Chapter 6

HEAT STRESS AND POLLEN-PISTIL INTERACTIONS

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INTRODUCTION

For a number of plant species, sexual reproduction is substantially more sensitive to heat stress than vegetative processes (Zinn *et al.*, 2010). Consequently, the yield of crops with valuable reproductive structures used for food (i.e. grain crops and horticultural crops) and fiber (i.e. cotton) are especially sensitive to moderately elevated temperatures projected to result from global climate change (Peng *et al.*, 2004; Reddy *et al.*, 2002). Other than concerns over global climate change, another important consideration for many cotton producers is the extreme year-to-year variability in yields caused by temperature extremes. For example, a negative correlation has been reported between average maximum temperature during flowering and lint yields in cotton (*Gossypium hirsutum*; Oosterhuis, 2002). 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 reproduction was most vulnerable to average daily temperatures above 32.8° C to 34.4° C, and high temperature has been shown to substantially limit *in vivo* fertilization in thermosensitive cultivars (Snider *et al.*, 2009, 2011c; Fig. 1). 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). Depending upon the timing, duration and severity, heat stress could limit fertilization by inhibiting male (Jain *et al.*, 2007) and female (Saini *et al.*, 1983; Snider *et al.*, 2009) gametophyte development, inhibiting pollen germination (Burke *et al.*, 2004; Kakani *et al.*, 2005; Jain *et al.*, 2007), limiting pollen tube growth (Burke *et al.*, 2004; Kakani *et al.*, 2005; Hedhly *et al.*, 2004; Snider *et al.*, 2011a), or by altering the development of tissues required to carry out reproductive processes (i.e. anther and pistil tissues; Zinn *et al.*, 2010). Although literature concerning heat stress and reproductive development in sexual plants is extensive, the approaches used by various investigators to elucidate plant reproductive responses to high temperature vary substantially from study to study. Consequently, it is the aim of this review to characterize the impact of timing, duration and severity of heat stress on sexual processes occurring during the progamic phase. A special emphasis is placed on the biochemical response of the pistil to moderately high temperature and the resultant influence on *in vivo* pollen performance and fertilization. Although this chapter will focus on heat stress effects in cotton, inferences will also be drawn from work conducted with other species where information is lacking for cotton.

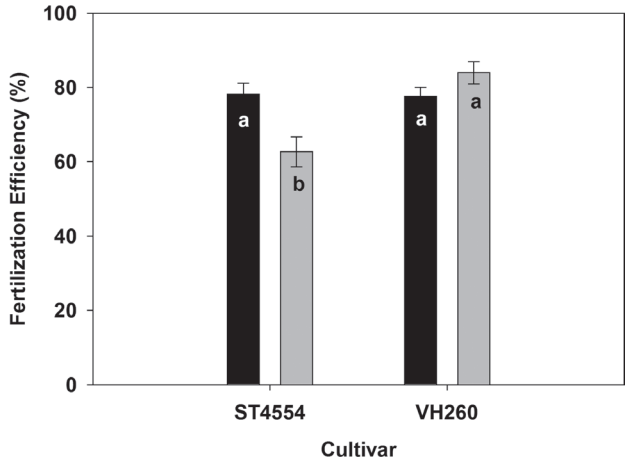

Figure 1: Fertilization efficiencies (B) of ST4554 and VH260 under a 30/20° C day/night temperature regime (black vertical bars; 30) and 38/20° C day/night temperature regime (gray vertical bars; 38). All values are means ± standard error (n = 9), and values not sharing a common letter are significantly different (LSD; $P < 0.05$). (Adapted from Snider *et al.*, 2011b)

