STRESS PHYSIOLOGY IN COTTON

NUMBER SEVEN
THE COTTON FOUNDATION
REFERENCE BOOK SERIES

Edited by
Derrick M. Oosterhuis

STRESS PHYSIOLOGY IN COTTON

THE COTTON FOUNDATION

Reference Book Series

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Monsanto is proud to be a member of the Cotton Foundation and sponsor of ***STRESS PHYSIOLOGY IN COTTON***, the seventh book in the Cotton Foundation's cotton reference book series. The sponsorship is a reflection of the company's belief that continued support of the development of technologies and materials that promote the production of more cost-effective, higher yielding cotton, will make a stronger cotton industry.

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STRESS PHYSIOLOGY IN COTTON

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COTTON PHYSIOLOGY BOOK SERIES

FOREWORD

The Cotton Foundation Reference Book Series started with the first publication ***COTTON PHYSIOLOGY*** in 1985, edited by J.R. Mauney and J.M. Stewart, followed by a second book, ***PHYSIOLOGY OF COTTON***, edited by J.M. Stewart, D.M. Oosterhuis, J.J. Heitholt, and J.R. Mauney published in 2010. This cotton physiology-related series is being continued using a smaller book format with each future book covering a specific pertinent topic. The smaller book format will facilitate timely publication and reduce the cost. The books will be published in book form as well as on CD's. Each book will incorporate a special symposium on a topic chosen by members of the National Cotton Council, Agronomy and Physiology Conference and held at the Beltwide Cotton Conferences. Prominent speakers will be invited to partake in the symposium, and together with additional invited authorities, will make up the subsequent book. The first of the new small book physiology of cotton series is on "Stress Physiology". The next symposium, to be held at the Beltwide Cotton Conferences in Atlanta in January 2011, and subsequent book, will be entitled "Flowering and Fruiting in Cotton".

PREFACE

If cotton production is to be sustainable and profitable, it is essential to know about the growth of the plant and how it responds to environmental stress. With its indeterminate growth habit the cotton plant self- stresses; that is, it grows and expands until some internal or external stresses begin to inhibit that growth and expansion. A sound understanding of physiological processes and how they respond to stress is needed to formulate strategies to manage those stresses to maximize production profitability. Choices about planting and harvest date, cultivar selection for soil and field location, fertility, pest management, and cultivation are all basically stress-management decisions. The effect of temperature, moisture, nutrition and pest attacks on cotton growth and yield depends upon the severity and timing of the stress and the ability of the plant to respond and adapt to it. While some of the effects of stress such as wilting have immediate cause and effect relationships, some effects such as pollen fertility are subtle and delayed in expression. Therefore, detailed knowledge of the effects of various stresses on the physiology of cotton is essential to an understanding of resistance and survival mechanisms for breeding for stress resistance and for formulation of improved management practices.

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Photography by Bill Robertson

Chapter 1

HIGH TEMPERATURE STRESS ON FLORAL DEVELOPMENT AND YIELD OF COTTON

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INTRODUCTION

Increased temperatures from global climate change are projected to cause substantial losses in crop productivity by the end of the twenty-first century. High temperature is predominant among the cardinal ecological factors that determine crop growth and productivity (Al-Khatib and Paulsen, 1999). In cotton, temperature is a primary controller of the rate of plant growth, developmental events, and fruit maturation (Baker, 1965). An optimum temperature range of 20 to 30°C has been reported for cotton (Reddy *et al*, 1991), but cotton is successfully grown at temperatures in excess of 40°C in India and Pakistan for example. There is no clear consensus about the optimum temperature for cotton as plant response varies with plant developmental stage and plant organ (Burke and Wanjura, 2009). The effects of high temperature on germination, seedling growth, vegetative growth and crop development have been well documented (e.g., Hodges *et al*., 1993; Reddy *et al*., 1996). Although adverse temperatures can affect all stages of development, the crop seems to be particularly sensitive to adverse temperatures during reproductive development (Oosterhuis, 2002). The objective of this review is to compile the literature of the effects of high temperature on reproductive development in cotton with emphasis on events occurring in the flower following pollination leading to fertilization and seed set.

TEMPERATURE REQUIREMENTS OF COTTON

Cotton in its native state grows as a perennial shrub in a semi-desert habitat, and as such requires warm temperatures. However, despite originating from hot climates, cotton does not necessarily yield best at excessively high temperatures, and a negative correlation has been reported between yield and high temperature during flowering and early boll development (Oosterhuis, 1999) (Fig. 1). Ninety years ago, Balls (1919) reported that cotton in the field in Egypt seemed to grow best around 32°C, and that prolonged temperatures above 35°C were harmful. Work in growth chambers in Mississippi showed that the ideal temperature range for cotton was from 20 to 30°C (Reddy *et al*, 1991). Comparison of long-term temperature data and average yearly cotton yields from eastern Arkansas showed that yields decreased significantly when the mean maximum day temperature for July exceeded 32°C (Oosterhuis, unpublished). The thermal kinetic window (TKW) for enzyme activity strongly correlates with optimal temperatures for gen-

eral metabolism and growth for various species (Burke *et al*., 1988; Burke, 1990). The TKW for cotton is between 23.5 and 32°C (Burke *et al*., 1988). Because typical daily high temperatures are often in excess of this range during the growing season, high temperature represents a major limitation to crop development and productivity.

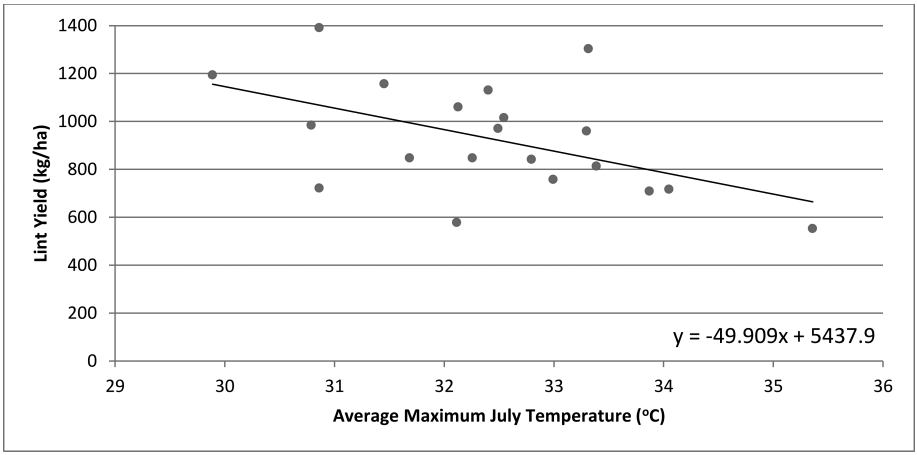

Figure 1. Negative correlation between cotton yield and high temperature during July when flowering and early boll development occur in Arkansas. (Adapted from Oosterhuis, 1999).

The optimum temperature for stem and leaf growth was about 30°C (Hodges *et al*., 1993). Once temperatures reach about 35ºC, growth rate and photosynthesis of cotton begins to decrease (Bibi *et al*., 2008, 2010). However, average daily maximum temperatures during flowering and boll development in the US Cotton Belt are almost always above 35°C, and well above the optimum for photosynthesis. Reddy *et al*. (1991) observed a 50% decline in total shoot biomass for Upland cotton plants grown under a 40/30°C day/night temperature regime relative to plants grown under the optimal day/night temperature condition (30/20°C). Similarly, a decline in dry matter production at day temperatures in excess of 30°C was observed for Pima cotton (Reddy *et al*., 1995). Temperatures in excess of the optimum also result in significant declines in leaf area. For example, leaf expansion is optimal under a 30/22°C day/night temperature regime for Upland cotton and declines at temperatures in excess of this growth temperature regime (Reddy *et al*., 1992c). Reddy *et al*. (1995) observed a comparable trend for Pima cotton with leaf area declining significantly at high temperatures above 31.3°C. Recently, Bibi *et al*. (2010) showed that leaf extension growth in Upland cotton declined significantly at temperatures above 35°C.

High temperatures can have both direct inhibitory effects on growth and yield, and indirect effects due to high evaporative demand causing more intense water stress (Hall, 2001). Plant water-deficit stress often coincides with high temperatures, but with irrigation and adequate precipitation this is not always a problem. Even though it is difficult to separate the exacerbating effects of water deficit on temperature stress, this review will only focus on the effects of elevated temperatures.

EFFECTS OF HIGH TEMPERATURE ON PLANT GROWTH AND DEVELOPMENT

All stages of vegetative development from germination to initiation of floral structures are affected by high temperature (Paulsen, 1994). Cotton developmental events occur much more rapidly as maximum temperatures increase (Reddy *et al*., 1996). Temperature plays a vital role in germination and emergence, and also in subsequent stand development, fruiting patterns and final yield. Roots generally have a lower optimum temperature range for growth than shoots, with optimum temperatures reported to be 30°C (Arndt, 1945; Pearson *et al*., 1970). McMichael and Burke (1994) showed that root growth was enhanced when the root temperatures were within or below cotton's thermal kinetic widow. The number of vegetative and fruiting branches produced per plant was strongly influenced by temperature, with an increase in vegetative branches and a decrease in fruiting branches with high temperatures (Fig. 2; Hodges et a., 1993). The number of fruiting sites was shown to increase by 50% as the temperature was raised from 30 to 40°C, however, the number of squares and bolls decreased dramatically above 35°C to zero at 40°C. Reddy *et al*. (1996) reported that young bolls shed when grown at average daily temperatures of 32°C or higher.

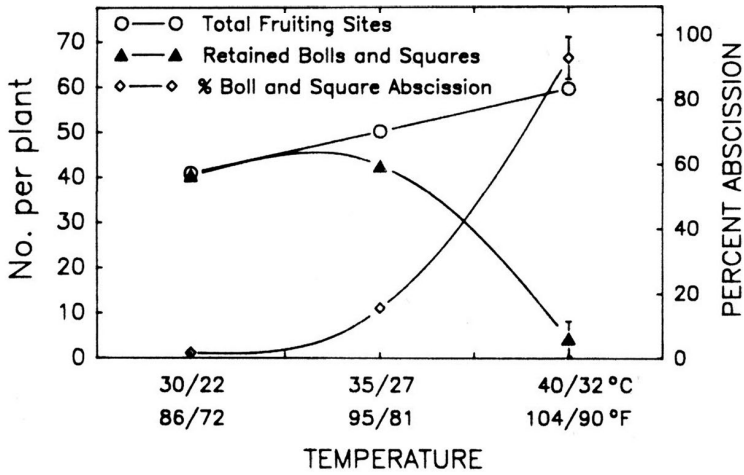

Figure 2. The effect of increasing day/night temperature on fruiting sites produced, bolls and squares retained, and percent boll and square abscission. (From Hodges *et al*., 1993).

Limitations to normal growth and development in cotton under heat stress result from numerous adverse effects on the physiology of the cotton plant. For example, photosynthesis in cotton is highly sensitive to temperatures above 35°C (Crafts-Brandner and Salvucci, 2000; Wise *et al*., 2004; Bibi *et al*., 2008; Snider *et al*., 2009). High temperature in cotton influences photosynthesis by decreasing quantum efficiency of the photosynthetic apparatus (Law and Crafts-Brandner, 1999; Bibi *et al*., 2008; Snider *et al*., 2009; Snider *et al*., 2010), decreasing chlorophyll content (Reddy *et al*., 2004; Snider *et al*., 2009; Snider *et al*., 2010), inhibiting rubisco activase (Feller

et al., 1998; Law and Crafts-Brandner, 1999; Crafts-Brandner and Salvucci, 2000), decreasing membrane integrity (Rahman *et al*. 2004; Schrader *et al*., 2004; Bibi *et al*., 2008), and increasing photorespiration (Perry *et al*., 1983). Additionally, high temperature significantly increases dark respiration rates in a variety of species and can ultimately result in lower translocation rates to developing sinks. For example, Cowling and Sage (1998) found that *Phaseolus vulgaris* plants exposed to high day/night temperature regimes had respiration rates nearly twice those of plants under the control temperature regime. Timlin *et al.* (2006) found that photosynthate partitioning to developing potato tubers decreased when temperatures increased above the optimum (20°C), and the decrease in carbon allocation to the tubers was strongly associated with high respiratory carbon losses. Studies investigating the effect of high night temperature in cotton have shown that high night temperature increases respiration rates (Arevalo *et al*., 2008; Loka and Oosterhuis, 2010), decreases soluble carbohydrate concentrations in source leaves (Arevalo *et al*., 2008; Loka and Oosterhuis, 2010), increases abscission (Arevalo *et al*., 2008), and results in significantly lower yield (Arevalo *et al*., 2008; Gipson and Joham, 1968).

HIGH TEMPERATURE AND REPRODUCTIVE DEVELOPMENT

Reproductive development is particularly sensitive to high temperature both before and after anthesis. This has been clearly demonstrated in cotton (Reddy *et al*., 1996; Oosterhuis, 2002) and other crops such as cereals (Paulsen, 1994). The sequence of reproductive development is also hastened as temperatures increase, i.e., the time to the appearance of first square, first flower and first mature open boll decreased as the average temperature for each event increased (Reddy *et al*., 1996). In addition, the development of flowers up the main stem, the vertical flowering interval, decreases with increasing temperature (Hodges *et al*., 1993). The total number of fruiting sites produced increased approximately 50% as the temperature increased from 30°C to 40°C, whereas at temperatures above 35°C abscission increased sharply with near zero retention of bolls at 40°C (Hodges *et al*., 1993). Boll retention decreases significantly under high temperature (Reddy *et al*., 1991; Reddy *et al*., 1992b; Reddy *et al*., 1995; Reddy *et al*., 1999; Zhao *et al*., 2005) and is reported to be the most heat sensitive component of cotton growth and development. For example, Reddy *et al*. (1991) observed that temperatures in excess of a 30/20°C day/night temperature regime resulted in significantly lower boll retention due to enhanced abortion of squares and young bolls. Subsequently, Reddy *et al*. (1992a) and Reddy *et al*. (1992b) observed declines in boll retention at temperatures in excess of a 30/22°C day/night temperature regime for both Pima and Upland cotton, respectively. An additional study showed even greater sensitivity of boll retention to increasing temperatures, where boll retention was negatively impacted at day temperatures in excess of 26.6°C (Reddy *et al*., 1995). Recently, Zhao *et al*. (2005) found that cotton plants exposed to a 36/28°C day/night growth temperature regime retained approximately 70% fewer bolls than plants grown under a 30/22°C day/night temperature regime. In this study, there was a strong correlation between high abscission rates and low nonstructural carbohydrate contents of the floral buds. Pima cotton appears to be more tolerant to higher temperatures than Upland Delta-type cotton (Hodges *et al*., 1993).

There is no exact identification of the most heat-sensitive aspect of the reproductive process in cotton, but Reddy *et al* (1996) concluded that there was a short period associated with flowering when the reproductive process is most vulnerable to average daily temperatures above 32.8°C to 34.4°C. Because a number of reproductive processes must occur in a highly concerted fashion during flowering for fertilization to occur, sexual reproduction is only as tolerant to heat stress as the most thermosensitive process (Hedhly *et al*., 2009; Zinn *et al*., 2010), and depending upon the timing, duration and severity, heat stress can limit fertilization by inhibiting male (Jain *et al*., 2007) and female (Saini *et al*., 1983) gametophyte development, pollen germination (Burke *et al*., 2004; Kakani *et al*., 2005; Jain *et al*., 2007), and pollen tube growth (Burke *et al*., 2004; Hedhly *et al*., 2004; Kakani *et al*., 2005; Snider *et al.,* 2011a).

Anthesis

The day of anthesis is a critical event in the reproductive development of *Gossypium hirsutum*. The flower opens as a white flower at dawn (Stewart, 1986) with pollination reported to occur between 0700 and 1100 h (Pundir, 1972) and germination within 30 minutes after pollination (Stewart, 1986). The pollen tube extends through the transmitting tissue of the style and fertilization occurs between 12 and 24 h later (Stewart, 1986). Successful *in vivo* pollen tube growth and subsequent fertilization of the ovule is a prerequisite for seed formation in *G. hirsutum*, and seeds with their associated fibers are the basic components of yield. Therefore, any abiotic stress that inhibits directional pollen tube growth from the stigma to the ovules on the day of anthesis and limits fertilization will also limit yield.

Pollination and Pollen Germination

Pollination of a receptive stigma on the day of anthesis requires that the anthers dehisce and release their mature pollen grains on the stigmatic surface. Heat stress has been shown to inhibit pollination by limiting anther dehiscence in rice (Matsui and Omasa, 2002) and the amount of pollen available for pollination in tomato (Peet *et al*., 1998). Anther indehiscence under excessively high temperature may also occur in cotton, but reports specifically addressing heat stress-induced anther indehiscence in cotton are lacking. Meyer (1966) reported a positive correlation between anther sterility and the maximum temperatures at 15 and 16 days prior to anthesis, suggesting that microgametophyte development was exceptionally sensitive to high temperature immediately after meiosis of the microspore mother cells had occurred. Much of the sensitivity of reproductive organs to heat stress has been attributed to the sensitivity of pollen grains to high temperature extremes. In contrast with female reproductive tissues, mature pollen grains of various species do not exhibit an acclimative response to heat stress (Dupuis and Dumas, 1990; Mascarenhas and Crone, 1996), and Kakani *et al*. (2005) has suggested that pollen grains on the exposed surface of the stigma would be more sensitive to high temperature than the more deeply seated ovules. Data from *in vitro* studies have shown that the optimal temperature range for cotton pollen germination is between 28 and 37°C (Burke *et al*., 2004; Kakani *et al*., 2005). Typical summer temperatures experienced in cotton growing regions normally exceed the optimal temperature, and adverse effects on pollen germination can be expected. However, this has not been clearly documented in the field.

Pollen Tube Growth and Fertilization

Due to the inability of mature pollen grains to effectively respond to high temperature, recent studies with cotton have focused on pollen tube elongation responses to high temperature using *in vitro* systems (Burke *et al*., 2004; Kakani *et al*., 2005; Liu *et al*., 2006). For example, Burke *et al*. (2004) and Kakani *et al.* (2005) showed that the optimal temperature across a range of *G. hirsutum* cultivars for pollen tube growth was from 28 to 32°C and 31.8°C, respectively. Liu *et al*. (2006) reported a 27.8°C temperature optimum for pollen tube growth and showed a strong correlation between maximum pollen tube growth and boll retention in *G. hirsutum*. In a previous study, Barrow (1983) compared techniques to evaluate the response of cotton pollen to high temperature, including pollen viability staining, pollen germination, pollen tube penetration of the stigma, penetration to the base of the style, and penetration of the ovules. This author showed that viability, and germinability were unaffected by pre-treating pollen with temperatures as high as 40°C. However, penetration of the stigma, style, and ovules was negatively impacted at 33°C and above, where cotton pollen exposed to temperatures ≥ 35°C for 15 h prior to anthesis was unable to penetrate the ovules. These findings suggested that pollen fertility under high temperature could not be directly inferred from pollen viability and germination measurements (Barrow, 1983). Using style penetration by the pollen tubes as a criterion for pollen fertility, Rodriguez-Garay and Barrow (1988) showed that heat tolerance could be genetically transferred to heat-sensitive lines by performing crosses with pollen that had been exposed to temperatures ≥ 35°C for 15 h, thereby only pollinating with pollen that survived the high temperature treatment. The maximum daily temperatures experienced by cotton plants during the flowering period often exceed the optimal temperature for successful pollen tube growth, with afternoon temperatures in excess of 38°C. Recently, Snider *et al*. (2009) reported that growing cotton plants under a 38/20°C day night temperature regime beginning one week prior to flowering was sufficient to cause a 32.9% decline in *in vivo* fertilization efficiency (Fig. 3). Poor fertilization efficiency under high temperature (Snider *et al*., 2009) likely accounts for the decline in seed set observed for cotton exposed to high temperature conditions in both the field (Pettigrew, 2008) and the growth chamber (Bibi *et al*., 2010). In a subsequent study, Snider *et al*. (2011a) reported that diurnal pollen tube growth rate through the transmitting tissue of the cotton style was slowed by moderately high temperature (34.6°C) under field conditions, whereas the number of ovules, number of fertilized ovules, fertilization efficiency, and pollen germination were unaffected. It was concluded that *in vivo* pollen tube growth rate was more sensitive to high temperature than any of the other measured parameters.

Carbohydrates and ATP

In contrast with *in vitro* pollen tube growth, *in vivo* pollen tube growth and fertilization depend not only upon the status of the male gametophyte but also upon the status of the pistil. For example, numerous studies have shown that *in vivo* pollen performance under heat stress is strongly influenced by pistil genotype (Gawel and Robacker, 1986; Hedhly *et al*., 2004;

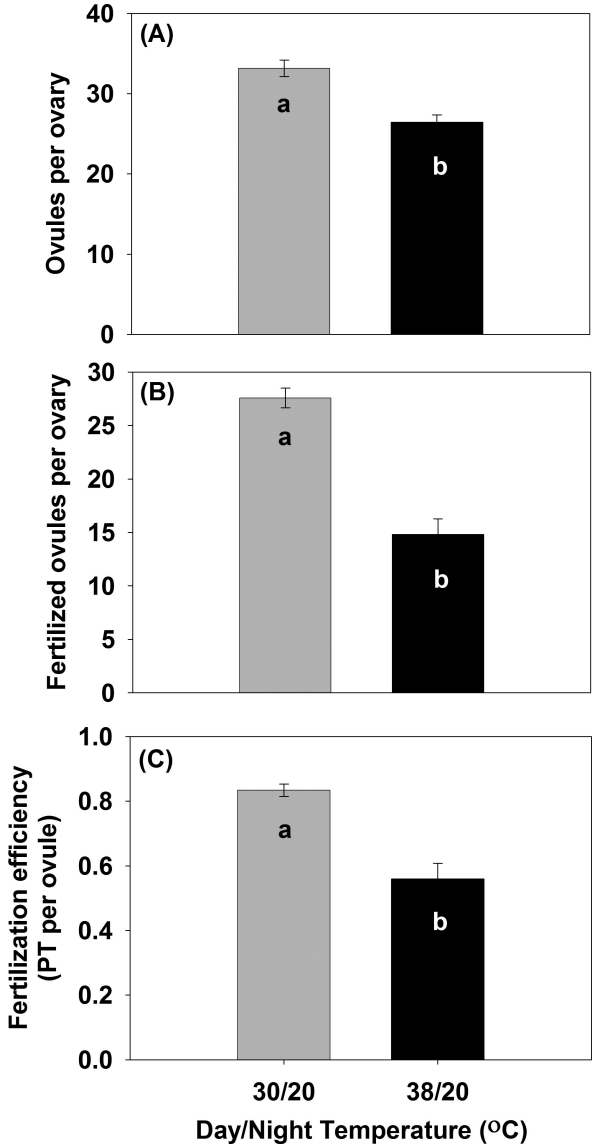

Figure 3. The number of total ovules (A), fertilized ovules (B) and fertilization efficiency expressed as pollen tubes per ovule (PT per ovule) (C) in *Gossypium hirsutum* pistils under normal (30/20°C) and high (38/20°C) day temperature regimes. Heat stress induced significant reductions in all three parameters measured. All values are means ± SE (n = 15), and values not sharing a common letter are significantly different (Student's t-test; $P < 0.05$). (From Snider *et al*., 2009).

Hedhly *et al*., 2005). Also, a number of physical and biochemical pollen-pistil interactions are required for successful pollen tube growth and fertilization of the ovule (Lord, 2003; Herrero and Hormaza, 1996). In various plant species, a readily available supply of carbohydrates in the pistil is essential in promoting a number of key events during plant reproductive development, including gametophyte development (Rodrigo and Herrero, 1998; Castro and Clemente, 2007; Jain *et al*., 2007), pollen germination (Jain *et al*., 2007), pollen tube growth (Herrero and Arbeloa, 1989; Gonzalez *et al*., 1996), and fertilization (Snider *et al*., 2009). For example, studies with peach (Herrero and Arbeloa, 1989) and kiwifruit (Gonzalez *et al*., 1996) have shown that *in vivo* pollen tube growth utilizes protein and carbohydrate reserves supplied to the pollen tube from the transmitting tissue of the style as tube growth transitions from an autotrophic phase (utilizing carbohydrates accumulated in the pollen grain) to a heterotrophic phase (utilizing external carbohydrates present in the style). In tobacco, germinating pollen grains and growing pollen tubes have been shown to exhibit a high energy requirement relative to vegetative tissues with respiration rates 10 times those of vegetative tissues (Tadege and Kuhlemeier, 1997).

Heat stress results in substantial alterations in the carbohydrate balance of reproductive tissues, causing poor reproductive success under high temperature. For example, Zhao *et al*. (2005) reported that high temperature conditions resulted in significantly lower levels of nonstructural carbohydrates in one day old cotton bolls and significantly higher abscission rates of young bolls; abscission rates were negatively correlated with the nonstructural carbohydrate content of the young boll. Some authors have shown that heat-tolerant cultivars of tomato (defined as cultivars with greater seed set under high temperatures) retain higher carbohydrate concentrations in the pollen grains and anther walls following chronic heat stress than do less heat-tolerant cultivars (Pressman *et al*., 2002; Firon *et al*., 2006). Additionally, Jain *et al*. (2007) reported that season-long high temperature in grain sorghum resulted in poor pollen germination and reduced seed set concomitant with non-detectable levels of sucrose and 50% reductions in starch content of microspores during late developmental stages relative to optimal temperature conditions. For cotton, Snider *et al*. (2009) recently reported that soluble carbohydrate and adenosine triphosphate (ATP) concentrations in pistils exposed to high ambient temperature conditions (38/20°C) one week prior to flowering were approximately 20.3 and 55% lower, respectively, on the day of anthesis than under control temperature conditions (30/20°C) (Fig. 4). Because the decline in energy reserves occurred concomitantly with a decline in fertilization efficiency (Fig. 3), these authors concluded that the energy demands for proper gametophyte development or pollen tube growth were insufficient and thereby limited the fertilization process. Subsequent research has shown that a cotton cultivar known to exhibit reproductive thermotolerance (VH260), as evidenced by good boll retention and stable fertilization efficiency under high temperature, also had higher pistil ATP concentration than a conventional cultivar (ST4554 B2RF) widely utilized by cotton farmers in the Mississippi river delta in 2008 (Snider *et al*., 2011b). These findings suggest that the energetic status of the pistil may be a strong determinant of reproductive thermotolerance in cotton.

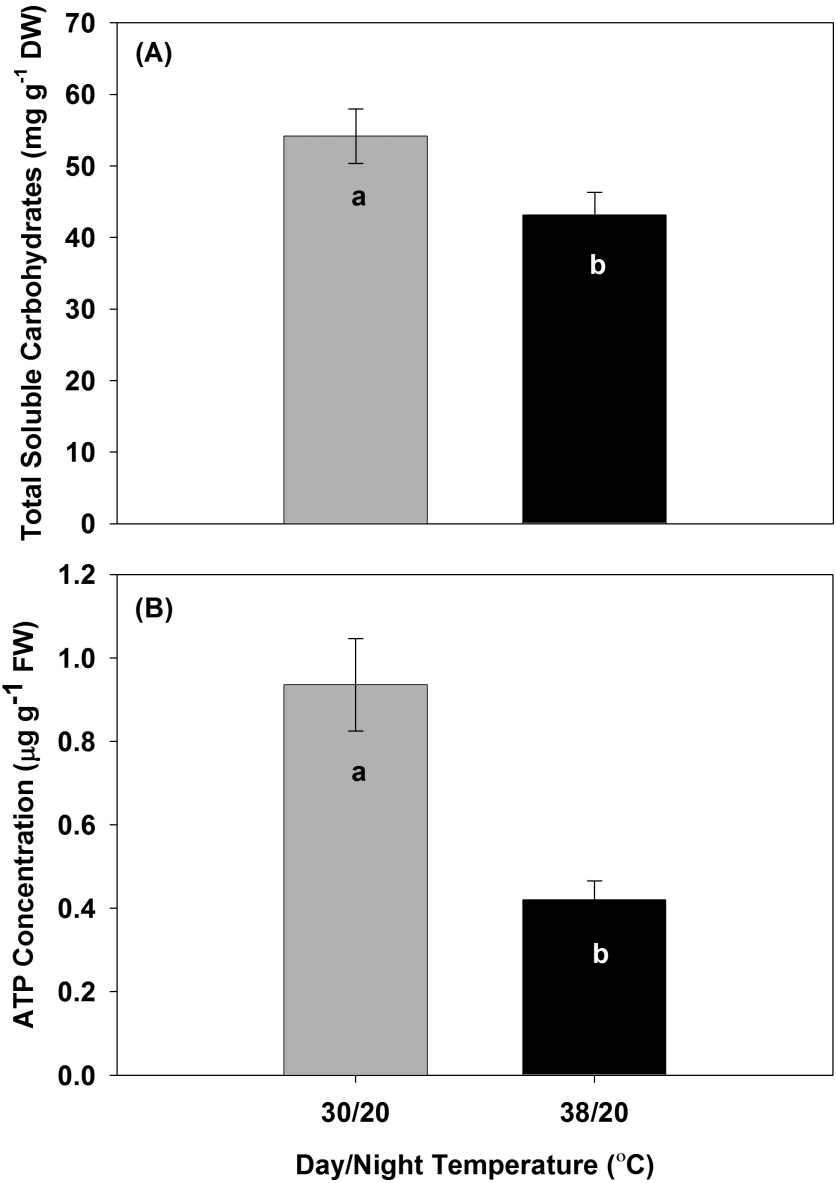

Figure 4. Total soluble carbohydrate and ATP concentrations of *Gossypium hirsutum* pistils exposed to high day temperatures (38/20°C) and optimal day temperatures (30/20°C). Heat stress reduced both soluble carbohydrate (A) and ATP levels (B). All values are means ± SE (n = 15), and values not sharing a common letter are significantly different (Student's t-test; $P < 0.05$). (From Snider *et al*., 2009).

The Leaf Subtending the Fruit

Because the carbohydrate balance of reproductive tissues strongly influences reproductive success in cotton (Zhao *et al*., 2005; Snider *et al*., 2009), it is also important to discuss the influence of high temperature on source strength. In *G. hirsutum*, most of the carbohydrate required for boll development is obtained from leaves subtending the reproductive unit (Ashley, 1972; Wullschleger and Oosterhuis, 1990). The importance of the subtending leaf in maintaining carbohydrate supply in the pistil was also demonstrated by Pettigrew (2001) who showed that exposure of cotton plants to shaded conditions (~70% of full sunlight) resulted in significant declines in nonstructural carbohydrate contents of both subtending leaves and ovules on the day of anthesis. The relationship between source leaf thermostability and reproductive success was recently demonstrated in a report showing that *Arabidopsis* mutants exhibiting thermostable photosynthesis also yield more seeds under high temperature than thermosensitive variants (Kurek *et al*., 2007). For cotton, Snider *et al*. (2009) reported that poor fertilization efficiency (Fig. 3) was associated with lower soluble carbohydrate and ATP content in the pistil under heat stress (Fig. 4) and lower photosynthetic rates, lower quantum yield, and lower total chlorophyll content in the subtending leaves. Subsequently, Snider *et al*. (2010) evaluated the subtending leaf photosynthetic response of two cotton cultivars known to exhibit differences in reproductive thermal stability: VH260 (thermotolerant) and ST4554 (thermosensitive). Although photosynthesis was significantly lower for ST4554 exposed to a 38/20°C day/night temperature regime relative to a 30/20°C day/night temperature regime, subtending leaf photosynthesis was unaffected by high temperature in VH260 (Snider *et al*., 2010). Using rapid leaf temperature changes and quantum efficiency measurements at a range of temperatures (15-50°C), these authors further reported a 7.5°C higher optimal temperature (T_{opt}) and a 5.5°C higher threshold temperature for quantum efficiency ($T_{15\Phi PSII}$) of VH260 subtending leaves relative to ST4554 subtending leaves (Snider *et al*., 2010; Fig. 5). These findings suggest that genotypic differences in reproductive thermotolerance are closely associated with the thermal stability of the subtending leaf.

Calcium, Antioxidants, and ROS.

Another factor essential for reproductive success is calcium. For example, calcium is known to promote pollen germination *in vitro* (Brewbaker and Kwack, 1963), and accumulation of high levels of loosely bound calcium in the transmitting tissue of the style prior to the passage of the pollen tube through that tissue is thought to promote pollen tube growth through the style in cotton (Zhang *et al*., 1997) and other species (Zhao *et al*., 2004; Ge *et al*., 2009) because calcium uptake by pollen tube tips *in vitro* is required for pollen tube growth by promoting vesicle fusion at the tip of the elongating tube (Pierson *et al*., 1996). Furthermore, calcium is known to promote fertilization (Faure *et al*., 1994; Tian and Russell, 1997) and egg activation (Digonnet *et al*., 1997). During heat stress, potentially damaging reactive oxygen species (ROS) accumulate in plant tissues (Foyer and Noctor, 2005; Tang *et al*., 2006) along with a concomitant increase in cytosolic calcium (Jiang and Huang, 2001; Gong *et al*., 1998). Calcium is essential in enhancing the antioxidant enzyme activity required to protect the plant under oxidative stress conditions via ROS scavenging (Gong *et al*., 1998; Jiang and Huang, 2001). In contrast with

antioxidant enzymes, NADPH oxidase (NOX) produces O_2^- in a calcium-augmented fashion, which is needed to soften cell walls and promote cell expansion during pollen tube growth (Potocky *et al.*, 2007).

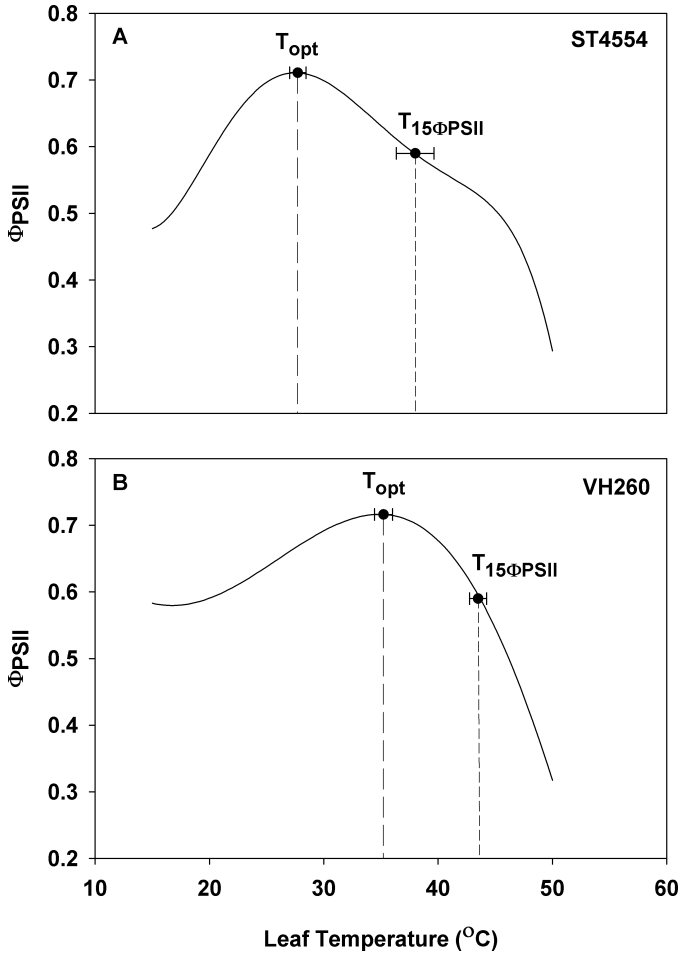

Figure 5. Effect of temperature on _PSII in *G. hirsutum* cv. ST4554 (A) and cv. VH260 (B). Leaves were illuminated with 500 μmol photons m^{-2} s^{-1} and incubated at temperatures ranging from 15 to 50°C for 5 min at each temperature prior to *Φ*PSII determination. In each graph, a representative curve illustrates how T_{opt} (the temperature at which the highest quantum efficiency was obtained for a given leaf) and $T_{15\Phi PSII}$ (the temperature causing a 15% decline in *Φ*PSII from the value at T_{opt}) were determined for a given cultivar. *G. hirsutum* cv. ST4554 had a 7.5 and 5.5°C lower (Student's *t*-test; $P < 0.05$) mean T_{opt} (27.7°C) and $T_{15\Phi PSII}$ (38°C), respectively, than VH260 (35.2 and 43.5°C, respectively). Horizontal bars = standard error. (From Snider *et al*., 2010).

Snider *et al*. (2009) recently reported increases in the water soluble calcium concentration (Fig. 6) and glutathione reductase activity (Fig. 7B) of heat-stressed cotton pistils, but a decline in NOX activity of pistils exposed to high day temperature (Fig. 7C). These authors suggested that a calcium-augmented antioxidant response to high temperature interfered with NOX activity required for successful pollen tube growth *in vivo*. Further research has shown that cotton pistils from a cultivar with known reproductive thermotolerance (VH260) also had significantly higher levels of total and water soluble calcium content than a more sensitive cultivar (ST4554 B2RF), and genotypic thermotolerance was associated with higher antioxidant enzyme (superoxide dismutase and glutathione reductase) activity in the pistil under optimal growth temperatures (Snider *et al*., 2011b). These findings suggest that calcium content and pre-stress antioxidant enzyme activity of the pistil may be important criteria for identifying thermotolerant cultivars. Additionally, the genotypic differences in subtending leaf thermostability discussed previously were shown to be dependent upon pre-stress antioxidant enzyme activity, where the thermotolerant cultivar had significantly higher levels of pre-stress antioxidant enzyme activity in the subtending leaf than the thermosensitive cultivar (Snider *et al*., 2010; Fig. 8).

