Cotton Catchment Communities CRC

SUMMER SCHOLARSHIP - 2007/2008 SEASON

Project title
Cultivar cold tolerance screening using germination chill protocols

Aims and milestones

- To test cotton cultivars for cold tolerance using physiologically based germination chill tests.
- The project seeks to test the hypothesis that germination chill tests can provide an indication of cold tolerance and provide recommendations for cultivar choice if sown in conditions where cold weather may be encountered early in crop establishment.

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Project Summary

This summer scholarship project was undertaken by Charles Tuck. Charles is in his fourth and final year of Bachelor of Science in Agriculture at the University of Sydney and is majoring in Agronomy. The scholarship commenced on 15th January, 2008 and was completed on 1st December 2008.

Abstract

Cotton (*Gossypium hirsutum*) is sensitive to cold conditions during germination and establishment. Identifying cultivars with cold tolerance offers opportunities for future breeding efforts and provides growers with increased flexibility for sowing date options. Developing cultivars with chilling tolerance will allow cultivars to withstand the cold shocks during early cotton growth (15th September – 30th November), allowing better establishment under cool conditions, and reducing the costs associated with replanting. This project aims to utilise germination chill protocols, and investigate their applicability in determining genetic variation in seedling chilling tolerance. Seeds of ten cultivars (DP16, Namcala, Pima A-8, Sicala 350B, Sicot 289RR, Sicot 71, Sicot 75, Sicot 81, Siokra V-18 and TL) were germinated at four temperatures (14ºC, 18ºC, 22ºC and 30ºC) and germination probability (percentage) was determined after 4, 7 and 10 d. Ten seedlings were selected randomly and the length from the hook of the hypocotyl to the tip of the radicle (seedling length) was measured. Laboratory tests were correlated to an early planted (August) field experiment conducted 40 km west of Narromine, central west NSW. Seedling length provided a better indication of cold tolerance than germination probability. The best correlation with field emergence was with the Cool-Warm Seedling Length test (average of seedling length between cool temperature 14°C at Day 7 and warm temperature 30°C at Day 4) which provided an R^2 of 0.73. The Cool-Warm Vigour Index (average of seedling germination between cool temperature14°C at Day 7 and warm temperature 30°C at Day 4) also provided a correlation with field emergence only when Namcala (outlier) was removed (R^2 = 0.62). The electrolyte leakage test provided a negative correlation with field emergence. The laboratory and field experiments indicated that there was some genetic variation in the selected cotton cultivars, with Namcala, DP16, Sicot 75, Sicala 350B and Siokra V-18 showing some degree of cold tolerance during field emergence and laboratory tests. Further research is needed to test more cultivars to validate the Cool-Warm Seedling Length test (Tuck Test).

Background

Australia is regarded as a supplier of high quality cotton and produced 274,200 tons of cotton, valued at $471.2 million, in 2006-07 (ABARE 2007). There are two main cotton production states in Australia, New South Wales (NSW) and Queensland (QLD). These areas are divided into hot, central and cool zones of cotton production, based on average daily growing degree days (McMahon and Low 1972). The average frequency of cold shocks (minimum temperature ≤11°C) in major Australian cotton producing regions during the early growth of cotton (15th September – 30th November) ranges from 40 at Hillston (NSW) to 4 in Emerald (QLD) (Bange and Milroy 2004).

Cotton (*Gossypium hirsutum spp.*) is sensitive to cold conditions during germination and establishment (Wanjura *et al.* 1969). To germinate and establish successfully, cotton needs the soil temperatures to be 14°C or greater for three consecutive days (Constable and Shaw 1988). The importance of germination and establishment is vital to the development of a high yielding cotton crop (Christiansen and Rowland 1981). A cotton stand that is established with a sufficient number of vigorous seedlings is an important step in the production cycle as it sets a limit on yield potential. All the steps after the emergence and establishment can only maintain or decrease potential yield of the developing cotton stand (Wanjura 1981). In addition, unfavourable soil temperatures and moisture conditions will have considerable effect on the variability in emergence time leading to greater differences in the variability in growth and development of the subsequent crop (Wanjura and Buxton 1972b).

A seed is considered germinated if it has a radicle length of 3mm (Wanjura and Buxton 1972a). Chilling injury can occur in cotton seedlings when the temperature drops below a range of 15°C to 20°C during germination (Cole and Wheeler 1974; Lauterbach *et al.* 1999). Prolonged exposure to temperatures below 15°C will slow the metabolic activity of the seed as well as make the seed susceptible to plant pathogens and other stresses when the soil temperatures start to rise (Borth *et al.* 1997; Buxton and Sprenger 1976). At temperatures below 10°C, the root tip can be damaged permanently (Christiansen 1968).

Chilling tolerance refers to the plants' ability to withstand injuries arising from severe chilling stress and avoidance of severe and persisting growth inhibitors caused by mild chilling stress (Stamp 1984). Developing cultivars with chilling tolerance is important as this will allow cultivars to withstand the cold shocks during early cotton growth (15th September – 30th November), allowing better establishment under cool conditions and reducing the costly need to replant. Identifying cultivars with cold tolerance may offer opportunities for future breeding efforts and provide growers with increased flexibility for sowing dates.

Although some work has been carried out on American cultivars, few germination chill experiments have been carried out on modern Australian cultivars to assess their potential for cold tolerance. It is hypothesised that there is genetic variation in cotton seedling chilling tolerance and that germination chill tests are correlated to field emergence.

Aims and objectives

This project aims to refine germination chill protocols for cotton germination and emergence, while also investigating whether germination chill tests can provide an indication of current cultivar differences in chilling tolerance. These will be tested through correlations between field emergence and laboratory germination experiments.

Hypothesis

It is hypothesised that there is genetic variation in cotton seedling chilling tolerance and that germination chill tests are correlated to field emergence.

Methodology

There was one laboratory experiment which was conducted in the two locations (Narrabri and Sydney) and one field experiment conducted at Narromine. An electrical leakage test and seed weight experiment was also conducted. Details of the ten cotton cultivars tested are presented in Table 1.

Table 1. Cotton cultivars used to assess the different germination tests and for identifying cold tolerance (Stiller 2008, *pers. comm.*).

Cotton cultivar	Origin	Soil type	Maturity
DP16	Mississppi, USA	Light textured loam-clay loam	Medium
Namcala	Arizona, USA	Clay loam	Medium-full
Pima A-8	Arizona, USA	Clay loam	Medium-full
Sicala 350B	CSIRO, Australia	Vertosol	Full
Sicot 289RR	CSIRO, Australia	Vertosol	Full
Sicot 71	CSIRO, Australia	Vertosol	Medium-full
Sicot 75	CSIRO, Australia	Vertosol	Medium-full
Sicot 81	CSIRO, Australia	Vertosol	Full
Siokra V-18	CSIRO, Australia	Vertosol	Medium
TL	Zimbabwe	Vertosol	Full

Laboratory experiment

The laboratory experiment was conducted at the Australian Cotton Research Institute (ACRI) and at the University of Sydney using germination incubators. The germination assessments used four replicates of 50 seeds placed on two sheets of wet paper towel with another sheet placed on top and rolled (Duesterhaus 2000; Hall and Gannaway 2005; Hopper *et al.* 1994). The rolled sheets were placed in covered plastic containers in the incubator/growth cabinet held at constant temperature. Seeds were germinated at four temperatures 14ºC, 18ºC (hereafter referred to as cool germination test), 22ºC and 30ºC (hereafter referred to as warm germination test).

Seedling germination protocol

The germination protocol used four replicates of 50 seeds placed on two sheets of wet paper towel with another sheet placed on top and rolled (Duesterhaus 2000; Hall and Gannaway 2005; Hopper *et al.* 1994). The rolled sheets were placed in covered plastic containers in the incubator/growth cabinet at a constant temperature. Seeds were germinated at four temperatures 14ºC, 18ºC (hereafter referred to as cool germination test), 22ºC and 30ºC (hereafter referred to as warm germination test). Seedlings with a radicle of 3 mm or longer were counted as germinated (Wanjura and Buxton 1972a) and germination counts were determined after Day 4, 7 and 10 (i.e., days after sowing). In addition, ten seedlings were selected randomly and the length from the hook of the hypocotyl to the tip of the radicle was measured (hereafter referred to as seedling length) (Fig. 1).

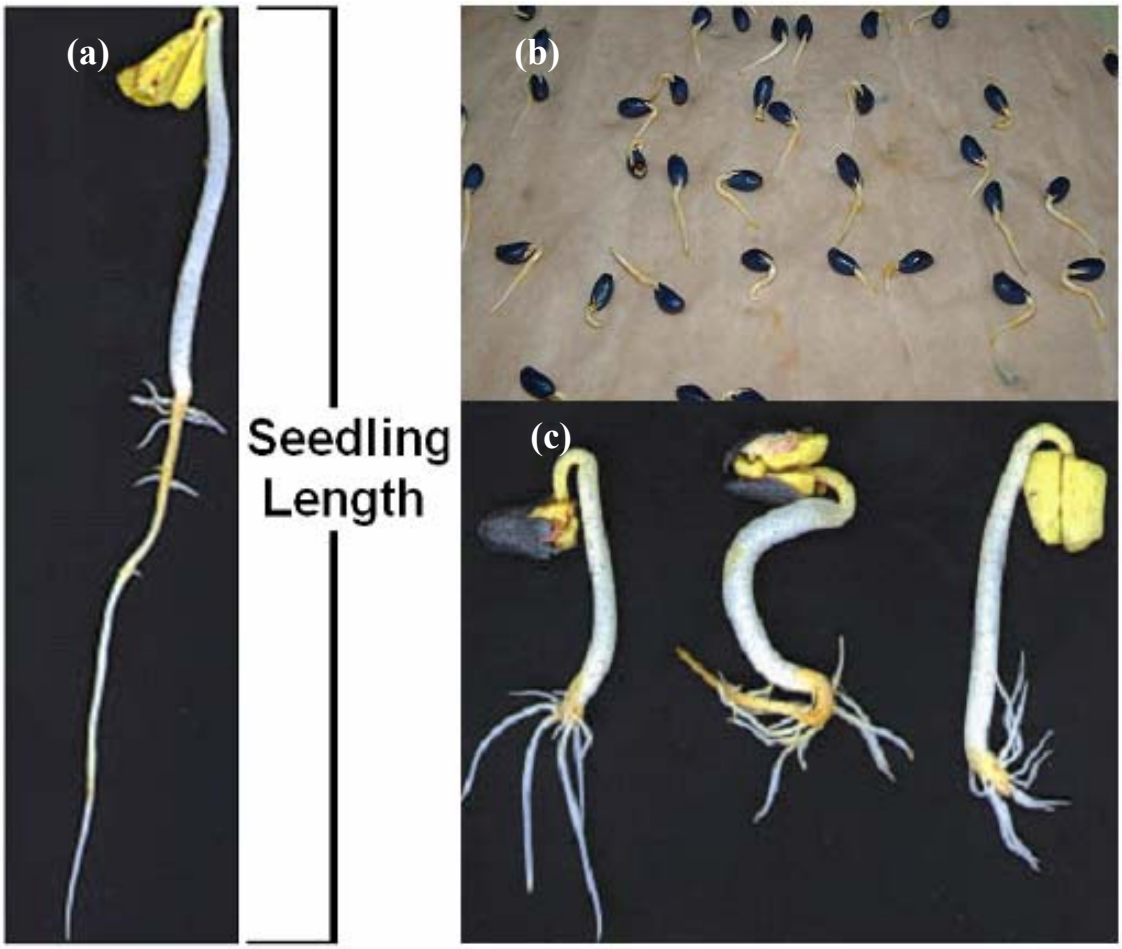


Fig. 1. (a) Diagram showing how the germinated cotton seedling length was measured (Burke *et al.* 2008). (b) Germinating cotton seeds on wet paper towel. (c) Germinated cotton seedlings showing chilling damage (Burke *et al.* 2008).

Electrolyte Leakage Test

Seed imbibition can result in cellular damage at chilling temperatures and this can be assessed through the measurement the electrical conductivity of electrolytes released during initial imbibition (Schulze *et al.* 1996). Increase in electrolyte leakage indicates the increase in cellular damage and the lack of cold tolerance. Cotton seed is sensitive to chilling injury during imbibition especially at the time of initial hydration and during the 18 to 30 hours after imbibition begins (Christiansen 1967). Fifty seeds from each cultivar were washed with 30 ml of deionised water twice. The seeds were then placed in 30 ml of 5ºC water and allowed to imbibe for 24 hours (Schulze *et al.* 1997). After 24 hours, the water was removed from the tube and allowed to return to room temperature where the electrical conductivity of the water was measured.

Seed Weight Test

The weight of 200 seeds was determined to see if seed weight influenced the ability of different cotton cultivars to germinate under chilling temperatures.

Calculations

Cool-Warm Vigour Index

A combination of the warm germination test and the cool germination test was reported to be reliable indicator of field performance (Hake *et al.* 1996, Smith and Varvil 1984, Buxton *et al.* 1977). In the Cool-Warm Vigour Index, seeds are germinated separately at 30ºC and 18ºC and counted after 4 and 7 days, respectively. The germination probabilities are then added together and divided by two (see Equation 1) for each cultivar (hereafter referred to as the Cool Warm Vigour Index) (Bird and Reyes 1967; Hopper *et al.* 1994; Schulze *et al.* 1997).

$$Cool\ \ Warm\ \ Vigour\ \ Index = \frac{Cool\ \ Temperature + Warm\ \ Temperature}{2} \qquad (1)$$

The Cool-Warm Seedling Length test ("Tuck Test") was developed specifically for this study and measures the average of ten randomly selected seedlings from the hook of the hypocotyl to the tip of the radicle from the same germination protocol. The cool temperature seedling length (14ºC or 18ºC) is combined with the warm temperature seedling length (22ºC or 30ºC) and divided by two (hereafter referred to as the Cool-Warm Seedling Length (see Equation 2)).

$$Cool\ \ Warm\ \ Seedling\ \ Length = \frac{Cool\ \ Temperature\ \ Seedling\ \ Length + Warm\ \ Temperature\ \ Seedling\ \ Length}{2} \qquad (2)$$

Field Experiment

The laboratory tests were correlated to a field experiment conducted in a cotton field in the Lower Macquarie Valley, 40 km west of Narromine (32º13'55 S, 148º14'23 E). This was located in a semi-arid environment on a Grey Vertosol in central west New South Wales, Australia. Cotton seeds were planted on two different dates, 23 August

2008 and 6 September 2008 in a split plot design with four replicates. The ten cotton cultivars were randomly allocated to the subplots. Each cotton cultivar had 50 seeds planted in a 5m row.

The field experiment had emergence counts taken 14, 21, 28 and 35 days after planting. The soil temperature was recorded at 9am daily at the nearby Trangie Research Station which is approximately 40km north of the experimental field site. The temperature station is located on clay loam soil, 5km north-west of Trangie (32º01'55 S, 147º59'02 E) (Fig. 2).

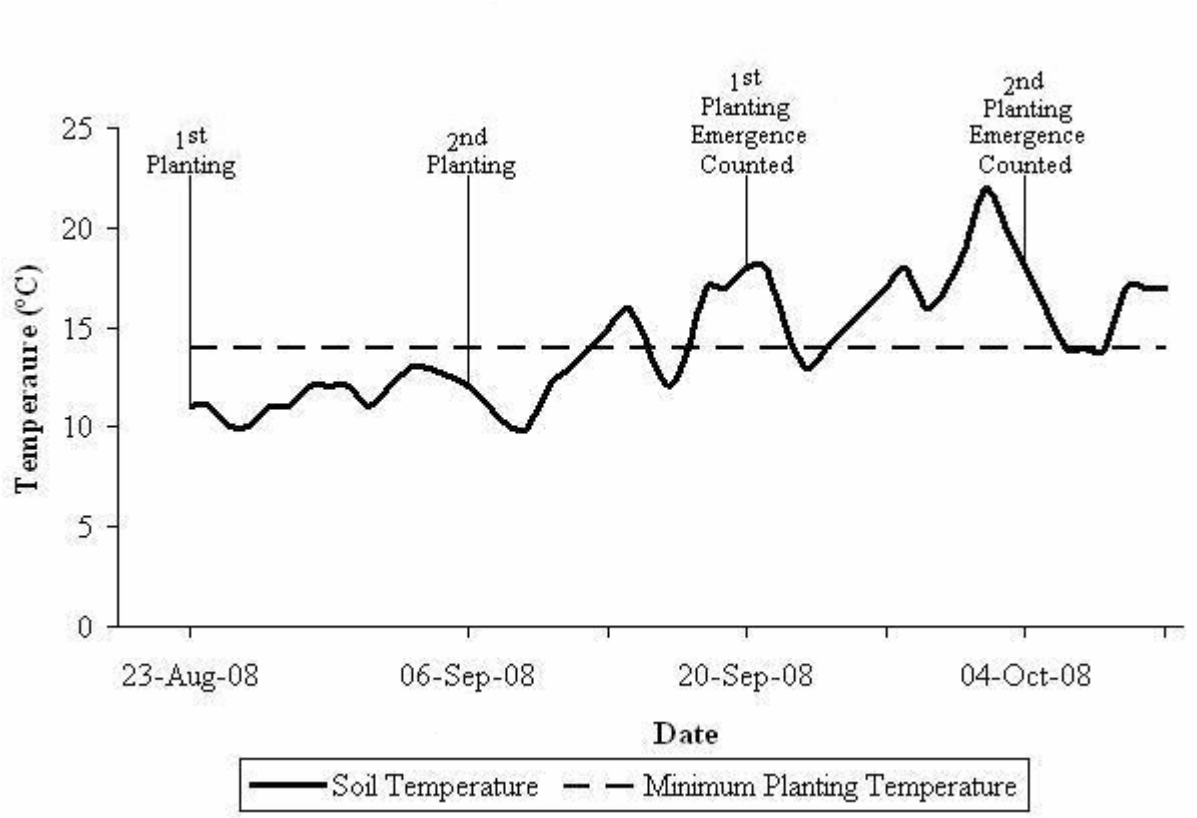


Fig. 2. The soil temperature at 10 cm at the Trangie Research Station for the period of the field experiment (Ceeney 2008).

Field emergence was counted at 28 days after sowing (hereafter referred to as field emergence). As the field emergence probabilities of both plantings were similar, only the emergence probabilities of the first planting were used for correlations with the laboratory tests, as they encountered the coldest field temperatures.

Data Analysis

The data collected during the germination protocols were analysed using analysis of variance (ANOVA) and regression (Genstat 10th edition, VSN International Ltd). Emergence counts from the field and germination counts in the laboratory were analysed using binary logistic regression. The seedling length of germinated cotton seeds, electrolyte leakage tests and seed weight were analysed using analysis of variance (ANOVA).

Results

Seedling germination protocol

There was an interaction in seedling germination probability between cultivar and temperature on Day 4 ($P<0.001$). Germination on Day 7 had no interaction between cultivar and temperature, but there were main effect differences for cultivar ($P<0.001$) and temperature ($P<0.001$). There was an interaction between cultivar and temperature on Day 10 ($P<0.05$) (Table 2).

Table 2. Table of significant Chi-tests for germination probability for cultivar (DP16, Namcala, Pima A-8, Sicala 350B, Sicot 289RR, Sicot 71, Sicot 75, Sicot 81, Siokra V-18 and TL) and temperature (14ºC, 18ºC, 22ºC and 30ºC) combinations on 4, 7 and 10 days after sowing.

Day	Cultivar	Temperature	Cultivar x Temperature
4	***	***	***
7	***	***	n.s.
10	***	***	*

* represents $P<0.05$, ** represents $P<0.01$, *** represents $P<0.001$ and n.s. represents not significant at $P=0.05$

On Day 4 at 14ºC the cultivars Namcala, Pima A-8, Sicot 289RR, Sicot 71 and Sicot 81 had germination probabilities below 0.50 (Fig. 3). All cultivars had germination probabilities above 0.75 for temperatures of 18ºC and above. At Day 7, cultivars with germination probabilities above 0.90 were Namcala, Sicot 289RR, Sicot 71, Sicot 81 and TL (Fig. 4). On Day 10, cultivars DP16, Pima A-8, Sicala 350B and Sicot 75 had a germination probabilities greater than 0.90 for temperature 14ºC and all cultivar interactions with temperatures 18ºC and above had germination probabilities above 0.90 (Fig. 5).

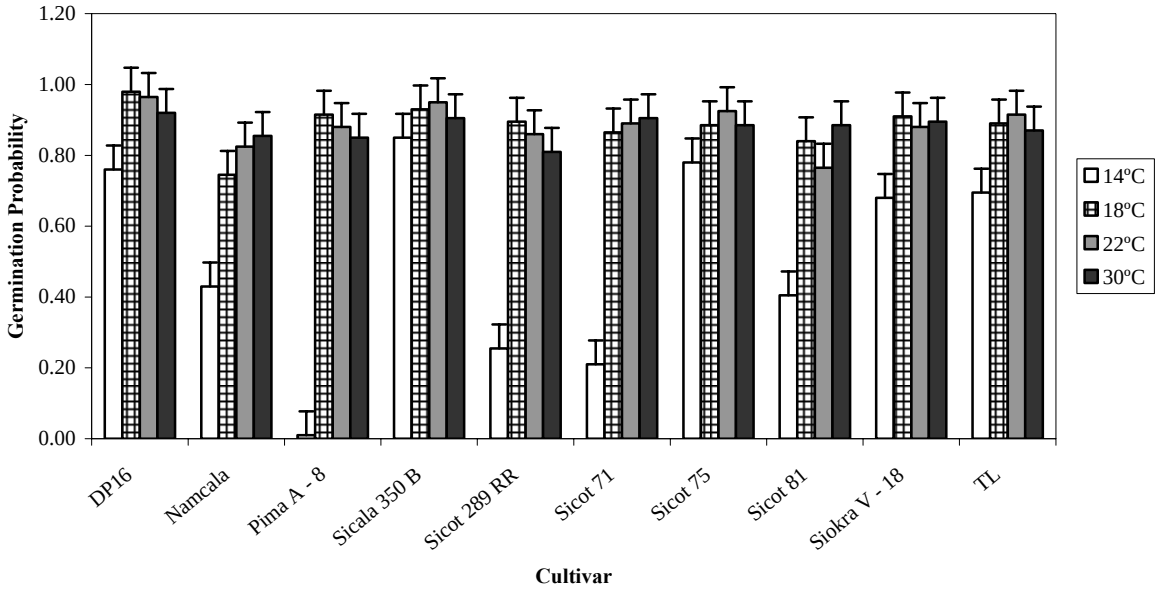


Fig. 3. Day 4 germination probability of all cultivars in four temperature treatments (14ºC, 18ºC, 22ºC and 30ºC). Vertical bars represent l.s.d.s for cultivar by temperature interaction at *P*=0.05.

(*a*) (*b*)

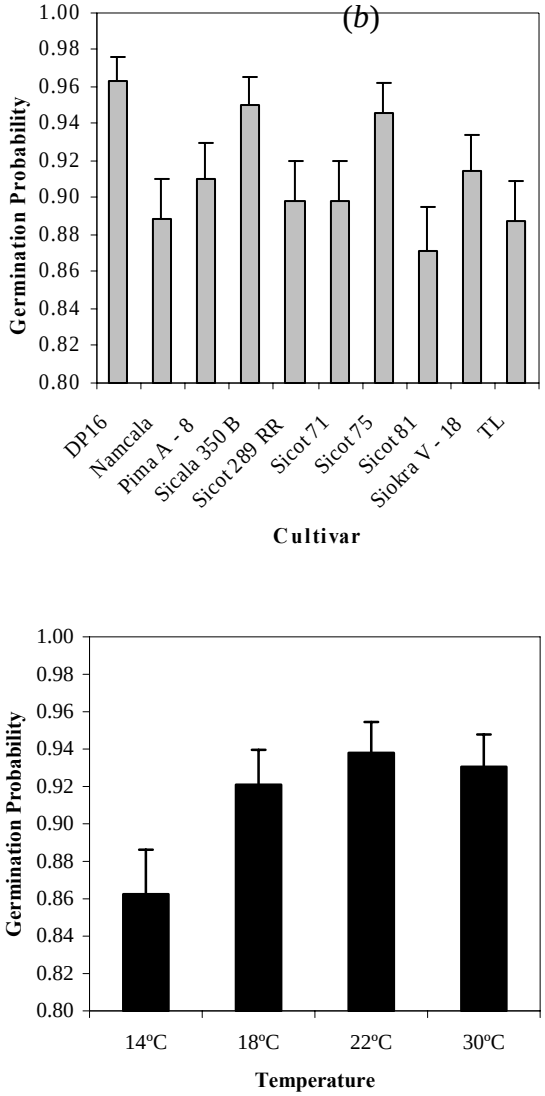


Fig. 4. Day 7 germination probability of (*a*) cultivars and (*b*) four temperature treatments (14ºC, 18ºC, 22ºC and 30ºC). Vertical bars represent l.s.d.s for (*a*) cultivar and (*b*) temperature main effect, respectively at *P*=0.05.

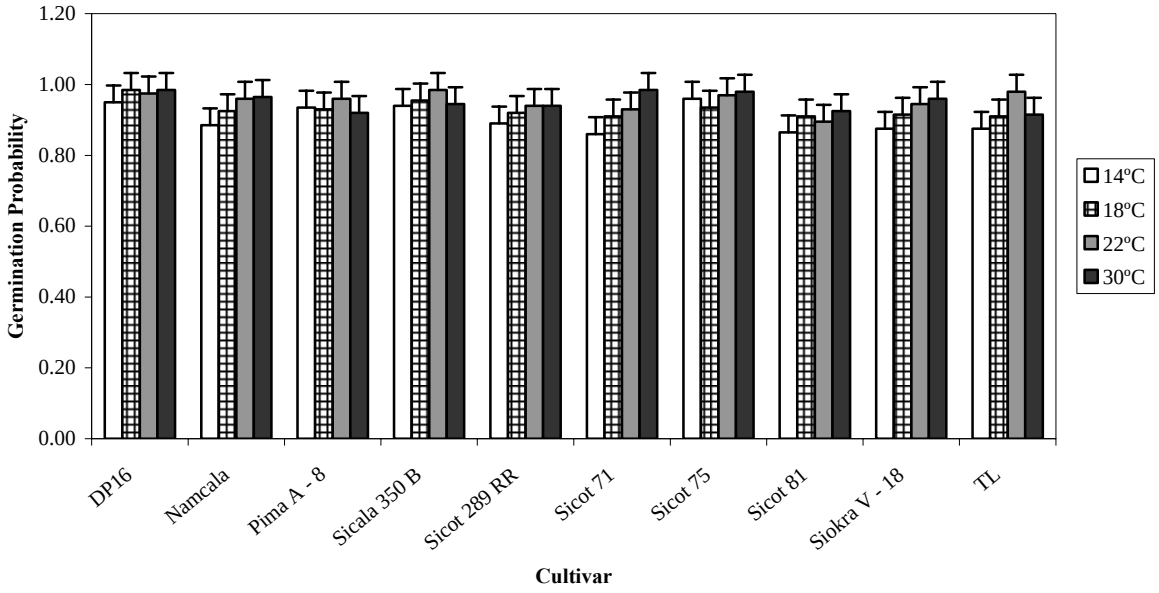


Fig. 5. Day 10 germination probability of cultivars in the four temperature treatments (14ºC, 18ºC, 22ºC and 30ºC). Vertical bars represent l.s.d.s for cultivar by temperature interaction at *P*=0.05.

