Effect of High Temperature on Pollen Tube Growth and Energetics in the Cotton Pistil

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RESEARCH PROBLEM AND BACKGROUND INFORMATION

Environmental stress during floral development is a major reason for the disparity between actual and potential yields in crops with valuable reproductive structures (Boyer, 1982). For Arkansas-grown cotton a correlation exists between high temperature and low yield (Oosterhuis, 2002). The day of anthesis is a critical event in reproductive development for *G. hirsutum*. The flower opens as a white flower at dawn; pollination occurs a few hours later, and fertilization of the ovule occurs 12 or more hours later, after successful pollen tube growth through the style has occurred (Stewart, 1986). Therefore, any abiotic stress that inhibits pollen tube growth from the stigma to the ovules on the day of anthesis will decrease reproductive success. Because actively growing pollen tubes have a high energy requirement relative to vegetative tissues (Tadege and Kuhlemeier, 1997), one major type of support provided by the pistil during active pollen tube growth through the style (in the absence of heat stress) is a readily-available supply of carbohydrates (Herrero and Arbeloa, 1989) In *G. hirsutum*, greater than 60% of the total carbohydrate requirement of developing reproductive tissue is provided by adjacent, subtending leaves (Ashley, 1972). Heat stress limits source leaf strength and carbohydrate allocation to developing sinks by decreasing photosynthesis (Bibi et al., 2008), increasing dark respiration (Cowling and Sage, 1998) and photorespiration (Jiao and Grodzinski, 1996), and inhibiting carbohydrate translocation (McNairn, 1972). The first objective of this study was to measure the effect of high day temperature on pollen tube growth, soluble carbohydrate content, and energy levels of the pistil on the day of anthesis; the second objective was to quantify the effect of heat stress on subtending leaf physiology.

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**RESEARCH DESCRIPTION**

Two consecutive experiments were conducted to evaluate the effects of heat stress on reproductive development and source leaf activity in *Gossypium hirsutum* L. Experiments were initiated in January and repeated in April 2008 using the cotton cultivar cv. ST4554 B2RF planted in two-liter pots and placed in two walk-in growth chambers (Model PGW 36; Controlled Environments Limited, Winnipeg, Canada) at the Altheimer Laboratory, Arkansas Agricultural Research and Extension Center, Fayetteville, Ark., under 0/0°C day/night temperature regimes. Plants were grown under a 12/12 hr photoperiod at a 500 μmol m⁻² s⁻¹ photosynthetically active radiation (PAR) and were watered daily with half-strength Hoagland's solution.

At approximately one week prior to flowering, plants were randomly transferred from one growth chamber to the other, and the day temperature in one of the growth chambers was gradually increased at a rate of 2°C/day until a 38/20°C day night temperature regime had been reached. Only flowers and subtending leaves between main stem nodes 5 and 10 in the first fruiting position along a sympodial branch were analyzed. Because there was no significant effect of experiment date on any of the parameters measured, data were pooled from the two consecutive experiments. Pistils used for pollen tube growth analysis were collected 24 h after anthesis and stored in formalin-acetic acid-alcohol (FAA) for future microscopic evaluation. All other pistils were collected at midday (1200 to 1300 h) and stored at -80°C for subsequent ATP analysis or dried at 47°C for three days for soluble carbohydrate determination.

Pollen tubes were observed in ovules using UV microscopy, and pollen performance was expressed as the number of ovules in an ovary containing a clearly distinguishable pollen tube (fertilized ovules) divided by the total number of ovules in each ovary. Soluble carbohydrates were quantified at 340 nm using a microplate reader and glucose assay reagent after all soluble carbohydrates in the sample extract had been enzymatically converted to glucose. ATP content in pistils was quantified using a luminometer and the firefly luciferase assay, where light production by luciferase is directly proportional to the ATP content of the sample. Photosynthesis of subtending leaves was determined using an LI-6200 portable photosynthesis system. Chlorophyll content was estimated spectrophotometrically, and maximum quantum yield was quantified using a portable fluorometer.

**RESULTS**

Pollen tube growth declined significantly under high day temperature. For example, the pollen tube/ovule ratio was 32.9% lower under the 38/20°C day/night temperature regime than under the 30/20°C day/night temperature conditions ($P < 0.0001$; Fig. 1A). High day temperature resulted in significant declines in total soluble carbohydrate and ATP concentrations in cotton pistils. Heat stress resulted in a 20.3% decline in total soluble carbohydrate content (Fig. 1B), and ATP concentrations in pistils exposed to heat stress were approximately 55% lower than in pistils maintained at optimal temperature conditions throughout the sampling period ($P = 0.0003$; Fig. 1C).
High day temperatures resulted in a significant reduction in net photosynthesis of leaves. Net CO$_2$ fixation rates under high day temperature conditions (8.9 μmol CO$_2$ m$^{-2}$ s$^{-1}$) were approximately 16.8% lower than fixation rates (10.7 μmol CO$_2$ m$^{-2}$ s$^{-1}$) observed for subtending leaves grown under optimal day temperature conditions ($P < 0.0001$; Fig. 2A). Total chlorophyll content was also significantly reduced in subtending leaves exposed to heat stress, where heat stressed leaves exhibited a 11.3% reduction in total chlorophyll content relative to the control ($P < 0.0001$; Fig. 2B). Significant declines ($P < 0.0001$) in maximum quantum yield (3.83%; Fig. 2C) were observed for leaves exposed to high day temperature relative to optimal day temperature conditions.

**DISCUSSION AND PRACTICAL APPLICATION**

Decreased *in vivo* pollen tube growth was a major cause of low reproductive success, as evidenced by the decline in the pollen tube/ovule ratio (Fig. 1A). Lower carbon fixation rates by heat-stressed subtending leaves (Fig. 2) decreased source strength and reduced soluble carbohydrate allocation to developing flowers as evidenced by lower soluble carbohydrate contents in heat stressed pistils relative to pistils exposed to optimal day temperatures (Fig. 1B). Because pollen tube growth declined concomitantly with carbohydrate and ATP content in cotton pistils (Figs. 1A-C), we conclude that the energy requirements of growing pollen tubes cannot be sufficiently met under heat stress. Our conclusions are substantiated by the fact that *Arabidopsis* mutants exhibiting thermostable photosynthesis also yield a much higher number of seed under heat stress than thermosensitive variants (Kurek et al., 2007). These findings suggest that selection of cultivars with greater thermotolerance of the photosynthetic apparatus might be an important method for decreasing heat stress-induced year to year variability in yield.

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**LITERATURE CITED**


Fig. 1. Pollen tube/ovule ratio, soluble carbohydrate, and ATP contents of *G. hirsutum* pistils exposed to high (38/20°C) day temperatures. All values are means ± standard error (n = 15), and values sharing a common letter are significantly different (Student’s t-test; P < 0.05).
Fig. 2. Photosynthesis, chlorophyll content, and quantum yield of *G. hirsutum* leaves under normal (30/20°C) and high (38/20°C) day temperature regimes. All values are means ± standard error (n = 42), and values sharing a common letter are significantly different (Student's t-test; P < 0.05).