SEXUAL REPRODUCTION IN COTTON

Sexual reproduction in flowering plants occurs in essentially three stages: gametophyte development (from meiosis to pollination), the progamic phase (from pollination to zygote formation), and embryo development (from zygote to seed; Herrero and Hormaza, 1996). During the progamic phase a number of reproductive processes must occur in a highly concerted fashion for successful fertilization to occur. 1) Anther dehiscence allows mature pollen grains to be transferred to a receptive stigmatic surface; 2) pollen grains germinate and pollen tubes penetrate the stigmatic surface of the pistil; 3) pollen tubes grow through the transmitting tissue of the style and towards a sexually competent ovule; 4) finally, double fertilization produces a zygote and its associated endosperm. The precise timing and coordination of events during the progamic phase is to a large extent genetically predetermined and varies substantially between plant species (Lankinen *et al.*, 2007; Williams, 2008). For example, *Quercus rubra* has a progamic phase lasting 57 weeks, whereas *Cichorium intybus* completes the progamic phase in 15 to 20 min (Williams, 2008).

There is a wealth of literature available on the pattern of reproductive development in cotton (e.g. Beasley, 1975; Stewart, 1986), and the sequence and timing of events during sexual reproduction have been well defined. For example, the first flower is produced approximately 8 weeks after plant emergence, and due to the indeterminate growth habit of cotton, flowers are continually produced in a 3 day vertical flowering interval and a 6 day horizontal flowering interval throughout the growing season (Oosterhuis, 1990). The day of anthesis is a critical event in the overall reproductive development of cotton, where a white flower opens at dawn (Stewart, 1986), pollination occurs approximately between 0700 and 1100 h (Pundir, 1972) and pollen 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 after pollination (Stewart, 1986). Successful *in vivo* pollen tube growth and subsequent fertilization of the ovule is a prerequisite for seed formation in cotton, and seeds with their associated fibers are the basic components of yield. Although events during the progamic phase occur in a relatively consistent manner for cotton, the temperatures encountered either before or during the progamic phase also exert considerable control over the fertilization process (Snider *et al.*, 2009; Oosterhuis and Snider, 2011; Snider *et al.*, 2011b), and can strongly influence yield (Oosterhuis, 2002; Pettigrew, 2008; Oosterhuis and Snider, 2011).

HEAT STRESS AND YIELD

Final yield has 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, 2002). 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° C 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 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 the 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. It is also important to note that a large proportion of the ovules available in a given ovary must be fertilized to ensure boll retention (Stewart, 1986). Consequently, limitations to the fertilization process could be at least partially responsible for poor boll retention under high temperature conditions.

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 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. Similarly, Lewis (2000) 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.

HEAT STRESS AND GAMETOPHYTE DEVELOPMENT

Poor fertilization has been cited as a likely cause of reduced yields and seed production in cotton exposed to high temperature during reproductive development (Pettigrew *et al.*, 2008; Snider *et al.* 2009, 2011b). Although a number of elegant studies have been conducted to specifically identify the most thermosensitive stage of reproduction in other species, no consensus currently exists regarding the most heat-sensitive stage of reproduction leading up to fertilization in cotton. In an early study with cotton, 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. Studies with other species utilizing moderately high temperature exposure at different reproductive stages of development have implicated early pollen development as the most heat-sensitive process in plant sexual reproduction (Peet *et al.*, 1998; Porch and Jahn, 2001; Sato *et al.*, 2002). For example, Peet *et al.* (1998) showed that reproductive output (i.e. fruit and seed production) could be completely abolished when pistils of male sterile tomato plants grown under an optimal day/night temperature regime (28/22° C) were pollinated with pollen that had developed under a high (32/26° C) day/night temperature regime. In contrast, when male sterile pistils exposed to the high day/night temperature regime were pollinated with pollen that had developed under optimal conditions, fruit set and seed per fruit were maintained at 40 and 87% of the levels observed when both pollen and pistils were kept at the optimal growth temperature.

