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 30ºC has been reported for cotton (Reddy *et al*, 1991), but cotton is successfully grown at temperatures in excess of 40ºC 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 35ºC were harmful. Work in growth chambers in Mississippi showed that the ideal temperature range for cotton was from 20 to 30ºC (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showed that yields decreased significantly when the mean maximum day temperature for July exceeded 32°C (Oosterhuis, unpublished).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 general 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.

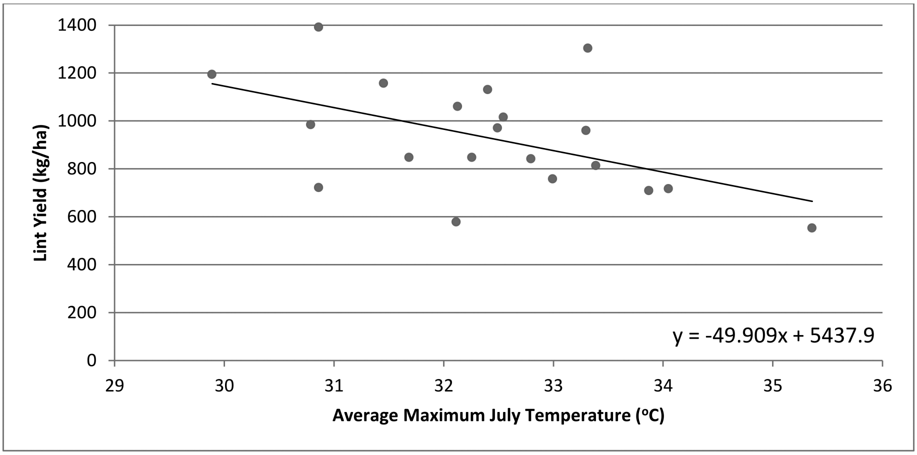

Figure 1. Negative correlation between cotton yield and high temperature during July when flowering and early boll development occur in Arkansas. (Adapted from Oosterhuis, 1999).

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.

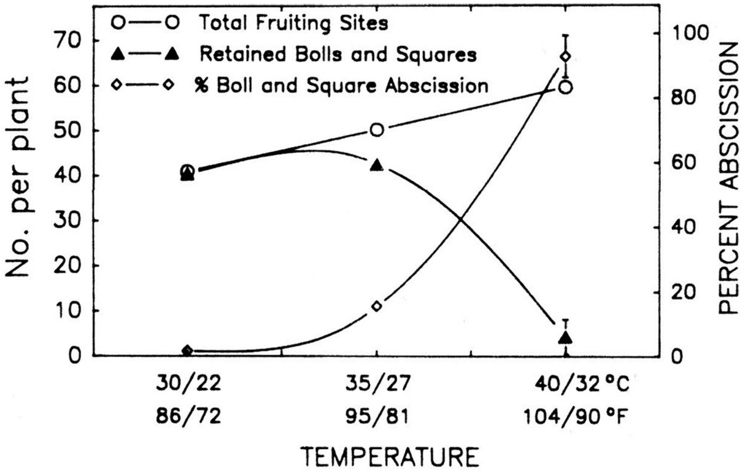

Figure 2. The effect of increasing day/night temperature on fruiting sites produced, bolls and squares retained, and percent boll and square abscission. (From Hodges *et al.*, 1993).

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;

