, such as the height of a plant at which a substantial number of bolls begin setting (Feaster and Turcotte, 1985), boll weight, and boll retention during reproductive development stage (Brown and Zeiher, 1998), as well as stomatal conductance (Radin et al., 1994; Lu et al., 1998), and cellular membrane thermostability (Rahman et al., 2004). Although screening for heat tolerance in cotton for the breeding of improved cultivars is a positive step towards the amelioration of heat stress, it is still an ongoing process.
Another option for alleviating the effects of heat stress in cotton crops is to use irrigation. The predominant methods of supplemental water to cotton are furrow and overhead sprinkler irrigation (Bauer, 1994). To alleviate the effects of heat stress, the overhead sprinkler irrigation system is the more applicable solution as it also cools the canopy through evaporation from the leaves. One of the most efficient physical methods to alleviate heat stress is to sprinkle water to cool the plant canopy (Chesness et al., 1979). Sprinkler irrigation to reduce heat stress has been studied in several vegetable crops such as watercress (*Nasturtium officinale*) (McHugh and Nishimoto, 1980), tomato (*Lycopersicon esculentum*) (Carolus, 1971), bean (*Phaseolus lunatus*) (Krogman and Hobs, 1973), muskmelon (*Cucumis melo*) and cucumber (*Cucumis sativus*) (Bible et al., 1968). In an experiment conducted by Jenni et al. (2008), it was found that sprinkler irrigation applied to endive when ambient air temperatures were greater than 28°C resulted in temperature reductions of 2.9 to 11°C. While sprinkler irrigation shows great results for alleviating heat stress, this is not an economical solution. In the U.S. Cotton Belt, temperatures reach levels above 35°C on a daily average during reproductive development (Reddy et al., 1991; Boykin et al., 1995). This would require daily irrigation to alleviate the heat stress and would result in excess water and deleterious effects. Also with the rising cost of fuel, producers would not be able to endure the added cost of production.

Of the different options for alleviating heat stress, agrochemicals appear to provide the best option. The use of agrochemicals in crops has become a common practice around the world to control weeds, insects, and to regulate plant growth. If agrochemicals could effectively reduce heat stress, they would provide a more cost effective option than the use of irrigation. Most agrochemical applications are able to be scheduled further apart, unlike irrigation that would need to be done daily to maintain reduced heat stress. Also producers already have applicator
systems or hire private applicators to apply the wide array of agrochemicals used in production. This would allow for a simple solution to deal with heat stress.

As previously mentioned the use of agrochemicals might be a simple and efficient way to alleviate heat stress. In cotton, agrochemicals such as plant growth regulators are used to affect the physiological processes of the plants (Bauer, 1994). A common plant growth regulator used in cotton is mepiquat chloride. Mepiquat chloride reduces leaf expansion and shortens internodes (Bauer, 1994). Another plant growth regulator being researched for cotton is 1-Methylcyclopropene (1-MCP). This compound blocks the action of the stress hormone ethylene, and thereby alleviates stress. If ethylene in plants is increased under high temperature stress, then application of 1-MCP would provide an economical and practical means of alleviating the detrimental effects of heat. This agrochemical is already widely used in horticulture, so this would be a rather inexpensive application for the producer.

**Conclusion**

In cotton significant factors affecting boll retention or shedding are linked to the duration of exposure to high temperatures (Reddy et al., 1992), thus decreasing yield. The process of fruit abscission is mainly triggered by ethylene, ethylene is a plant growth regulator usually produced under stress conditions, such as drought and high temperature. Therefore, 1-MCP could be an economical tool in the control of fruit abscission, through its function of inhibiting the action of ethylene. There is also some evidence for preventing boll shedding (Kawakami et al., 2006) and some evidence of decreasing the severity of heat stress in cotton with the use of 1-MCP (Storch, 2010). As a result of this project we expect to understand the physiological and yield effects of 1-MCP on cotton plants.
REFERENCES


CHAPTER I

Physiological Effects of 1-Methylcyclopropene on Cotton Flowers under Normal and High Temperatures

ABSTRACT

With global warming, the realization of increased high temperature stress in crops has become a major factor affecting crop growth and yield. Cotton (Gossypium hirsutum L.) is affected at all stages of development, but the crop seems to be particularly sensitive to adverse temperatures during reproductive development. The objective of these growth chamber studies was to quantify the effects of high temperature alone and in combination with applications of the plant growth regulator 1-Methylcycloprope (1-MCP) on cotton reproductive organs. Treatments consisted of two temperature regimes; normal at 32°C/24°C (day/night) and high at 38°C/24°C (day/night), with 1-MCP applied to white flowers on the day of anthesis. High temperature had significant effect on glutathione reductase activity, glucose, sucrose, and starch in both the reproductive organs and subtending leaves of cotton. The high temperature regime increased glutathione reductase (GR) activity, while the 1-MCP treatment had no significant effect in the flowers collected one day after anthesis. Both glucose and starch levels of the flowers showed decreased concentrations in the high temperature regime, whereas the subtending leaves concentrations of sucrose was decreased and the starch concentration was increased. These results indicated that although high temperatures significantly affected the GR levels and carbohydrate concentrations, 1-MCP treatments had no significant effects on reproductive organs or subtending leaves collected one day after anthesis.
Cotton is one of the world’s most important crops and provides fiber, feed, and soil enrichment. A popular belief among many producers and the general public is that cotton favors high temperatures, although it has been shown that high temperatures can detrimentally affect cotton plants (Oosterhuis, 2002; Hall, 2004; Pettigrew, 2008). The optimum range for cotton growth and development is 20-30°C (Reddy et al., 1991, 1992). Unfortunately, in the U.S. cotton producing regions, temperatures are usually well above the optimum during reproductive development (Reddy et al., 1991; Bibi et al., 2005; Pettigrew, 2008). With extreme year-to-year yield variability in cotton is a common occurrence that is difficult to explain, and has been related to high temperatures during flowering (Oosterhuis, 1999; Snider et al., 2009).

Studies have shown that high temperature stress during reproductive development can lead to poor fertilization and fruit abscission (Reddy et al., 1991, 1992; Oosterhuis, 1999; Bibi et al., 2006; Pettigrew, 2008; Snider et al., 2009). Abeles et al. (1992) reported that plants experiencing stress conditions produced an increase in ethylene, which has been shown to be a major factor in the regulation of the abscission process in cotton (Guinn, 1982a, 1982b; Lipe et al., 1972).

1-Methylcyclopropene (1-MCP) is a plant growth regulator produced by the company Agrofresh (Philadelphia, PA), which decreases or delays the effect of ethylene by occupying the ethylene receptor sites (Blankenship and Dole, 2003). 1-MCP has been shown to reduce, prevent, or delay abscission in horticulture (Byers et al., 2005; Zhong et al., 2001; Sisler et al., 1999; Moualem et al., 2004). Studies have also indicated an enhanced tolerance to heat stress in both
wheat (*Triticum aestivum* L.) (Hayes et al., 2007) and cotton (*Gossypium hirsutum* L.) (Kawakami, 2008).