Based on the aforementioned reports and those of other researchers, the meiotic phase of pollen development has been widely regarded as an exceptionally thermosensitive stage of the reproductive process (Ahmed *et al.*, 1992; Porch and Jahn, 2001; Erickson and Markhart, 2002; Pressman *et al.*, 2002; Sato *et al.*, 2002). High temperature exposure during this stage of pollen development can limit fertilization and subsequent seed development by 1) decreasing the number of mature pollen grains available for pollination (Ahmed *et al.*, 1992; Porch and Jahn, 2001; Pressman *et al.*, 2002); 2) causing abnormal pollen development, resulting in decreased viability and germinability of available pollen grains (Ahmed *et al.*, 1992; Porch and Jahn, 2001; Erickson and Markhart, 2002; Sato *et al.*, 2002); and 3) resulting in abnormal anther morphology, thereby limiting anther dehiscence at anthesis (Ahmed *et al.*, 1992; Porch and Jahn, 2001; Erickson and Markhart, 2002; Sato *et al.*, 2002). Under moderately elevated temperature exposure extending from microsporogenesis to anthesis, altered carbohydrate metabolism of developing anthers and pollen grains prevents the accumulation of carbohydrates needed to drive the initial, autotrophic phase of pollen tube growth and accounts for poor pollen viability at anthesis (Aloni *et al.*, 2001; Pressman *et al.*, 2002; Firon *et al.*, 2006; Sato *et al.*, 2006; Jain *et al.*, 2007).

Although the effects of heat stress on male reproductive development have been well documented in a number of species, including cotton (Oosterhuis and Snider, 2011), comparably little is known about the sensitivity of female gametophyte development. Snider *et al.* (2009) reported declines in ovule number and fertilization efficiency when cotton plants were exposed to a 38/20° C temperature regime prior to flowering; the declines in fertilization efficiency observed in that study could have been explained by limitations to *in vivo* pollen performance or by decreased pollen tube guidance due to a higher proportion of defective ovules. For example, Saini *et al.* (1983) reported that exposing wheat plants to high temperature (30° C) for three days during the meiotic stage of pollen and megaspore mother cell meiosis did not alter pollen germinability, but pollen tube guidance to the ovules was prevented due to an increase in ovule abnormalities and a decrease in the proportion of functional ovules.

HEAT STRESS EFFECTS ON ISOLATED POLLEN GRAINS

Many of the available reports investigating the effects of high temperature on plant sexual reproduction have been *in vitro* studies of pollen performance (i.e. pollen germination and pollen tube growth) and have allowed researchers to identify the temperature sensitivity of the fully mature male gametophyte in isolation from either parental or female reproductive tissues. In contrast with vegetative and pistil tissues, mature pollen does not exhibit acquired thermotolerance via a typical heat shock response and is extremely sensitive to high temperature exposure (Frova *et al.*, 1989; van Herpen *et al.*, 1989; Dupuis and Dumas, 1990; Hopf *et al.*, 1992). For example, Dupuis and Dumas (1990) reported that pre-exposure of mature maize pollen to high temperature (40° C) for 4 h prior to pollination abolished *in vitro* fertilization even when pollination was performed on spikelets maintained at 28° C throughout the experiment. In contrast, when spikelets, previously exposed to 40° C for 4 h, were pollinated with unstressed pollen, a 43% fertilization rate was obtained (Dupuis and Dumas, 1990). Because of this study and other reports (reviewed in Zinn *et al.*, 2010; Hedhly *et al.*, 2009), it has been proposed that mature pollen grains on the exposed surface of the stigma would be more sensitive to high temperature than the more deeply seated ovules (Kakani *et al.*, 2005), and

recent studies with cotton have focused on pollen germination and tube growth responses to high temperature using *in vitro* systems (Burke *et al.*, 2004; Kakani *et al.*, 2005; Liu *et al.*, 2006).

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). 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 (Fig. 2). 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*. Recently, Burke (2011) showed that the germinability of otherwise viable cotton pollen exposed to 39° C decreased to ~40% of the levels observed under control temperature conditions (28° C). 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, and negative impacts on the male gametophyte can be expected.

