

AGRONOMY & SOILS

The Impact of Variable, Cold Minimum Temperatures on Boll Retention, Boll Growth, and Yield Recovery of Cotton

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ABSTRACT

A previous field experiment reported that cotton grown in the tropical dry season, where minimum temperatures during flowering can be cold ($< 10^{\circ}\text{C}$), yielded a greater proportion of cotton from bolls pollinated when minima were warmer. The retention and growth of bolls was postulated to be reduced by cold night temperatures. However, biotic stresses (e.g., pests) might have confounded this response. Reported herein is a pot experiment where biotic stresses were effectively removed. Over two seasons, (i) ambient and (ii) ambient plus 5 to 6°C (range $10\text{--}24^{\circ}\text{C}$), night thermal conditions were imposed from 1 wk prior to first flower to 2 wk after last effective flower. Day temperatures were the same. Average ambient minimum temperature for the treatment period was 12.6°C (range $5.9\text{--}21.1^{\circ}\text{C}$) and 10.2°C (range $2.6\text{--}21.0^{\circ}\text{C}$) in 2003 and 2004. Flowers were damaged by low ambient minimum temperatures occurring near anthesis, which led to shedding or lower seed number per boll which reduced boll size. The latter could be due to poor pollination and competition for assimilates. Shedding was correlated ($p < 0.01$) with minimum temperature at anthesis with $< 40\%$ survival when minima were $< 6^{\circ}\text{C}$. Seed cotton yield was the same. It was concluded that the yield recovery from variable, low minimum temperatures during flowering was (1) similar to where damage to fruit was biotic with compensation occurring on later flowering fruiting sites provided temperatures were warm;

and (2) unlikely to be impacted by damage to photosynthetic apparatus due to the minimum temperatures observed here.

Minimum temperatures below the lowest reported base temperature for cotton development of 12°C (Constable and Shaw, 1988) during flowering might be detrimental to boll retention and growth. In tropical northwestern Australia, growing irrigated cotton during the dry or winter season (lat. 13 to 17°S) is being evaluated because key insect pests and high temperatures during flowering are avoided. However, the photothermal pattern of the dry season is the reverse of the wet (summer) season and that of spring-sown cotton in temperate latitudes. Cold night temperatures are possible mid season and high temperatures are likely early and late in the season (Yeates et al., 2010a).

Field experiments in the tropical winter season found a reduced retention of bolls and a lower contribution to cotton yield from flowers when anthesis coincided with the greatest frequency of cool minima ($< 12^{\circ}\text{C}$) (Yeates et al., 2010a). In these experiments, cool minima occurred early in flowering; hence flowers on the first position on sympodia nearer the bottom of the plant were most affected. However, these crops also produced high lint yields ($2,064$ kg lint/ha) due to an increased contribution from later flowers, which occurred on upper and outer fruiting sites of the plant when temperatures warmed (Yeates et al., 2010a), and a larger biomass (Yeates et al., 2010b). In a field environment it was difficult to determine whether cool minimum temperatures were a factor in the early fruit loss as other possible causes such as insect pests, water logging, and nutrient stress can occur. Moreover, in the field there was no “warm night” control for comparison.

Less is known about the impact of cold minimum temperatures during flowering and boll growth of cotton than on early growth (Cothren, 1999; Mauney, 1986). In controlled environment studies where fixed temperatures were used, there was a quadratic response of boll weight to temperature with the optimum occurring at a minimum of approximately

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22°C (mean of 25°C) with a rapid fall in boll weight when night temperature was below 16°C or above 25°C (Gipson and Ray, 1970; Hesketh and Low, 1968). Hesketh and Low (1968) also found boll retention was correlated with minimum temperature, although square retention was more tolerant to cooler temperatures than bolls.

Controlled environment research might not be completely transferable to field-grown crops because most published studies of cotton have been conducted with fixed day and/or night temperature regimes (e.g., Gipson and Joham, 1968; Hesketh and Low, 1968; McDowell et al., 2007; Reddy et al., 1991, 1992). Moreover, many of these studies have been run for a set calendar-day period and, hence, have not allowed for developmental thermal time differences between treatments when measuring boll weights in response to temperature. For example, Gipson and Joham (1968) measured the effect of night temperatures from 10°C to 26.5°C on boll growth for the first 45 d of boll growth. Reddy et al. (1991) used five different fixed minimum and maximum temperatures, the coldest being 10-20°C, to measure boll weight 49 d after flowering. Not surprisingly boll weights under cool temperatures were less at the time of measurement in both studies.

Low minimum temperatures can reduce boll growth and retention due to: (1) lower net photosynthesis following reduced growth and respiration at night, which suppresses sink demand and inhibits the export of starch from leaves (Warner et al., 1995), although this response can be negated when acclimation to low temperatures occurs (Singh et al., 2005); (2) permanent injury to photosynthetic apparatus (Berry and Bjokman, 1980); (3) prevention of pollen germination and tube growth, where the minimum that stops growth or germination depended on genotype and ranged from 9.8 to 20.1°C (Kakani et al., 2005; Stewart, 1986).

The climate of the tropical dry season adds a further dimension to mid-season cold temperature effects because temperatures at sowing are hot then fall until flowering when the coldest minima occur; temperatures then rise rapidly during late boll fill as the days become longer (Yeates et al., 2010a). The positive correlation between yield and fruit on the upper and outer fruiting sites of the plant measured in field experiments (Yeates et al., 2010a) suggest climatic conditions were more favorable later in crop growth. Fruit removal studies elsewhere have found

that yield compensation might occur in cotton where climatic conditions become more favorable following removal (Kennedy et al., 1986; Pettigrew et al., 1992; Wilson et al., 2003). Minimum temperatures are also variable during the dry season and there are not extended periods of extreme cold (Cook and Russell, 1983). Hence, it is possible greater boll retention and growth might occur during periods when minima are warm, which also can occur early in flowering.