Seedling length

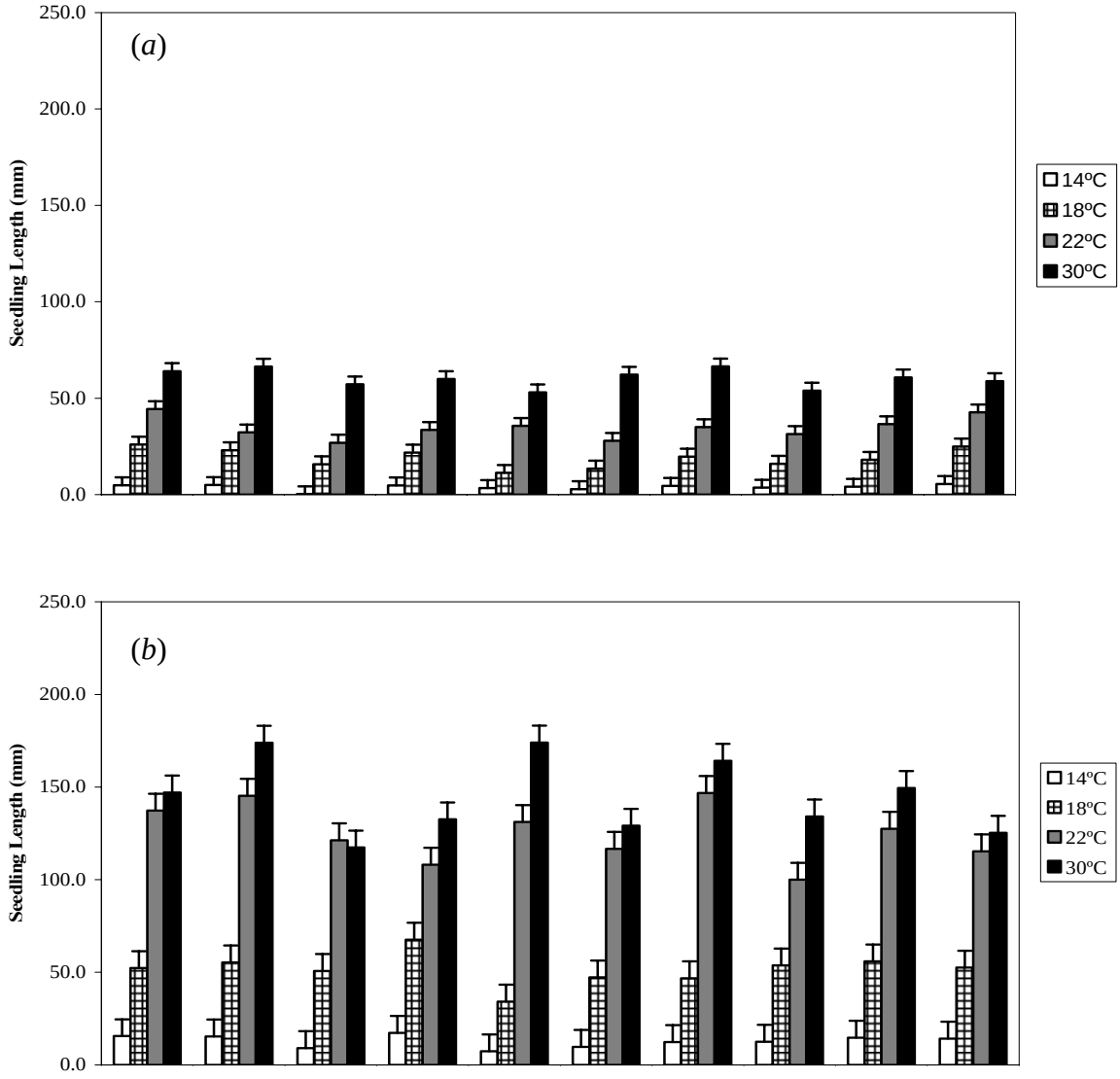
Seedling length in the germination tests showed interactions between cultivar and temperature on Day 4, 7 and 10 (*P*<0.001) (Table 3). The seedling length showed cultivar by temperature interactions on each day while the germination probability only had interactions on Day 4 and 10.

Table 3. Table of significant F-test for seedling length for cultivar (DP16, Namcala, Pima A-8, Sicala 350B, Sicot 289RR, Sicot 71, Sicot 75, Sicot 81, Siokra V-18 and TL) and temperature (14ºC, 18ºC, 22ºC and 30ºC) combinations on 4, 7 and 10 days after sowing.

Day	Variety	Temperature	Variety x Temperature
4	***	***	***
7	***	***	***
10	***	***	***

* represents *P*<0.05, ** represents P<0.01, *** represents *P*<0.001 and n.s. represents not significant at *P*=0.05

All cultivars had greater seedling lengths as the temperature increased from 14ºC to 30ºC on Day 4 (Fig. 6). On Day 7, seedling lengths for all cultivars at 22ºC and 30ºC were greater than 100mm, with Namcala, Sicot 289RR and Sicot 75 having seedling lengths greater than 150mm at 30ºC (Fig 6). At 14ºC there were three cultivars, Pima A-8, Sicot 289RR and Sicot 71 under 10mm on Day 7 (Fig 6). On Day 10, the seedling length increased as temperature increased for all cultivars except Pima A-8 which decreased from 22ºC to 30ºC. Sicot 289RR had the longest seedling length at 30ºC and the shortest seedling length at 14ºC on Day 10 (Fig. 6).



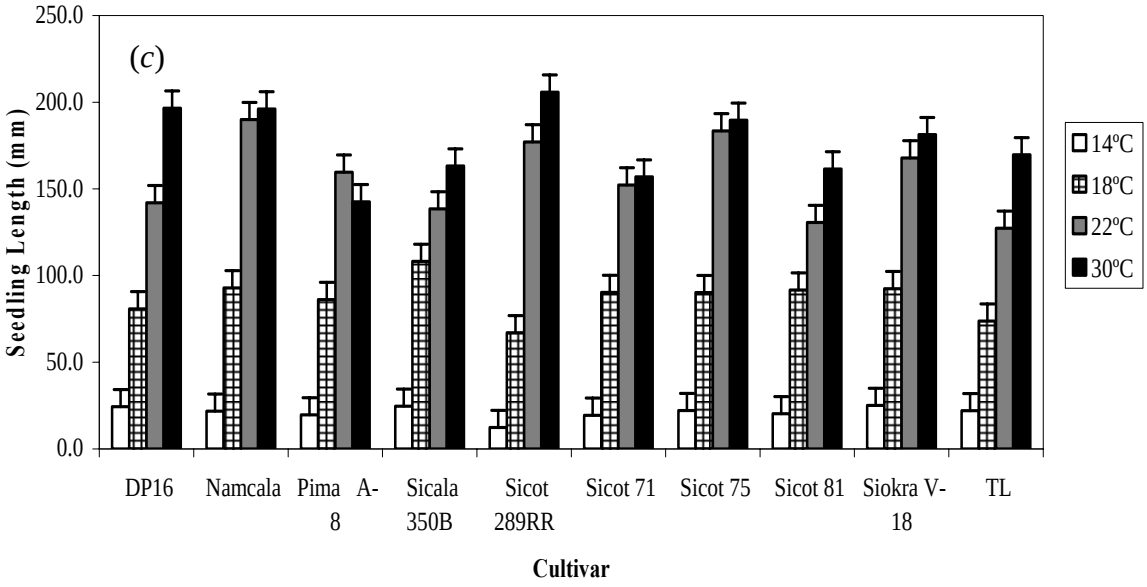


Fig. 6. (*a*) Day 4, (*b*) Day 7 and (*c*) Day 10 seedling length of cultivars (DP16, Namcala, Pima A-8, Sicala 350B, Sicot 289RR, Sicot 71, Sicot 75, Sicot 81, Siokra V-18 and TL) and temperature (14ºC, 18ºC, 22ºC and 30ºC) in the four temperature treatments (14ºC, 18ºC, 22ºC and 30ºC). Vertical bars represent l.s.d.s for cultivar by temperature interaction at *P*=0.05.

Field experiment

The field experiment had emergence counts taken 14, 21, 28 and 35 days after planting. Comparing the two sowings, there were significant differences for both sowings at 28 d after sowing (Table 4).

Table 4. F-test of emergence probability of ten cultivars in the field at Narromine planted on 23rd August and 6th September 2008

Days After Planting	Planting Date: 23/08/08	Planting Date: 6/09/08
14	n.s.	*
21	*	n.s.
28	**	*
35	*	n.s.

* represents *P*<0.05, ** represents P<0.01, *** represents *P*<0.001 and n.s. represents not significant at *P*=0.05

The field experiment showed that cotton seeds were able to germinate at temperatures below 14°C although field emergence probability was less than 0.30 on Day 28, except for Namcala which was 0.35 (data not presented). The soil temperature at 10cm, measured at 9am daily, was below the minimum plant temperature (14°C) for the first 19 days of the 1st planting (Fig. 2), which is the reason the 1st planting took longer to emerge. The strong variation in field emergence among cultivars in the 1st planting after 28 days was due to the soil temperature increasing to just over the minimum planting temperature (14°C) for periods of the 4th week.

Regression analysis

This section presents results of the regression analysis comparing various germination and germination seedling emergence tests with field emergence (28 days after sowing).

Cool germination tests

There were no correlations between the field emergence and germination probability in the cool germination tests (14 and 18 °C). The seedling length in the cool germination test at 14ºC at 7 and 10 days was however significantly correlated with field emergence 2 at 7 and 10 days (Table 5).

Table 5. Cool germination test correlation with field emergence probability after 28 days.

	Cool	Germination Probability		Seedling Length	
Day	**Temperature**	**R^2**	***P* Value**	**R^2**	***P* Value**
4	14	0.2619	n.s.	0.3117	n.s.
7	14	0.0000	n.s.	0.5358	*
10	14	0.0274	n.s.	0.4082	*
4	18	0.1692	n.s.	0.3572	n.s.
7	18	0.0113	n.s.	0.2562	n.s.
10	18	0.0812	n.s.	0.2685	n.s.

* represents $P<0.05$, ** represents $P<0.01$, *** represents $P<0.001$ and n.s. represents not significant at $P=0.05$

Warm germination tests

The germination probability in the warm germination tests were not correlated to field emergence. The only significant correlation was between field emergence and seedling length in the warm germination test at 30ºC at 4 days (Table 6).

Table 6. Warm germination test correlation with field emergence probability after 28 days.

	Warm	Germination Probability		Seedling Length	
Day	**Temperature**	**R^2**	**P-Value**	**R^2**	**P-Value**
4	22	0.0001	n.s.	0.0262	n.s.
7	22	0.1144	n.s.	0.2489	n.s.
10	22	0.0566	n.s.	0.1386	n.s.
4	30	0.1186	n.s.	0.5634	*
7	30	0.2378	n.s.	0.2217	n.s.
10	30	0.2284	n.s.	0.1581	n.s.

* represents $P<0.05$, ** represents $P<0.01$, *** represents $P<0.001$ and n.s. represents not significant at $P=0.05$

Cool-Warm Vigour and Seedling Length Index

The Cool-Warm Vigour Index (Equation 1) did not provide any significant correlation with field emergence with any combination of cool or warm germination test (Table 7). However, the Cool-Warm Seedling Length Index (Equation 2) provided significant correlations with field emergence ($P<0.05$).

Table 7. Different combinations of the Cool-Warm Vigour Index and correlation with field emergence after 28 days.

Cool		Warm		Germination Probability		Seedling Length	
Day	Temperature	Day	Temperature	R^2	*P* Value	R^2	*P* Value
4	14	4	30	0.2661	n.s.	0.6326	**
4	14	7	30	0.2873	n.s.	0.2467	n.s.
4	14	10	30	0.2923	n.s.	0.1767	n.s.
7	14	4	30	0.0221	n.s.	0.7293	**
7	14	7	30	0.0497	n.s.	0.3371	n.s.
7	14	10	30	0.0381	n.s.	0.2473	n.s.
10	14	4	30	0.1031	n.s.	0.6175	**
10	14	7	30	0.1402	n.s.	0.3669	n.s.
10	14	10	30	0.1374	n.s.	0.2687	n.s.
4	14	4	22	0.2050	n.s.	0.0673	n.s.
4	14	7	22	0.2655	n.s.	0.2920	n.s.
4	14	10	22	0.2576	n.s.	0.1701	n.s.
7	14	4	22	0.0000	n.s.	0.1813	n.s.
7	14	7	22	0.0161	n.s.	0.4048	*
7	14	10	22	0.0076	n.s.	0.2419	n.s.
10	14	4	22	0.0041	n.s.	0.1840	n.s.
10	14	7	22	0.0646	n.s.	0.4006	*
10	14	10	22	0.0462	n.s.	0.2438	n.s.
4	18	4	30	0.0322	n.s.	0.5741	*
4	18	7	30	0.0161	n.s.	0.3626	n.s.
4	18	10	30	0.0344	n.s.	0.2535	n.s.
7	18	4	30	0.0718	n.s.	0.5283	*
7	18	7	30	0.1229	n.s.	0.5494	*
7	18	10	30	0.1194	n.s.	0.4291	*
10	18	4	30	0.1413	n.s.	0.4684	*
10	18	7	30	0.1991	n.s.	0.5305	*
10	18	10	30	0.2133	n.s.	0.5352	*
4	18	4	22	0.0540	n.s.	0.1599	n.s.
4	18	7	22	0.0372	n.s.	0.3802	n.s.
4	18	10	22	0.0619	n.s.	0.2718	n.s.
7	18	4	22	0.0009	n.s.	0.2566	n.s.
7	18	7	22	0.0569	n.s.	0.6538	**
7	18	10	22	0.0375	n.s.	0.3790	n.s.
10	18	4	22	0.0064	n.s.	0.4316	*
10	18	7	22	0.1111	n.s.	0.6446	**
10	18	10	22	0.0858	n.s.	0.3347	n.s.

* represents $P<0.05$, ** represents $P<0.01$, *** represents $P<0.001$ and n.s. represents not significant at $P=0.05$

When the cool temperature in the Cool-Warm Seedling Length was changed to 14ºC instead of 18ºC, the R^2 increased from 0.53 to 0.73 (P<0.01) (Fig 7). The correlation between the traditional Cool-Warm Vigour Index (cool temperature 18ºC) and field emergence (Fig 7) were not significant (P>0.05) with an R^2 of 0.07. When the outlier (Namcala cultivar) was removed, the correlation between field emergence and laboratory germination (cool temperatures, 14ºC and 18ºC) becomes significant (P<0.05) (Fig 8).

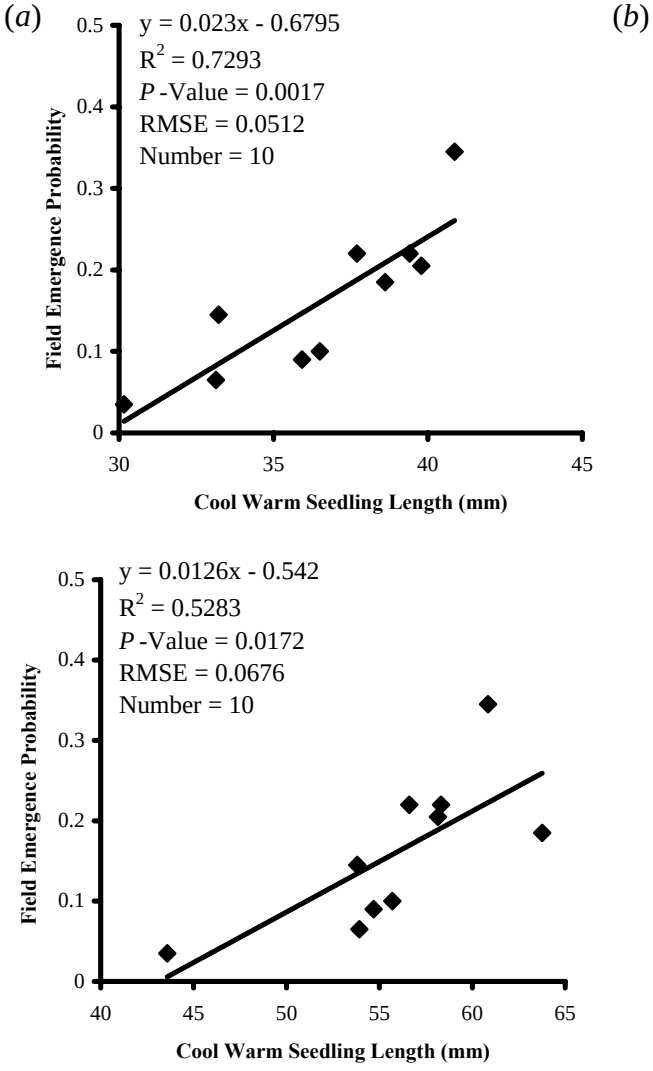


Fig. 3. (*a*) Correlation between field emergence probability and Cool-Warm Seedling Length at Day 7 at 14ºC (Cool) and Day 4 at 30ºC (Warm). (*b*) Correlation between field emergence probability and Cool-Warm Seedling Length at Day 7 at18ºC (Cool) and Day 4 at 30ºC (Warm).

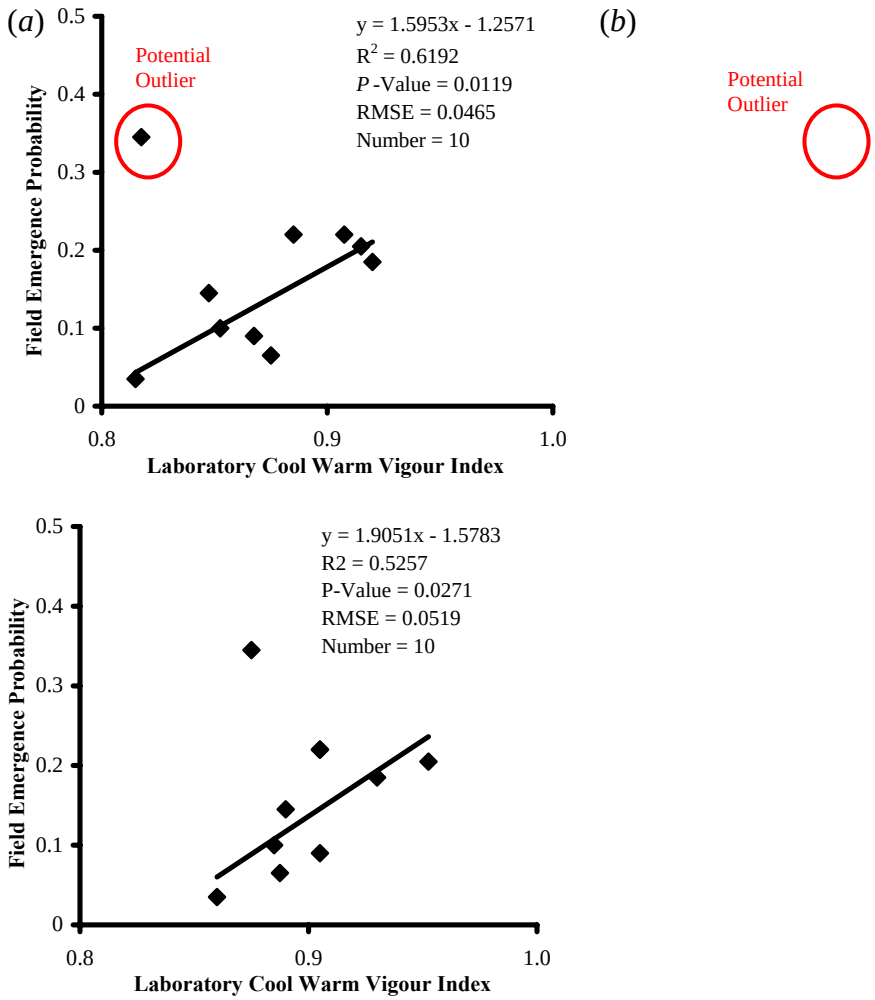


Fig. 4. (*a*) Correlation between field emergence probability and the Cool-Warm Vigour Index at Day 7 at 14ºC (Cool) and Day 4 at 30ºC (Warm) (with outlier Namcala cultivar circled). (*b*) Correlation between field emergence probability and Cool-Warm Vigour Index at Day 7 at 18ºC (Cool) and Day 4 at 30ºC (Warm) (with outlier Namcala cultivar circled).

The Cool Warm Vigour Index correlated to field emergence probability indicated that Sicot 289 RR had the lowest tolerance of cool temperatures. DP16, Sicala 350 B, Sicot 75, Siokra V-18 and Sicot 71 cultivars all performed well in the Cool-Warm Vigour Index with an index greater then 0.90 (Fig. 9). Namcala performed better than the other cultivars with a field emergence greater then 0.30 but had a Cool-Warm Vigour Index below 0.90.

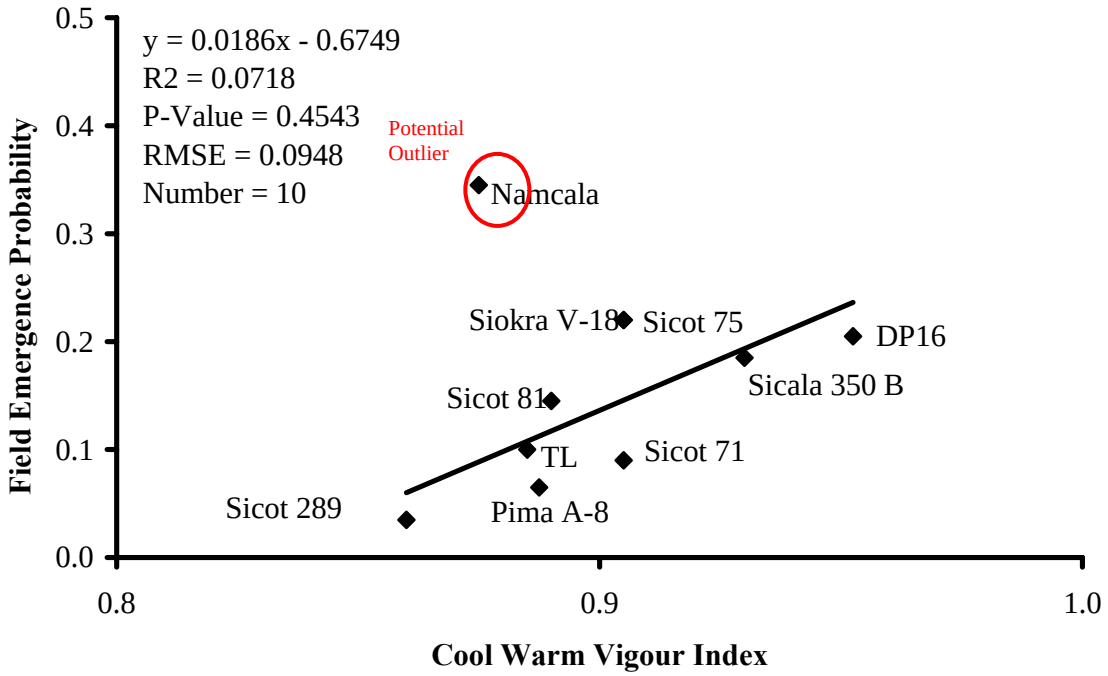


Fig. 5. Cultivar performance in the traditional Cool-Warm Vigour Index correlated to field emergence probability.

The Cool-Warm Seedling Length test also indicated that Sicot 289 RR had the lowest tolerance of cold temperatures. Sicot 289 RR, Pima A-8 and Sicot 81 all performed poorly in the Cool-Warm Seedling Length test (less than 35mm), and the field emergence probabilities were less than 0.20 (Fig. 10). DP16, Namcala and Sicot 75 performed well, with Cool-Warm Seedling Length greater than 38mm.

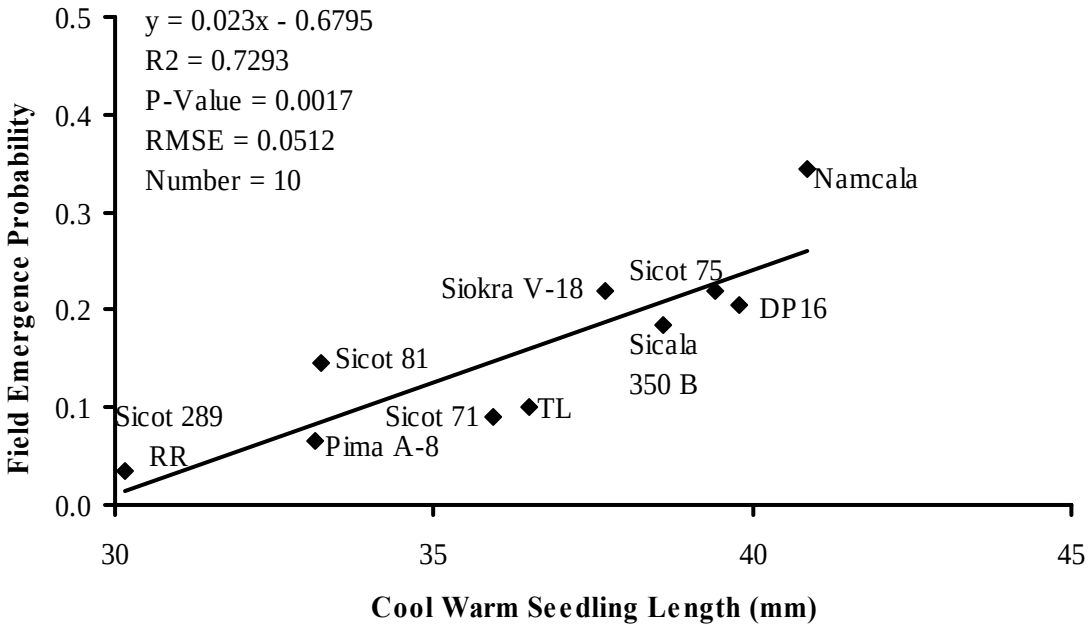


Fig. 6. Cultivar performance in the proposed Cool-Warm Seedling Length test when correlated to field emergence probability.

Electrolyte leakage test

The electrical conductivity of the electrolytes released during imbibition has a correlation with field emergence (P<0.05). There was a negative correlation as cultivars with a lower electrical conductivity reading had a greater probability of field emergence. Namcala and DP16 had EC readings of 100 µs/cm or below (Fig. 11).

