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Diurnal pollen tube growth rate is slowed by high temperature in field-grown *Gossypium hirsutum* pistils

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ABSTRACT

For *Gossypium hirsutum* pollination, germination, and pollen tube growth must occur in a highly concerted fashion on the day of flowering for fertilization to occur. Because reproductive success could be influenced by the photosynthetic activity of major source leaves, we hypothesized that increased temperatures under field conditions would limit fertilization by inhibiting diurnal pollen tube growth through the style and decreasing subtending leaf photosynthesis. To address this hypothesis, *G. hirsutum* seeds were sown on different dates to obtain flowers exposed to contrasting ambient temperatures while at the same developmental stage (node 8 above the cotyledons). Collection and measurement were conducted at 06:00, 09:00, 12:00, 15:00, and 18:00 h on August 4 (34.6 °C maximum air temperature) and 14, 2009 (29.9 °C maximum air temperature). Microclimate measurements included photosynthetically active radiation, relative humidity, and air temperature. Pistil measurements included pistil surface temperature, pollen germination, pollen tube growth through the style, fertilization efficiency, fertilized ovule number, and total number of ovules per ovary. Subtending leaf measurements included leaf temperature, photosynthesis, and stomatal conductance. Under high temperatures the first measurable pollen tube growth through the style was observed earlier in the day (12:00 h) than under cooler conditions (15:00 h). Also, high temperature resulted in slower pollen tube growth through the style (2.05 mm h^{-1}) relative to cooler conditions (3.35 mm h^{-1}), but there were no differences in fertilization efficiency, number of fertilized ovules, or ovule number. There was no effect of sampling date on diurnal photosynthetic patterns, where the maximum photosynthetic rate was observed at 12:00 h on both dates. It is concluded that, of the measured physiological and reproductive processes, pollen tube growth rate showed the greatest sensitivity to high temperature under field conditions.

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1. Introduction

The day of anthesis is a critical event in the reproductive development of cotton (*Gossypium hirsutum*). The first flower is produced approximately 8 weeks following plant emergence, and flowers are continually produced by the same plant throughout the growing season due to the indeterminate growth habit of the cotton plant (Oosterhuis, 1990). On the day of anthesis, a white flower opens at dawn (Stewart, 1986) with pollination reported to occur between 07:00 and 11:00 h (Pundir, 1972) and germination within 30 min following pollination (Stewart, 1986). The pollen tube extends through the transmitting tissue of the style and fertilization of the ovule occurs between 12 and 24 h later (Stewart, 1986).

Because a number of reproductive processes must occur in a highly concerted fashion 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). As a consequence, the yield of plant species with reproductive structures of agricultural importance is exceptionally sensitive to high temperature stress during flowering (Sato et al., 2001; Oosterhuis, 2002; Pettigrew, 2008).

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). Recent reviews by Hedhly et al. (2009) and Zinn et al. (2010), have suggested that pollen development and function may be the most thermosensitive reproductive processes to high temperature. For example, chronic high temperature exposure during the meiotic phase of microgametophyte development results in poor pollen germination and seed set in *Sorghum bicolor* (e.g. Jain et al., 2007). Both *in vivo* (Hedhly et al., 2004) and *in vitro* (Burke et al., 2004; Kakani et al., 2005) studies utilizing short-

Abbreviations: PAR, photosynthetically active radiation; PFD, photon flux density; RH, relative humidity; T_{air}, air temperature; T_{leaf}, leaf temperature; T_{pistil}, pistil temperature.

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term high temperature exposure encompassing only the time of pollen germination and pollen tube elongation have shown that both pollen germination and tube growth are strongly influenced by high temperature. Hedhly et al. (2004) reported that high temperatures in excess of the optimum (30 °C) resulted in an increase in the rate of pollen tube growth through the style for *Prunus avium*, but decreased the number of pollen tubes to reach the base of the style. In contrast, using a semi-*in vivo* system in *G. hirsutum*, Gawel and Robacker (1986) reported the highest pollen tube growth rates at 30 °C with declines in pollen tube growth rate observed at temperatures in excess of the optimum. Kakani et al. (2005) reported temperature optima of 31.8 and 28.6 °C for pollen germination and maximum tube length *in vitro*, respectively. A previous *in vitro* study by Burke et al. (2004) showed a comparable optimal temperature range for cotton pollen tube elongation (28–32 °C) but a much broader temperature range for optimal pollen germination (28–37 °C), suggesting pollen germination may not be as sensitive to high temperature as pollen tube growth.

Although the effect of high temperature on male gametophyte development and function have been well documented (reviewed in Hedhly et al., 2009; Zinn et al., 2010), reports on the effects of heat stress on female reproductive development are limited. For example, high temperature can also limit pollen tube guidance to the ovules by increasing ovule abnormalities and decreasing the proportion of functional ovules (Saini et al., 1983). Snider et al. (2009b) showed that exposure of *G. hirsutum* plants to long-term high temperature prior to anthesis decreased ovule number and limited fertilization efficiency, but it was uncertain in this experiment if high temperature more severely compromised male or female reproductive development. To our knowledge, the effect of above-optimal temperature on pollen germination, diurnal pollen tube growth, and fertilization efficiency has not been quantified in a field setting.