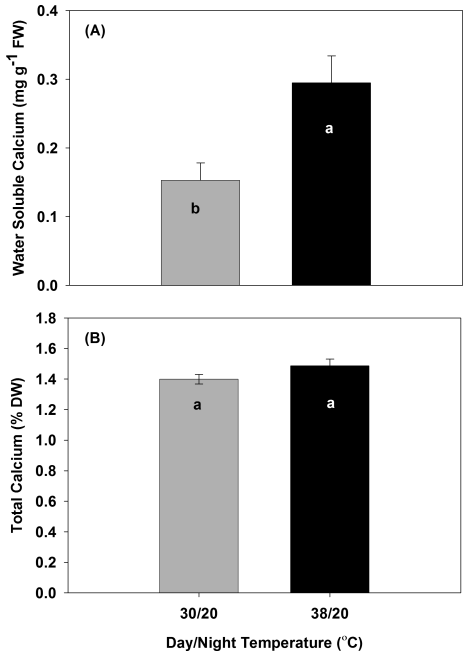

Figure 6. Water soluble (A) and total calcium (B) responses to high day temperature in *Gossypium hirsutum* pistils exposed to heat stress (38/20°C) and optimal (30/20°C) temperature conditions. Heat stress induces a significant increase in water soluble calcium (A) levels but does not alter total calcium (B) content. All values are means ± SE (n = 15 for total calcium and n = 10 for water soluble calcium). Values not sharing a common letter are significantly different (Student's t-test; $P < 0.05$). (From Snider *et al*., 2009).

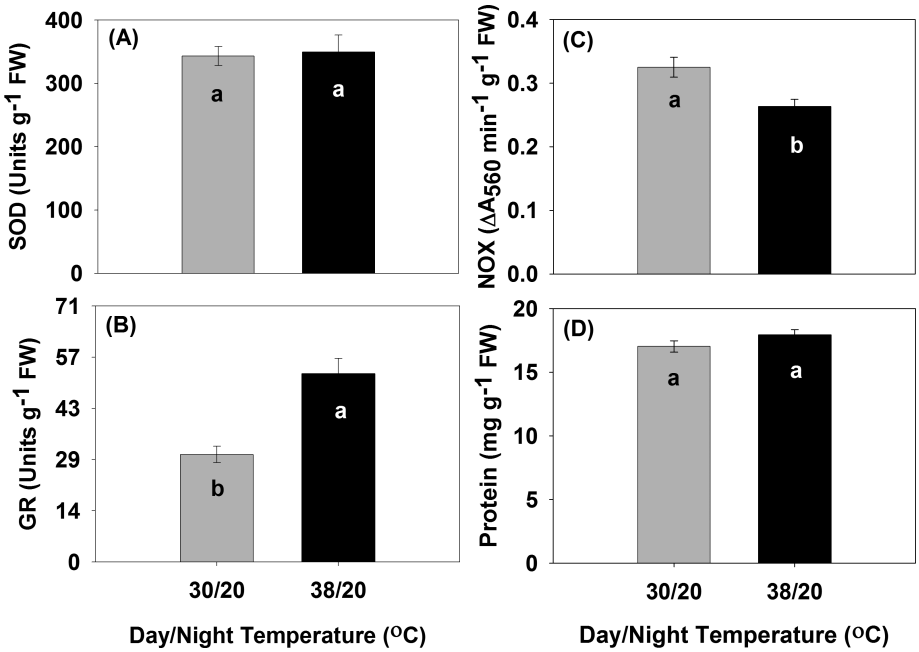

Figure 7. In *Gossypium hirsutum* high day temperature does not alter superoxide dismutase (SOD) activity (A), increases glutathione reductase (GR) activity (B), decreases NADPH Oxidase (NOX) activity (C) and does not change soluble protein content (D). All values are means ± SE (n = 15). Values not sharing a common letter are significantly different (Student's t-test; $P < 0.05$). (From Snider *et al*., 2009).

Polyamines

Polyamines have been associated with a large number of plant growth and developmental processes. In particular, they have been associated with floral initiation with increased polyamines concentration occurring during flowering in horticulture plants. Polyamines play an important role in flowers and seed induction and have been shown to decrease under high temperature stress. However, there is limited information about polyamines in cotton (*Gossypium hirsutum* L.) and no reports of effects on the flowering process and heat stress. Bibi *et al*. (2007) reported a negative correlation of temperature and polyamines, with polyamine content in cotton ovaries decreasing with increased canopy temperature. Subjecting the plants to high temperatures (38°C) compared to the optimum (30°C) significantly decreased spermidine and spermine levels but not putrescine (Bibi *et al*., 2010a). Successful seed fertilization was significantly decreased by the high temperature, and significantly increased by exogenous application of putrescine (Bibi *et al*., 2010a). The authors suggested the possibility of ameliorating high temperature stress in cotton flowers through exogenous application of putrescine.

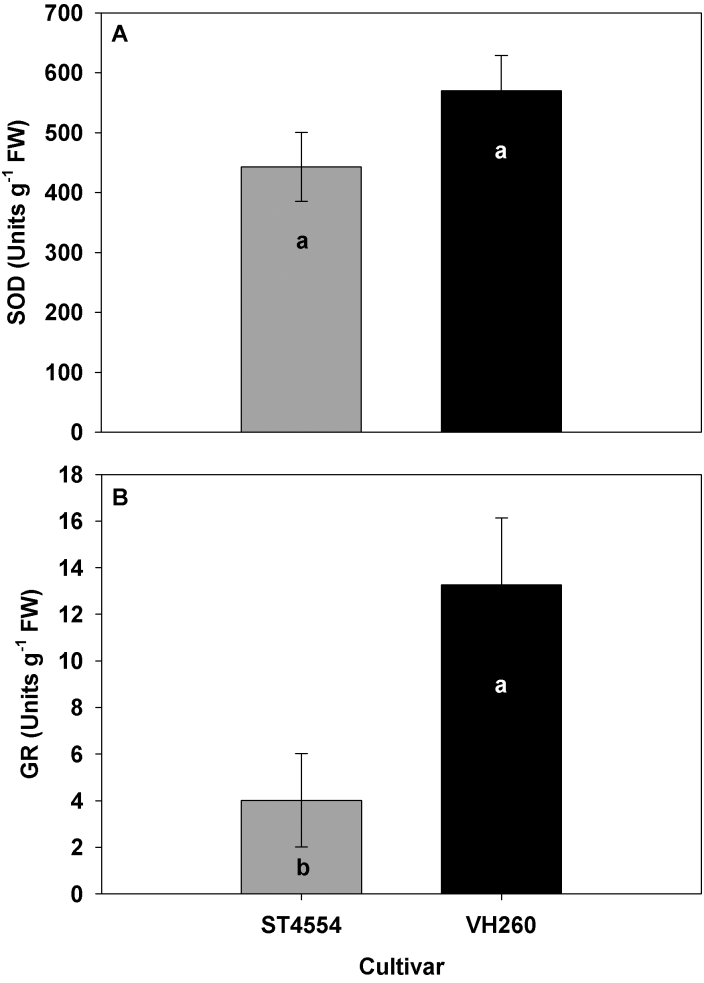

Figure 8. Effect of cultivar on SOD (A) and GR (B) activity of *G. hirsutum* grown under 30/20°C day/night temperature regime. GR was significantly higher in VH260 compared with ST4554 (B), whereas SOD was not significantly different (B). All values are means ± standard error (n = 6). Values not sharing a common letter are significantly different (Student's *t*-test; $P < 0.05$). (From Snider *et al*., 2010).

Genotypic Thermotolerance

Higher temperatures adversely influence the growth, development and yield of cotton, and with the increased concern about global warming, this has focused attention on the need for enhanced thermotolerance in commercial cultivars. A number of researchers have documented

genotypic thermotolerance in cotton (Cottee *et al*., 2007; Taha *et al*., 1981; Brown and Zeiher, 1998; Snider *et al*., 2010). However, although substantial genotypic variation exists in the cotton germplasm pool, this has generally not been exploited in breeding programs. Oosterhuis *et al*. (2009) reported that there does not appear to be sufficient genotypic differences in the current Upland cotton breeding trials grown in the US Cotton Belt for exploitation by plant breeders for improved thermotolerance.

Breeders have improved yields in Pima cotton (*Gossypium barbadence* L.) by increasing high temperature tolerance (Kittock *et al*., 1988), however little has been done to improve high temperature tolerance in Upland cotton (*G. hirsutum* L.). A possible solution to this problem is to utilize ruderal genetic material collected from the areas where cotton grows under conditions of extreme heat such as southern Mexico. Bibi *et al*. (2010) showed that a wild type cotton (*G. hirsutum* L. race Palmeri, PI681044) from coastal Oaxaca, Mexico exhibited significantly more thermotolerence than four commercial Upland Mid-south cotton cultivars (Tamcot Sphinx, FiberMax 960BR, Stoneville 474, and Deltapine 444BR). The ruderal *G. hirsutum* race Palmeri was significantly more tolerant to high temperature stress than the commercial cultivars (higher quantum yield of PSII, leaf extension growth, and antioxidant enzymes). Amongst the commercial cultivars tested, only Tamcot Sphinx showed some tolerance to high temperature. It has been speculated that year-to-year variability in yield of modern cotton cultivars is due to modern cultivars being more sensitive to environmental stress conditions compared to obsolete cultivars. Brown and Oosterhuis (2010) showed that modern cultivars (*G. Hirsutum* Stoneville 474 and Suregrow 747) had improved physiological responses under ideal temperature environments (30°C), however obsolete cultivars (*G. Hirsutum* Stoneville 213 and Deltapine 16) were less sensitive in leaf photosynthesis, chlorophyll fluorescence, and membrane integrity of leaves to high temperatures (38°C).

Snider *et al*. (2010) showed that genotypic differences in reproductive thermotolerance of upland cotton are closely associated with the thermal stability of the subtending leaf. These authors used two cotton cultivars: VH260 from Pakistan (thermotolerant) and ST4554 for the US Mid-south (thermosensitive), and found a 7.5°C higher optimal temperature for quantum efficiency of VH260 subtending leaves relative to ST4554 subtending leaves (Fig. 5).

EFFECT OF HIGH TEMPERATURE ON YIELD

Final yield has also been shown to be strongly influenced by temperature in cotton (Wanjura *et al*., 1969) and a negative correlation between cotton lint yield and high temperature was reported for the Mississippi Delta (Oosterhuis, 1999). Year-to-year variation in cotton yields, a major concern of cotton producers, has been associated with unpredictable variation in seasonal temperatures (Oosterhuis, 1999). Oosterhuis (unpublished) compared final lint yields with average maximum temperatures weekly after flowering for cotton in eastern Arkansas, and showed a significant decline in yield when average maximum temperatures exceeded 32°C during the flowering period. Reddy *et al*. (1996) reported a sharp decline in fruit efficiency (boll weight per total dry weight produced) when temperatures exceeded about 29°C. It is interesting that as long ago as ninety years, Balls (1919) reported that cotton in Egypt seemed to grow and yield best

around 32°C, and that prolonged temperatures above 35^0C were harmful. High, above average, temperatures during the day can decrease photosynthesis and carbohydrate production (Bibi *et al.*, 2008), and high night temperatures will increase respiration and further decrease available carbohydrates (Gipson and Joham, 1968; Loka and Oosterhuis, 2010), resulting in decreased seed set, reduced boll size and decreased number of seeds per boll, and the number of fibers per seed (Arevalo *et al.*, 2008).

Boll number and boll size, the basic yield components, are negatively impacted by high temperature. Boll retention has been shown to decrease significantly under high temperature (Reddy *et al*., 1991; Reddy *et al*., 1992b; Reddy *et al*., 1995; Reddy *et al*., 1999; Zhao *et al*., 2005) and was reported to be most heat sensitive yield component of cotton. For example, Reddy *et al*., (1991) observed that temperatures in excess of a 30/20°C day/night temperature regime resulted in significantly lower boll retention due to enhanced abortion of squares and young bolls. Subsequently, Reddy *et al*. (1992a) and Reddy *et al*. (1992b) observed declines in boll retention at temperatures in excess of a 30/22°C day/night temperature regime for both Pima and Upland cotton. An additional study showed even greater sensitivity of boll retention to increasing temperatures, where boll retention was negatively impacted at day temperatures in excess of 26.6°C (Reddy *et al*., 1995). Recently, Zhao *et al*. (2005) found that cotton plants exposed to a 36/28°C day/night growth temperature regime retained approximately 70% fewer bolls than plants grown under a 30/22°C day/night temperature regime. In this study, there was a strong correlation between high abscission rates and low nonstructural carbohydrate contents of the floral buds. In addition to negatively impacting boll retention, temperatures in excess of the optimum also result in decreased boll size (Reddy *et al*., 1999; Pettigrew, 2008). The cotton crop, due to its perennial nature and indeterminate growth habit can compensate for short periods of stress, such that variation in temperatures during the cropping season allows some flowers during the flowering period to escape exposure to damaging temperatures so that some bolls are eventually produced.

The number of seeds per boll is an important basic component of cotton yield. Groves (2009) emphasized the importance of seed number in determining yield by reporting that the number of seeds per acre accounted for more than 80% of total yield variability in cotton. Seed number is a function of the number of locules (carpels) per boll and the number of ovules per locule (Stewart, 1986). Several factors such as the lack of seed fertilization, post-fertilization termination of embryo growth, cultivar and environment can also contribute to variation in the number of seeds per boll (Turner *et al*., 1977). Researchers have shown that high temperature stress is a major factor negatively impacting seed development. For example, Reddy *et al*. (1999) showed that temperatures higher than 26.0°C increased short fiber mote frequency in Upland cotton and suggested that either fertilization had been negatively impacted due to insufficient pollen/ovule development or that fertilized ovules aborted soon after the fertilization event had occurred. Pettigrew (2008) reported that slight elevations in temperature (approximately 1°C above control temperatures) under field conditions were not sufficient to cause a decline in seed weight but were sufficient to cause a significant decline in seed number per boll, which was the primary cause of reduced yield under high temperature conditions. This was confirmed by observations of Lewis (2000) who compared a cool year 1990 in the Mid-south (mean maximum daily temperature of 32.2°C for July) with a hot year 1996 (mean maximum daily temperature of 36.6°C

for July) and showed that the number of seeds decreased in the hot year from 2.987 to 2.093 million per hectare. This was associated with a lower average number of seeds per boll, i.e. 23.6 seeds/boll in the hot year compared to 28 seeds/boll in the cool year. Lewis (2000) concluded that about 99 percent of the variation in number of seeds per hectare in his three year study was explained by changes in the mean maximum July temperatures. Although Pettigrew (2008) also observed declines in boll size and lint percent, boll size was more negatively affected than was lint percent; therefore, the author concluded that decreased seed number caused a decline in boll size and lint yield. Furthermore, Pettigrew (2008) speculated that heat stress may have decreased seed number by compromising ovule fertilization, which was subsequently confirmed by Snider *et al*. (2009) (Fig. 3).

SUMMARY

Cotton originates from hot climates, but does not necessarily yield best at excessively high temperatures, and a negative correlation has been reported between yield and high temperature during early boll development. Although cotton is sensitive to high temperature at all stages of growth, it is particularly sensitive to high temperatures during reproductive development, and environmental stress during floral development represents a major limitation to crop development and productivity. There is no clear consensus about the optimum temperature for cotton as plant response varies with plant developmental stage and plant organ, and the environment in which the cultivar was developed. The optimal thermal window for Upland cotton is 23-32°C in which metabolic activity is most efficient. In *Gossypium hirsutum* L., canopy growth and reproductive development are severely inhibited at temperatures in excess of the optimal day/night temperature regime of 30/20°C, which commonly occur in the US Cotton Belt during flowering and boll development.

Because a number of reproductive processes must occur in highly concerted fashion during the progamic phase (from pollination to fertilization) for successful fertilization and seed production to occur, final yield in cotton is exceptionally sensitive to high temperatures during the flowering period. High temperatures typical of those experienced during a normal growing season in the U.S. Cotton Belt are sufficient to significantly inhibit fertilization, seed set, and yield in thermosensitive cotton cultivars. Depending upon the duration, timing and severity of the stress, fertilization could be limited by poor gametophyte development, decreased pollen germination, and limited pollen tube growth. Under field conditions, diurnal pollen tube growth rate is extremely sensitive to moderately high temperatures, where exposure to moderately high ambient temperatures (34.6°C) results in slower pollen tube growth rates despite no change in pollen germination or ovule fertilization. Heat stress limits fertilization by decreasing subtending leaf photosynthesis, depleting pistil ATP and carbohydrates, increasing oxidative stress in the pistil, and altering pistil calcium concentrations. Having higher pistil concentrations of ATP and calcium is related to genotypic fertilization thermostability. Furthermore, reproductive thermotolerance in cotton is also associated with having elevated pre-stress antioxidant enzyme activity in both the subtending leaf and the pistil, which is likely an innate mechanism for coping with rapid temperature changes that are common under field conditions.

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Chapter 2

COTTON FLOWERS: POLLEN AND PETAL HUMIDITY SENSITIVITIES DETERMINE REPRODUCTIVE COMPETITIVENESS IN DIVERSE ENVIRONMENTS

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INTRODUCTION

Crop species grown throughout the world experience environmental stresses that limit their growth, development, and full expression of their genetic potential for agronomic yield. Comparison of average crop yields with reported record yields has shown that the major crops grown in the U.S. exhibit annual average yields three- to seven-fold lower than record yields due to unfavorable environmental conditions (Boyer, 1982). Analysis of yields from corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), soybean (*Glycine max* L.), sorghum (*Sorghum vulgare* L.), oat (*Avena sativa* L.), barley (*Hordeum vulgare* L.), potato (*Solanum tubersosum* L.), and sugar beet (*Beta vulgaris* L.) revealed that the average yield represented only 22% of the mean record yield. Crops with economically valuable reproductive structures showed the greatest discrepancy between average and record yields. Those crops having marketable vegetative structures exhibited approximately three-fold reductions in yield (Boyer, 1982). These data suggest that plants have high productivity potential, but are operating well below their genetic potential.

Yield loss might be lessened by identifying and optimizing those plant protective mechanisms that could be used to improve stress-resistant germplasm stocks. One such protective mechanism is acquired thermotolerance, a process postulated to be closely linked to the heat shock response. Plants are frequently exposed to elevated soil and air temperatures resulting in a reduction in their growth, development and ultimately productivity. Subjecting them to a period of sub-lethal elevated temperatures induces a transient state of thermotolerance, which raises the injury threshold and protects the plants from subsequent, otherwise lethal, high temperatures (Vierling, 1991). This acquisition of thermotolerance is a complex physiological phenomenon that has been shown to involve at least some heat shock proteins (HSPs). Although varying in magnitude among plant cultivars, most vegetative tissues exhibit an inducible heat shock response. Germinating pollen, however, has not been found to exhibit the heat shock protein induction pattern upon exposure to elevated sub-lethal temperatures and concomitantly exhibits rapid losses in viability upon heat exposure (Hopf *et al*., 1992). This may explain Boyer's observation that crops with economically valuable reproductive structures show the greatest discrepancy between average and record yields (Boyer, 1982).

Modest progress has been achieved in selecting cotton cultivars with improved heat tolerance by heat treatment of pollen prior to pollination allowing only the more heat tolerant pollen to be effective in subsequent crosses (Rodriguez-Garay and Barrow, 1988). The process of selecting pollen with improved heat tolerance could be accelerated with a rapid and reliable method of germinating cotton pollen to measure viability across a range of environmental stresses. Current pollen germination techniques include "hanging drop culture", "sitting drop suspension culture", "suspension culture" and "surface culture" (Shivanna and Rangaswamy, 1992). The hanging drop and sitting drop cultures use only small volumes of germination media and small amounts of pollen, and are therefore of limited usefulness in physiological and biochemical studies.

Cotton pollen has proved to be recalcitrant to traditional in vitro germination and pollen tube growth protocols. Kearney and Harrison (1932) described the failure of in vitro techniques and went so far as to use the percentage of pollen grains that burst when placed in weak sugar solutions as a measure of viability. Failures to germinate cotton pollen in vitro drove Iyenger to dissect cotton pollen tubes from in situ germinated pollen (Iyenger, 1938). Bronkers (1961) first described a reliable technique for in vitro cotton pollen germination. Miravalle (1965) has since reported that the pollen tubes grown in this media were short, the cytoplasm was cloudy and granular, and the process required 24 h or longer. Taylor (1972) described a medium that overcame many of the limitations outlined by Miravalle (1965). Taylor reported rapid pollen germination (2 to 3 h), more normal appearing cytoplasm, and longer pollen tubes. Wauford (1979) further improved upon Taylor's medium and averaged 47% germination and 2.6 mm pollen tube lengths. Although Wauford's protocol was an improvement upon Taylor's medium, the 2.6 mm pollen tube length achieved in vitro does not compare with the 20 to 40 mm tube lengths reported in vivo. The most recent in vitro cotton pollen germination report by Barrow (1981) described the use of a hanging drop technique to forcefully eject pollen tube-like structures. Recent findings in our laboratory revealed that the pollen tube-like structures were not tubes but were pollen cytoplasm ejected from the pollen as it osmotically ruptured in a way similar to that reported by Kearney and Harrison (1932).

Burke *et al*. (2004) described the development of a pollen germination media and technique that provides high pollen germination levels and improved pollen tube growth. In developing the media it was necessary to evaluate the following variables: temperature, humidity, pH, and carbon source. The temperature effect on pollen germination and pollen tube elongation was evaluated over a range of temperatures from 20 to 43˚C. Pollen germination was high across the range of temperatures from 20 to 37˚C. The percent pollen germination declined from a mean of 71% at 37˚C to 23% at 40˚C, with little germination occurring at 43˚C. Pollen tube elongation rate was low at 20˚C and increased with increasing temperature up to 28˚C. The 28 and 31˚C samples exhibited similar pollen tube lengths with significant (0.05 level) declines in tube length observed at 34˚C and above. Kakani *et al.* (2005) showed optimal pollen germination and elongation at 30˚C when evaluating pollen responses to temperature in five-degree increments.

The effect of humidity levels on pollen germination and pollen tube elongation was evaluated at 35, 50, 80, and 100% relative humidity (RH) (Burke *et al*., 2004). Pollen that germinated on media in 35% RH had short pollen tubes located at the interface between the pollen grain and

the germination medium. The 50% RH resulted in increased pollen tube length, while the best elongation occurred at 80% RH. Although germination levels were high, most pollen tubes remained short as they ruptured when incubated under 100% RH. A range of humidity (50 to 80%) can be used during pollen germination; however, if humidity levels are too low (35% or less), germination occurs, but only short tubes are observed. If the humidity level is too high (100%), germination occurs and tubes rupture shortly thereafter.

The present study investigated genetic variability in the abiotic stress tolerance of mature pollen. Heat stress was imposed on pollen in situ and evaluated in vitro for germination and pollen tube development responses. The importance of humidity levels on pollen viability and germination was also investigated. Laboratory-based tests permitting rapid evaluation of the overall abiotic stress tolerance of the pollen were developed. Our findings provide breeders with a previously unexplored reservoir of genetic diversity associated with reproductive abiotic stress tolerance.

HEAT SENSITIVITY

Cotton seeds were planted into 5 gallon pots containing 900 g of Sunshine Mix #1 soil (Sun Gro Horticulture Distributors Inc., Bellevue, WA). Three seeds were planted per pot pots were placed on benches in a greenhouse set to provide a 30/25°C day/night cycle. Plants were grown throughout the year and 430 W high-pressure sodium lights (P. L. Light Systems, 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. Flowers were harvested between 0930 and 1030 h from the greenhouse plants and were placed on moistened Model 583 Gel Dryer Filter Paper (Bio-Rad Laboratories, Hercules, CA) in a Pyrex baking dish. The flowers and filter paper were covered with CO_2 permeable Glad ClingWrap (The Glad Products Company, Oakland, CA). Temperature incubations were performed in the presence of high humidity from the wet filter paper in an attempt to separate temperature stress from humidity responses. Replicate samples were placed in the dark in VWR Model 2005 incubators (Sheldon Manufacturing, Inc., Cornelius, OR) set to 39 or 28°C. The trays containing the flowers were incubated for 5 h, the flowers were then removed from the trays and the pollen collected by gently tapping the inverted flower. The pollen was germinated in vitro at 28°C according to the procedure of Burke *et al*. (2004). The pollen was incubated on the media for 1 h prior to analysis. Pollen germination was determined microscopically using a Leica MZ6 modular stereomicroscope (Leica Microsystems Inc., Bannockburn, IL). The percent germination was determined for 16 replicate samples harvested over a two-month period.

Temperature incubations (39°C 5h) under high humidity in an attempt to separate temperature stress from humidity responses showed no significant difference in the heat induced decline in pollen germination among the Suregrow 248, Stoneville 474, Deltapine 565, NM67, Acala Maxxa and Phytogen 72 cotton lines (Fig. 1). All of the lines exhibited a 55 to 65% decline in pollen germination following the heat treatment.

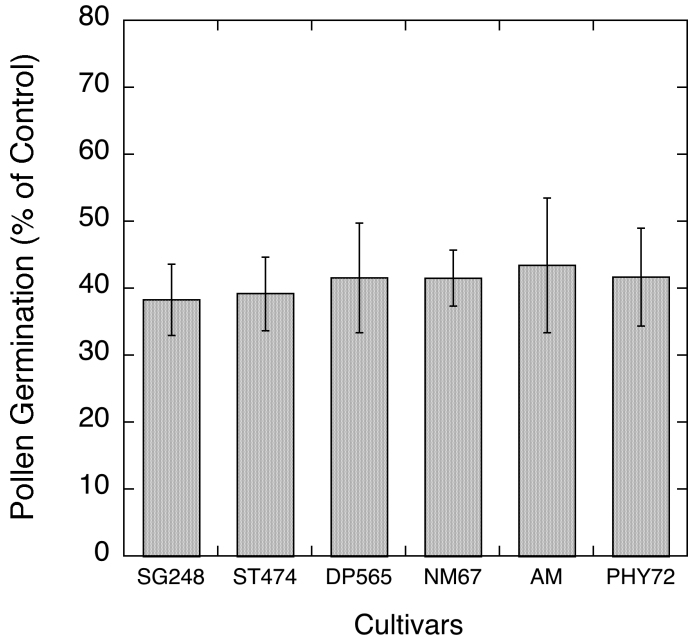

Figure 1. High temperature sensitivity of cotton pollen from Suregrow 248, Stoneville 474, Deltapine 565, NM67, Acala Maxxa, and Phytogen 72 cotton cultivars. Flowers were incubated at 28 or 39°C in high relative humidity chambers for 5 h, the pollen removed, and in vitro germination evaluated at 28°C and 80% relative humidity. Error bars represent the standard error of twelve replications.

IN SITU POLLEN DEHYDRATION

Pollen drying was evaluated in a laboratory with an ambient 25% RH environment. Humidity level was monitored using a Model H08-004-02 HOBO RH and temperature sensor (Onset Corporation; Bourne, MA) and found to be 25% RH throughout the experiment. Flowers were harvested between 0930 and 1030 h from the greenhouse plants and placed in Ziploc plastic bags for transport into the laboratory. Upon returning to the laboratory, the petals of the flowers were removed and the flowers with exposed pollen were placed on a bench top for 6.5 h. Following the treatment, the pollen was germinated in vitro at 28°C according to the procedure of Burke *et al*. (2004). The pollen was incubated on the media for 1 h prior to analysis. Pollen germination was determined microscopically using a Leica MZ6 modular stereomicroscope (Leica Microsystems Inc., Bannockburn, IL). The percent germination was determined for 17 replicate samples harvested over a two-month period. The Stoneville 474 pollen showed a 44% reduction in pollen germination, the Suregrow 248 pollen showed a 31% reduction, Deltapine 565 pollen showed a 20% reduction, NM67 pollen showed a 33% increase, Acala Maxxa showed a 45% reduction and Phytogen 72 showed a 42% reduction in pollen germination (Fig. 2).

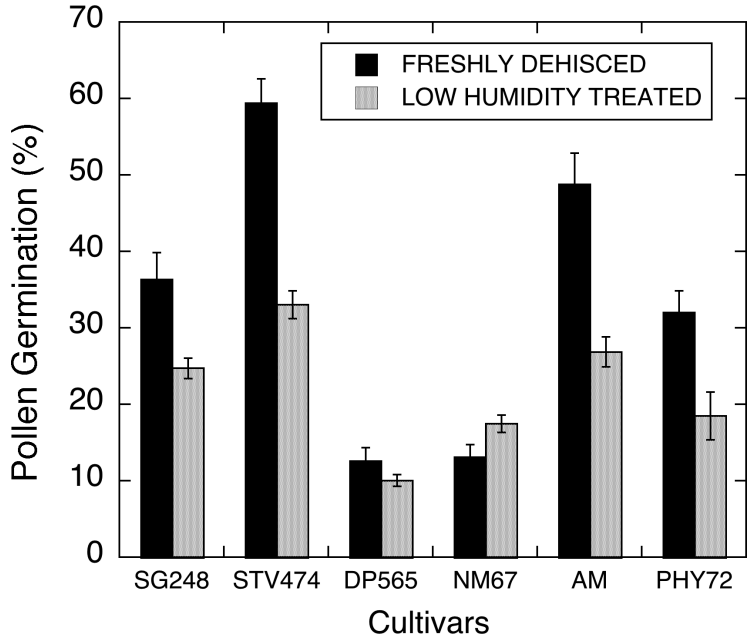

Figure 2. In situ pollen dehydration of cotton pollen from Suregrow 248, Stoneville 474, Deltapine 565, NM67, Acala Maxxa, and Phytogen 72 cotton cultivars. Flower petals were removed and the exposed anthers and pollen were incubated for 6.5 h in a 25% relative humidity. In vitro pollen germination was evaluated at 28°C and 80% relative humidity. The percent pollen germination of the low humidity treated pollen (grey bar) was compared with the germination of freshly dehisced pollen (black bar). Error bars represent the standard error of seventeen replications.

It is interesting to note that the germination percentages of the NM67 and DP565, the two lines showing the greatest dehydration resistance were also the two lines showing the lowest percent germination prior to the dehydration treatment. These results suggested the possibility of genetic differences in the pollen's ability to retain internal moisture or in their ability to take up moisture from the in vitro pollen germination medium. Before testing this hypothesis further it was necessary to determine if the pollen from these lines had similar moisture contents at the beginning of the study.

POLLEN WATER CONTENT

The water content of the pollen grains was evaluated according to the procedure of Nepi *et al*. (2001). Fresh pollen was weighed, dried in an oven at 104°C, and reweighed to determine the amount of water loss. Drying was continued until no further change in pollen weights was observed. The percent pollen water content was determined for 5 replicate samples per line. Replicate experiments showed no significant differences in water contents among the pollen from all lines immediately following dehiscence. The lines exhibited water contents of 51% (Fig. 3).

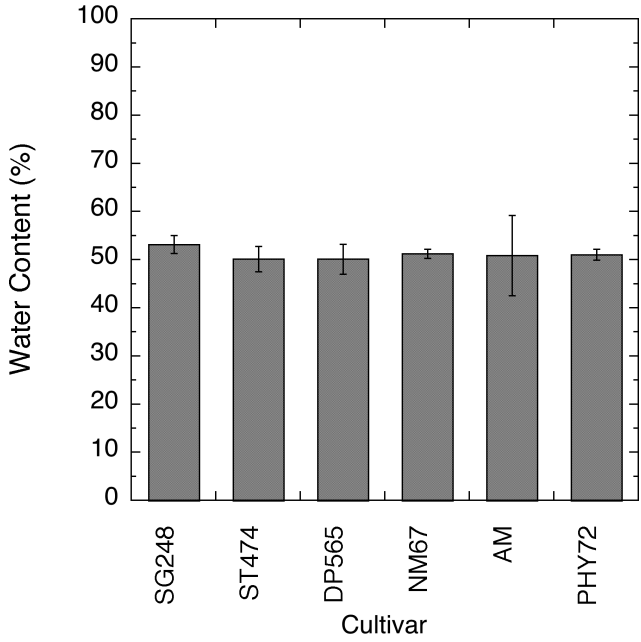

Figure 3. The water content of cotton pollen at dehiscence from Suregrow 248, Stoneville 474, Deltapine 565, NM67, Acala Maxxa and Phytogen 72 cotton cultivars. Error bars represent the standard error of five replications.

Having shown that the pollen started with equal internal water contents, experiments were performed to determine if pollen tube length development was impacted by the humidity surrounding the pollen during germination.

HUMIDITY EFFECTS ON POLLEN TUBE LENGTH DEVELOPMENT

Pollen germination was evaluated in 25 and 80% RH environments. The 80% RH level was obtained by using a 1.7 liter storage container (Rubbermaid Save and Serve) containing a saturated solution of NH_4SO_4 as described by Gawel and Robacker (1986). The 25% RH was the humidity level of the laboratory in which the experiments were performed. Flowers were harvested between 0930 and 1030 h from the greenhouse plants and placed in Ziploc plastic bags for transport into the laboratory. Upon returning to the laboratory, the pollen was collected by gently tapping the inverted flower. The pollen was germinated in vitro at 28°C according to the procedure of Burke *et al*. (2004) with half of the pollen placed in a 25% RH environment and the other half placed in an 80% RH environment. The pollen was incubated on the media for 1 h prior to analysis. Pollen germination was determined microscopically using a Leica MZ6 modular stereomicroscope. The percent germination was determined for samples from

3 replicate experiments. The SG248, STV474, DP565, and NM67 showed 35-40% reductions in pollen tube length when germinated in a 25% RH environment compared with the 80% RH environment (Fig. 4). The low humidity was more deleterious to the Acala Maxxa and PHY72 pollen as shown by the 60-65% reductions in pollen tube lengths in the 25% RH environment. These results support the hypothesis that the Acala Maxxa and PHY72 may lose internal water more rapidly than the SG248, STV474, DP565, and NM67. This water loss appears to reduce germination and pollen tube development in vitro.

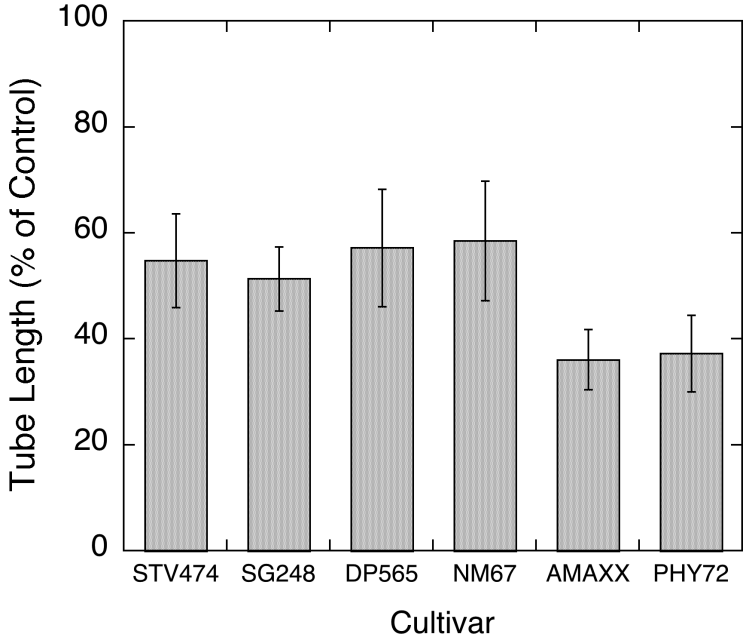

Figure 4. The effect of humidity on in vitro pollen tube length development of cotton pollen from Suregrow 248, Stoneville 474, Deltapine 565, NM67, Acala Maxxa and Phytogen 72 cotton cultivars. Germination at 25% relative humidity was compared with germination at 80% relative humidity. Error bars represent the standard error of three replications.

POLLEN WATER UPTAKE

The rate of water movement into pollen was evaluated by monitoring the time required for the pollen to rupture in an aqueous medium. Flowers were harvested between 0930 and 1030 h from the greenhouse plants and placed in Ziploc plastic bags for transport into the laboratory. Upon returning to the laboratory, the flower petals were folded back and the anthers dipped into 3-4 drops of a 0.8 M sucrose solution on a glass microscope slide. A cover slip was immediately placed on the slide and the time required for the pollen grains (a minimum of 100 grains per field of view) to rupture was determined microscopically using a Leica MZ6 modular stereomicro-

scope. The time to the first pollen grain rupture was determined for 41 flowers for each cultivar. The pollen from field-grown cotton was evaluated using flowers harvest at 0930 and 1330 h to determine if the time to first rupture changed over time.

If the assumption that water is lost more readily from the Acala Maxxa and PHY72 pollen than the SG248, STV474, DP565, and NM67 pollen is correct, then it is reasonable to hypothesize that water movement into the Acala Maxxa and PHY72 pollen may occur more rapidly than the SG248, STV474, DP565, and NM67 pollen. We chose to test this hypothesis by evaluating the rate of water uptake into the pollen. This was accomplished by monitoring the swelling and rupturing of the pollen grains in aqueous media. Burke (2002) reported the hypersensitivity of cotton pollen to water and that pollen grains placed in water would swell and rupture in seconds to minutes. In order to optimize the detection of genetic differences in pollen water uptake between cotton lines, we evaluated a range of osmotic media to slow the rate of pollen popping and maximize the difference should they exist. We observed optimum differences in the rate of pollen popping using a 0.8 M sucrose solution. A 2 to 4-fold difference in the time required to rupture the pollen was observed among these six cotton lines (Fig. 5). Although the absolute time required to rupturing of the pollen grain varied between the greenhouse and field-grown cotton, the ranking of the lines was identical. These findings further support the hypothesis that there exists genetic difference in the ability of pollen to retain internal water levels and maintain pollen viability.

