Cold shock at 10 °C for 10 and 20 nights does not reduce cotton tissue viability

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Abstract

Cold shock at temperatures below 11 °C was previously believed to delay cotton development. This paper tested whether exposure to 10 °C for 10 and 20 nights affected tissue viability. Experiments were conducted using plants grown in the glasshouse at the 14-node stage and plants grown outdoors at the 7-node stage. Simple tissue viability tests using tetrazolium (TTC) and relative electrical conductivity (REC) were conducted following treatments. Leaf photosynthesis and chlorophyll fluorescence were also measured to determine whether there were changes in photosynthetic function. Plants treated with cold shock at 10 °C for 10 and 20 nights did not show any significant impact on tissue viability or cell membrane integrity in the TTC and REC tests, respectively. Photosynthesis and chlorophyll fluorescence levels fell slightly in the 20 night treatment compared to the control, but recovered quickly outdoors at the end of the 20 nights. There was no evidence in the data that exposure to 10 °C for 10 and 20 nights will reduce cotton tissue viability or plant growth.

Introduction

Temperature is the dominant factor affecting yield and development of cotton (*Gossypium hirsutum* L). The variability of the Australian climate often accounts for difficulties in crop establishment and development, with events of extreme cold likely to occur at the start or end of the growing season. Low temperatures after sowing slow cotton development. The potential problem of cold shock has been accentuated in recent years due to the geographical expansion of the industry.

Early work identified cold shock (or chilling injury) as being a further factor affecting growth rates of cotton (Constable and Shaw 1988). Cold shock was defined as being an event where the minimum daily temperature falls to or below 11 °C. Each event (one night at below 11 °C) reduces the plants' growth and development regardless of the maximum temperature reached the following day, and also extends duration to flowering by 5.2 day degrees (Constable and Shaw 1988). The definition of cold shock was derived from the day degree function by re-analysis of data from a field experiment. Hence, the temperature threshold has never been experimentally ascertained. Furthermore, this work was done in the 1970s using different cultivars to those used today.

More recent studies have also suggested that there is less impact on cotton following cold exposure than previously thought. A study showed that short duration (night) exposure to cold temperatures had little impact on growth up to flowering, and that effects were only found to occur after treatment at 10 °C for 10 consecutive nights and at 5 °C for 5 nights (Bange and Milroy 2004). The base temperature in cotton is also most likely closer to 15 °C rather than 12 °C (Bange and Milroy 2001). The use of cold shock in estimating development when using a base temperature of 12°C would most likely compensate for inaccuracies in the base temperature in the past.

This paper presents the results of an investigation into the effects of cold temperatures on the viability of cotton tissue. It builds on previous studies, aiming to improve the sensitivity within the window where cold shock is thought to occur (around 11 °C), and to quantify the effects of cold shock on growth and development. The hypothesis tested was that exposure to 10 °C for 10 and 20 nights does not affect tissue viability; and that this lack of impact of cold on cotton growth and development is because cold treatment is not affecting tissue function and survival. A viability test with 2,3,5-triphenyl tetrazolium chloride (TTC) was used to detect the existence of cold shock.

Materials and methods

The experiment used glasshouse and cold room facilities at the Australian Cotton Research Institute, Narrabri (30° 12' S and 149° 36' E). Two experiments were conducted. Experiment 1 was conducted in the glasshouse and used plants that were of the 14-node stage. Experiment 2 was conducted outdoors on plants at the 7-node stage. Experiment 1 had 40 pots, with 13 plants per treatment. Experiment 2 had 51 plants with 17 per treatment. Seedlings of cultivar Sicala V3i were planted in late 2003 and thinned out to one plant per pot in 9 L, 250 mm diameter pots. Pots were filled with soil (Grey Vertosol) from nearby fields. Drip irrigation was set up and plants were fertilised with Miracle Gro® (an all purpose fertiliser) when required.