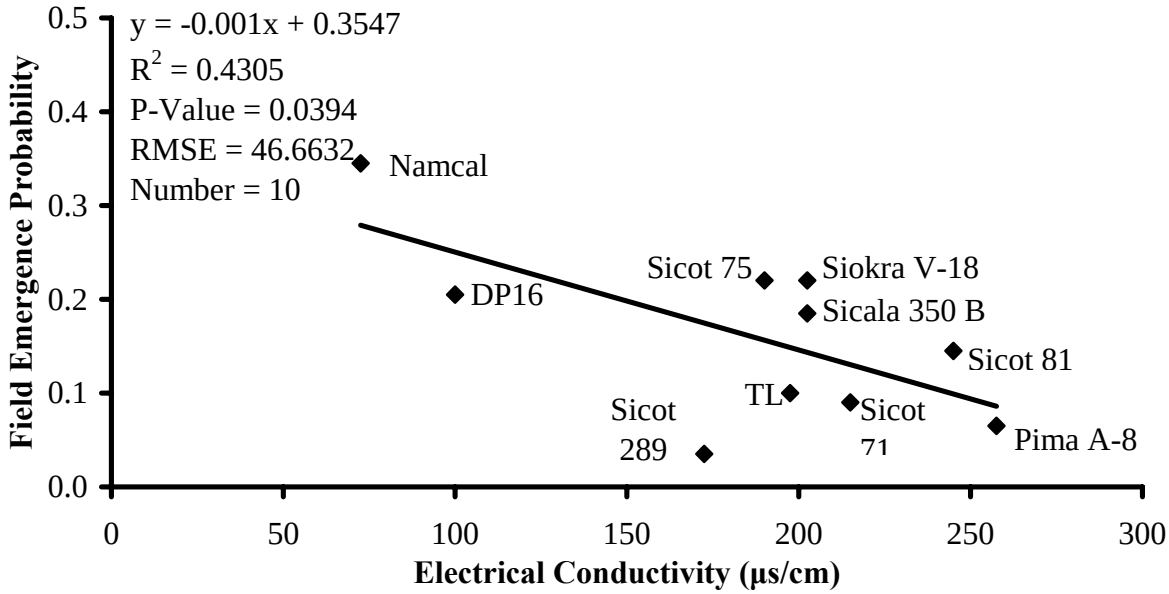


Fig. 7. Correlation between field germination and the electrical conductivity of seeds soaked for 24 hours.

Seed Weight Test

There was no correlation between the weight of 200 seeds and the field emergence probability (Fig 12).

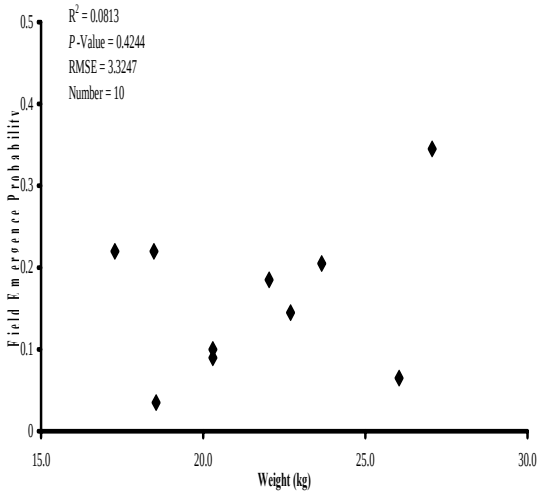


Fig. 8. Correlation of field emergence probability to the weight of 200 seeds.

Discussion

Seedling germination protocol

Germination protocols in the laboratory can be used to help assess chilling tolerance in cotton. The seedling germination protocol showed variation in cultivar response to chilling at germination. There were interactions between temperature and cultivar on Days 4 and 10. This is similar to work by Schulze *et al.* (1997) and Duesterhaus (2000) who showed there was genetic variation of cold tolerance in cotton cultivars in the USA.

Cool germination test

The standard 18ºC cool germination test has been used in many studies (Duesterhaus 2000; Hopper *et al.* 1994; Schulze *et al.* 1996). The cool germination test in the current study consisted of two different temperatures (14ºC and 18ºC), but they provided no correlation to field emergence. These results are similar to Schulze *et al.* (1996) who found no significant relationship between the cool germination test and early planted field emergence ($R^2 = 0.26$) or late planted field emergence ($R^2 = 0.31$) after 4 weeks. In contrast, Bolek (2006) reported a significant correlation between field emergence (Day 7) and germination probability at 18ºC after 7 days ($R^2 = 0.57$, $P<0.05$). Another study in Texas, USA also found a significant correlation between field emergence and the cool germination test in 1998 ($R^2 = 0.70$) but no correlation in 1999 ($R^2 = 0.20$) (Duesterhaus 2000). In 1998, a warm spring resulted in a high correlation with the cool germination test, were as in 1999 extremely stressful environmental factors resulted in a poor correlation (Duesterhaus 2000).

The cool germination test provided no correlation with field germination at 14ºC on Days 4, 7 and 10 in the current study. This is consistent with Bolek (2006) who also reported that there was no significant relationship between field emergence and germination probability at 15ºC and 13ºC. These findings were supported by Lauterbach *et al.* (1999) who suggested that chilling injury occurs in cotton seedlings whenever the temperature drops below 15ºC during germination. Cool temperatures below 20ºC may cause chilling injury to cotton seedlings and reduce germination and emergence (Cole and Wheeler 1974) and stand establishment (Duesterhaus, Hopper *et*

al. 1999). A sufficient quantity of vigorous seedlings is essential to stand establishment, as this is the step in the production cycle where maximum yield is set (Wanjura 1981).

Seedling length at the 18ºC cool germination test had no significant correlation to field emergence in the current study. The seedling length in the 14ºC cool germination test successfully predicted the field emergence after 28 days. The recommended temperature for farmers to plant cotton in Australia is three consecutive days of 14ºC (Constable and Shaw 1988), however, the recommended cool germination test for cotton breeders is 18ºC (Duesterhaus *et al.* 2000; Schulze *et al.* 1997). This poises the question as to why cotton breeders use 18ºC when 14ºC would provide a better indication of cold tolerance. Hence, a cool temperature test at 14°C may provide a better indication of cold tolerance than 18°C.

Warm germination test

The warm germination test of 30°C had no correlation to field emergence in this study. Due to the early field planting in this study, almost 2 months before the recommended planting date (Muldoon 2008, *pers. comm.*), the soil temperature (Fig. 2) was below the recommended planting temperature of 14ºC for three consecutive days (Constable and Shaw 1988). This could have resulted in the lack of correlation between the warm germination test and field emergence, especially when cool temperatures are encountered during germination and emergence (Bourland 1992). Duesterhaus (2000) found significant correlations between warm germination test and field emergence in an excellent warm spring, while in the following year, cool temperatures resulted in no correlation with field emergence after 4 weeks.

The importance of using vigorous seed was evident with the strong relationship between seedling length at 30ºC on Day 4 and field emergence in the current study. Vigorous cultivars create rapidly emerging and growing seedlings that produce plants with higher boll set percentages, while low vigour causes slower seedling emergence, delayed stand establishment and reduced competitive effectiveness (Douglas *et al.* 1974). Low temperatures slow emergence and this is often associated with seedling disease, which usually results in the development of a poor cotton stand with reduced vigour (Constable 1976). Pima A-8 was the only cultivar that had a significant

decrease in seedling length at 30ºC on Day 10. This suggests that *Gossypium barbadense* (Pima cotton cultivars) may have a lower optimal radicle and hypocotyl elongation temperature than *Gossypium hirsutum*, which is 34.4ºC (Wanjura and Buxton 1972a).

Cool-Warm Vigour Index

Research has indicated that a combination of the warm and cool germination test is a more reliable indicator of field performance (Buxton *et al.* 1977; Hake *et al.* 1996; Smith and Varvil 1984). Cool-Warm Vigour Index was developed by Bird and Reyes (1967) and is also known as the "Texas Cool Test". The current study also found that a combination of the cool and warm seedling length test provided a better predictor of field emergence. However, there was no correlation between field emergence and the Cool-Warm Vigour Index in this study. There was no correlation ($R^2 = 0.07$) between the Cool-Warm Vigour Index and field emergence before an outlier, Namcala cultivar was removed. However, this suggests that this index is not robust as there was no real physiological reason to remove Namcala. The relationship of Cool-Warm Vigour Index with field emergence (with outlier Namcala cultivar removed) had a stronger correlation when the 14ºC ($R^2 = 0.62$) was used instead of 18ºC ($R^2 = 0.53$). Similarly the Cool-Warm Seedling Length increased R^2 from 0.53 to 0.73 when 14ºC was used instead of 18ºC in the correlation with field emergence. This indicates that the cool temperature in the Cool-Warm Vigour Index and the Cool-Warm Seedling Length should be 14ºC. Similar to this study, Klos and Brummer (2000) found that seedling height of lucerne was a better trait than germination time to predict field performance when the traits are measured in a laboratory or greenhouse.

Combinations of the Cool-Warm Vigour Index for both the seedling length and germination probability were better at predicting field emergence than either the cool germination test or warm germination test on their own. The top four predictors for seedling length and germination were combinations of cool and warm temperature tests. This is supported by Kerby *et al.* (1989) who concluded that the Cool-Warm Vigour Index was a better indicator of field emergence than either component alone. Duesterhaus (2000) found that the Cool-Warm Vigour Index was a good predictor of field stand establishment ($R^2 = 0.80$) when there were excellent conditions at planting,

while adverse conditions at planting resulted in no correlation (R^2 = 0.22). This indicates that the Cool-Warm Vigour Index may be a good predictor of field emergence under ideal conditions, but may not be as good a predictor under cool conditions.

Cold tolerant cultivars

The proposed 'Tuck Test' (Cool-Warm Seedling Length index at a cool temperature of 14°C on Day 7 and warm temperature of 30°C on Day 4) provided the best correlation between field emergence and seedling length (R^2 = 0.73). The poor performance of Sicot 289 RR in the Cool-Warm Seedling Length test and field emergence under chilling conditions is due to it being a bred for a tropical climate (Table 1). The tolerance of Pima A-8 (*Gossypium barbadense*) to chilling conditions is low, similar to low chilling tolerance reported in many pima cultivars (Bolek 2006; Buxton *et al.* 1976). Siokra V-18, Sicala 350B, Sicot 75, Namcala and DP16 cultivars performed well in the field emergence with Cool-Warm Seedling Lengths greater than 38mm and these cultivars could be recommend to farmers looking to plant early in the season. Cultivars that have a Cool-Warm Seedling Length greater then 40mm are considered to have excellent cold tolerance, with Namcala being the only cultivar to achieve this. Namcala was bred in Arizona, USA in an arid and semi-arid climate that experiences extreme hot and cold temperatures.

Electrolyte leakage test

The electrolyte leakage test provided a correlation between field emergence after 28 days and sugars released during the first 24 hours of imbibition. However, the R^2 was below 0.50 indicating that this relationship was not strong. This work agrees with Schulze *et al.* (1996) who also found there was a negative correlation (Early planting R^2 = -0.31; late planting R^2 = -0.61) with field emergence after 4 weeks. Schulze *et al.* (1996) also suggested that the electrical conductivity test was a good indicator of chilling tolerance in cotton and the low R^2 values were probably due to other uncontrolled field variables other than temperature. Similar this study, Yaklich *et al.* (1979) reported that field emergence and vigour in soybeans were negatively related to electrolyte leakage. Both the electrolyte leakage test and the "Tuck Test" (Cool-

Warm Seedling Length) consistently showed that Namcala and DP16 have greater tolerance of chilling temperatures.

Weight Test

The weight test provided no correlation with field emergence after 28 days. Hopper *et al.* (1994) found that seed weight had no correlation with field emergence, but instead was significantly correlated to lint length ($R^2 = 0.45$) and strength ($R^2 = 0.61$). Larger seeds tend to produce longer and stronger fibres (Hopper *et al.* 1994).

Further work

Future studies are needed to:

- Test more cultivars to determine the accuracy of the Cool-Warm Seedling Length test (Tuck Test).
- Grow seeds from the same batch of seeds produced in the same year so that it can be confirmed that seedling vigour and cold tolerance is due to the cultivar, and not confounded by seed storage time and conditions.

Conclusion

From this investigation into the cold tolerance of cotton it can be concluded:

- A cool temperature of 14°C will provide a better indication of cold tolerance than 18°C, which is currently used by industry as a predictor of cold tolerance.
- Seedling length provides a better indication of cold tolerance than germination probability.
- The “Tuck Test” (Cool-Warm Seedling Length at a cool temperature of 14°C on Day 7 and warm temperature of 30°C on Day 4) provided the best correlation ($R^2 = 0.73$) to field emergence under cool conditions.
- When testing for cold tolerance, field experiments need to be planted early to ensure seeds or seedlings encounter a cold snap or drop in temperature similar to those experienced in cooler cotton producing area.
- The electrical conductivity test provides a negative correlation with field emergence under cool conditions.

Reference

ABARE (2007) 'Australian Commodity Statistics.' Australian Bureau of Agricultural and Resource Economics, Canberra.

Bange MP, Milroy SP (2004) Impact of short-term exposure to cold night temperatures on early development of cotton (*Gossypium hirsutum* L.). *Australian Journal of Agricultural Research* **55**, 655-664.

Bird LS, Reyes AA (1967) Effects of cottonseed quality on seed and seedling characteristics. In 'Proceedings of the Beltwide Cotton Production Research Conference' pp. 199-206

Bolek Y (2006) Predicting cotton seedling emergence for cold tolerance: *Gossypium barbadense*. *Journal of Agronomy* **5**, 461-465.

Borth TD, Krieg DR, Jividen GM (1997) Genetic and environmental factors affecting the fatty acid composition of polar and non-polar lipids of cottonseed. In 'Proceedings of the Beltwide Cotton Conference' pp. 1455-1458

Bourland FM (1992) Characterization and improvement of seed and seedling vigor in cotton. In 'Proceedings of the Beltwide Cotton Conference'. Memphis, Tennessee pp. 1326-1329. (National Cotton Council)

Burke J, Boman R, Dotray P, McMichael BL, Kaufman H, Oosterhuis DM, Zak J, Wheeler T, Hopper NW (2008) Cotton Root Disorder. In. (Ed. B Calame). (Cotton Incorporated: Lubbock, Texas)

Buxton DR, Melick PJ, Patterson LL, Pegelow EJ (1977) Relationships among cottonseed vigor and emergence. *Agronomy Journal* **69**, 677-681.

Buxton DR, Sprenger PJ (1976) Genetic variability for cottonseed germination at favourable and low temperatures. *Crop Science* **16**, 243-246.

Buxton DR, Sprenger PJ, Pegelow EJ (1976) Periods of chilling sensitivity in germinating pima cottonseed. *Crop Science* **16**, 471-474.

Ceeney S (2008) 'Soil Temperatures; Day degree accumulation; Strategic management of weeds in a flex system.' NSW Department of Primary Industries, Trangie.

Christiansen MN (1967) Periods of sensitivity to chilling in germinating cotton. *Plant Physiology* **42**, 431-433.

Christiansen MN (1968) Induction and prevention of chilling injury to radicle tips of imbibing cottonseed. *Plant Physiology* **43**, 743-746.

Christiansen MN, Rowland R (1981) Cotton physiology – Seed and germination. In 'Proceedings of the Beltwide Cotton Conference'. New Orleans, LA pp. 315-318. (National Cotton Council of America)

Cole DF, Wheeler JE (1974) Effect of pregermination treatments on germination and growth of cottonseed at suboptimal temperatures. *Crop Science* **14**, 451-454.

Constable GA (1976) Temperature effects on the early field development of cotton. *Australian Journal of Experimental Agriculture and Animal Husbandry* **16**, 19-24.

Constable GA, Shaw AJ (1988) 'Temperature requirements for cotton.' New South Wales Department of Agriculture and Fisheries P5.3.5.

Douglas AG, Flores JA, Andrews CH (1974) Effects of seed vigor and competition on performance of cotton. In 'Proceeding of the Beltwide Cotton Production Research Conference'. Memphis, Tennesse pp. 75-77. (National Cotton Council)

Duesterhaus B (2000) Laboratory screening test for the evaluation of cold tolerance in cotton. Texas Tech University.

Duesterhaus B, Hopper NW, Gannaway JR, Jividen GM (1999) Development of a laboratory screening test for the evaluation of cold tolerance in cotton seed germination. In 'Proceeding of the Beltwide Cotton Conference'. Memphis, Tennessee pp. 621-623. (National Cotton Council)

Duesterhaus B, Hopper NW, Gannaway JR, Valco TD (2000) A screening test for the evaluation of cold tolerance in cottonseed germination and emergence. In 'Proceedings of the Beltwide Cotton Conference'. Memphis, Tennessee pp. 596-599. (National Cotton Council)

Hake SJ, Kerby TA, Hake KD (1996) 'Cotton production manual.' (Universtiy of California, Division of Agriculture and Natural Resources)

Hall AJ, Gannaway JR (2005) Evaluation of fatty acid composition of cotton germplasm and association with cold tolerance. In 'Proceeding of the Beltwide Cotton Conference'. New Orleans, Louisiana pp. 923-931

Hopper NW, Gannaway JR, Jividen GM, Struve TH (1994) Selection of cold tolerant lines of cotton based on seedling response. In 'Proceeding of the Beltwide Cotton Conference'. Memphis, Tennessee p. 1375. (National Cotton Council)

Kerby TA, Keeley M, Johnson S (1989) Weather and seed quality variables to predict cotton seedling emergence. *Agronomy Journal* **81**, 415-419.

Klos KLE, Brummer EC (2000) Field response to selection in alfalfa for germination rate and seedling vigor at low temperatures. *Crop Science* **40**, 1227-1232.

Lauterbach B, Krieg DR, Jividen GM (1999) Fatty acid composition of lipid fractions in germinating cotton as affected by temperature. In 'Proceedings of the beltwide cotton conference'. Memphis, Tennessee pp. 564-565. (National Cotton Council of America)

McMahon J, Low A (1972) Growing degree days as a measure of temperature effects on cotton. *Cotton Growing Review* **49**, 39-49.

Schulze DH, Hopper NW, Gannaway JR, Jividen GM (1996) Laboratory tests used to screen for chilling tolerance in cotton genotypes. In 'Proceedings of the Beltwide Cotton Conference'. Memphis, Tennessee pp. 1240-1243. (National Cotton Council)

Schulze DH, Hopper NW, Gannaway JR, Jividen GM (1997) Evaluation of chilling tolerance in cotton genotypes. In 'Proceedings of the Beltwide Cotton Conference'. Memphis, Tennessee pp. 1383-1385. (National Cotton Council)

Smith CW, Varvil JJ (1984) Standard and cool germination tests compared with field emergence in upland cotton. *Agronomy Journal* **76**, 587-589.

Stamp P (1984) Chilling tolerance of young plants demonstrated on the example of maize (*Zea mays* L.). *Advances in Agronomy and Crop Science 7*, 1-83.

Wanjura DF (1981) Germination and strand establishment. In 'Proceeding of the Beltwide Cotton Conference'. pp. 321. (National Cotton Council Memphis, Tennessee)

Wanjura DF, Buxton DR (1972a) Hypocotyl and radicle elongation of cotton as affected by soil environment. *Agronomy Journal* **64**, 431 - 434.

Wanjura DF, Buxton DR (1972b) Water uptake and radicle emergence of cottonseed as affected by soil moisture and temperature. *Agronomy Journal* **46**, 427 - 431.

Wanjura DF, Hudspeth EBJ, Bilbro JDJ (1969) Temperature effect on emergence rate of cotton *(Gossypium hirsutum* L.) under field situations. *Agronomy Journal* **61**, 387-389.

Yaklich RW, Kulik MM, Anderson JD (1979) Evaluation of vigor tests in soybean seeds: relationship of ATP, conductivity, and radioactive tracer multiple criteria laboratory tests to field performance. *Crop Science* **19**, 806-810.

Presentations and public relations

An article, "Assessing cultivar cold tolerance using germination chill protocols – preliminary studies" was published at the 14th Australian Cotton Conference:

Tuck CA, Bange MP, Tan DKY, Stiller WN (2008). Assessing cultivar cold tolerance using germination chill protocols – preliminary studies.
http://australiancottonconference.com.au/index.php?url=/cottons/showpage/3/179/198

Charles Tuck presented a seminar and poster on his work at the inaugural Stepping Out with Fresh Ideas Conference held at the Veterinary Science Conference Centre, The University of Sydney in November 2008, showcasing the work of fourth year students in agriculture. The conference was attended an audience of academic staff, industry representatives, including Cotton Australia and fellow students.

Assessing cultivar cold tolerance using germination chill protocols – preliminary studies

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Summary

The potential for chilling conditions in many Australian cotton growing regions during early growth and development stages demonstrates the need for assessing cold tolerance of cultivars. Preliminary studies have been initiated to assess a range of simple techniques to detect cold tolerance between cultivars. Early results have been positive in detecting some differences between cultivars. The laboratory germination test where seeds are germinated at temperatures of 14 °C for 4 days showed some potential for providing an indication of differences in germination and early establishment.

Introduction

Cotton (*Gossypium hirsutum* L.) is sensitive to cold conditions during germination and establishment. Chilling injury can occur in cotton seeds and reduce germination and emergence whenever the temperature drops below a range of 15°C to 20°C for a few hours during the first few days of germination (Lauterbach *et al.* 1999, Cole and Wheeler 1974). The average frequency of cold shocks (minimum temperature ≤11°C) in major Australian cotton producing regions during the early growth of cotton (15th September – 30th November) ranges from 40 at Hillston (NSW) to 4 in Emerald (QLD) (Bange and Milroy 2004). This emphasises the importance of developing understanding of the differences in cultivars with cold tolerance or management practices that will avoid the potential cold periods during early cotton growth thus allowing for better crop establishment and in severe cases reduce the costly need to replant.

Although some work has been carried out on American cultivars, few germination chill experiments have been carried out on modern Australian cultivars to assess their potential for cold tolerance. Studies have been initiated to develop methodologies to assess chilling and cold tolerance at germination and early growth and establish whether there is variation in Australian cultivars. In identifying cultivars that may offer cold tolerance it may offer opportunities for future breeding efforts and for growers to select these cultivars specifically for these traits to mitigate

chilling damage. Here we present some preliminary assessment of data assessing only a few of the techniques that we are investigating. Studies are continuing to assess a broader range of approaches to assess variation in cold tolerance of cultivars.

Materials and methods

Measurements were conducted at the Australian Cotton Research Institute (ACRI) and at the University of Sydney using germination incubators on ten cotton cultivars (Table 1).

Table 1: Cotton cultivars used to assess techniques to detect differences in cold tolerance.

- Sicala 350B
- Pima A-8
- Namcala
- Sicot 81
- Sicot 71
- DP16
- Sicot 289RR
- Sicot 75
- Siokra V-18
- TL

There were four replicates for each cultivar and the experiments were repeated in two locations (Narrabri and Sydney). Seeds were germinated at four differing temperatures 14ºC, 18ºC (cool germination test), 22ºC and 30ºC (warm germination test). Germination percentages where determined after 4, 7 and 10 d. In addition ten seedlings were selected randomly and the length from the hook of the hypocotyl to the tip of the radicle was also measured.

A cool-warm vigour index test was calculated using the 18ºC and 30ºC germination percentages taken at 4 and 7 d. The germination percentages were added together and divided by two to provide a cool-warm vigour index for each cultivar. Research has indicated that a combination of the warm germination test and the cool germination test are a more reliable indicator of field performance (Hake et al. 1996, Smith and Varvil 1984, Buxton et al. 1977).

The techniques described above were then correlated to field data of seedling vigour, which assessed the number of true leaves on ten plants per plot (averaged over four plots) 31 d. after planting at Myall Vale, Narrabri (NSW). Only six of the ten cultivars were tested in the field study. Germination tests were analysed using analysis of variance (ANOVA) and regressions with the Genstat statistical program.

Preliminary results

Preliminary results have suggested that there may variability in cultivar cold tolerance (Table 2, Figure 1). It is interesting that DP16 (95.25) had a higher cool-warm vigour index than Namcala (87.50). Namcala was originally developed in New Mexico, USA with a climate ranging from arid

to semi-arid, and average summer temperatures ranging from 26ºC at high elevations to around 33ºC with maximum temperatures of up to 50ºC (Constable G.A., pers. comm..). DP16 was first developed in Mississippi, USA, where the climate is cooler and more humid. Hence, DP16 seedlings may be more adapted to chilling conditions during germination and establishment than Namcala which originated from a hotter, dryer climate.

We also see that some of the tests show some relationship with the number of true leaves 31 d after sowing indicating that the tests may reflect tolerance that translates into differences in the field (Figure 2). Work in continuing to assess a broader range of germination tests to see whether they have the potential to detect differences in cultivar cold tolerance. We are also investigating techniques that will account for seed viability due to differences in seed age.

Table 2: Warm germination, cool germination and cool-warm vigour index for selected cultivars

Cultivar	Cool warm vigour index	Cool germination test (%)	Warm germination test (%)
DP16	95.25	92.00	98.50
Sicala 350 B	93.00	90.50	95.50
Siokra V – 18	90.50	89.50	91.50
Sicot 71	90.50	90.50	90.50
Sicot 75	90.50	88.50	92.50
Sicot 81	89.00	88.50	89.50
Pima A – 8	88.75	85.00	92.50
TL	88.50	87.00	90.00
Namcala	87.50	85.50	89.50
Sicot 289 RR	86.00	81.00	91.00
L.s.d at $P = 0.05$	3.66	-	4.99
F test	$P < 0.001$	Not significant	$P < 0.05$

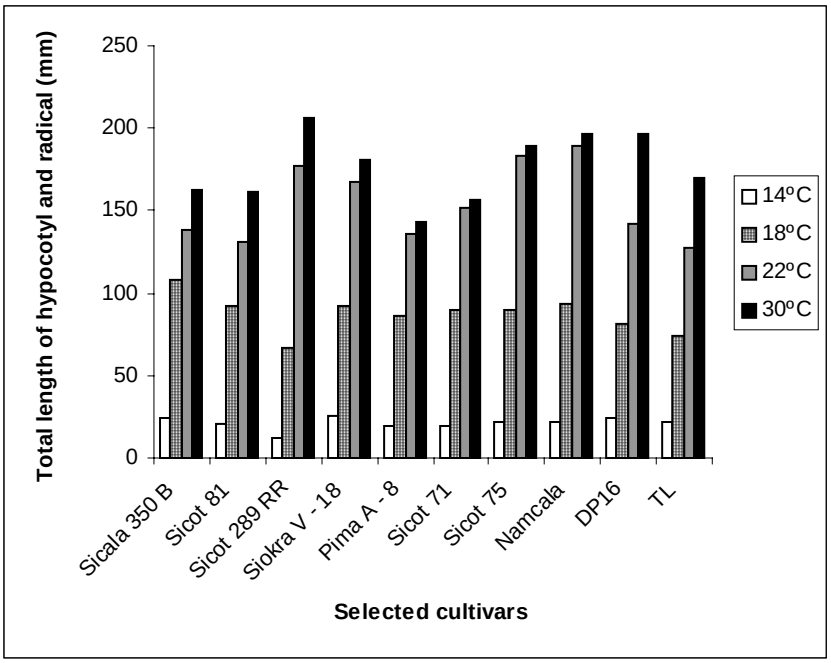

Figure 1: The effect of temperature on hypocotyl and radicle length after 10 days.