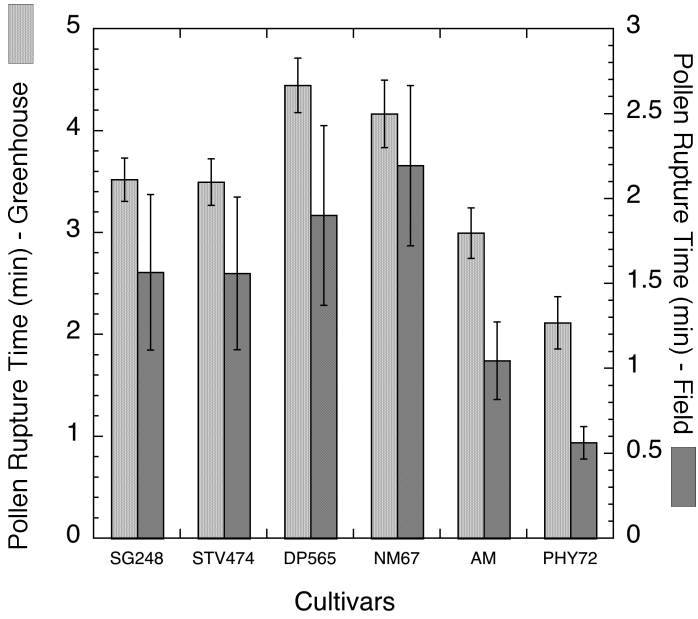

Figure 5. The time to first pollen grain rupture when cotton pollen from greenhouse-grown (light grey) and field-grown (dark grey) Suregrow 248, Stoneville 474, Deltapine 565, NM67, Acala Maxxa, and Phytogen 72 cotton cultivars were placed in 0.8 M sucrose. Error bars represent the standard error.

COMPETITIVE POLLINATION

The relative time required for the pollen to germinate and pollen tubes to reach the ovules was evaluated by competitive pollination. Competitive pollination was evaluated in a greenhouse with well-watered plants. Pollen from Suregrow 248, Stoneville 474, Deltapine 565, NM67, Acala Maxxa, and Phytogen 72 were co-pollinated with pollen from the glandless cotton Gregg 65. Gregg 65 flowers were sterilized according to the procedure of Burke (2002). Anthers on a flower from Gregg 65 and a test line were simultaneously rubbed on the recipient stigma. The resulting boll was allowed to mature and seed were harvested for analysis. Seeds were planted in soil flats, placed in a growth chamber set to 30°C, and hypocotyls were evaluated for gossypol glands two weeks after planting. Only bolls with 20 or more seeds were evaluated.

Figure 6 shows the percentage of glandless offspring. The results showed that pollen from PHY72, Acala Maxxa, and SG248 germinated more rapidly and/or pollen tubes grew more rapidly than STV474, DP565, and NM67 allowing approximately 70% of the resulting seeds to be glanded. The STV474, DP565, and NM67 pollen had similar germination and growth rates to those of the Gregg 65. This is shown by the 50:50 split in glanded and glandless offspring. The results suggest that pollen that is sensitive to relative humidity levels (Fig. 4) not only will lose water more rapidly in dry environments but will hydrate and germinate more rapidly in moist environments (Fig. 6).

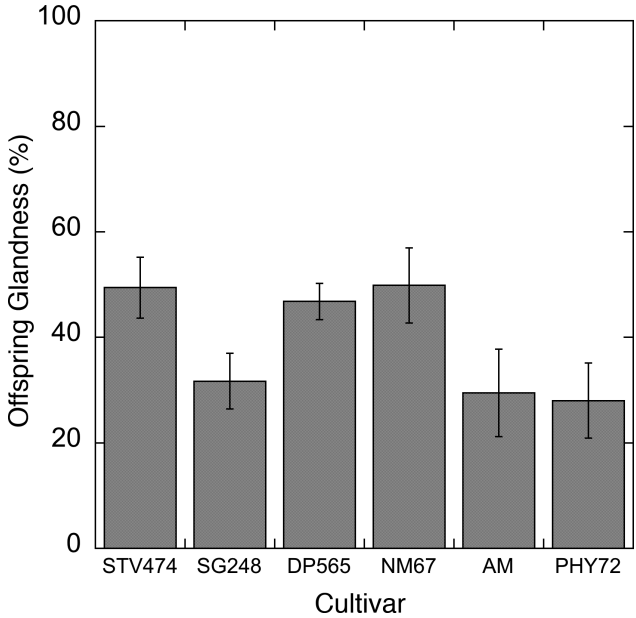

Figure 6. The percent glandless cotton plants obtained from Gregg 65 glandless cotton that was co-pollinated with pollen from Gregg 65 and pollen from either Suregrow 248, Stoneville 474, Deltapine 565, NM67, Acala Maxxa or Phytogen 72 cotton cultivars. Error bars represent the standard error.

SUMMARY

Genetic diversity in reproductive abiotic stress tolerance has been reported for cotton based upon the percentage of anther dehiscence of mature pollen in adverse environments. This study investigated the abiotic stress tolerance of mature pollen and identified genetic variability among six cotton lines. Similar high temperature sensitivities were observed for the SG248, STV474, DP565, NM67, Acala Maxxa, and Phy72 pollen. Genetic diversity in pollen viability was observed following a 6.5 h exposure to 25% RH. NM67, DP565, and SG246 exhibited less inhibition of pollen germination than STV474, Acala Maxxa and PHY72. Similar pollen water contents were observed for all lines. Genetic diversity in pollen tube length development at 25% RH compared with 80% RH was observed. Acala Maxxa and Phy72 pollen produced tube lengths of 35-40% of controls at 80% RH, while STV474, SG248, DP565, and NM67 exhibited tube lengths 50-60% of controls. Pollen water uptake studies showed faster uptake in PHY72 and Acala Maxxa than the other lines. Competitive pollinations showed faster germination of PHY72, Acala Maxxa and SG248 pollen compared to STV474, DP565 and NM67. These findings show genetic differences in cotton pollen sensitivities to water uptake and water loss. Our findings provide breeders with a previously unexplored reservoir of genetic diversity associated with reproductive abiotic stress tolerance.

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Chapter 3

WATER-DEFICIT STRESS IN COTTON

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INTRODUCTION

Water deficit is the major abiotic factor limiting plant growth and crop productivity around the world (Kramer, 1983). Approximately one third of the cultivated area of the world suffers from chronically inadequate supplies of water (Massacci *et al*., 2008). In all agricultural regions, yields of rain-fed crops are periodically reduced by drought (Kramer, 1983), and the severity of the problem may increase due to changing world climatic trends (Le Houerou, 1996). Advances in irrigation technology have helped reduce the gap between potential and actual yield, but irrigation costs and limited water supplies constrain irrigation throughout the world.

Water availability and quality affect the growth and physiological processes of all plants since water is the primary component of actively growing plants ranging from 70-90% of plant fresh mass (Gardner *et al*., 1984). Due to its predominant role in plant nutrient transport, chemical and enzymatic reactions, cell expansion, and transpiration, water stresses result in anatomical and morphological alterations as well as changes in physiological and biochemical processes affecting functions of the plants (Hsiao, 1973; Kramer, 1980).

Plant water deficits depend both on the supply of water to the soil and the evaporative demand of the atmosphere. In general, plant water stress is defined as the condition where a plant's water potential and turgor are decreased enough to inhibit normal plant function (Hsiao *et al*., 1973). The effects of water stress depend on the severity and duration of the stress, the growth stage at which stress is imposed, and the genotype of the plant (Kramer, 1983).

This review discusses the effects of water-deficit stress on several facets of growth and development in domestic cotton (*Gossypium hirsutum* L.).

EFFECTS OF WATER-DEFICIT STRESS ON MORPHOLOGICAL CHARACTERISTICS

Water-deficit stress adversely affects plant performance and yield development throughout the world (Boyer, 1982). Water-deficit stress reduces cell and leaf expansion, stem elongation, and leaf area index (Jordan *et al*., 1970; McMichael and Hesketh, 1982; Turner *et al*., 1986; Ball *et al*., 1994; Gerik *et al*., 1996). Leaf, stem and root growth rate are very sensitive to water stress because they are dependent on cell expansion (Hsiao, 1976; Hearn, 1994).

Krieg and Sung (1986) reported that water stress caused a reduction in the whole plant leaf area by decreasing the initiation of new leaves, with no significant changes in leaf size of leaf abscission. Both the main stem and sympodial branches developed significantly less leaves; however, the effect was less severe on the main-stem leaves. Pettigrew (2004) reported that water-deficit stress resulted in a decrease in leaf size, but noted that this decrease was accompanied by an increase in the specific leaf weight (SLW), a phenomenon also observed by Wilson *et al.* (1987). Significantly fewer nodes and lower dry weights of stems and leaves of water-stressed plants compared to those of the control were reported by Pace *et al.* (1999) (Table 1), while McMichael and Quisenberry (1991) observed decreased shoot-to-root ratios of plants grown under conditions of severe water stress. Malik *et al*. (1979) reported that root growth appears to be less affected by drought than shoot growth. Several researchers (Creelman *et al*., 1990; McMichael and Quisenberry, 1991; Ball *et al*., 1994; Pace *et al*., 1999) observed that seedlings of water-stressed cotton showed increased root elongation, accompanied by a reduction in root diameter.

Table 1. Plant height, stem and leaf dry weight, leaf area, and node number in drought-stressed and well-watered control plants of Stonevelle 506 and Tamcot HQ95 at the end of the drought, 49 days after planting. The drought treatment was imposed by withholding water for 13 d. (From Pace *et al*., 1999).

Plant Part	Treatment	
	Drought	Control
Plant height (cm)	20.0*	27.9
Stem dry weight (g)	1.13*	1.39
Leaf dry weight (g)	1.41*	2.16
Leaf area (cm^2)	56*	153
Node number	7.8*	9.4

* Means in a row are significantly different at the 0.05 probability level.

A correlation between leaf abscission and low plant water potentials has been commonly reported (Addicott and Lynch, 1955; Bruce *et al*., 1965), and McMichael *et al.* (1972) identified a linear relationship between the rates of leaf abscission and the levels of the imposed water-deficit stress; however, leaf abscission occurred after the stress was relieved and not during the period of stress. Addicott and Lynch (1955) speculated that formation of the abscission layers requires sufficient plant turgor. In addition, McMichael *et al*. (1973) observed that younger leaves were not as prone to abscission as older ones.

Water-deficit stress has also been shown to alter cell ultrastructure. Ackerson *et al*. (1981) observed that leaves of adapted plants contained large starch granules in the chloroplast wherein the structure of the thylakoid membranes appeared to be damaged. In addition, Berlin *et al.* (1982) indicated that water stress caused significant changes in the grana and stroma lamellae, palisade cell walls, number and size of chloroplasts, and the structure of mitochondria. In support of that observation, Bondada and Oosterhuis (2002) reported loss of chloroplast membrane integrity accompanied by an increase in leaf wax production (Bondada and Oosterhuis, 2002;

Oosterhuis *et al*., 1991; Meek and Oosterhuis, 2010). Changes in the chemical composition of epicuticular wax and lipid content were also observed. The wax from water-stressed leaves contained more long-chain alkanes compared to the control (Oosterhuis *et al*., 1991; Bondada *et al*, 1996). Conversely, water-deficit stress decreased glycolipids and, to a lesser effect, phospholipids, while the triacylglycerols increased (Pham Thi *et al*., 1985; Wilson *et al*., 1987).

EFFECTS OF WATER-DEFICIT STRESS ON PHYSIOLOGICAL CHARACTERISTICS

The effects of water deficit on different plant physiological processes are complex and interrelated. Cellular water content largely controls stomatal aperture, and stomatal conductance directly affects CO_2 diffusion and photosynthetic carbon fixation, which in turn affects metabolic functions such as respiration. However, for ease of discussing these physiological functions, we have addressed each function separately.

Photosynthesis

Photosynthesis plays a major role in determining crop productivity in all species and is directly affected by water stress. Photosynthetic rates of the leaves decrease as the relative water content and leaf water potential decrease (Lawlor and Cornic, 2002) (Fig. 1). The effects of water stress on photosynthesis are complex, and may include a combination of stomatal closure (Sharkey, 1990; Chaves, 1991; Cornic, 1994) and the inhibition of metabolic processes, including ribulose bisphosphate synthesis and adenosine triphosphate synthesis.

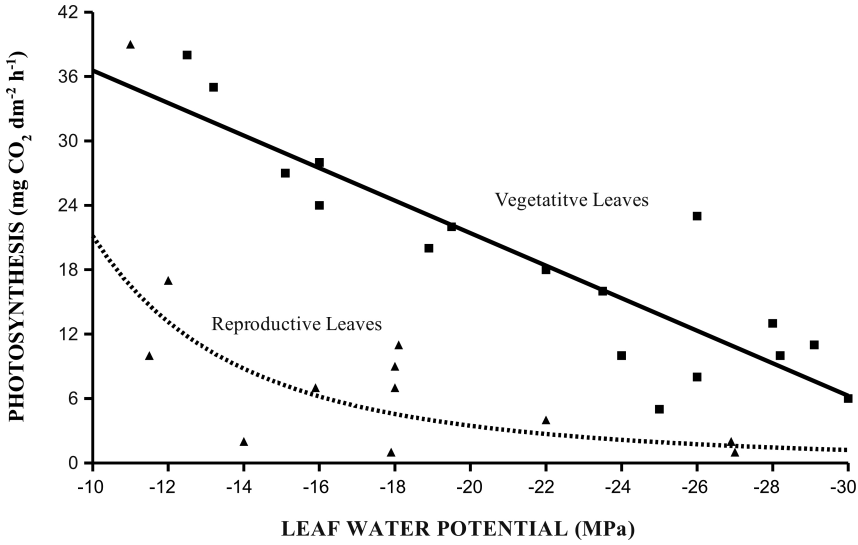

Figure 1. Relationship between photosynthesis and leaf water potential of vegetative and reproductive cotton leaves. (Redrawn from Ackerson *et al*., 1977a).

In cotton, several reports have indicated that water stress causes a reduction in photosynthesis rates due to a combination of stomatal and non-stomatal limitations (Pallas *et al*., 1967; McMichael and Hesketh, 1982; Turner *et al*., 1986; Sung and Krieg, 1986; Genty *et al*., 1987; Ephrath *et al*., 1990; Faver *et al*., 1996, Lacape *et al*., 1998; Leidi *et al*., 1999). However, there has been some controversy concerning the relative importance of these two processes responsible for photosynthetic impairment under water deficit (Flexas and Medrano, 2002; Lawlor and Cornic, 2002; Chaves *et al*., 2002, Lawlor, 2002).

The relative contributions of stomatal opening and metabolic processes to the decrease of photosynthesis in drought-stressed plants are still being studied and debated. According to Chaves and Oliveira (2004) and Flexas *et al*. (2004a), decreased CO_2 diffusion from outside the plant to the site of carboxylation is the main cause for reduced photosynthetic rates under most water-stress conditions. Reduced CO_2 diffusion has been attributed to stomatal closure, reduced mesophyll conductance, or a combination of these factors (Flexas *et al*., 2002; Warren *et al*., 2004). Additionally, other factors, such as time of day, ambient CO_2 concentrations, nutrient levels, leaf type, growth stage, genotypic differences and abscisic acid (ABA) concentrations may affect photosynthetic rate in drought-stressed plants.

Stomatal Factors

Stomatal closure decreases water loss, but also decreases the movement of CO_2 into the plant. Significant correlations between leaf water potential and stomatal conductance under conditions of water-deficit stress have been reported (Socias *et al*., 1997), but diverse reports exist for cotton. Experiments with potted plants have shown stomatal closure due to water stress in cotton to be similar to other crops. Kanemasu and Tanner (1969) and Boyer (1970) quantified stomatal resistance on a variety of crops, including cotton, and found that stomatal resistance due to stomatal closure increased dramatically at between -0.8 and -1.2 MPa. Harris (1973) and Bielorai *et al*. (1975) also reported that in potted experiments stomatal conductance was significantly decreased under conditions of water-deficit stress.

However, field experiments have shown cotton stomatal conductance to be adaptable to water stress. Ackerson *et al*. (1977) reported that leaf stomatal conductance of field-grown cotton was slightly affected and leaf stomata did not completely close even under very low water potentials, and they speculated that light intensity is probably more of a controlling factor than leaf water status. Jordan and Ritchie (1971) observed that cotton plants that had been adapted to low water conditions were capable of stomatal conductance and photosynthesis at very negative leaf water potentials. Complete stomatal closure did not occur even at leaf water potentials approaching -3.0 MPa and it was suggested that stomatal closure in field-grown plants is prevented in order for the plants to maintain water flux.

Ackerson (1981) and Ackerson and Herbert (1981) expanded on this discovery, finding that water stress adapted plants had similar leaf conductance under wet conditions, but maintained turgor at more negative leaf potentials than non-adapted plants. Wullschleger and Oosterhuis (1990) reported that while both moderate and severe water stress significantly decreased leaf stomatal conductance, bract stomatal conductance remained unaffected.

Non-Stomatal Factors

Changes in the photosynthetic apparatus under drought through metabolic impairment are far more complicated than those resulting from inhibition of stomatal function, and they are predicted to occur under conditions of severe drought stress. Gimenez *et al*. (1992) reported that capacity of ribulose 1,5-bisphosphate (RuBP) regeneration could be a metabolic process that could be a limiting step in photosynthesis under water-deficit stress, while Medrano *et al*. (1997) speculated over the activity of ribulose 1,5-bisphosphate carboxylase/oxidase (Rubisco). Additionally, adenosine 5-triphosphate (ATP) synthesis or ATP-synthase activity could be severely inhibited resulting in a decrease in photosynthetic rates (Younis *et al*., 1979; Tezara *et al*., 1999). Leaf photochemistry (Cornic and Massacci, 1996) and permanent photoinhibition (Bjorkman and Powles, 1984) have also been suggested to be affected under limiting water conditions.

Pettigrew (2004) speculated that the higher photosynthetic rates and increased PSII quantum efficiency (ΦPSII) with rehydrated plants could be attributed to the higher chlorophyll content per unit leaf area that was observed. Similar results were reported from Massacci *et al*. (2008) who observed that photosynthetic electron transport was enhanced under conditions of water stress due to an increased efficiency in the open PSII reaction centers. They also observed that photorespiration increased at the onset of water stress in order to prevent an inhibition of the photosynthetic apparatus and over-production of damaging reactive oxygen species. Massacci *et al*. (2008) attributed this to an increase in photorespiration rates in order to prevent an inhibition of the photosynthetic apparatus and over-production of damaging reactive oxygen species. Genty *et al*. (1989) also reported that ΦPSII is positively correlated with the quantum efficiency of CO_2 fixation. They also noted that photon receptors were not impaired under conditions of water stress. Similarly photon distribution and PSII photochemistry was not affected, however electron transport through PSI was inhibited. In contrast, Enahli and Earl (2005), Inamullah and Isoda (2005), and Kitao *et al*. (2007) observed that quantum efficiency of PSII decreases under conditions of water stress. Additionally, Enahli and Earl (2005) observed in their study, where water stress levels varied from moderate to severe, that even though photosynthetic rates remained unaffected under moderate stress rates, significant decreases were observed in the velocity of carboxylation of Rubisco and at the CO_2 concentration at the site of carboxylation. Those responses became more prominent under severe water-deficit stress where both photosynthetic rates as well as concentration of CO_2 at the site of carboxylation decreased. Upon relief from the water stress, CO_2 concentrations returned to control levels however, photosynthetic rates remained low indicating metabolic and non-stomatal inhibition, which is in contrast with Pettigrew (2004). The explanation for these contrasting results has been suggested to lie in the heterogeneity of the photosynthetic apparatus across the cotton leaf (Wise *et al*., 1992). However, Massacci *et al*. (2008) indicated that leaf patchiness is significantly decreased under conditions of water deficit.

Other Factors Affecting Photosynthesis in Drought-Stressed Plants

In addition to stomatal closure and changes in metabolic rates and leaf photochemistry, several other factors have been linked to decreases in photosynthesis in drought-stressed cotton plants. Ackerson *et al*. (1977) observed differences between photosynthesis rates at different

times of the day (morning vs. afternoon) as well as between leaves of different age, which agreed with previous reports (Jordan and Ritchie, 1971; Jordan *et al*., 1975).

Furthermore, Ackerson (1981) and Ackerson and Herbert (1981) observed that the older leaves in plants adapted through successive drought cycles, contained up to five times more starch than corresponding leaves in non-adapted plants. In addition, photosynthetic rates of the adapted older leaves were lower under wet conditions compared to non-adapted plants while no effect was observed on the photosynthetic rates of the young leaves by the adaptation. The reduction in photosynthetic rates was attributed to feedback inhibition of photosynthesis due to carbohydrate accumulation and not to stomatal restriction.

Other factors such as abscisic acid (ABA) concentration, ambient CO_2 concentrations and nutrient deficiencies have been shown to have an effect on leaf stomatal conductance under limited water conditions. Radin and Ackerson (1981) in potted experiments with different CO_2 concentrations and nitrogen rates indicated that water-deficit stress significantly decreased both stomatal and mesophyll conductance compared to the control. They also reported that nitrogen deficiency significantly increased stomatal sensitivity to the intercellular CO_2 concentrations at low water potentials, a result which was similar to the effect of ABA application. They concluded that behavior of stomata is closely controlled by ABA concentrations under conditions of water deficit. Similar responses of stomatal conductance were reported for phosphorus-deficient cotton plants (Radin, 1984).

In experiments with different cotton genotypes, Pettigrew (1993) found that okra and super okra leaf type plants had lower stomatal conductance values than normal leaf type isolines at high water potentials and this was attributed to the lower abaxial stomatal density of okra leaf types (Wells *et al*., 1986). Similar findings were reported by Karami *et al*. (1980) and Nepomuceno *et al*. (1998) who also noticed that super okra was able to maintain higher leaf and turgor potentials at lower osmotic potentials compared to the normal leaf plants under water deficit. In addition, okra and super okra leaf type plants exhibited higher photosynthetic rates at similar low water potentials compared to the normal leaf type plants in greenhouse and field experiments (Karami *et al*., 1980; Nepomuceno *et al*., 1998; Pettigrew, 2004).

Marani *et al*. (1985) reported reduced canopy photosynthetic rates under conditions of water stress which they attributed to decreased leaf expansion and hence, leaf area as well as to the leaf age of the canopy and the increased senescence rates due to reduced supply of water. However, Constable and Hearn (1981) observed in field experiments that net assimilation rate was not affected by irrigation treatments, whereas Pettigrew (2004) reported that leaf photosynthetic rates increased in the morning for water-stressed field-grown cotton plants in the Mississippi Delta before decreasing in the afternoon. Those different responses however, could be attributed to the different stages of growth that water-deficit stress was imposed, the different genotypes, the different leaf ages and position of leaves in the canopy.

As Karami *et al*. (1980) reported, photosynthesis during the reproductive stage was less sensitive to water stress compared to the vegetative stage while young leaves had higher photosynthetic rates compared to older ones at the same leaf water potentials. Pettigrew (2004) also speculated that the higher photosynthetic rates could be attributed to the hydraulic conductivity of the soils that allowed the plants to rehydrate during the night, hence enabling their photosynthetic apparatus to operate more efficiently during the morning.

Photosynthetic responses under conditions of water-deficit stress appear to be affected by several factors. Even though recently, Flexas *et al*. (2006) observed that photosynthetic rates are mostly limited by decreased stomatal conductance as well as reduced mesophyll conductance that ultimately result in a general metabolic impairment due to lower carbon substrate concentration, a conclusion over which photosynthetic metabolic process or factor is most sensitive under water-deficit stress has yet to be made.

WATER-DEFICIT STRESS EFFECTS ON RESPIRATION, ATP, AND CARBOHYDRATES

Respiration is the process by which a plant obtains energy by reacting oxygen with sugars (glucose) to produce water, carbon dioxide and adenosine 5-triphosphate (ATP). Dark respiration (in contrast to photorespiration and photosynthesis) occurs during the day and night, and its rates during the day vary between 25 and 100% of the respiratory activity during the night (Krômer, 1995). Of the CO_2 fixed each day by net photosynthesis, about 30-70% is released back to the atmosphere through dark respiration (Atkin *et al*., 1996) with 50-70% of whole plant respiration occurring in leaves (Atkin *et al*., 2007). However, Flexas *et al*. (2005, 2006) pointed out that the percentage of daily fixed carbon that is respired is expected to be higher in water-stressed plants, mainly because of the inhibitory effect of water deficit has on photosynthesis.

According to Atkin *et al*. (2009) the responses of respiration rates to water deficit vary by plant genotype, the type and the age of tissue (mature or still actively growing), the duration and severity of stress, changes in activity of respiratory enzymes, substrate availability, and ATP demand. De Vries *et al*. (1979) conducted studies in maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) and observed that while respiration rates remained unaffected at low or moderate water stress, they decreased at severe water stress. A similar pattern was also observed by McCree *et al*. (1984) in sorghum (*Sorghum bicolor* L.), and Boyer (1970) and Ribas-Carbo *et al*. (2005) in soybean (*Glycine max* L.). However, Boyer (1970, 1971) in studies with sunflower (*Helianthus annuus* L.), found a decrease in respiration rates when drought stress was imposed, while Ghashgaie *et al*. (2001) noticed an increase, and Lawlor and Fock (1977) reported no change.

Limited data exists concerning water- deficit stress effects on respiration of cotton. Pallas *et al*. (1967) reported that respiration initially decreased with increasing severity of the water stress and eventually increased at more severe stress. Loka and Oosterhuis (unpublished data) observed that respiration rates of water-stressed plants decreased compared to unstressed plants in controlled environment experiments. Wullschleger and Oosterhuis (1990) reported that boll respiration remained unaffected under moderate water stress and significantly decreased once the stress became more severe.

ATP Content

Adenosine 5-triphosphate (ATP) constitutes the molecular currency of intracellular energy transfer for plant metabolism. Photosynthesis and respiration are the main plant processes through which ATP is produced, and specifically through the pathways of (a) photophosphorylation (cyclic and non-cyclic) in the chloroplasts, (b) glycolysis in the cytosol, and the most important pathway, and (c) oxidative phosphorylation in the mitochondria (Raymond and Pradet, 1983).

Measurements of ATP in water-stressed tissues show considerable variation. Flexas and Medrano (2002) reported a decrease in ATP content of leaves with a relatively small decrease in relative water content; however, Tezara *et al*. (1999) observed that ATP content was not depleted completely even at very low relative water content and when photosynthesis had stopped. Sharkey and Seeman (1989) found no differences in the ATP content of mildly-stressed bean (*Phaseolus vulgaris*, L.) leaves, while Meyer *et al*. (1992) indicated that ATP content progressively decreased as the relative water contents decreased.

Recently, Lawlor and Tezara (2009) speculated that drought stress might also result in an increased ATP content through the respiratory pathway in order to compensate for reduced rates of chloroplast ATP synthesis. Pandey *et al*. (2002) conducted studies to determine the effect of water-deficit stress on the photosynthetic metabolites on cotton during the reproductive stage. They reported that water-stress resulted in a decrease in leaf ATP content while, nicotinamide adenine dinucleotide phosphate (NADP) content was increased. Leaf 3-phosphoglyceric acid (3-PGA) and pyruvate content remained unaffected by the water stress treatments.

Carbohydrate Metabolism and Translocation

As mentioned above, photosynthesis has been shown to be adversely affected by water stress. Photosynthesis is the fundamental function through which plants fix carbon and produce carbohydrates, so it is expected that water stress would also affect carbohydrate metabolism.

An early study by Eaton and Ergle (1948) showed that water stress significantly reduced starch concentrations and increased hexose sugars in cotton leaves, with variable effects on sucrose accumulation. Parida *et al*. (2007) found that total leaf soluble carbohydrate and leaf hexose concentrations were increased, while leaf starch contents decreased in both drought-tolerant and drought-sensitive cultivars. Increase in hexose and depletion of leaf starch concentration have also been reported in soybean (*Glycine max* L. Merr.) (Huber *et al*., 1984; Liu *et al*., 2004) and pigeonpea (*Cajanus cajan* L.) (Keller and Ludlow, 1993).

In contrast, Ackerson (1980) observed that higher quantities of starch were accumulated in water-stressed cotton leaves compared to those of the control. Additionally, acclimated young cotton leaves had the ability to export sucrose, whereas non-acclimated plants did not at the same low leaf water potential. He speculated that translocation of photosynthates was greatly inhibited under conditions of water stress. In support of this observation, Timpa *et al*. (1986) reported that drought stress caused no change in leaf sucrose concentrations of non-flowering cotton strains, while glucose levels were significantly higher in the drought-stressed leaves compared to the control, indicating that the source sink-relationships are affected by drought.

Impairment of the photoassimilate translocation mechanism under conditions of water-deficit stress has been reported for other crops as well, such as sugarcane (*Beta vulgaris* L.) (Hartt, 1967), maize (*Zea mays* L.) (Boyer and McPherson, 1977), and wheat (*Triticum aestivum*, L.) (Johnson and Moss, 1976). Liu *et al*. (2004) made a similar observation for soybean source-sink relationships and reported that sucrose and leaf starch concentrations decreased significantly under water stress resulting in a decrease in the rate of sucrose export from the leaves.

Sung and Krieg (1979) conducted experiments with different leaf-type cotton genotypes and water stress at different stages of development to study the effect of water stress on the rate of assimilate export from the leaf by measuring the disappearance of labeled ^{14}C from the leaf. They reported that translocation of assimilates was reduced under much lower water potential values compared to photosynthesis, concluding that photosynthesis is more sensitive to water-deficit stress than translocation (Fig. 2), which is in accordance with Wardlaw (1967), who also concluded that main consequence of water stress on translocation is on the availability of photosynthate. Sung and Krieg (1979) however, observed that water-deficit stress altered the assimilate export pattern of the upper canopy leaves allocating more photosynthate to vegetative growth and fruits while the water stress had no effect on the export pattern of the lower canopy leaves.

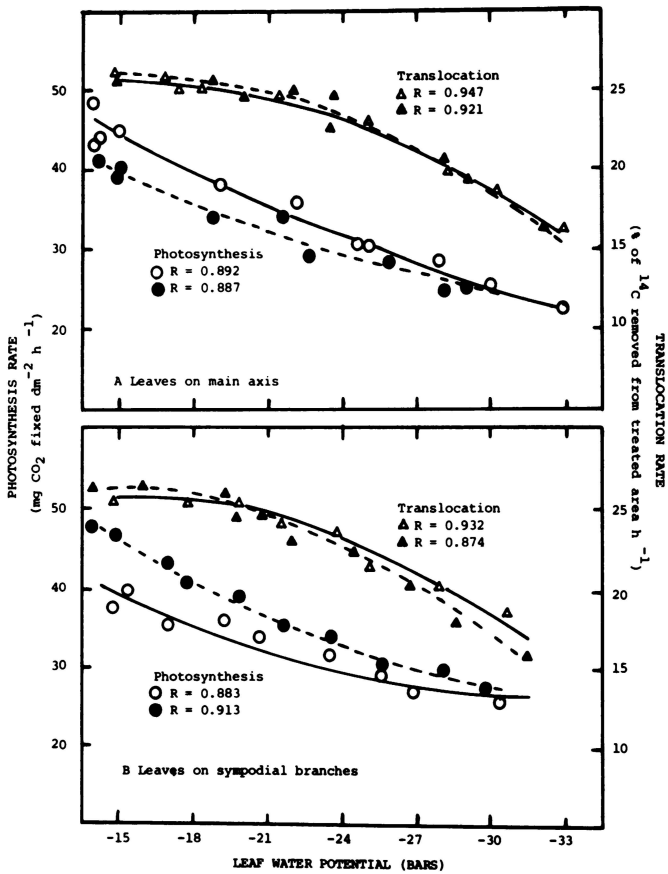

Figure 2. Response of photosynthesis and translocation rates to increasing water stress in cotton as a function of leaf type and growth stage (○, ▲: flower bud development; ●, Δ: boll-filling period). (From Sung and Krieg, 1979).

Guinn (1976) however, did not notice any difference in carbohydrate accumulation in 4-day-old bolls in cotton plants that had been subjected to water stress compared to those that had been properly watered. Additionally, Heitholt *et al*. (1994) reported that carbohydrate concentrations of the receptacle and ovary had no relationship with subsequent retention of 5-day-old floral buds or two-day-old bolls in cotton, however, the plants were not subjected to water stress. Similarly, Liu *et al*. (2004) failed to correlate pod abortion of water-stressed soybeans with pod carbohydrate concentrations. Zinselmeier *et al*. (1995, 1999) observed that accumulation of sucrose in young water-stressed maize ovaries paralleled the cessation of ovary growth and an additional decrease in hexose concentration. They speculated that the ratio of hexose to sucrose could play an important role in ovary development. An inhibition of invertase activity due to drought stress could also result in an increase in ovary sucrose content (Schussler and Westgate, 1991; Liu *et al*., 2004). This was also noted by Weber *et al*. (1998) for legume seed development. Further explanation of carbohydrate metabolism in cotton flowers and developing bolls during drought stress is needed.

PLANT MECHANISMS UNDERLYING RESILIENCE TO WATER-DEFICIT STRESS

Antioxidants

Drought stress has been reported to induce an oxidative stress due to inhibition of photosynthesis (Smirnoff, 1993) resulting from the production and accumulation of toxic oxygen species such as peroxide radicals, hydrogen peroxide and hydroxyl radicals (Foyer *et al*., 1997). The accumulation of reactive oxygen species (ROS) originates mainly from the decline in CO_2 fixation which leads to higher leakage of electrons to O_2 (Foyer *et al*., 1997), while other factors triggering formation of free radicals involve fatty acid β-oxidation (del Rio *et al*., 1998), membrane associated oxidases (Desikan *et al*., 1996) and photorespiration (Faria *et al*., 1999).

These reactive oxygen species produced during water-deficit stress can damage many cellular components including lipids, proteins, carbohydrates and nucleic acids (Monk *et al*., 1987). Membrane lipid peroxidation and protein oxidation constitute the simplest criteria of assessing the extent of oxidative damage in the tissue (Noctor and Foyer, 1998; Mittler 2002). Efficient antioxidant systems in the plant can minimize the level of oxidative stress and protect the tissues. Such antioxidant systems can be enzymatic or non-enzymatic. The major antioxidant species in the plants are superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (AP), and glutathione reductase (GR), along with carotenoids and α-tocopherol (Gaspar *et al*., 2002). Additionally, polyamines and flavonoids have been shown to provide some protection from free radical injury (Bouchereau *et al*., 1999), while the photosynthetic system through the xanthophyll-zeaxanthin cycle can also contribute in the relief of oxidative stress. The levels of these antioxidant systems, however, have shown increases, decreases or no effect, depending on the species, duration of drought stress and the specific antioxidants investigated (Reddy *et al*., 2004).

Mahan and Wanjura (2005) performed field studies to identify changes in antioxidant metabolism in cotton. They observed that even though the glutathione amount and form changed during the season, the changes were not in response to the water-stresses, and they concluded that cotton has a limited ability to alter glutathione metabolism in response of drought stress. In contrast, ascorbate peroxidase activity was increased in water-stressed plants compared to the well-watered plants, while no significant change was reported in the levels of malondialdehyde (MDA), an indicator of cell-membrane damage, leading them to speculate that the oxidative stress was alleviated before membrane damage could occur. However, Kawakami *et al*. (2010) also reported that glutathione reductase of potted grown plants was not affected by water-deficit stress, whereas superoxide dismutase of water-stressed plants was significantly decreased compared to the control.

Proteins

Plants have been shown to accumulate specific stress-associated proteins in order to survive adverse environmental conditions (Vierling, 1991; Ingram and Bartels, 1996). Heat shock proteins (HSPs) and late embryogenesis abundant (LEA)-type proteins are two major types of stress-induced proteins that are produced upon the induction of drought stress and are considered to play a role in cellular protection during the stress (Ingram and Bartels, 1996; Zhu *et al*., 1997).

Heat shock proteins have been observed to be produced at any stage of crop development and under different environmental factors such as water-deficit stress (Bray, 1993), UV-radiation (Dohler *et al.*, 1995), or heavy metal accumulation (Neumann *et al.*, 1994). Their molecular weights and proportions differ among species, and they are considered as molecular chaperones essential for the maintenance of protein homeostasis and prevention of denaturation (Vierling, 1991), even though the mechanism by which they contribute to drought tolerance is still not certain. One hypothesis is that they are involved in energy dependent protein unfolding or assembly/disassembly reactions, and they prevent protein degradation under adverse conditions (Pelham, 1986). Another hypothesis is that they are related to the protection and stabilization of particular organelles such as chloroplasts, ribosomes and mitochondria. Additionally, some members of the HSPs have been shown to aid in the maintenance and restoration of enzymes (Sun *et al.*, 2001).

In arid and semi-arid regions, dryland crops may synthesize and accumulate substantial levels of HSPs in response to elevated leaf temperatures due to decreased rates of transpiration. Burke *et al.*, (1985) conducted experiments with field-grown cotton, where soil water deficits resulted in canopy temperatures of 40°C or greater for two to three weeks. At least eight new polypeptides accumulated in about half of the water-stressed leaves while no polypeptides were accumulated in the irrigated cotton leaves. In another study, Kuznetsov *et al.* (1999) imposed a short-term heat shock treatment to cotton plants at flowering, prior to water-deficit stress imposition, and they observed that heat-treated plants accumulated greater quantities of two HSPs (70 and 80kDa) as well as amino acids (asparagine, proline and arginine especially). Additionally, larger osmotic adjustment values were observed, and the authors speculated that HSPs have a protective role in cotton under condition of water-deficit stress. However, in a similar experiment with field-grown soybean (*Glycine max* (L.) Merr.), several HSPs were observed in both irrigated and water-deficit stress plants (Kimpel and Key, 1985).

Late embryogenesis abundant proteins, the second major type of stress-induced proteins, have been found in a wide range of plant species in response to desiccation or drought stress (Ingram and Bartels, 1996). Even though they were first identified in cotton seeds during their maturation and desiccation phases (Baker *et al.*, 1988), it has since been recognized that they also accumulate in vegetative tissues under conditions of water stress (Bray, 1993). According to Bray *et al.* (2000) most LEA proteins exist as random coiled α-helices. They are characterized by their high hydrophilicity index and glycine content (Garay-Arroyo *et al.*, 2000). They are considered to act as water-binding molecules, participate in ion sequestration, and contribute in membrane stabilization (Ingram and Bartels, 1996).