I hypothesized that high temperature stressed cotton plants would experience higher levels of stress during early reproductive development and that the application of 1-MCP will partially alleviate the stress levels. Therefore, the objective of this study was to quantify the effects of high temperature stress on reproductive development in cotton, while investigating 1-MCP’s ability to ameliorate high temperature stress in cotton flowers and young fruit.

**MATERIAL AND METHODS**

Two consecutive growth chamber experiments were conducted in the Altheimer Laboratory, located at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR. Cotton (*Gossypium hirsutum* L.) cultivar ST4288B2F (PVP 201000309) was planted in 2 liter pots filled with Sunshine potting mix (Sun Gro Horticultural Distribution Inc., Bellevue, WA). The pots were randomly arranged in two large walk-in growth chambers (Model PGW36, Conviron, Winnipeg, Canada) with day/night temperatures of 32/24°C (day/night), 14 hour photoperiods and a relative humidity of 60%. After 6 weeks (one week prior to flowering), the temperature of one growth chamber was increased 38/24°C, the temperature of the other chamber was maintained at 32/24°C. Plants were re-randomized and watered daily with a half-strength Hoagland’s nutrient solution (Hoagland and Arnon, 1933). The chambers were presumed identical in all variables (e.g. light and relative humidity) with differences only in daytime temperatures (32°C and 38°C). The experiments were arranged in a completely randomized
design with two factors and six replications. The factors consisted of 1-MCP (formulation A17492E) treatments (treated and untreated) and sample day (1 day after anthesis for 1 week).

In the 1-MCP treatment, white flowers from the first sympodial position located between nodes 5 to 10 were sprayed using a 25 ml spray bottle. Flowers were sprayed at 10:00 AM with 0.05482 ml of a solution containing 9.5 g of 1-MCP active ingredient per liter. This application corresponded approximately to the recommended field application of 10 g of active ingredient per hectare. Parameters collected were antioxidant enzymes of the ovary, and carbohydrates of both the ovary and subtending leaf.

**Antioxidant Glutathione Reductase (GR) Activity**

Cotton flower ovaries were collected at 1 day after white flower for determinations of GR. The ovary extraction procedure for enzyme determination followed descriptions by Gomez et al. (2004) with modifications. A fresh ovary sample was ground using a mortar and pestle with liquid nitrogen, and placed into a 35 ml centrifuge tube. An extraction solution was prepared by mixing 3.02 g of PIPES (Sigma Company, St. Louis, MI) buffer in 150 ml of distilled water (50nM final concentration), 0.189 g of DL-cysteine hydrochloride (6mM) (Sigma Company, St. Louis, MI), 0.352 g of D-isoascorbic acid (10mM) (Sigma Company, St. Louis, MI), 0.074 g of EDTA (1mM) (Sigma Company, St. Louis, MI), and 2 g of polyvinylpyrrolidone-10 (1%) (Sigma Company, St. Louis, MI). The resulting solution was mixed thoroughly and the pH was adjusted to 6.8, and 0.6 ml of Triton X-100 (0.3%) (Sigma Company, St. Louis, MI) was added to the buffer solution, and the volume was adjusted to 200 ml with deionized water. The tube containing the ovary sample received 0.5 g of polyvinylpyrrolidone, one drop of antifoam A, and 4 ml of extraction buffer solution, and was
homogenized for 3 min with a Polytron homogenizer (Brinkmann Instruments Inc., Palo Alto, CA). The samples were centrifuged for 20 min at 13000 rpm (21000 x g) at 4°C in a Hermle centrifuge (Labnet International, Inc, Edison, NJ) and the supernatant was collected and stored at -80°C until enzyme measurement.

The glutathione reductase (GR) assay of Schaedle and Bassham (1977) was followed. The assay was initiated by placing 950 µl of a reaction solution and 50 µl of plant extracted sample in a 1-ml quartz cuvette. The reaction solution was prepared by adding 0.303 g of Tris (50mM) (Sigma Company, St. Louis, MI), 0.007 g of NADPH+H (0.15 mM) (Sigma Company, St. Louis, MI), 0.016 g of oxidized glutathione (0.5mM) (Sigma Company, St. Louis, MI), and 0.031 g of MgCl$_2$ (3mM) (Sigma Company, St. Louis, MI) in 40 ml of distilled water. The pH was adjusted to 7.5 and the final volume was adjusted to 50 ml with distilled water. The GR activity was measured with an Ascent Multiscan microplate reader (Molecular Devices Corporation, Sunnyvale, CA). The instrument was regulated to display a wavelength of 340 nm and measurements were made during a period of 1 min. Glutathione quantities were expressed as mmol g$^{-1}$ of fresh weight.

**Carbohydrate Extraction and Analysis**

Soluble carbohydrate content was measured according to a modification of the Hendrix (1993) protocol. Cotton flowers and subtending leaves were collected at 1 day after white flower for determinations of carbohydrates. The samples were oven dried for 3 days at 50°C and then ground with a mortar and pestle. The ground tissue was extracted 3 times with 80°C aqueous ethanol (800ml ethanol /L) and the samples were centrifuged after each extraction at 5000 rpm and finally the fractions were pooled, while the remaining pellet was used for the determination.
of starch content. Active charcoal was then added to the pooled fractions to remove substances that could interfere with the carbohydrate measurements and the samples were centrifuged again at 3500 rpm. The supernatant was immediately stored at -80°C for later determination of sucrose and hexose (fructose and glucose) with a MultiScan Ascent Microplate Reader (Thermo Fisher Scientific Inc., Waltham, MA). The glucose HK-assay kit (Sigma Chemical Company, St Louis, MO) was used. A 10μl aliquot of each extract was pipetted into a well of a microtitration plate and the plate was incubated at 50°C for 40 min to evaporate ethanol. Ten microliters of water were then added to each well along with 100 μl of glucose assay reagent and the plate was incubated again for 15min at 30°C. The absorbance was measured three times a 340 nm using a Microplate reader (Molecular Devices Corporation, Sunnyvale, CA). Subsequently, 0.25 enzyme units of phosphoglucose isomerase was added to the extracts in each well of the plate and the absorbance was again measured at 340nm, after which, 83 enzyme units of invertase were added to the extracts and the microtitration plate was incubated at 30°C for 60 min. The absorbance was measured three times at 340nm.

For the determination of starch content, the remaining pellet was treated with 0.1N KOH and the pH of the samples was adjusted to 7.2 with 1N CH₃COOH. Tris buffer and α-amylase were added subsequently and the samples were kept in an 85°C waterbath for 30 min. The pH of the samples was again decreased to 5 with 1N CH₃COOH and 1ml of amyloglucosidase preparation was added. After incubation in a 55°C waterbath for 60 min, the samples were centrifuged at 5000 rpm for 15 min and the supernatant was stored in a 1.5ml microcentrifuge tubes at -80°C. For the determination of starch concentrations, 10μl of each sample and 10μl of water was pipetted into each well of a microtitration plate. After which, 100 μl of glucose assay reagent was added to each well and, after incubation at 30°C for 15 min, the absorbance was
measured three times at 340nm. The quantification of carbohydrates concentration was done with the construction of a glucose standard curve with concentrations of 0, 0.005, 0.0125, 0.025, 0.05, 0.125, 0.25, 0.50 μg glucose/μl. All chemicals used were provided by Sigma (Sigma Chemical Company, St Louis, MO).