The objective of these experiments was to measure the effects of variable, cold minimum temperatures (averaging 10-12°C) during flowering on cotton fruit growth, fruit retention, fruiting dynamics (compensation), and yield, while minimizing confounding effects such as insects and disease.

MATERIALS AND METHODS

The experiments were performed at the Katherine Research Station, 4 km east of Katherine (14°28' S, 132°18' E), Northern Territory, Australia. Due to greater distance from the ocean, Katherine has a higher probability of lower dry season minimum temperatures than Ord River, Western Australia (15°39' S, 128°43' E), where previous field experiments were located (Yeates et al., 2010a). Katherine also has similar maximum temperatures, photoperiod, and monthly solar radiation as Ord River (Cook and Russell, 1983). The project was conducted over two seasons with sowing occurring on 28 April 2003 and 30 April 2004.

Minimum temperature treatments were created by removing plants to a glasshouse at night during the flowering phase ("warm" plants) and comparing these with plants grown at ambient temperatures (i.e., outside) at night during flowering ("cool" plants). During the treatment period all plants were grown at ambient temperatures and solar radiation during the day.

The timing of plant growth stages and treatment period are shown in Table 1. To ensure plants were exposed to the same minimum temperatures prior to flowering, all plants were grown outside until 6 and 7 d prior to first flower in 2003 and 2004, respectively. The treatment period then commenced and the warm-night plants were removed to the glasshouse at night for the next 60 and 53 d in 2003 and 2004, respectively; that is at a minimum of 15 d after flowering was completed. The warm plants were then returned outside at night until maturity.

Table 1. Dates of plant growth stages and the timing of the treatment period.

Growth Stage	2003	2004
Sow	30 April	30 April
1 st Square	5 June	3 June
Start treatment period	23 June	21 June
1 st Flower	29 June	28 June
<u>Last effective flower</u>		
Warm	24 July	22 July
Cool	29 July	29 July
End treatment period	20 August	13 August
<u>Maturity</u>		
Warm	26 September	21 September
Cool	30 September	7 October

Glasshouse minimum temperatures were maintained approximately 5°C above ambient to ensure similar daily variation in minimum temperature to plants grown at ambient temperatures. The only exceptions were when ambient temperatures were < 6°C; on these nights glasshouse temperatures were not permitted to fall below 10.3°C. When ambient temperatures were warm, glasshouse minimum temperatures did not exceed 24°C. The glasshouse used adjustable roof vents to regulate night temperatures.

In each year, three groups of two night temperature treatments (cool and warm) were grown, each group was physically separated. Cotton was grown in 17-L square pots (20-cm sides) containing one plant per pot. Within a group, a temperature treatment consisted of eight pots arranged in two rows of four, each on its own trolley with the edges of the pots separated by ≤ 1 cm. The groups were aligned in an east-to-west direction to ensure even light interception, as the sun was most northerly in the winter months, and to account for a dry easterly breeze that is common during the day during June to September. Temperature treatments were randomized within each group. The base of the pots were 15 cm above the ground, 1 m separated each group, and there was a 1.5-m walkway between trolleys within a group. The walkway ensured that the treatment on the north side of each group did not shade the southern treatment. Shade cloth (80% radiation reduction) was placed on the north side of the trolleys to reduce solar heating of the pots. The cloth was perpendicular to the ground from the surface to 3 cm above the lip of the pots with a 5-cm gap to allow air flow. The soil surface below the pots was covered with

sabi (*Urochloa mosambiensis* (Hack.) Dandy), dry weight approximately 3 t/ha, killed 1 wk prior to sowing, and mulched with a flail mower.

Four seeds of the Bt transgenic cultivar Siokra V-16B (containing the Monsanto Cry1Ac and Cry2Ab proteins) were sown per pot and thinned to one per plot 15 d later. This cultivar produced consistently high yields in cultivar comparisons made over the previous 6 yr in the dry season at Ord River (S.J. Yeates, unpublished data).

Movement in and out of the glasshouse was timed so plants were exposed to a temperature change of at most 2°C. Accordingly, plants were moved outside on the coldest mornings approximately 45 min after sunrise and returned 30 min prior to sunset. The same east-west direction of the trolleys was maintained at night. For the period when plants were inside after sunrise, 50% of ambient, photosynthetically active radiation passed through the glasshouse. The average conditions of each treatment (Fig. 1) were calculated using a model of potential photosynthesis (Constable, 1986). The additional time inside the glasshouse accounted for less than 3% of daily photosynthesis because of cooler temperatures after sunrise. Within a trolley, pots were rotated clockwise weekly, from 15 d after sowing until approximately 1 month prior to picking as there was risk of damaging the plants and large bolls when moved. Temperatures were recorded (Tinytag Plus™, TGP-0050, Gemini Data Loggers, Chichester, West Sussex, UK; www.gemindataloggers.com) half hourly at canopy height inside and outside the glasshouse with a logger also placed on a trolley. The glasshouse and trolley loggers were placed in an ACS50 Stevenson Screen (Gemini Data Loggers) and the outside logger was placed inside a standard Stevenson Screen.

To determine the location within the glasshouse where temperature was least variable between the three groups of pots prior to commencement of the experiment, spatial variation in night temperature was logged within the glasshouse. Sensors were placed at 1-m separation in each direction from the center of the glasshouse at plant height. Plants were located within the middle section where temperatures varied less than 0.5°C and were at least 2 m from the walls. The most uniform area to locate the groups of pots was ± 3 m east-west and ± 1.5 m north-south of the center of the glasshouse.