Osmotic Adjustment and Compatible Osmolytes

Plants experiencing stressful conditions, such as drought, tend to actively accumulate highly soluble organic compounds of low molecular weight, called compatible solutes, as well as inorganic ions, i.e. K, in order to prevent water loss, maintain water potential gradients and reestablish cell turgor (Hsiao, 1973). This process is called osmotic adjustment and according to Boyer (1982) enables plants to: (1) continue normal leaf elongation but at a reduced rate, (2) adjust their stomatal and photosynthetic functions, (3) maintain the development of their roots and subsequently continue soil moisture extraction, (4) postpone leaf senescence, and (5) achieve better dry matter accumulation and yield production under adverse conditions. Osmotic adjustment has been reported in the leaves of a number of crops such as wheat (*Triticum aestivum* L.) (Morgan, 1977), maize (*Zea mays* L.) (Acevedo *et al.*, 1979), sorghum (*Sorghum bicolor* L.) (Jones *et al.*, 1978; Turner *et al.*, 1978), rice (*Oryza sativa* L.) (Cutler *et al.*, 1980), barley (*Hordeum vulgare* L.) (Matsuda *et al.*, 1981), pearl millet (*Pennisetum americanum* L.) (Henson *et al.*, 1982), sunflower (*Helianthus annuus* L.) (Turner *et al.*, 1978), as well as cotton (*Gossypium hirsutum* L.) (Acevedo *et al.*, 1979, Oosterhuis and Wullschleger, 1987). Interestingly, cotton appears to have a greater ability to osmotically adjust to water stress compared to other major crops (Ackerson *et al.* 1977; Oosterhuis and Wullschleger, 1988). Additionally, Oosterhuis *et al.* (1987) observed that primitive landraces and wild types of cotton exhibited higher osmotic adjustment compared to commercial cultivars. They investigated the osmotic adjustment of cotton roots under water deficit and demonstrated that cotton roots show a considerably larger percentage adjustment than the leaves (Fig. 3), reinforcing the ability of the plant to maintain a positive turgor and hence continue normal growth under water stress. A similar pattern was observed in cotton flowers (Trolinder *et al.*, 1993) and bolls (Van Iersel and Oosterhuis, 1996) wherein both flowers and fruits were found to be less affected by the water stress imposed than the subtending leaves. These authors concluded that cotton flowers and bolls are largely independent on the xylem connections for their water supply and that the phloem is the most important factor in water transport to the flowers and developing bolls. Ackerson and Hebert (1980) observed that cotton plants that had been subjected to consecutive water-stress cycles exhibited increased osmoregulation compared to plants that had not been subjected to stress previously. They reported that photosynthetic rates were higher due to higher stomatal conductance at low water potentials, but the opposite was observed under high water potentials.

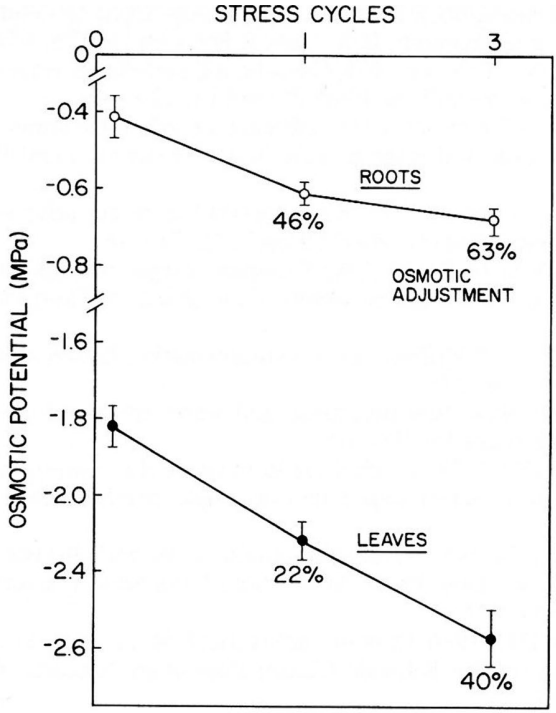

Figure 3. Effect of number of water stress cycles of osmotic adjustment in cotton leaves and roots. The percentage osmotic adjustment was calculated as the percentage decrease in treatment osmotic adjustment compared to the unstressed control. (From Oosterhuis and Wullscheleger, 1987).

Osmolytes are organic compounds that exist in a stable form inside the cells and are not easily metabolized. In general, they do not have an effect on cell functions, even when they have accumulated in high concentrations, i.e. more than 200mM (Hare *et al.*, 1998; Sakamoto and Murata, 2002). Compatible solutes include sugars and sugar alcohols (polyols) (Yancey *et al.*, 1982), amino acids such as proline (Aspinall and Paleg, 1981; Bonhert *et al.*, 1995) and its analogues (Naidu *et al.*, 1987), quaternary ammonium compounds (betaines) and tertiary sulfonium compounds (Rhodes and Hanson, 1993). Production of osmolytes is a general method in plants to maintain osmotic potential and cell turgor, as stated above; however, they also have secondary roles such as stabilization of membranes and maintenance of proper protein conformation at low leaf water potentials (Papageorgiou and Morata, 1995), protection of cells by scavenging for ROS (Pinhero *et al.*, 2001), as well as regulation and integration in the metabolism of stressed photosynthetic tissues (Lawlor and Cornic, 2002). Their synthesis and accumulation varies among plant species, as well as among cultivars of the same species. They are most often confined to the chloroplasts and cytoplasmic compartments and according to Ain-Lhout *et al.* (2001) occupy less than 20% of the total volume of mature cells.

EFFECT OF WATER-DEFICIT STRESS ON YIELD

Water deficit significantly compromises plant development and productivity around the world (Boyer, 1982). In many crops, reproductive development is most sensitive period to drought stress following seed germination and seedling establishment (Saini, 1997). In cotton, however, there is still debate about the most sensitive period to water-stress during development in relation to yield, even though water sensitivity during flowering and boll development has been well established (Constable and Hearn, 1981; Cull *et al.*, 1981a,b; Turner *et al.*, 1986).

According to Reddell *et al.* (1987) the early flowering period is the most sensitive to water stress, whereas Orgaz *et al.* (1992) concluded that water stress during peak flowering had the most detrimental effects on cotton yield. On the other hand, a number of reports (Radin *et al.*, 1992; Plaut *et al.*, 1992; de Cock *et al.*, 1993) state that boll development, particularly well after the end of effective flowering, is the most water-deficit-sensitive period for cotton. Additionally, in an earlier experiment, Harris and Hawkins (1942) reported that delaying irrigation at fruiting could increase yield by inhibiting excessive vegetative growth, a result reinforced by Singh (1972), who reported increased number of flowers and bolls per plant as well as increased yield when cotton plants were stressed during the pre-flowering season. Conversely, Stockton *et al.* (1961) and Lashing *et al.* (1970) observed that increased irrigation resulted in increased flowering. Guinn *et al.* (1981) concluded that a moderate water-deficit stress early in the season could be beneficial to the plants by slowing vegetative growth, but that the risk of negative results meant that these practices should be approached with caution. Other crops such as wheat, rice, barley and maize vary in drought sensitivity by growth stage (Saini and Westgate, 2000). However, this variable sensitivity of growth stages in cotton, together with the perennial nature and indeterminate growth pattern, which makes distinct growth stages indistinguishable, may explain the poor understanding of the effects of water deficit on cotton seed set and development.

According to Grimes *et al.* (1969) there is a positive correlation between the yield and the number of bolls produced. However, the biochemical or metabolic functions affecting boll retention have not been adequately investigated. Both irrigation rate and application type have both been shown to affect boll production and retention (Ritchie *et al.*, 2009; Whitaker *et al.*, 2008; Dumka *et al.*, 2004) but the physiological explanations are not clear. The majority of studies have focused on the consequences of water stress on dry matter, boll number and weight, as well as lint yield and their correlations to leaf photosynthesis and plant water relations, without any emphasis on the biochemical and metabolic processes of the reproductive units themselves. Guinn *et al.* (1976, 1981, 1984, 1988, and 1990) focused mainly on the hormonal aspects of water-stressed cotton fruiting forms and specifically on the responses of abscisic acid (ABA), indole-3-acetic acid (IAA) and ethylene. They observed that water stress increased ethylene evolution from young bolls as well as their ABA content while it decreased the concentrations of free IAA. However, they were unable to conclude as to which hormone was solely responsible for causing boll abscission and ultimately yield reduction. Research in other crops however, has indicated that ABA caused pollen sterility in barley, wheat and rice (Saini and Westgate, 2000). McMichael *et al.* (1973) also reported a strong, linear correlation between boll shedding rates and decreasing pre-dawn leaf water potentials. However, they speculated that boll abscission

was also controlled by endogenous factors that were dependent on plant water status such as increased ethylene production (McMichael *et al.*, 1972).

Lint yield is generally reduced under water stress because of reduced boll production primarily due to the production of fewer flowers and bolls (Stocton *et al.*, 1961; Grimes, 1969; Gerik *et al.*, 1996), but also because of increased rates of boll abortion when the stress is extreme during the reproductive growth stage (Grimes and Yamada, 1982; McMichael and Hesketh, 1982; Turner *et al.*, 1986). In addition, Pettigrew (2004) reported that the distribution of the bolls, both vertically and horizontally, was affected by water-deficit stress with the water-stressed plants retaining more bolls at the first fruiting position and producing less bolls above main-stem node 11 compared to the control. He speculated that the reduction observed in lint yield production was due to the loss of these fruiting positions as well as reduced lint per seed (Fig. 4).

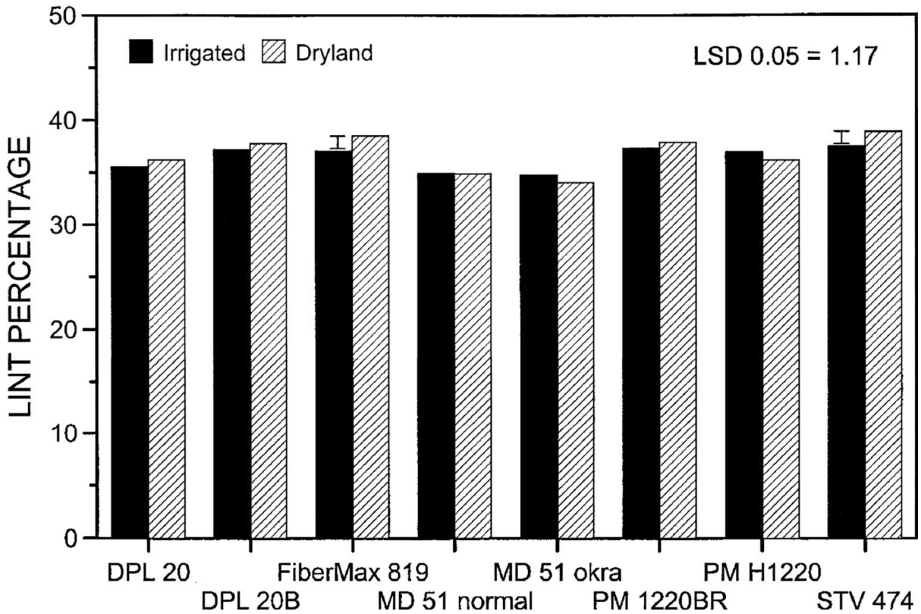

Figure 4. Lint percentage response of eight cotton genotypes when grown under either dryland or irrigated conditions. Genotype means averaged across the years 1998 to 2001. Vertical bars denote LSD values at the 0.05 level and are present only when the differences between soil moisture treatments for the individual genotypes are significant at P=0.05. (From Pettigrew, 2004).

Fiber properties have been reported to be insensitive to water-deficit stress (Bennett *et al.*, 1967; Marani and Amara, 1971, Hearn, 1976, 1995), unless the water-deficit stress is extremely severe. Leaf water potentials of -2.8 MPa have been shown to reduce fiber length (Bennett *et al.*, 1967). Water-deficit stress has also been reported to cause a significant reduction in micronaire (Eaton and Ergle, 1952; Marani and Amirav, 1971).Timing of water-deficit stress is also a significant factor since Marani and Amirav (1971) showed that stress early in the flowering season had no

effect on fiber quality. However, when the stress occurred shortly after flowering, it significantly decreased fiber length. Since the extension of the cotton fiber is a process primarily dependent on turgor (Dhindsa *et al.*, 1975) and carbohydrate supply, the reductions in plant water status and photosynthesis that occur under conditions of water-deficit stress would result in decreases in fiber growth. This was supported by Cosgrove *et al.* (1993) who reported that increased volume of growing plant cells depends on the water uptake by the vacuole. However, lint yield is a function not only of fiber qualities but also a function of number of fibers/seed and number of seeds/unit area (Lewis *et al.*, 2000). According to Rabadia *et al.* (1999) a strong correlation exists between plant water content and accumulation of dry matter in the developing fiber and seed, which implies that rapid water uptake is required for supporting seed growth. Additionally, the number of motes (unfertilized ovules) has also been demonstrated to increase under conditions of water-stress deficit (Saranga *et al.*, 1998) leading to further yield reduction.

COTTON WATER USE EFFICIENCY

Water use efficiency (WUE) quantifies plant biomass production based on water consumption. Physiological WUE is calculated as the ratio of carbohydrate fixation to transpiration, while agronomically, it is defined as the ratio between dry matter produced and quantity of water used. Because high WUE results in increased biomass production per unit of water, WUE is attractive as a trait to estimate drought tolerance.

Measurements of WUE are difficult to obtain, particularly when attempting to quantify efficiency and needs throughout the growing season. Crop WUE can be influenced by a number of environmental and management factors including radiation load, temperature, humidity, ambient CO_2 concentration, soil type and structure, soil water availability, nutrition, and genetic makeup (Lin and Ehleringer, 1982; Constable and Rawson, 1980; Reich *et al.*, 1985; Zur and Jones 1984; Reddy *et al.*, 1995; Loveys *et al.*, 2004).

Numerous physiological factors need to be considered when dealing with improving WUE, including stomatal regulation of gas exchange, regulation of plant development and functioning, increased photosynthetic capacity of the mesophyll, increased root hydraulic conductivity, and osmotic adjustment (Bacon, 2004). Additionally, WUE depends upon plant morphological characteristics, such as leaf size and position and canopy structure along with management practices, such as row spacing and plant density (Rosenow *et al.*, 1983; Krieg, 2000).

Measurements of WUE are further complicated by the difficulty in measuring whole-plant carbon dry matter accumulation and transpiration in the field, as well as the inaccuracies associated with scaling from occasional leaf photosynthesis measurements to estimates of whole-plant growth and water use. Hence, agronomic whole plant WUE evaluations are mostly based on general measurements of total dry matter production at the end of the season, as well as the combined total of the soil water bank, irrigation, and rainfall over a growing season. Despite the inherent errors and difficulties in measurement, a number of studies have evaluated cotton WUE; with values ranging from 0.1 to 0.3 kg lint/m^3 water (Hearn, 1979).

It has been suggested that WUE depends primarily on photosynthesis (Radin, 1992). Because photosynthesis is tied so closely to stomatal opening, it is not surprising that genes involved in

stomatal opening and closing regulate WUE (Chaves *et al.*, 2004). Hence, any discussion of WUE centers around gas exchange via the stomata. According to Bjorkman and Pearcy (1982) photosynthetic WUE in C3 plants could be expected to double with a doubling of the CO2 in the atmosphere, due to decreases in stomatal conductance required to meet CO2 demand in elevated CO_2 environments and the increase of intrinsic WUE (Morison, 1993; Drake *et al.*, 1997).

Farquhar and his colleagues pioneered the carbon isotope ratio technique ($\delta^{13}C$), and demonstrated its value by relating a low level of discrimination with enhanced WUE (Farquhar and Richards, 1984; Farquhar *et al.*, 1988; Condon *et al.*, 1987; Hubrick and Farquhar, 1989). Therefore, a possible solution to the difficulty in measuring WUE is to use $\delta^{13}C$ discrimination ratio between intercellular and ambient CO_2 concentration to estimate WUE (Farquhar *et al.*, 1982b;, Ehleringer *et al.*, 1989, 1993), but even this method is influenced by other factors that may change Ci concentration and affect $\delta^{13}C$ discrimination. Researchers using carbon isotope analysis have found varying relationships between WUE and drought tolerance in cotton. Positive relationships between isotope measurements of WUE and productivity were found by Gerik *et al.* (1996b). However, Leidi *et al.* (1993, 1999) reported inconsistent results across years, and multiple researchers have reported no correlation between carbon isotope measurements of WUE and plant productivity (Yakir *et al.*, 1990; Saranga *et al.*, 1998a). Lu *et al.* (1996) reported a positive association between carbon isotope discrimination and stomatal conductance. Saranga *et al.* (2004) observed no correlation between carbon isotope discrimination and yield production under water-deficit stress conditions and concluded that WUE needed to be combined with other physiological parameters for more accurate results. Therefore, the relationship between physiological WUE and cotton productivity is still unsettled.

Water use efficiency has been shown to vary substantially among species, genotypes and within species (Yoo *et al.*, 2009). Roark and Quisenberry (1977) and Quisenberry *et al.* (1981, 1984) found significant variability among exotic strains of *Gossypium hirsutum*, indicating possible improvements in growth stress characteristics. Chaves and Oliveira (2004) pointed out that it is important to understand the mechanism of drought tolerance, since different genotypes adapt to water deficit in different ways. Breeding crop varieties for higher WUE is a solution for improving water use in both rainfed and irrigated crop production (Condon *et al.*, 2004).

Water use efficiency also varies between cultivars and growth habits. Eaton and Belden (1929) and Gustein (1969) reported that Acala cultivars had lower water requirements than Pima cultivars. Quisenberry *et al.* (1976, 1991) reported that primitive cultivars, characterized by indeterminate growth patterns, had much higher WUE's compared to the modern determinate cultivars. They concluded that WUE was positively correlated with the indeterminate growth habit. According to a review by Gerik *et al.* (1995), relatively little progress had been made in increasing productivity of cotton or other crops per unit of water, i.e. by enhanced WUE, even though dryland and irrigated yields have increased. These yield increases have been mainly due to improved partitioning of carbohydrate to fruit (Gifford *et al.*, 1984).

Water use efficiency can vary by leaf age, node, and fruiting position, with variations occurring from one leaf to another in a cotton plant (Rawson and Constable, 1980; Wullschleger and Oosterhuis, 1989; Quisenberry *et al.*, 1976; Quisenberry *et al.*, 1991). Leaf shape, surface features, and position in the canopy can influence WUE. Picotte *et al.* (2007) using isotope discrimination reported that WUE was increased in plants with smaller, narrower leaves that had

higher trichome densities. Loveys *et al.* (2004) maintained that changes in leaf size may change the CO_2 and H_2O fluxes in and out of the leaf due to modification in the boundary layer. Differences in leaf thickness could significantly affect WUE, with thinner leaves decreasing WUE (Stanhill, 1980). Rapid leaf development in annuals influences WUE due to more efficient use of soil water through minimizing surface evaporation (Lopez-Castaneda, 1996). Leaves shaded in the canopy may have greater WUE than leaves in the sun, as was shown for *Betula pendula* (Sellin *et al.*, 2011), because of more conservative stomatal behavior and lower hydraulic conductance. There has not been any related work on cotton. Diaheliotropic leaves, that track the sun, like in cotton can increase carbon gain and WUE while not intensifying photoinhibition (Zhang *et al.*, 2009). These diaheliotropic leaf movements of cotton may reduce heat stress under dry conditions (Wang *et al.*, 2004), which could improve WUE.

Cultural practices have been shown to have an effect on WUE in some cases. Raven *et al.* (2004) concluded that restricted availability of soil nutrients decreases, or frequently has no effect, on plant WUE, mainly because decreased growth rate parallel decreased WUE. Ahmed *et al.* (1990) reported that WUE improved with increasing Zn fertilization through enhanced gas exchange. Blum (2005) said that maximizing soil moisture use is a crucial component of drought resistance and generally expressed in lower WUE. Salinity has also been shown to decrease both photosynthesis and transpiration (Hoffman and Phene, 1971) resulting in lower WUE indicating an effect on stomatal aperture. Additionally, failure to control insect pests in cotton early in the season results in yield losses and lower WUE (Jordan, 1986).

AMELIORATION OF WATER-STRESS DEFICIT

Alleviation of water-deficit stress through management practices such as early planting and irrigation has been known to farmers for a long time. Recent technological advances have provided scientists with a better understanding of the physiology of crops, thereby enabling them to make predictions and schedule management practices to minimize yield losses due to water stress.

Plant Growth Regulators

Amelioration of water-stress deficit through the use of plant growth regulating (PGR) substances has been suggested as a potential solution to water-deficit stress. Glycine betaine, a quaternary ammonium compound that is naturally accumulated in higher plants, has been shown to protect functional enzymes and lipids of the plant photosynthetic apparatus and maintain electron flow through thylakoid membranes (Xing and Rajashekar 1999; Allakverdiev *et al.*, 2003). Foliar application of glycine betaine has been reported to enhance drought tolerance and yield in maize (Agboma *et al.*, 1997a), tomato (Makela *et al.*, 1998), tobacco (Agboma *et al.*, 1997b), and wheat (Diaz-Zarita *et al.*, 2001). In cotton however, there are contrasting results depending upon the growing region. For instance, Gorham *et al.* (2000) found that glycine betaine aids in drought tolerance of cotton grown in Pakistan, while Meek *et al.* (2003) reported that foliar application of glycine betaine in Arkansas had no significant effect on yield.

Salicylic acid, a plant hormone that has been shown to increase the production of antioxidants, has also been observed to induce drought tolerance and improve yield in wheat (Singh

and Usha, 2003; Waseem *et al.*, 2007) and sunflower (Hussain *et al.*, 2008c, 2009). Application of salicylic acid however, has yet to be tested in cotton.

PGR-IV is a plant growth regulator that contains gibberellic acid (GA) and indolebutyric acid (IBA) that has been reported to increase root growth, nutrient uptake, boll retention and lint yield of well-watered cotton (Hickey, 1992; Oosterhuis, 1995; Oosterhuis and Zhao, 1994). In a 4-year field study, foliar application of PGR-IV was shown to increase yield under dryland conditions (Livingston *et al.*, 1992). Zhao and Oosterhuis (1997) conducted growth chamber experiments and indicated that application of PGR-IV before the onset of water stress could result in enhanced photosynthesis and dry matter accumulation. The increase in photosynthesis was attributed to either an increase in the nutrient absorption or improved carbohydrate translocation (Oosterhuis, 1995).

1-Methylcyclopropene (1-MCP), an ethylene inhibitor (Binder and Bleecker, 2003) has also been demonstrated to have a positive effect on stomatal resistance of water-stressed cotton leaves but with no significant changes in yield (Kawakami *et al.*, 2010). However, in another experiment, no significant effect of 1-MCP application on leaf stomatal conductance was observed in cotton plants experiencing water stress during flowering (Loka and Oosterhuis, 2011). However, the sucrose concentrations of water-stressed pistils though were lower compared to the control indicating that 1-MCP may improve the activity of sucrose cleaving enzymes resulting in better utilization of pistil carbohydrates.

It would appear that the use of PGRs has the potential to ameliorate water-deficit stress in cotton production. However, there is insufficient information on the use of these chemicals for such a purpose, specifically, how they influence metabolism to offset the adverse effect of drought and help maintain yield potential.

Selection for Drought Tolerant Genotypes

Drought tolerance is a quantitative trait, which means that it is controlled by more than one gene and has a complex inheritance. Since cotton originates from areas that are often exposed to water-deficit stress, considerable genetic variability in drought tolerance exists (Saranga *et al.*, 1998b; Pettigrew and Meredith, 1994; Quisenberry *et al.*, 1981). Past research focused on physiological traits such as photosynthesis and stomatal conductance (Leidi *et al.*, 1993; Nepomuceno *et al.*, 1998; Jones *et al.*, 1999), transpiration rates (Quisenberry *et al.*, 1982; Leidi *et al.*, 1993), canopy temperature (Hatfield and Quisenberry,1987; Jackson *et al.*, 1988), specific leaf weight (Morey *et al.*, 1974; Kumar *et al.*, 1987, Lopez *et al.*, 1995), excised leaf water loss (Roark *et al.*, 1975; Quisenberry *et al.*, 1982), leaf turgor maintenance (Quisenberry *et al.*, 1983), leaf carbon isotope discrimination (Yakir *et al.*, 1990; Saranga *et al.*, 1998a, 1999; Leidi *et al.*, 1999), leaf and root osmotic adjustment (Wullschleger and Oosterhuis, 1987; Nepomuceno *et al.*, 1998; Saranga *et al.*, 2001), leaf fluorescence (Burke, 2007; Longenberger *et al.*, 2009), WUE (Quisenberry and McMichael, 1991; Saranga *et al.*, 1998, 1999), biomass accumulation (Quisenberry *et al.*, 1981; Hatfield *et al.*, 1987), root growth and root-to-shoot ratio (Quisenberry *et al.*, 1981; Cook, 1985; McMichael and Quisenberry, 1991), cell membrane stability (Rahman *et al.*, 2008) and fruiting habit (Burke *et al.*, 1985a; Sharp and Davies, 1989; Lopez *et al.*, 1995). However, none of the above physiological traits has so far been consistently correlated positively with drought toler-

ance. Molecular studies have also been conducted for identifying quantitative trait loci (QTLs) responsible for improved cotton production under water limiting conditions (Saranga *et al.*, 2004, 2008) while use of genetic engineering and transgenic plants has been shown to result in helpful correlations (Lv *et al.*, 2007; Parkhi *et al.*, 2009).

SUMMARY

Water-deficit stress has a significant effect on cotton's growth and development, with primary affects on plant structure, leaf morphology and cell ultrastructure. Physiological processes such as stomatal conductance, photosynthesis and respiration are consequently impaired with further implications on the metabolic functions such as carbohydrate and energy production as well as carbohydrate translocation and utilization. Even though cotton possesses mechanisms to anticipate the negative effects of water-deficit stresses (i.e., accumulation of antioxidants, osmolytes and heat shock proteins) their protective capacity depend not only on the extent of the stress, but also on the timing of the stress as well as on the way the stress occurs (sudden or gradual). Yield reductions and fiber quality compromises are inescapable when water-deficit stress conditions override the plant's protective mechanisms. However, advances are being made at the physiological level entailing identification of exogenous or endogenous substances that can ameliorate the negative effects of drought and at the molecular level identification of genes involved with increased drought tolerance.

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Chapter 4

LIGHT AND THE COTTON PLANT

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INTRODUCTION

Light is the energy that supports all life on earth. It is also the energy that photosynthesis of ancient plants for creating the carbon that was the basis of fossil fuels. Today the sun's energy also powers photosynthesis and is involved in a myriad of plant processes that influence how plants develop and grow. This review will explore these processes.

WHAT IS LIGHT?

To humans, light is that portion of the electromagnetic radiation spectrum (ERS) that our eyes can detect. It is in fact only a small portion of the ERS (Fig. 1) that includes radiation wavelengths ranging from gamma rays to TV and radio. This small sliver of the ERS closely corresponds to the wavelengths that are utilized to drive plant photosynthesis, namely 400 to 700 nm or photosynthetically active radiation (PAR). However, this small range is not the only part of the radiation spectrum that affects plant growth and development. The presence of neighboring plants will alter the light environment through reflection from, and transmission through, plant tissues. Reflected and transmitted light will be enriched in far-red light in relation to red light. Both far-red and red light can be detected through phytochrome, a photoreceptor pigment in plants that changes form in response to red or far-red light and their ratio (Fig. 2). There are also blue light receptors, phototropins and cryptochromes, which mediate responses to the blue wavelengths of light. Mediation of growth and development in response to light environment is called photomorphogenesis and may include germination, (de)etiolation, and shade avoidance. Much of the initial knowledge concerning photomorphogenesis was obtained from experiments that utilized the Beltsville Spectrograph developed by Drs. Harry Borthwick, Marion Parker, and Sterling Hendricks at the USDA research center at Beltsville, MD (Kasperbauer, 1992). They passed a light source through two prisms that separated the light beam into its constituent components, much like a rainbow (Fig. 3). They placed plants into these separate light zones and observed the effect on plant growth. Through this work and other experiments the theory of phytochrome was developed and subsequently led to its discovery.

LIGHT INTERACTION WITH COTTON LEAVES

Leaves of *Gossypium hirsutum* "track" the light throughout the day. In other words, the leaves remain perpendicular, or mostly so to the impinging sunlight (Fig. 4). Ehleringer and Hammond (1987) found that the cosine of incidence of impinging radiation on *G. hirsutum* leaves was nearly one for the core hours of 0700 to 1500 hours. This observation is significant

due to Lambert's Cosine Law which states that the intensity of radiation is a function of the cosine of the angle from the perpendicular. If the angle of light impingement is 0 (the angle light is striking the surface is perpendicular), the cosine is 1. The law is also known as the cosine emission law or Lambert's emission law. It is named after Johann Heinrich Lambert, from his Photometria (Lambert, 1760).

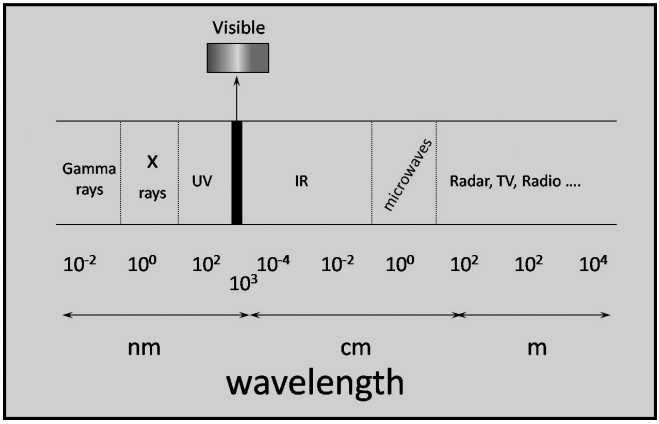

Figure 1. The electromagnetic radiation spectrum contains the relatively small range visible to the human eye, which closely corresponds to the wavelengths utilized in photosynthesis.

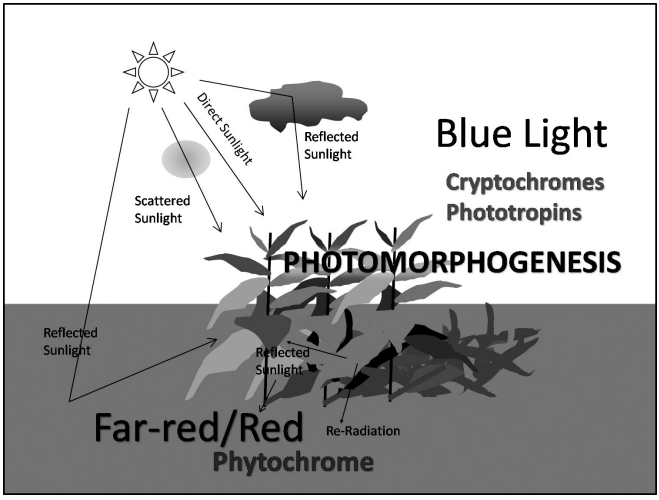

Figure 2. The various ways plants interact with light as affected by both the environment and neighboring vegetation and results in reflected, reradiated, scattered, and direct sunlight. Sensing mechanisms include both red/far-red (phytochrome) and blue (cryptochrome and phototropin) absorbing pigments.

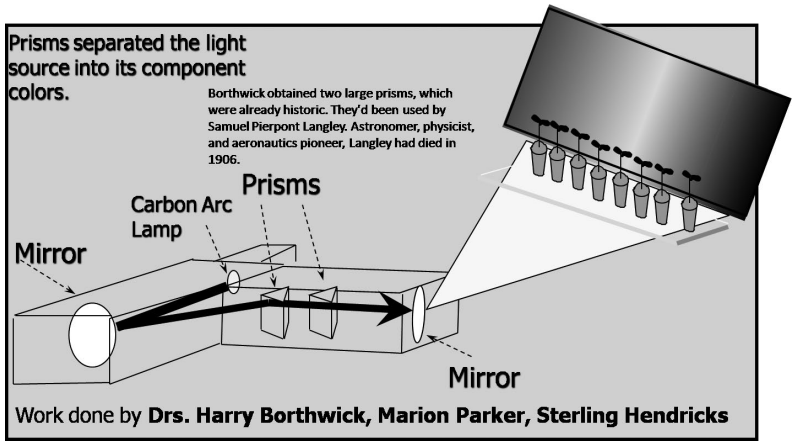

Figure 3. The Beltsville spectrograph used prisms to separate light into its component parts, thus allowing the study of finite wavelength ranges on plant growth and development.

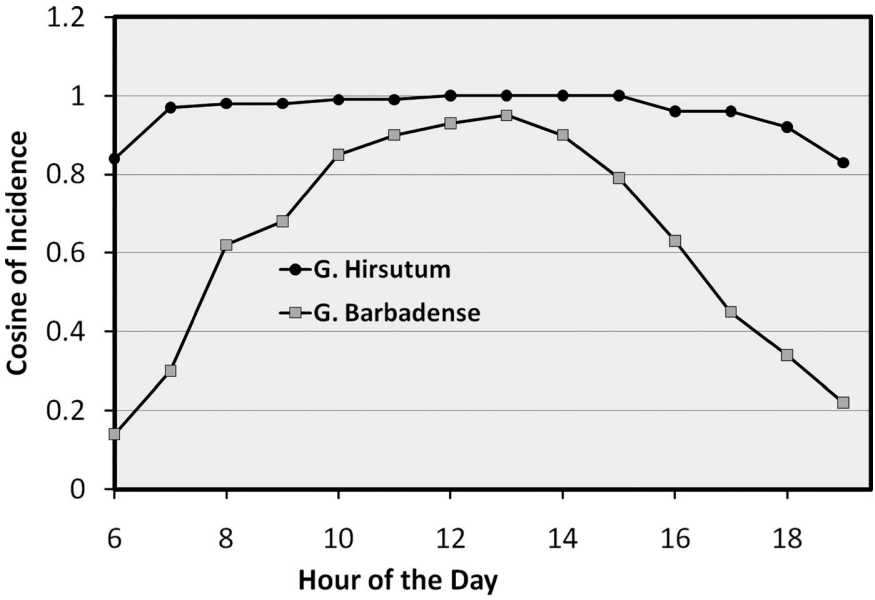

Figure 4. The diurnal course of the cosine of the angle of incidence for leaves of *Gossypium hirsutum* cv. Stoneville 825, and *G. barbadense* cv. Pima S5. Data are from Phoenix, AZ. (Adapted from Ehleringer and Hammond, 1987).

Such movement of upland cotton with the sun's position maximizes the available sunlight to power photosynthesis. Interestingly, *Gossypium barbadense* does not show a change in leaf position throughout the day. We know Lambert's Cosine Law through our own life experiences. The change of seasons is due to the changing angle of incidence that sunlight exhibits due to the earth's tilting and further explains the differing daylengths seen with changing latitude throughout the year.

The photosynthetic response of cotton leaves to PAR intensity may be seen in Figure 5. Although the okra-leaf cotton photosynthetic rates found by Pettigrew (2004) were greater than the normal-leaf, all showed little increase at a PPFD above 1,000 µmol m^{-2} s^{-1}. This threshold also exists for canopy photosynthesis rates which are maximized at a PPFD of 1,200 µmol m^{-2} s^{-1} (Wells, unpublished). These photosynthetic responses to light are typical of C3 photosynthetic metabolism.

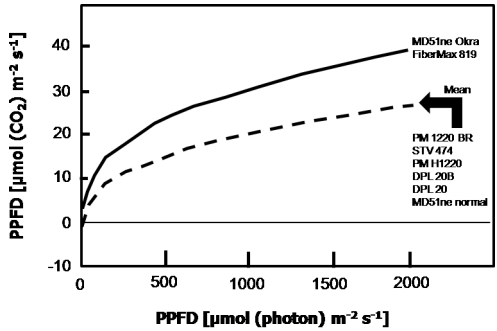

Figure 5. Mean photosynthetic response of two okra-leaf and six normal-leaf cotton genotypes to varying photosynthetic photon flux density (PPFD) in 1998 at Stoneville, MS. (Adapted from Pettigrew, 2004).

TOO LITTLE LIGHT

Goodman (1955) stated "this inability of some varieties (cotton) to respond to all cloud events may provide evidence in favour of the usually postulated mechanism of temporary carbohydrate shortage within the plant as being the fundamental cause of shedding during periods of cloudy weather". He was explaining the consequences of too little light during the season in Sudan. The work of Zhao and Oosterhuis (1998) using shading shelters in field-grown cotton during squaring, flowering and boll development, supported this. They showed that reduced light (63%) significantly decreased photosynthesis and carbohydrate concentrations in leaves and bolls, resulting in increased fruit abscission and decreased yield and fiber quality (Zhao and Oosterhuis, 1994). This phenomenon was also addressed in research of Pettigrew (1994) in which 30% shade was imposed on cotton during reproductive development (Table 1). The un-shaded control treatment exhibited lint yield, percentage yield at first harvest, and bolls per m^2 values that were 24, 10 and 26 % greater than the 30% shade treatment, respectively. In the same study, light was increased to lower canopy strata by either reflection or opening the canopy by restraint of neighboring rows. These treatments led to 6 and 17% greater fiber yield than observed in the untreated control, respectively. Both shade and enhancement of light penetration to lower canopy leaves demonstrate that photosynthate production is the ultimate determinant of cotton productivity.

Table 1. Lint yield, first harvest percentage boll mass, lint percentage, seed mass, and boll number averaged over 1991 and 1992.

Treatment	Lint Yield kg/ha	First Harvest %	Boll Mass g	% Lint %	Seed Mass mg	Boll Number no./m^2
Open canopy	1397	95.6	4.47	37.6	97	83
Reflectors	1261	93.6	4.44	37.7	98	76
Control	1190	92.7	4.41	37.8	99	72
Shaded Plot (30%)	957	84.0	4.44	37.7	98	57
LSD (0.05)	68	1.9	ns	ns	ns	4

(Adapted from Pettigrew, 1994).