Each experiment was a completely randomised block design with 2 factors (developmental stage of the plant and cold shock treatments). Within each experiment, pots were evenly and randomly allocated to one of three cold shock treatments. The cold shock treatments were: 10 °C at 10 nights, 10 °C at 20 nights (hereafter known as 10 night and 20 night treatments) and a control. During the cold shock treatment, plants were placed into the cool room overnight. Cold shock duration was for 16 hours. Artificial lights were used to ensure consistent day length (approximately 12 hours). Plants were taken and returned to glasshouse/outdoors after each night.

Data collection

Within each treatment, 5 plants were designated as plants for destructive sampling and a separate 4 as plants for non-destructive measurements. Plants for destructive sampling were those used for tests where leaves had to be removed and tested at the end of treatment (tetrazolium and relative electrical conductivity). Plants for non-destructive sampling were for the continual measurement of

photosynthesis and chlorophyll fluorescence. For both sets of measurements, a leaf at the same node position on each plant was used.

Climate data

Air and soil temperature and atmospheric humidity was measured frequently for each treatment, using data loggers (Gemini Data Loggers® Pty., 'Tiny Tag Plus'). Measurements were taken every half hour.

Relative electrical conductivity

Leaves from the cold treated and control plants were removed for relative electrical conductivity (REC) testing after the appropriate treatment, that is, after the 10 and 20 night treatments (Table 1). REC is measured to indicate the level of plasma membrane integrity. A high REC is indicative of structural damage. Five leaf discs were cut from each leaf and submerged in 10 mL of distilled water. Contents were shaken and allowed to stand for 30 minutes. Electrical conductivity (EC) was measured using a calibrated conductivity meter (TD Scan3). The same tubes were then autoclaved at 120°C at a pressure of 103kPa for 15 minutes to achieve total electrolyte leakage. Once cooled, EC was measured again and relative electrical conductivity (REC) calculated to give the final measurement of damage. REC is the initial EC divided by final EC.

Tetrazolium viability test

The test is based on the presence of respiratory enzymes in the leaf mitochondria which reduce the tetrazolium (TTC) salt to a red product, formazin. If there is tissue damage following cold shock, these enzymes are destroyed and tissue will not stain as red. The level of redness (or optical density) was measured using a spectrophotometer. Leaves from cold treated and control plants were also removed for testing after appropriate treatment (Table 1). 'Pre-treatment' tests were also done. Two leaf discs were cut from each leaf and submerged in a glass test tube of 0.8% TTC solution in phosphate buffer (pH 7.4). The contents of each were then vacuum-infiltrated to ensure thorough uptake of TTC into the leaf. Tubes were then stored overnight in complete darkness. Following this, leaf discs were removed, rinsed three times with water then submerged in ethanol (95%) and incubated again in the dark overnight. Ethanol extracts the red formazin from the leaf. The absorbance or optical density of the ethanol substance was measured at 530nm using a double beam spectrophotometer (Smart Spec 3000), with 95% ethanol as the blank.

Table 1. Timing of data collection for the tetrazolium viability tests and relative electrical conductivity measurements. ✔ - Data was collected on this day., X – Data was not collected on this day.

	Treatment		
Day	Control	10 night treatment	20 night treatment
0	✔	✔	✔
10	X	✔	X
20	X	X	✔

Photosynthesis and chlorophyll fluorescence

Measurements were taken using an infra-red gas analyser (IRGA) Portable Photosynthesis System; Li-COR® model 6400-40, with a Leaf Chamber Fluorometer attachment. Measurements taken two to three times a week, before the treatment was imposed, during treatment and after treatment until the plants have recovered.