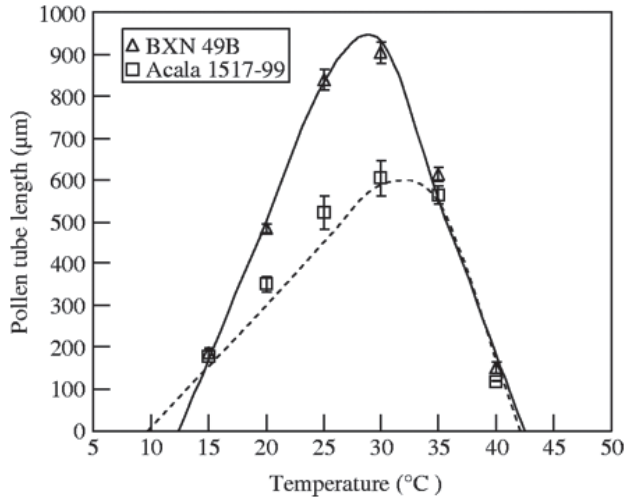

Figure 2: Pollen tube length responses to temperature for two cotton cultivars (BXN 49B and Acala 1517-99). Error bars indicate ± s.e. (Adapted from Kakani *et al.*, 2005)

HEAT STRESS AND POLLEN-PISTIL INTERACTIONS

Although pollen development and function are considered exceptionally heat-sensitive processes, it is also important to note that under natural conditions, high temperature will simultaneously impact male and female reproductive tissues, resulting in a synergistic effect on reproductive output (Reviewed in Zinn *et al.*, 2010; Oosterhuis and Snider, 2011; Snider and Oosterhuis, 2011). Furthermore, it has been reported that *in vivo* pollen performance under high temperature can be modulated by the pistil tissue through which the pollen tubes grow (Pundir, 1972; Hedhly *et al.*, 2005). For example, Pundir (1972) reported that pollen tube growth rates could be increased in *G. hirsutum* (♀) x *G. arboreum* (♂) crosses or slowed in *G. arboreum* (♀) x *G. hirsutum* (♂) crosses, relative to the rates typically obtained by the pollen donor. Given these results, Stewart (1986) suggested that the nutritional or hormonal balance of the transmitting tissue of the style was important for *in vivo* pollen tube growth. Several researchers have since confirmed in a variety of species that a number of complex physical and biochemical pollen-pistil interactions are required for successful pollen tube growth from the stigma to the ovules (Herrero and Arbeloa, 1989; Gonzalez *et al.*, 1996; Herrero and Hormaza, 1996; Lord and Russell, 2002; Lord, 2003). As a result, heat-induced changes in the pistil can be expected to exert considerable control over pollen performance *in vivo*.

Because mature pollen grains lacking a heat shock response and exposed on the stigmatic surface of the pistil are considered more vulnerable to heat stress than the deeply seated ovules, many authors have utilized *in vitro* pollen germination and tube growth assays to screen for heat tolerant genotypes in a number of species (Kakani *et al.*, 2002; Kakani *et al.*, 2005; Liu *et al.*, 2006; Reddy and Kakani, 2007; Salem *et al.*, 2007). However, it has been reported that *in vitro* pollen germination and tube growth responses to high temperature are not necessarily predictive of *in vivo* pollen performance under elevated temperature (Hedhly *et al.*, 2005a; Barrow, 1983; Young *et al.* 2004), and high temperature can also limit pollen germination through loss of stigmatic receptivity (Hedhly *et al.*, 2005b). Furthermore, under field conditions, it was recently reported that the diurnal pattern of pollen tube growth was strongly altered by moderately elevated temperature in *G. hirsutum*, where pollination occurred earlier in the day under higher diurnal temperatures (Snider *et al.*, 2011a; Fig. 3C). Interestingly, pistil and air temperatures were comparable during the estimated time of pollination, and pollen germination was unaffected by high temperature (Snider *et al.*, 2011a; Fig. 3A-B). It is interesting to speculate that temperature-dependent anther dehiscence may have allowed for pollen to be deposited on the stigmatic surface when temperatures were favorable for pollen germination in *G. hirsutum*.