Statistical Analyses

A fit model statistical analysis with six replications was used to evaluate the results. The chamber effect was also added to a model as a fixed effect, and significant values in chamber interactions or chamber main effect were inferred to temperature treatment (normal and high). The software JMP version 9 (SAS Institute Cary, NC) was used to perform the statistical analyses. Means and standard errors values were assessed to assemble graphs using the Microsoft Office Excel 2007 software (Microsoft Corporation, Redmond, WA). Analysis of variance and conventional Students’ t-tests were used to analyze statistical significance between means (Appendix I). A probability less than 0.05 was considered significant.

RESULTS

Glutathione Reductase Activity

The GR activity results showed no significant main interaction between 1-MCP and temperature treatments, However chamber temperature effect showed a significant main effect (P= 0.0081). Thus, treatments were analyzed by averaging 1-MCP treatments over chambers and only an analysis of means comparison of the main effects (normal and high temperature chamber
treatments) was made. The high-temperature (38°C) significantly increased GR activity in the ovary compared to the normal-temperature (32°C) (Fig. 1).

![Figure 1. Effect of temperature on glutathione reductase activity of cotton ovaries. Columns with the same letters are not significantly different (P=0.05). Errors bars represent ± one standard error. Data was averaged across chambers and sampling days.](image)

As previously mentioned, 1-MCP treatments showed no significant interaction with temperature. The application of 1-MCP had no effect on GR activity in the ovary collected 1 day post-anthesis (P = 0.9732; Fig. 2). This effect may be associated with the short time interval between ovary stress detection and the ovary stress response, while the 1-MCP treated flowers maintain their GR activity the untreated flowers have yet to respond to the temperature stress.
Figure 2. Effect of 1-MCP treatment on glutathione reductase activity of cotton ovaries measured one day after treatment. Columns with the same letters are not significantly different (P=0.05). Errors bars represent ± one standard error. Data was averaged across chambers.

**Carbohydrates**

Total soluble carbohydrates (glucose, fructose, and sucrose) had no significant interaction between 1-MCP and temperature treatments in both the cotton ovaries and subtending leaves. High temperature decreased glucose (P = 0.0153; Fig. 3) and starch (P = 0.0385; Fig. 4) content of the ovary, but had no significant effect on the ovary fructose (P = 0.1152; Fig. 5) and sucrose (P = 0.9673; Fig. 6) concentration. In the subtending leaf, high temperature decreased sucrose (P = 0.0005; Fig. 7) and increased starch (P<0.0001; Fig. 8) concentration. The decline in soluble carbohydrate content was primarily attributed to high temperature stress during reproductive development.
Figure 3. Effect of temperature on glucose concentration in cotton ovaries measured at 1 day after treatment. Columns with the same letters are not significantly different (P=0.05). Errors bars represent ± one standard error. Data was averaged across chambers and sampling days.

Figure 4. Effect of temperature on starch concentration in ovaries measured at 1 day after treatment. Columns with the same letters are not significantly different (P=0.05). Errors bars represent ± one standard error. Data was averaged across chambers and sampling days.
Figure 5. Effect of temperature on fructose concentration in cotton ovaries measured at 1 day after treatment. Columns with the same letters are not significantly different (P=0.05). Errors bars represent ± one standard error. Data was averaged across chambers and sampling days.

Figure 6. Effect of temperature on sucrose concentration in cotton ovaries measured at 1 day after treatment. Columns with the same letters are not significantly different (P=0.05). Errors bars represent ± one standard error. Data was averaged across chambers and sampling days.
Figure 7. Effect of 1-MCP on sucrose concentration in subtending leaves measured at 1 day after treatment. Columns with the same letters are not significantly different (P=0.05). Errors bars represent ± one standard error. Data was averaged across chambers and sampling days.

Figure 8. Effect of temperature on starch concentration in subtending leaves measured at 1 day after treatment. Columns with the same letters are not significantly different (P=0.05). Errors bars represent ± one standard error. Data was averaged across chambers and sampling days.
The high temperature (38°C) treatment produced a significant effect on the starch concentrations in the plant, with ovary starch concentrations decreasing (P=0.0385; Fig. 4) and subtending leaf concentrations increasing (P<.0001; Fig. 8) compared to the control temperature (32°C).

There was also no significant interaction between 1-MCP and temperature treatments on starch, in both the cotton ovaries and leaves (data not shown). The increase of starch in the subtending leaves could be related to weak sink activity under the high temperature regime, i.e., the assimilate supply of the subtending leaf exceeded the demand of the ovary. 1-MCP had no significant effect on ovary concentrations of glucose (P = 0.5769; Fig. 9), fructose (P = 0.6017; Fig. 10), and sucrose (P = 0.9673; Fig. 11).

![Figure 9](image-url)

Figure 9. Effect of 1-MCP on glucose concentration in cotton ovaries measured at 1 day after treatment. Columns with the same letters are not significantly different (P=0.05). Errors bars represent ± one standard error. Data was averaged across chambers and sampling days.
Figure 10. Effect of 1-MCP on fructose concentration in cotton ovaries measured at 1 day after treatment. Columns with the same letters are not significantly different (P=0.05). Errors bars represent ± one standard error. Data was averaged across chambers and sampling days.

Figure 11. Effect of 1-MCP on sucrose concentration in cotton ovaries measured at 1 day after treatment. Columns with the same letters are not significantly different (P=0.05). Errors bars represent ± one standard error. Data was averaged across chambers and sampling days.
DISCUSSION

Currently high temperatures limit growth and development processes in much of the cotton producing areas (Reddy et al., 2002). Change to warmer climates in the future can shorten development stages and change crop suitability areas (Craufurd and Wheeler, 2009).

My results showed that glutathione reductase (GR) activity significantly increased with high temperature (Fig. 1), a result also observed by Sudhakar et al., (2001) in Morus alba, Lee et al., (2000) in Cucumis sativas, Keles et al., (2002) in Triticum aestivum, and Kawakami et al., (2007) in Gossypium hirsutum L. Glutathione reductase is located mainly in the chloroplast where it represents about 80% of the total GR activities in leaf tissues, but is also found in cytosol, glyoxysomes, and peroxisomes (Edwards et al., 1990; Jimenez et al., 1997). Glutathione reductase ensures efficient recycling of glutathione in the ascorbate-glutathione cycle, which allows for a re-reduction of ascorbate (Foyer et al., 1976; Nakano et al., 1980). In the ascorbate-glutathione cycle, glutathione acts as a recycled intermediate in the reduction of H\textsubscript{2}O\textsubscript{2} using electrons derived from H\textsubscript{2}O (Foyer et al., 1997). This suggests that GR plays an important role in the protection of plants against oxidative stress. It has been observed that stress-tolerant plants have high GR activity (Kocsy et al., 1996, 2000; Mittova et al., 2003; Snider et al., 2011). Furthermore, it has been shown that enhanced chloroplastic GR activity in transgenic plants results in increased protection against oxidative stress (Foyer et al., 1995; Pilon-Smit et al., 2000).