PHYTOCHROME MEDIATED RESPONSES

A classic shade avoidance is seen in many plant species. This response entails photomorphogenesis including an increased nodal length, increased height, and a reduction in branching in response to shade which is enriched in far-red (FR) light. Ouedraogo and Hubac (1982) described such a response when the 9-hour light period was ended with 30 min. of FR light exposure. In a subsequent experiment, they found lower root mass and higher shoot dry weight/root dry weight ratio when the light period ended with 30 min. of FR light (Ouedraogo *et al*., 1986). The ending FR would convert the FR form of phytochrome (Pfr) to the R form of phytochrome (Pr) thus resulting in the morphological alterations observed. Smith *et al*. (1990) showed that the R/FR light ratio increased as a sensor was moved away from an artificial canopy of tobacco. In addition, the ratio of Pfr/Ptotal increased as a cuvette containing purified phytochrome was moved in a similar manner. These results showed that it was possible for plants to sense neighboring plants despite not being directly shaded by their presence, an important bit of knowledge when comprehending population effects on crop growth.

Light environment may also be altered by using different colored mulches. Kasperbauer (1994) used white, red, and green mulches in field-grown cotton (Table 2). He found the plant height, fiber yield and boll number were increased 10, 27, and 26% when red mulch was utilized instead of white. These same parameters were increased by 13, 23, and 21% when green mulch was used instead of white. In another study, fiber length was almost 4 mm longer in plants that were grown over the high FR/R reflectors (3.79 and 3.78 mm for green and red, respectively) than when grown over surfaces that reflected more photosynthetic light (1.07 and 0.94 mm for aluminum and white, respectively). This elongation response is very similar to stem elongation responses reported by Ouedraogo and Hubac (1982).

Table 2. Characteristics of mature cotton plants after growth from emergence through maturity over different colored soil covers (mulches) in trickle irrigated field plots near Florence, SC. Values are 2-year means ± SE. Seed cotton includes seed with fibers attached. Fiber and seed weights were determined after ginning.

Plant Characteristic	White	Red	Green
Height (cm)	79±1	87±2	89±2
Bolls (no)	19±1	24±1	23±1
Seed cotton (g)	78±4	98±6	95+5
Fiber (g)	30±2	38±2	37+2
Seed (g)	45±2	57±4	56±3
Seed weight (mg)	94±3	105±4	I07±4
Fiber seed^{-1} (mg)	62±3	70+4	70+3

(Adapted from Kasperbauer, 1994).

ULTRAVIOLET MEDIATED RESPONSES

Ultraviolet light is light that comprises the wavelengths of 10-400 nm. The range most often studied in relation to plant growth is ultraviolet B (UV-B) or medium wave. Zhao *et al*. (2004) examined the effects of both elevated CO_2 concentrations and UV-B levels on cotton growth and development (Fig. 6). The intensities of UV-B chosen were zero, that which would be experienced on a sunny day in Mississippi (8 kJ m^{-2} d^{-1}), and that which would be equivalent to a 30% increase associated with loss of stratospheric ozone (16 kJ m^{-2} d^{-1}). They found that only the greatest intensity of UV-B resulted in reduced photosynthesis, namely decreases of 56 and 45% at 360 and 720 µL L^{-1} CO_2, respectively when compared with the 0 and 8 kJ m^{-2} d^{-1} UV-B intensities. Gao *et al*. (2003) showed that cotton plant growth was negatively affected by radiation supplemented with UV-B at 4.8 and 9.5% above that found in ambient light (Table 3). The 4.8% enhanced level decreased plant height, leaf area per plant, net assimilation rate, relative growth rate, and biomass per plant by 5, 19, 37, 29,and 12%, respectively. These same variables were reduced by the 9.5% enhanced UV-B by 24, 29, 42, 45, 34%, respectively.

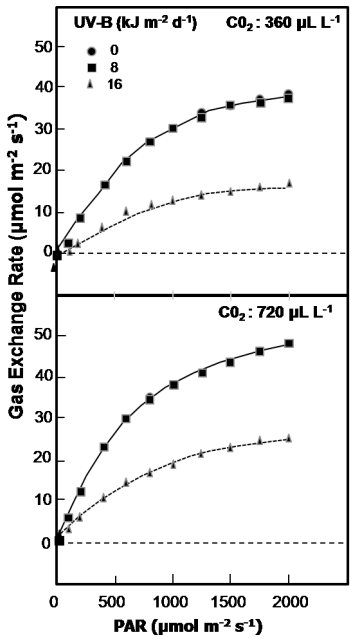

Figure 6. Photosynthetic light-response curves of cotton uppermost fully expanded mainstem leaves at first flower stage as affected by elevated [CO_2] and UV-B radiation. Data are means ± SE of three measurements. (Adapted from Zhao *et al*., 2004).

Table 3. The comparisons of cotton growth under different UV-B treatments.

UV-B Treatment	Plant Height (cm)	Leaf Area (cm^2/plant)	Net Assimilation Rate ($g/m^2/d$)	Relative Growth Rate (g/g/d)	Biomass (g/plant)
Ambient	104	3709	8.3	0.068	168
+ 4.8%	99.5	3000	5.2	0.048	148
+ 9.5%	89.5	2626	4.4	0.044	110

(Adapted from Gao *et al*., 2003).

TOO MUCH LIGHT

While maximizing light exposure is desirable for maximum plant productivity, there are situations when too much light can cause physiological harm to cotton. Payton *et al*. (1997) found about a 60 % inhibition of photosynthesis in response to a 3 hour exposure to 1,300 μmol m^{-2} s^{-1} at 5-7°C in both a normal genotype and a genotype with overexpressed Mn superoxide dismutase activity (Fig. 7). These 'photoinhibitory' effects have been also observed at milder temperatures. Königer and Winter (1993) observed reductions in photosynthetic rate of about 30 to 40% at a temperature as high as 20°C when exposed to either 1,000 or 1,800 μmol m^{-2} s^{-1} (Fig. 8). Photoinhibition can be caused by visible light (V), by ultraviolet light (UV), and by the interaction (UV-V) (Powles, 1984). These wavelengths may be involved with another pigment often seen in cotton, anthocyanin. Anthocyanin is the red color that appears in cotton especially in later stages of growth. It has been implicated in the absorption of UV light. In a study we conducted (Wells, unpublished) in North Carolina, plastic frames were attached to main-stem leaves and they were inverted and held in place so the abaxial surface was exposed to sunlight (Fig. 9A). The result was an increase in anthocyanin concentration in leaf tissue that were not covered by the frames (Fig. 9B). The anthocyanin concentration in the inverted leaves exhibited 20 to 50% increases over that in the non-inverted leaves. It has been suggested that anthocyanin plays the role of a sunscreen Gould (2004) reported that anthocyanins offer protection by two processes, by acting as a mask for filtering green light and by scavenging reactive oxygen species, thereby reducing the losses from photoinhibition after leaves are exposed to strong light. Purified anthocyanin scavenges almost all species of reactive oxygen and nitrogen with an efficiency up to four times greater than those of ascorbate and α-tocopherol. He also proposed that higher incidence of anthocyanins in stress environment is the last line of defense against ROS and photoinhibition after all other mechanisms of protections have been exhausted. Hoch *et al*. (2003) theorized that anthocyanins protect foliar nutrient resorption during senescence in certain tree species by protecting photosynthetic tissues from excess light. Using wild type and anthocyanin-deficient mutants of three deciduous woody species, they found wild type plants maintained higher photochemical efficiencies than mutants and were able to recover more easily from the effects of a high light, low temperature environment than could the mutants. Based on these reports it is possible that the anthocyanin increase in the inverted leaves is induced as a photo-protectant from light directed at tissue that is normally unexposed.

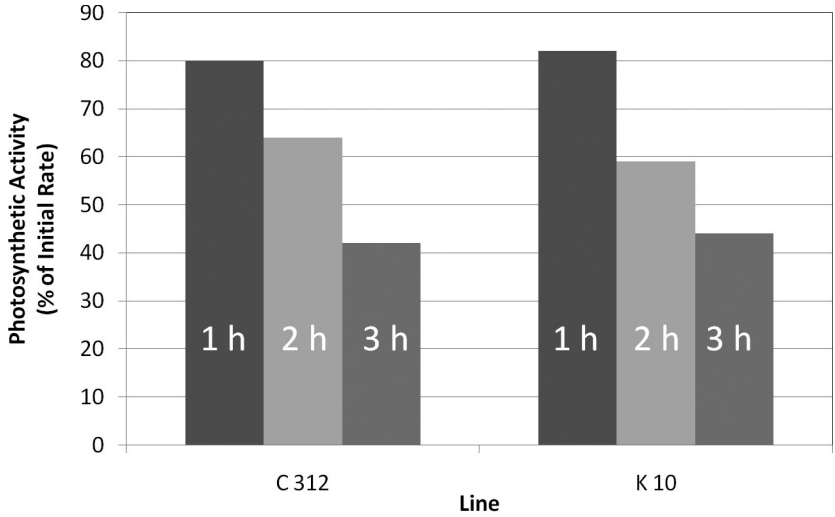

Figure 7. Steady-state photosynthetic activity at 25°C as a percentage of the activity prior to an exposure to a PPFD of 1300 µmol m^{-2} s^{-1} at 5-7°C for 1 h, 2 h, and 3 h for leaf discs from plants of Coker 312 and Mn superoxide dismutase transgenic line, K10. (Adapted from Payton *et al*., 1997).

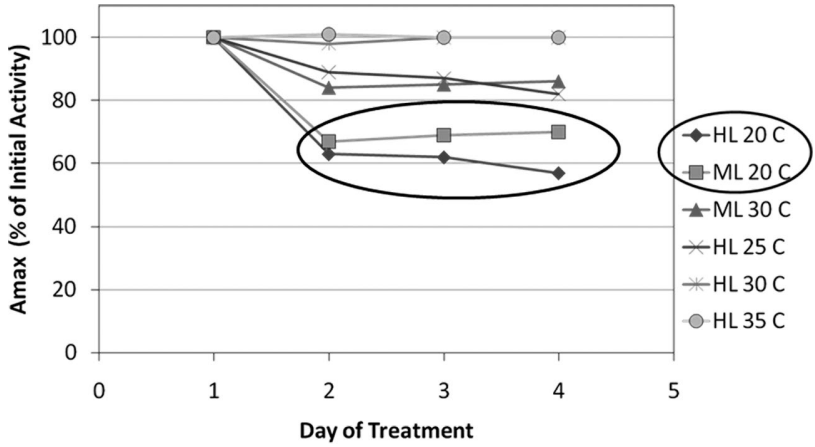

Figure 8. Changes in gas exchange parameters of sun leaves of *Gossypium hirsutum* during 4-d treatments. Leaves were exposed to either 1,800 µmol m^{-2} s^{-1} (HL) or 1,000 µmol m^{-2} s^{-1} (ML) at air temperatures of 35, 30, 25, and 20°C, respectively. Maximum CO_2 assimilation rates (A_{max}) of each quantum level are expressed as percent of the initial values measured at the onset of treatments. (Adapted from Königer and Winter, 1993).

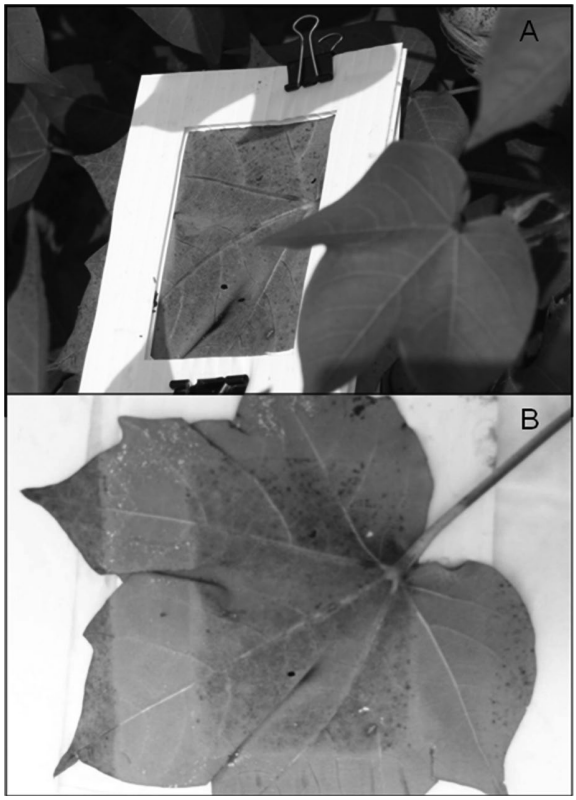

Figure 9. A. The plastic frame used to hold the leaf in a position that presents the adaxial to sunlight, and B. the leaf with the frame removed. Note the lack of red color where the frame was situated.

SUMMARY

Man's eye can detect only a small portion of the electromagnetic radiation spectrum (ERS) and this range closely corresponds to the wavelengths that are utilized to drive plant photosynthesis, namely 400 to 700 nm or PAR. There other wavelengths of light that fall outside of the PAR range that are involved in altering growth and development in response to the environment. Light will be reflected by and will be transmitted through neighboring plants and will alter the light environment. Reflected and transmitted light will be enriched in far-red light in relation to red light. Both far-red and red light can be detected through phytochrome, a photoreceptor pigment in plants that changes form in response to red or far-red light and their ratio. Phytochrome plus the blue light receptors, cryptochromes and phototropins, cause alterations in plant growth and development called photomorphogenesis. In addition, UV light can negatively affect cotton photosynthesis and growth when present at a sufficient intensity. Both too little light and too much light can have negative effects on cotton growth through effects on photosynthesis. Too little light fails to produce photosynthate in sufficient quantity to maximize growth thus leading to fruiting form shedding. Too much light, especially in the presence of low temperatures, causes reduced photosynthesis through photoinhibition. In either case, crop productivity is reduced. One thing is for sure, changing light environments will bring about change either through direct effects on photosynthetic capacity or through photomorphogenesis.

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Chapter 5

EFFECT OF ENVIRONMENT ON ETHYLENE SYNTHESIS AND COTTON

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INTRODUCTION

Utah is not the first state that comes to mind for cotton research, and, in fact, the total 2009 cotton acreage for Utah came in at 0.02 acres (Fig. 1). On a per acres basis, this is some of the most expensive cotton ever gown in North America. All of it was in either our research greenhouse, or under electric lights in a growth chamber. In this article I describe the past 2 years of research with the plant hormone ethylene. My students and I have found the responses of cotton to be far more interesting than corn or soybeans. Here we explain why.

Figure 1. A photograph of the Utah State University Research Greenhouse complex. The inset shows cotton plants in electronic balances for studies on transpiration rate.

Controlled environments provide a critical intermediate step in scaling from petri dishes to the field (Fig. 2). They allow the separation of individual environmental effects and provide an environment that can be reproduced at any time and in any location around the world. When plants are grown under electric lights the environmental conditions are nearly identical from day to day and from week to week.

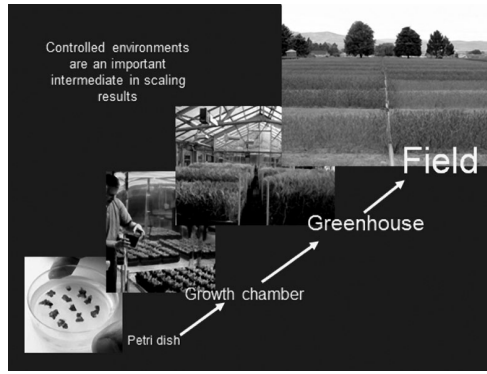

Figure 2. A diagram showing typical locations for scaling research results from a petri dish to the field.

Cotton: A Physiological Paradox

All students in introductory Plant Physiology learn that plants with C3 metabolism are favored by cool temperatures, and plants with C4 metabolism are favored by warm temperatures. Except cotton. The C3 pathway of photosynthesis is universal in cotton and yet it's temperature optimum exceeds many crop plants with C4 photosynthesis.

Part of the explanation for this comes from the high transpiration rates of cotton and the associated evaporative cooling. Figure 3 shows that the canopy temperature of cotton can be 11°C cooler than the air temperature. This astonishing difference only occurs in hot dry climates, but it indicates that the stomates of cotton stay open even in the middle of the day and keep the plant well below the air temperature. Most crop plants would be doing well to achieve a canopy temperature that was 5ºC below air temperature. Cotton has a unique ability to cool itself on hot days.

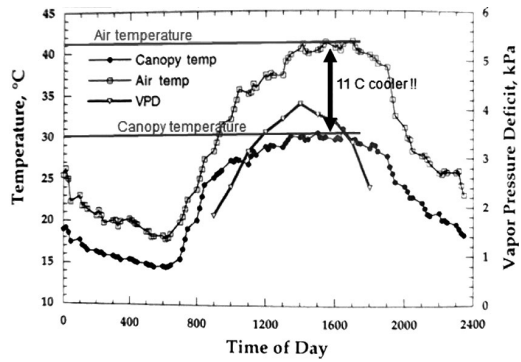

Figure 3. A 24-hour graph of the canopy temperature of cotton leaves (solid circles, left hand axis) and air temperature (open circles, right hand axis), and the driving gradient for transpiration vapor pressure deficit (open circles, right hand axis).

Hormones and Developmental Signaling

Hormones have the same definition in human, animal, and plant systems. A hormone is a molecule that signals responses at extremely low concentrations. In many ways a hormone is like a drum major leading a thousand piece marching band through a complicated parade route.

In animals hormones must be produced in one location and transported to another location, but this definition does not fully apply in plant systems where some hormones can be synthesized on one group of cells and signal an effect a few cells away.

There are five classic plant hormones. Among the most powerful is ethylene, which is widely used to alter plant responses, primarily through the commercial product Ethephon. Ethylene is widely regarded as a growth inhibitor and has long been thought to provide a signal leading to senescence and early aging in plants (Abeles *et al*., 1992). Ethylene is typically called the ripening hormone because of its role in ripening climateric fruit like avacados and banannas. Ethylene, however, is produced by all cells of a plant at all stages of the life cycle (Abeles *et al*., 1992).

Effects of Water Stress on Ethylene Synthesis

Ethylene production is commonly thought to increase during water stress, but there is considerable controversy on this topic. Part of the problem is the result of differences among experimental methods in ethylene research. Several studies have examined desiccation of detached leaves. These studies indicate that water stress increases ethylene production. Studies using intact plants indicate decreased ethylene synthesis (Morgan *et al*., 1990; Narayana *et al*., 1991). Ethylene synthesis was unaffected in maize mutants with variable internal concentrations of abscisic acid (Voisin *et al*., 2006). However, in this study the tissue was detached and placed in a sealed vial for capture of the ethylene. Thus, ethylene synthesis was not captured from the whole plant. The current understanding is that the effect of water stress on ethylene synthesis depends on the rate at which the plants are stressed. Rapid induction of water stress should promote ethylene production and slow induction should inhibit production (Morgan and Drew, 1997; Xu and Qi, 1993). Despite a lack of consistency in the technique used for whole-plant measurements, molecular techniques suggest that abscisic acid (ABA) influences ethylene effects in plant organs leading to a decrease in synthesis (Chaves *et al*., 2003). Several transcription factors that link ABA levels and ethylene production have been identified (Manavella *et al*., 2006). Members of this same family have also been influenced by light (Manavella *et al*., 2006). Reduced ethylene production is expected in the field since drought stress typically occurs slowly over the course of weeks. However, water-deficit stress occurs rapidly in highly porous media, especially when the root-zone volume is restricted (Morgan and Drew, 1997). Given observations made with different techniques and the molecular data, it seems likely that ethylene synthesis would decrease as a result of water-deficit stress.

Wheeler *et al*. (2004) quantified the ethylene synthesis rate of four crop plants in a large sealed chamber at the NASA-Kennedy Space Center (Fig. 4). They were surprised to find that ethylene synthesis peaked at anthesis in wheat and soybeans. This is likely due to its

role in signaling pollination and anther dehiscence. The ethylene synthesis rate of lettuce closely followed its growth rate, indicating that ethylene synthesis in this vegetative plant is a constant fraction of the photosynthetic rate. Although the potatoes in this study grew to be large, high yielding plants, they produced only minimal amounts of ethylene. These results are contrary to the widely held belief that ethylene synthesis is highest just prior to physiological maturity.

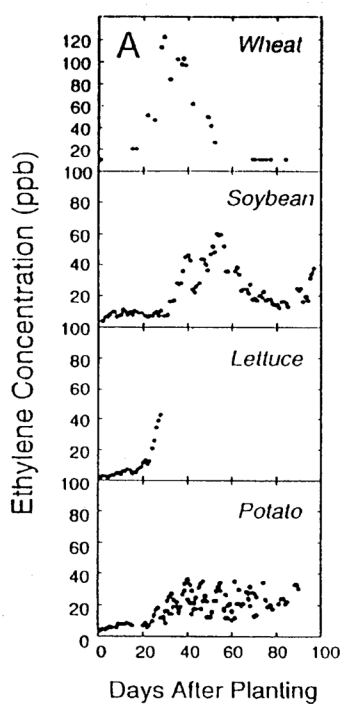

Figure 4. The relative production of ethylene over the life cycle of four crop plant species; wheat, soybean, lettuce and potato. The data are the increase in ethylene in a closed plant growth chamber at the NASA-Kennedy Space Center. Contrary to conventional wisdom, many crops have a peak ethylene synthesis at anthesis, and none of these crops had a peak ethylene synthesis rate during senescence. (From Wheeler *et al.*, 2004).

Effects of Atmospheric Ethylene on Growth and Yield

Ethylene is often called a self-extracting hormone because it is a gas at room temperature. This feature, however, can cause significant problems for the growth of plants in sealed environments where the ethylene cannot disperse with the wind. We recently completed a series of studies to determine the threshold levels at which ethylene alters plant growth. We were surprised to find that ethylene is 10,000 times more toxic to plants than carbon monoxide is to people.

In people, 50 ppm carbon monoxide toxicity starts to cause headaches. In plants, pollination is impaired by only 5 ppb (0.005 ppm; Klassen and Bugbee, 2002, 2004).

Hypothesis: Ethylene Decreases Cell Expansion and Internode Elongation

Ethylene is listed in textbooks as a growth inhibitor based on the classic effects of atmospheric ethylene on cell expansion and internode elongation. In both corn and cotton, ethylene acts like a dwarfing hormone and dwarfs the plants without any visible symptoms of stress (Fig. 5a and 5b). It is likely that the plants have the same number of cells, but cell expansion is inhibited. In soybeans, however, the effect of continuous ethylene exposure was radically different: ethylene inhibited leaf expansion, but increased internode elongation (Fig. 6). We clearly have much to learn about the effects of ethylene on plant development.

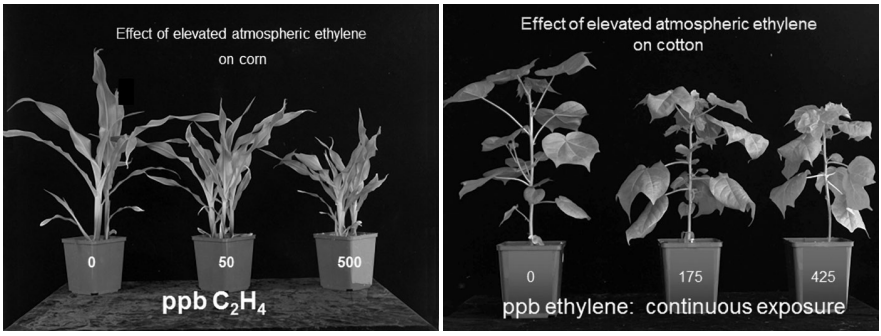

Figure 5 a and b. The effect of elevated ethylene levels on corn and cotton plants continuously exposed to ethylene in the air. In both species the internode elongation and the leaf expansion rates were decreased.

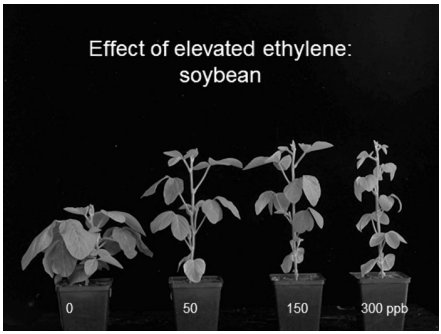

Figure 6. The effect of continuous exposure to ethylene on soybeans. Contrary to other crops, note that ethylene caused an increase in internode elongation. Similar to other crops, however, leaf expansion decreased with increasing ethylene.

The Effects of Stress in Plants

We know that some stress in our lives can lead to our most creative moments. A life without any stress rarely inspires people to greatness. Similarly, one can hypothesize that some stress in plants is also beneficial to trigger reproductive growth and optimal yields. However, this view is controversial and generally not accepted in cotton production. Notwithstanding, the effects of stress are hard to predict: in both plants and people.

Some of our reactions to stress are detrimental to the rapid healing. Our bodies over-react to stress. Swelling at the site of an injury is a good example of this because it reduces blood flow and slows healing. We reduce the swelling of a sprained ankle by using ice packs to cool the tissue, wrapping to compress the tissue, and elevation to reduce the blood pressure. We also use anti-inflammatory drugs to minimize inflammation.

Plants might also overreact to stress – and if they do, oversensitivity to ethylene is a good candidate for signaling the overreaction. In the past few years several groups have been studying a relatively new compound called 1-MCP (1-methylcyclopropene) (Kawakami *et al*., 2010), which blocks the perception of ethylene in plants (Sisler and Serek, 1997). The development of this product has provided physiologists a tool to study the effects of ethylene on growth and development of a wide range of plants. This is a major breakthrough because a biological over-reaction to stress in crop plants has the potential to cause billions of dollars in yield losses. 1-MCP has the potential to act like an anti-inflammatory agent in plants.

Ethylene, Leaf Elongation and Water Stress

We studied the effects of mild drought stress, with and without 1-MCP on leaf elongation in corn (Fig. 7). Our hypothesis was that application of 1-MCP would restore at least some of the normal leaf elongation in the drought-stressed plants. The 1-MCP was applied at 0.5 grams per liter of active ingredient (AFxRD). There was no beneficial effect of the 1-MCP in these studies.

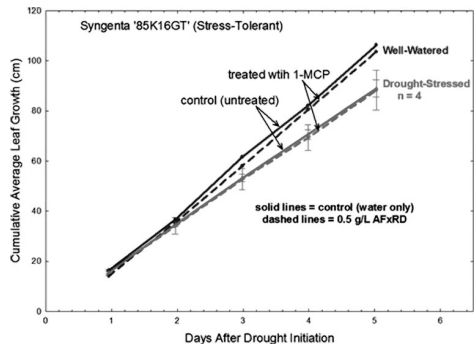

Figure 7. The effect of mild drought stress on cumulative leaf growth in corn. The data is the average of the most recently expanded four leaves. Plants with the dashed lines were treated with a technical grade of 1-MCP called AFxRD.

Similarly, Loka and Oosterhuis (2010) reported that application of 1-MCP to water-stressed cotton plants had no significant effect on leaf gas exchange functions, although carbohydrate metabolism of the pistil was significantly affected. Kawakami *et al*. (2010) reported that there was no significant effect on water-use efficiency and dry matter production water-stressed cotton plants treated with 1-MCP, but individual leaves had higher stomatal resistance and better maintenance of membrane integrity. An antagonistic relationship between ethylene and ABA on stomatal closure of water-stressed plants has also been reported (Wilkinson and Davies, 2010).

We subsequently studied the effect of more severe stress, but with a gradual onset, and with intermittent stress. Again, there was no beneficial effect of the 1-MCP application. The line labeled UTC in Figure 8 is the untreated control plant that was also the well-watered control treatment.

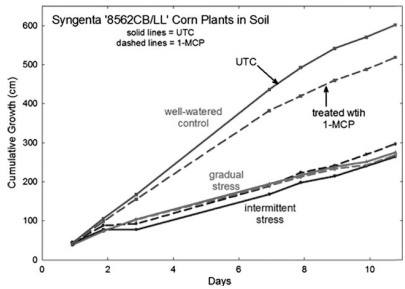

Figure 8. The effect of severe drought stress on cumulative leaf elongation in corn. Blocking the perception of ethylene with sprays of 1-MCP did not restore leaf elongation regardless whether or not the drought stress was gradually imposed or intermittent.

Ethylene, Leaf Elongation and Heat Stress

We subsequently studied the potentially beneficial effect of 1-MCP applications in heat-stressed corn plants. Plants were grown in three matching plant growth chambers (Fig. 9) that had four, 1000 W high pressure sodium lamps in each chamber to provide the equivalent of close to full sunlight at the top of the plant canopy (a photosynthetic photon flux (PPF) of 1600 μmol/m^2/s).

Figure 9. Three matching growth chambers at Utah State University that provide the equivalent of 80% of full sunlight at solar noon in the summer. Each chamber has been modified to include 4, 1000W high pressure sodium lamps and a recirculating, chilled water filter below the lamps. Cotton growth and development in this high light environment was excellent and representative of the field.

After some preliminary studies, we found that the air temperature needed to be above 33°C to be hot enough to reduce leaf expansion. The three chambers were thus set to 33, 37, and 40°C. There were 12 replicate plants per treatment and the CO_2 was elevated to 900 ppm to partially close stomates and reduce evaporative cooling of the leaves. The elevated temperatures effectively reduced leaf expansion (measured as daily leaf elongation; Fig. 10), but there was no significant effect of blocking ethylene on the restoration of leaf elongation.

Collectively, these studies do not indicate a significant role for ethylene in mediating the effects of either drought or heat stress, at least in corn plants. This is contrary to the conventional wisdom in most textbooks, which suggest that ethylene plays a key role in mediating plants responses to a wide range of environmental stresses.

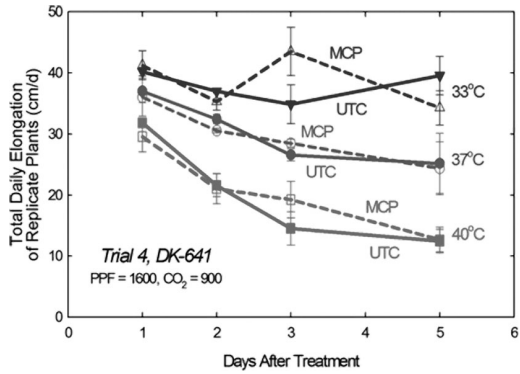

Figure 10. The effect of high temperature stress on leaf elongation of corn, with and without treatment of 1-MCP to block ethylene perception. 1-MCP did not result in a significant increase in leaf elongation at any of the three temperatures.

Development of Techniques for the Real-time Measurement of Whole-plant Transpiration

We have long sought improved techniques for the measurement of whole plant transpiration rates. We recently coupled digital balances to a data acquisition system (Campbell Scientific, model CR1000). This merger of balances and datalogger has allowed us to measure changes in mass of 1 gram and transpiration rates over 10 minute intervals. Figure 11 shows five cotton plants on five balances in a growth chamber. This is what we call a mini-lysimeter system.

We used this mini-lysimeter system to determine the effect of blocking ethylene perception on stomatal aperture. Figure 12 shows the diurnal transpiration rate of cotton plants over a 6 day period. There were two control plants that were sprayed with water and two plants sprayed with 1-MCP at field rates. Plants were initially sprayed with 1x of the field rate (10 g a.i./ha), and then sprayed with 3x the field rate. There was no significant effect on transpiration with either of the two spray treatments.

Figure 11. Five cotton plants on five balances in a growth chamber. This is what we call a mini-lysimeter system.

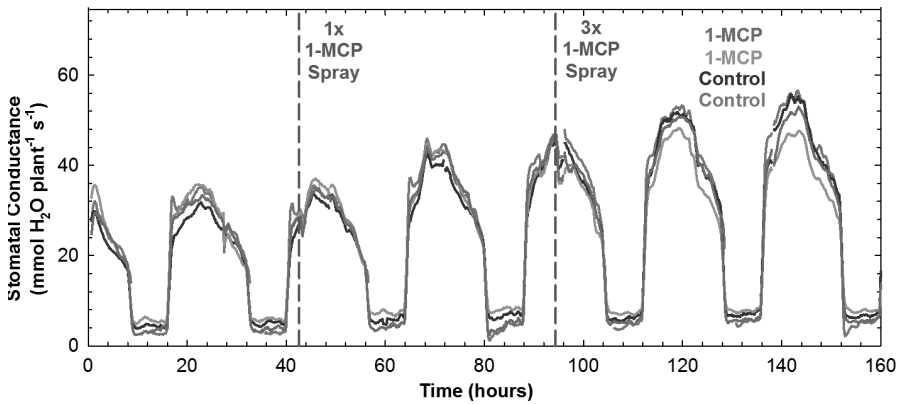

Figure 12. The diurnal transpiration rate of cotton plants over 6 days. Two of the plants were sprayed with 1-MCP to examine the effect of blocking ethylene on transpiration rate.

Jet Lag in Cotton

We used this mini-lysimeter system to determine possible circadian rhythms in several plants. Figure 13 shows the effect of changing the photoperiod on transpiration rate of cotton. Plants were grown with an 8 hour light period and a 16 hour dark period (days 1, 2, and 3 in Fig. 13). The photoperiod was then abruptly changed to a 16 hour light period. The stomates closed by about 2/3 after 8 hours of light, even though the environmental conditions remained exactly the same in this controlled growth chamber.

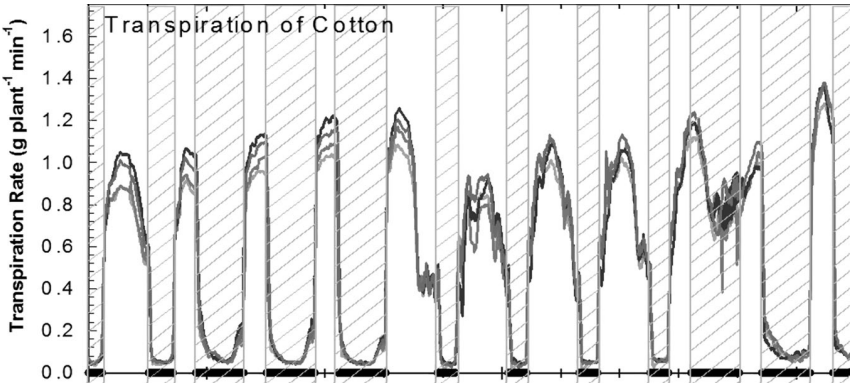

Figure 13. The effect of changing the photoperiod on transpiration rate in cotton.

The plants began to adapt to this longer photoperiod after only one day, and after three days of 16 hour light periods, the photoperiod was changed back to an 8 hour light period. The stomates opened in the dark for the first night, but then quickly adapted so that they closed almost normally in the second consecutive long night. Among the 3 primary crop plants we have studied (corn, soybeans and cotton), cotton has the most profound circadian rhythm. We have called this phenomenon: Jet Lag.

SUMMARY

Cotton has been a fascinating crop to work with. It is highly responsive to environmental signals and has significantly higher transpiration rates per unit leaf area than any other plant we have studied. These high transpiration rates likely help cotton leaves stay cool in environments with high air temperature.

We have not been able to find a role for ethylene in signaling a reduction in leaf elongation caused by either drought stress or heat stress. These studies do not prove that ethylene never has a role in signaling stress in these conditions, but they do indicate that it does not have the universal role that is suggested by textbooks.

Finally, ethylene does not appear to play a role in mediating stomatal aperture in well-watered plants. Cotton does have a profound circadian rhythm, however, which may help it stabilize transpiration rates in variable environmental conditions.

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Chapter 6

STRESS RESPONSE IN COTTON ROOT SYSTEMS

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INTRODUCTION

The development of the root system of the cotton (*Gossypium hirsutum* L.) plant is under genetic control but may be modified by environmental factors. The root system is an integral part of the "soil-plant" environment and as such provides the means for both water and nutrient absorption as well as the production of key plant hormones such as abscisic acid, cytokinins, and certain gibberellins. Also, part of this environment, the root system is subjected to a myriad of influences such as different soil properties as well as soil micro-flora and fauna that act alone or in combination to impact root development and plant productivity.

Since cotton has a taproot system, the extensibility of root development is dependent on the initiation and growth of the lateral or secondary roots. Therefore, these roots can extend outward from the taproot to a distance of over two meters (Taylor and Klepper, 1974). These roots also remain fairly shallow (less than one meter deep, Hayward, 1938). The lateral roots are formed from the cambial layer of the taproot and are arranged in a row according to the number of vascular bundles present in the primary root. The depth of root penetration depends on a number of factors, but in general the taproot can reach depths of over three meters and can elongate at a rate from less than one to over six centimeters per day. In general, the root system continues to grow and increase in length until young bolls (fruit) begin to form (Taylor and Klepper, 1974), at which time root length declines as older roots die. New roots continue to be formed past this point but the net result is a decline in total length (Hons and McMichael, 1986).

The concept of root stress in cotton as the plant develops, as will be discussed in subsequent sections of this chapter, centers generally around the impact of soil conditions on root system performance and growth, since roots grow in the soil matrix and are subject to factors that change in the soil environment. One particular aspect of cotton root development, however, that might be categorized as strictly root stress, would be the infection of roots by plant pathogens such as Verticillium wilt (*Verticillium dahliae* L.), and other pathological organisms. Although these organisms live in the soil, they can have a more direct effect on root system growth as contrasted to other soil factors such as water and nutrient stress

Therefore, the overall objectives of this chapter are to discuss individually some of the major soil factors that influence root development, how these factors affect plant productivity in gen-

eral, and the extent of genetic variability in the response of cotton to soil (root) stress. A more in-depth presentation of the factors that impact cotton root development is discussed in the chapter by McMichael *et al*. (2010) on growth and development of root systems.

STRESS RESPONSES IN COTTON ROOT SYSTEMS

There are many different kinds of stress that can influence root development in cotton. As indicated earlier, most of these stress factors originate as a result of changes that occur in the soil surrounding the roots that indirectly impact root function as well as root growth. Each of these factors will be discussed in terms of how they affect cotton root development with the realization that the interactions between these factors are extremely important in determining the final result.