In vivo pollen tube growth is sensitive to high temperature, where above optimal temperatures accelerate tube growth in some species (Buchholz and Blakeslee, 1927; Pasonen *et al.*, 2002; Hedhly *et al.*, 2004) and slow tube growth in others (Gawel and Robacker 1986; Snider *et al.*, 2011a). Given the importance of pollen-pistil interactions in determining successful pollen tube growth to the ovules, biochemical responses of the pistil to high temperature will necessarily influence pollen tube growth and fertilization. For example, Snider *et al.* (2009) reported that heat-induced declines in fertilization efficiency for *G. hirsutum* were associated with increased oxidative stress in the pistil, declines in the soluble carbohydrate and ATP content of the pistil, and decreased subtending leaf photosynthesisunder high temperature (38/20° C). Subsequent investigations have identified biochemical parameters of the pistil and subtending leaf that significantly impact pollen tube growth and fertilization under high temperature in *G. hirsutum*.

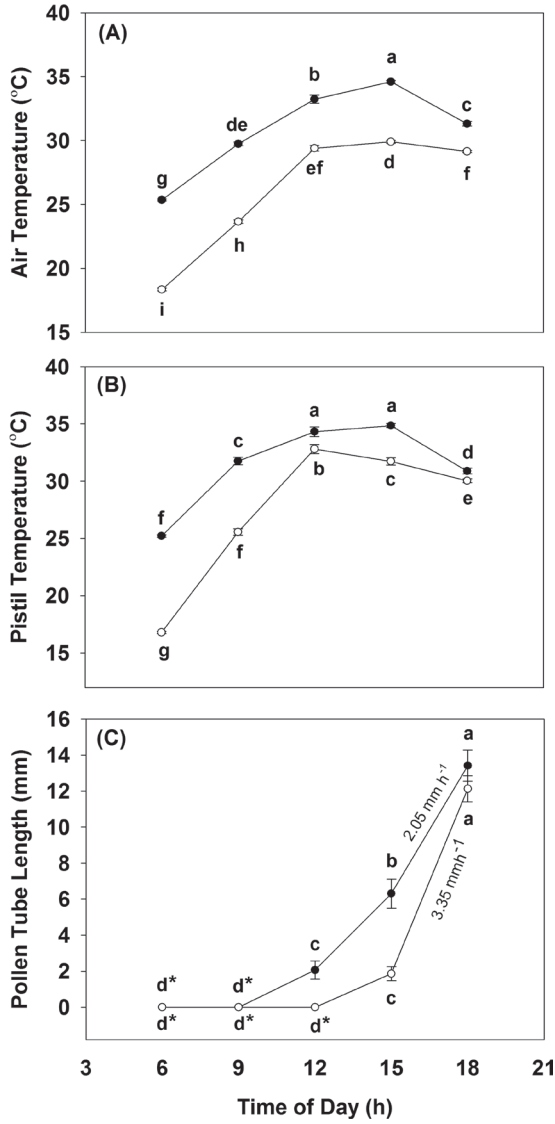

Figure 3: Diurnal air temperature (A) pistil temperature (B) and *in vivo* pollen tube growth (C) under optimal (T_{max} = 29.9° C; open circles) and high (T_{max} = 34.6° C; closed circles) air temperature conditions from 06:00 to 18:00 h in 3 h increments. An asterisk next to a data point indicates that no pollen grains were present on the stigmatic surface at that time of day (pollen tube length = 0). All values are means ± standard error (n = 6), and values not sharing a common letter are significantly different (LSD; $P < 0.05$). Pollen tube growth rates (in mm h^{-1}) under optimal and high temperature conditions are shown adjacent to the corresponding line. (Adapted from Snider *et al.*, 2011a)

The Subtending Leaf

Because the carbohydrate balance of reproductive tissues strongly influences reproductive success in cotton (Zhao *et al.*, 2005; Snider *et al.*, 2009), it is 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 carbohydratecontents 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 was associated with lower soluble carbohydrate and ATP content in the pistil under heat stress and lower 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 temperaturefor quantum efficiency ($T_{15\Phi PSII}$) of VH260 subtending leaves relative to ST4554 subtending leaves (Snider *et al.*, 2010). 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 (Gong *et al.*, 1998; Jiang and Huang, 2001). 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).