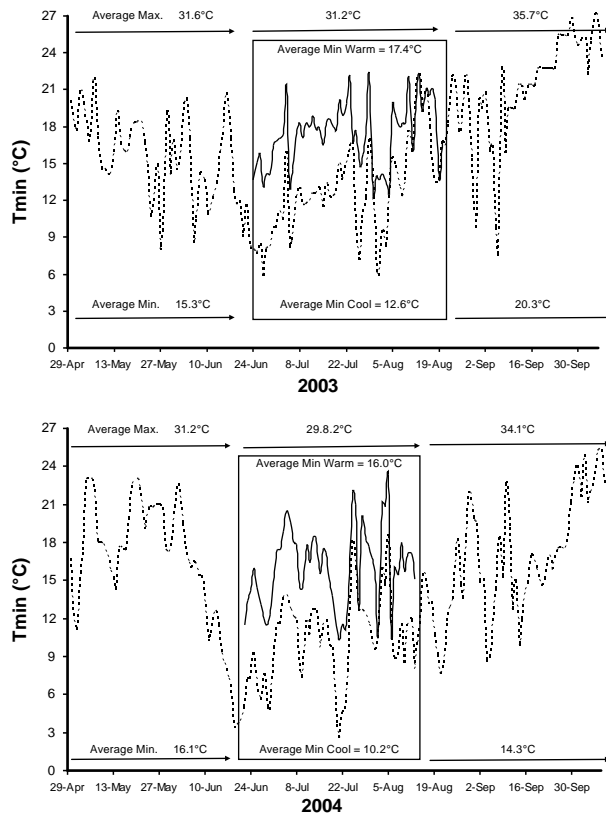


Figure 1. Observed daily minimum and average maximum temperatures; the box indicates when the temperature treatments were imposed and the average minimum for this period. Broken and solid lines are ambient and glasshouse minimum temperatures respectively. Arrows show the period before, during and after the treatments were imposed; average maximum and minimum temperature for each period is shown above the arrows.

Each pot was irrigated at 1000 h by a single 2-L/h emitter until the drainage occurred from exit holes at the base. From 14 d after first flower the plants were watered twice daily, at 1000 h and 1500 h. The potting mix consisted of a mixture of sand, peat, and vermiculite at a ratio by volume of 6:5:1, respectively. Slow release fertilizer 45 g Ozmocote™ + Moeco™ 10 g/pot (i.e., 8.4 g N, 1.8 g P, 5.1 g K, 1.1 g S, 0.5 g Mg, 0.1 g Ca, 0.2 g Fe, 9 mg Zn, 1 mg each of Cu, B, Mn, Mo) was incorporated with the potting mix. Aquasol™ liquid fertilizer 0.8 g was applied to the soil surface of each pot in 250 ml of water on three occasions 14 d apart in June and July each year (i.e., 184 mg N, 32 mg P, 144 mg K, 0.4 mg Zn, 0.48 mg Cu, 0.01 mg Mo, 1.2 mg Mn, 0.5 mg Fe, and 0.1 mg B per application).

Insect damage was prevented by: (1) the location at Katherine, which is not a cotton production area, so many cotton specific pests were not present or present in low numbers due to the winter growing season; (2) scouting twice weekly for insect pests and

applying insecticide when required; and (3) growing of Bt cotton, which in field studies provided excellent protection against the main local pests *Helicoverpa armigera* (Hübner), *Helicoverpa punctigera* (Wallengren), *Earias huegeli* Rog., and *Earias vittella* (Fab.) (Annells and Strickland, 2003; Yeates et al., 2010a).

Measurements. The plants were mapped for fruit retention weekly, from early squaring to the start of the treatment period, then twice weekly from first flower to the end of flowering, then weekly to maturity. Fruit were characterized as squares, white flowers, pink flowers, and bolls. Fruit position on the plant was also recorded, where for each sympodia, P1, P2, and P3 are first, second, and third fruiting positions laterally from the main stem, respectively. Monopodia fruit were recorded for each node. The anthesis date was recorded for each fruit from the mapping data, where a white flower denoted anthesis today, red flower denoted anthesis 1 d ago, and a small boll (< 5 mm) with a withered petal denoted anthesis 2 d ago. These characterizations were based on experience from previous experiments in the tropical winter season (S. J. Yeates, unpublished data) and observations from spring-sown crops at temperate latitudes (Hake et al., 1996). It was observed that when minimum temperatures were < 10°C for at least three nights, the red flower stage lasted 1 d longer (2 d total) than when minimum temperatures were > 10°C; hence, in estimating the day of anthesis for red flowers, a 2-d period and withered petals at 3 d was recorded. At maturity, fruit retention, seed cotton weights, seed cotton weight per boll, and number of bolls and seeds were measured for each fruiting position on all plants.

All plant data were combined for the eight plants on each trolley. The only exception was the calculation of the proportion of flowers surviving to maturity on each day of the flowering period. To have a sufficient number of flowers to calculate survival, all plants in the three trolleys of each temperature treatment ($n = 24$) were used and a 3-d moving average was calculated for each anthesis date. The relationship between flower survival and minimum temperature was then tested using the moving 3-d average for each variable.

Statistical analysis. Analysis of variance was made using Genstat, (Release 14.2; Lawes Agricultural Trust, IACR, Rothamsted, UK). The three groups of two trolleys can be regarded as three replicates, where the treatment provided to the warm trolleys was to remove them to a glasshouse for specific periods during the experiment. Running the experiment over

2 yr provided replication. Because there were observations for different parts of the same plants and we were looking for whether the 'warm' treatment was greater than the 'cool' treatment for certain parts, the temperature x plant-part interaction was calculated to find out when warm > cool. Two separate analysis were made: (1) the variables that were measured for the whole plants (e.g., seed cotton weight), were separated into the values measured from all P1, P2, P3 (sympodia), and MP (monopodia); and (2) the same variables measured from P1 fruit on individual main-stem nodes (5 to 14). The analysis of variance was structured as follows: *Whole plant data*: "Year" is difference between the years (1 d.f.); "Time x Group" is the difference between the three groups of two trolleys within each year, 2 d.f. for each year gives 4 d.f. "Temp" is the overall difference between cool and warm (1 d.f.); the "Year x Temp" interaction, that is, whether the magnitude of the difference between cool and warm is significantly different between years (1 d.f.); and "Residual" (4 d.f.). *Within plant data*: "Position" is the factor in the analysis for plant positions, the interaction of interest being "Temp X Position" (3 d.f.), that is, are the differences between warm and cool the same for all positions. The analysis description and philosophy for the P1 data measured on individual fruiting branches are the same as for whole plant analyses, except that there were 10 nodes rather than four plant positions and the "Year x Temp x Node" interaction (9 d.f.) was of most interest.