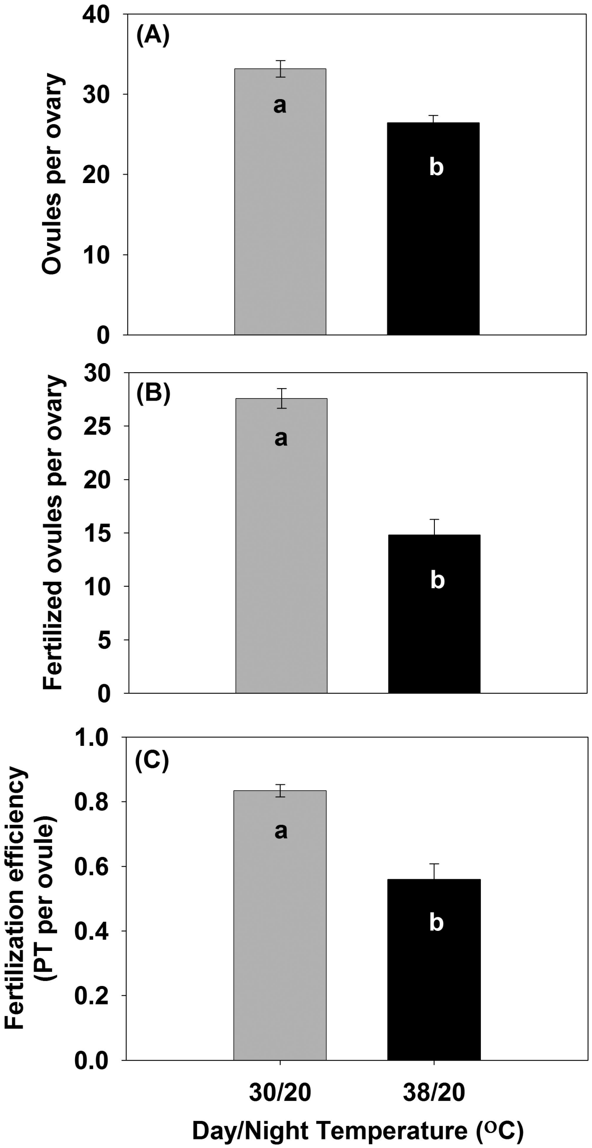

Figure 3. The number of total ovules (A), fertilized ovules (B) and fertilization efficiency expressed as pollen tubes per ovule (PT per ovule) (C) in *Gossypium hirsutum* pistils under normal (30/20°C) and high (38/20°C) day temperature regimes. Heat stress induced significant reductions in all three 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.

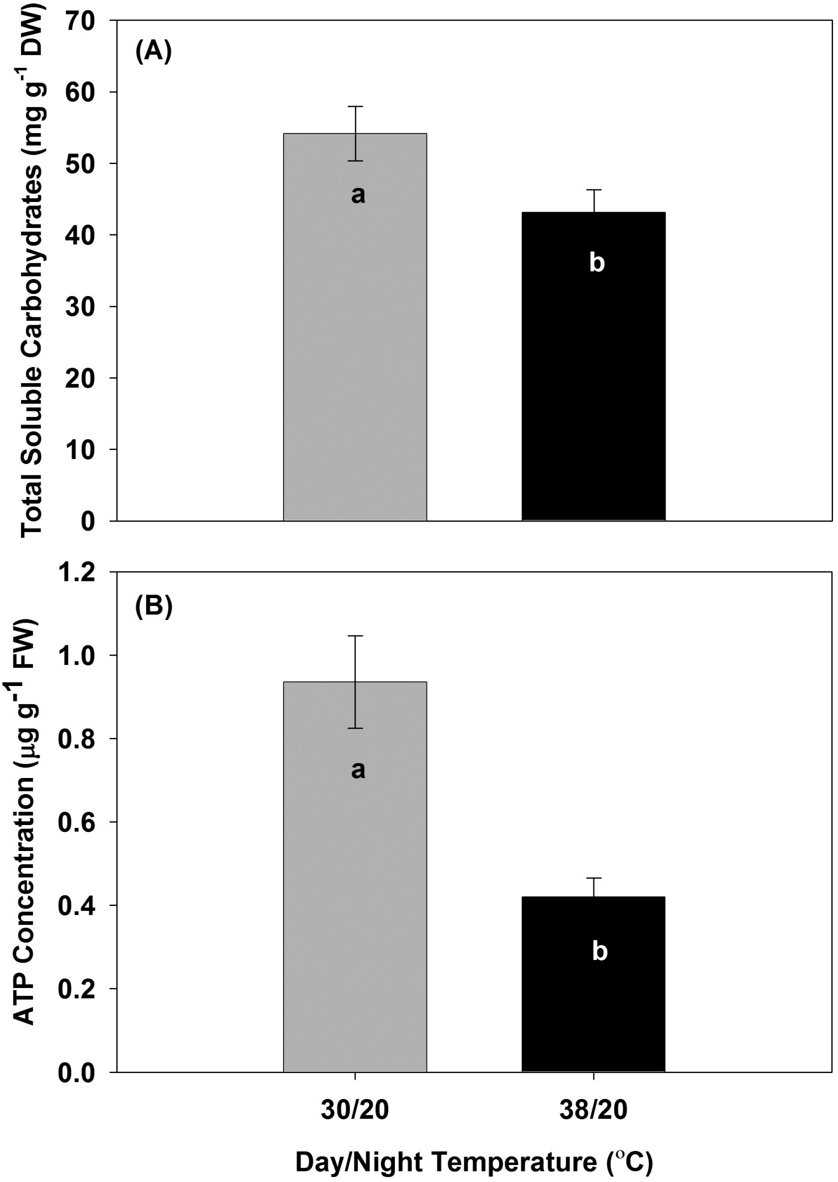

Figure 4. Total soluble carbohydrate and ATP concentrations of *Gossypium hirsutum* pistils exposed to high day temperatures (38/20°C) and optimal day temperatures (30/20°C). Heat stress reduced both soluble carbohydrate (A) and ATP levels (B). All values are means ± SE (n = 15), and values not sharing 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).