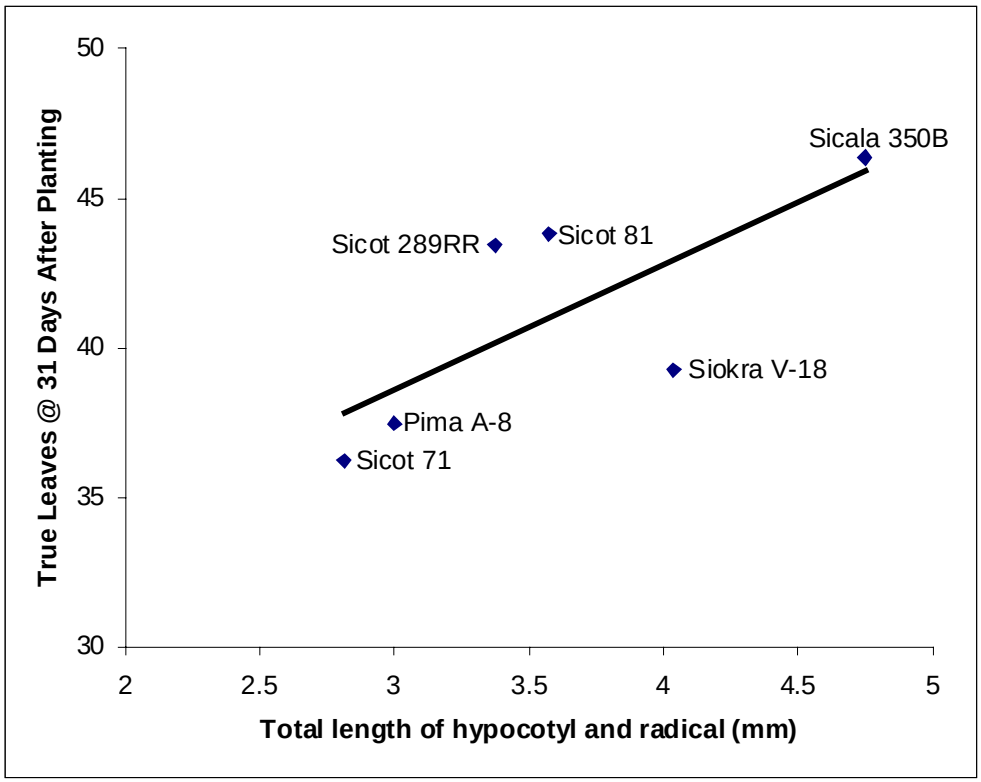

Figure 2. Relationship between total length of hypocotyl and radical (mm) after 4 days at 14 °C (laboratory test) and the number of true leaves at 31 days after planting (in the field).

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References

Bange MP, Milroy SP (2004) Impact of short-term exposure to cold night temperatures on early development of cotton (*Gossypium hirsutum* L.). *Australian Journal of Agricultural Research* **55**, 655-664.

Buxton, D. R., Melick, P. J., Patterson, L. L. & Pegelow, E. J. (1977). Relationships among cottonseed vigor and emergence. *Agronomy Journal* **69**, 677-681.

Cole DF, Wheeler JE (1974) Effect of pregermination treatments on germination and growth of cottonseed at suboptimal temperatures. *Crop Science* **14**, 451-453.

Hake SJ, Kerby TA, Hake KD (1996) Cotton production manual. University of California, Division of agriculture and natural resources. Publication 3352.

Lauterbach B, Krieg DR, Jividen GM (1999) Fatty acid composition of lipid fractions in germinating cotton as affected by temperature. In 'Proceedings of the beltwide cotton conference'. Memphis, Tennessee pp. 564-565. (National Cotton Council of America).

Smith CW, Varvil JJ (1984). Standard and cool germination tests compared with field emergence in upland cotton. *Agronomy Journal* **76**, 587-589.

Cold tolerance screening for cotton cultivars using germination chill protocols

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Abstract. Cotton (*Gossypium hirsutum*) is sensitive to cold conditions during germination and establishment. Identifying cultivars with cold tolerance offers opportunities for future breeding efforts and provides growers with increased flexibility for sowing date options. Developing cultivars with chilling tolerance will allow cultivars to withstand the cold shocks during early cotton growth (15th September – 30th November), allowing better establishment under cool conditions, and reducing the costs associated with replanting. This project aims to utilise germination chill protocols, and investigate their applicability in determining genetic variation in seedling chilling tolerance. Seeds of ten cultivars (DP16, Namcala, Pima A-8, Sicala 350B, Sicot 289RR, Sicot 71, Sicot 75, Sicot 81, Siokra V-18 and TL) were germinated at four temperatures (14ºC, 18ºC, 22ºC and 30ºC) and germination probability (percentage) was determined after 4, 7 and 10 d. Ten seedlings were selected randomly and the length from the hook of the hypocotyl to the tip of the radicle (seedling length) was measured. Laboratory tests were correlated to an early planted (August) field experiment conducted 40 km west of Narromine, central west NSW. Seedling length provided a better indication of cold tolerance than germination probability. The best correlation with field emergence was with the Cool-Warm Seedling Length test (average of seedling length between cool temperature 14°C at Day 7 and warm temperature 30°C at Day 4) which provided an R^2 of 0.73. The Cool-Warm Vigour Index (average of seedling germination between cool temperature14°C at Day 7 and warm temperature 30°C at Day 4) also provided a correlation with field emergence only when Namcala (outlier) was removed (R^2 = 0.62). The electrolyte leakage test provided a negative correlation with field emergence. The laboratory and field experiments indicated

that there was some genetic variation in the selected cotton cultivars, with Namcala, DP16, Sicot 75, Sicala 350B and Siokra V-18 showing some degree of cold tolerance during field emergence and laboratory tests. Further research is needed to test more cultivars to validate the Cool-Warm Seedling Length test (Tuck Test).

Additional Keywords: Cotton, *Gossypium hirsutum*, cold tolerance, germination, establishment, cold germination assays, electrolyte leakage

Introduction

Australia is regarded as a supplier of high quality cotton and produced 274,200 tons of cotton, valued at $471.2 million, in 2006-07 (ABARE 2007). There are two main cotton production states in Australia, New South Wales (NSW) and Queensland (QLD). These areas are divided into hot, central and cool zones of cotton production, based on average daily growing degree days (McMahon and Low 1972) (Appendix 1). The average frequency of cold shocks (minimum temperature ≤11°C) in major Australian cotton producing regions during the early growth of cotton (15th September – 30th November) ranges from 40 at Hillston (NSW) to 4 in Emerald (QLD) (Bange and Milroy 2004).

Cotton (*Gossypium hirsutum spp.*) is sensitive to cold conditions during germination and establishment (Wanjura *et al.* 1969). To germinate and establish successfully, cotton needs the soil temperatures to be 14°C or greater for three consecutive days (Constable and Shaw 1988). The importance of germination and establishment is vital to the development of a high yielding cotton crop (Christiansen and Rowland 1981). A cotton stand that is established with a sufficient number of vigorous seedlings is an important step in the production cycle as it sets a limit on yield potential. All the steps after the emergence and establishment can only maintain or decrease potential yield of the developing cotton stand (Wanjura 1981). In addition, unfavourable soil temperatures and moisture conditions will have considerable effect on the variability in emergence time leading to greater differences in the variability in growth and development of the subsequent crop (Wanjura and Buxton 1972b).

A seed is considered germinated if it has a radicle length of 3mm (Wanjura and Buxton 1972a). Chilling injury can occur in cotton seedlings when the temperature drops below a range of 15°C to 20°C during germination (Cole and Wheeler 1974; Lauterbach *et al.* 1999). Prolonged exposure to temperatures below 15°C will slow the metabolic activity of the seed as well as make the seed susceptible to plant pathogens and other stresses when the soil temperatures start to rise (Borth *et al.* 1997; Buxton and Sprenger 1976). At temperatures below 10°C, the root tip can be damaged permanently (Christiansen 1968).

Chilling tolerance refers to the plants' ability to withstand injuries arising from severe chilling stress and avoidance of severe and persisting growth inhibitors caused by mild chilling stress (Stamp 1984). Developing cultivars with chilling tolerance is important as this will allow

cultivars to withstand the cold shocks during early cotton growth (15th September – 30th November), allowing better establishment under cool conditions and reducing the costly need to replant. Identifying cultivars with cold tolerance may offer opportunities for future breeding efforts and provide growers with increased flexibility for sowing dates.

Although some work has been carried out on American cultivars, few germination chill experiments have been carried out on modern Australian cultivars to assess their potential for cold tolerance. This project aims to refine germination chill protocols for cotton germination and emergence, while also investigating whether germination chill tests can provide an indication of current cultivar differences in chilling tolerance. These will be tested through correlations between field emergence and laboratory germination experiments. It is hypothesised that there is genetic variation in cotton seedling chilling tolerance and that germination chill tests are correlated to field emergence.

Materials and Methods

There was one laboratory experiment which was conducted in the two locations (Narrabri and Sydney) and one field experiment conducted at Narromine. An electrical leakage test and seed weight experiment was also conducted. Details of the ten cotton cultivars tested are presented in Table 1.

Table 1. Cotton cultivars used to assess the different germination tests and for identifying cold tolerance (Stiller 2008, *pers. comm.*).

Cotton cultivar	Origin	Soil type	Maturity
DP16	Mississppi, USA	Light textured loam-clay loam	Medium
Namcala	Arizona, USA	Clay loam	Medium-full
Pima A-8	Arizona, USA	Clay loam	Medium-full
Sicala 350B	CSIRO, Australia	Vertosol	Full
Sicot 289RR	CSIRO, Australia	Vertosol	Full
Sicot 71	CSIRO, Australia	Vertosol	Medium-full
Sicot 75	CSIRO, Australia	Vertosol	Medium-full
Sicot 81	CSIRO, Australia	Vertosol	Full
Siokra V-18	CSIRO, Australia	Vertosol	Medium
TL	Zimbabwe	Vertosol	Full

Laboratory experiment

The laboratory experiment was conducted at the Australian Cotton Research Institute (ACRI) and at the University of Sydney using germination incubators. The germination assessments used four replicates of 50 seeds placed on two sheets of wet paper towel with another sheet placed on top and rolled (Duesterhaus 2000; Hall and Gannaway 2005; Hopper *et al.* 1994). The rolled sheets were placed in covered plastic containers in the incubator/growth cabinet held at constant temperature. Seeds were germinated at four temperatures 14ºC, 18ºC (hereafter referred to as cool germination test), 22ºC and 30ºC (hereafter referred to as warm germination test).

Seedling germination protocol

The germination protocol used four replicates of 50 seeds placed on two sheets of wet paper towel with another sheet placed on top and rolled (Duesterhaus 2000; Hall and Gannaway 2005; Hopper *et al.* 1994). The rolled sheets were placed in covered plastic containers in the incubator/growth cabinet at a constant temperature. Seeds were germinated at four temperatures 14ºC, 18ºC (hereafter referred to as cool germination test), 22ºC and 30ºC (hereafter referred to as warm germination test). Seedlings with a radicle of 3 mm or longer were counted as germinated (Wanjura and Buxton 1972a) and germination counts were determined after Day 4, 7 and 10 (i.e., days after sowing). In addition, ten seedlings were selected randomly and the length from the hook of the hypocotyl to the tip of the radicle was measured (hereafter referred to as seedling length) (Fig. 1).

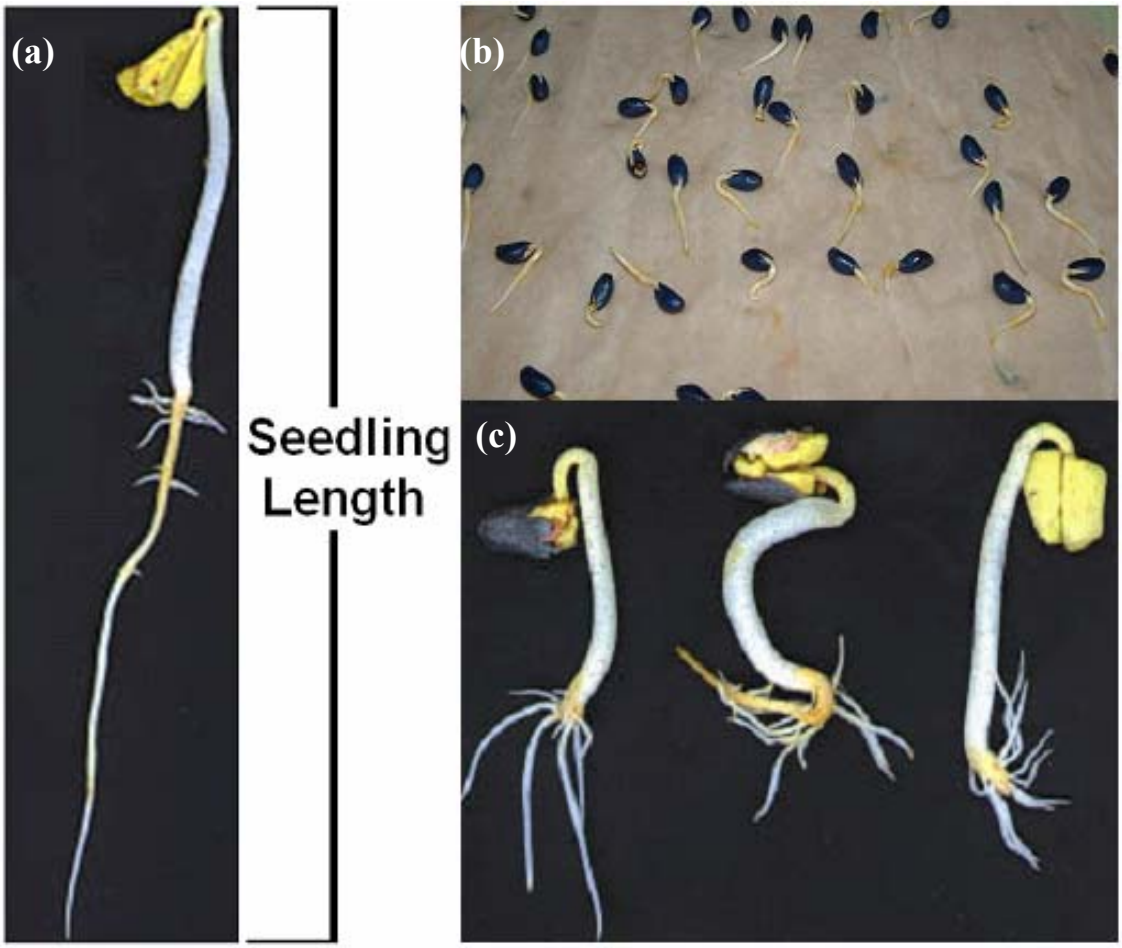


Fig. 1. (a) Diagram showing how the germinated cotton seedling length was measured (Burke *et al.* 2008). (b) Germinating cotton seeds on wet paper towel. (c) Germinated cotton seedlings showing chilling damage (Burke *et al.* 2008).

Electrolyte Leakage Test

Seed imbibition can result in cellular damage at chilling temperatures and this can be assessed through the measurement the electrical conductivity of electrolytes released during initial imbibition (Schulze *et al.* 1996). Increase in electrolyte leakage indicates the increase in cellular damage and the lack of cold tolerance. Cotton seed is sensitive to chilling injury during imbibition especially at the time of initial hydration and during the 18 to 30 hours after imbibition begins (Christiansen 1967). Fifty seeds from each cultivar were washed with 30 ml of deionised water twice. The seeds were then placed in 30 ml of 5ºC water and allowed to imbibe for 24 hours (Schulze *et al.* 1997). After 24 hours, the water was removed from the tube and allowed to return to room temperature where the electrical conductivity of the water was measured.

Seed Weight Test

The weight of 200 seeds was determined to see if seed weight influenced the ability of different cotton cultivars to germinate under chilling temperatures.

Calculations

Cool-Warm Vigour Index

A combination of the warm germination test and the cool germination test was reported to be reliable indicator of field performance (Hake *et al.* 1996, Smith and Varvil 1984, Buxton *et al.* 1977). In the Cool-Warm Vigour Index, seeds are germinated separately at 30ºC and 18ºC and counted after 4 and 7 days, respectively. The germination probabilities are then added together and divided by two (see Equation 1) for each cultivar (hereafter referred to as the Cool Warm Vigour Index) (Bird and Reyes 1967; Hopper *et al.* 1994; Schulze *et al.* 1997).

$$Cool\ \ Warm\ \ Vigour\ \ Index = \frac{Cool\ \ Temperature + Warm\ \ Temperature}{2} \qquad (1)$$

The Cool-Warm Seedling Length test (“Tuck Test”) was developed specifically for this study and measures the average of ten randomly selected seedlings from the hook of the hypocotyl to the tip of the radicle from the same germination protocol. The cool temperature seedling length (14ºC or 18ºC) is combined with the warm temperature seedling length (22ºC or 30ºC) and divided by two (hereafter referred to as the Cool-Warm Seedling Length (see Equation 2)).

$$Cool\ \ Warm\ \ Seedling\ \ Length = \frac{\underset{Seedling\ \ Length}{Cool\ \ Temperature} + \underset{Seedling\ \ Length}{Warm\ \ Temperature}}{2} \qquad (2)$$

Field Experiment

The laboratory tests were correlated to a field experiment conducted in a cotton field in the Lower Macquarie Valley, 40 km west of Narromine (32º13’55 S, 148º14’23 E). This was located in a semi-arid environment on a Grey Vertosol in central west New South Wales, Australia. Cotton seeds were planted on two different dates, 23 August 2008 and 6 September 2008 in a split plot design with four replicates. The ten cotton cultivars were randomly allocated to the subplots. Each cotton cultivar had 50 seeds planted in a 5m row.

The field experiment had emergence counts taken 14, 21, 28 and 35 days after planting. The soil temperature was recorded at 9am daily at the nearby Trangie Research Station which is approximately 40km north of the experimental field site. The temperature station is located on clay loam soil, 5km north-west of Trangie (32º01'55 S, 147º59'02 E) (Fig. 2).

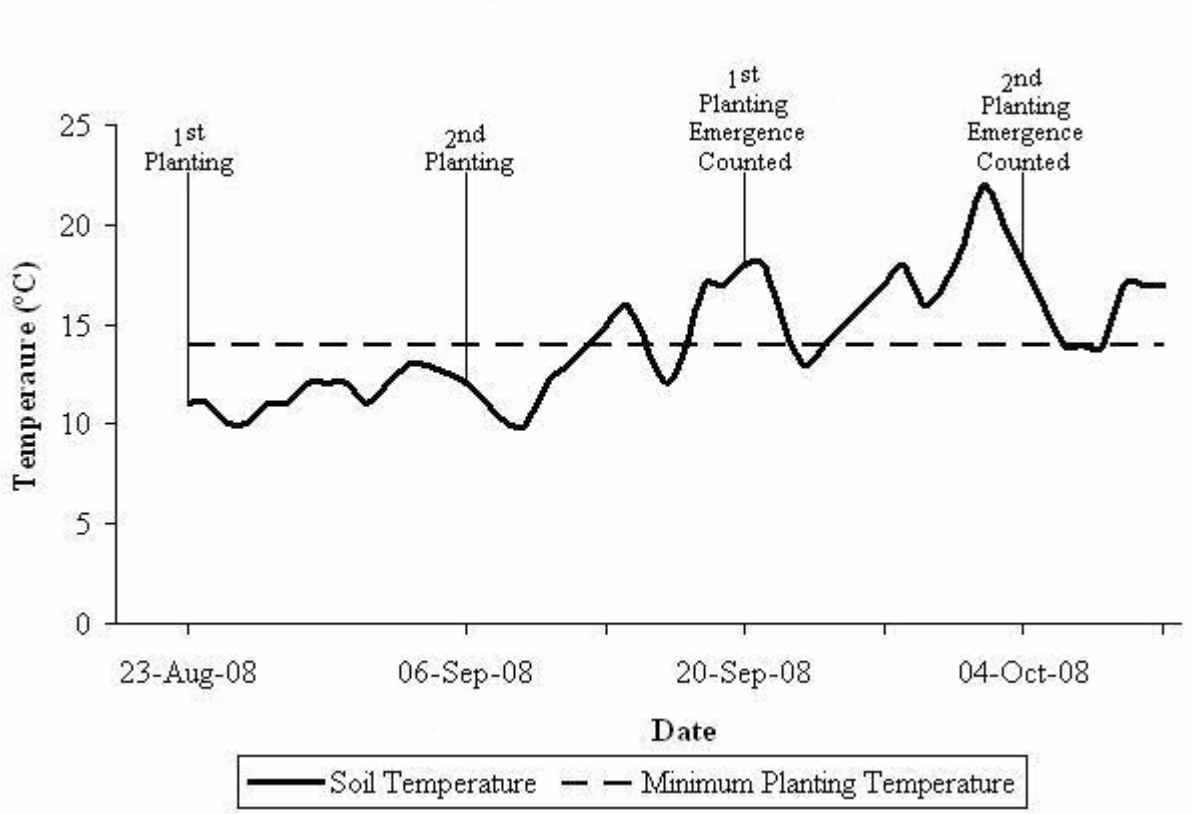


Fig. 2. The soil temperature at 10 cm at the Trangie Research Station for the period of the field experiment (Ceeney 2008).

Field emergence was counted at 28 days after sowing (hereafter referred to as field emergence). As the field emergence probabilities of both plantings were similar, only the emergence probabilities of the first planting were used for correlations with the laboratory tests, as they encountered the coldest field temperatures.

Data Analysis

The data collected during the germination protocols were analysed using analysis of variance (ANOVA) and regression (Genstat 10th edition, VSN International Ltd). Emergence counts from the field and germination counts in the laboratory were analysed using binary logistic regression. The seedling length of germinated cotton seeds, electrolyte leakage tests and seed weight were analysed using analysis of variance (ANOVA).

Results

Seedling germination protocol

There was an interaction in seedling germination probability between cultivar and temperature on Day 4 ($P<0.001$). Germination on Day 7 had no interaction between cultivar and temperature, but there were main effect differences for cultivar ($P<0.001$) and temperature ($P<0.001$). There was an interaction between cultivar and temperature on Day 10 ($P<0.05$) (Table 2).

Table 2. Table of significant Chi-tests for germination probability for cultivar (DP16, Namcala, Pima A-8, Sicala 350B, Sicot 289RR, Sicot 71, Sicot 75, Sicot 81, Siokra V-18 and TL) and temperature (14ºC, 18ºC, 22ºC and 30ºC) combinations on 4, 7 and 10 days after sowing (Appendix 2).

Day	Cultivar	Temperature	Cultivar x Temperature
4	***	***	***
7	***	***	n.s.
10	***	***	*

* represents $P<0.05$, ** represents $P<0.01$, *** represents $P<0.001$ and n.s. represents not significant at $P=0.05$

On Day 4 at 14ºC the cultivars Namcala, Pima A-8, Sicot 289RR, Sicot 71 and Sicot 81 had germination probabilities below 0.50 (Fig. 3). All cultivars had germination probabilities above 0.75 for temperatures of 18ºC and above. At Day 7, cultivars with germination probabilities above 0.90 were Namcala, Sicot 289RR, Sicot 71, Sicot 81 and TL (Fig. 4). On Day 10, cultivars DP16, Pima A-8, Sicala 350B and Sicot 75 had a germination probabilities greater than 0.90 for temperature 14ºC and all cultivar interactions with temperatures 18ºC and above had germination probabilities above 0.90 (Fig. 5).

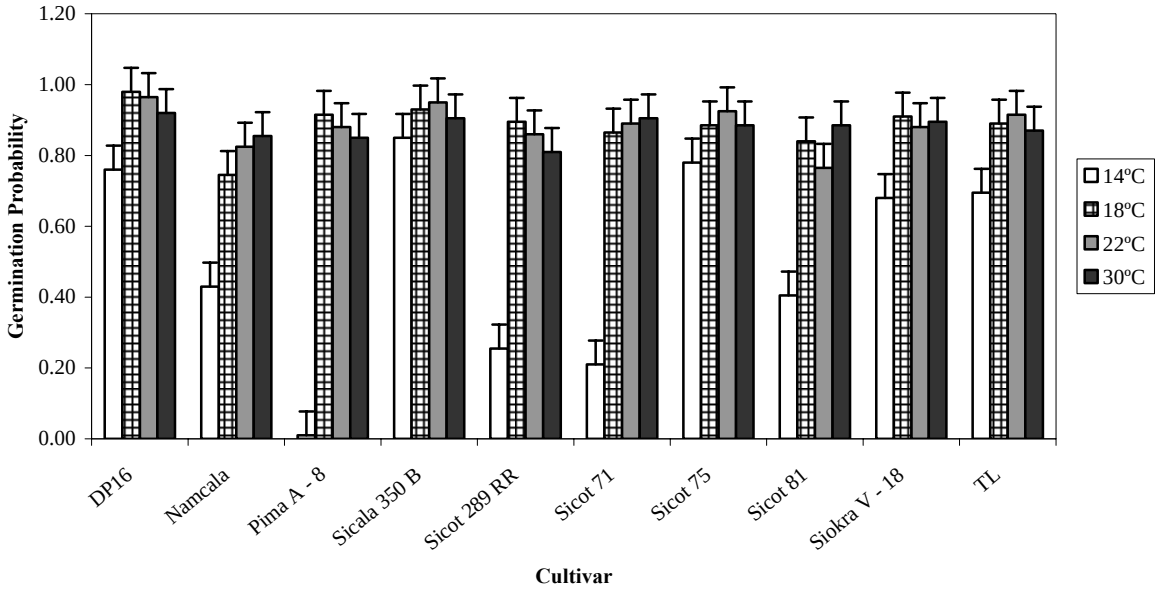


Fig. 3. Day 4 germination probability of all cultivars in four temperature treatments (14ºC, 18ºC, 22ºC and 30ºC). Vertical bars represent l.s.d.s for cultivar by temperature interaction at *P*=0.05.