Regression analysis using Statistical Analysis System (SAS, 2001) was used for the relationships between temperature and boll survival, and between seed cotton weight and seed number or boll number. Regression coefficients calculated between the latter three variables were tested for homogeneity. The correlations between yield components were from the combined bolls harvested at each node at each plant position.

RESULTS

Observed Temperatures. During the treatment period, ambient minimum temperatures (cool plants) averaged 12.6°C (range 5.9-21.1°C) and 10.2°C (range 2.6-20.1°C) in 2003 and 2004, respectively (Fig. 1). The minimum temperatures in the glass-house for the warm plants averaged at least 4.8°C higher during the treatment period, 17.4°C (range 12.3-23.4°C) and 16.0°C (range 10.3-23.4°C) in 2003 and 2004. Warm plants were also exposed to

average minimum temperatures that were at least 1 to 5°C (Fig. 1) above the reported base for cotton development of 12 to 15.5°C (Dippenaar et al., 1990; Hearn and Constable, 1984; Mauney, 1986; Viator et al., 2005). The number of ambient minima below 12°C during the treatment period was 22 and 38 compared with 0 and 8 for the warm plants in 2003 and 2004, respectively. Temperatures increased following the treatment period (Fig. 1).

Ambient minimum temperatures were cold for the first 10 d of the treatment period, which coincided with early flowering. The average minimum for the first 10 d of the treatment period in 2003 was 8.8°C and 15.4°C, and in 2004 was 6.8°C and 13.4°C for the cool and warm plants, respectively.

Fruit Retention at Start of the Treatment Period. All plants were gown outside in ambient conditions until the start of the treatment period and plant mapping confirmed there was no significant ($p < 0.05$) difference between the treatments in fruit retention. Square retention was high for all treatments: 98.3% and 98.8% in 2003 and 2004, respectively. There was also no significant difference in total square number, or their location on the plant, or the average node number for the uppermost fruiting branch P1, P2, and P3 squares, which was 12, 10, and 7, respectively, for both years.

Yield and Boll Position on Plant. Surprisingly there was no seed cotton yield difference ($p < 0.05$) between the cool and warm plants in both seasons (Table 2, Fig. 2). However, temperature significantly ($p < 0.05$) changed the contribution of boll number and weight per boll to the yield (Table 2); the cool plants producing more bolls, whereas the warm plants had heavier bolls (Fig. 2). This was because the cool plants produced yield on different fruiting positions than the warm plants. The temperature x position (within the plant) interaction was highly significant for seed cotton weight, boll number, and weight per boll (Table 2) and the means are shown in Fig. 2. The year x temperature x position was not significant for these variables. The temperature x position interaction was significant and reflected the differences in average temperature between the seasons (Fig. 1). For the warm plants, P1 bolls were the major contributor to seed cotton yield (46 %), whereas for cool plants the contribution of P1 bolls was significantly less (30%). The greatest contribution to yield on cool plants came from fruiting positions other than P1 or P2, that is, P3 and MP+ (monopodia + fourth

position + adventitious bolls). The P3 and MP+ bolls accounted for 47% of seed cotton yield on the cool plants compared with 28% for the warm plants. The majority of the MP+ yield comprised monopodia bolls with the proportion of total seed cotton yield as adventitious and P4 bolls being 3% in the warm treatment and 6% in the cool treatment.

Table 2. Analysis of variance for seed cotton yield, boll number, and boll weight. Variables calculated for all P1, P2, P3, and MP+ positions within plants. F values in parentheses when $p > 0.0001$. Where *, **, * is $p < 0.05$, $p < 0.01$ and $p < 0.0001$ respectively. Means are shown in Fig. 2.**

Source of variation	Variable		
	Seed Cotton	Boll No.	Boll Weight
Temperature	ns (0.243)	** (0.003)	** (0.005)
Year x Temp.	ns (0.968)	ns (0.824)	ns (0.918)
Boll Position	***	***	* (0.033)
Year x Position	***	***	ns (0.051)
Temp. x Position	***	***	** (0.001)
Year x Temp. x Position	ns (0.767)	ns (0.431)	ns (0.825)

The warm plants produced more P1 bolls that were heavier than the cool plants. The significantly higher seed cotton yield from P3 and MP+ bolls for the cool treatment were due to greater boll number (Fig. 2). Boll weight was generally less for the cool treatment at all fruiting positions. Although there was no significant difference in the total yield from P2 bolls between the temperature treatments (Fig. 2), the warm plants produced more seed cotton on nodes 5 to 8 but less seed cotton on nodes 9 to 13 that flowered later (data not presented).

Anthesis Date for Different Fruit Positions and Yield Accumulation. As expected, due to the temperature difference between the treatments, the median anthesis date at the same fruiting position became greater as the treatment period progressed. Approximately 14 d after minimum temperature differences were imposed (6 July), the delay in flowering was small; hence, there was only a 0- to 2-d delay in flowering of P1 bolls on the lowest four nodes (Fig. 3). By late in July, the same fruiting position flowered 4 to 7 d later on the cool treatment. For the cool treatment, many of the P3 + bolls (P3 + monopodia + P4 + adventitious) flowered after 18 July and at least 45% of the monopodia yield was produced on bolls that flowered after 26 July compared with no monopodia yield after this date on the warm treatments (Fig. 3).