1-MCP applications had no significant effect on GR activity (Fig. 2). As mentioned previously this is primarily attributed to the short time interval allowed for ovary stress response between 1-MCP application and measurement of the ovary stress (i.e. GR activity), while the 1-
MCP treated flowers maintain their GR activity the untreated flowers have yet to respond to the stress. Kawakami (2008) observed similar results showing no effects of 1-MCP application on flowers until the second day after application.

Carbohydrates are considered to be the basic building components for the majority of crops and especially cotton where the fiber consists of 99% carbohydrates (Constable and Oosterhuis, 2010). Furthermore, 60% of the total carbohydrate requirement of developing reproductive tissue is provided by adjacent, subtending leaves (Ashley 1972, Wullschleger and Oosterhuis 1990). The high-temperature ovaries showed a significant decrease of the carbohydrates glucose (Fig. 3) and starch (Fig. 10), while in the subtending leaf there was a significant increase in starch concentrations (Fig. 11). Again, the 1-MCP applications had no significant affects on either the carbohydrate concentrations in the ovaries or leaves of the cotton plants. The high starch concentrations in the subtending leaves are attributed to a weak sink activity under high temperatures (Snider et al., 2010; 2011). Heat stress limits source strength and carbohydrate allocation to developing sinks by decreasing photosynthesis, increasing dark respiration and photorespiration, and inhibiting translocation (Snider et al., 2009). Snider et al., (2009) also reported decreased subtending leaf activity inhibited pollen development, tube growth through the style, or guidance to ovules due to insufficient energy supply. These adverse effects of high temperature on cotton reproductive development result in decreased fertilization and lower seed numbers per boll.

In conclusion, antioxidant enzyme results indicated that GR activity in ovaries increased under high temperatures, and carbohydrate activity of ovaries and leaves decreased under high temperatures. The 1-MCP treatments had no significant effects on either the GR or carbohydrate activities of the reproductive organs. Overall, high temperatures have negative impacts on cotton
during reproductive development and 1-MCP treatments showed no effect one day after application. The study needs to be continued for further quantification of 1-MCP and high temperature effects on cotton with measurements taken at two days or later after 1-MCP treatment.

REFERENCES


CHAPTER II

Effects of 1-Methylcyclopropene on the Physiology and Yield of Field-Grown Cotton

ABSTRACT

Cotton (Gossypium hirsutum L.) is an important industrial crop but suffers from extreme sensitivity to environmental stress. The current projects were designed to evaluate the effectiveness of the plant growth regulator 1-Methylcyclopropene (1-MCP) to alleviate the effects of stress, maintain fruit and seed numbers for increased yield. Two field studies were conducted in Marianna and Fayetteville Arkansas in 2010 and repeated in 2011. The field study conducted in Marianna, AR consisted of five treatments; an untreated control, 1-MCP @ 10 g ai/ha applied at first flower (FF) and FF + 1 week, 1-MCP @ 10 g ai/ha applied at FF + 1 and FF + 2, 1-MCP @ 10 g ai/ha applied at FF + 2 and FF + 3, 1-MCP @ 10 g ai/ha applied when the daily maximum temperature exceeded 95°F starting at FF. Measurements were made of boll weight, boll number and yield, as well as on plant physiological responses. The field study conducted in Fayetteville, AR consisted of two treatments an untreated control and 1-MCP applied @ 10 g ai/ha applied at first flower (FF). These treatments were applied to cotton planted at two different planting dates in order to give two temperature regimes during the same development stage. These two planting dates produced temperature averages of 91°F and 99°F in 2010, and 99°F and 104°F in 2011. Measurements were made of boll weight, boll number and yield, as well as on plant physiological responses. Yield and physiological measurement results for Marianna, AR indicated no significant effect and possible negative effects on cotton plants
not experiencing stress. While yield and physiological results from Fayetteville, AR indicated 1-MCP applications resulted in the positive influence of the plant growth regulator on the cotton plants, results showed significant effects on the fiber and seedcotton yields, seed weight, seed number, and boll number. Overall, the studies indicated that foliar application of 1-MCP has the potential to be used in cotton production to overcome environmental stress problems and achieve higher and more stable yields due to reduced plant stress.

INTRODUCTION

Cotton (Gossypium hirsutum L.) is an annual row crop grown in warm climates for fiber, oil, and feed production. The Mississippi River Delta of Arkansas, cotton is mainly grown for fiber production. Cotton yields in the United States are substantially lower than the theoretical maximum according to Baker and Hesketh (1969). Cotton yield is affected by genetics, management practices, and unfavorable weather conditions (Arevelo, 2004). Though overall yields have increased overtime, there is a negative correlation between high temperatures and cotton yields since 1980 (Oosterhuis, 2000). Despite originating from warmer environments, the cotton crop prefers a temperature range of 20-30°C, and has optimum metabolic rates between 23-32°C (Burke et al., 1988). Extreme year-to-year variability is becoming an increasing concern for cotton farmers (Lewis et al., 2000; Johnson and Bourland, 2003).

Decreased and variable cotton yields have been associated with environmental stresses. The woody, indeterminate and perennial biology of the cotton plant is the main reason why under conditions of environmental stress the plant focuses on survival rather than on increased production (Krieg, 2002). Among all stress factors, temperature and drought appear to play the
most significant role in decreasing crop yields in the world. In August 2000, a combination of high temperature and dry weather was estimated to cause damage to US agriculture that extrapolated to a loss of $4.2 billion dollars (Mittler, 2006).

The main components of cotton yield are boll number per unit of land area and seed number per boll (Worley et al., 1974). Cotton typically abscises about 65 percent of the total flowers developed (Addicott, 1982), which is one of the main reasons it does not reach its theoretical yield potential. Although the relationship of temperature stress is well documented in boll abscission, high temperature stress has also indicated a role in flower senescence and pollination (Abeles et al., 1992; Snider et al., 2009).

A common response of plants under stress is increased ethylene synthesis (Abeles et al., 1992). Ethylene is an endogenous phytohormone associated with senescence, abscission and pollination processes (Abeles et al., 1992). Abeles et al., (1992) reported plants experiencing stress conditions produced an increase in ethylene, which has been shown to be a major factor in the regulation of the abscission process in cotton (Guinn, 1982a, 1982b; Lipe et al., 1972). Studies have shown that high temperature stress in cotton during reproductive development can lead to poor fertilization and abscission (Reddy et al., 1991; 1992; Oosterhuis, 1999; Bibi et al., 2006; Pettigrew, 2008; Snider et al., 2009).

1-Methylcyclopropene (1-MCP) is a plant growth regulator produced by the company Agrofresh (Philadelphia, PA), which inhibits the ethylene response in plants by inhibiting the ethylene receptor sites (Blankenship and Dole, 2003). 1-MCP has also been widely used to improve shelf life and quality of agriculture products. Furthermore, the affinity of 1-MCP for the receptor sites is approximately 10 times greater than that of ethylene. In addition, compared with ethylene, 1-MPC is active at much lower concentrations. 1-MCP was also reported, in some
species, to decrease ethylene biosynthesis through feedback inhibition (Blankenship and Dole, 2003).