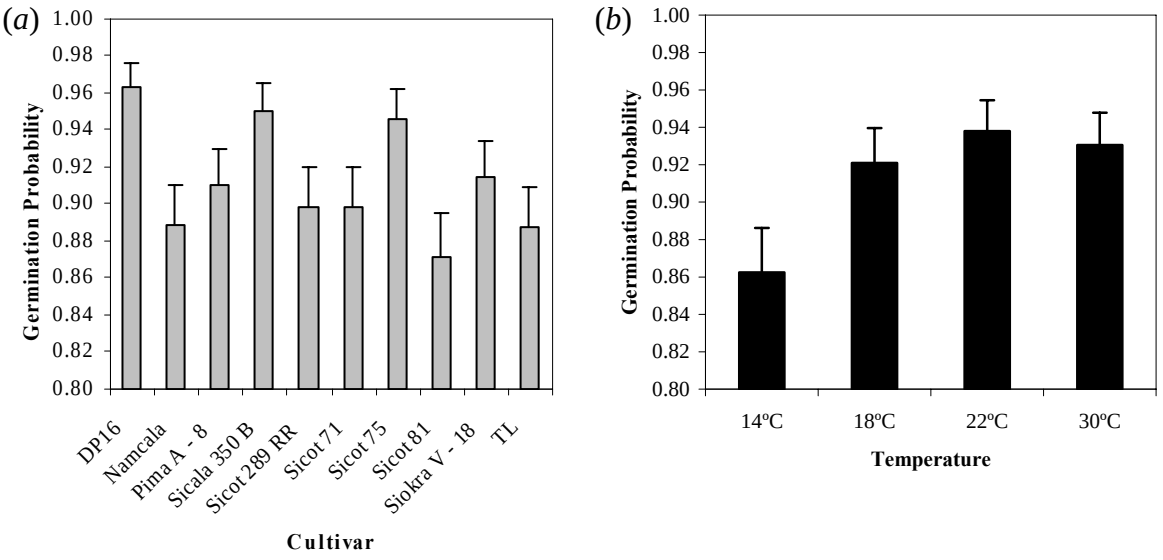


Fig. 4. Day 7 germination probability of (*a*) cultivars and (*b*) four temperature treatments (14ºC, 18ºC, 22ºC and 30ºC). Vertical bars represent l.s.d.s for (*a*) cultivar and (*b*) temperature main effect, respectively at *P*=0.05.

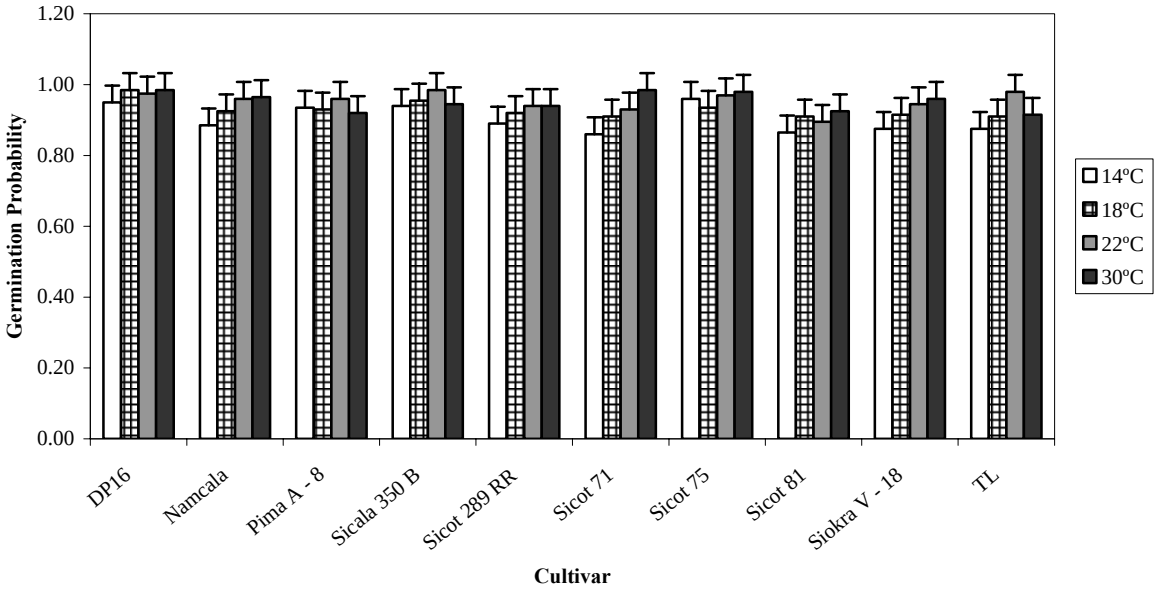


Fig. 5. Day 10 germination probability of cultivars in the four temperature treatments (14ºC, 18ºC, 22ºC and 30ºC). Vertical bars represent l.s.d.s for cultivar by temperature interaction at *P*=0.05.

Seedling length

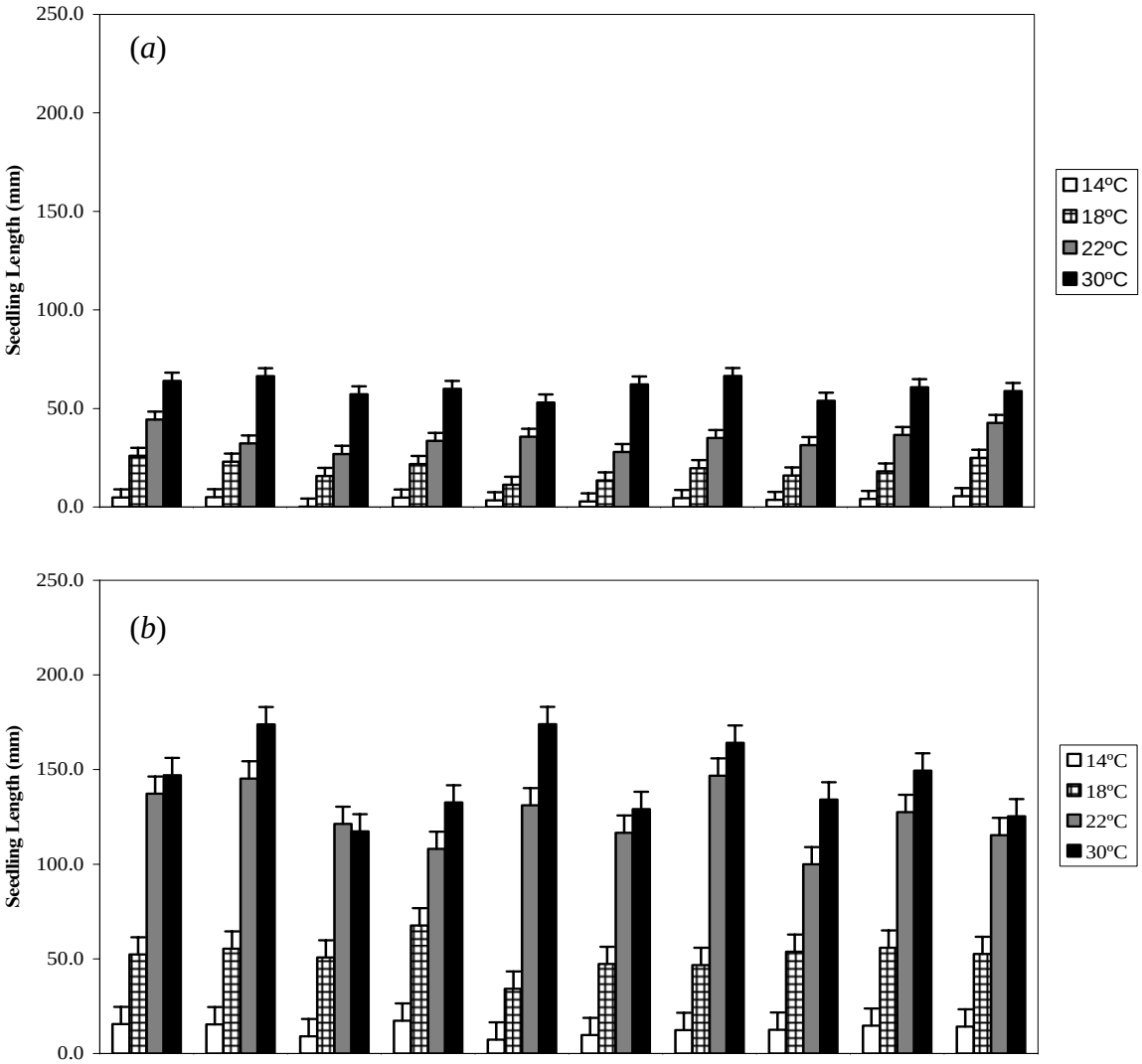
Seedling length in the germination tests showed interactions between cultivar and temperature on Day 4, 7 and 10 (*P*<0.001) (Table 3). The seedling length showed cultivar by temperature interactions on each day while the germination probability only had interactions on Day 4 and 10.

Table 3. Table of significant F-test for seedling length for cultivar (DP16, Namcala, Pima A-8, Sicala 350B, Sicot 289RR, Sicot 71, Sicot 75, Sicot 81, Siokra V-18 and TL) and temperature (14ºC, 18ºC, 22ºC and 30ºC) combinations on 4, 7 and 10 days after sowing (Appendix 3).

Day	Variety	Temperature	Variety x Temperature
4	***	***	***
7	***	***	***
10	***	***	***

* represents *P*<0.05, ** represents P<0.01, *** represents *P*<0.001 and n.s. represents not significant at *P*=0.05

All cultivars had greater seedling lengths as the temperature increased from 14ºC to 30ºC on Day 4 (Fig. 6). On Day 7, seedling lengths for all cultivars at 22ºC and 30ºC were greater than 100mm, with Namcala, Sicot 289RR and Sicot 75 having seedling lengths greater than 150mm at 30ºC (Fig 6). At 14ºC there were three cultivars, Pima A-8, Sicot 289RR and Sicot 71 under 10mm on Day 7 (Fig 6). On Day 10, the seedling length increased as temperature increased for all cultivars except Pima A-8 which decreased from 22ºC to 30ºC. Sicot 289RR had the longest seedling length at 30ºC and the shortest seedling length at 14ºC on Day 10 (Fig. 6).



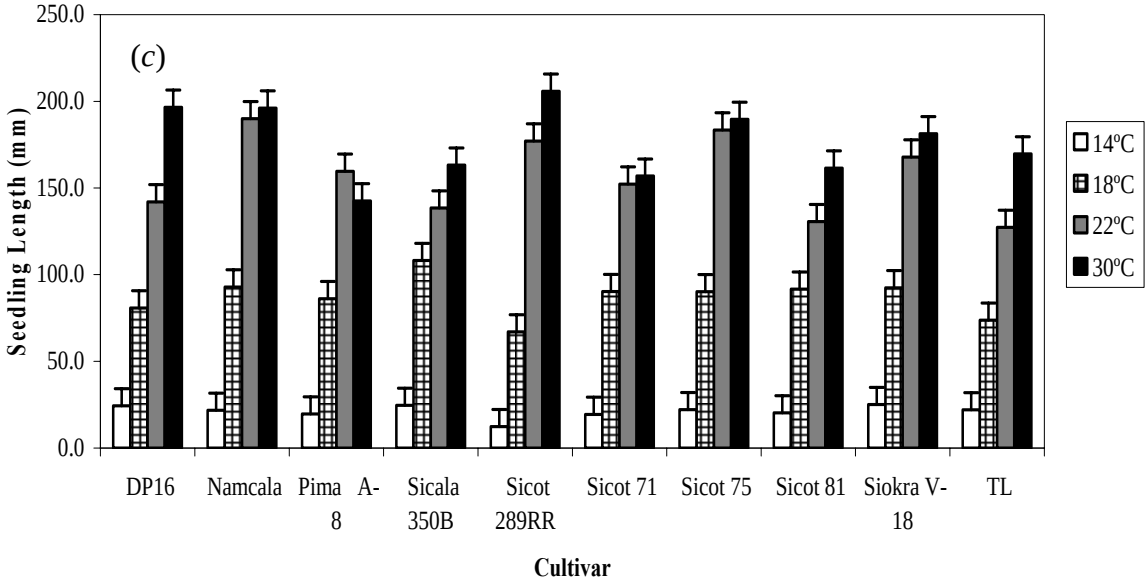


Fig. 6. (*a*) Day 4, (*b*) Day 7 and (*c*) Day 10 seedling length of cultivars (DP16, Namcala, Pima A-8, Sicala 350B, Sicot 289RR, Sicot 71, Sicot 75, Sicot 81, Siokra V-18 and TL) and temperature (14ºC, 18ºC, 22ºC and 30ºC) in the four temperature treatments (14ºC, 18ºC, 22ºC and 30ºC). Vertical bars represent l.s.d.s for cultivar by temperature interaction at *P*=0.05.

Field experiment

The field experiment had emergence counts taken 14, 21, 28 and 35 days after planting. Comparing the two sowings, there were significant differences for both sowings at 28 d after sowing (Table 4).

Table 4. F-test of emergence probability of ten cultivars in the field at Narromine planted on 23rd August and 6th September 2008 (Appendix 4)

Days After Planting	Planting Date: 23/08/08	Planting Date: 6/09/08
14	n.s.	*
21	*	n.s.
28	**	*
35	*	n.s.

* represents *P*<0.05, ** represents P<0.01, *** represents *P*<0.001 and n.s. represents not significant at *P*=0.05

The field experiment showed that cotton seeds were able to germinate at temperatures below 14°C although field emergence probability was less than 0.30 on Day 28, except for Namcala which was 0.35 (data not presented). The soil temperature at 10cm, measured at 9am daily, was below the minimum plant temperature (14°C) for the first 19 days of the 1st planting (Fig. 2), which is the reason the 1st planting took longer to emerge. The strong variation in field emergence among cultivars in the 1st planting after 28 days was due to the soil temperature increasing to just over the minimum planting temperature (14°C) for periods of the 4th week.

Regression analysis

This section presents results of the regression analysis comparing various germination and germination seedling emergence tests with field emergence (28 days after sowing).

Cool germination tests

There were no correlations between the field emergence and germination probability in the cool germination tests (14 and 18 °C). The seedling length in the cool germination test at 14ºC at 7 and 10 days was however significantly correlated with field emergence 2 at 7 and 10 days (Table 5).

Table 5. Cool germination test correlation with field emergence probability after 28 days.

	Cool	Germination Probability		Seedling Length	
Day	**Temperature**	**R^2**	***P* Value**	**R^2**	***P* Value**
4	14	0.2619	n.s.	0.3117	n.s.
7	14	0.0000	n.s.	0.5358	*
10	14	0.0274	n.s.	0.4082	*
4	18	0.1692	n.s.	0.3572	n.s.
7	18	0.0113	n.s.	0.2562	n.s.
10	18	0.0812	n.s.	0.2685	n.s.

* represents $P<0.05$, ** represents $P<0.01$, *** represents $P<0.001$ and n.s. represents not significant at $P=0.05$

Warm germination tests

The germination probability in the warm germination tests were not correlated to field emergence. The only significant correlation was between field emergence and seedling length in the warm germination test at 30ºC at 4 days (Table 6).

Table 6. Warm germination test correlation with field emergence probability after 28 days.

	Warm	Germination Probability		Seedling Length	
Day	Temperature	R^2	P-Value	R^2	P-Value
4	22	0.0001	n.s.	0.0262	n.s.
7	22	0.1144	n.s.	0.2489	n.s.
10	22	0.0566	n.s.	0.1386	n.s.
4	30	0.1186	n.s.	0.5634	*
7	30	0.2378	n.s.	0.2217	n.s.
10	30	0.2284	n.s.	0.1581	n.s.

* represents $P<0.05$, ** represents $P<0.01$, *** represents $P<0.001$ and n.s. represents not significant at $P=0.05$

Cool-Warm Vigour and Seedling Length Index

The Cool-Warm Vigour Index (Equation 1) did not provide any significant correlation with field emergence with any combination of cool or warm germination test (Table 7). However, the Cool-Warm Seedling Length Index (Equation 2) provided significant correlations with field emergence ($P<0.05$).

Table 7. Different combinations of the Cool-Warm Vigour Index and correlation with field emergence after 28 days.

Cool		Warm		Germination Probability		Seedling Length	
Day	Temperature	Day	Temperature	R^2	*P* Value	R^2	*P* Value
4	14	4	30	0.2661	n.s.	0.6326	**
4	14	7	30	0.2873	n.s.	0.2467	n.s.
4	14	10	30	0.2923	n.s.	0.1767	n.s.
7	14	4	30	0.0221	n.s.	0.7293	**
7	14	7	30	0.0497	n.s.	0.3371	n.s.
7	14	10	30	0.0381	n.s.	0.2473	n.s.
10	14	4	30	0.1031	n.s.	0.6175	**
10	14	7	30	0.1402	n.s.	0.3669	n.s.
10	14	10	30	0.1374	n.s.	0.2687	n.s.
4	14	4	22	0.2050	n.s.	0.0673	n.s.
4	14	7	22	0.2655	n.s.	0.2920	n.s.
4	14	10	22	0.2576	n.s.	0.1701	n.s.
7	14	4	22	0.0000	n.s.	0.1813	n.s.
7	14	7	22	0.0161	n.s.	0.4048	*
7	14	10	22	0.0076	n.s.	0.2419	n.s.
10	14	4	22	0.0041	n.s.	0.1840	n.s.
10	14	7	22	0.0646	n.s.	0.4006	*
10	14	10	22	0.0462	n.s.	0.2438	n.s.
4	18	4	30	0.0322	n.s.	0.5741	*
4	18	7	30	0.0161	n.s.	0.3626	n.s.
4	18	10	30	0.0344	n.s.	0.2535	n.s.
7	18	4	30	0.0718	n.s.	0.5283	*
7	18	7	30	0.1229	n.s.	0.5494	*
7	18	10	30	0.1194	n.s.	0.4291	*
10	18	4	30	0.1413	n.s.	0.4684	*
10	18	7	30	0.1991	n.s.	0.5305	*
10	18	10	30	0.2133	n.s.	0.5352	*
4	18	4	22	0.0540	n.s.	0.1599	n.s.
4	18	7	22	0.0372	n.s.	0.3802	n.s.
4	18	10	22	0.0619	n.s.	0.2718	n.s.
7	18	4	22	0.0009	n.s.	0.2566	n.s.
7	18	7	22	0.0569	n.s.	0.6538	**
7	18	10	22	0.0375	n.s.	0.3790	n.s.
10	18	4	22	0.0064	n.s.	0.4316	*
10	18	7	22	0.1111	n.s.	0.6446	**
10	18	10	22	0.0858	n.s.	0.3347	n.s.

* represents $P<0.05$, ** represents $P<0.01$, *** represents $P<0.001$ and n.s. represents not significant at $P=0.05$

When the cool temperature in the Cool-Warm Seedling Length was changed to 14ºC instead of 18ºC, the R^2 increased from 0.53 to 0.73 (P<0.01) (Fig 7). The correlation between the traditional Cool-Warm Vigour Index (cool temperature 18ºC) and field emergence (Fig 7) were not significant (P>0.05) with an R^2 of 0.07. When the outlier (Namcala cultivar) was removed, the correlation between field emergence and laboratory germination (cool temperatures, 14ºC and 18ºC) becomes significant (P<0.05) (Fig 8).

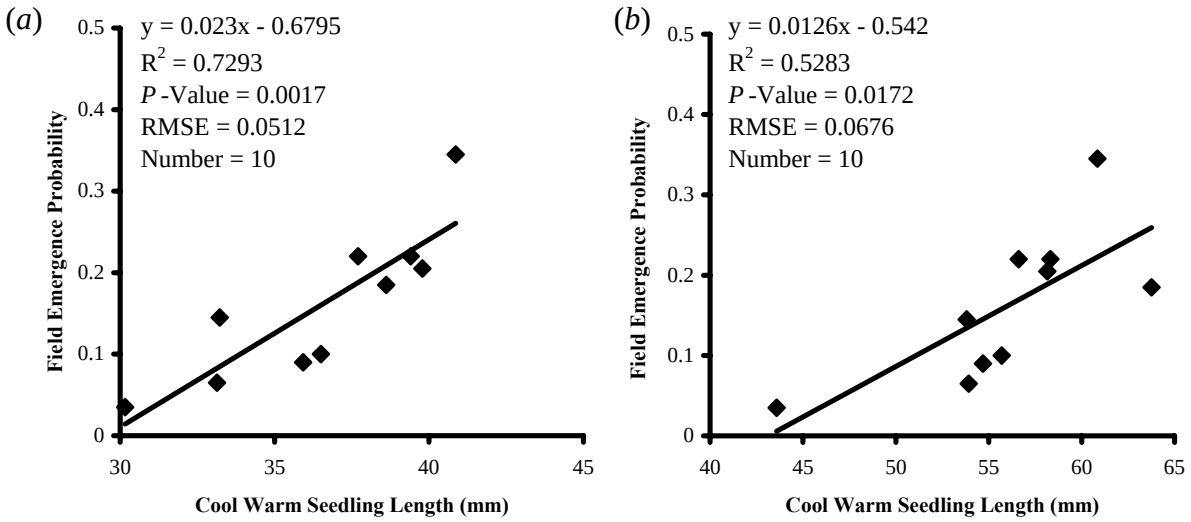


Fig. 3. (*a*) Correlation between field emergence probability and Cool-Warm Seedling Length at Day 7 at 14ºC (Cool) and Day 4 at 30ºC (Warm). (*b*) Correlation between field emergence probability and Cool-Warm Seedling Length at Day 7 at18ºC (Cool) and Day 4 at 30ºC (Warm).

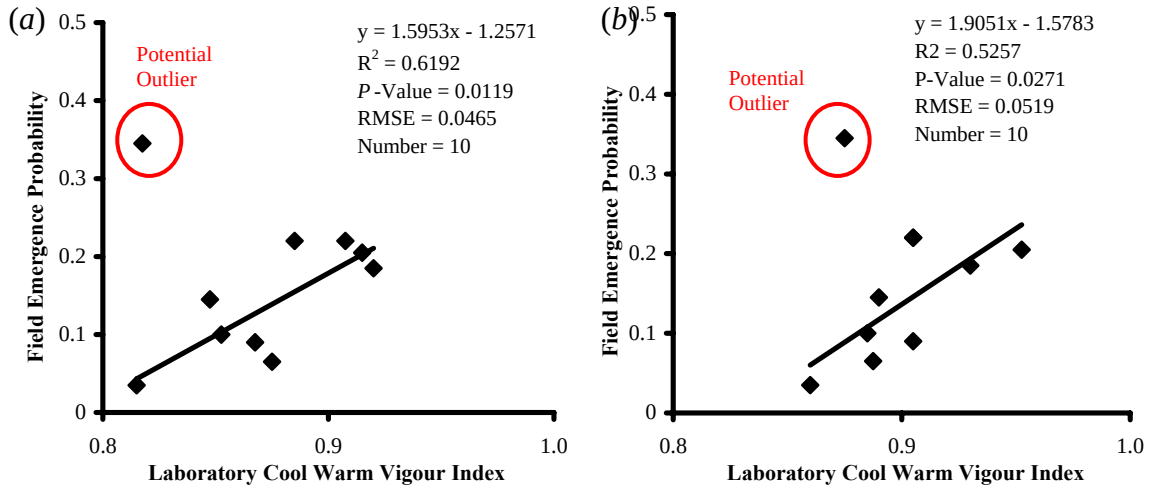


Fig. 4. (*a*) Correlation between field emergence probability and the Cool-Warm Vigour Index at Day 7 at 14ºC (Cool) and Day 4 at 30ºC (Warm) (with outlier Namcala cultivar circled). (*b*)

Correlation between field emergence probability and Cool-Warm Vigour Index at Day 7 at 18ºC (Cool) and Day 4 at 30ºC (Warm) (with outlier Namcala cultivar circled).

The Cool Warm Vigour Index correlated to field emergence probability indicated that Sicot 289 RR had the lowest tolerance of cool temperatures. DP16, Sicala 350 B, Sicot 75, Siokra V-18 and Sicot 71 cultivars all performed well in the Cool-Warm Vigour Index with an index greater then 0.90 (Fig. 9). Namcala performed better than the other cultivars with a field emergence greater then 0.30 but had a Cool-Warm Vigour Index below 0.90.

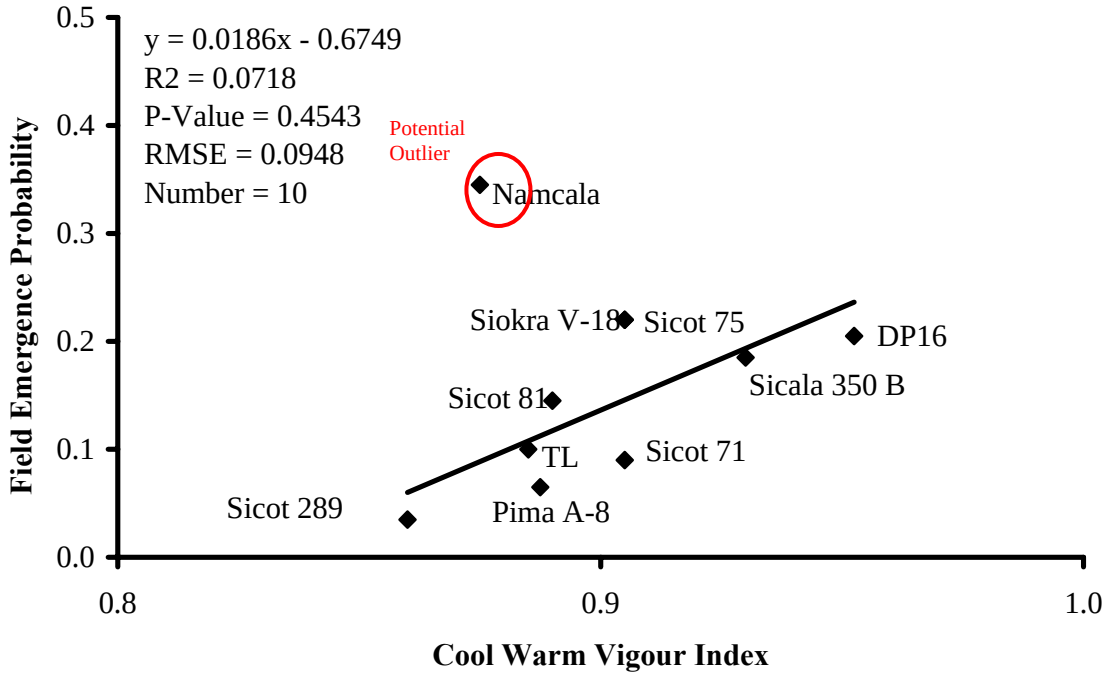


Fig. 5. Cultivar performance in the traditional Cool-Warm Vigour Index correlated to field emergence probability.

The Cool-Warm Seedling Length test also indicated that Sicot 289 RR had the lowest tolerance of cold temperatures. Sicot 289 RR, Pima A-8 and Sicot 81 all performed poorly in the Cool-Warm Seedling Length test (less than 35mm), and the field emergence probabilities were less than 0.20 (Fig. 10). DP16, Namcala and Sicot 75 performed well, with Cool-Warm Seedling Length greater than 38mm.