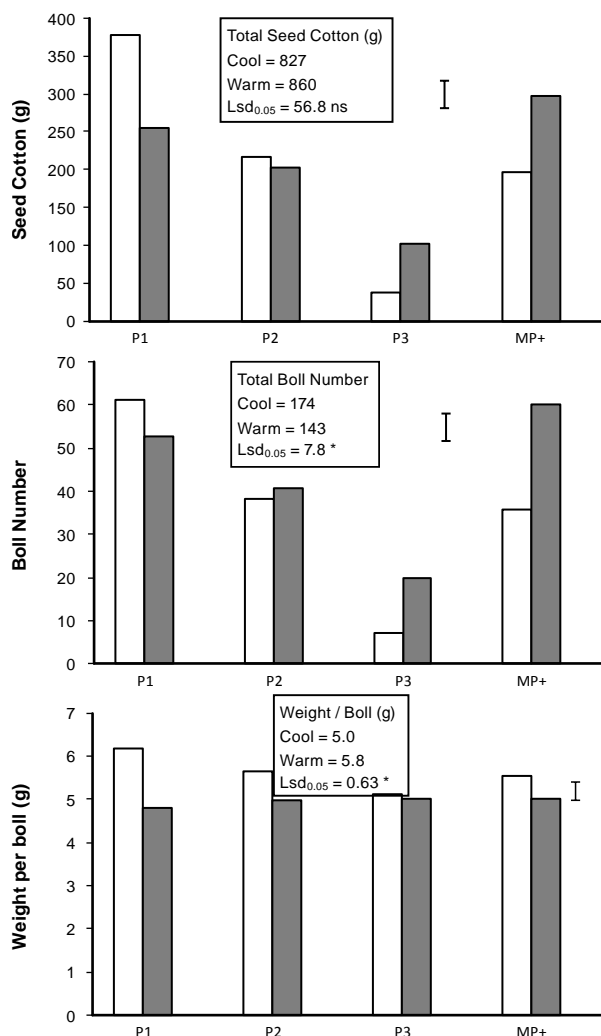


Figure 2. Temperature effect on total seed cotton, boll number, and weight per boll (per 8 plants), and the temperature x plant position interaction for these variables on all P1, P2, P3, and MP+ (monopodia + P4 + adventitious) bolls. Open bars are warm nights, filled bars are cool nights, and error bar is lsd_{0.05}.

All adventitious flowers producing seed cotton were located on nodes 8 to 11 and flowered on the same day as the P2 bolls at that node. Mature P4 bolls were only produced on the cool treatment on nodes 5 to 7 with their anthesis date coinciding with the P1 flowers on nodes 12 to 14.

Flowers pollinated after 18 July contributed 60 to 70% of yield in the cool treatment compared with 15 to 30% of yield for the warm treatment (Fig. 4) and these flowers were on fruiting sites on the outside of the plant (Fig. 3). The slower accumulation of yield on the cool plants between 18 July and 25 July in 2004 coincided with much lower minima over the period, 7.8°C compared with 14.2°C in 2003 (Fig. 4), despite similar fruiting positions flowering at this time in both seasons (Fig. 3).

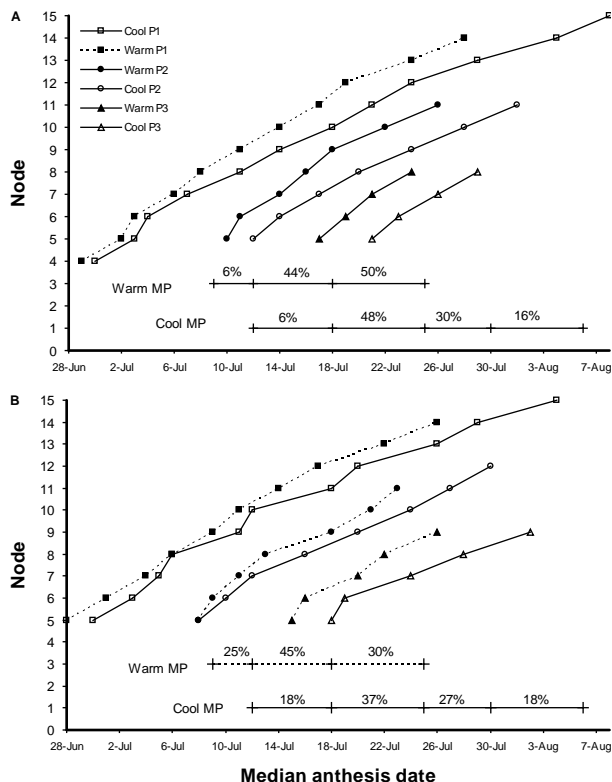


Figure 3. The median anthesis date for P1, P2, and P3 flowers. Horizontal lines show the range of anthesis dates for monopodial bolls (MP) and the percentage of the mature MP bolls that flowered within these dates. For A) 2003 and B) 2004. NB the range in anthesis dates for any fruiting position was ± 2 to 8 d from the median.

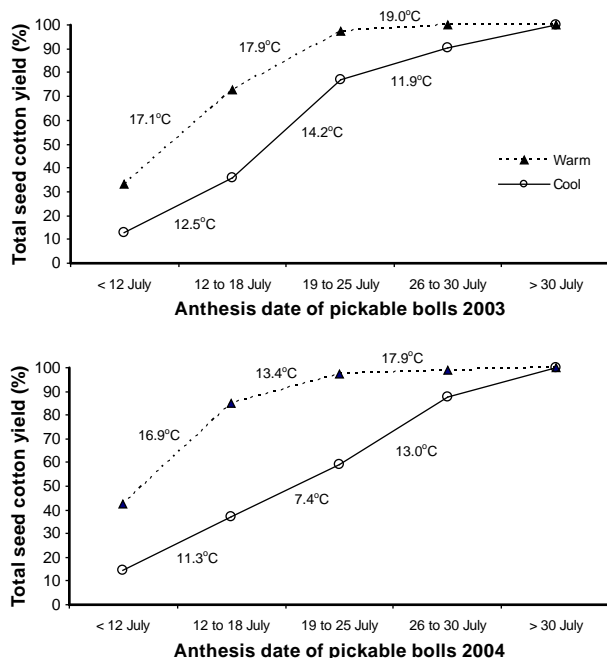


Figure 4. The accumulation of seed cotton yield by the anthesis date of pickable bolls. Average minimum temperatures for each range of anthesis dates up to 30 July is also shown.