It is hypothesized that 1-MCP sprayed on cotton plants will decrease the high temperature stress response of the cotton plant. It was expected that plants treated with 1-MCP would have less fruit abscission, which would result in higher yields. The current studies were designed to evaluate the possible use of 1-MCP to alleviate the adverse effect of environmental stresses experienced during the season, on square and boll development, and therefore reduce yield variability and result in higher yields.

MATERIALS AND METHODS

Field studies were conducted at two locations; Marianna and Fayetteville, AR, in 2010 and 2011. Both studies measured yield parameters and physiological measurements were taken during reproductive development. To evaluate the effect of 1-MCP on the parameters, these treatments were combined and analyzed with statistical software.

A field study was conducted at the University of Arkansas Lon Mann Cotton Research Station at Marianna, AR. The cotton (*Gossypium hirsutum* L.), cultivar ST4288B2F (PVP 201000309) was planted on May 13, 2010 and May 11, 2011. Fertilizers were applied according to preseason soil tests and recommended rates. Weed and insect control were performed according to state extension recommendations and furrow irrigated. The plot size was 4 rows by 15 m, with a row spacing of 0.96 m and plant density of 10 plants/m. The experiment was arranged in a Randomized Complete Block design with five replications. Treatments consisted of: (T1) an untreated control, (T2) 1-MCP @ 10 g ai/ha applied at the first flower (FF) and FF +
1 week stage, (T3) 1-MCP @ 10 g ai/ha applied at FF + 1 week and FF + 2 weeks, (T4) 1-MCP @ 10 g ai/ha applied at FF + 2 weeks and FF + 3 weeks, and (T5) 1-MCP @ 10 g ai/ha applied when the daily air maximum temperature exceeds 95°F starting at FF, temperatures were measured by Watch Dog (Spectrum Technologies, Plainfield, IL) weather data loggers.

A second field study was conducted at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR had two planting dates for both 2010 and 2011 to ensure different temperature regimes during the same cotton growth stage. The two planting dates for 2010 were May 24 and June 8, while the 2011 planting dates were May 31 and June 14. Fertilizers were applied according to preseason soil tests and recommended rates. Weed and insect control were performed according to state extension recommendations and furrow irrigated. The plot size was 4 rows by 15 m, with a row spacing of 0.96 m and plant density of 10 plants/m. The experiment was arranged in a Randomized Complete Block design with five replications. Treatments consisted of: (T1) an untreated control, (T2) 1-MCP @ 10 g ai/ha applied at the first flower (FF). All 1-MCP treatments were sprayed with a backpack CO₂ sprayer calibrated to deliver 1-MCP (A17492E) @ 10 g ai/ha.

1-MCP concentrations were the recommended rates by Agrofresh Inc. (Philadelphia, PA). The CO₂ backpack sprayer was set at 22psi with Tee Jet 8002VS spray nozzles in order to apply 10 gallons of water/chemical solution to the acre. The application was applied over the top of the cotton canopy as fine particle size droplets. The small droplet size prevented pollen bursting in the flower and since 1-MCP is a gas it would distribute throughout the cotton plants more equally.
Yield Parameters

All yield parameters were calculated from a one meter length of row from each plot. The total numbers of bolls were counted and harvested for determination of seedcotton yield, boll size, gin turnout and lint yield. Seed size was calculated by weighing and counting 400 seeds from each plot harvest, and the number of seed per sample was estimated by dividing the weight of the total amount of seeds by the seed size.

Fiber Quality

Cotton fiber samples from both studies conducted in Marianna, AR, were sent for fiber analysis to the Louisiana State University Cotton Fiber Testing Laboratory, AgCenter, Baton Rouge, LA. The following parameters were analyzed: micronaire, length, strength, uniformity, short fiber index, and elongation.

Antioxidant Glutathione Reductase (GR) Activity

Cotton flower ovaries and subtending leaves collected at two days after white flower, were used for determinations of GR. The ovary extraction procedure for enzyme determination followed descriptions by Gomez et al. (2004) with modifications. A fresh ovary sample was ground using a mortar and pestle with liquid nitrogen, and placed into a 35 ml centrifuge tube. An extraction solution was prepared by mixing 3.02 g of PIPES (Sigma Company, St. Louis, MI) buffer in 150 ml of distilled water (50nM final concentration), 0.189 g of DL-cysteine hydrochloride (6mM) (Sigma Company, St. Louis, MI), 0.352 g of D-isoascorbic acid (10mM) (Sigma Company, St. Louis, MI), 0.074 g of EDTA (1mM) (Sigma Company, St. Louis, MI), and 2 g of polyvinylpyrrolidone-10 (1%) (Sigma Company, St. Louis, MI). The resulting
solution was mixed thoroughly and the pH was adjusted to 6.8 and a 0.6 ml of Triton X-100 (0.3%) (Sigma Company, St. Louis, MI), was added to the buffer solution, and the volume was adjusted to 200 ml with deionized water. The tube containing ovary sample, 0.5 g of polyvinylpyrrolidine, one drop of antifoam A, and 4 ml of extraction buffer solution, was homogenized for 3 min with a Polytron homogenizer (Brinkmann Instruments Inc., Palo Alto, CA). The samples were centrifuged for 20 min at 13000 rpm (21000 x g) at 4°C in a Hermle centrifuge (Labnet International, Inc, Edison, NJ) and the supernatant was collected and stored at -80°C until the day of the enzyme.

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**Carbohydrate Extraction and Analysis**

Soluble carbohydrate content was measured two days after treatment according to a modification of the Hendrix protocol (1993). Cotton flowers and subtending leaves were
collected at 1 day after white flower, and were selected for determinations of carbohydrates. The samples were oven dried for 3 days at 50°C and then ground with a mortar and pestle. The ground tissue was extracted 3 times with 80°C aqueous ethanol (800 ml ethanol/L) and the samples were centrifuged after each extraction at 5000 rpm and finally the fractions were pooled, while the remaining pellet was used for the determination of starch content. Active charcoal was then added to the pooled fractions to remove substances that could interfere with the carbohydrate measurements and the samples were centrifuged again at 3500 rpm. The supernatant was immediately stored at -80°C for later determination of sucrose and hexose (fructose and glucose) with a MultiScan Ascent Microplate Reader (Thermo Fisher Scientific Inc., Waltham, MA). The glucose HK-assay kit (Sigma Chemical Company, St Louis, MO) was used. A 10 μl aliquot of each extract was pipette into a well of a microtitration plate and the plate was incubated at 50°C for 40 min to evaporate ethanol. Ten microliters of water were then added to each well along with 100 μl of glucose assay reagent and the plate was incubated again for 15 min at 30°C. The absorbance was measured three times a 340 nm using a Microplate reader (Molecular Devices Corporation, Sunnyvale, CA). Subsequently, 0.25 enzyme units of phosphoglucone isomerase was added to the extracts in each well of the plate and the absorbance was again measured at 340 nm which, 83 enzyme units of invertase were added to the extracts and the microtitration plate was incubated at 30°C for 60 min. The absorbance was measured three times at 340 nm.