Water Stress and Root Water Relations

The water content of the soil can have a significant influence on rooting depth and rooting density and therefore on function of cotton roots (Klepper *et al.*, 1973). Root activity can also change as the soil dries since root proliferation may occur at lower depths to maintain water uptake rates (Klepper *et al.*, 1973). Recently, McMichael and Lascano (2010) demonstrated the occurrence of "hydraulic lift" in cotton roots where water is transmitted to the roots in the drier upper soil layers through the root system. The water moves from the wetter lower layers to the upper layers to maintain the viability of the roots in the drier layers to reduce overall root stress. In general, soils with a small water-holding capacity have deeper roots while those with a larger capacity have shallow roots (Glinski and Lipiec, 1990). McMichael (unpublished data) showed that rooting densities of cotton increased significantly at lower depths and decreased in upper soil layers in several commercial cotton cultivars when the upper soil profile dried. Klepper *et al.* (1973) also observed that the rooting patterns of cotton in a drying soil shifted as the soil dried. Initially more roots were in the upper layers, but as a result of the death of the older roots in the upper soil levels due to the soil drying and production of new roots at the lower depths, the rooting density increased with depth. Cotton plants grown in uniformly moist soil did not show this reversal. Malik *et al.* (1979) also showed that emergence of cotton roots from soil cores of different water contents into a soil zone where water was freely available to the roots increased as the soil dried.

The root/shoot ratios also increased as the water content increased due to an absolute increase in root weight with shoot weight not being affected. Changes in water distribution as a result of irrigation practices can also impact the growth of cotton roots. Radin *et al*. (1989) noted that long irrigation cycles tended to trigger more rapid deterioration of the root system during periods of heavy fruiting above the normal net reduction in root growth as fruit develops. This trend was slow to be reversed. Carmi *et al.* (1992) observed that in cotton irrigated with a drip system a shallow root system with a high percentage of the roots less than one millimeter in diameter were concentrated around the emitters which resulted in a strong dependence on a frequent supply of water for continued growth. In other studies, Carmi *et al*.

(1993) also showed that the capability of more mature cotton plants to adjust rooting patterns to large changes in water distribution was slow and that preferential root growth relative to shoot development did not occur in response to progressive soil drying in their case. Carmi and Shalhevet (1983) also observed that dry matter production by cotton roots was less severely inhibited than shoots under decreasing soil moisture. This implies that changes in the root dry weight/root length relationships can change in response to changes in soil moisture. In terms of water extraction, Taylor and Klepper (1975) observed that water uptake in cotton was proportional to the rooting density as well as the difference in water potential between the root xylem and the bulk soil. Jordan (1983) showed that rooting densities may decrease to as low as 0.2 cm/cm^3 and still extract water. Taylor and Klepper (1974) showed that root length did not increase in a soil layer when the water content fell below 0.06 cm^3/cm^3 which was equivalent to a soil water potential of -0.1 MPa. In other work, Taylor and Klepper (1971) also observed that water extraction per unit length of root was greater in wet soil and decreased exponentially with soil water potential. In general, they found that deep roots were as effective as shallow roots in extracting water. Interactions between soil water status and soil temperature can also influence the function of cotton roots. Radin (1990) showed that the hydraulic conductance of cotton roots declined at cooler temperatures which would affect water uptake. Bolger *et al*. (1992) also showed that conductance decreased when the root temperatures were reduced from 30°C to 18°C. These results would suggest that under certain conditions the water uptake by cotton roots may decrease as a result of low soil temperatures even though water was not a limiting factor. Oosterhuis (1981) showed that root hydraulic conductivity was decreased by mild water deficit. The importance of the water relations of cotton roots *per se (i.e.,* axial vs. radial water flow and cell water relations) is certainly not to be overlooked in any discussion of the impact of water on root development. Oertli (1968) has provided an excellent review of water transport through the root systems of plants and soil-root interactions. Since much of this information is directly related to other factors mentioned in this chapter, a more comprehensive rendering is included in the next discussion on osmotic adjustment.

Most of the studies on the water relations of cotton have focused on the whole plant (e.g., Ackerson *et al*., 1977). Field research using mini-rhizotrons has shown that non-irrigated cotton had a deeper root length than irrigated cotton (McMichael, 1990; Keino *et al*., 1994). Furthermore, only non-irrigated cotton showed cultivar differences in root length density (Keino *et al*., 1994). These results suggested that cotton cultivars express large differences in root length distribution under water stress, and therefore, deep rooting cultivars should be selected within environments where water is limiting. Carmi *et al*. (1992) showed that a shallow and restricted root system resulted in strong dependence of the plants on frequent and sufficient water supply, such that temporary minor changes in irrigation affected plant water status and productivity. However, a shallow root system allowed maximum flexibility for using irrigation to quickly and efficiently affect plant water status and influence processes which determine productivity. In the last ten years there have been a number of new methods introduced to measure the water relations of roots. In cotton, thermocouple psychrometers have been used to measure root water potential (Oosterhuis, 1987; Yamauchi *et al*., 1995) and osmotic potential (Oosterhuis, 1987). The vapor pressure osmometer has also been used to

record osmotic potential (Ball and Oosterhuis, 2004) in excised roots. There are few reports on the nature of the osmotica in cotton and the importance of proline (McMichael and Elmore, 1977) and glycine betaine.

Root resistance accounts for a significant fraction of the hydraulic resistance in most plants (e.g., Fiscus, 1983). Radial root resistance is usually substantially higher than the axial resistance (Yamauchi *et al*., 1995). Hydraulic conductivity in cotton roots is reduced under conditions of water-deficit stress (Oosterhuis and Wiebe, 1980). Methods to measure cotton root hydraulic conductance were compared by Yang and Grantz (1996) with the reverse flow and transpirational methods appearing to have more physiological validity than the root exudation method. There have been reports of oscillations of 30 to 50 minutes in apparent hydraulic conductance in cotton plants (Passioura and Tanner, 1985), which is similar to the oscillations in stomatal conductance of cotton leaves (Barrs, 1971). Water deficit decreased cotton root pressure by 51% compared to a well-watered control, but had no effect on the exponential pressure-flux relationship (Oosterhuis and Wiebe, 1986).

Osmotic adjustment, or osmoregulation, is a plant mechanism for drought tolerance and the maintenance of water (ψ_w) potential gradients (Wyn Jones and Gorham, 1983). Osmotic adjustment involves the active accumulation of osmotic (e.g. sugars, organic acids and mineral ions) in the cytosol during periods of water deficit or salt stress to lower the osmotic potential (ψ_s) (Munns and Termaat, 1986). The lowered ψ_s response to decreasing ψ_w allows for the maintenance of pressure potential (ψ_p) for turgor (Hsiao, 1973). Turgor maintenance under water stress allows continuation of growth, although at a reduced rate in comparison to optimal conditions (Sharp and Davies, 1979). Osmotic adjustment may be an important mechanism in plant tolerance although some crops do not undergo adjustment (Morgan, 1980; Oosterhuis and Wullschleger, 1988). Osmotic adjustment is a well accepted phenomenon in higher plants (Morgan, 1984). The occurrence of osmotic adjustment, however, is not universal. Varying degrees of adjustment will depend on the nature of the applied stress, and also on the crop or species, cultivar, organ, and developmental age of the organ (Morgan, 1984; Turner and Jones, 1980). In cotton, as in most other crops, research on osmotic adjustment has focused on the leaves (Ackerson, 1981; Ackerson and Herbert, 1981; Cutler and Rains, 1977, 1979), and there are few reports of adjustments in the water relations of cotton roots in response to water stress (Oosterhuis and Wullshleger, 1987a). Cotton appears to have a greater ability to osmotically adjust to water stress than most other major row crops (Oosterhuis and Wullschleger, 1988) (Table 1). The magnitude of osmotic adjustment in cotton was greater in leaves (0.41 MPa) than roots (0.19 MPa), although the percentage change was greater in roots (46%) than leaves (22%) (Oosterhuis and Wullschleger, 1987a). The authors related this to the drought tolerance and survival capabilities of cotton. There is only one reported study of the role of osmotic adjustment with the growth of a root system in droughted field plants (Ball *et al*., 1994). This study showed only a small, limited amount of osmotic adjustment in the roots of field-grown cotton and a substantial adjustment in the leaves in agreement with Oosterhuis and Wullschleger (1987a). Osmotic potential of leaves varies diurnally (Hsiao, 1973), independently of daily cycles of leaf hydration. Therefore, leaves can maintain turgor during the daytime at the same level as during the night (Acevedo *et al*., 1979). Radin *et al*. (1989) inter-

preted the diurnal cycling of osmotic potential in cotton as an indication of a "sink-limited" condition within the plant during the boll development period. However, there have not been any similar studies on cotton roots. There is only a small range of genetic diversity of this trait in commercial cotton cultivars (Oosterhuis *et al*., 1987), although Nepumeceno *et al*. (1998) recently reported significant drought tolerance in an Australian commercial cultivar, CS-50. However, a more substantial range of osmotic adjustment exists in the primitive landraces and wild types of cotton (Oosterhuis *et al*., 1987). However, the role of osmotic adjustment in a cultivar bred for production as an annual crop may be quite different from that of osmotic adjustment in a perennial wild type. Osmotic adjustment has been favored as a trait offering potential for manipulation in the breeding of drought resistant crops (Sharp and Davies, 1979; Morgan and Condon, 1986; Turner, 1986). Work in Australia on wheat (*Tritium aestivum* L.) (Morgan and Condon, 1986) and sorghum (*Sorghum bicolor* L.) genotypes (Ludlow and Muchow, 1988; Ludlow *et al*., 1989) has shown increased yield in high osmotic adjusting phenotypes. The yield increase in sorghum of nearly 30% over low adjusting phenotypes was related to deeper rooting resulting in more carbon fixation and increased harvest index. A clear yield advantage from osmotic adjustment in cotton has not been demonstrated. The role of osmotic adjustment in maintaining root growth, allowing water uptake longer in drying soil, has been emphasized by Acevedo and Hsiao (1974). The premise that osmotic adjustment allows for turgor maintenance and increased root growth at low water potentials implies that the plant will be able to exploit a greater and deeper soil volume for water. The role of the root system during drought is receiving current research attention as a possible sensing organ and in root-to-shoot ratios. Jones and Turner (1978) cautioned that the capacity to tolerate drought may be attributed to factors other than plant water relations, such as rooting habit, conductance of water through the xylem, and desiccation tolerance.

Table 1. Magnitude and percentage osmotic adjustment in response to water stress by various crop plants. (From Oosterhuis and Wullschleger, 1988).

Crop	Osmotic adjustment			
	Magnitude		Percentage[z]	
	Leaves	Roots	Leaves	Roots
	----------(MPa)----------		----------(%)----------	
Cotton	0.41a[y]	0.21a	22.4	46.3
Sorghum	0.31a	0.19a	25.1	37.1
Sunflower	0.17b	0.16a	13.9	25.2
Wheat	0.08c	0.03b	6.6	4.4
Soybean	0.05c	0.00b	4.0	-0.8

[z] Percentage osmotic adjustment refers to the percentage decrease in osmotic potential compared to the well-watered control.

[y] Means within columns followed by the same letter are not significantly different at the 5% level of probability.

Soil Temperature

The temperature of both the soil and air can have a significant influence on the growth of cotton root systems. Most research has shown that in general, the growth of cotton roots increases with increasing soil temperature until an optimal temperature is reached beyond which growth declines. Early work suggested that the optimal soil temperature for the growth of cotton roots was approximately 35°C (Bloodworth, 1960; Lety *et al*., 1961; Pearson *et al*., 1970; Taylor *et al*., 1972). Pearson *et al*. (1970) showed that root elongation increased to a maximum of 32°C and then declined sharply as soil temperature increased in 80-hour-old seedlings. Research by Bland (1993) in controlled environment experiments showed that the rate of cotton root growth increased with the rate at which the soil warmed. His experiments indicated that the root system grew at progressively lower rates of elongation as the rate of soil warming was reduced from isothermal conditions. In research on the growth of roots of cotton seedlings at various soil temperatures, McMichael and Burke (1994) showed that the optimal temperature for root elongation may depend on the level of available substrate or stored seed reserves. They suggested that the measured root length at 10 DAP (days after planting), for example, represented a composite of both narrow and broad metabolic temperature responses. Analysis of mitochondrial electron transport showed that the temperature optimum for root metabolism at 10 DAP (days after planting) for example, was lower than that obtained from the measure of accumulated root growth during the same time period. Kaspar and Bland (1992) indicted that changes in soil temperature can affect growth of a number of root system components. For example, low temperatures generally reduced cotton root branching (Brower and Hoagland, 1964), while higher temperatures approaching the optimum tend to increase branching (Nielsen, 1974). The uptake of water by roots is reduced at low temperature (Nielsen, 1974) while higher temperatures result in increased uptake. Bolger *et al*. (1992) demonstrated that the hydraulic conductance of cotton roots declined as the root zone temperature decreased below 30°C and that conductance at 18°C averaged 43% of that at 30°C. Differences in the response of different root types to temperature were also apparent. Research conducted by Arndt (1945) indicated that the cotton taproot may be more adapted to adverse soil temperatures than subsequent branch roots at least until the taproot had developed to approximately 10 cm in length. Later work on seedling development of a number of exotic cotton strains grown in hydroponics showed similar results (McMichael *et al*., 2010) (Fig 1.). Steiner and Jacobsen (1992) also noted differences between two cotton cultivars in their sensitivity to soil temperature. When the root temperature was low (20°C), root growth was reduced regardless of the temperature of the air (McMichael and Burke, 1994). The root-shoot interaction in response to temperature may be related to changes in source-sink relationships. Guinn and Hunter (1968), for example, showed changes in carbohydrate levels in shoots and roots in response to temperature with a build-up of sugars occurring at low root temperatures. The successful emergence and initial growth of cotton seedlings is important for the establishment of healthy and improved productivity. Wanjura and Buxton, (1972 a, b) showed that when the minimum soil temperature at planting depth dropped from approximately 20°C to l2°C, the hours required for initial seedling emergence increased from 100 to approximately 425 hours. In many cotton-growing areas the soil temperature can be significantly lower than the optimum when seeds are planted thus impacting the final yield. Therefore the

development of cultivars that possess a root system that can grow and function at low temperatures could improve plant performance. However, since the exact mechanism(s) of the response of cotton roots to temperature are not known, further research, perhaps in the molecular area, is needed to elucidate the nature of the response.

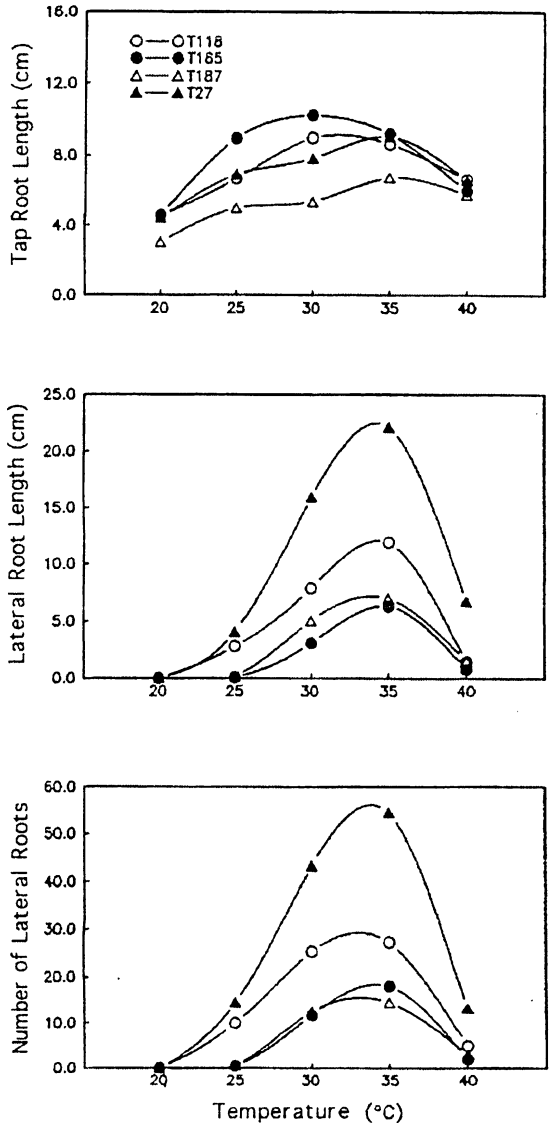

Figure 1. The influence of temperature on the growth of primary (tap) roots and lateral roots of 10-day-old cotton seedlings of four exotic strains of cotton. (From McMichael *et al.*, 2010)

Salinity

Cotton is a relatively salt tolerant species, but growth can still decline when the plant is exposed to saline stress. Germination and emergence (El-Zahab, 1971) and seedling growth (Zhong and Lauchli, 1993) are particularly salt-sensitive. Salinity generally reduces root growth (Silberbush and Ben-Asher, 1987), but there have been reports of mild salinity enhancing root growth (Jafri and Ahmad, 1994; Leidi, 1994). The ions Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- are the common constituents involved in high salinity and altered plant growth and root expression. Primary root growth of cotton seedlings was severely inhibited by high concentrations of NaCl in the growing medium, but supplemental Ca reduced Na influx and improved root growth (Cramer *et al*., 1987; Zhong and Lauchli, 1993). The protective effect of supplemental Ca on root growth under high salinity has been associated with improved Ca status and maintenance of K/Na selectivity (Cramer *et al*., 1987) and improved cell production (Kurth *et al*., 1986). Obviously high soil salinity can cause effects similar to water-deficit stress on plant growth (Kramer and Boyer, 1995). The degree of salinity influences the plant's ability to osmotically adjust to the altered water potential gradient between the soil solution and the plant root. According to Zhong and Lauchli (1993), cotton is a relatively salt tolerant plant, but can be very sensitive to salt conditions in the seedling stage. Water stress and ion toxicity are most likely the result of high salt conditions that reduce plant growth. Cramer *et al*. (1987) observed that the growth of the taproot of cotton seedlings was reduced in the presence of NaCl but that the effects could be countered somewhat by the addition of Ca to the growing media. Zhong and Lauchli (1993) found that the elongation of the taproot of cotton seedlings was reduced by 60% over the control plants when the roots were exposed to 150 mol / m^3 NaCl. The addition of Ca increased the elongation rate to within 80% of the controls. They also observed that the growth zone (the region of root cell elongation) of the taproot was shortened by the increased salt content of the media. Kurth *et al*. (1986) showed that the rate of cell production declined in cotton roots in the presence of high salt and that the shape of the cortical cells were affected. Reinhardt and Rost (1995d) also observed that high salt reduced the width and length of metaxylem vessels in cotton seedlings which increase with plant age. These changes in root morphology along with changes in osmotic relationships as a result of high salt, can result in a significant reduction in root growth and root activity to reduce plant productivity.

Pathogens

The presence of soil-borne pathogens can impact the growth and function of cotton root systems. Pathogens such as *Phymatotrichopsis omnivera* are common agents that cause root rot in cotton (Rogers, 1937). Domsch *et al*. (1980) have indicated that cotton seedlings may be more resistant to attack by this organism than older plants due to a reduced carbon content of the root bark. An increase in the carbon content of the roots due to loss of branches and fruit tends to reverse this effect. King and Presley (1942) reported that a disease of cotton that was characterized by a swollen taproot and internal black rot of the vascular tissue was found in Arizona in 1922.

The organism was identified as *Thielaviopsis basicola* and was found to be most damaging to the cotton root system in the seedling stage. Rothrock (1992) later showed an interaction of this organism with soil temperature, soil water, and soil texture on the infection of cotton roots. Burke and Upchurch (unpublished data) observed that cotton plants grown at low temperatures in the absence of pathogens had increased lateral root production even at the low temperatures (13°C). Other studies have shown that infection of cotton roots by nematodes may impact the growth and development of the plant (Kirkpatrick *et al*., 1991). These authors indicated that the effects of the infection were similar to water stress. The hydraulic conductivity was reduced and drought resistance was increased.

Recently Liu (1995) demonstrated the effect of VAM (vesicular arbuscular mycorrhizae) on *Verticillium wilt* in cotton. His data indicated that when the cotton roots are colonized by VAM, the incidence of *Verticillium* is reduced resulting in improved yields.

Genetic Potential

The growth of the root system of cotton is under genetic control (McMichael *et al*., 1987) but may be modified by the environment as discussed in previous sections of this chapter. McMichael (1990) has shown variability for root weight in a number of exotic cotton accessions. Variability in root/shoot ratios was also observed in these studies. Earlier, McMichael *et al*. (1985) showed genetic differences in the number of vascular (xylem) bundles in cotton taproots and suggested that variability in lateral root production was associated with the differences in vascular arrangement. Later research indicated this to be the case (McMichael *et al*., 1987; Quisenberry *et al*., 1981). McMichael *et al*. (unpublished data) also found genetic differences in the response of cotton seedlings to changes in temperature. Quisenberry *et al*. (1981) found differences in older plants in lateral root production as well as taproot growth. It was further suggested by McMichael *et al*. (1985) that the observed increase in the vascular system and enhanced lateral root production could lead to improved water status of the plant in drought conditions since the potential for additional water uptake and utilization might be possible. Work by Cook and El-Zik (1992) suggested that cotton genotypes having deep roots and increased lateral root production would be more drought resistant based on the variability in root traits. Oosterhuis and Wullschleger (1987b), however, were unable to show significant improvement in hydraulic properties of the plants with the increased vascular arrangement. In field studies, Hons and McMichael (1986) showed that water extraction patterns from fallow rows of a 2x2 skip row pattern were significantly less than cotton planted in every row. This suggested that there was not sufficient rooting density in the cultivar used to use the additional water in the fallow rows. This led Quisenberry and McMichael (1996) to use a more extensive skip-row planting technique to show significant, differences in rooting potential in a number of cotton genotypes by measuring differences in yield as a function of the ability of the plant root systems to extract water. This approach can be utilized to rapidly evaluate genetic differences in root development under field conditions.

Genetic Variability for Improving Root Growth

Genetic variability in a number of root parameters in cotton has been shown to occur across a range of environmental conditions. Quisenberry and McMichael (1996) indicated that genetic differences in rooting potential was related to plant productivity and that an increase in potential (primarily increases in root branching and distribution) could result in increases in yield of cotton under conditions of a drying soil profile. Greenhouse studies conducted using twenty-five cotton genotypes ranging from exotic accessions to commercial cultivars showed significant variability in the dry weights of root systems of sixty day-old plants (Table 2). The variability was greater in the exotic accessions than in the commercial cultivars (McMichael and Quisenberry, 1993). McMichael *et al*. (1985) showed that the increased root xylem (vascular bundle) arrangements in the taproot of some of the exotic cotton accessions resulted in a significant increase in total vessel cross-sectional area and an increased number of lateral roots. This increase suggested an overall decrease in axial resistance to water flow in the root system which may be associated with characteristics of drought tolerance in plants with the increased xylem vessels. Oosterhuis and Wullschelger (1987a) supported the finding that increased water flux was associated with increased xylem cross sectional area. However, an increased number of vessel elements in the xylem of the primary root did not result in any apparent decrease in axial resistance to water flow. The increased number of lateral roots associated with increased vascular bundles resulting in increased xylem vessels may be important characteristics associated with drought tolerance in plants with the increased xylem vessels which may lead to improved yields.

SUMMARY

The growth and development of the root system of cotton has been shown to be genetically controlled, but subject to modifications by a wide range of both above and below-ground environmental conditions. The overall productivity of the plant is, therefore, influenced by the integrated response of the roots to environmental stimuli. In this chapter we have briefly touched on how the cotton root system initiates and grows as well as discussed a number of major factors that influence root development. We have also presented some strategies for enhancing root growth in cotton such as taking advantage of genetic variability. Since current techniques are readily available and can be incorporated into most cotton research programs, future work should not neglect the importance of taking into account the development of the root system in evaluating cotton growth and productivity. As molecular biology continues to make inroads into our understanding of plant development and presents the possibilities for genetic engineering of plant growth processes, the opportunity also exists for manipulating the growth and development of the root system. These advances coupled with the new concepts of precision farming for example, may provide the means for maximizing cotton root system function for maximum plant productivity.

Table 2. Mean root dry weights averaged over experiments for 25 cotton genotypes grown in the greenhouse. Plants were 60 days old at time of harvest. (From McMichael and Quisenberry, 1992).

Genotype	Root dry weight (g)
T184	3.95
T141	3.86
T252	3.30
T283	3.12
T1	3.11
T171	3.08
T256	3.07
T461	2.83
T25	2.80
T115	2.79
T15	2.74
T1236	2.73
T185	2.47
T80	2.45
T45	2.36
Paymaster 145	2.16
Deltapine 61	2.15
G. herbaceum	2.15
Coker 5110	2.05
T151	2.03
T50	2.01
Tamcot CAMD-E	1.91
T169	1.87
Pima S-5 (*G. barbadense*)	1.77
Lubbock dwarf	1.63
LSD (0.05)	0.47

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Chapter 7

PHYSIOLOGY OF BORON STRESS IN COTTON

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INTRODUCTION

Boron is the most deficient essential micronutrient in cotton (*Gossypium hirsutium* L.) fields (Rosolem *et al*., 2001). Cotton has a relatively high requirement for B (Zhao and Oosterhuis, 2002), requiring an average of 340 g B ha^{-1} and exporting in seedcotton around 12% of the B accumulated in the plant (Rochester, 2007). Nutritional disorders caused by B deficiency in cotton are quite common in tropical soils, where soil organic matter and/or clay content are low (Rosolem *et al*, 2001), and in other areas of the world where B availability is significantly reduced in calcareous soils (Shorrocks, 1997). For instance, it was estimated that 37% of Indian soils are B deficient (Singh, 2009). Boron is prone to leach through the soil profile, depending on soil texture, another factor leading to B deficiency (Communar and Keren, 2006; Rosolem and Biscaro, 2007) as well as posing an environmental threat to water tables. Conversely, B toxicity to crops is most commonly found in arid and semi-arid regions or soils developed from marine sediments, as a consequence of high B concentration in irrigation water and use of high B compost material or fly ash (Nable *et al*., 1997). Hence, B deficiency or toxicity can be found throughout cotton growing regions worldwide.

The range between boron deficiency and toxicity is very narrow. It is known that B deficiency can significantly limit cotton yields without any visible foliage and flower symptoms, characterizing the occurrence of "hidden hunger" (Satya *et al*., 2009). Boron deficiency is not easily recognized even in foliar diagnosis, since cotton plants showing 11 mg kg^{-1} of B in the most recently mature leaves yielded the same dry matter as non-deficient plants, but the number of reproductive structures was lower (Rosolem *et al*., 1999). Reported B sufficiency levels in cotton leaves range from 16 to 80 mg kg^{-1} (Rosolem *et al*., 2001; Zhao and Oosterhuis, 2002), and toxicity may be observed with B concentrations over 100 mg kg^{-1} (Bergmann, 1992). Because of this narrow range, plant analysis is not a highly effective tool for monitoring plant B nutritional status and estimating plant response to fertilizers.

Despite positive yield responses to B applied either to the soil or sprayed directly on cotton leaves, a controversy remains as to when to apply B, as well as the best means of doing so. The low mobility of B in cotton phloem is an additional complication in this equation, because a temporary deficiency may lead to some yield loss. In this chapter the onset, development and physiology of B deficiency in cotton will be discussed aiming at a better understanding of the role of B in cotton production.

FUNCTIONS OF BORON IN PLANTS

The unusual nature of boron chemistry suggests the possibility of a wide variety of biological functions for the micronutrient. However, the exact metabolic functions are not yet fully understood (Hansch and Mendel, 2009). Boron is essential for the formation of meristematic tissues: its uptake is fast but its mobility in cotton plants is low. Most of the currently known processes involving B are based on its role in the formation of reversible diester bonds with *cis*-diol containing molecules, but it may play a role in membrane stabilization crosslinking glycoproteins, and may be also involved in their recruiting to membrane domains (Wimmer *et al*., 2009). Boron may stimulate or inhibit enzymes and participate in phenol metabolism avoiding toxicity problems (Römheld and Marschner, 1991).

One of the primary functions of B in higher plants is based on the formation of borate esters with apiose residues of rhamnogalacturonan II (RG-II) in the cell wall (Kobayashi *et al*., 1996), which is essential to its structure and function and contributes significantly to the control of cell wall porosity and strength (Fleischer *et al*., 1999, Ryden *et al*., 2003). Boron was reported to be involved in sugar transport, plant respiration, metabolism of RNA, carbohydrates and plant hormone (indole acetic acid) metabolism (Camacho-Cristótal *et al*., 2008). It promotes structural integrity of bio-membranes and the formation of lipid rafts. Since all these functions are fundamental to meristematic tissues, boron deficiency is predominantly damaging in actively growing organs such as shoot and root tips (Hansch and Mendel, 2009). The transport of chlorine and phosphorus are increased as a result of plasmalemma ATPase induction, and it has been shown that boron can stimulate proton pumping that causes hyperpolarization of the membrane potential (Camacho-Cristóbal, 2008). Hence, B may affect ionic absorption and its deficiency would decrease the uptake of several nutrients (Dugger, 1983).

No membrane-bound molecules interacting with B have been isolated so far, but deficiency symptoms point to additional functions of B in cell membranes. Binding of mitochondrial ATP synthase, several beta-glucosidases, a luminal binding protein and fructose bisphosphate aldolase to B was significantly reduced with B deprivation (Wimmer *et al*., 2009).

Boron is particularly important during the plant reproductive phase as pollen germination and growth of the pollen tube are impaired when B is deficient (Agarwala *et al*., 1981). In cotton, B deficiency during flowering and fruit formation increases shedding, decreasing fiber yields and also fiber quality (Miley *et al*., 1969; Rosolem and Costa, 2000). Given the rather high proportion of B present in the non-cell wall fraction of pollen and silk, the high B requirement for plant reproduction suggests an additional role for B other than in cell wall formation. However, the identity of non-cell wall B binding substrates in pollen and carpel tissue awaits further study. The higher sensitivity of plant reproduction to B deficiency is also related to weaker B transport into floral organs, especially where transpiration is suppressed in reproductive plant parts by enclosure of sheaths (e.g. wheat ear) or husks (e.g. maize ear) during the critical stage of development (Huang *et al*., 2009).

There is increasing evidence that B is required for the maintenance of the structure and functions of membranes and, especially, plasma membrane (Camacho-Cristobal *et al*., 2008). For example, B deficiency altered the membrane potential and reduced the activity of proton-pumping

ATPase in roots (Ferrol and Donaire 1992), and it has been also reported that B deficiency alters plasma membrane permeability for ions and other solutes (Cakmak *et al*., 1995). Therefore, B action in membranes might not be restricted to stabilizing membrane molecules with *cis*-diol groups, but also by regulating the expression of genes involved in membrane structure and function.

BORON UPTAKE AND MOBILITY

Boron is present in soil solution in several forms. However, at common soil pH values, the most abundant is the undissociated boric acid. It is accepted that it is the only essential nutrient that plants take up from soil as an uncharged molecule (Marschner, 1995). Boron in soil solution moves towards plant roots mainly through mass flow (Barber, 1966), then its uptake can be carried out by three different molecular mechanisms, depending on B availability: (i) passive diffusion across lipid bilayers, where B can cross membranes by a passive process to satisfy plant B requirements (Brown *et al*., 2002); (ii) facilitated transport by major intrinsic protein (MIP) channels; and (iii) an energy-dependent high affinity transport system induced in response to low B supply, which is mediated via BOR transporters (Tanaka and Fujiwara, 2008).

The first experimental evidence suggesting the involvement of channel proteins in B transport was provided by Dordas *et al*. (2000), when they described that B permeation across root plasma-membrane vesicles was partially inhibited by channel blockers. Another boric acid channel has been identified in *Arabidopsis* (AtNIP5;1), which belongs to the nodulin 26-like intrinsic proteins (NIP), subfamily of the MIPs family (Takano *et al*., 2006). At-NIP5;1 is localized and expressed in the plasma membrane of root epidermal, cortical, and endodermal cells, and it is upregulated in B-deficient roots, suggesting a crucial role of this channel for B uptake under low availability (Takano *et al*., 2006).

Physiological studies also have shown the occurrence of active B uptake by roots under low B conditions (Dannel *et al*., 2002). One BOR transporter (OsBOR1 in rice) has been suggested to be involved in the efficient uptake of B into root cells under B deficiency (Nakagawa *et al*., 2007). A BOR transporter was also indentified as capable of increasing B toxicity tolerance by pumping excess boric acid out of the cell (Miwa *et al*., 2007). Plants may be tolerant to B excess or deficiency through the expression of these transporters. Most of the results were obtained in model plants but could be applied to other plant species and may be helpful in developing crops tolerant either to B toxicity or deficiency (Miwa and Fujiwara, 2010).

More recently, accumulating evidence suggests that non-sugar-alcohol-producing plants can transport boric acid preferentially to young tissues. This translocation was detected under B limitation, but not under conditions of normal B supply, and B transporters and channels may be involved. The fact that this translocation occurs only under boron limitation suggests that plants are capable of sensing boron levels and regulating boron transport (Tanaka and Fujiwara, 2008).

The mobility of B within the plant is an important characteristic and is determined by the plant species and B availability. Knowledge of B mobility in plants is useful for the management of B application in agricultural systems where nutrient supply may be limiting or excessive. The remobilization is generally defined as the movement of nutrients from a plant tissue to another, through the phloem.

BORON IN COTTON

More than 90% of B in plants is found in cell walls, and if there is any B remobilization in cotton phloem, it is low (Rosolem and Costa, 2000). When cotton was exposed to a temporary deficiency and B was sprayed on new or old leaves, the responses varied. Boron applied to young immature leaves increased B concentration locally, with no further effects. However, despite the effects of B deficiency inhibiting meristematic growth and its low mobility within the plant, there was a positive response to B application to mature leaves. As there was no new development of cell walls to incorporate the nutrient, it could eventually be available for mobilization (Rosolem and Costa, 2000). The authors argued that foliar application of B to mature leaves may have prevented, at least in part, xylem malformation, and when the nutrient was replaced in the solution, the preservation of a better vascular system allowed for near-normal plant growth. Bogiani and Rosolem (unpublished) observed that B remobilization in cotton was low, but there were differences among cotton cultivars in mobilizing B from roots, stems and leaves to reproductive structures. Furthermore, when B concentration in the nutrient solution was low, but enough to avoid severe shedding, the reproductive structures received more B from other plant parts, but when there was plenty of B for plant growth, most of the nutrient accumulated in cotton leaves. Hence, B remobilization occurred under low B supply, and under high B supply, the nutrient was transported mainly in the transpiration stream, accumulating in organs with high transpiration rates. In China, it has been shown that B uptake by cotton roots is faster than B uptake by leaves and translocation (Xie *et al*., 1992). During vegetative growth, B is mobilized mainly to growing points and young leaves, whereas during reproductive growth, it is mobilized preferentially towards the main-stem leaves and leaves subtending reproductive structures. Though B is not easily remobilized from old leaves, it may be remobilized from photosynthetic active leaves (Xie *et al*., 1992).

These results are consistent with the findings of Tanaka and Fujiwara (2008) on B transport in non-sugar-alcohol-producing plants, mediated by B transporters and channels. For instance, OsBOR1, a B efflux transporter in rice was found to mediate efficient B translocation from root to shoot under B deficiency (Uraguchi *et al*., 2009). Conversely, a temporary deficiency of B leads to xylem malformation, which may decrease the translocation of B, carbohydrates, etc, to new tissues in cotton (Oliveira *et al*., 2006).

The possibility of some B translocation out of the leaves would explain some responses of cotton to foliar B application observed in the field in Brazilian acidic soils (Carvalho *et al*., 1996; Ferreira and Carvalho, 2005) and in calcareous soils in Greece (Dordas, 2006), among others.

Boron Deficiency

The appearance and severity of B deficiency symptoms in cotton are a function of soil nutrient availability, time of plant exposure to deficiency and cultivar (Silva *et al*., 1982; Rosolem *et al*., 1999). Considering the role of B in cell wall and membrane formation and in carbohydrate transport (Tanada, 1983; Agarwala *et al*., 1981), the first symptoms appear in young parts of

the plant, in vessel tissues and reproductive organs (Hinkle and Brown, 1968). As a result of the critical role of B in expanding tissues and its limited mobility in cotton, it must be supplied continuously throughout the plant's life. If it is withdrawn from the nutrient medium, even for a short period, a deficiency is established and reproductive structures shed (Rosolem and Costa, 2000; Oliveira *et al*., 2006). When B is replaced in the nutrient solution after a temporary deficiency, full growth recovery does not occur and, therefore, a temporary B deficiency causes permanent damage to the plant (Rosolem and Costa, 2000). This is important in the field because B uptake and transport to new tissues depends on the transpiration stream, which may be impaired by a very low evaporative demand, stomata closure in hot, dry days, low temperatures, etc. This may lead to a temporary B deficiency in cotton, even when there is plenty of soil B available.

Boron deficiency can result in shorter fruit branches and poor fruit set, deformed, chlorotic leaves and development of dark green bands (often excessively hairy) on the petioles and stems (Hinkle and Brown, 1968; Rosolem and Bastos, 1997). The pith in such regions of the petioles is characteristically necrotic, the terminal bud often dies and many lateral branches develop, which have short internodes and enlarged nodes. Under B deficiency there is significant square and boll shedding (Zhao and Oosterhuis, 2002; Rosolem and Bastos, 1997, Oliveira *et al*., 2006). Abnormal fibers have also been observed in cultured ovules (Birnbaum *et al*., 1974) and shorter fibers in the field (Sankaranarayanan *et al*., 2010). The petals are frequently crumpled and misshapen. Discoloration of the extra-floral nectaries is quite common. Cracks may develop on the stems, at the base of the squares or bolls, and there may be some exudation (Shorrocks 1997). The accumulation of chlorogenic and caffeic acids caused by B deficiency inhibits the *enzyme auxi*n oxidase, resulting in auxin accumulation in the plant tissue (Gupta, 2006), over proliferation of the cambium (Oliveira *et al*., 2006), and a fast and unproportional elongation and collapse of the nearby cells (Srivastava and Gupta, 1996). Therefore, morphological changes during B deficiency development may be due to auxin accumulation in the tissue.