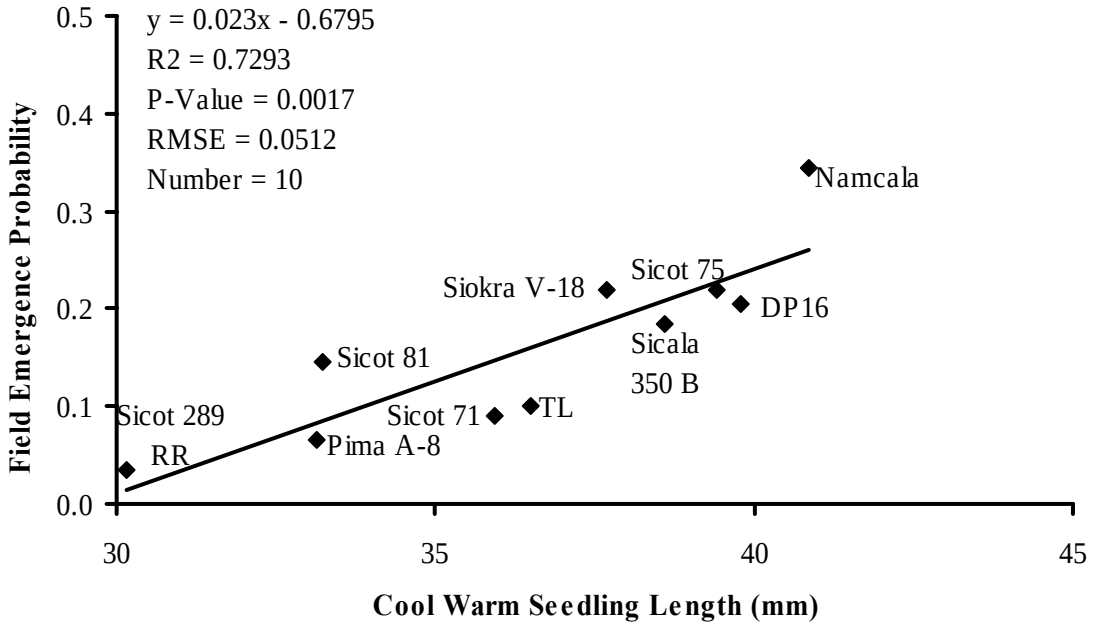


Fig. 6. Cultivar performance in the proposed Cool-Warm Seedling Length test when correlated to field emergence probability.

Electrolyte leakage test

The electrical conductivity of the electrolytes released during imbibition has a correlation with field emergence (P<0.05). There was a negative correlation as cultivars with a lower electrical conductivity reading had a greater probability of field emergence. Namcala and DP16 had EC readings of 100 µs/cm or below (Fig. 11).

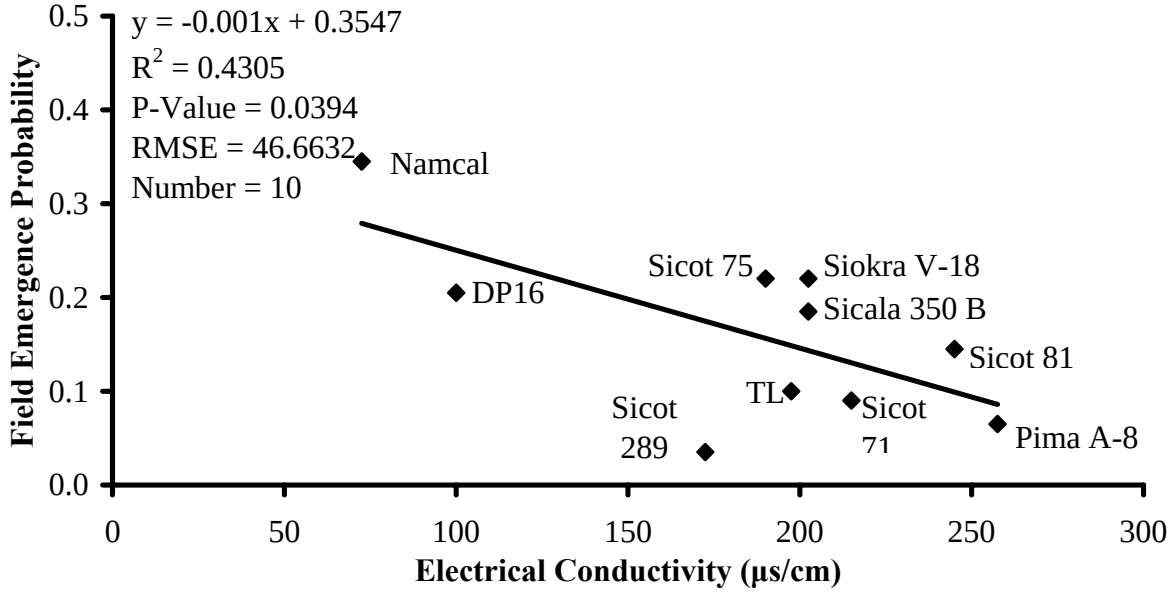


Fig. 7. Correlation between field germination and the electrical conductivity of seeds soaked for 24 hours.

Seed Weight Test

There was no correlation between the weight of 200 seeds and the field emergence probability (Fig 12).

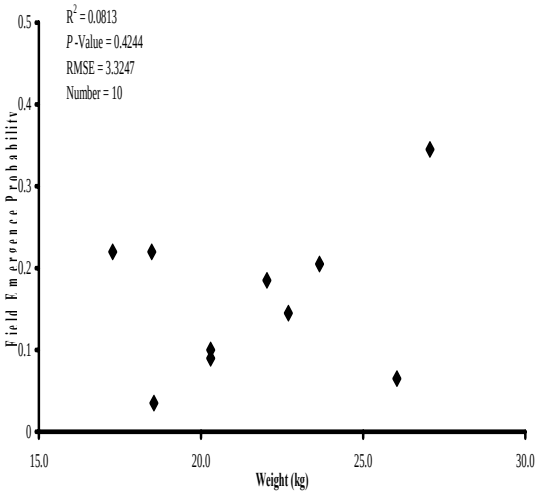


Fig. 8. Correlation of field emergence probability to the weight of 200 seeds.

Discussion

Seedling germination protocol

Germination protocols in the laboratory can be used to help assess chilling tolerance in cotton. The seedling germination protocol showed variation in cultivar response to chilling at germination. There were interactions between temperature and cultivar on Days 4 and 10. This is similar to work by Schulze *et al.* (1997) and Duesterhaus (2000) who showed there was genetic variation of cold tolerance in cotton cultivars in the USA.

Cool germination test

The standard 18ºC cool germination test has been used in many studies (Duesterhaus 2000; Hopper *et al.* 1994; Schulze *et al.* 1996). The cool germination test in the current study consisted of two different temperatures (14ºC and 18ºC), but they provided no correlation to field emergence. These results are similar to Schulze *et al.* (1996) who found no significant relationship between the cool germination test and early planted field emergence ($R^2 = 0.26$) or late planted field emergence ($R^2 = 0.31$) after 4 weeks. In contrast, Bolek (2006) reported a significant correlation between field emergence (Day 7) and germination probability at 18ºC after 7 days ($R^2 = 0.57$, $P<0.05$). Another study in Texas, USA also found a significant correlation between field emergence and the cool germination test in 1998 ($R^2 = 0.70$) but no correlation in 1999 ($R^2 = 0.20$) (Duesterhaus 2000). In 1998, a warm spring resulted in a high correlation with the cool germination test, were as in 1999 extremely stressful environmental factors resulted in a poor correlation (Duesterhaus 2000).

The cool germination test provided no correlation with field germination at 14ºC on Days 4, 7 and 10 in the current study. This is consistent with Bolek (2006) who also reported that there was no significant relationship between field emergence and germination probability at 15ºC and 13ºC. These findings were supported by Lauterbach *et al.* (1999) who suggested that chilling injury occurs in cotton seedlings whenever the temperature drops below 15ºC during germination. Cool temperatures below 20ºC may cause chilling injury to cotton seedlings and reduce germination and emergence (Cole and Wheeler 1974) and stand establishment (Duesterhaus, Hopper *et al.* 1999). A sufficient quantity of vigorous seedlings is essential to stand establishment, as this is the step in the production cycle where maximum yield is set (Wanjura 1981).

Seedling length at the 18ºC cool germination test had no significant correlation to field emergence in the current study. The seedling length in the 14ºC cool germination test successfully predicted the field emergence after 28 days. The recommended temperature for farmers to plant cotton in Australia is three consecutive days of 14ºC (Constable and Shaw 1988), however, the recommended cool germination test for cotton breeders is 18ºC (Duesterhaus *et al.* 2000; Schulze *et al.* 1997). This poises the question as to why cotton breeders use 18ºC when 14ºC would provide a better indication of cold tolerance. Hence, a cool temperature test at 14°C may provide a better indication of cold tolerance than 18°C.

Warm germination test

The warm germination test of 30°C had no correlation to field emergence in this study. Due to the early field planting in this study, almost 2 months before the recommended planting date (Muldoon 2008, *pers. comm.*), the soil temperature (Fig. 2) was below the recommended planting temperature of 14ºC for three consecutive days (Constable and Shaw 1988). This could have resulted in the lack of correlation between the warm germination test and field emergence, especially when cool temperatures are encountered during germination and emergence (Bourland 1992). Duesterhaus (2000) found significant correlations between warm germination test and field emergence in an excellent warm spring, while in the following year, cool temperatures resulted in no correlation with field emergence after 4 weeks.

The importance of using vigorous seed was evident with the strong relationship between seedling length at 30ºC on Day 4 and field emergence in the current study. Vigorous cultivars create rapidly emerging and growing seedlings that produce plants with higher boll set percentages, while low vigour causes slower seedling emergence, delayed stand establishment and reduced competitive effectiveness (Douglas *et al.* 1974). Low temperatures slow emergence and this is often associated with seedling disease, which usually results in the development of a poor cotton stand with reduced vigour (Constable 1976). Pima A-8 was the only cultivar that had a significant decrease in seedling length at 30ºC on Day 10. This suggests that *Gossypium barbadense* (Pima cotton cultivars) may have a lower optimal radicle and hypocotyl elongation temperature than *Gossypium hirsutum*, which is 34.4ºC (Wanjura and Buxton 1972a).

Cool-Warm Vigour Index

Research has indicated that a combination of the warm and cool germination test is a more reliable indicator of field performance (Buxton *et al.* 1977; Hake *et al.* 1996; Smith and Varvil 1984). Cool-Warm Vigour Index was developed by Bird and Reyes (1967) and is also known as the "Texas Cool Test". The current study also found that a combination of the cool and warm seedling length test provided a better predictor of field emergence. However, there was no correlation between field emergence and the Cool-Warm Vigour Index in this study. There was no correlation ($R^2 = 0.07$) between the Cool-Warm Vigour Index and field emergence before an outlier, Namcala cultivar was removed. However, this suggests that this index is not robust as there was no real physiological reason to remove Namcala. The relationship of Cool-Warm Vigour Index with field emergence (with outlier Namcala cultivar removed) had a stronger correlation when the 14ºC ($R^2 = 0.62$) was used instead of 18ºC ($R^2 = 0.53$). Similarly the Cool-Warm Seedling Length increased R^2 from 0.53 to 0.73 when 14ºC was used instead of 18ºC in the correlation with field emergence. This indicates that the cool temperature in the Cool-Warm Vigour Index and the Cool-Warm Seedling Length should be 14ºC. Similar to this study, Klos and Brummer (2000) found that seedling height of lucerne was a better trait than germination time to predict field performance when the traits are measured in a laboratory or greenhouse.

Combinations of the Cool-Warm Vigour Index for both the seedling length and germination probability were better at predicting field emergence than either the cool germination test or warm germination test on their own. The top four predictors for seedling length and germination were combinations of cool and warm temperature tests. This is supported by Kerby *et al.* (1989) who concluded that the Cool-Warm Vigour Index was a better indicator of field emergence than either component alone. Duesterhaus (2000) found that the Cool-Warm Vigour Index was a good predictor of field stand establishment ($R^2 = 0.80$) when there were excellent conditions at planting, while adverse conditions at planting resulted in no correlation ($R^2 = 0.22$). This indicates that the Cool-Warm Vigour Index may be a good predictor of field emergence under ideal conditions, but may not be as good a predictor under cool conditions.

Cold tolerant cultivars

The proposed 'Tuck Test' (Cool-Warm Seedling Length index at a cool temperature of 14°C on Day 7 and warm temperature of 30°C on Day 4) provided the best correlation between field emergence and seedling length ($R^2 = 0.73$). The poor performance of Sicot 289 RR in the Cool-

Warm Seedling Length test and field emergence under chilling conditions is due to it being a bred for a tropical climate (Table 1). The tolerance of Pima A-8 (*Gossypium barbadense*) to chilling conditions is low, similar to low chilling tolerance reported in many pima cultivars (Bolek 2006; Buxton *et al.* 1976). Siokra V-18, Sicala 350B, Sicot 75, Namcala and DP16 cultivars performed well in the field emergence with Cool-Warm Seedling Lengths greater than 38mm and these cultivars could be recommend to farmers looking to plant early in the season. Cultivars that have a Cool-Warm Seedling Length greater then 40mm are considered to have excellent cold tolerance, with Namcala being the only cultivar to achieve this. Namcala was bred in Arizona, USA in an arid and semi-arid climate that experiences extreme hot and cold temperatures.

Electrolyte leakage test

The electrolyte leakage test provided a correlation between field emergence after 28 days and sugars released during the first 24 hours of imbibition. However, the R^2 was below 0.50 indicating that this relationship was not strong. This work agrees with Schulze *et al.* (1996) who also found there was a negative correlation (Early planting R^2 = -0.31; late planting R^2 = -0.61) with field emergence after 4 weeks. Schulze *et al.* (1996) also suggested that the electrical conductivity test was a good indicator of chilling tolerance in cotton and the low R^2 values were probably due to other uncontrolled field variables other than temperature. Similar this study, Yaklich *et al.* (1979) reported that field emergence and vigour in soybeans were negatively related to electrolyte leakage. Both the electrolyte leakage test and the “Tuck Test” (Cool-Warm Seedling Length) consistently showed that Namcala and DP16 have greater tolerance of chilling temperatures.

Weight Test

The weight test provided no correlation with field emergence after 28 days. Hopper *et al.* (1994) found that seed weight had no correlation with field emergence, but instead was significantly correlated to lint length (R^2 = 0.45) and strength (R^2 = 0.61). Larger seeds tend to produce longer and stronger fibres (Hopper *et al.* 1994).

Further work

Future studies are needed to:

- Test more cultivars to determine the accuracy of the Cool-Warm Seedling Length test (Tuck Test).
- Grow seeds from the same batch of seeds produced in the same year so that it can be confirmed that seedling vigour and cold tolerance is due to the cultivar, and not confounded by seed storage time and conditions.

Conclusion

From this investigation into the cold tolerance of cotton it can be concluded:

- A cool temperature of 14°C will provide a better indication of cold tolerance than 18°C, which is currently used by industry as a predictor of cold tolerance.
- Seedling length provides a better indication of cold tolerance than germination probability.
- The “Tuck Test” (Cool-Warm Seedling Length at a cool temperature of 14°C on Day 7 and warm temperature of 30°C on Day 4) provided the best correlation (R^2 = 0.73) to field emergence under cool conditions.
- When testing for cold tolerance, field experiments need to be planted early to ensure seeds or seedlings encounter a cold snap or drop in temperature similar to those experienced in cooler cotton producing area.
- The electrical conductivity test provides a negative correlation with field emergence under cool conditions.

Acknowledgments

I would like to thank Dr Daniel Tan for his guidance, enthusiasm and patience during this study. Without his assistance and encouragement this thesis would never have been possible. I would especially like to thank Dr Mike Bange and Dr Warwick Stiller for their guidance, support and help with germination protocol, access to cotton germplasm and manuscript editing. The work received partial financial support from a summer scholarship from the Cotton Catchment Communities Cooperative Research Centre. I would also like to thank Jane Caton, Jo Price, Dave Shann, and Nicola Cottee and others from CSIRO for technical assistance and advice.

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Reference

ABARE (2007) 'Australian Commodity Statistics.' Australian Bureau of Agricultural and Resource Economics, Canberra.

Bange MP, Milroy SP (2004) Impact of short-term exposure to cold night temperatures on early development of cotton (*Gossypium hirsutum* L.). *Australian Journal of Agricultural Research* **55**, 655-664.

Bird LS, Reyes AA (1967) Effects of cottonseed quality on seed and seedling characteristics. In 'Proceedings of the Beltwide Cotton Production Research Conference' pp. 199-206

Bolek Y (2006) Predicting cotton seedling emergence for cold tolerance: *Gossypium barbadense*. *Journal of Agronomy* **5**, 461-465.

Borth TD, Krieg DR, Jividen GM (1997) Genetic and environmental factors affecting the fatty acid composition of polar and non-polar lipids of cottonseed. In 'Proceedings of the Beltwide Cotton Conference' pp. 1455-1458

Bourland FM (1992) Characterization and improvement of seed and seedling vigor in cotton. In 'Proceedings of the Beltwide Cotton Conference'. Memphis, Tennessee pp. 1326-1329. (National Cotton Council)

Burke J, Boman R, Dotray P, McMichael BL, Kaufman H, Oosterhuis DM, Zak J, Wheeler T, Hopper NW (2008) Cotton Root Disorder. In. (Ed. B Calame). (Cotton Incorporated: Lubbock, Texas)

Buxton DR, Melick PJ, Patterson LL, Pegelow EJ (1977) Relationships among cottonseed vigor and emergence. *Agronomy Journal* **69**, 677-681.

Buxton DR, Sprenger PJ (1976) Genetic variability for cottonseed germination at favourable and low temperatures. *Crop Science* **16**, 243-246.

Buxton DR, Sprenger PJ, Pegelow EJ (1976) Periods of chilling sensitivity in germinating pima cottonseed. *Crop Science* **16**, 471-474.

Ceeney S (2008) 'Soil Temperatures; Day degree accumulation; Strategic management of weeds in a flex system.' NSW Department of Primary Industries, Trangie.

Christiansen MN (1967) Periods of sensitivity to chilling in germinating cotton. *Plant Physiology* **42**, 431-433.

Christiansen MN (1968) Induction and prevention of chilling injury to radicle tips of imbibing cottonseed. *Plant Physiology* **43**, 743-746.

Christiansen MN, Rowland R (1981) Cotton physiology – Seed and germination. In 'Proceedings of the Beltwide Cotton Conference'. New Orleans, LA pp. 315-318. (National Cotton Council of America)

Cole DF, Wheeler JE (1974) Effect of pregermination treatments on germination and growth of cottonseed at suboptimal temperatures. *Crop Science* **14**, 451-454.

Constable GA (1976) Temperature effects on the early field development of cotton. *Australian Journal of Experimental Agriculture and Animal Husbandry* **16**, 19-24.

Constable GA, Shaw AJ (1988) 'Temperature requirements for cotton.' New South Wales Department of Agriculture and Fisheries P5.3.5.

Douglas AG, Flores JA, Andrews CH (1974) Effects of seed vigor and competition on performance of cotton. In 'Proceeding of the Beltwide Cotton Production Research Conference'. Memphis, Tennesse pp. 75-77. (National Cotton Council)

Duesterhaus B (2000) Laboratory screening test for the evaluation of cold tolerance in cotton. Texas Tech University.

Duesterhaus B, Hopper NW, Gannaway JR, Jividen GM (1999) Development of a laboratory screening test for the evaluation of cold tolerance in cotton seed germination. In 'Proceeding of the Beltwide Cotton Conference'. Memphis, Tennessee pp. 621-623. (National Cotton Council)

Duesterhaus B, Hopper NW, Gannaway JR, Valco TD (2000) A screening test for the evaluation of cold tolerance in cottonseed germination and emergence. In 'Proceedings of the Beltwide Cotton Conference'. Memphis, Tennessee pp. 596-599. (National Cotton Council)

Hake SJ, Kerby TA, Hake KD (1996) 'Cotton production manual.' (Universtiy of California, Division of Agriculture and Natural Resources)

Hall AJ, Gannaway JR (2005) Evaluation of fatty acid composition of cotton germplasm and association with cold tolerance. In 'Proceeding of the Beltwide Cotton Conference'. New Orleans, Louisiana pp. 923-931

Hopper NW, Gannaway JR, Jividen GM, Struve TH (1994) Selection of cold tolerant lines of cotton based on seedling response. In 'Proceeding of the Beltwide Cotton Conference'. Memphis, Tennessee p. 1375. (National Cotton Council)

Kerby TA, Keeley M, Johnson S (1989) Weather and seed quality variables to predict cotton seedling emergence. *Agronomy Journal* **81**, 415-419.

Klos KLE, Brummer EC (2000) Field response to selection in alfalfa for germination rate and seedling vigor at low temperatures. *Crop Science* **40**, 1227-1232.

Lauterbach B, Krieg DR, Jividen GM (1999) Fatty acid composition of lipid fractions in germinating cotton as affected by temperature. In 'Proceedings of the beltwide cotton conference'. Memphis, Tennessee pp. 564-565. (National Cotton Council of America)

McMahon J, Low A (1972) Growing degree days as a measure of temperature effects on cotton. *Cotton Growing Review* **49**, 39-49.

Schulze DH, Hopper NW, Gannaway JR, Jividen GM (1996) Laboratory tests used to screen for chilling tolerance in cotton genotypes. In 'Proceedings of the Beltwide Cotton Conference'. Memphis, Tennessee pp. 1240-1243. (National Cotton Council)

Schulze DH, Hopper NW, Gannaway JR, Jividen GM (1997) Evaluation of chilling tolerance in cotton genotypes. In 'Proceedings of the Beltwide Cotton Conference'. Memphis, Tennessee pp. 1383-1385. (National Cotton Council)

Smith CW, Varvil JJ (1984) Standard and cool germination tests compared with field emergence in upland cotton. *Agronomy Journal* **76**, 587-589.

Stamp P (1984) Chilling tolerance of young plants demonstrated on the example of maize (*Zea mays* L.). *Advances in Agronomy and Crop Science 7*, 1-83.

Wanjura DF (1981) Germination and strand establishment. In 'Proceeding of the Beltwide Cotton Conference'. pp. 321. (National Cotton Council Memphis, Tennessee)

Wanjura DF, Buxton DR (1972a) Hypocotyl and radicle elongation of cotton as affected by soil environment. *Agronomy Journal* **64**, 431 - 434.

Wanjura DF, Buxton DR (1972b) Water uptake and radicle emergence of cottonseed as affected by soil moisture and temperature. *Agronomy Journal* **46**, 427 - 431.

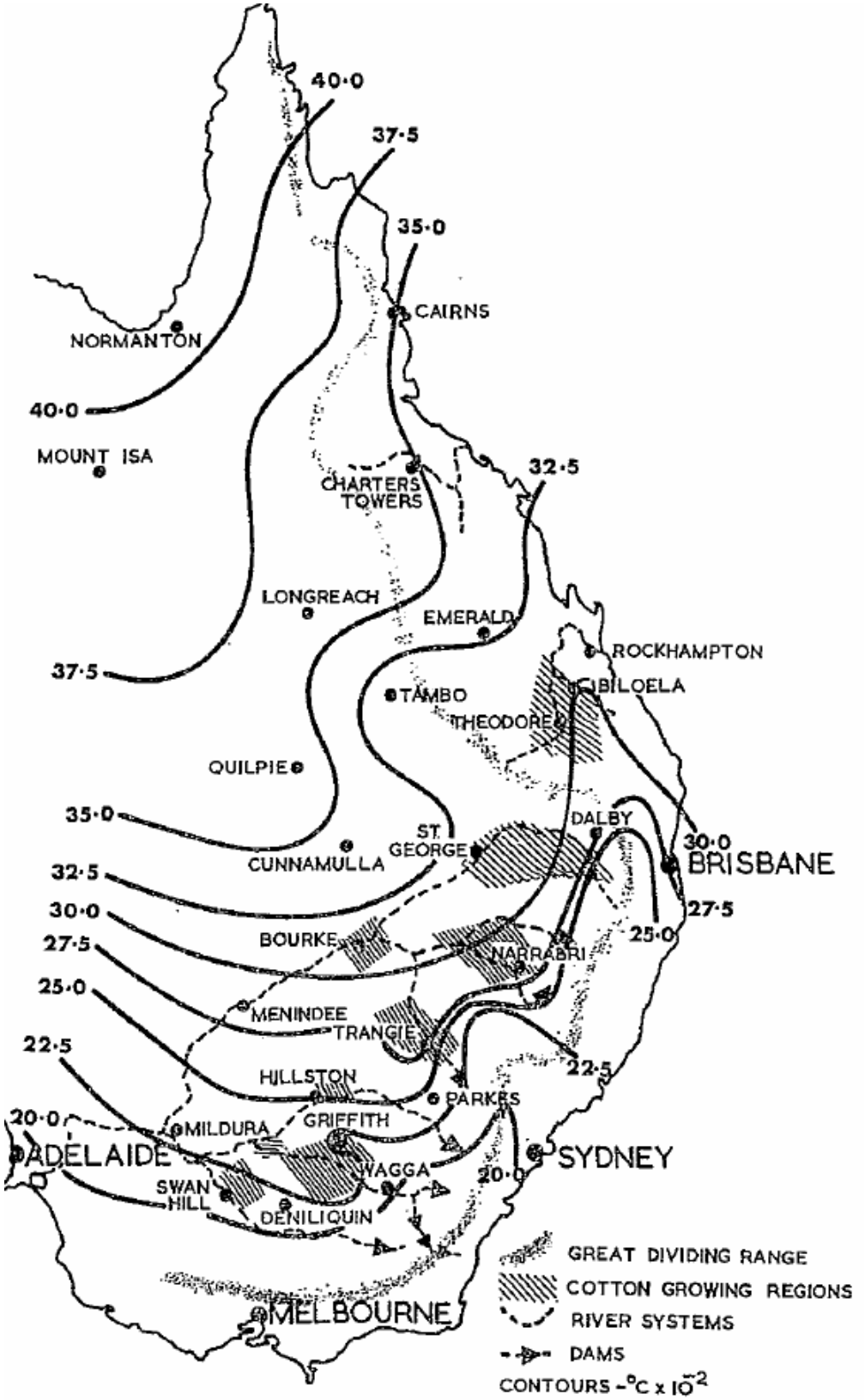
Wanjura DF, Hudspeth EBJ, Bilbro JDJ (1969) Temperature effect on emergence rate of cotton *(Gossypium hirsutum* L.) under field situations. *Agronomy Journal* **61**, 387-389.

Yaklich RW, Kulik MM, Anderson JD (1979) Evaluation of vigor tests in soybean seeds: relationship of ATP, conductivity, and radioactive tracer multiple criteria laboratory tests to field performance. *Crop Science* **19**, 806-810.