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preparation was added. After incubation in a 55°C waterbath for 60 min, the samples were centrifuged at 5000 rpm for 15 min and the supernatant was stored in a 1.5ml microcentrifuge tubes at -80°C. For the determination of starch concentrations, 10μl of each sample and 10μl of water was pipette into each well of a microtitration plate. After which, 100 μl of glucose assay reagent was added to each well and, after incubation at 30°C for 15 min, the absorbance was measured three times at 340nm. The quantification of carbohydrates concentration was done with the construction of a glucose standard curve with concentrations of 0, 0.005, 0.0125, 0.025, 0.05, 0.125, 0.25, 0.50 μg glucose/μl. All chemicals used were provided by Sigma (Sigma Chemical Company, St Louis, MO).

**Total and soluble calcium extraction and analysis**

One dried, ground ovary was extracted for total calcium analysis via the wet ashing procedure described by Plank (1992) using a nitric acid digest followed by the complete combustion of organic matter via the addition of 30% H₂O₂. For determination of water soluble calcium content, one ovary was rinsed in distilled water and homogenized in 20:1 ratio of distilled water:g fresh weight. Samples were subsequently centrifuged at 21000 x g for 20 min and the supernatant was used for quantification of water soluble calcium analysis. Both total calcium samples and water soluble calcium samples were analyzed via the inductively coupled plasma spectrometer (ICP) (Model CIROS; Spectro Analytical Instruments GmbH & Co., Germany).
Statistical Analyses

A fit model statistical analysis with six replications was used to evaluate the results. The chamber effect was also added to a model as a fixed effect, significant values in chamber interactions or chamber main effect were inferred to temperature treatment (normal and high). The software JMP version 9 (SAS Institute Cary, NC) was used to perform the statistical analyses. Means and standard errors values were assessed to assemble graphs using the Microsoft Office Excel 2007 software (Microsoft Corporation, Redmond, WA). Analysis of variance and conventional Students’ t-tests were used to analyze statistical significance between means (Appendix II). A probability less than 0.05 was considered significant.

RESULTS

Yield Parameters

No significant differences were found in yield parameters at Marianna in 2010 (Table 1), or in 2011 (Table 2). Numerically higher yields were observed in the untreated control in 2011 compared to the 1-MCP treatments.

Table 1. Effect of 1-MCP on seedcotton yield and lint yield. Experiment conducted at Marianna, AR, in 2010.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Seedcotton Yield</th>
<th>Lint Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1- Untreated Control</td>
<td>3885</td>
<td>1600</td>
</tr>
<tr>
<td>T2 – 1-MCP at FF and FF+1</td>
<td>3745</td>
<td>1531</td>
</tr>
<tr>
<td>T3 – 1-MCP at FF+1 and FF+2</td>
<td>3457</td>
<td>1383</td>
</tr>
<tr>
<td>T4 – 1-MCP at FF+2 and FF+3</td>
<td>3876</td>
<td>1625</td>
</tr>
<tr>
<td>T5 – 1-MCP when Temp. &gt; 95°F</td>
<td>3843</td>
<td>1527</td>
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<tr>
<td>P-value &gt; (0.05) Significant</td>
<td>0.682</td>
<td>0.493</td>
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Table 2. Effect of 1-MCP on seedcotton yield and lint yield. Experiment conducted at Marianna, AR, in 2011.

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<th>Treatment</th>
<th>Seedcotton Yield</th>
<th>Lint Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1- Untreated Control</td>
<td>4664 kg/ha</td>
<td>1753 kg/ha</td>
</tr>
<tr>
<td>T2 – 1-MCP at FF and FF+1</td>
<td>4513 kg/ha</td>
<td>1627 kg/ha</td>
</tr>
<tr>
<td>T3 – 1-MCP at FF+1 and FF+2</td>
<td>4564 kg/ha</td>
<td>1673 kg/ha</td>
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<tr>
<td>T4 – 1-MCP at FF+2 and FF+3</td>
<td>4090 kg/ha</td>
<td>1537 kg/ha</td>
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<tr>
<td>T5 – 1-MCP when Temp. &gt; 95°F</td>
<td>4306 kg/ha</td>
<td>1624 kg/ha</td>
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<tr>
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<td>0.501 kg/ha</td>
<td>0.803 kg/ha</td>
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In Fayetteville yields were significantly increased in 2010 and 2011 (P = 0.0043; Fig. 1) and (P = 0.0045; Fig. 2), this was attributed to a significant increase in the number of bolls (P = 0.0071; Fig. 3) and (P = 0.0040; Fig. 4) produced on cotton in 1-MCP treatments. Yield parameters showed increased seedcotton yield, lint yield (P = 0.0021; Fig. 5) and (P = 0.0065; Fig. 6), and seed weight (P = 0.0068; Fig. 7) and (P = 0.0038; Fig. 8) in both 2010 and 2011, respectively.
Fig. 1. Effect of 1-MCP on seedcotton yield for both planting dates, a three day average maximum temperature during the treatment period is shown next to the planting date. Results of the experiments conducted at Fayetteville in 2010. Columns with same letter are not significantly different (P=0.05). Errors bars represent ± one standard error.

Fig. 2. Effect of 1-MCP on seedcotton for both planting dates, a three day average maximum temperature during the treatment period is shown next to the planting date. Results of the experiments conducted at Fayetteville in 2011. Columns with same letter are not significantly different (P=0.05). Errors bars represent ± one standard error.
Fig. 3. Effect of 1-MCP on boll number for both planting dates, a three day average maximum temperature during the treatment period is shown next to the planting date. Results of the experiments conducted at Fayetteville in 2010. Columns with same letter are not significantly different (P=0.05). Errors bars represent ± one standard error.

Fig. 4. Effect of 1-MCP on boll number for both planting dates, a three day average maximum temperature during the treatment period is shown next to the planting date. Results of the experiments conducted at Fayetteville in 2011. Columns with same letter are not significantly different (P=0.05). Errors bars represent ± one standard error.
Fig. 5. Effect of 1-MCP on lint yield for both planting dates, a three day average maximum temperature during the treatment period is shown next to the planting date. Results of the experiments conducted at Fayetteville in 2010. Columns with same letter are not significantly different (P=0.05). Errors bars represent ± one standard error.

Fig. 6. Effect of 1-MCP on lint yield for both planting dates, a three day average maximum temperature during the treatment period is shown next to the planting date. Results of the experiments conducted at Fayetteville in 2011. Columns with same letter are not significantly different (P=0.05). Errors bars represent ± one standard error.
Fig. 7. Effect of 1-MCP on seed weight for both planting dates, a three day average maximum temperature during the treatment period is shown next to the planting date are. Results of the experiments conducted at Fayetteville in 2010. Columns with same letter are not significantly different (P=0.05). Errors bars represent ± one standard error.

Fig. 8. Effect of 1-MCP on seed weight for both planting dates, temperatures shown with planting date are a three day average high during treatment period. Results of the experiments conducted at Fayetteville in 2011. Columns with same letter are not significantly different (P=0.05). Errors bars represent ± one standard error.
Fiber Quality

Fiber quality was only measured at the Marianna location for both 2010 and 2011. Fiber qualities measured were length, uniformity, strength, elongation, and micronaire. There indicated no significant effect of 1-MCP treatment on any fiber quality in either year (P > 0.05; Tables 3 and 4).