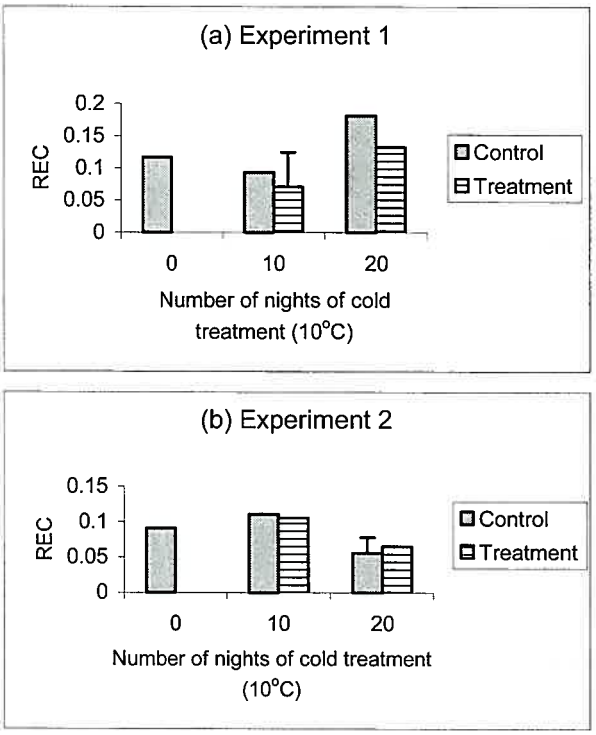
Data for TTC viability tests and REC were analysed using an analysis of variance (ANOVA) with Genstat 7th Edition. The photosynthesis and chlorophyll fluorescence readings were analysed using REML (Residual Maximum Likelihood) with correlated error terms, with Genstat 7th Edition.

Results

Relative electrical conductivity

In both Experiments 1 and 2, the results for REC showed no significant difference between the 10 and 20 night treated plants and the corresponding controls following cold treatment (Figures 1a and 1b). Hence, there is little evidence of structural damage in leaf cellular membrane.

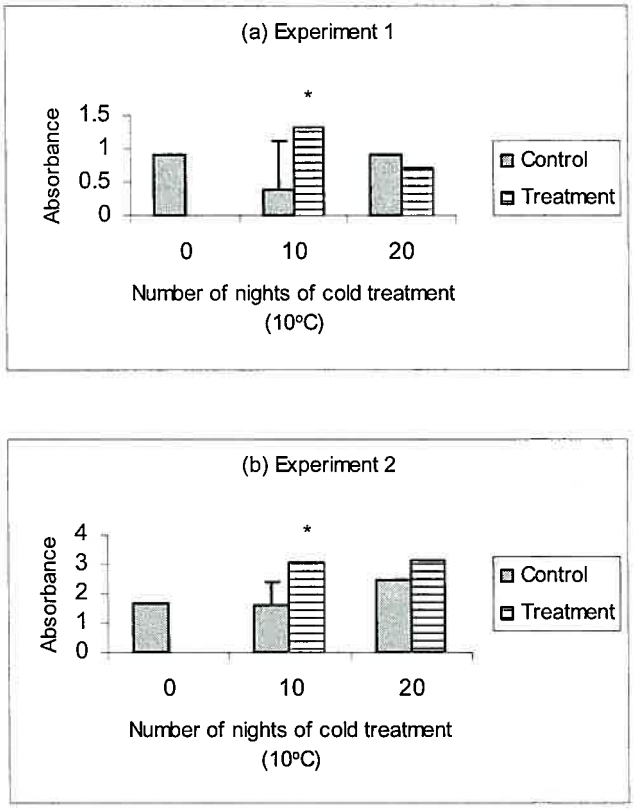
Figure 1. Effect of cold shock treatment at 10 °C for 10 and 20 nights on the relative electrical conductivity (REC) levels of cold treated and control plants for (a) Experiment 1 and (b) Experiment 2. Vertical lines indicate lsd values at P = 0.05.



Tetrazolium viability tests

In both Experiments 1 and 2, absorbance levels for the 10 night treated plants were slightly higher ($P<0.05$) than the control (Figure 2a and 2b). However, this can be disregarded as absorbance levels in the treated plants were only slightly higher than the controls and thus were not damaged by cold shock. Following cold treatment for 20 nights, there was no significant reduction in tissue viability in both experiments.