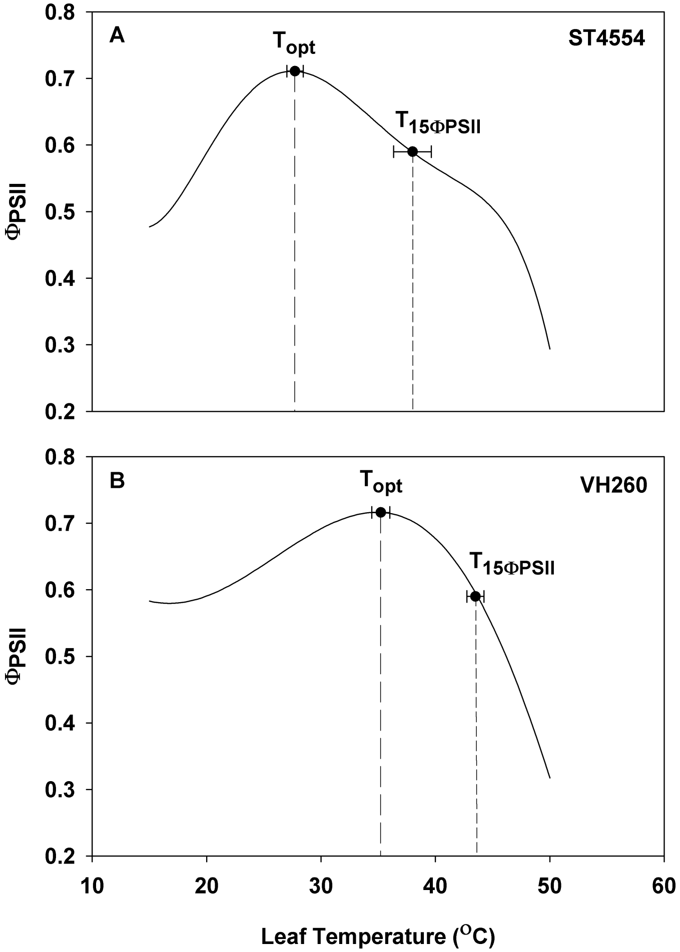

Figure 5. Effect of temperature on _PSII in *G. hirsutum* cv. ST4554 (A) and cv. VH260 (B). Leaves were illuminated with 500 μmol photons m^{-2} s^{-1} and incubated at temperatures ranging from 15 to 50°C for 5 min at each temperature prior to *Φ*PSII determination. In each graph, a representative curve 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).

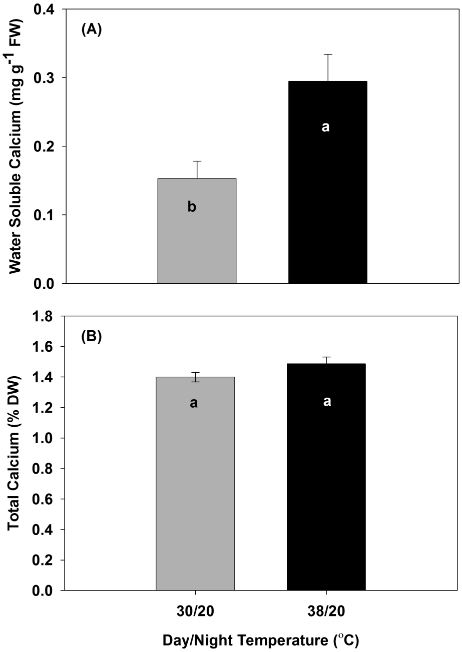

Figure 6. Water soluble (A) and total calcium (B) responses to high day temperature in *Gossypium hirsutum* pistils exposed to heat stress (38/20°C) and optimal (30/20°C) temperature conditions. Heat stress induces a significant increase in water soluble calcium (A) levels but does not alter total calcium (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).