Although B deficiency decreases photosynthesis (Zhao and Oosterhuis, 2003), sugars and starch accumulate in leaves of deficient plants (Dugger, 1983). According to Dugger (1983), B deficiency decreases photosynthesis by decreasing the activity of nitrogenous compounds such as uracil, a precursor of UDPG (uridine diphosphate glucose), which is involved in sucrose synthesis (Birnbaum *et al*., 1977). With less UDPG, translocation is decreased and starch and photoassimilate accumulate. However, it is worth noting that while B deficiency increased nonstructural carbohydrates in deficient cotton leaf blades and depressed photosynthate export from leaves, leaf intercellular CO_2 concentration of cotton plants changed little with increasing leaf-blade B concentrations (Zhao and Oosterhuis, 2003).

It has often been observed that reproductive growth, especially flowering, fruit and seed set and seed yield, is more sensitive to B deficiency than vegetative growth. This is due to several reasons such as: each flower develops over a very narrow window of time, some reproductive structures (e.g., pollen chamber, embryo sac) have poorer access to the vascular system than any vegetative organ (van Iersel *et al*., 1994), and sexual reproduction involves a large number of specialized cell types, many of which have distinctive cell walls (Huang *et al*., 2009). Phloem elements in the peduncle vascular cylinder of B-deficient plants have no clear differentiation and the number of vascular bundles of the petiole and peduncle is decreased in B-deficient

cotton and the few xylem elements formed are disorganized. Moreover, in B-deficient cotton plants, the xylem vessel walls were thickened and vessels were observed in lower number, with an irregular perimeter (Oliveira *et al*., 2006). Boron deficiency during the early growth of cotton has been reported to decrease the leaf CO_2-exchange rate, increase leaf blade non-structural carbohydrate concentration, and decrease photosynthate export out of leaves (Zhao and Oosterhuis, 2002). The decrease in carbohydrate transport to fruiting sites results in square and flower abscission (Rosolem *et al*., 2001; Zhao and Oosterhuis, 2002, 2003). Squares remaining are deformed, with chlorotic bracts and stunted corolla (Silva *et al*., 1982).

With fewer reproductive structures the sink for carbohydrates is decreased and excess carbohydrate is available for vegetative growth, resulting in rank-growth, self-shading, delayed maturity and less yield.

Boron Toxicity

Boron toxicity is a serious concern for sustainable crop production in irrigated agriculture throughout the world. Boron is transported within the plant mainly in the transpiration stream through the xylem and accumulates at the leaf tips and margins of older leaves (Bennet, 1993; Sestren and Kroplin, 2009). Hence, toxicity symptoms (yellowing and necrosis in patches between veins and tips and margins of leaves) first appear on older leaves. As severity of the disorder increases, the chlorotic areas later become necrotic, and the necrosis progresses from the leaf tips and margins towards the midrib and base of the leaf (Ahmed *et al*., 2008). This gives the leaf a scorched appearance and eventually the entire leaf dies and falls from the plant (Silva *et al*., 1979). Cassman (1993) reported that in cotton the necrotic areas of the leaves suffering B toxicity contained 2700-6400 mg B kg^{-1}, and Silva *et al*. (1979) observed that cotton plants showing symptoms of B toxicity contained over 590 mg B kg^{-1}. Boron concentration may vary 100 fold within a single leaf, hence, results of foliar diagnosis represent only an average of the actual concentration. Boron concentration usually increases with leaf age (Brown and Shelp, 1997) and in some cases may reach toxic levels in old leaves and be deficient in newly developed leaves (Oertli, 1994).

Boron accumulation in old leaves could unbalance cell wall constituents leading to tissue necrosis and death (Sestren and Kroplin, 2009). In excess, B concentration increases in the cytosol, causing metabolic dysfunctions through the formation of complexes with NAD^+ and eventually affecting the RNA structure (Loomis and Durst, 1992). However, toxicity of mature tissues may be due rather to the accumulated retardation of many cellular processes, enhanced in light by photo-oxidative stress (Reid *et al*., 2004).

Boron toxicity negatively affects very diverse processes in vascular plants, such as photosynthetic rates, leaf chlorophyll contents, root cell division and lignin and suberin levels (Reid, 2007). Accordingly, a reduced growth of shoots and roots is typical of plants exposed to high B levels (Nable *et al*., 1990). According to Camacho-Cristóbal (2008) three main causes have been proposed taking into account our knowledge of B chemistry (i.e. the ability of B to bind compounds with two hydroxyl groups in the *cis*-configuration): (i) alteration of cell wall structure; (ii) metabolic disruption by binding to the ribose moieties of molecules such as adenosine

triphosphate (ATP), nicotinamide adenine dinucleotide, (reduced form) (NADH) or nicotinamide adenine dinucleotide phosphate, (reduced form) (NADPH); and (iii) disruption of cell division and development by binding to ribose, either as the free sugar or within RNA (Reid *et al*., 2004).

There are genotypic differences in tolerance to high B, e.g. in wheat, characterized by a decreased B concentration in leaf tissues (Nable *et al*., 1990), probably due to a reduced uptake of B. The basis for B-tolerancein plants has been explained by plant ability to efflux B, and two models have been proposed for this mechanism: borate exchange or an anion channel (Hayes and Reid, 2004). BOR1 is an efflux-type borate transporter required for the transport of B from roots to shoots under low B supply (Takano *et al*., 2002). However, in the presence of toxic levels of B, BOR1 is degraded via endocytosis (Takano *et al*., 2005), and its' over expression does not result in better plant growth (Miwa *et al*., 2006), suggesting that BOR1 is not involved in B tolerance. More recently it was found that overproduction of another B transporter in *A. thaliana*, BOR4-GFP, improved growth under conditions of B toxicity through B efflux (Miwa *et al*., 2007). This enhanced B efflux from the roots of crop plants is expected to result in improved crop productivity in B-toxic soils. Another gene, Bot1 (a BOR1 ortholog), has been identified as responsible for B-toxicity tolerance in barley (Sutton *et al*., 2007), and it has been suggested that the BOR2 gene encodes an efflux type borate transporter responsible for tolerance to B toxicity in wheat and barley (Reid, 2007).

SUMMARY

Boron deficiency and toxicity can be observed in many cotton regions worldwide. Considering the low remobilization of B within cotton plants, even a temporary deficiency occurring with enough available B in soil may lead to some degree of reproductive structure shedding, either decreasing cotton yields or delaying plant maturity and increasing costs. Although foliar fertilization has not been regarded as effective in correcting B deficiency in low B soils, it may help to overcome a temporary B deficiency, with some improvement in cotton yields in tropical soils (Rosolem *et al*., 2001) and significant increases in Mediterranean soils (Dordas, 2006). This would only be possible as a consequence of some B translocation in cotton. Over 90 % of B is bound to cell walls and membranes, while some of the remaining 10 % could be available for remobilization. In addition, B applied to mature leaves does not bind to the previously formed cell walls and could also be available for mobilization within the plant. Therefore, some B could be mobilized from mature leaves into actively growing reproductive organs via phloem, as recently demonstrated in white lupin (Huang *et al*., 2008). This remobilization was promoted by specific boron transporters. This was not demonstrated in cotton, but accumulating evidence suggests that non-sugar-alcohol-producing plants can transport boric acid preferentially to young tissues, which would explain the observed responses of cotton to foliar applied B. Moreover, some differences have been observed in B remobilization among cotton cultivars. In addition to B fertilization, the selection of cultivars or the introduction of the ability to remobilize B would be important steps in better dealing with B deficiency and toxicity in cotton. The natural genetic variability in this trait and the introduction of B transporter genes are tools to be used in plant breeding towards improved B use in cotton.

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Chapter 8

GEMINIVIRAL DISEASES OF COTTON

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INTRODUCTION

Plant viruses infect cotton in most parts of the world and can lead to decreased yield, or loss of the entire crop. While over 20 virus diseases of cotton have been described in the American Phytopathological Society "Cotton Disease Compendium" (Kirkpatrick and Rothrock, 2001), only a few have actually been shown to be of virus etiology. The main viruses, for which a causative relationship has been proven, include several geminiviruses of the genus *Begomovirus* (Briddon and Markham, 2001; Idris and Brown, 2004) and a luteovirus belonging to the genus *Polerovirus* (Corrêa *et al*., 2005; Distéfano *et al*., 2010; Silva *et al*., 2008). Among these the geminiviruses are the most destructive and a potential threat to cotton cultivation all over the world. The ubiquitous presence of white fly (*Bemisia tabaci Gennadius*), the insect vector of begomoviruses in all major cotton production areas, and human activity that has disseminated these viruses into geographical locations where they were not found earlier has compounded the problem. Geminiviruses are single-stranded (ss)DNA viruses with small circular genomes encapsidated in characteristic twinned (geminate) particles that are transmitted by insect vectors. They infect either monocotyledonous or dicotyledonous plants and are taxonomically divided into four genera based on insect vectors, genome organization and host range (Stanley *et al*., 2005). Geminiviruses that are transmitted by *Bemisia tabaci*, are classified in the genus *Begomovirus***.** These are the most numerous and the most important due to their emergence as a major limiting factor in the production of many dicotyledonous crops, including cotton, in the warmer parts of the World (Seal *et al*., 2006). Begomoviruses may be further divided into two distinct groups, those originating from the Old World (OW) and those prevalent in the New World (NW). Begomoviruses from the NW have genomes consisting of two genomic components, known as DNA A and DNA B (each 2600 – 2800 nucleotides [nt]), and both are required to systemically infect plants. In the OW, although a few bipartite begomoviruses have been identified, the majority of begomoviruses are monopartite, with a genome consisting of a single circular ssDNA component homologous to the DNA a component of the bipartite begomoviruses (Fig.1). Furthermore, the majority of monopartite begomoviruses are associated with additional small (approx. 1350 nt) circular ssDNA molecules. The first is a satellite, known collectively as betasatellites, which is often required by the helper begomovirus to successfully infect host plants and induce disease symptoms. The second is satellite-like molecule, collectively named alphasatellites, which is not essential for the virus to infect plants. The two viral diseases of cotton of confirmed begomovirus etiology are cotton leaf curl disease (CLCuD)

(Fig. 2) and cotton leaf crumple disease (CLCrD). Cotton leaf curl disease has been reported from the Indian subcontinent and Africa affecting tetraploid cotton (*Gossypium hirsutum* and *G. barbadense*) introduced from the Old World while diploid cottons, that have their origins in the OW, are completely immune to the disease. Cotton leaf crumple is found in the Americas and is, in most years, not a significant problem.

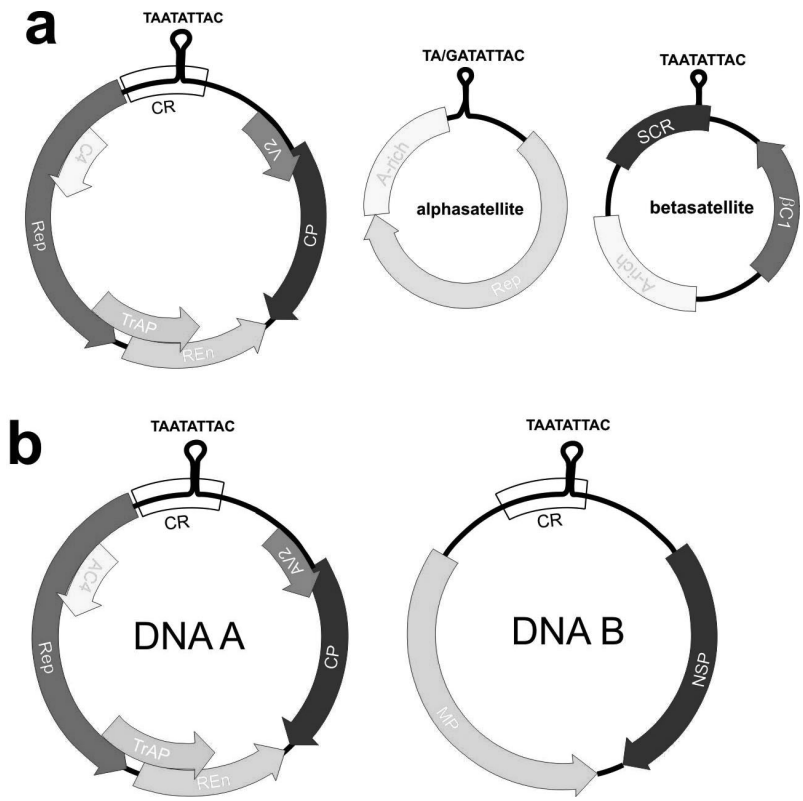

Figure 1. Arrangements of the genomes of bipartite (a) and monopartite begomoviruses with their associated betasatellites and alphasatellites (b).The positions and orientations of genes are shown by arrows. The genes encode the coat protein (CP), the replication-associated protein (Rep), the transcriptional activator protein (TrAP), the replication enhancer protein (REn), the movement protein (MP) and the nuclear shuttle protein (NSP). The products encoded by open readings frames (A)V2 and (A)C4 have yet to be named. Characteristically, begomoviruses native to the New World lack the AV2 gene. The alphasatellites encode a Rep whereas the single gene encoded by the betasatellites, which is encoded in the complementary-sense, is known as βC1. Bipartite begomoviruses contain a sequence of ~200 nt which is conserved between the DNA A and DNA B components and is known as the common region (CR) The hairpin structure is shown as position zero for each component. This contains the nonanucleotide sequence, which is highly conserved.

COTTON LEAF CURL DISEASE

A phylogenetic tree based on complete nucleotide sequences of all cotton begomoviruses is shown in Figure 3. Cotton leaf curl is a destructive disease of cotton and several other malvaceous plant species that is transmitted by *B. tabaci*. Presently the disease is prevalent throughout Pakistan and northwestern India. Infected cotton plants display a range of symptoms such as leaf curling, stunting and a poor yield of cotton fiber. Besides, affected plants may develop enations on the veins on the undersides of leaves which may develop into cup-shaped, leaf-like structures (Fig. 2). Symptoms in cotton usually appear within 2–3 weeks of inoculation by *B. tabaci* (Singh *et al*., 1997) and are primarily characterized by a deep downward cupping of the youngest leaves. This is followed by either upward or downward curling of the leaf margins, swelling and darkening of the veins as well as the formation of enations on the veins, which frequently (dependant on variety) develop into cup-shaped, leaf-like structures.

Figure 2. Symptoms induced by cotton leaf curl disease in cotton. Note the vein swelling, vein darkening (often CLCuD affected plants appear darker green than non-affected plants) and enations on the veins. Frequently these enations develop into cup-shaped leaf-like structures. In this case the leaves show upward leaf curling. However, downward curling may also occur.

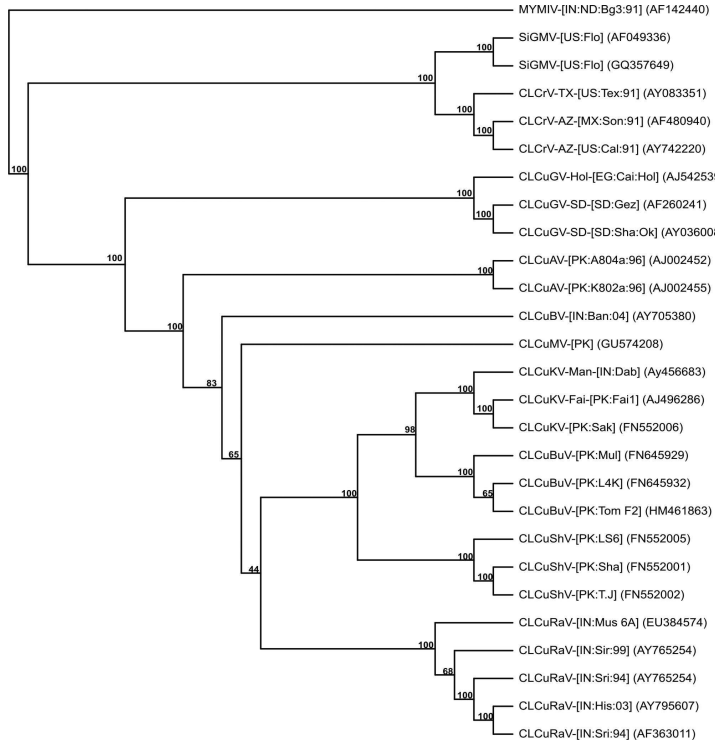

Figure 3. Phylogenetic dendrogram, based upon an alignment of the full length genome (or DNA A genomic component) sequences of selected begomoviruses. The figures at nodes indicate percentage bootstrap confidence values (1000 replicates). The viruses shown are Cotton leaf crumple virus (CLCrV), Cotton leaf curl Alabad virus (CLCuAV), Cotton leaf curl Burewala virus (CLCuBuV), Cotton leaf curl Gezira virus (CLCuGV), Cotton leaf curl Kokhran virus (CLCuKoV), Cotton leaf curl Multan virus (CLCuMuV), Cotton leaf curl Rajasthan virus (CLCuRaV), Cotton leaf curl Shadadpur virus (CLCuShV) and Sida golden mosaic virus (SiGMV). The tree was rooted on an outgroup, the DNA A component of Mungbean yellow mosaic virus (MYMV), a bipartite begomovirus that occurs in southern Asia and is only distantly related to the remaining monopartite viruses. Of the species shown, only MYMV and SiGMV do not cause disease in cotton. The geographical origins of the viruses are indicated. In each case the database accession number of the sequence used is given. (Isolate descriptors are as given in Fauquet *et al*., 2008).

CLCuD has been recorded from several countries in Africa. In fact it was first named as leaf curl by Kirkpatrick (Kirkpatrick, 1931) who also described the symptoms of the disease as definite curling of the leaf margins, either upward or downward and a peculiar crinkled appearance (enations) may be produced by the veins. Veins of the leaves become thickened which are more pronounced on the underside. Two types of vein thickening are commonly seen, small vein thick-

ening (SVT) and main vein thickening (MVT). Small vein thickening is more common in field conditions and is characterized by small green bead-like thickening on the young leaves. These irregular thickenings gradually extend and coalesce to form a continuous reticulation of the small veins. Main vein thickening is characterized by the green thickening of the distal ends of the larger veins of young leaves. The thickening first appears near the leaf margin and then extends inward to form a network of dark green thickened main vein (Watkins, 1981). In extreme but not infrequent cases, formation of the cup- shaped, leaf-like outgrowths appear on the underside of the leaves. Enations has however been observed even on leaves of plants with only mild bead-like vein thickening (Mahmood, 1999). According to Tarr (1951), severely infected plants may show spirally twisted petioles, fruiting branches and, to a lesser extent, the main stem, which tends to grow tall with elongated internodes in *Gossypium barbadense*. All the varieties show a dwarfing effect, more so in the dwarf varieties, internodal distance is reduced and the affected plants become stunted in early infection with adverse effect on fruiting. There is reduction in boll number and boll weight resulting in loss of yield. In extreme cases, the plants succumb to its attack and some growers had to plough-up their crop during 1991-1992 in Pakistan.

History of Cotton Leaf Curl Disease

In Africa, CLCuD was first reported 1912 from Nigeria affecting *G. barbadense*. In 1924, it was recorded in Sudan and subsequently from Tanzania 1926 (Kirkpatrick, 1931). It was one of the most important diseases of cotton in these countries and had potential to cause significant losses. On the Indian subcontinent CLCuD was first observed near Multan in 1967 (Hussain and Ali, 1975) on a few individual plants and has been noted consistently since then. In the beginning, the disease did not attract serious attention because it was sporadic and of minor economic importance. The disease, however, become prominent in 1973 when it was observed on several varieties, including 149-F and B-557, with incidences of 5% of the field. The disease occurred only late in the season on the upper portion of the plant. Hussain and Mahmood (1988) reported that in 1987, the incidence was up to 80% in certain fields. In 1988, the disease damaged the cotton crop on 60 hectares in the Multan district. In the following years the affected area increased. It affected 200 hectares in 1989 and 800 hectares in 1990. The incidence increased substantially and caused losses from 22.3% to 68.5% in the affected fields in some areas of Punjab depending upon the variety, time of infection and environmental conditions. However, in 1991, the disease reached epidemic proportion, affecting an area of 14,000 hectares in Multan, Khanewal and Vehari Districts. In 1992, the disease spread to more than 48,500 hectares causing a decrease in production and significant monetary loss to the country. In 1993, the disease spread to the entire cotton belt of the Punjab with varying intensity causing losses across 889,000 hectares. The disease was also reported from Dera Ghazi Khan district of Punjab and Sindh province, during 1996-97. The loss in yield varied with the intensity of the disease and with the crop stage at which it occurred. In the severest cases, farmers were forced to plough-up their fields. Cotton production in Pakistan decreased from 1.938 million metric tons in 1991 to 1.445 million metric tons in 1992 and fell further to 1.105 million metric tons in 1993. CLCuD was the main force behind yield decline in these years. The first 3 years of the disease epidemic (1992-1994) in Pakistani Punjab were the most severe in terms of disease intensity. The epidemic of CLCuD in Pakistan is one of the best examples of the dramatic shift in importance of a previously insignificant endemic disease.

The introduction of resistant cotton varieties in the late 1990s (as described later) restored cotton production in Pakistan to above the levels seen before the epidemic of CLCuD. However, during 2001 typical disease symptoms were seen in resistant cotton varieties, suggesting the appearance of a resistance breaking strain of CLCuD (Mansoor *et al*., 2003a) which has now spread into northwestern India.

Spread of the Disease by the Insect Vector

CLCuD is transmitted by feeding of the whitefly (*B. tabaci*) which can complete the entire cycle, from the acquisition of the virus to infection of a new host plant, within 6.5 hours. The disease is not mechanically transmissible and is not carried in soil or seed. *B. tabaci* is the only known vector of begomoviruses (Brown, 1997). *B. tabaci* is capable of establishing high population levels, particularly in crops grown under irrigated, arid conditions in both field and greenhouse systems. In addition, this whitefly has the potential to colonize a wide range of dicotyledonous species, among which are primarily vegetables and fiber species of great importance to worldwide agricultural production. Recent studies indicate that there are numerous populations of *B. tabaci* that vary somewhat in their capacity to develop high population densities and cause feeding damage within their host ranges and the efficacy with which they can transmit geminiviruses (Bedford *et al*., 1994; Brown and Bird, 1992; Brown *et al*., 1995; Maruthi *et al*., 2002).

Aetiology of CLCuD

The aetiology of CLCuD from Sudan and the Indian subcontinent has been determined. In both regions the disease is caused by begomovirus complexes consisting of monopartite begomoviruses, a disease-specific, symptom determining satellite and frequently also involves an additional satellite-like molecule.

Diversity of Monpartite Begomoviruses Associated with CLCuD

Cotton leaf curl disease (CLCuD) occurring on the Indian subcontinent has been shown to be associated with several begomoviruses (Harrison *et al*., 1997; Kirthi *et al*., 2004; Mansoor *et al*., 1993; Mansoor *et al*., 2003b; Nadeem *et al*., 1997; Zhou *et al*., 1998). Initial studies of the disease in Pakistan in the early 1990s determined that the disease was associated with begomoviruses (Mansoor *et al*., 1993a). At that time only bipartite and monopartite begomoviruses were known, the satellites associated with some monopartite begomoviruses were not identified until 1999-2000 (Briddon *et al*., 2001; Mansoor *et al*., 1999). No evidence for the presence of a DNA B component was found, leading to the conclusion that the disease was caused by a monopartite begomovirus, yet the monopartite begomovirus identified (now known as Cotton leaf curl Multan virus [CLCuMV]) was experimentally only poorly infectious to cotton and did not induced the symptoms typical of CLCuD (Briddon *et al*., 2000). This indicated that some component or factor, essential for induction of the disease symptoms, remained to be identified. The fact that the disease was shown experimentally only to be transmissible by *B. tabaci*, the vector of begomoviruses, strongly suggested that the additional component must consist of ssDNA.

An early study into the diversity of begomoviruses associated with CLCuD concluded that there were essentially four begomovirus variants infecting cotton in Pakistan (Zhou *et al*., 1998). Three of the viruses identified are now classified as species; CLCuMV, Cotton leaf curl Alalabad virus (CLCuAV) and Cotton leaf curl Khokhran virus (CLCuKV). Further investigation identified additional species – Papaya leaf curl virus (Mansoor *et al*., 2003b) and Tomato leaf curl Bangalore virus (Kirthi *et al*., 2004). In northwestern India many of the viruses identified in Pakistan were subsequently found to be present and an additional distinct species, Cotton leaf curl Rajasthan virus (CLCuRaV) was also identified (Kirthi *et al*., 2004). This species was later also identified in Pakistan infecting both cotton and tomato (Nawaz-ul-Rehman *et al*., 2010; Shahid et. al., 2007).

More recently the genetic make-up of begomoviruses in Pakistan has changed dramatically. The virus associated with resistance breaking in cotton across Pakistan has been shown to be a distinct recombinant begomovirus, Cotton leaf curl Burewala virus (CLCuBuV) (Amrao *et al*., 2010b). This virus consists of sequences derived from two of the begomovirus species associated with the CLCuD epidemic during the 1990s, CLCuMuV and CLCuKoV. Surprisingly this virus lacks one of the usual complement of genes encoded by begomoviruses, as will be discussed later. As was the case with the epidemic in the 1990s, the virus associated with resistance breaking in cotton (CLCuBuV) has spread into India (Kumar *et al*., 2010) and there are now problems with CLCuD in previously resistant varieties with particularly severe losses to the crop during 2009-2010.

Throughout the epidemic of CLCuD in most of Pakistan during the 1990s, the cotton growing region of southern Sindh province remained largely unaffected by the disease. For this reason, the farmers there were not growing resistant cotton varieties. However, during 2003-2004 the disease appeared in central and lower Sindh, causing substantial yield losses. This coincided with introduction of cotton varieties not approved by the Government authorities for cultivation, since they are highly susceptible to CLCuD. Analysis of the begomoviruses associated with the outbreak has shown the presence in Sindh of CLCuKoV and a newly identified recombinant species for which the name Cotton leaf curl Shadadpur has been proposed (Amrao *et al*., 2010a). The reason for the differences between Sindh and the rest of Pakistan, with respect to the incidence of CLCuD and the diversity of associated begomoviruses, remains unclear. However, the presence in Sindh of a distinct biotype of *B. tabaci*, with possible distinct host ranges and virus vectoring specificities, has been suggested.

In Sudan, where cotton production was severely affected by CLCuD during the early parts of the 20th century, a single begomovirus species (Cotton leaf curl Gezira virus) has been shown associated with CLCuD in Africa (Idris and Brown, 2002). The virus has been characterized from several malvaceous hosts including cotton, okra and *Sida alba*. Recent investigation into diversity revealed that limited diversity exist in cotton begomoviruses in Africa. A related but distinct species has been reported from hollyhock named as Hollyhock leaf crumple virus. These viruses are only distantly related to CLCuD begomoviruses found in the Indian subcontinent, being instead more closely related to other begomoviruses originating from Africa and the Mediterranean region. These results suggest that distinct begomovirus complexes were mobilized from indigenous hosts to susceptible cotton upon their cultivation

in Africa and Asia. Nevertheless, the begomovirus components from Asia and Africa complement each other under experimental conditions (R.W. Briddon, unpublished data). Thus, human activity may disseminate these begomoviruses within the Old World where begomovirus components have been reported.

DNA Satellites Associated with CLCuD

Satellites are defined as viruses or nucleic acids (DNA or RNA) that depend on a helper virus for their replication but lack extensive nucleotide sequence identity to their helper virus and are dispensable for its proliferation (Murant and Mayo, 1982). The majority of satellites consist of RNA and are associated with viruses with RNA genomes and have no discernable effects on the symptoms caused by their helper viruses in plants (Hu *et al*., 2009). However, some satellite module symptoms, either ameliorating or exacerbating the symptoms induced by the helper virus.

The first ssDNA satellite was identified in association with Tomato leaf curl virus (ToLCV) in tomatoes from Australia (Dry *et al*., 1997). The satellite, known as the ToLCV-sat, is a circular molecule of approx. 700 nucleotides that has little similarity to its helper virus other than a predicted hairpin structure containing within the loop the sequence TAATATTAC (the so called nonanucleotide motif) that (for geminiviruses) forms part of the virion-strand origin of replication). This molecule has no discernable effect on ToLCV infections and encodes no proteins. At the time this was a novel oddity. Its significance, being related to a much larger group of begomovirus-associated satellites, was not realized until later.

Alphasatellites Associated with Cotton Leaf Curl Disease

The search for additional components associated with begomoviruses that cause CLCuD first identified a class of molecules that were named DNA 1 and that we now refer to as alphasatellites (Mansoor *et al*., 1999). The alphasatellites comprise a group of closely related ssDNA molecules that encode a single protein, a rolling-circle replication initiator protein (the replication-associated protein [Rep]). As a consequence, alphasatellites are capable of autonomous replication in cells of host plants. Since, by definition, satellites depend on a helper virus for their replication, alphasatellites are best described as satellite-like. For all other functions alphasatellites depend on their helper begomoviruses, including movement within plants and insect transmission between plants (Mansoor *et al*., 1999; Saunders *et al*., 2002; Saunders and Stanley, 1999). This likely requires the alphasatellite ssDNA to be encapsidated within the coat protein of the helper virus.

Alphasatellites share no significant levels of sequence identity to geminiviruses but contain a predicted hairpin structure with, in the loop, the nonanucleotide sequence. Surprisingly, the alphasatellite Rep exhibits high levels of sequence identity to the Reps encoded by components of nanoviruses (Saunders *et al*., 2000; Saunders and Stanley, 1999). The family Nanoviridae is a second family of DNA viruses with circular single-stranded genomes that replicate by a rolling-circle mechanism (Gronenborn, 2004). Their genomes are multipartite,

consisting of 6 to 8 components that are encapsidated is small icosahedral particles and are transmitted plant-to-plant by aphids. It has been suggested that begomoviruses may have captured a nanovirus Rep encoding component during a co-infections of a nanovirus and a begomovirus (Mansoor *et al*., 1999; Saunders *et al*., 2002; Saunders and Stanley, 1999). The benefit, to the helper begomovirus, of the presence of an alphasatellite remains unclear. Initially it was suggested that the alphasatellite may act as a "dampener", mopping up cellular resources and ameliorating the symptoms induced, thus extending the life of the plant and thereby benefitting the virus by extending the period during which it may be transmitted by the insect vector to new hosts. Although some evidence in support of this has been forthcoming (Wu and Zhou, 2005), evidence recently obtained suggests that the Rep encoded by alphasatellites may be a suppressor of host silencing (post-transcriptional gene silencing [PTGS], also known as RNA interference [RNAi]) and thus involved in overcoming host defences (Nawaz-ul-Rehman *et al*., 2010). RNAi is, amongst its many functions, an RNA induced defence mechanism that is involved in the destruction of foreign and aberrant RNA (Voinnet, 2001; Voinnet *et al*., 1999).

Interestingly, some nanovirus infections are associated with multiple Rep encoding components. In addition to the *bona fide* virus Rep encoding component, known as the master Rep component (Timchenko *et al*., 1999; Timchenko *et al*., 2000), there are additional components that are not related to the components of the virus. These molecules are satellite-like and may be the form of the progenitor of the begomovirus-associated alphasatellites that was originally captured during a co-infection. A proposal is being prepared to classify these molecules with the begomovirus satellite-like components as alphasatellites (R.W. Briddon, manuscript in preparation). The begomovirus-associated satellite-like molecules differ little from those associated with nanoviruses other than being larger in size (by 300-400 nt). Most of this size increase is due to the presence, in the begomovirus-associated satellite-like molecules of a sequence rich in adenine (the A-rich region: Fig. 1). This is believed to be required for effective encapsidation of the molecule in the begomovirus coat protein. Begomoviruses have a strict size selection for movement in plants and encapsidation; selecting for unit length (~2800 nt; begomovirus genomes and genomic components), half unit length (~1400 nt; alphasatellites, betasatellites defective molecules derived from the virus genome) and one quarter unit length (~600-700nt; the ToLCV-sat and defective molecules derived from the virus genome) (Frischmuth *et al*., 1997; Frischmuth *et al*., 2001; Frischmuth and Stanley, 1991; Rojas *et al*., 1998).

Betasatellites Associated with CLCuD

The identification of an alphasatellite associated with CLCuD affected cotton spurred the search for further half-unit length ssDNA molecules. This led to the identification of a diverse set of molecules which we now call betasatellites (Briddon *et al*., 2008). Betasatellites are approximately half the size of their helper virus genomes (~1350 nt) and have a highly conserved structure, despite the fact that their sequences may show as little as 45% nucleotide sequence identity (Briddon *et al*., 2003; Bull *et al*., 2004) They encode a single gene (known

as βC1) in the complimentary sense, have a sequence rich in adenine and sequence of 80-100 nt that is highly conserved between all betasatellites so far identified (Briddon *et al*., 2003; Briddon *et al*., 2001)(Fig. 1).

The Rep encoded by geminiviruses is a sequence specific DNA binding protein. The protein binds to specific sequence motifs, known as iterons, adjacent to the nonanucleotide-containing stem loop structure, to initiate virion-strand viral DNA replication. The iterons of geminivirus species differ, meaning that the Rep of one species will not recognise the origin of replication (iterons) of another species. The DNA A and DNA B components of bipartite begomoviruses share a sequence of high sequence identity, known as the common region, that encompasses the origin of replication (iterons and stem-loop structure). This serves to maintain the integrity of the split genome, allowing the DNA A-encoded Rep to initiate rolling circle replication of both components. Although transreplicated by the Rep encoded by their helper begomoviruses, betasatellites do not contain the iterons sequences of their helper begomoviruses, rasing the question of how the interaction functions. A single virus may transreplicate numerous betasatellites (for example the begomovirus Ageratum yellow vein virus can transreplicate the majority of betasatellites tested; Briddon *et al*., 2003) and a single betasatellite may be transreplicated by numerous distinct begomovirus species (as is the case for Cotton leaf curl Multan betasatellite [CLCuMB] that is associated with CLCuD across the Indian sub-continent; Mansoor *et al*., 2003b). This indicates that betasatellites have a much looser relationship with their helper viruses than the DNA B components of bipartite begomoviruses have with their cognate DNA A components. Although far from resolved, recent evidence suggests that betasatellites contain a hyper-variable region of sequence lying between the SCR and the A-rich region. This vairable sequence contains (in most cases) numerous sequences that are similar to (differing by only a few nucleotides) the iterons of begomoviruses. It is possible that the sepseud-iterons allow betasatellites to interact with the Rep proteins of multiple begomoviruses for their replication. In the field this relaxed relationship leads to frequent exchanges of betasatellites between distinct begomoviruses (as has been proposed for CLCuD where a single betasatellite [CLCuMB] is capable of interacting with numerous begomoviruses to induce the disease; Mansoor *et al*., 2003b) and the presence in some plants of multiple betasatellites apparently maintained by single begomoviruses (Mubin *et al*., 2010). Overall this means that betasatellite-associated begomoviruses may rapidly adapt to changing conditions by interacting with different betasallites.

In contrast to the alphasatellites, for which there is some indication of their possible evolutionaty origins, the orgins of betasatellites remain unclear. There are no sequences with significant sequence similarities in the databases. However, the presence in betasatellites of an A-rich sequence suggests that, like alphasatellites, they may have originated with another group of, as yet unidentified, single stranded circular DNA replicons.

Since they were first identified, research on betasatellites, to identify possible functions encoded by this satellite, has moved at a rapid pace. As well as being required (by some begomoviruses, including those associated with CLCuD) to infect the host plants from which they were isolated and induce typical disease symptoms, they were shown in some cases to elevate virus DNA levels (Briddon *et al*., 2001; Saunders *et al*., 2000). This suggested either that the

betasatellite enhanced virus replication (more viral DNA per infected cell) or that the betasatellite enhanced virus movement in plants (thus more cells infected). So far all functions of betasatellites have been attributed to the product of the single gene they encode, known as βC1 (Fig. 1). βC1 is a pathogenicity (symptom) determinant (Saeed *et al*., 2005; Saunders *et al*., 2004), a suppressor of PTGS, may facilitate virus movement (Saeed *et al*., 2007), binds DNA (Cui *et al*., 2005), and interacts with a variety of host and virus encoded factors including a host ubiquitin-conjugating enzyme (part of the host ubiquitin proteasome pathway that is involved in protein turnover)(Eini *et al*., 2009), ASYMMETRIC LEAVES 1 (a host factor involved in controlling leaf development) (Yang *et al*., 2008), attenuates the expression of jasmonic acid responsive genes implicated in plant defence against insects (suggesting that βC1 may enhance virus transmission by making the plant more "palatable" for the vector; Yang *et al*., 2008) and the helper virus coat protein (Kumar *et al*., 2006). Recently studies of a βC1 protein have shown that it has the capacity to self-interact and form higher order multimers *in vitro* and *in vivo* (Cheng *et al*., 2011). Mutant βC1 proteins that lack the capacity to self-interact, and that do not form multimers, were also unable to induce typical symptoms in plant, suggesting that βC1 acts, *in planta*, as a multimer. However, the precise significance of this finding remains unclear.

In addition to being shown to be the dominant pathogenicity determinant in begomovirus-betasatellite infections, expression of the βC1 of Cotton leaf curl betasatellite from a Potato virus X (PVX) vector, has shown that this is able to induced all the symptoms typical of CLCuD in tobacco in the absence of all helper virus encoded factors (Qazi *et al*., 2007). Constitutive expression of CLCuMB βC1 in transgenic plants under the control of the Cauliflower mosaic virus 35S promoter induces virus-like symptoms but these do not resemble typical CLCuD symptoms. Since PVX, in common with the begomoviruses that cause CLCuD, is phloem limited, this indicates that βC1 determines symptoms, but the virus contributes by ensuring the gene is expressed in the correct tissues.

CLCuD occurring in Pakistan during the 1990s, although associated with multiple distinct begomovirus species, involved only a single species of betasatellite (CLCuMB). However, following resistance breakdown in cotton during the early 2000s, a distinct variant of CLCuMB became prominent (referred to as the Burewala strain of CLCuMB [CLCuMB^{Bur}]), with the earlier variant (referred to as the Multan strain of CLCuMB [CLCuMB^{Mul}) no longer encountered (Amin *et al*., 2006). CLCuMB^{Bur} differs from CLCuMB^{Mul} in containing some sequence (~80 nt) in the SCR derived from a tomato betasatellite. The significance of this recombinant sequence remains unclear but is characteristic of the resistance breaking strain of CLCuD. The recombinant betasatellite CLCuMB^{Bur} was earlier detected in tomato from India and indicates a close relationship between the begomovirus diseases of cotton and tomato.