P1 Yield and Its Components per Fruiting Branch Node. Seed cotton yield, boll number, and weight per boll. Whether minimum temperature impacted P1 yield on different nodes each season was of interest. The year x node x temperature interaction was significant for P1 seed cotton yield and boll number (Table 3); means are shown in Fig. 5. Node and temperature x node interaction were significant for seed cotton weight, boll number, and weight per boll (Table 3). The temperature main effect is the difference in total P1 bolls as shown in Fig. 2 except that seed cotton weight is calculated per node in Table 3. The P1 seed cotton yield at each node was significantly less on the cool plants for nodes 6 to 11 in 2003, and 5 to 12 in 2004 (Fig. 5). However, the contribution of boll number and weight per boll to P1 yield was different between nodes (Fig. 5). In 2003, P1 boll weight was significantly less on nodes 6, 9, 10, and 11 of the cool plants, whereas on node 7, boll number and weight were significantly reduced. After node 13, greater boll number and weight contributed to a higher P1 seed cotton yield in the cool treatment. In 2004, boll number explained P1 seed cotton yield differences on nodes 10 and above with a mixture of boll number and boll weight contributing to higher P1 seed cotton yield from nodes 5 to 9 on the warm plants. Examples of differences in P1 boll size due to minimum temperature are shown in Fig. 6.

Table 3. Analysis of variance for P1 seed cotton yield, boll number, and boll weight from each node. F values in parentheses when $p > 0.0001$. Where *, **, *** is $p < 0.05$, $p < 0.01$ and $p < 0.0001$ respectively. Means are shown in Fig. 5.

Source of variation	Seed Cotton	Boll No.	Boll Weight
Temperature	** (0.004)	ns (0.083)	***
Year x Temp.	ns (0.533)	ns (0.290)	ns (0.522)
Node	***	***	* (0.021)
Year x Node	ns (0.336)	ns (0.973)	** (0.005)
Temp x Node	***	***	* (0.033)
Year x Temp x Node	* (0.043)	* (0.011)	ns (0.067)

Comparison of early flowers when treatments overlapped for date of flowering. As there was little difference in the median flowering date between the temperature treatments for P1 bolls on nodes 5 to 7 and 5 to 8 in 2003 and 2004 (Fig. 3), these bolls were exposed to the same daily pattern of temperature changes early in growth. Flowering occurred prior to distal-position flowers on these nodes, thus minimizing competition for assimilate soon after flowering. The P1 seed cotton

yield at these nodes was significantly reduced on the cool plants in both years, the only exception being node 5 in 2003 (Fig. 5). In 2004, the weight per boll and boll number was reduced, whereas in 2003, lower weight per boll contributed most to yield differences at node 6 and boll number and weight per boll were significantly lower on the cool plants at node 7.

These differences in P1 seed cotton yield on each node could be explained by the minimum temperature at flowering (Figs. 5, 6 and 7). In 2003, there was no P1 yield difference between the warm and cool treatments at node 5 and flowering coincided with higher ambient minimum temperatures on 4 July. The lower P1 yield on the cool treatment on nodes 6 and 7 was associated with lower temperatures near anthesis, that is 10°C on 5 and 6 July. These temperatures occurred

soon after anthesis at node 6 and boll weight was less in the cool treatment, whereas for node 7, the median anthesis date coincided with these cold temperatures and boll number and weight were reduced. In 2004, P1 flowering of the cool treatment on nodes 5, 6, and 7 coincided with minimum temperatures $\leq 9^{\circ}\text{C}$ and boll number was significantly less than the warm treatment at these nodes. Warmer temperatures $> 12^{\circ}\text{C}$ on the later flowering cohort of bolls from 4 to 7 July would explain the greater boll weight on node 6. These warmer temperatures also coincided with pollination of most surviving P1 flowers at node 8 and boll survival was equal to the warm treatment. After 7 July minimum ambient temperatures were $< 8^{\circ}\text{C}$ and weight per boll at node 8 was significantly less compared with the warm treatment.

