Effects of Moderately High Temperature on Diurnal Pollen Tube Growth and Fertilization in Field-Grown Cotton

J.L. Snider¹, D.M. Oosterhuis², and E.M. Kawakami²

RESEARCH PROBLEM

A number of events must occur in a highly concerted fashion during flowering for successful fertilization and seed production to occur. As a result, the yield of crop species with reproductive structures of agronomic importance is substantially more sensitive to environmental stress than the yield of crops with vegetative structures of agricultural importance. High temperature during flowering is known to limit fertilization and lint yield in cotton, but information regarding the temperature sensitivity of key reproductive processes under field conditions during flowering is limited.

BACKGROUND INFORMATION

The day of flowering is a critical event in the reproductive development of cotton. A white flower opens at dawn, pollination occurs within a few hours after flower opening, and pollen germination occurs within 30 minutes 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). Abiotic stress that limits any of the aforementioned processes leading to successful fertilization and seed development will necessarily limit yield since the number of seeds produced and the amount of fiber per seed are the basic components of yield in cotton. Consequently, high temperature has been shown to limit fertilization in cotton (Snider et al., 2009), and a negative correlation has been reported between high temperature during flowering and lint yield (Oosterhuis, 2002).

RESEARCH DESCRIPTION

To evaluate the effects of high temperature on in vivo diurnal pollen tube growth and fertilization, cotton (Gossypium hirsutum L.) cv. ST4554B2RF seeds

¹Research scientist, USDA-ARS, Dale Bumpers Small Farms Research Center, Booneville.
²Distinguished professor and graduate student, respectively. Department of Crop, Soil, and Environmental Sciences, Fayetteville.
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, Ark. in 1-m rows. Seeds were planted on different dates (28 May and 5 June 2009) to obtain flowers at the same developmental stage (i.e. same node) that had developed under different environmental conditions. Only pistils collected on 4 and 14 August 2009 (from plants corresponding to the 28 May and 5 June planting dates, respectively) were subsequently used for anatomical analysis because air temperatures from these dates showed the greatest contrast with minimal differences in other climatological parameters.

Diurnal quantification of air temperature, pistil temperature, and pollen tube growth was performed at five different times throughout the day: 0600, 0900, 1200, 1500, and 1800 h. Air and pistil temperatures were measured using a digital thermometer and a type K thermocouple. Styles from pistils collected at each time of day were fixed in a 3:1 solution of ethanol:acetic acid, cleared and softened in 1M NaOH, and stained in decolorized aniline blue. Pollen tubes were visualized within the style using UV microscopy, pollen tube length was measured in mm, and pollen tube growth rate was expressed in mm h⁻¹. Pollen germination was expressed as a percent and calculated as follows: (number of germinated pollen grains)/(30 pollen grains scored on the stigmatic surface). For fertilization efficiency determination, flowers were collected 24 h after anthesis to allow sufficient time for fertilization to occur (Stewart, 1986) and prepared for UV microscopy as described above. Ovules containing a pollen tube were considered fertilized and fertilization efficiency was calculated as follows: [(number of fertilized ovules per ovary)/(total number of ovules per ovary)] × 100.

RESULTS

Air temperature was significantly higher on 4 August at all sample times throughout the day than on 14 August (Fig. 1A). For example, the maximum air temperatures were recorded at 1500 h and were 34.6 and 29.9 °C on 4 and 14 August, respectively (Fig. 1A). Compared with diurnal air temperatures recorded on 14 August, air temperatures recorded on 4 August ranged from 7.1 °C higher at 0600 h to 2.2 °C higher at 1800 h. There was a significant two-way interaction between time of day and sample date for both pistil temperature (Fig. 1B; \( P < 0.0001 \)) and pollen tube length through the style (Fig. 1C; \( P < 0.0001 \)). Pistil temperature was significantly higher on 4 August at all sample times throughout the day than on 14 August (Fig. 1B). The maximum pistil temperatures observed were recorded at 1500 h and 1200 h on 4 August (34.9 °C) and 14 August (32.8 °C), respectively. Compared with diurnal pistil temperatures recorded on 14 August pistil temperatures recorded on 4 August ranged from 8.4 °C higher at 0600 h to 0.85 °C higher at 1800 h (Fig. 1B).

Figure 1C shows that pollen tubes were first measurable within the style at 1200 h on 4 August and at 1500 h on 14 August. These were also the first of the sample times utilized in this study in which pollen grains were first visible on the
stigmatic surface. Pollen tubes continued to elongate through the style throughout the day, and the final pollen tube lengths observed at 1800 h were statistically indistinguishable at 13.4 and 12.1 mm on 4 and 14 August, respectively (Fig. 1C). Pollen tube growth rate through the style was significantly slower on the warmer sample date (4 August) than on the cooler sample date (14 August), where pollen tube growth rates from 1500 to 1800 h were 2.05 mm h⁻¹ on 4 August and 3.35 mm h⁻¹ on 14 August (Fig. 1C; $P = 0.0058$). In contrast with pollen tube growth, pollen germination on the stigmatic surface was not significantly affected by sample date (Fig. 2A; $P = 0.088$), and fertilization efficiency was unaffected by sample date (Fig. 2B; $P = 0.412$).

DISCUSSION AND PRACTICAL APPLICATION

Because high temperature slowed pollen tube growth rate through the style without declines in pollen germination or ovule fertilization, we conclude that diurnal pollen tube growth rate may be more sensitive than either of these processes to moderately high temperature. Identification of the most heat-sensitive stages of reproduction in cotton is an important first step in developing strategies for mitigating the negative impacts of high temperature on yield.

LITERATURE CITED


Fig. 1. Diurnal air temperature (A) pistil temperature (B) and *in vivo* pollen tube growth (C) on 14 August ($T_{\text{max}} = 29.9 \, ^\circ\text{C}$; open circles) and 4 August ($T_{\text{max}} = 34.6 \, ^\circ\text{C}$; closed circles) 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.
Fig. 2. *In vivo* pollen germination (A) and fertilization efficiency (B) for *Gossypium hirsutum* pistils collected on 4 August (gray bars) and 14 August 2009 (black bars). All values are means ± standard error (n = 6), and values not sharing a common letter are significantly different (Student’s *t*-test; *P* < 0.05).