Snider *et al.* (2009) recently reported increases in the water soluble calcium concentration and glutathione reductase activity of heat-stressed cotton pistils, but a decline in NOX activity of pistils exposed to high day temperature. 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), as evidenced by higher fertilization efficiencies under high temperature (Fig. 1), 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; Fig. 4). 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).

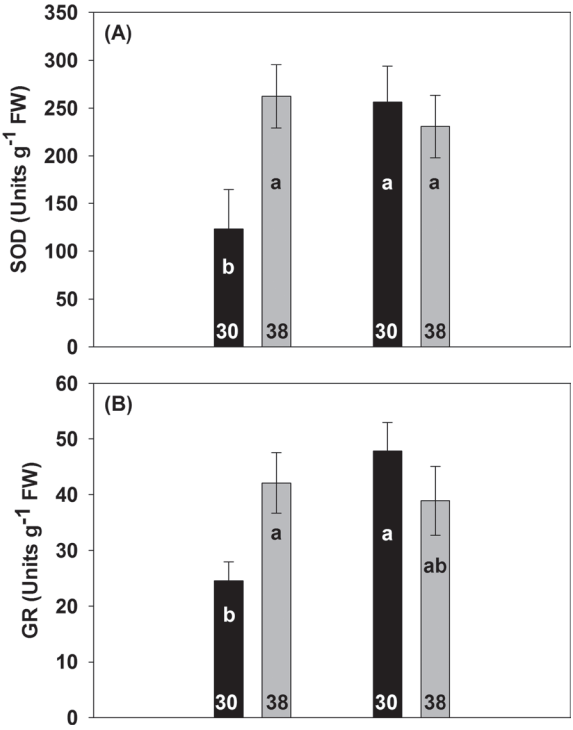

Figure 4: The effect of cultivar and temperature regime on superoxide dismutase (SOD) activity (A), and glutathione reductase (GR) activity (B) in *Gossypium hirsutum* pistils. For VH260, both SOD and GR activities were no different under the 38/20° C day/night temperature regime (gray bars; 38) when compared with the 30/20° C day/night temperature regime (black bars; 30), whereas ST4554 had significantly higher SOD and GR activities under high day temperature when compared with control temperature pistils. All values are means ± standard error (n = 10 for SOD and 16 for GR). Values not sharing a common letter are significantly different (LSD; $P < 0.05$). (Adapted from Snider *et al.*, 2011b)

Carbohydrates and ATP

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). However, 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 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). Because the decline in energy reserves occurred concomitantly with a decline in fertilization efficiency, 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.

Under field conditions and much more moderate high temperature exposure (T_{max} = 34.6° C), diurnal pollen tube growth rates were significantly slowed in *G. hirsutum* pistils (Snider *et al.*, 2011a; Fig. 3) without any alterations pollen germination, or fertilization, suggesting that *in vivo* pollen tube growth may be more sensitive than the other reproductive processes. Subtending leaf photosynthesis, pistil oxidative status, and pistil ATP content (Snider *et al.*, 2011a, 2011c) were also unaffected by the moderately elevated temperatures observed under field conditions. In contrast, high temperature significantly decreased soluble carbohydrate supply in the pistil during pollen tube growth through the style (Snider *et al.*, 2011c; Fig. 5), and pollen tube growth rates were highly correlated (r^2 = 0.932) with the soluble carbohydrate

content of the pistil during pollen tube growth (Fig. 6). It is well established that pollen tube growth transitions from an autotrophic growth phase (utilizing carbohydrate reserves preexisting within the pollen grain at the time of anthesis) to a heterotrophic growth phase (utilizing carbohydrate reserves within the transmitting tissue of the style) (Herrero and Hormaza, 1996; Herrero and Arbeloa, 1989). Given that the energy requirements of actively growing pollen tubes are approximately 10 fold higher than those of vegetative tissues (Tadege and Kuhlemeier, 1997), it is to be expected that heat-induced declines in pistil carbohydrate supply should have a pronounced affect on *in vivo* pollen tube growth. Consequently, the carbohydrate balance of the pistil should be strongly influenced by the moderate temperature increases projected to result from global climate change.