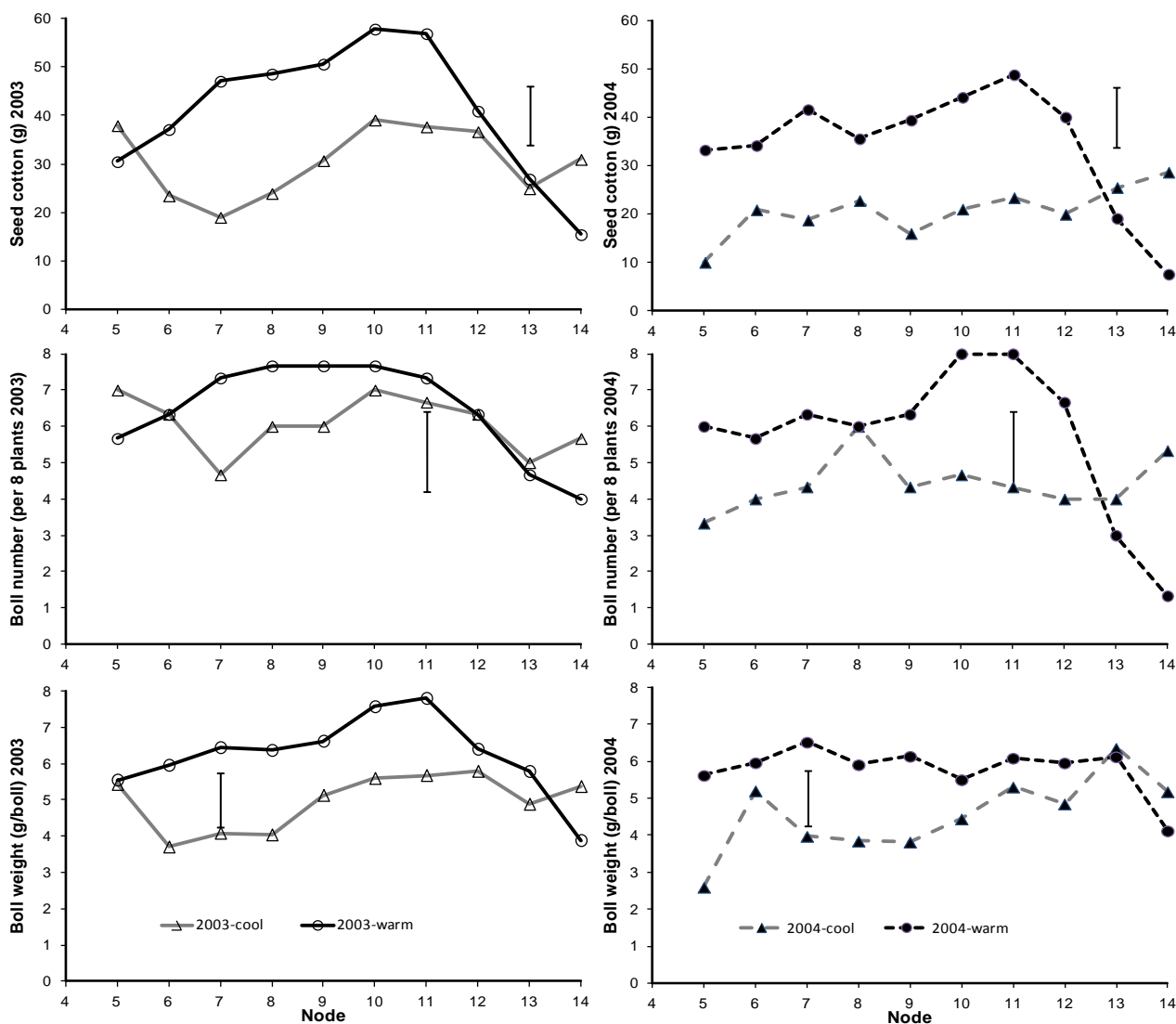


Figure 5. Seed cotton weight, boll number, and average boll weight on the first position (P1) sympodium flower at each main-stem node; Open bars are warm nights, filled bars are cool nights, the bar is the $Lsd_{0.05}$. The interaction between these factors and temperature and year is shown.



Figure 6. Top: A P1 boll damaged by low ambient minimum temperature. Bottom: the adjacent P2 boll flowered in warmer temperatures+.



Figure 7. Warm treatment plants showing large P1 bolls in the lower canopy.

Seed number. Seed cotton yield was best explained by seed number ($r^2 \geq 0.97$) (Table 4). The relationship between seed number and seed cotton yield appears not to be affected by minimum temperature. Testing for homogeneity found no significant ($p < 0.05$) difference in slopes of the fitted lines between the P1 bolls exposed to warm or cool minimum temperatures or the inclusion of bolls from non-P1 positions (e.g., P2 and P3). This contrasted with the relationship between boll number and seed cotton yield where the regression coefficient with seed cotton yield was lower when minima were cool and slopes of fitted lines were

significantly different. The regression coefficient between boll number and seed number was less than the relationship either component had with seed cotton yield, although the slopes of the regression lines were not significantly different.

Effect of Minimum Temperature on Flower Survival. The survival of flowers to become harvestable bolls was significantly ($p < 0.01$) correlated with minimum temperature near anthesis (Fig. 8). The relationship was curvilinear with a rapid fall in flower survival when minima were $< 11^\circ\text{C}$, with survival of between 20 and 40% of flowers when minimum temperatures were $< 6^\circ\text{C}$ near anthesis.

Table 4. The regression coefficients and fitted linear relationships between seed cotton weight (Sc) and seed number (Sn) or boll number (Bn), for P1 bolls and all fruiting positions. All regression coefficients were highly significant ($p < 0.001$); n = the sample number; standard errors of slope and intercept are in parentheses.

Fruiting Sites	Sc v Bn		Sc v Sn		Bn v Sn	
	r^2	function	r^2	Function	r^2	function
All - P1 (n=49)	0.89	$y = 6.28x - 3.47$ (0.352) (1.92)	0.98	$y = 0.193x + 0.94$ (0.0045) (0.719)	0.84	$y = 0.026x + 1.3$ (0.0019) (0.295)
Cool - P1 (n=26)	0.86	$y = 5.2x - 1.30$ (0.473) (2.33)	0.97	$y = 0.198x + 0.57$ (0.0075) (0.911)	0.79	$y = 0.031x + 1.2$ (0.0039) (0.474)
Warm - P1 (n=23)	0.94	$y = 6.54x - 1.83$ (0.355) (2.10)	0.98	$y = 0.193x + 0.88$ (0.0068) (1.291)	0.84	$y = 0.027x + 0.7$ (0.0019) (0.371)
All fruiting positions (n=117)	0.90	$y = 5.79x - 1.61$ (0.172) (0.84)	0.98	$y = 0.191x + 0.28$ (0.0032) (0.455)	0.85	$y = 0.030x + 0.71$ (0.0011)(0.162)

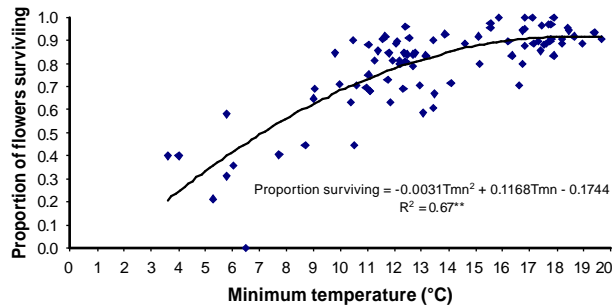


Figure 8. The effect of minimum temperature (T_{mn}) near anthesis on the proportion of flowers from all fruiting sites surviving to produce pickable bolls. Where minimum temperature was calculated as the average for the day of anthesis plus 2 d.