Table 3. Effect of 1-MCP on fiber quality parameters in Marianna 2010.

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<thead>
<tr>
<th>Treatment</th>
<th>Length (mm)</th>
<th>Uniformity (%)</th>
<th>Strength (g/tex)</th>
<th>Elongation (%)</th>
<th>Micronaire</th>
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</thead>
<tbody>
<tr>
<td>T1 - Untreated Control</td>
<td>1.13</td>
<td>81.94</td>
<td>29.9</td>
<td>6.2</td>
<td>4.82</td>
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<td>T2 – 1-MCP at FF and FF+1</td>
<td>1.13</td>
<td>83.18</td>
<td>29.68</td>
<td>6.38</td>
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<tr>
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<td>29.54</td>
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*N.S.= Not Significant P>0.05

Table 4. Effect of 1-MCP on fiber quality parameters in Marianna 2011.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Length (mm)</th>
<th>Uniformity (%)</th>
<th>Strength (g/tex)</th>
<th>Elongation (%)</th>
<th>Micronaire</th>
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</thead>
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<td>1.21</td>
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<td>T3 – 1-MCP at FF+1 and FF+2</td>
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<td>1.19</td>
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<td>32.32</td>
<td>6.72</td>
<td>3.52</td>
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<tr>
<td>T5 – 1-MCP when Temp. &gt; 95°F</td>
<td>1.22</td>
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*N.S.= Not Significant P>0.05
Glutathione Reductase Activity

Overall, 1-MCP application in Marianna did not significantly affect the GR enzyme activity in cotton plants in 2010 or 2011 (P = 0.8023; Fig. 9) and (P = 0.1089; Fig. 10).

Fig. 9. Effect of 1-MCP on GR activity at Marianna, AR 2010. Columns with same letter are not significantly different (P=0.05). Errors bars represent ± one standard error.
Fig. 10. Effect of 1-MCP on GR activity at Marianna, AR 2011. Columns with same letter are not significantly different (P=0.05). Errors bars represent ± one standard error.

At Fayetteville, 1-MCP showed no significant changes in GR activity in 2010 in the first planting date (P = 0.4199; Fig. 11 average temperature 91°F) and in the second planting date (P = 0.4199; Fig 12 average temperature 97°F). However in 2011 1-MCP was close to a significant effect (P = 0.0674; Fig. 12) when temperatures were 99°F and 104°F.
Fig. 11. Effect of 1-MCP on GR activity for both planting dates. Results of the experiments conducted in Fayetteville in 2010. Columns with same letter are not significantly different (P=0.05). Errors bars represent ± one standard error.

Fig. 12. Effect of 1-MCP on GR activity for both planting dates, a three day average maximum temperature during the treatment period is shown next to the planting date. Results of the experiments conducted in Fayetteville in 2011. Columns with same letter are not significantly different (P=0.05). Errors bars represent ± one standard error.
Calcium and Carbohydrates

Calcium and carbohydrate measurements of the ovary were only taken in the Fayetteville, AR location in 2010 and 2011. Calcium and carbohydrates were not significantly affected by 1-MCP applications in both years of the experiment (data not shown).

DISCUSSION

The plant growth regulator 1-MCP is widely used for improving the quality and shelf life of fruits, vegetables and flowers. However 1-MCP has not been used commercially on crops during the season, for preventing stress and improving yield. There have been some reports of 1-MCP improving cotton yields (Storch, 2010) but this was not conclusive..

The current studies in Fayetteville showed that 1-MCP improved the yield of high temperature stressed field-grown cotton (Figs. 1 through 8). This was due to the effect of 1-MCP increasing the retention of cotton bolls. Increased yield at the Fayetteville location was attributed to applications of 1-MCP preventing the ethylene action to allow fruit abscission. Studies have reported that applications of 1-MCP reduced leaf abscission of mung beans (Phaseolus aureus) (Sisler et al., 1999) and citrus (Citrus sinensis L.) (Sisler et al., 1999, Pozo and Burns 2000; Zhong et al., 2001). In addition, 1-MCP also had been shown to affect the process of fruit abscission in cherry tomatoes (Lycopersicon esculentum) (Moualem et al., 2004), in apples (Malus sylvestris) (Dal Cin et al, 2005; Byers et al., 2005), and in citrus (Citrus sinensis L.) (Pozo et al., 2004). Planting date effects (Appendix II) were also analyzed to show differences between the two planting dates.
In the Marianna field study, the application of 1-MCP had no significant effect on yield. This lack of effect was attributed to there not being high temperature stress occurring during the study. Temperatures above 35°C have been shown to significantly decrease photosynthesis (Bibi et al., 2006). Temperatures were milder in Marianna, AR during the 2010 and 2011 growing season, whereas temperatures were much higher in the Fayetteville, AR location over both growing seasons (Appendix II).

A common plant response to stress is the production of ethylene, a stress hormone. Ethylene induces senescence, abscission and a variety of adverse plant responses. Blankenship and Dole (2003) reported 1-MCP reduces the effect of ethylene by occupying the receptor sites. Therefore, the application of 1-MCP to field-grown cotton under heat stress conditions should reduce the level of ethylene and help alleviate the abiotic stress. In my studies the application of 1-MCP produced the expected positive result in Fayetteville but not in Marianna. This is explained by the different temperature regimes experienced at each location.

Quantifications of plant stress in our experiments using antioxidant enzymes and carbohydrates indicated that 1-MCP did not significantly reduce the level of measurable stress in the cotton plant. Cotton is very sensitive to environmental stresses (Krieg, 2002), and therefore, the ability to reduce the impact of abiotic stress with 1-MCP application is of major importance in cotton production for protection of yield.

In conclusion, the use of 1-MCP proved to have a positive effect on the physiology and yield of field-grown cotton in Fayetteville, AR two years in a row. Significant yield increases were observed in the treatments where 1-MCP was applied at first flower during both planting dates. While, there was no significant effect and possibly a negative effect in the Marianna, AR location. This effect could be explained by the fact that applications of 1-MCP lowered cotton
stress responses in Fayetteville experienced by low antioxidant activities and higher quantum yield during high temperature stress, Marianna did not experience high temperature stress for any long duration causing no significance and possibly a negative effect. The study needs to be continued and future research should be designed to clarify the effect of 1-MCP on the both high temperature stressed and non-stressed cotton plants to determine the best rates and timing of 1-MCP applications.

REFERENCES


Cotton (*Gossypium hirsutum* L.) is a major crop grown for fiber, oil and feed. Concerns about high year-to-year yield variability have been on the rise. This has been related to the extreme sensitivity of cotton to environmental stress conditions, drought and high temperatures in particular, which causes fruit shed, reduces photosynthate assimilation, and decreases yield. High temperatures adversely affect plant growth, particularly during anthesis (Snider et al., 2011) and increase ethylene production by plants. Ethylene is the key plant growth regulator that is produced during stress and triggers physiological processes that include increased levels of antioxidant enzyme activity which may act to increase tolerance to the stress conditions. Although, some ethylene is necessary for normal plant growth, increased endogenous ethylene levels are associated with fruit shed, pollen sterility and poor fertilization.