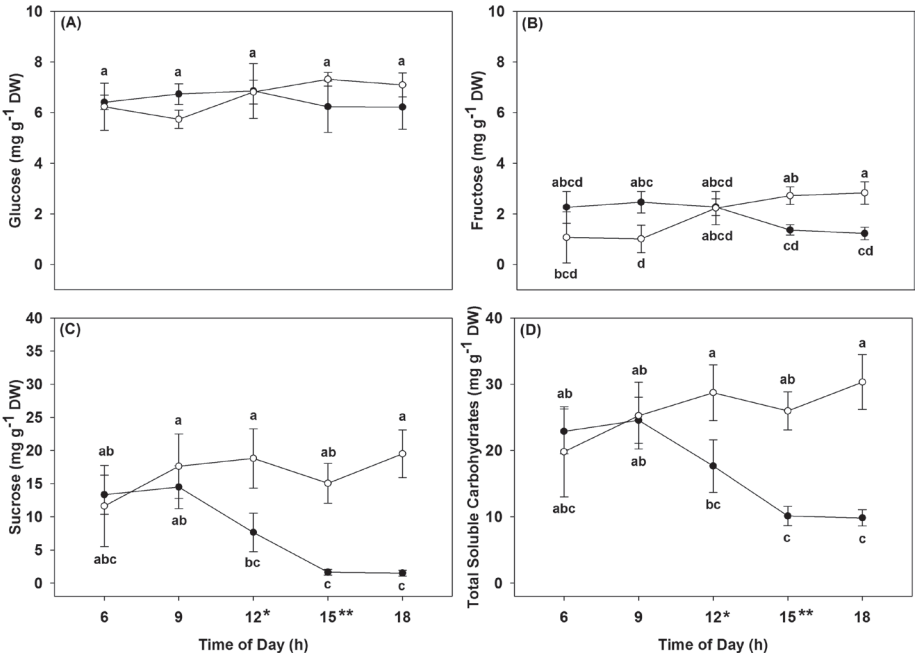

Figure 5: Diurnal levels of glucose (A), fructose (B), sucrose (C), and total soluble carbohydrates (D) for *Gossypium hirsutum* pistils sampled on August 4 (closed circles) and August 14, 2009 (open circles) from 0600 to 1800 h in 3 h increments. All values are means ± standard error (n = 12 for 0600 and 0900 h, 10 for 1200 and 1800 h, and 8 for 1500 h on August 4; n = 12 for 1500 h, 10 for 1800 h, 8 for 0900 and 1200 h, 4 for 0600 h on August 14), and values not sharing a common letter are significantly different (LSD; $P < 0.05$). Single or double asterisks indicate the sample time at which pollen tubes were first observed in the style on August 4 and 14, respectively. (Adapted from Snider *et al.*, 2011c)