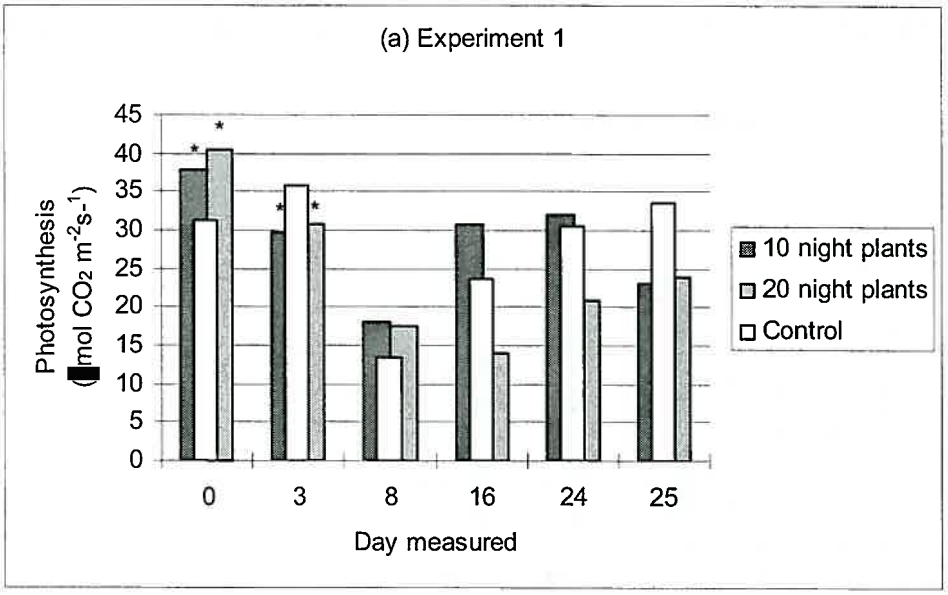
Figure 2. Effect of cold shock treatment at 10 °C for 10 and 20 nights, on the absorbance levels of cold treated and control plants in (a) Experiment 1 and (b) Experiment 2. Vertical lines indicate lsd values at P = 0.05. * indicates treatments which are significantly different (P<0.05) from the control measured on the same day.