DISCUSSION

These experiments indicate that low minimum temperatures during early boll growth and not biotic causes such as pests, contributed to the reduced yield from early first-position flowers observed in the tropical winter season (Yeates et al., 2010a). The ambient minimum temperatures observed during flowering were below those observed in the experiments of Yeates et al. (2010a). Hence, these experiments also demonstrate that full yield recovery from daily minima $< 6^{\circ}\text{C}$ during flowering was possible provided such minima are episodic.

Yield compensation from cold minima during flowering occurred via two processes. First, early in flowering plants capitalized on periods of warmer minima by retaining more flowers (Fig 8). Second, the general increase in temperature and radiation later in flowering that typifies the tropical dry season (Yeates et al., 2010a), favored the retention and growth of later pollinated flowers (Fig. 5). When temperatures are warmer early in flowering, boll retention would be higher, and later fruiting sites would not have been produced or their flowers shed because of competition for assimilate from the earlier pollinated bolls (Hearn, 1994). This was the case for the warm night temperature treatment in these experiments.

The full-yield recovery from low minimum temperatures observed in these experiments was similar to the recovery reported when fruit removal was biotic (i.e., insect pests, real and simulated) (Holman and Oosterhuis, 1999; Jones et al., 1996; Kennedy et al., 1986; Kletter and Wallach, 1982; Lei and Gaff, 2003; Pettigrew et al., 1992; Wilson et al., 2003). These studies and our experiments demonstrated lost fruits were replaced with later flowering fruit

from other sites, with recovery greatest when temperatures remained favorable for boll growth later in the growing season. However, a key difference between our experiments and the aforementioned experiments was that damage to fruit was abiotic and vegetative organs were also exposed to the low minimum temperatures.

The full-yield recovery suggests that any damage to photosynthetic apparatus by the low minimum temperatures observed was minor. This is consistent with controlled temperature studies by McDowell et al. (2007), where fixed minimum temperatures from 5 to 10°C for two and 20 nights, respectively, did not reduce photosynthetic capacity. Only a 2°C minimum for two nights of 16 h duration caused permanent damage to photosynthetic apparatus. In our study, the lowest recorded temperature was 2.6°C , however, being an ambient temperature this minimum was only sustained for the hour prior to sunrise.

For the warm treatment we were able to generate minimum temperatures during flowering that were less detrimental to early fruit retention or growth than when temperatures were cool (ambient). This was shown by the higher contribution to yield by P1 and P2 bolls on warm plants (Fig. 2). There was greater than 70% yield from P1 and P2 bolls on the warm plants, which was similar to that reported for cotton sown in spring at temperate latitudes and having similar minimum temperatures (Heitholt, 1993; Mauney, 1986).

Seed number per boll reflects the number of ovules and their fertilization efficiency (Stewart, 1986). Cold night temperatures are likely to have reduced seed number in two ways. First, by preventing ovule fertilization, as many of the minimum temperatures observed during flowering were below 11.1 and 9.8°C , the lowest temperatures where pollen germination and tube growth was prevented in *in vitro* studies (Kakani et al., 2005). Second, by reducing assimilate supply to ovules. Net photosynthesis also could have been lower during cold minimum temperatures (Burke et al., 1988; Hearn and Constable, 1984; Lu et al., 1997; Reddy et al., 1992), and combined with competition from other bolls, the number of surviving ovules per boll would have been reduced. For example, the lower weight per boll from the flowers on distal fruiting sites and on monopodia in the cool treatment, despite greater boll numbers (Fig. 5), indicates the weight per boll was reduced by competition for assimilates. Hence extreme cold,

which could prevent pollination, combined with lower assimilate supply to individual flowers could explain the low flower survival to maturity when minimum temperatures were $\leq 6^{\circ}\text{C}$ (Fig. 8).

The delay in maturity due to cool minima (Table 1) was due to the combined impact of slower heat unit accumulation and the extended flowering period required for compensation from boll shedding and damage. This delay was 16 d in the coolest season (2004) when damage to early fruit was greatest. The implications for crop management will be broadly similar to when compensation from insect pest damage occurs. First, the period of crop water use will be longer, which could require additional irrigation. However, unlike pest damage, cool temperatures will also reduce evaporative demand, so any additional water requirement might be less. Further research is required to measure water use of crops compensating for cool temperatures. Second, achieving a balance between yield compensation via fruiting sites toward the top and outside of the plant and appropriate vegetative growth will require careful management. Over use of growth regulators or insufficient irrigation or nutrient deficiency will inhibit compensatory growth and reduce yield. On the other hand, ample supply of water and/or nutrients combined with an insufficient amount of growth regulator could lead to excessive or rank vegetative growth. Concurrent growth regulator research has shown that avoidance of high doses of mepiquat chloride during fruiting site production is essential to permit compensation via the production of additional fruiting sites (Yeates et al., 2002). Third, in regions where late season pests can occur later, flowering delays to maturity might increase pest control costs.

CONCLUSIONS

Low minimum temperatures during early flowering ($< 9^{\circ}\text{C}$) contributed to a reduced proportion of yield from early first-position fruiting sites. Flowers produced on these sites were more likely to be shed or produce smaller bolls. Yield recovery from variable, low minimum temperatures during flowering was: (1) enhanced by plants retaining more flowers during periods when minima were higher; (2) similar to where damage to fruit was biotic, with compensation occurring on later flowering fruiting sites provided temperatures were warm; and (3) unlikely to be impacted by damage to photosynthetic apparatus

due to observed minimum temperatures.

ACKNOWLEDGMENTS

This work was supported by the Australian Cotton Catchment Communities CRC. We also thank the NT Department of Resources for the provision of the glasshouse at Katherine Research Station and Dr. Greg Constable, CSIRO, in simulating potential photosynthesis to determine optimum time to remove the plants from the glasshouse and for helpful suggestions to the document. This work forms part of a PhD study by the senior author and the contribution of Professor Shu Fukai of the University of Queensland is acknowledged.

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