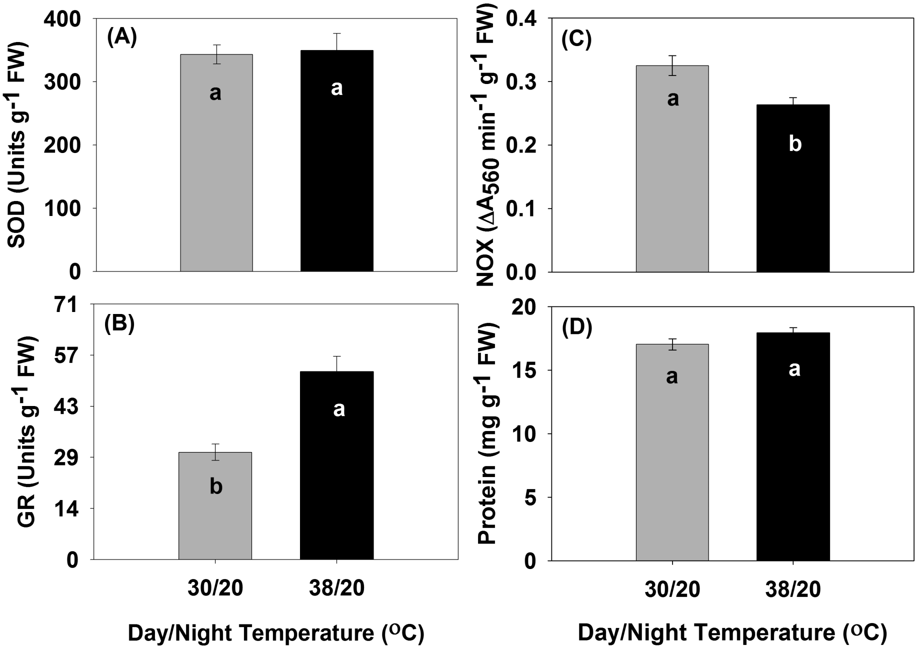

Figure 7. In *Gossypium hirsutum* high day temperature does not alter superoxide dismutase (SOD) activity (A), increases glutathione reductase (GR) activity (B), decreases NADPH Oxidase (NOX) activity (C) and does not change soluble protein content (D). All values are means ± SE (n = 15). Values not 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.

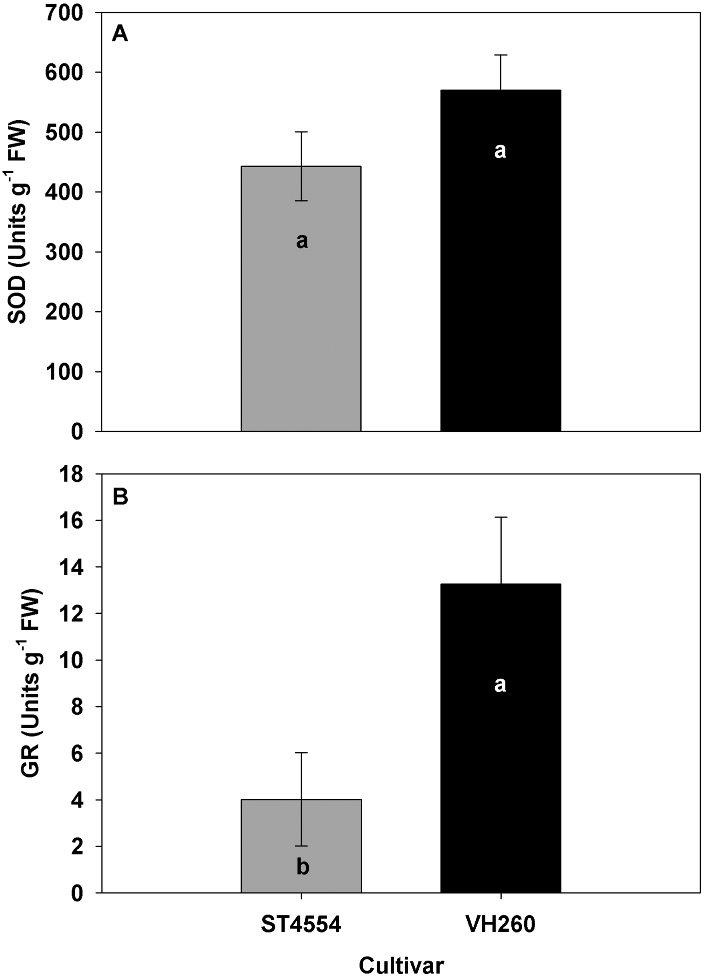

Figure 8. Effect of cultivar on SOD (A) and GR (B) activity of *G. hirsutum* grown under 30/20°C day/night temperature regime. GR was significantly higher in VH260 compared with ST4554 (B), whereas SOD was not significantly different (B). All values are means ± standard error (n = 6). Values not sharing 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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