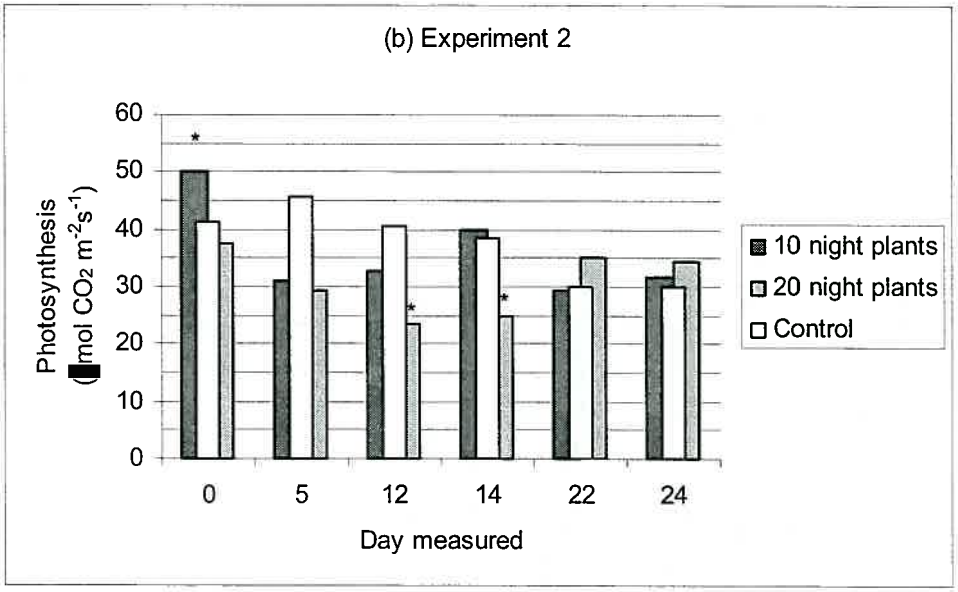


Photosynthesis

In Experiment 1, photosynthetic rates of the 10 night and 20 night plants were slightly reduced (P<0.05) on day 3 of treatment compared to the control plants (Figure 3a). Following this, there were neither significant differences nor any consistent trend for the photosynthetic rates in the cold treated plants to be lower than the control. In Experiment 2, photosynthetic rates of plants in the 20 night treatment were slightly reduced (P<0.05) on days 12 and 14, compared to corresponding controls (Figure 3b). In both experiments, such effects appeared to be temporary as the plants recovered to a consistent rate that did not differ significantly from the controls at the end of the cold shock treatments.

Figure 3. Leaf photosynthesis (μmol $CO_2m^{-2}s^{-1}$) of the control, 10 night and 20 night treatments at 10 °C, measured using an infra-red gas analyser (IRGA) in (a) Experiment 1 and (b) Experiment 2. * identifies treatments which are significantly different ($P<0.05$) from the control measured on the same day. Note that day number zero refers to the day on which the first measurement was taken, which was the day before cold treatments were first imposed. Days were subsequently numbered after this.