CLCuD in Sudan is similarly associated with a betasatellite (Idris *et al*., 2005). This betasatellite, Cotton leaf curl Gezira betasatellite (CLCuGB), is distinct from that occurring on the Indian subcontinent (Figure 4). CLCuGB may be transreplicated and maintained by CLCuMV to induce typical disease symptoms. Interestingly, CLCuGB is widespread across Africa and, together with distinct begomoviruses, causes disease in other species, including okra (Kon *et al*., 2009) and the non-malvaceous crop tomato (Chen *et al*., 2009). This contrasts with the situ-

ation on the Indian sub-continent. Although CLCuMB is occasionally identified in other plant species, it is only consistently found in ornamental *Hibiscus* and the fiber crops *Hibiscus cannabinus* and *Hibiscus sabdariffa* (Das *et al*., 2008; Paul *et al*., 2008; Roy *et al*., 2009). Disease in, for example, okra (Jose and Usha, 2003), chillies (Hussain *et al*., 2009) and tomato (Sivalingam *et al*., 2010) are associated with distinct betasatellites.

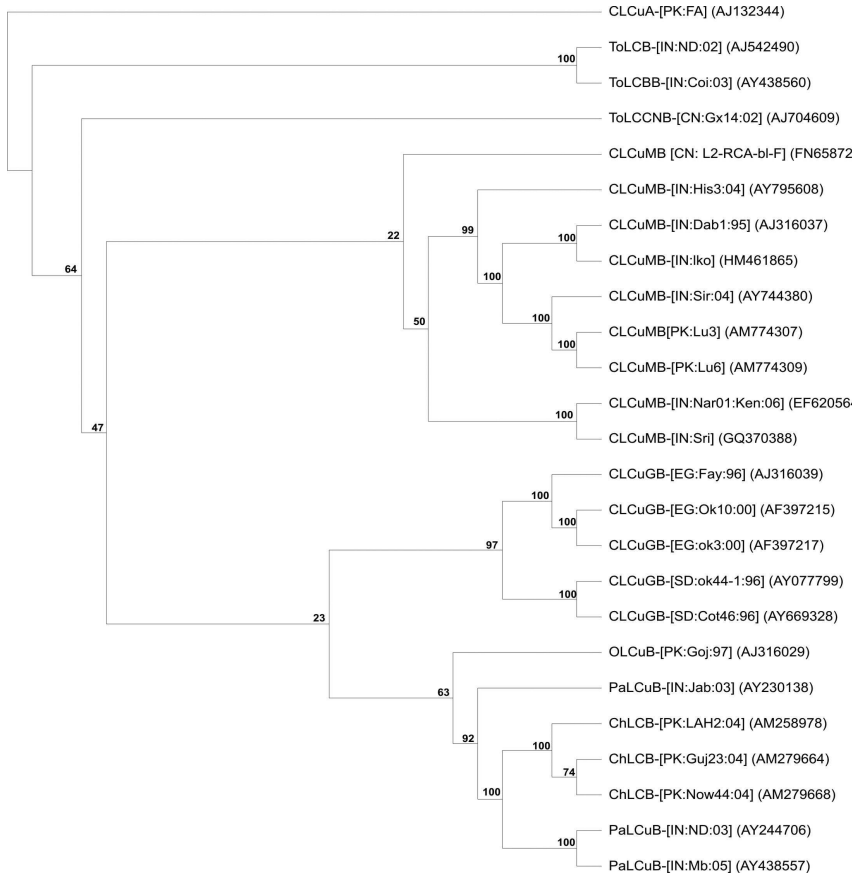

Figure 4. Phylogenetic dendrogram, based upon an alignment of the full length sequences of selected betasatellites. The figures at nodes indicate percentage bootstrap confidence values (1000 replicates). The betasatellites shown are Chilli leaf curl betasatellite (ChLCB), Cotton leaf curl Multan betasatellite (CLCuMB), Okra leaf curl betasatellite (OLCuB), Papaya leaf curl betasatellite (PaLCuB). The tree was rooted on an outgroup, the cotton leaf curl alphasatellite (CLCuA); an unrelated sequence of a similar size.. The geographical origins of the betasatellites involved in CLCuD (CLCuMB and CLCuGB) are indicated. In each case the database accession number of the sequence used is given. (Isolate descriptors are as given in Briddon *et al*., 2008).

COTTON LEAF CRUMPLE DISEASE

Cotton leaf crumple disease (CLCrD) is a disease of cotton that occurs in the New World. CLCrD was first reported from California (Dickson *et al*., 1954) and in Arizona a few years later (Allen *et al*., 1960). The symptoms of the disease are characteristically floral distortion, hypertrophy of interveinal tissue resulting in downward curling of leaves, and a foliar mosaic accompanied by vein clearing and frequent vein distortion (Brown and Nelson, 1987). Losses resulting from CLCrD infection range from 21 to 86%, depending on the age of plants at the time of infection (Allen *et al*., 1960; Brown *et al*., 1987; van Schaik *et al*., 1962). CLCrD can be transmitted experimentally by *B. tabaci* to numerous species within the families Malvaceae and *Fabaceae* families (Brown and Nelson, 1987). The disease mainly occurs in the Sonoran Desert of Arizona and Sonora, Mexico. The disease also occurs in southern California, the Rio Grande Valley of Texas, and Guatemala. The disease is among the earliest known where the causal agent was suspected to be a begomovirus.

Although geminate virus particles typical of geminiviruses were observed in CLCrD-affected cotton in the early 1980s (Brown and Nelson, 1984), the complete sequence of the virus concerned (Cotton leaf crumple virus [CLCrV]) was not determined until 2004 (Idris and Brown, 2004).

CLCrV is a typical bipartite begomovirus that characteristically (for New World begomoviruses) lacks the V2 gene (Fig. 1). It is closely related to other begomoviruses occurring in the New World. The DNA A component shares the highest levels of nucleotide sequence identity with Squash leaf curl virus, whereas the DNA B component has the highest levels of identity with Abutilon mosaic virus and Bean calico mosaic virus. CLCrV is only distantly related to the viruses causing CLCuD in the Old World.

MANAGEMENT OF BEGOMOVIRUS DISEASES OF COTTON

The diploid species of cotton (*G. arboreum* and *G. herbaceum*), that were grown across Asia and Africa prior to the introduction of tetraploid cottons (*G. hirsutum* and *G. barbadense*) are immune to CLCuD. A recent study on cotton species grown in a living herbarium being maintained at CCRI Multan has identified other sources of resistance in wild species of cotton (Azhar *et al*., 2010). The major obstacle however, is the ploidy barriers and therefore several steps are necessary to introduce characters from diploid to tetraploid cotton. The task has been complicated due to the lack of understanding of mechanism of resistance in diploid Asiatic species and the lack of DNA markers linked to disease resistance. Two strategies have been employed to incorporate useful characters from *G. arboreum*; one is the introduction of useful traits from *G. hirsutum* into *G. arboreum* (often termed as hirsutization of *G. arboreum*) and the other is to clone useful genes from *G. arboreum*. However, both strategies require long-term commitment.

During early 1990s, to counter the first epidemic of CLCuD, conventional selection and breeding was used to identify existing *G. hirsutum* cultivars in Pakistan with resistance to the disease and transfer this resistance to other, elite varieties. Varieties CP-15/2 and LRA-5166 were identified with stable resistance to the disease. Efforts concentrated on hybrids having

these two elite varieties as parents. The progenies 1098 and 1100, out of the cross 492/87 x CP-15/2, in the crop season 1992-93 emerged as the first instalment of lines resistant to CLCuD. Ali (1997) laid out a study to determine the mode of inheritance of host plant resistance mechanism against CLCuD. Crosses were made between the most susceptible cotton genotype, S-12 and the resistant variety, LRA-5166. Their F1s and backcross to LRA-5166 showed complete resistance against the disease. The F2 segregating population showed good fit to a ratio of 3:1 resistant/ susceptible. Thus, it was concluded that the disease is under the control of single dominant gene. It was reported that F1s of a cross between resistant parent (CIM-443) and susceptible parent (CIM-240) were often tolerant. He further observed that the cross between two tolerant parents produced a resistant F1 with one dominant gene coming from each parent. Thus it was indicated that two dominant genes governed resistance against the CLCuD. On the other hand, Rahman (Rahman *et al*., 2002) screened 22 genotypes of cotton for resistance against CLCuD. Out of these 22 genotypes only six, LRA-5166, Cedix, FVH-53, CIM-1100, CP-15/2 and CIM-443, were found to be extremely resistant. The resistance sources (LRA-5166 and CP-15/2) were employed for crosses with the most susceptible variety, S-12 (Rahman *et al*., 2005). The plants in the F_2 generation of crosses S-12 X LRA-5166, S-12 X CP15/2 and S-12 X CIM-443 and their reciprocals demonstrated a 13:3 (non-susceptible:susceptible) ratio. However, on the basis of F_3 progeny test, he suggested that two dominant genes at two loci acting epistatically might have conditioned the CLCuD resistance and a third gene known as suppressor gene is also involved which inhibits the expression of major genes. In spite of substantial efforts, advancement in breeding cotton for resistance to CLCuD has been slow. The main bottleneck is that the breeders have had to rely on field inoculation by whiteflies to screen for resistance.

Further efforts, involving crosses between local varieties and exotic virus resistant cultivars at the Central Cotton Research Institute, Multan (Pakistan) led to the development of several CLCuD resistant varieties. Subsequently further inbred lines, including Cedix, MS-40 and Reba, were found to be resistant to the disease. It has been shown that the resistance of Cedix, a cotton cultivar highly resistant to CLCrV from El Salvador, is controlled by two dominant and supplementary genes, which must occur together in order to confer full resistance (Wilson and Brown, 1991). Recently, more efforts were made to find resistance in *G. hirsutum* to CLCrV.

In the late 1990s, the widespread use of resistant varieties essentially removed CLCuD as a significant factor in cotton production in Pakistan. However, during the 2001 cropping season, symptoms of CLCuD appeared on all previously resistant cultivars at Burewala, district Vehari and by 2002 the disease reached epidemic proportions. This indicated the emergence of a resistance breaking strain of the virus (Mansoor *et al*., 2003a). Recently the begomovirus complex associated with resistance breakdown has been characterization. The so called "Burewala" resistance breaking strain of CLCuD is associated with a novel recombinant begomovirus, CLCuBuV, that lacks one of the usual complement of genes encoded by begomoviruses, C2 (Amrao *et al*., 2010b). The C2 protein has, amongst other functions, a suppressor of gene silencing activity. This may suggest that resistance breaking is due to the lack of C2, in turn suggesting that host resistance, in resistant cotton varieties, is due to recognition of the C2 protein (the so called avirulence determinant recognised by the host encoded resistance gene). However, this hypothesis has yet to be tested and it remains possible that resistance breaking is due to the

recombinant betasatellite (mentioned above) associated with the Burewala strain of CLCuD. At this time no reliable source of resistance to the resistance breaking strain of the disease has been identified and efforts are mainly concentrated on the transfer of resistance from diploid sources.

A number of reports on the resistance in *G. barbadense* to CLCuD have been published. Hutchinson and Knight (1950) developed resistance against the leaf curl disease in *G. barbadense* by repeated cycles of selection and, from the nature of response to selection, it was inferred that resistance to leaf curl was controlled by minor genes. Tarr (1951) was of the opinion that resistance against the virus in *G. barbadense* may not always be a stable quality. He reported that no major gene was involved in conferring resistance to the disease, and he suggested that resistance may be due to the cumulative effect of minor genes. On the other hand, Siddig (1968) suggested that the resistance was under the control of a single gene or very closely linked genes.

In addition to the use of natural resistance, it is hoped that, in the future, genetically engineered resistance will be useful for achieving resistance to begomoviruses in cotton and efforts are underway to achieve this objective. The advent of transgenesis offers many ways of obtaining virus resistant plants. It provides the ability to produce crop varieties inherently resistant to pathogen infection. The strategies which have been investigated for their usefulness in providing transgenic resistance against phytopathogenic viruses, including geminiviruses, can be grouped under the terms-pathogen derived resistance (PDR; in which a nucleic acid sequence, which may or may not encode a functional protein, derived from the pathogen is used as the source of resistance) and non pathogen derived resistance (NPDR; in which the source of sequence for resistance is other than the pathogen). Both these strategies have been used to develop transgenic resistance against viruses with a varied level of success. The first report of transgenic resistance against a plant virus involved the expression of the CP of *Tobacco mosaic virus* (Abel *et al*., 1986) and this strategy was subsequently also tried for geminiviruses. Tomato plants expressing the CP of the monopartite begomovirus Tomato yellow leaf curl virus (TYLCV) exhibited delayed symptom development and subsequently showed recovery of symptoms which was dependent on the expression level of the CP (Kunik *et al*., 1994).

Resistance using RNAi has also been achieved against geminiviruses by targeting either coding or non-coding regions of the genome. Transient expression of the bipartite begomovirus *Mungbean yellow mosaic virus* (MYMV) IR sequences as an intron spliced hairpin resulted in complete recovery in blackgram plants infected with MYMV (Pooggin *et al.*, 2003). Similarly an intron spliced hairpin construct containing sequences of the IR conserved between the monopartite begomoviruses TYLCV, Tomato yellow leaf curl Sardinia virus (TYLCSV) and *Tomato yellow leaf curl Malaga virus* yielded a broad spectrum resistance when transiently expressed in tomato and *N. benthamiana* plants challenged with these viruses by *Agrobacterium*-mediated inoculation or whitefly transmission. No virus could be detected in plants which were challenged with virus, that had earlier been inoculated with the hairpin construct, using PCR and a positive correlation between resistance and the accumulation of TYLCV-specific siRNAs (the effector of the RNAi response) was observed in silenced plants (Abhary *et al*., 2006).

Similarly various NPDR strategies have been used. For example, dianthin, a potent ribosome inactivating protein isolated from *Dianthus caryophyllus*, has been exploited to engineer transgenic resistance to the bipartite begomovirus African cassava mosaic virus in *N. benthamiana*

(Hong *et al*., 1997). Similarly the RNAse barstar (Zhang *et al*., 2003), an insect symbiont derived virus binding protein (GroEl) (Edelbaum *et al*., 2009) and peptide aptamers (short peptides that interfere with enzyme activity) (Lopez-Ochoa *et al*., 2006) have also shown promise as strategies to obtain resistance against geminiviruses.

Unfortunately there are not many success stories in engineering resistance cotton against begomoviruses. Asad *et al*. (2003), in a proof of concept study, showed that an antisense construct containing partial Rep sequences of CLCuKoV could provide resistance against the virus in tobacco using RNAi. This construct has been transformed into cotton and performs well in small-scale field trials (Shaheen Aftab, personal communication). One limitation of gene silencing based technologies is that they are sequence specific – thus small changes in the targeted virus sequence can overcome the resistance. Thus, it is essential to identify those targets which remain conserved among these viruses. This is no easy task, particularly for CLCuD, where numerous distinct viruses can cause the disease.

SUMMARY

Virus diseases of cotton are an important factor limiting production in some major cotton-growing countries. Whitefly-transmitted viruses are the most important and are currently causing significant losses to cotton production in Pakistan and northwestern India. These viruses are potentially a threat to all cotton-growing areas where the whitefly (*Bemisia tabaci* Gennadius) occurs. Human activity is disseminating both the viruses and their vector to new geographical locations. Exciting progress has been made in understanding the biology of the causal agents of these viral diseases from Asia, Africa and Americas. Cotton-infecting begomoviruses in the Old World are invariably monopartite and are associated with DNA satellites. Two types of DNA satellites, known as alphasatellites and betasatellites, have been identified, although only a betasatellite is essential for symptomatic virus infection of cotton. Cultivated diploid cotton species of Asian/African origin, *Gossypium arboreum* and *G. herbaceum,* are immune to leaf curl disease. Sources of resistance in cultivated tetraploid cotton species (*G. hirsutum* and *G. barbadense*) are limited and emerging virus strains often overcome the available resistance. Recent progress in developing genetically-engineered resistance against begomoviruses is encouraging but commercial exploitation of transgenic cotton varieties will depend on our ability to develop broad-spectrum resistance.

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Chapter 9

ABIOTIC STRESS AND COTTON FIBER DEVELOPMENT

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INTRODUCTION

Abiotic stresses, particularly water deficit, salinity, and temperature extremes, are the primary factors limiting crop productivity, accounting for more than a 50% reduction in crop yields worldwide (Boyer, 1982). Areas affected by drought are expanding and this trend is expected to accelerate (Burke *et al*., 2006). Growth of the world's population combined with an increase in global prosperity and decrease in arable land are creating increasing demands for food, fiber and biomaterials (Ragauskas *et al*., 2006). More than 80% of available fresh water is consumed by agriculture (Delmer, 2005), and the need for sustainable agricultural methods is ever increasing. Drought is a perennial environmental constraint, affecting an estimated 25 percent of all crops worldwide at enormous cost. Therefore, increasing food and fiber quantity and quality through biotechnology for improved stress tolerance and biomass production has the potential to impact the complex and interrelated issues of globalization, poverty, hunger, population growth, climate change, energy, biodiversity, and environmental degradation. The task of identifying gene functions and developing effective strategies to use these functions for crop improvement is daunting and much more knowledge is needed to achieve the promise of plant biotechnology.

Plant Responses to Water-Deficit Stress

Although cotton is considered to be a drought tolerant plant, like most major agricultural crops, its production is negatively impacted by water-deficit stress. Cotton, being a perennial with an indeterminate growth habit and a complex fruiting pattern, is considered to have the most complicated response to environmental conditions and management practices of the major row crops grown in the United States (Oosterhuis, 1990). Cotton yield is generally proportional to the amount of water available and acceptable yield enhancements from irrigation are typically seen in arid and semi-arid environments such as Arizona, California and West Texas (Radin *et al*., 1992.

Cotton fiber initiation, elongation, secondary cell wall development and maturation are genetically regulated, but are also affected by the environmental conditions faced by the plant during its lifecycle. Throughout cotton development, the plant perceives both internal and external cues that alter the physiological, metabolic, and cellular programs that ultimately determine the final characteristics of the fiber. Understanding fiber biology in terms of these cues has been slow in coming. Water deficit induces a variety of plant responses, including changes in gene

expression, accumulation of the phytohormone abscisic acid (ABA), production of osmotically active compounds, and the synthesis of protective proteins that scavenge oxygen radicals or act as molecular chaperones (Wang *et al*., 2003). These responses are controlled by molecular networks that activate stress responsive mechanisms to re-establish homeostasis and to protect and repair damaged proteins and membranes (Ramachandra-Reddy, 2004). Comparative analysis of gene expression profiles in cotton leaf and root tissues under well-watered and water-deficit conditions indicated extensive tissue-specific and stress-responsive changes in gene expression (Payton *et al*., 2010). While many of these stress induced genes fall into known functional categories, including, protective factors such as heat shock proteins, desiccation response proteins (dehydrins) and antioxidant enzymes, along with known stress responsive regulatory factors, the majority of stress-responsive transcripts identified in both tissues have functions that are not yet known. Thus, much remains to be learned about abiotic stress responses in cotton.

In recent years, our understanding of the regulatory mechanisms that control stress acclimation mechanisms in the model plant Arabidopsis has dramatically increased. A full review of these findings is outside the scope of this article but readers are directed to Hirayama and Shinozaki (2010) for a recent review. It is clear that research to uncover the basic mechanisms used by plants to respond to stressful environmental conditions will provide a strong foundation for more focused research aimed at understanding comparable mechanisms in cotton and other crops.

METABOLIC ASPECTS OF FIBER DEVELOPMENT

Comparisons between the cotton fiber transcriptome and metabolome at different stages of development have shown that stage-specific events can be characterized by their transcript and metabolite profiles (Gou *et al*., 2007). The up- and down-regulation of genes is dependent on the stage of fiber development as are the metabolic pathways that are utilized. For example, during fiber initiation and elongation, fiber cells must synthesize primary cell walls while maintaining a balance between turgor and extensibility. During the transitional phase from primary to secondary cell wall synthesis, a shift in cell metabolism occurs to meet the demand for cellulose synthesis by re-directing energy to carbohydrate metabolism and secondary cell wall synthesis. This shift in cellular function corresponds with the unique metabolic demands of the two major events in the fiber cell, namely, cell elongation and cellulose deposition.

Using a gene expression and GC/MS-based metabolite profiling approach, Gou *et al*. (2007) identified seven metabolic pathways, including secondary metabolites, fatty acid and carbohydrate metabolism, that function during cotton fiber development. At three days post anthesis (DPA), metabolite analysis revealed high levels of sucrose, which correlate with elevated expression of eight aquaporin-like genes. This combination promotes the build-up of turgor by increasing the osmotic potential and accelerating the rate of water uptake, respectively. Aquaporins are present in the plasma membrane (PIPs) and the tonoplast (TIPs) and are essential for cell expansion. Liu, *et al*. (2008), characterized the expression of cotton aquaporin genes *GhPIP1-2* and *GhTIP1*and found these genes to be highly and preferentially expressed at 5 DPA, further supporting their important roles during cotton fiber cell

expansion. Fiber cell elongation also requires that the cell wall be loosened for expansion. α-expansins play a major role in cell-wall weakening and disassembly in processes such as ripening, abscission and certain developmental pathways including pollen-tube growth and xylem formation (McQueen-Mason *et al*., 2007). In cotton fiber, four genes that belong to the α-expansin family were highly expressed during the outgrowth and rapid elongation stages, but were down-regulated when cells entered the secondary cell wall synthesis stage (Gou *et al*., 2007). Similarly, genes encoding putative xyloglucan endotransglycosylases (XTHs), which are involved in cell-wall remodeling, have recently been characterized in cotton and some *XTH* genes were shown to be preferentially expressed during the early stages of fiber elongation (Michailidis *et al*., 2008; Lee *et al*., 2010).

Based on the activity measurements of malate-synthesizing enzymes such as phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase (MDH), Thaker *et al*. (1999), demonstrated that the osmolyte, malate, plays an important role during rapid cell elongation. PEPC and MDH activities were elevated during the elongation stage of fiber development, whereas NADPH-MDH activity (an antagonist of PEPC) was reduced. This is consistent with findings that *PEPC* and *MDH* expression levels are higher in fibers from long staple cultivars than in those from short staple cultivars (Basra and Malik, 1983). Other genes implicated in the elongating cell are the plasma membrane proton translocating-ATPase (PM-H^+-ATPase), and vacuolar proton translocating-ATPase (V-ATPase) (Benedict *et al*., 1999). V-ATPase is known to be involved in driving solute movement into vacuoles for maintaining turgor, whereas PM-H^+-ATPase transports H^+ out of the cytosol, acidifying the apoplast and changing the extensibility of the cell wall.

Lipids are an integral part of membrane and cell wall synthesis. Gou *et al*. (2007) reported the upregulation of lipid biosynthetic genes and lipid metabolism at 6 DPA that was maintained throughout the elongation phase. In accordance with the amounts of fatty acids in fiber cells, genes that encode enzymes such as acyl-CoA-binding protein, fatty acid elongase, 3-keto-acyl-CoA synthase, β-ketoacyl-CoA synthase, and ω-3 fatty acid desaturase and very-long-chain fatty acid condensing enzyme, were upregulated at this stage and greatly reduced at 21 DPA. This is consistent with findings that lipid metabolizing enzymes and lipid transfer proteins, which have recently been shown to induce cell wall extension in in vitro assays (McQueen-Mason *et al*., 2007), are particularly highly expressed in fiber cells (Song and Allen, 1997; Orford and Timmis, 1998; and Ji *et al*., 2003). During fiber elongation, two predominant respiratory pathways, the oxidative pentose phosphate pathway (OPPP) and glycolysis, provide energy and the conversion of substrates to intermediates required for biosynthesis. The enzyme activity levels in these pathways vary with the demand for respiratory products (Thaker *et al*., 1999). For example, measured activity of glucose-6 phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) was high during the cell elongation up to 15 DPA, before falling to negligible levels at 24 DPA and 30 DPA, respectively. Thus, increased activity of OPPP enzymes could reflect the demand for NADPH and intermediates in the regulation of carbon channeling during the elongation phase and this is further supported by increased hexose kinase activity (Thaker *et al*., 1999).

At the onset of secondary cell wall formation, data gathered from transcript and metabolite profiles clearly demonstrate dynamic changes in metabolism that center on cellulose synthesis (Gou *et al*., 2007). Thus, metabolic pathways that are active during fiber elongation are down-regulated with the onset of secondary wall formation. This is evident in the reduction of G6PDH and 6PGDH activity, indicating a transition in metabolic priorities (Thaker *et al*., 1999). To illustrate this, pectin, a polysaccharide component of primary cell walls, is synthesized in part by UDP-glucose-6-dehydrogenase and UDP-D-glucuronate 4-epimerase. These enzymes, which convert UDP-glucose into UDP-D-glucuronate and then UDP-galacturonate, are down-regulated during the secondary wall synthesis stage. In light of the view that UDP-glucose serves as an immediate substrate for cellulose polymerization in cotton fiber, down-regulation of enzymes that compete for UDP-glucose makes metabolic sense (Guo *et al*., 2007). Interestingly, the activity of the glycolytic enzymes, aldolase and pyruvate kinase increase upon the shift to secondary cell wall deposition, indicating a role in cellulose synthesis (Thaker *et al*., 1999). Metabolite profiling showed that glucose, and to some extent, fructose accounts for about 50% of the total polar phase metabolites in rapidly elongating fiber cells, but decreases to 9% at 21 DPA, indicating an increase in carbohydrate utilization for cellulose synthesis (Guo *et al*., 2007). The demand for carbon in secondary cell wall synthesis is further supported by an increase of both gene expression and activity of pectin degrading enzymes, such as β-galactosidase and β-arabinosidase.

In-depth reviews by Delmer (1999) and, more recently, Haigler (2007), discussed the carbon flux into cellulose. In the models presented by these authors, UDP-glucose, derived from a variety of enzymatic reactions, is the immediate substrate for cellulose synthesis. One source of UDP-glucose is the hydrolysis of sucrose by sucrose synthase (SuSy). Although it is not conclusively determined whether the cytosolic (S-SuSy) or the membrane-associated (M-SuSy) enzyme supplies the substrate for cellulose synthesis, substantial evidence indicates that M-SuSy is likely to be the predominant enzyme that channels UDP-glucose to cellulose while S-SuSy partitions carbon for general metabolic needs (Haigler, 2007). This evidence comes from the observation that more than 50% of total SuSy protein is tightly associated with the plasma membrane, paralleling the patterns of cellulose deposition during secondary wall synthesis (Amor *et al*., 1995; Salnikov *et al*., 2003). Furthermore, it was shown that sucrose and not UDP-glucose was the preferred substrate for cellulose synthesis, indicating that a direct, energy-saving mechanism for channeling UDP-glucose to cellulose synthase is in place. However, UDP-glucose for cellulose synthesis could also be supplied by UDP-glucose pyrophosphorylase (Carpita and Delmer, 1981; Waefler and Meiser, 1994). It should be noted, however, that production of UDP-glucose through this reaction requires more energy input than from SuSy (Haigler, 2007).

Ultimately, all carbon comes from imported sucrose or re-synthesized sucrose within the cell. Besides SuSy, cell wall and vacuolar invertases also catalyze the break-down of sucrose into glucose and fructose. On the other hand, sucrose-phosphate synthase (SPS) can exert control over carbon allocation by irreversibly re-synthesizing sucrose-6-phosphate followed by the production of sucrose by sucrose-phosphate phosphatase (SPP). This sucrose cycling may be useful for efficiently controlling metabolic processes at the different stages of fiber development (Haigler *et al*., 2001; 2007).

ENVIRONMENTAL EFFECTS ON FIBER DEVELOPMENT

Cotton plants grown under unsuitable environmental conditions such as temperature extremes, water deficit, and salinity stress face reduced growth and productivity resulting from loss of fruit and altered fiber development. McMichael *et al*. (1973) found that water-deficit stress before 14 DPA leads to boll abscission, but beyond that "window of susceptibility" abscission generally does not occur. However, water-deficit stress during fiber elongation or secondary wall synthesis leads to decreased fiber length and maturity, respectively (reviewed in Cothren, 1999). Although it is not fully known how fiber quality is affected by stress, it could be due, at least in part, to the accumulation of signaling molecules such as abscisic acid (ABA). In cotton, as in other plants, ABA produced in response to water deficit and heat stress, for example, induces stomatal closure and lowers leaf water potential, these responses negatively affect photosynthesis and accumulation of carbon assimilate (Cothren, 1999; Finkelstein *et al*., 2002). Many of these biochemical and physiological changes result from ABA-induced changes in gene expression patterns. Moreover, Dasani and Thaker *et al*. (2006) reported an inverse correlation between final fiber length and ABA levels in three different cultivars. In a long staple cultivar, rapid ABA accumulation started after the fiber had attained peak elongation while, in a short staple cultivar, ABA accumulation was observed during elongation. Greater inhibition of fiber elongation was observed in cultured ovules of short and middle staple cultivars than in ovules of a long staple cultivar when the media were supplemented with ABA. It is yet to be determined if these changes ultimately affect cellulose synthesis in the fiber.

There are substantial data that show cotton fiber cellulose deposition and the degree of polymerization are affected by cool temperatures and, furthermore, that this process might be more sensitive than respiration (Haiger, 2007). Fibers exposed to cool temperatures have a prolonged period of elongation and reduced rate of secondary wall thickening, giving rise to growth rings (Basra and Saha, 1999). Temperatures below 27º C can negatively affect cellulose deposition in the secondary wall through the disruption of photoassimilate production, transport and uptake, the availability of respiration-derived energy, or direct and/or indirect effects on enzyme activity and kinetics (Roberts *et al*., 1992). The decrease in cell wall synthesis during cool nights could relate to the metabolic pathways that partition the substrate for cellulose synthesis at different developmental stages (Haigler, 2007). For example, Haigler *et al*. (2001) proposed a model to indicate that, under stress conditions, cells could shift from a M-SuSy (thought to channel UDP-glucose to cellulose synthase) to the soluble isoform (S-SuSy), reflecting a down-regulation of cellulose synthase.

IMPROVEMENT OF ABIOTIC STRESS TOLERANCE USING BIOTECHNOLOGY

The development of more stress-tolerant crops has been hindered by our limited knowledge of the precise physiological parameters that reflect the genetic potential for improved productivity under water-limited and thermally stressful environments. The potential to identify key traits that limit yield under abiotic stress conditions hinges upon an understanding of the crop at

the physiological and molecular levels. Moreover, an understanding of physiological processes that result in crop yield is paramount to accurate identification and introgression of candidate genetic material for yield improvement. Identification and characterization of quantitative trait loci (QTL) associated with improved fiber quality and yield under stressful growing conditions and selective introgression of QTL into elite cotton cultivars using a molecular breeding approach is underway (Paterson, *et al*., 2003; Saranga *et al*., 2004). While this approach is likely to bring improvements in stress tolerance, it only allows breeders to tap the genetic diversity existing within the species and, perhaps, its close relatives. QTL introgression can also introduce undesirable agronomic characteristics from the donor parents. Therefore, the development of transgenic plants by the introduction of selected genes provides a more focused approach for the creation of plants with improved abiotic stress tolerance and use of transgenes allows for the transfer of genes from any source, including non-plant species. Transgenic technology also allows for the expression of the introduced gene to be precisely controlled both temporally and spatially. This capability can be critical if expression of a given gene is needed only at a specific developmental stage, in a specific organ or tissue, or in response to specific environmental conditions. Although promoters that are constitutively expressed at high levels are still widely used, they are not appropriate for all transgenes. This is especially true for genes that encode stress responsive regulatory factors, which can have serious deleterious effects when constitutively expressed. Generation and testing of transgenic cotton plants that express gene cassettes controlled by stress-inducible promoters is now underway and it seems possible that this approach will allow for the enhancement of stress tolerance phenotypes without negative agronomic consequences. Therefore, while we are likely to see steady progress using traditional and molecular breeding strategies, transgenic modifications will provides a wider variety of options for the improvement of stress tolerance in crop plants.

More than a decade has passed since the first commercially successful transgenic agricultural crops were launched. These first products were based, in large part, on simple monogenic traits, such as herbicide tolerance or insect resistance, which did not require manipulation of complex molecular pathways in the transgenic plant (Century *et al*., 2008). Engineering crops with improved abiotic stress tolerance has proven to be much more difficult due to the multiple complex pathways involved in controlling the native stress responses. Most strategies for engineering abiotic stress tolerance in plants used so far have relied primarily on the expression of genes that encode protective molecules, such as dehydrins and antioxidant enzymes or enzymes involved in the synthesis of functional metabolites and ion pumps (for examples see Roxas *et al*., 2000; Kornyeyev *et al*., 2001; Payton *et al*., 2001; Yan *et al*., 2003; Park *et al*., 2004; He *et al*., 2005). More recently, strategies that employ genes involved in signaling and regulatory pathways for engineering plant stress responses have been developed and show great promise (Umezawa *et al*., 2006). Manipulation of these types of genes can affect a broad range of downstream events, which may result in superior tolerance to multiple stressful environments. An attractive target for manipulation and gene regulation is transcription factors (TFs) that bind to promoter regulatory elements and activate cascades of genes that act together in response to internal or external signals (Bhatnagar-Marthur *et al*., 2008). One the most well studied groups of TFs involved in drought and cold tolerance are the *CBF* (C-repeat binding factor) genes (also known

as dehydration-responsive element-binding protein [*DREB1*] genes). These ABA-independent transcription factors belongs to the ERF/AP2 family that binds to the DRE/CRT motif with a conserved (A/G)CCGACNT sequence within the promoters of a suite of genes known to establish stress tolerance (Shinozaki and Yamaguchi-Shinozaki, 2007). As reviewed by Century *et al*. (2008), overexpression of these genes, specifically *CBF3*, in Arabidopsis and ectopic expression in wheat (Pellegrineschi *et al*., 2004), tomato (Wang *et al*., 2003; Chaves and Oliveira, 2004), tobacco (Kasuga *et al*., 2004), rice (Oh *et al*., 2005), and potato (Benham *et al*., 2005; Pino *et al*., 2007) produced enhanced tolerance to one or more types of abiotic stress. However, in some of these examples, the benefits of constitutively over-expressing *CBF3* were overwhelmed by undesirable side effects such as growth retardation. In some cases, these negative effects were mitigated with the use of the stress-inducible promoters while still providing increased stress tolerance (Bhatnagar-Marthur *et al*., 2008).

Another group of TF shown to confer multiple stress resistance is the ABA-dependent TF from Arabidopsis, *ABF3*. ABA regulates seed desiccation tolerance and dormancy and inhibits the phase transition from embryonic to germinative growth and from vegetative to reproductive growth. In addition, ABA acts as an internal signal to mediate some physiological responses to environmental stresses. ABA has been shown to regulate plant responses to drought, cold, and high temperature (reviewed in Marion-Poll and Leung, 2006). ABA levels increase in vegetative tissues during exposure to these stresses, triggering adaptive responses that are essential for their survival and productivity. For example, under drought conditions, ABA induces stomatal closure, minimizing water loss through transpiration (Finkelstein *et al*., 2005). Many of the biochemical and physiological changes that result from ABA-induced changes in gene expression patterns are dictated, in part, by a family of ABRE (Abscisic acid response elements)-binding transcription factors, or ABFs (ABRE-binding factors). Their expression is induced by ABA and by high salinity, cold or drought. Thus the ABF family of transcription factors is likely to be involved in ABA-dependent stress responses. Analysis of Arabidopsis that constitutively overexpress *ABF3* demonstrated that they are tolerant to chilling, freezing, heat and oxidative stress, with minimal inhibitory effect on germination and seedling growth (Kim *et al*., 2004). Moreover, Oh *et al*. (2005) and Vanjildorj *et al*. (2005) showed that constitutive ectopic expression of ABF3 in rice and lettuce resulted in increased tolerance to drought with normal growth in terms of whole plant morphology and seed development. These results indicate that tansgenes that express stress responsive transcription factors such as CBF3 and ABF3 may be good candidates for engineering multiple stress tolerance in cotton.

In addition to the technology used to generate transgenic plants that express their introduced genes in an appropriate way, it is also important to consider how these transgenic plants are evaluated to determine the effects of the introduced gene on stress tolerance characteristics. In most cases, transgenes have been tested only in model system plants such as *Arabidopsis* or tobacco. While these "proof-of-concept" experiments can give important clues about the potential usefulness of specific genes in crop plants such as cotton, in many cases the published work has depended on the assessment of transgenic plants under artificial environments that are unlikely to be faced by crops under field conditions. In addition, the physiological characterization in many of these studies does not extend beyond evaluation of growth or survival under

severe conditions. Therefore, rigorous physiological evaluation of the tolerance of transgenic crop plants to abiotic stresses and the effects of specific transgenes on agronomic traits such as yield and quality are generally lacking. Thus, as research in this area progresses and more stress tolerance candidate genes are tested, evaluation of plants that contain these genes in the field under "real world" conditions will, of course, become a priority. The effects of candidate stress tolerance genes on fiber yield and quality and the ability of these genes to provide agronomic improvements when introgressed into current cultivars will be critical to their eventual adoption by the cotton industry.

SUMMARY

Abiotic stresses, including water deficit and extreme temperatures, limit the yields and quality of cotton produced around the world. Efforts to develop new biotechnologies to improve abiotic stress tolerance in plants such as cotton are underway. While stress tolerance mechanisms are genetically and biochemically complex, tremendous progress is being made in our understanding of the regulatory pathways that regulate these mechanisms in model plants such as *Arabidopsis*. This research will undoubtedly uncover dozens, if not hundreds, of new candidate genes with the potential to provide improved stress tolerance characteristics in crop plants, including cotton. Evaluation of these genes in crop plants may take many years and the development of commercial cultivars that incorporate the most successful of these technologies is likely to take decades.

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