Appendices

Appendix 1

Map of Eastern Australia showing Growing Degree Day contours (McMahon and Low 1972)



Appendix 2

Day 4 Germination binomial

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Variety	9	390.750	43.417	43.42	**<.001**
+ Temperature	3	1216.550	405.517	405.52	**<.001**
+ Variety.Temperature	27	357.139	13.227	13.23	**<.001**
Residual	120	136.924	1.141		
Total	159	2101.362	13.216		

Day 7 Germination binomial

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Variety	9	92.512	10.279	10.28	**<.001**
+ Temperature	3	83.643	27.881	27.88	**<.001**
+ Variety.Temperature	27	38.762	1.436	1.44	**0.067**
Residual	120	140.877	1.174		
Total	159	355.795	2.238		

Day 10 Germination binomial

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Variety	9	65.982	7.331	7.33	**<.001**
+ Temperature	3	53.617	17.872	17.87	**<.001**
+ Variety.Temperature	27	45.974	1.703	1.70	**0.013**
Residual	120	151.865	1.266		
Total	159	317.438	1.996		

Appendix 3

Analysis of variance – Day 4 Seedling length

Variate: Day_4_Root_Length_mm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	3	69657.003	23219.001	2718.31	**<.001**
Variety	9	1632.959	181.440	21.24	**<.001**
Temperature.Variety	27	1270.223	47.045	5.51	**<.001**
Residual	120	1025.005	8.542		
Total	159	73585.190			

Analysis of variance – Day 7 Seedling length

Variate: Day_7_Root_Length_mm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	3	459218.21	153072.74	3601.94	**<.001**
Variety	9	9255.89	1028.43	24.20	**<.001**
Temperature.Variety	27	17357.40	642.87	15.13	**<.001**
Residual	120	5099.68	42.50		
Total	159	490931.18			

Analysis of variance – Day 10 Seedling length

Variate: Day_10_Root_Length_mm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	3	599956.07	199985.36	3992.45	**<.001**
Variety	9	11974.31	1330.48	26.56	**<.001**
Temperature.Variety	27	26460.72	980.03	19.56	**<.001**
Residual	120	6010.90	50.09		
Total	159	644402.00			

Appendix 4
Planting date 23/08/08

Day 14 Field germination binomial

There no emergence of any cultivar

Day 21 Field germination binomial

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Variety	9	69.194	7.688	2.23	**0.048**
Residual	30	103.227	3.441		
Total	39	172.422	4.421		

Day 28 Field germination binomial

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Variety	9	118.081	13.120	3.18	**0.008**
Residual	30	123.916	4.131		
Total	39	241.997	6.205		

Day 35 Field germination binomial

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Variety	9	132.874	14.764	2.48	**0.030**
Residual	30	178.570	5.952		
Total	39	311.444	7.986		

Planting date 06/09/08

Day 14 Field germination binomial

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Variety	9	42.701	4.745	4.74	**<.001**
Residual	30	51.728	1.724		
Total	39	94.429	2.421		

Day 21 Field germination binomial

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Variety	9	61.881	6.876	1.32	**0.266**
Residual	30	155.762	5.192		
Total	39	217.643	5.581		

Day 28 Field germination binomial

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Variety	9	73.099	8.122	2.64	**0.022**
Residual	30	92.382	3.079		
Total	39	165.481	4.243		

Day 35 Field germination binomial

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Variety	9	35.260	3.918	0.68	**0.720**
Residual	30	172.636	5.755		
Total	39	207.896	5.331		

Impact of cold tolerance on germination and establishment of cotton (*Gossypium hirsutum*) and other summer crops – A review

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ABSTRACT

Temperature is the dominant environmental factor influencing cotton crop development and yield in Australia. Suboptimal temperatures reduce germination of cotton seed and seedling emergence. Cotton is considered a chilling sensitive plant because of its tropical origin. Chilling tolerant plants are able to tolerate temperatures between 0°C and 15°C, while chilling sensitive plants are susceptible to damage at these temperatures. Cotton seeds with low vigour experience slower seedling emergence, delayed stand establishment and reduced competitiveness. Suboptimal temperature affect the whole production cycle of cotton, from sowing to harvest. Cotton seed is susceptible to chilling injury from the initial imbibition of water. The morphological stages of a cotton plant have differing sensitivities to suboptimal or chilling temperature.

Phosphorus deficiency occurs when a cotton plant experiences a cool, wet and early start to the season. There are two main systems of nutrient uptake in a cotton plant, passive and active. The optimum soil temperature for cotton root growth is between 28°C and 35°C. At low temperatures, root growth of cotton is reduced, less branching occurs, there is reduced water uptake and nutrient uptake is altered. Temperatures of 5°C to 10°C during imbibition kill the embryo or cause abnormal development of the seedling, such as sloughing of the root cortex or formation of nub-roots symptoms, depending on the time of chilling.

Respiration in cotton plants is a temperature-sensitive process, which in turn affects photosynthesis and growth. There is genetic variation in cotton to chilling tolerance and there has been limited work on cotton plants and their acclimation to chilling temperatures. Planting date is determined by the soil temperature with a minimum of 14°C, required for 3 days. Temperature affects both the sowing date and the development of a cotton plant while also influencing its growth.

The warm germination test is a poor indicator of emergence if cooler temperatures are experienced during planting. The Texas cool test is good to establish seedling vigour, as the development of vigorous emergence and uniform establishment is paramount to maximising yields. Vigorous cotton cultivars with good seed quality have warm germination test (30°C) values greater then 80% and cool germination test (18°C) values greater then 60%. The metabolic chilling test establishes the response of cotton cultivars to temperatures below the efficient thermal window for metabolic activity. The metabolic chilling test and the imbibition chilling test are combined to provide different cultivars with a rating of their overall chilling tolerance. Initial injury stems from the imbibition of cold water, while secondary injury can occur 18 to 24 hours after the initiation of germination if temperatures remain below 18°C. Imbibition is an important part of germination as it is responsible for activating the metabolic processes of germination due to the number of hydrolytic enzymes involved. During the imbibition of water by the seed, there is also leakage of substances that contain mostly sugars with various organic and amino acids.

Attempts to develop a test that predicts field emergence under chilling stress have only been able to partially predict seedling emergence and vigour. Having an objective method for selecting chilling tolerance would be helpful to cotton breeders in cultivar development as well as provide producers with a choice of chilling tolerant cultivars for vigorous and uniform plant stands in early season plantings, hence maximising yield potential.

Key words: Chilling injury, imbibition, cotton, germination, establishment, screening techniques

I. INTRODUCTION

Cotton (*Gossypium hirsutum*) is a chilling-sensitive plant. Cotton production throughout the world is limited by the number of heat units available during the growing season. Temperature is the dominant environmental factor influencing crop development and yield (Constable & Shaw, 1988). The average frequency of cold shocks (minimum temperature ≤11°C) in major Australian cotton producing regions during the early growth of cotton (15th September – 30th November) ranges from 40 days at Hillston (NSW) to 4 days in Emerald (QLD) (Bange & Milroy, 2004).

Chilling injury can occur in cotton seedlings whenever the temperature drops below 15°C for a few hours during the first few days of germination (Lauterbach, Krieg & Jividen, 1999). Temperatures below 20°C reduce germination of cotton seed and seedling emergence (Cole & Wheeler, 1974). This emphasises the importance of developing cultivars with chilling tolerance as this will allow cultivars to withstand the cold shocks during early cotton growth (15th September – 30th November), allowing better establishment under cool conditions to reduce the costly need to replant.

(1) Cotton in Australia

There are two main cotton production states in Australia, New South Wales (NSW) and Queensland (QLD). The major cotton producing areas in New South Wales extend from the Macintyre River Valley in the north, to the Macquarie River Valley in the south and include the Gwydir and Namoi River Valleys (Cotton Australia, 2008). Cotton is also grown along the Barwon and Darling River in western New South Wales and the Lachlan and Murrumbidgee Rivers in southern New South Wales (Cotton Australia, 2008). While there is also some cotton produced in central Queensland near Emerald, Theodore and Biloela, most of the cotton production is in southern Queensland on the Darling Downs, St George, Dirranbandi and Macintyre Valley regions (Cotton Australia, 2008). Figure 1 shows the major cotton growing regions in Australia.

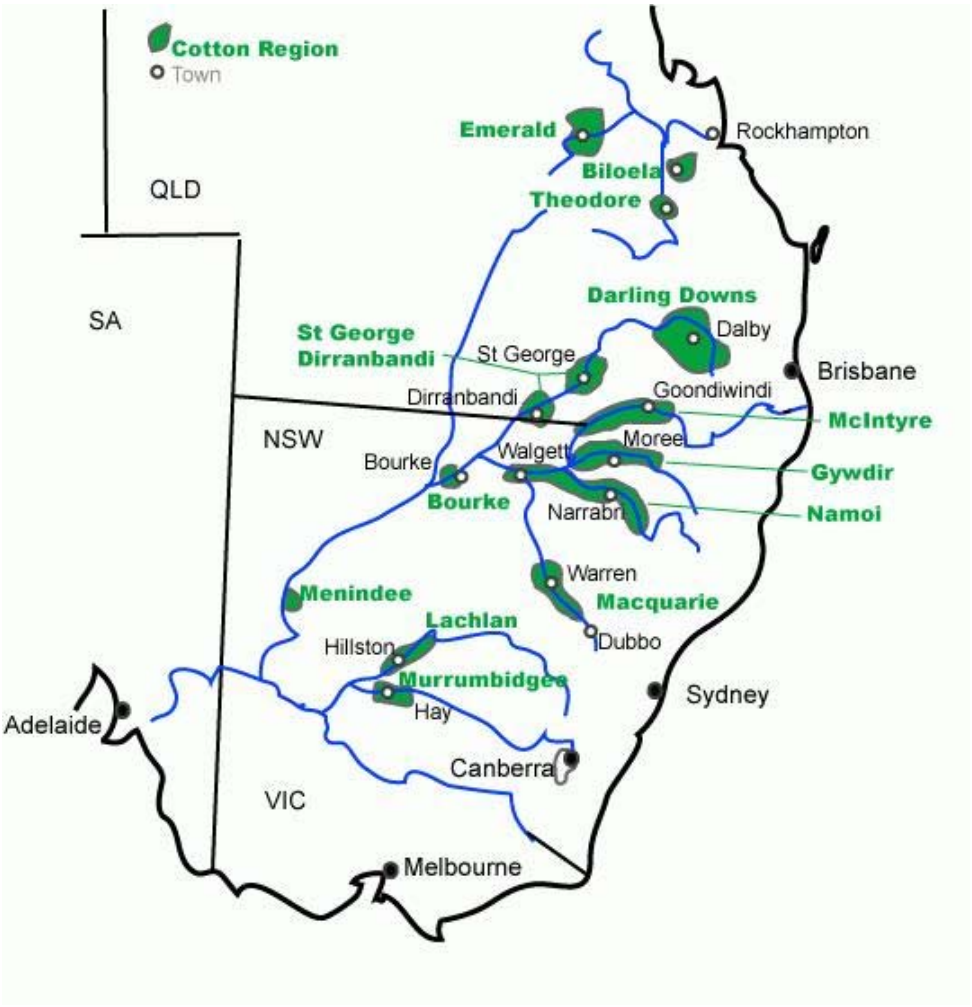

Figure 1. A map of South Eastern Australia showing the major cotton producing area

The Australian cotton crop in 2005-06 was 90% transgenic varieties with the remainder made up of conventional varieties and Pima cotton (Extra Long Staple cotton), with 84% of the 2005-06 crop grown under irrigation (Cotton Australia, 2008). In 2006-07, there were 108,700 ha harvested in New South Wales with lint yield averaging 8.9 bales/ha, and 34,900 ha harvested in Queensland with lint yield averaging 6.9 bales/ha (ABARE, 2007). Australia produced 274,200 tons of cotton, valued at $471.2 million, in 2006-07 (ABARE, 2007). The total cotton area harvested in the world in 2006-07 was 34.73 million ha with 26.62 million tons of lint produced world wide and lint yield averaging 3.4 bales/ha (ABARE, 2007).

Australia is regarded as a supplier of high quality cotton. The four main countries Australia exported to in 2006-07 were China (163,000 tons), Indonesia (126,400 tons), Thailand (75,000 tons) and Republic of Korea (53,800 tons), with exports totaling 486,500 tons in 2006-07 (ABARE, 2007). Australia's exports of cotton in 2006-07 were valued at $823.4

million down from $1,137.4 million in 2005-06 (ABARE, 2007). The major importer in 2006-07 was China with 2.3 million tons of cotton even though it produced 7.7 million tons domestically (ABARE, 2007). China is a key player in the global cotton industry as imports and production account for 38 % of the total 26.62 million tons of cotton lint produced in the world in 2006-07.

(2) Australian climate

Temperature is the dominant environmental factor influencing cotton production with longer, hotter summers providing the opportunity for higher yields. The temperatures experienced during the growing season has a major influence on sowing dates, growth rates, fruiting, yield and fibre quality (Constable & Shaw, 1988). The Australian growing season is determined by the last frost in spring and the first frost in autumn, as cotton is a frost sensitive plant (Hearn & Constable, 1984). Table 1 shows the three different cotton producing zones in Australia based on average daily growing degree days (McMahon & Low, 1972).

Table 1. Climatic data for Local Government Areas in Australian cotton producing regions (Bange & Milroy, 2004; BOM, 2008; McMahon & Low, 1972).

Local government areas	Cotton production zone	Average daily maximum / minimum temperature (°C) (Summer)	Number of cold shocks
Bourke, NSW	Hot	35.6 / 20.3	26
Dalby, QLD	Central	31.7 / 18.1	22
Emerald, QLD	Hot	34.1 / 21.0	4
Goondiwindi, QLD	Central	33.6 / 19.4	37
Gunnedah, NSW	Cool	33.3 / 17.7	33
Hillston, NSW	Cool	32.6 / 17.6	40
Moree, NSW	Central	34.4 / 18.7	25
Narrabri, NSW	Central	33.3 / 18.7	30
St George, QLD	Hot	34.1 / 20.9	16
Warren, NSW	Central	33.0 / 17.8	37

Day degrees are heat units calculated daily and accumulated from planting to harvest which is used to estimate crop development. The degree day calculation (see formula below) for

estimating cotton development in Australia uses a base temperature of 12°C (Constable 1976; Constable and Shaw 1988).

$$DayDegrees = \frac{(T_{Max} - 12) + (T_{Min} - 12)}{2}$$

T_{max} = Maximum daily temperature,

T_{min} = Minimum daily temperature, when

$T_{min} \leq 12°C$, then $T_{min} = 12$

There are several studies that agree with the equation proposed by Constable and Shaw (1988) and show that there is a relationship between cotton development and air temperature during the growing season (McMahon & Low, 1972; Roussopoulos, Liakatas & Whittington, 1998). This relationship can be shown as heat units (HU) or growing degree days (GDD). A cotton crop needs a certain number of accumulated day degrees to reach the different developmental stages in a cotton production cycle (Table 2) (Constable & Shaw, 1988).

Table 2. The cumulative day-degrees for different developmental stages of cotton (Constable & Shaw, 1988).

Crop Stage	Cumulative Day-degrees
Emergence	80
5 true leaves	330
First square	505
First flower	777
Peak flower	1302
Open boll	1527
60% open bolls	2050

The base temperature used to predict the number of day-degrees in Australia is 12°C (Constable & Shaw, 1988), which was suggested from the work by Constable (1976). Although more recent work by Bange and Milroy (2001; 2004) has proposed that the base temperature for predicting cotton production in Australia is closer to 15°C than to 12°C. The suggested change would see the base temperature used in Australia (12°C) move closer to the

base temperature of 15.5°C used in the USA (Mauney, 1986; Oosterhuis, 1990). Singh *et al.*, (2007) proposed that the germination of cotton had a base temperature closer to 12°C, while the development of cotton has a different base temperature of 15.5°C, as suggested by Oosterhuis (1990).

The Growing Degree Day (GDD) contours, illustrated in Figure 2, shows that the northern and western regions of Eastern Australia are hotter than the eastern and southern regions. McMahon and Low (1972) used the base temperature of 10°C, rounded up from 9.72°C, when developing the growing degree days as a measure of temperature effect on cotton.

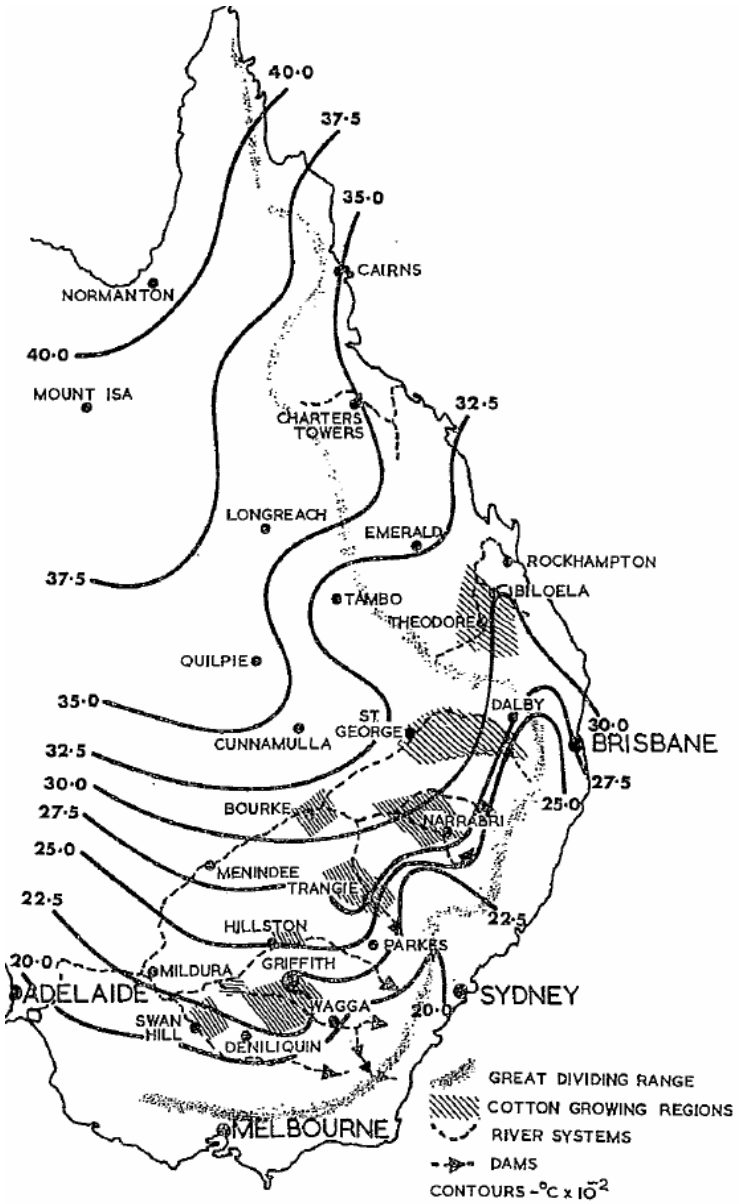

Figure 2. Map of Eastern Australia showing Growing Degree Day contours (1972).

(a) Cold shock in Australia

In the southern cotton growing regions of Australia, there can be up to 40 cold shocks during establishment and early development of cotton (15th of September to 30th of November) (Bange & Milroy, 2004). Cold shock is defined as an event where the minimum daily temperature falls to 11°C or less, and assumes chilling injury. The plant's growth and development the following day is reduced, regardless of the maximum temperature reached that day (Hearn & Constable, 1984; McDowell, Bange & Tan, 2007). Each cold shock event causes an increase of 5.2 day degrees, in the number of day degrees required by the plant to reach flowering (Constable & Shaw, 1988).

(b) Chilling and freezing

Chilling injury is a physiological disorder that can affect chilling sensitive plants at any developmental stage (Wang, 1990). Cotton is considered a chilling-sensitive plant because of its tropical origin (Wang, 1990). Chilling-tolerant plants are able to tolerate temperatures between 0°C and 15°C, while chilling sensitive plants are susceptible to damage at these temperatures (Wang, 1990).

Chilling tolerance refers to the plants' ability to withstand injuries arising from severe chilling stress. Chilling avoidance refers to the avoidance of severe and persisting growth inhibitors caused by mild chilling stress (Stamp, 1984). Chilling stress causes different visual symptoms due to metabolic dysfunctions and structural alterations (Lyons, 1973). Chilling injury can see a higher occurrence of disease as chilled roots display an increase in concentrations of reducing sugars and amino acids (Guinn, 1971). Prolonged exposure to temperatures below 15°C will slow the metabolic activity of the seed as well as make the seed susceptible to plant pathogens and other stresses when the soil temperatures start to rise (Borth, Krieg & Jividen, 1997; Buxton & Sprenger, 1976).

Cotton is extremely sensitive to frosts as it will stop plant growth and development, cause irreversible damage or even kill the plant (Constable & Shaw, 1988; Hearn & Constable, 1984). Frost tolerance is the ability of a plant to withstand temperatures below 0°C. Green and Roberts (2001) suggested that frost tolerance in cotton will only be possible through gene transfer.

(3) Germination and establishment

The process of germination and seedling emergence of cotton is illustrated in Figure 3. Cotton is sensitive to temperature during germination (Wanjura, Hudspeth & Bilbro, 1969). To germinate and establish successfully, cotton needs the soil temperature to be 14°C or greater for three consecutive days (Constable & Shaw, 1988). Therefore, soil temperature is the limiting factor when choosing the correct time to plant. Germination and crop establishment are vital to the development of a high-yielding cotton crop (Christiansen & Rowland, 1981). A cotton stand that is established with a sufficient quantity of vigorous seedlings is the most important step in the production cycle as it sets a limit on yield potential. All the steps after the emergence and establishment can only maintain or decrease potential yield of the developing cotton stand (Wanjura, 1981).

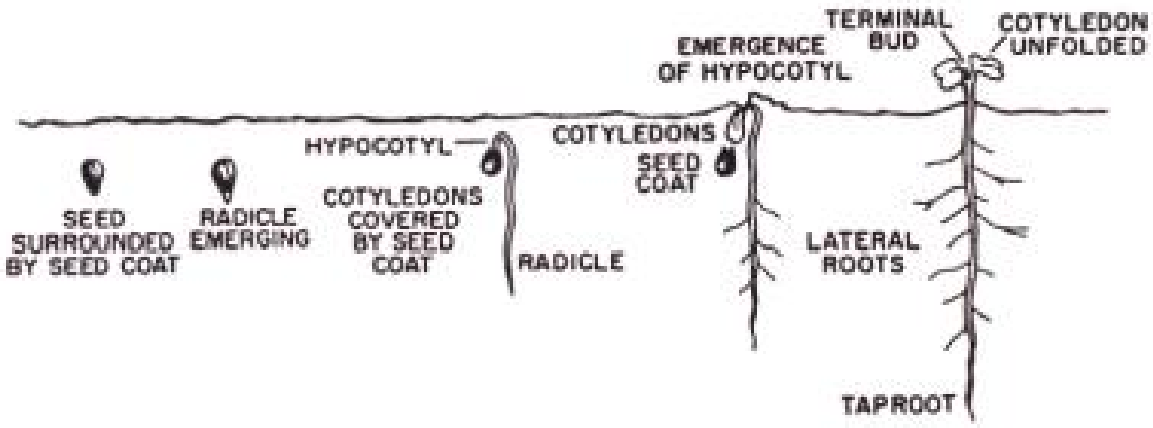

Figure 3. Germination and seedling emergence of cotton (Oosterhuis, 1990)

Cotton seed with high vigour creates rapidly emerging and growing seedlings which turn out plants with higher boll set percentages (Douglas, Flores & Andrews, 1974). Cotton seed. with low vigour cause slower seedling emergence, delayed stand establishment and reduced competitive effectiveness (Douglas et al., 1974). Low temperatures cause slow emergence that is often associated with seedling disease, and this usually results in the development of a poor cotton stand with reduced vigour (Constable, 1976).

A seed is considered germinated if it has a radicle length of 3 mm. The radicle, or primary root, develops rapidly and is the first organ to emerge from the seed coat during germination (Wanjura and Buxton (1972a). At temperatures below 10°C, the root tip can be damaged permanently (Christiansen, 1968). Christiansen and Thomas (1969) and Bradow (1990; 1991)

indicated that temperatures less than 10°C during the period of seed germination to early post emergence can reduce cotton plant growth and development. When the seed is in optimal growing conditions, the radicle can reach a depth of 25 cm or more by the time the cotyledons are unfolding (Taylor & Ratliff, 1969).

Cotton stands that emerge under unfavourable soil temperatures and moisture conditions will have considerable variability in emergence time that will lead to greater differences in the growth and development of the subsequent crop (Wanjura & Buxton, 1972b). Similarly, Constable and Shaw (1988) reported that an increase from 17°C to 18°C shortened the time from sowing to first square by 14 days, while an increase from 25°C to 26°C resulted in a reduction of only 3 days (Figure 4).

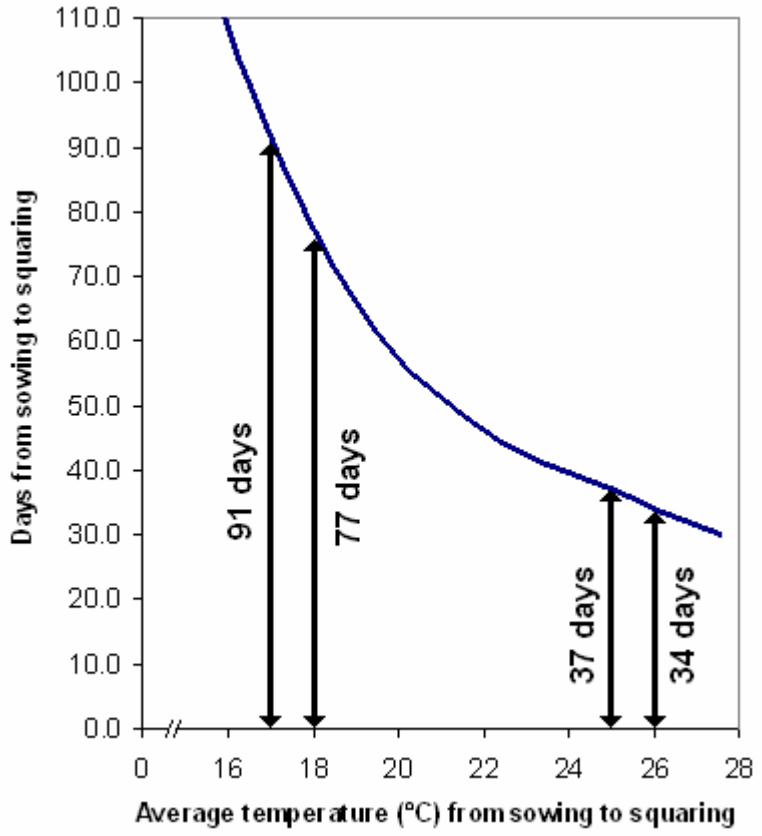

Figure 4. The effect of temperature on growth and development (Constable & Shaw, 1988).

The moisture content of the seed at the time of chilling determined the degree of injury to the seed and seeds with initial moisture content above 13% were protected against chilling injury (Christiansen, 1963). There are two time periods when cotton seeds can incur injury. The initial imbibition is the first period when injury can occur, which is known as imbibitional

chilling injury, while the commencement of the metabolic activity 18 or more hours after the germination has begun, the second period, is known as metabolic chilling injury (Christiansen, 1967). There are many factors that can affect germination and later stand establishment, and these include poor seed quality, drought, flooding, soil crusting, salinity, herbicide residues and cool temperatures (Oosterhuis, 1990).

II. TEMPERATURE EFFECTS ON COTTON AND OTHER SUMMER CROPS

The main constraints to summer crop production in Australia are water availability, the last frost in spring and the first frost in autumn. Summer crop production in Australia is primarily limited by temperature, with increases in soil temperatures and the beginning of the frost free summer months being the main factors affecting vigorous emergence and uniform establishment. Almost all summer crops are chilling sensitive as they require temperatures over 10°C (Wang, 1990) to achieve uniform emergence.