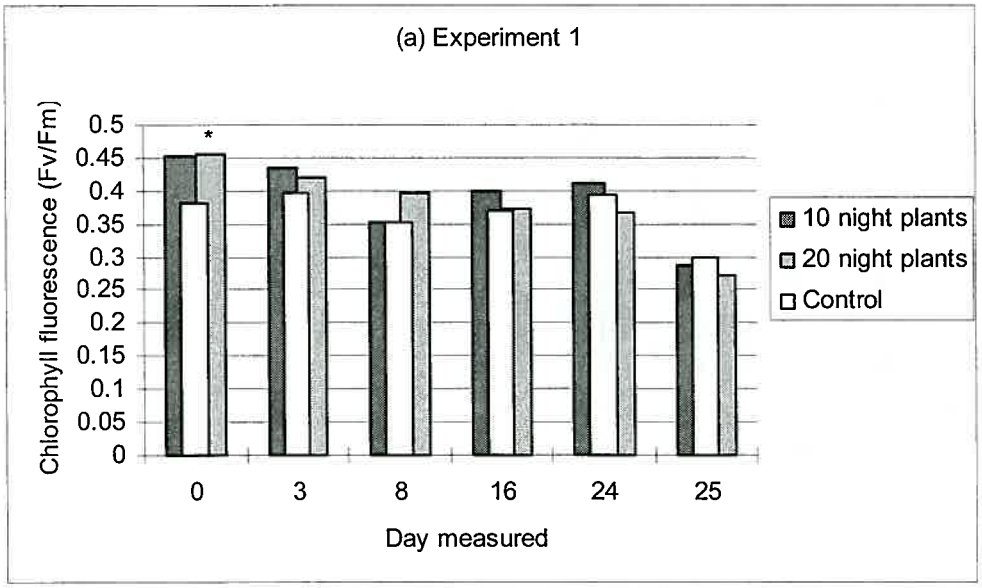


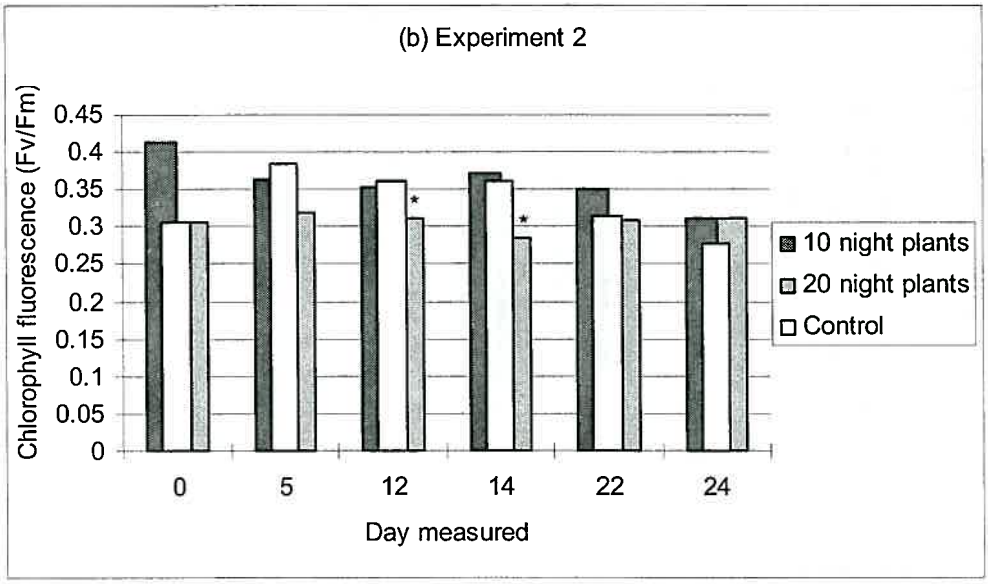
Chlorophyll fluorescence

Experiment 1 plants showed no evidence of reduced chlorophyll fluorescence following cold shock treatments for 10 and 20 nights (Figure 4a). Experiment 2 plants showed a significant reduction in

chlorophyll fluorescence on days 12 and 14; however recovery occurred at the end of the 20 night treatment (Figure 4b).

Figure 4. Chlorophyll fluorescence (Fv/Fm) of the control, 10 night and 20 night treatments at 10 °C measured using an infra-red analyser (IRGA) with a leaf chamber fluorometer attachment in (a) Experiment 1 and (b) Experiment 2. * identifies treatments that are significantly different ($P<0.05$) from the control measured on the same day.





Discussion

TTC and REC

Results for TTC tests and REC showed no significant reduction in plant tissue viability following both 10 and 20 nights of cold treatment at 10 °C. The two significant results of TTC tests in Experiment 1 and 2 were disregarded as the control plants were less viable than the treated plants. Such unusually low readings for the control plants may be due to plants having been sickly or water stressed on the day of testing.

The TTC viability test proved to be a reliable method to test the viability of cold affected tissue. The results corresponded to the additional method used (REC), as well as photosynthesis and chlorophyll fluorescence results; hence the method could be utilized to quantitatively assess cold damage in cotton plants. The TTC viability test specifically indicates the affect of cold shock on tissue function, whereas REC indicates damage to cellular structure. Therefore, the lack of significant damage in Experiment 1 and 2 suggests that the cellular function and structure of cotton tissue was not affected enough to reduce growth and development following cold treatments of 10°C for 10 and 20 nights duration.

These results indicate that cotton may be able to withstand colder night temperatures than previously thought, and that the temperature threshold for 'cold shock' may be lower than the currently accepted 11 °C. Recent studies showed that following cold treatment of 10 °C for 10 nights, growth up to flowering was affected (Bange and Milroy 2004).

Photosynthesis and chlorophyll fluorescence

Exposure of cotton plants to 10°C for 10 and 20 nights did not reduce photosynthetic rate or fluorescence significantly at the end of both experiments. In the photosynthesis measurements, there was a general trend with cold treated plants experiencing reduced photosynthesis during and immediately after treatment, and then recovering to a rate similar to the control plants. In Experiment 1, the final rate of photosynthesis was only slightly lower than original rates, suggesting that such cold treatments do not permanently reduce tissue function. Final reduction in photosynthetic rate may also be due to the ageing of the plants. These results suggest that cotton plants may be able to acclimatise to, or recover from, incidences of cold shock (10°C) of up to 10 and 20 nights duration. This has been shown to occur in plants at both a mature (14-node) stage and a more immature stage (7-node).

In conclusion, our experimental data showed evidence that exposure to 10°C for 10 and 20 nights does not affect cotton growth; and that this lack of impact could be because cold treatment did not affect tissue function and survival.

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