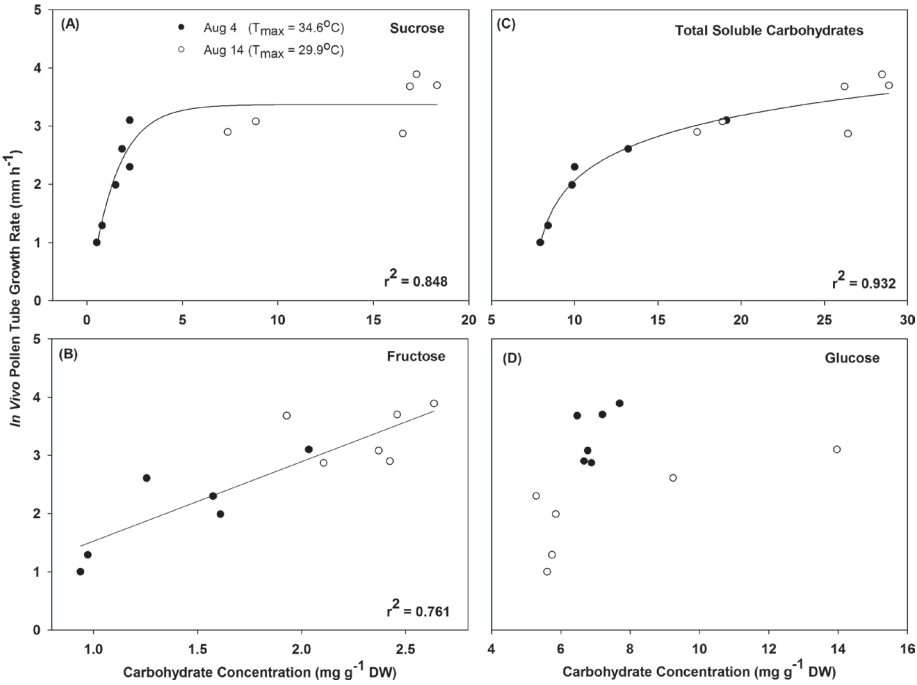

Figure 6: The relationship between pistil sucrose (A), fructose (B), total soluble carbohydrate (C), and glucose (D) concentrations and pollen tube growth rate in *Gossypium hirsutum* pistils sampled on August 4 (closed circles) and August 14, 2009 (open circles) between 1500 and 1800 h. (Adapted from Snider *et al.*, 2011c)

SUMMARY

For a number of plant species, sexual reproduction is substantially more sensitive to heat stress than vegetative processes, resulting in negative implications for the yield of agronomic crops with reproductive structures of economic importance. For example, a negative correlation has been reported between average maximum temperature during flowering and lint yields in cotton. Sexual reproduction in flowering plants occurs in essentially three stages: gametophyte development (from meiosis to pollination), the progamic phase (from pollination to zygote formation), and embryo development (from zygote to seed). For cotton, on the day of anthesis, the progamic phase lasts 12 to 24 h, and a number of events must occur in highly concerted fashion for successful fertilization and lint production to occur. Consequently, poor fertilization and seed set has been proposed as the likely cause of heat-induced yield reductions in cotton.

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. Based on the existing literature for a number of species, high temperature exposure

either during early pollen development or during the progamic phase of pollen development will negatively impact pollen performance and reproductive output, where both phases of pollen development are considered exceptionally sensitive to moderate heat stress. However, moderately elevated temperatures either before or during the progamic phase can limit fertilization by negatively impacting important pollen-pistil interactions required for successful pollen tube growth toward the ovules. In cotton, heat stress (38/20° C) has been shown to limit *in vivo* fertilization concomitant with decreases in subtending leaf photosynthesis, declines in pistil ATP and carbohydrates, increases in oxidative stress in the pistil, and alterations in pistil calcium concentration. Having higher pistil concentrations of ATP and calcium and having elevated pre-stress antioxidant enzyme activity in cotton pistils and subtending leaves has been related to genotypic fertilization thermostability. Under field conditions, diurnal pollen tube growth rate is more sensitive to moderately high temperatures (34.6° C) than either pollen germination or fertilization, where elevated temperature significantly slows pollen tube growth rates. Additionally, the same ambient temperature conditions (34.6° C) significantly decrease the soluble carbohydrate content of the pistil without influencing other biochemical parameters, and pollen tube growth rates are strongly and positively correlated with the soluble carbohydrate content of the pistil ($r^2 = 0.932$). Consequently, the carbohydrate balance of the pistil should be strongly influenced by the moderate temperature increases projected to result from global climate change.

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