I hypothesized that high temperatures would negatively affect cotton plants during reproductive development and that applications of the anti-ethylene compound 1-methylcyclopropene (1-MCP) to cotton plants could inhibit the physiological stress responses associated with higher levels of ethylene. It was expected that plants treated with 1-MCP would exhibit less fruit abscission. As a result, higher and less variable yields could be achieved without major changes in management and production costs. The objective of this study was to determine the effect 1-MCP on the physiology and yield of cotton in field and controlled environment conditions.

Three experiments were conducted including two under field conditions and one in controlled environment chambers. The objectives of the field studies were to evaluate the effect of 1-MCP on the physiology and yield of cotton. The objectives of the growth room studies were
to investigate the effects of both high temperatures and 1-MCP on the physiology of cotton plants under high temperature and normal temperature conditions.

The field studies showed that 1-MCP treatments at Marianna had no significant effect on the antioxidant activity glutathione reductase in the ovaries and yield parameters. The lack of effect on GR was surprising as GR activity was expected to be lower in 1-MCP treated plants. 1-MCP treatments at Fayetteville also showed no significant difference in glutathione reductase activity, as well as calcium and carbohydrate concentrations in the ovaries, but did have significant effects on the yield parameters. Yields from Fayetteville showed significant increases from the 1-MCP application applied at First Flower for both years. This yield increase resulted from an increased number of bolls in the 1-MCP treatment, indicating that 1-MCP had inhibited the negative effect of increased ethylene from the high temperature on boll abscission.

The growth room studies showed that plants under high temperature stress exhibited higher antioxidant glutathione reductase activity, decreased starch in the ovaries and increased starch in the subtending leaves. It was expected that high temperatures would increase GR as the plants attempted to counteract the stress, and decrease starch due to effects on leaf gas exchange. These responses were observed at one day post-anthesis. However, 1-MCP had no significant effects on glutathione reductase or starch, even though the 1-MCP would have decreased the ethylene levels and therefore less GR would be needed and less effect on gas exchange would result in less starch in leaves and more in the ovaries. This was not the case.

In conclusion, high temperatures negatively affect cotton plants during reproductive development and 1-MCP applications had significant effects on cotton yields at Fayetteville, which resulted from the positive influence 1-MCP had on reducing boll abscission. Results also showed that 1-MCP had no effect on the physiology and yield of plants in Marianna, the lack of
effect being attributed to lower temperatures during both growing seasons, i.e., the temperatures in Marianna were much lower than that of Fayetteville resulting in high temperature stress only at the Fayetteville location. Overall, our studies indicated that 1-MCP application could potentially be used in cotton production to overcome environmental stress problems and achieve higher and more stable yields, but may have no effect on plants not experiencing stress.

Future research should investigate the use of 1-MCP to elucidate the rate, frequency and timing of its application to positively and consistently impact yield. These studies should focus on the triggers for 1-MCP applications such as high temperature and upper temperature threshold for timing applications.
APPENDIX I

Chapter I

Ovary GR Activity analysis of variance

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Ovary Glucose analysis of variance

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Ovary Fructose analysis of variance

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Leaf Glucose analysis of variance

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Leaf Sucrose analysis of variance

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APPENDIX II

Chapter II

Ovary GR Activity analysis of variance Marianna, 2010 experiment

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Ovary GR Activity analysis of variance Marianna, 2011 experiment

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Subtending leaf GR Activity analysis of variance Marianna, 2010 experiment

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Membrane leakage analysis of variance Marianna, 2010 experiment

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Seedcotton yield analysis of variance Marianna, 2010 experiment

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<td>Planting Date</td>
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<tr>
<td></td>
<td>Treatment</td>
</tr>
<tr>
<td>Ovary total calcium analysis of variance Fayetteville, 2010 experiment</td>
<td>Planting Date</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
</tr>
<tr>
<td>Ovary water soluble calcium analysis of variance Fayetteville, 2010 experiment</td>
<td>Planting Date</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
</tr>
<tr>
<td>Ovary water soluble calcium analysis of variance Fayetteville, 2011 experiment</td>
<td>Planting Date</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
</tr>
</tbody>
</table>
Ovary glucose activity analysis of variance Fayetteville, 2010 experiment

Source | Prob > F
---|---
Planting Date | 0.3244
Treatment | 0.3347

Ovary fructose activity analysis of variance Fayetteville, 2010 experiment

Source | Prob > F
---|---
Planting Date | 0.7707
Treatment | 0.6157

Ovary sucrose activity analysis of variance Fayetteville, 2010 experiment

Source | Prob > F
---|---
Planting Date | 0.1045
Treatment | 0.4387

Ovary starch activity analysis of variance Fayetteville, 2010 experiment

Source | Prob > F
---|---
Planting Date | 0.1232
Treatment | 0.4199

Ovary glucose activity analysis of variance Fayetteville, 2011 experiment

Source | Prob > F
---|---
Planting Date | 0.2281
Treatment | 0.9260

Ovary fructose activity analysis of variance Fayetteville, 2011 experiment

Source | Prob > F
---|---
Planting Date | 0.2281
Treatment | 0.9260

Ovary sucrose activity analysis of variance Fayetteville, 2011 experiment

Source | Prob > F
---|---
Planting Date | 0.0139
Treatment | 0.3915

Leaf glucose activity analysis of variance Fayetteville, 2011 experiment

Source | Prob > F
---|---
Planting Date | 0.8848
Treatment | 0.3207

Leaf fructose activity analysis of variance Fayetteville, 2011 experiment

Source | Prob > F
---|---
Planting Date | 0.2644
Treatment | 0.7073

Leaf sucrose activity analysis of variance Fayetteville, 2011 experiment

Source | Prob > F
---|---
Planting Date | 0.6016
Treatment | 0.1789
Seedcotton yield analysis of variance Fayetteville, 2010 experiment
Source Prob > F
Planting Date 0.1887
Treatment 0.0043

Seedcotton yield analysis of variance Fayetteville, 2011 experiment
Source Prob > F
Planting Date <.0001
Treatment 0.0045

Lint yield analysis of variance Fayetteville, 2010 experiment
Source Prob > F
Planting Date 0.1686
Treatment 0.0021

Lint yield analysis of variance Fayetteville, 2011 experiment
Source Prob > F
Planting Date <.0001
Treatment 0.0065

Seed production analysis of variance Fayetteville, 2010 experiment
Source Prob > F
Planting Date 0.2055
Treatment 0.0068

Seed production yield analysis of variance Fayetteville, 2011 experiment
Source Prob > F
Planting Date <.0001
Treatment 0.0038

Boll number analysis of variance Fayetteville, 2010 experiment
Source Prob > F
Planting Date 0.7164
Treatment 0.0071

Boll number analysis of variance Fayetteville, 2011 experiment
Source Prob > F
Planting Date 0.0032
Treatment 0.0040
Marianna, AR 2010 daily temperatures

Fayetteville, AR 2010 daily temperatures
Marianna, AR 2011 daily temperatures

Fayetteville, AR 2011 daily temperatures