Reproductive development and yield are more sensitive to high temperature stress than photosynthesis in a number of plant species (Pettigrew, 2008; Prasad et al., 2008), but recent studies have suggested that the thermostability of major source leaves may correlate with reproductive thermostability by insuring sufficient photosynthate allocation to developing reproductive units under high temperature (Kurek et al., 2007; Snider et al., 2009b, 2010). In cotton, subtending leaves are the primary sources of carbohydrate supplied to subtended bolls (Ashley, 1972). However, high temperature ($T_{leaf} > 35$ °C) limits net photosynthesis by decreasing chlorophyll content (Snider et al., 2009b, 2010), limiting quantum efficiency (Bibi et al., 2008; Snider et al., 2009b, 2010), reducing electron transport (Wise et al., 2004), inactivating Rubisco activase (Salvucci and Crafts-Brandner, 2004), increasing dark respiration (Cowling and Sage, 1998), and increasing photorespiration (Jiao and Grodzinski, 1996).

Although high temperature is known to affect pollen tube growth patterns *in vivo* (Hedhly et al., 2004), studies characterizing the effect of high temperature on diurnal pollen tube growth, pollen germination, and fertilization *in vivo* along with quantification of the physiological status of major source leaves during the period of pollen tube growth are to our knowledge nonexistent. This omission is especially important given the role of pollen–pistil interactions in promoting successful pollen tube growth (Lord, 2003) and the role of major source leaves in producing photosynthate needed for reproductive development (Ashley, 1972; Kurek et al., 2007; Snider et al., 2009b). We hypothesized that increased temperature would limit fertilization efficiency by inhibiting diurnal pollen tube growth and by limiting net photosynthesis of the subtending leaf. The objectives of this study were to measure the effects of increased ambient temperature conditions on (1) diurnal patterns of pistil temperature, *in vivo* pollen tube growth, pollen germination, and fertilization in pistils of field-grown *G. hirsutum* and (2) diurnal patterns of subtending leaf temperature, photosynthesis, and stomatal conductance.

2. Materials and methods

2.1. Plant material and sampling method

To evaluate the effects of high temperature on diurnal pollen tube growth, and subtending leaf photosynthesis, *Gossypium hirsutum* L. (cv. ST4554B2RF) seeds were sown at a density of eight plants per meter in a Captina silt loam (Typic Fragidult) soil at the Arkansas Agricultural Research and Extension Center, Fayetteville AR in 1 m rows. Plots were 4 m × 7 m with 1 m borders between each plot. To maintain well-watered conditions, plants were irrigated to field capacity every 6 days in the absence of saturating rainfall. Fertilizer application, weed control, and insecticide applications were performed according to extension service's recommendations and practices. To insure that flowers and leaves selected for anatomical and physiological measurements would be in the same developmental stage (same first-position main-stem node above the cotyledons), but exposed to different ambient temperature conditions, seeds were planted on three different dates: May 28, June 5, and June 19, 2009. Only pistils collected and leaves measured on August 4 and 14, 2009 (from plants corresponding to the May 28 and June 5 planting dates, respectively) from flowering main-stem node 8 were subsequently used for anatomical and physiological analyses because air temperatures from these dates showed the greatest contrast with minimal differences in other climatological parameters observed (Figs. 1 and 2). For diurnal quantification of pollen tube growth and subtending leaf physiol-

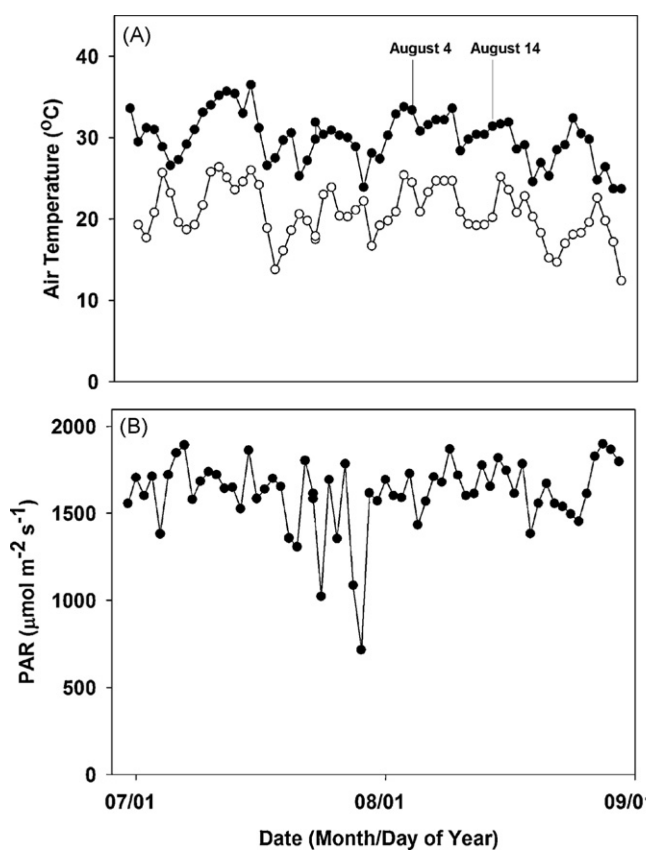

Fig. 1. Daily maximum (closed circles) and minimum (open circles) air temperature (A) and maximum photosynthetically active radiation (PAR; B) from June 30 to August 30, 2009. Diurnal sample dates (August 4 and August 14, 2009) are shown in (A).