(1) Cotton

Temperature is one of the most important variables affecting growth and developmental processes of cotton (Hodges et al., 1993). Suboptimal temperature affect the whole production cycle of cotton, from sowing to harvest. Cotton seed is susceptible to chilling injury from the initial imbibition of water. Seedling biochemical processes begin 18 to 30 hours after imbibition and it is affected by sub optimal temperature (Christiansen, 1967). The elongation of the radicle and hypocotyl during germination increases as temperature increases until an optimum of 34.4°C and further increases of temperature led to a decrease in elongation of the radicle and hypocotyl (Wanjura & Buxton, 1972a).

Biomass production in cotton has a optimum day/night temperature of 30°C/20°C indicating that cotton production can suffer from heat stress (Reddy, Baker & Hodges, 1991). Low temperature can affect the early development of cotton. Constable and Shaw (1988) illustrated the effects of temperature on the time taken from sowing to squaring in a cotton plants (Figure 4). The increase from 18°C to 25°C can reduce the time from sowing to squaring by 40 days. Temperature, photoperiod and genotype all influence floral initiation and these factors also affect the time between emergence and first fruiting branch (FFB) and its nodal position (Hearn & Constable, 1984). The optimum temperature for the number of nodes to first fruiting branch is 24°C (Thomson et al., 2001), which means that the plant has established the

balance between vegetative growth and reproductive development. Cotton plants favour the production of vegetative branches when the temperature is below the optimum (Hodges et al., 1993).

Temperature controls the rate of development of flower buds from initiation, through anthesis to maturity, which is expressed in growing day degrees (Table 3) (Hearn & Constable, 1984). Boll growth is more temperature-sensitive than vegetative growth (Hodges et al., 1993).

Table 3. Day degree requirement for bud and boll development (Hearn & Constable, 1984).

Fruit development process	**Growing Day degrees**
Initiation to 3mm	220
3mm to anthesis	300
Anthesis to maximum size	310
Maximum size to mature	365
Mature to fully open	75

The phenological stage of the cotton plant determines how sensitive it is to suboptimal or chilling temperature. Constable and Shaw (1988) reported that the most sensitive stage to low temperatures was the emergence to squaring stage. Many studies have exposed the plants to prolonged sub optimal temperatures (Bradow, 1990; Bradow, 1991; Christiansen & Thomas, 1969). More recent work has found that cotton plants have the ability to recover from prolonged night temperature of 10°C during vegetative growth and early flower stages (McDowell et al., 2007). The recent work by McDowell et al. (2007) showed that cotton plants are able to endure temperatures below 11.4°C which Constable (1976) suggested cotton plant development will cease.

(2) Cardinal temperatures for germination of selected summer crops

The cardinal temperatures for germination of selected summer crops are reported in Table 4. It emphasises that major broadacre summer crops are chilling sensitive, as only sunflowers had a temperature below the chilling-sensitive temperature of 10°C (Wang, 1990) Sunflowers (*Helianthus annuus*) are relatively frost tolerant until reaching the six to eight leaf stage, when they become frost sensitive (Kelleher, 2003). Table 4 also illustrates the frost tolerance of the selected summer crops. Summer crops, like soybeans and sunflowers, have frost tolerance at

the beginning and end of the production cycle which can allow them be planted with reduced risk of frost damage, although low temperatures will increase the time to emergence.

Table 4. The cardinal (minimum, optimum and maximum) temperatures for germination of selected summer crops.

Crop	Minimum Temperature (°C)	Optimum Temperature (°C)	Maximum Temperature (°C)	Tolerant to frost [a]	References
Gossypium hirsutum	14.4	34.4	41.9	--	(Kelleher, 2003; Wanjura, Buxton & Stapleto, 1970)
Zea mays	12	25-28	Not reported	+	(Farooq et al., 2008; Farrell, Serafin & Kneipp, 2007; Kelleher, 2003)
Oryza sativa	10	20	30	--	(Ali, Naylor & Matthews, 2006; Farrell et al., 2006; Kelleher, 2003)
Sorghum bicolor	16	23.3	Not reported	--	(Kanemasu, Bark & Choy, 1975; Kelleher, 2003; Wood et al., 2006)
Glycine max	13-15	19	Not reported	+	(Kelleher, 2003; Tyagi & Tripathi, 1983)
Helianthus annuus	5-8	24-27	Not reported	++	(Farrell et al., 2007; Kelleher, 2003)

[a] Tolerance during emergence and establishment.
Heavy = ++, Mild = +, Susceptible = -, Very susceptible = --

III. EFFECT OF COLD ON COTTON AND OTHER SUMMER CROPS

Chilling temperature not only effects the growth and development of cotton, it also affects the nutrition, root and shoot lengths, nutrient uptake, and respiration. All of the these factors are essential in maximising cotton yields, so to have them limiting or changing due to chilling temperatures can lead to low yields and ultimately low profitability. It is also important to understand that poor soil structure and chemical toxicities in the soil may limit root and shoot growth and nutrient uptake, although there may be adequate nutrients available.

(1) Nutrition

Nutrient supply is a major factor influencing crop yield and quality (Batten & Black, 2003). Yield penalties occur when there are deficiencies and imbalances of nutrient available to the plant. While excess fertiliser can be costly and lead to excessive vegetative growth, inadequate supplies of nitrogen at the start of the crop can led to reduced leaf expansion which resulted in smaller leaves and lower leaf area index (LAI) (Hearn & Constable, 1984). The average concentration of macronutrients and micronutrients in a cotton plant and their relative abundance are illustrated in Table 5 and Table 6.

Table 5. Average concentration and abundance of macronutrients in a cotton plant (Thomson et al., 2001).

Micronutrients	Concentration in Dry Matter (mg/kg)
Sodium	350
Chlorine	100
Silicon	100
Boron	75
Iron	32
Manganese	25
Zinc	25
Copper	8

Table 6. Average concentration and abundance of micronutrients in a cotton plant (Thomson et al., 2001).

Macronutrients	Concentration in Dry Matter (%)
Oxygen	45.00
Carbon	43.00
Hydrogen	6.00
Potassium	2.12
Nitrogen	1.96
Calcium	1.30
Magnesium	0.38
Sulfur	0.37
Phosphorus	0.27

Most cotton production in Australia is on cracking clay soils (Vertosols), with pH of 8.0 – 8.5, which may cause the availability of micro nutrients to be decreased (Thomson et al., 2001). The effect of pH on the availability of nutrients is indicated in Figure 5. The pH range 8.0 to 8.5, illustrates micro-nutrients like Zinc, Boron, Manganese and Iron are limiting. Phosphorus deficiency occurs when a cotton plant experiences a cool, wet and early start to the season (Thomson et al., 2001). When root growth is restricted due to cool wet periods, zinc deficiencies may be induced due to the low mobility of zinc in the soil solution and the slow mineralisation of zinc from soil organic matter (Thomson et al., 2001).

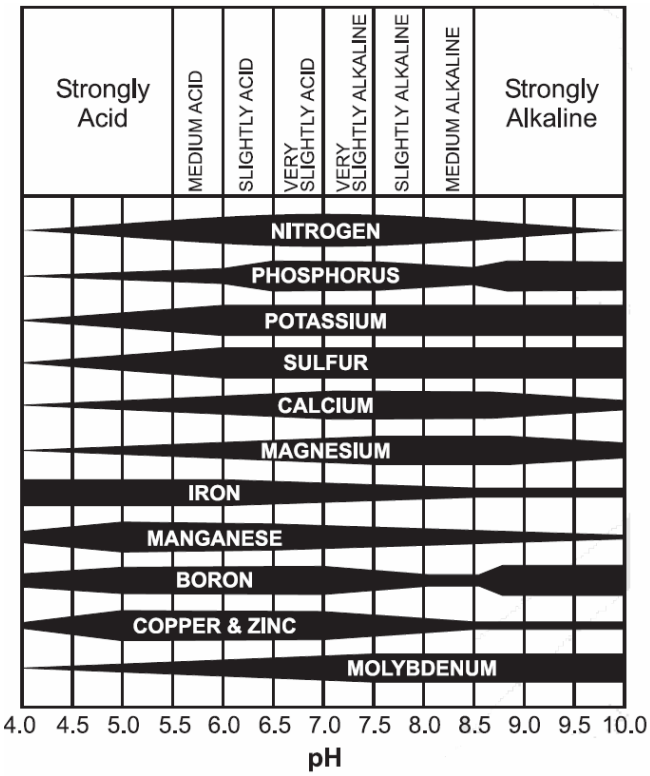

Figure 5. Soil pH influences the availability of most nutrients (Rochester & Larson, 2001; Thomson et al., 2001).

(2) Nutrient uptake

The greatest rate of nutrient uptake occurs between squaring and the end of flowering (Constable, Rochester & Cook, 1988) and most nitrogen uptake occurs 50 to 110 days after sowing (Thomson et al., 2001). Root growth is reduced at chilling temperatures (Thomson et al., 2001). This leads to lower nutrient uptake and thus reduced growth.

A plant's root has two main systems of nutrient uptake. One system is the passive uptake, which is where the ions diffuse into the free space in the root (Thomson et al., 2001). Active uptake, the other system, is the most important. Active uptake is a carrier-mediated process requiring energy to move substances against a concentration gradient (Knox et al., 2005). This is needed by the cotton plant as the concentration of ions in the xylem sap is greater than in the soil solution (Thomson et al., 2001). Table 7 illustrates the uptake and removal of essential nutrients during cotton production.

Table 7. Uptake and removal of essential nutrients during cotton production.

Nutrient	**Uptake**	**Removal in cotton seed**	**References**
Macro-nutrients	*kg/100 kg lint*	*kg/10 0kg lint*	
N	6.0 – 11.2	4.0 – 6.1	(Hodges, 1992; Thomson et al., 2001)
K	7.2 – 10.2	1.5 – 2.7	(Thomson et al., 2001)
P	1.4 – 2.6	0.8 – 1.2	(Thomson et al., 2001)
S	2.1 – 3.1	0.5	(Thomson et al., 2001)
Ca	6.2 – 13.1	0.3 – 0.5	(Thomson et al., 2001)
Mg	2.5 – 7.5	0.7 – 1.2	(Thomson et al., 2001)
Micro-nutrients	*g/100 kg lint*	*g/100 kg lint*	
Zn	7.5 – 9.0	5.7	(Constable et al., 1988)
Cu	0.6 – 3.1	1.3	(Constable et al., 1988)
Mn	28	5.2	(Rochester & Larson, 2001)
Fe	25 – 38	29.3	(Constable et al., 1988)
B	12.8	9.3	(Constable et al., 1988)

There are three ways in which a cotton plant absorbs nutrients from the soil solution. The first way is through root interception, where the roots grow through the soil absorbing any nutrients they find (Rochester & Larson, 2001; Thomson et al., 2001). The second option for roots to obtain nutrients is through mass flow, which is the movement of soil nutrients to the root surface. As water moves through the soil to the cotton plants roots, nutrients ions dissolves in the soil water and move to the root's surface (Rochester & Larson, 2001; Thomson et al., 2001). The third option is diffusion, which is the plant roots absorbing nutrients from the soil next to the root. Once the roots have absorbed some of the nutrients, it creates a concentration gradient and the higher concentration of ions further away from the roots move to the lower concentration and hence closer to the roots surface (Rochester & Larson, 2001; Thomson et al., 2001). Figure 6 demonstrates the processes that affect the availability of nutrients in the soil in cotton crops.

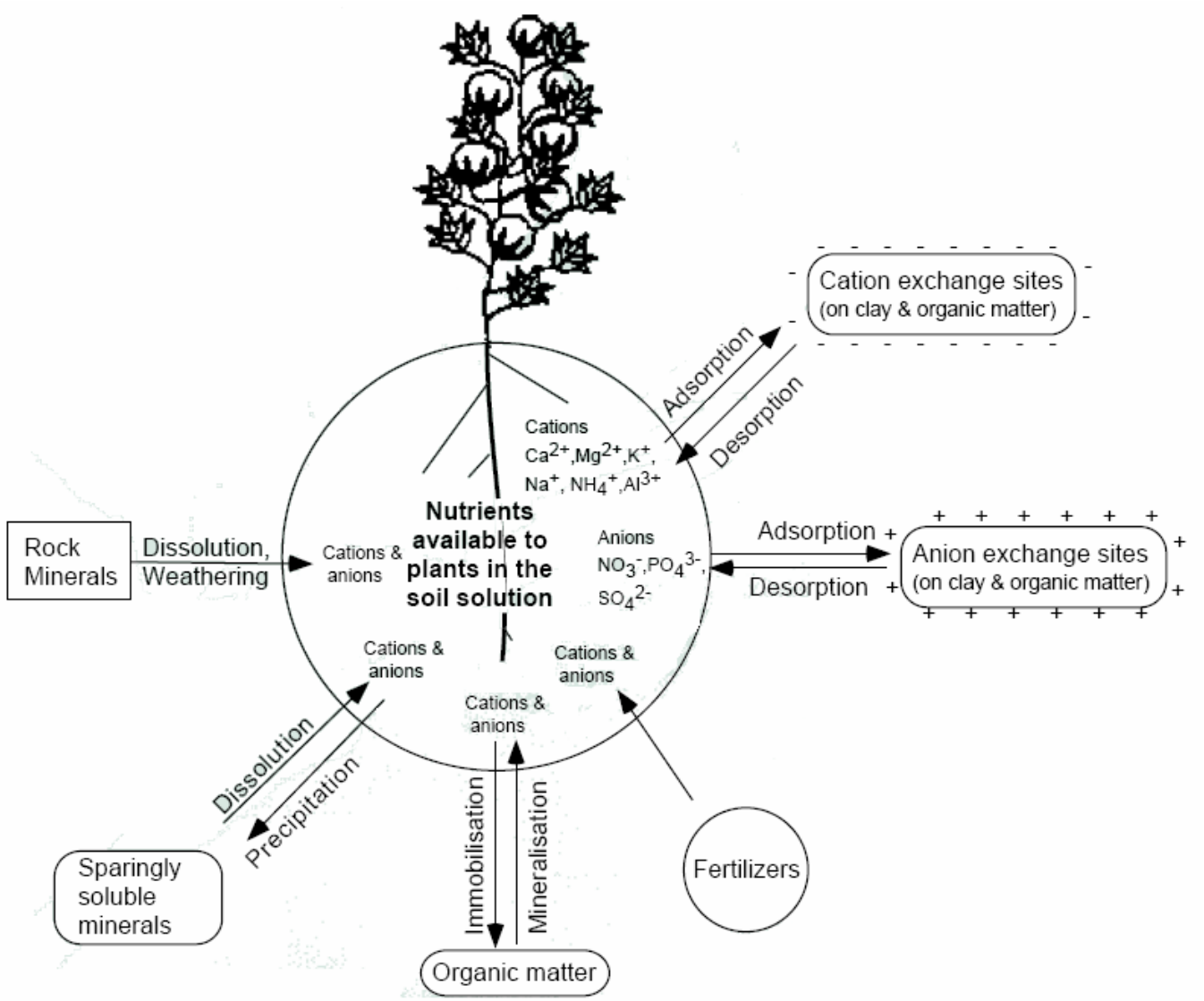

Figure 6. Processes affecting nutrient availability in the soil (Rochester & Larson, 2001).

(3) Root and shoot growth

Root growth occurs at a rate between 8 mm and 90 mm per day depending on temperature and soil strength (Hearn & Constable, 1984). Thomson et al. (2001) indicated that the optimum soil temperature for cotton root growth is between 28°C and 35°C. During the early vegetative stage, taproot length elongation can be between 12.5 mm and 50 mm per day depending on soil conditions, allowing roots to explore the soil to a depth of 900 mm while the height of the plant above the ground is only about 350 mm (Figure 7) (Oosterhuis, 1990).

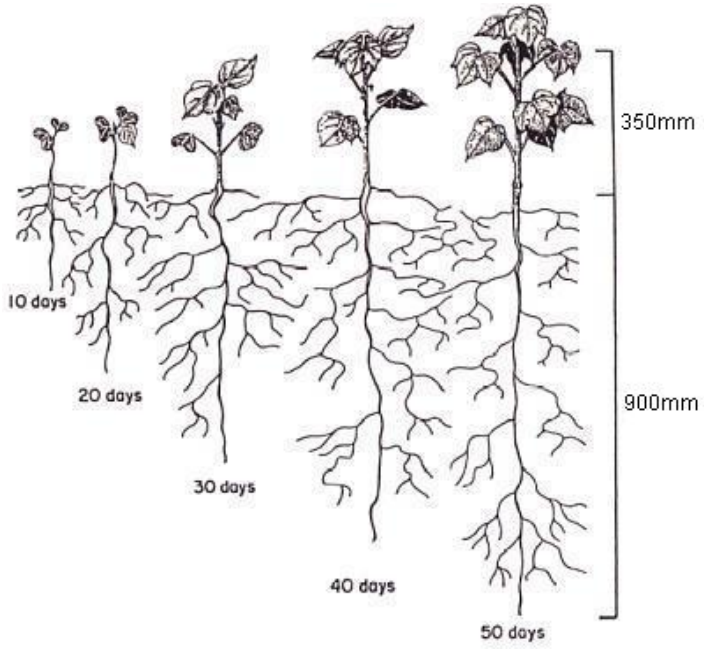

Figure 7. Root and shoot development of a cotton plant after emergence (Oosterhuis, 1990).

Cotton seedlings typically grow very slowly (Hodges et al., 1993). Therefore it is important to have warm soil that encourages rapid, uniform emergence, with the speed of plant growth directly related to temperature (Constable & Shaw, 1988). The relationship between temperature and emergence is illustrated in Table 7. Rapid seedling emergence is desirable since the health and vigour of seedlings is often correlated with the time required for the seedling to emerge after planting (Kerby, Keeley & Johnson, 1989; Steiner & Jacobson, 1992; Wanjura et al., 1970). Constable (1976) found that the most sensitive growth stage to cool temperature was the emergence to squaring, which emphasises the importance of getting rapid seedling emergence.

Table 8. The impact chilling soil temperatures have on germination time.

Crop	Cold Temperature (°C)	Days	Optimum Temperature (°C)	Days	References
Gossypium hirsutum	10	29	18	5	(Constable & Shaw, 1988)
Zea mays	12	14	25	4-5	(Kelleher, 2003)
Helianthus annuus	5-8	30	24-27	4	(Shaw, 1991)

Normal taproot growth (Figure 7) can cease if chilling occurs during germination. This chilling injury leads to curling, shortening and thickening of the roots and injury or death of the root tip. With the loss of the main taproot the plant has to develop lateral roots to compensate. At low temperatures, root growth of cotton is reduced, less branching occurs, reduced water uptake and nutrient uptake is altered (Bradow, 1990; McMichael & Burke, 1994; McMichael, Upchurch & Burke, 1996; Thomson et al., 2001).

The typical imbibitional chilling injury in cotton is the curling, shortening and thickening of the roots (Figure 8). The chilling during this phase of imbibition injures and typically kills the root tip meristematic tissue. This results in cessation of normal taproot growth (Figure 9), and leads to the development of lateral roots to compensate for the loss of the main tap root. These seedlings will survive if they do not experience water deficiency or disease. Low vigour plants, decreased germination, increased decay, production of abnormal plants and seed death are some of the symptoms of imbibitional chilling injury (Wang, 1990).

Figure 8. Germinated cotton seedlings showing chilling injury.

Figure 9. Healthy germinated cotton seedlings

(4) Respiration

Tropical species of plants are sensitive to suboptimal temperatures (Wang, 1990). Hodges et al. (1993) showed that respiration in cotton plants is a temperature sensitive process, which in turn affects gross photosynthesis and growth. Other studies have found that mitochondrial respiration is also deceased at chilling temperatures (Lawrence & Holaday, 2000; Lyons & Raison, 1970). At temperatures between 12°C to 15°C, tropical species exhibit a break in Arrhenius plots of mitochondrial function (Stewart & Guinn, 1971; Wang, 1990). Respiratory activity of isolated cotton mitochondria exhibits a break in Arrhenius plots at about 15°C (Stewart & Guinn, 1971), which is close to the base temperature of 15.5°C used in the USA for growing day degrees (Oosterhuis, 1990).

IV. GENETIC VARIATION AND ACCLIMATION

Temperature response in plants is governed by a complex interaction of genetic, developmental and environmental factors (Rahman, 2005). The complex genetic background of cotton allows breeders a diverse range of species to select for insect and disease tolerance or environmental stresses, like selecting for dry land production (Thomson et al., 2001). The development of short season varieties is important where environmental conditions are below optimal. The window for cotton production in the cooler areas (Table 1 and Figure 2), like Hillston and Gunnedah, is less then those in northern and or western areas. This creates further importance on the planting time in these areas as late planting may reduce yield.

Breeders use genetic variation in different lines to create superior lines for commercial production. The intra-varietal selection requires self-pollination by the breeders. In Australia, bacterial blight (*Xanthomonas campestris*) resistance was bred into the commercial lines now used in Australia. Scientists are currently using variation in genetics to develop cultivars that are tolerant to Fusarium wilt (*Fusarium oxysporum*) (Thomson et al., 2001). Similarly, Radin *et al.* (1994) have shown stomatal conductance varies genetically over a wide range and has increased with each release of new higher-yielding cultivars. The studies by Thomson *et al.* (2001) and Radin *et al.* (1994) showed that there is genetic variation in cotton. Therefore, selection of cultivars that are acclimatized to cooler conditions could allow for the development of cultivars with greater tolerance to frost and chilling injury.

Limited work has been carried out on cotton plants and there acclimation to cold temperatures. Cold acclimation is when plants undergo small changes to move from being chilling-sensitive to chilling-tolerant. Following day and night exposure of cold temperatures to 11-day old cotton seedlings, the cotton seedlings became hardened (Rikin, 1991). There was little evidence that short duration exposure of cotton to cold night temperatures had any significant impact on early crop development (Bange & Milroy, 2004). This was supported by McDowell et al. (2007) who showed that cotton plants can withstand temperatures below 11°C, with little or no effect on growth and development.

Bange and Milroy (2004) suggested that there is a lack of understanding on the effects of extreme temperatures on crop growth and development which impedes our capacity to explore management opportunities to improve crop yield and profitability under temperature extremes. Bange and Milroy (2004) also recommended that there should be further work carried out on the acclimation of cotton at cold temperatures. The selection and breeding of cold tolerant cultivars is important if cotton yields are to be maintained within regions where frequent cold shocks occur during early growth and development.

V. MANAGEMENT

It is important to limit the number of cold shocks that a cotton crop experiences, with management decisions like planting date, cultivar selection and season length affecting the date of maturity. If a cotton crop experiences cold shocks at either end of the production cycle, it can cause damage to either the emerging seedlings or the developing bolls which results in yield losses (Constable & Shaw, 1988). Management practices need to take into account the growth and development of the cotton plant and its associated requirements when trying to maximise yield (Oosterhuis, 1990). It is also essential for managers to be flexible as environmental conditions are always changing.

(1) Planting date

Planting date is determined by the soil temperature. Cotton can be planted when the soil minimum temperatures at 10 cm depth exceeds the critical 14°C for at least 3 days (Constable & Shaw, 1988). The planting dates for cotton vary for year to year as cotton is an environmentally-sensitive crop where early and late planting will decrease yields (Kittock, Taylor & Hofmann, 1987). Temperature affects both the sowing date and the seasonal

development of a cotton plant while also influencing its growth and development (Cottee et al., 2007).

Cole and Wheeler (1974) showed that germination and stand establishment will be reduced if cotton is planted at soil temperatures below 20°C. Soil temperature is the major factor when determining planting dates (Mahan & Gitz, 2007). Earlier planting to achieve longer growing seasons, better use of sunlight and rainfall and enhanced yield potential has led to a premium on selecting chilling-tolerant populations for major crop species (Yu & Tuinstra, 2001). Cotton is a chilling sensitive plant, and it is therefore important to avoid planting before the last expected frost (Constable & Shaw, 1988). Frosts may lead to the death or abnormal development of the seedling that will result in the need to re-sow the field. Re-sowing is a timely and costly practice that should be avoided wherever possible.

Quality and yield of the cultivar decreased when planting was after mid October in Australia, while a plateau effect stopped an increase in yield when planting very early (30th of September) (Constable, Harris & Paull, 1976). After the 20th of October yield falls by 20 kg ha^{-1} day, maturity is delayed and fibre micronaire declines (Constable & Shaw, 1988). The decrease in relative yield is illustrated in Figure 10.

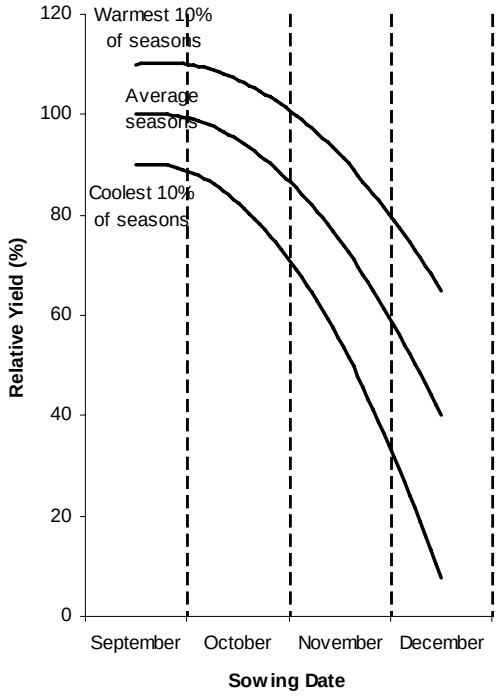

Figure 10. The effect of sowing date on relative yield (Constable & Shaw, 1988).

(2) Length of season

Early sowing allows maximum time for crop growth and development and ensures maximum yield potential at the earliest date. Although early sowings are ideal, they come with risks. Planting early leaves the stand open to a greater risk of frost damage, and it may suffer from slow emergence, seedling disease, poor early vigour and exposure to herbicide damage (Constable & Shaw, 1988).

(3) Cultivar Selection

It is critically important to choose cultivars of high quality and vigour that have the ability to germinate and emerge uniformly and quickly under a wide range of conditions, like moisture and temperature stress, commonly seen in the field environment (Bennett, 2004). In the cool cotton production zone of Australia they can be limited by the length of the season, which makes it imperative to select a short season cultivar in that region. A long season cultivar planted in a short season would see major reductions in yield (Constable & Shaw, 1988).

VI. SCREENING TECHNIQUES FOR COLD TOLERANCE IN GERMINATION AND ESTABLISHMENT

(1) Screening techniques in cotton

When selecting for cold tolerance, cotton breeders have planted cultivars early in the season, and selecting those that germinate vigorously and emerge uniformly. It would be beneficial to cotton breeders if they were able to also test for cold tolerance with a simple laboratory test. Many studies have attempted to develop tests that predict field emergence under chilling stress. These studies have only been able to show significant correlations (R^2) with field emergences, when affected by chilling stress (Table 8).

An early indicator of crop establishment is seedling vigour (Bennett, 2004). Although, cotton breeders have traditionally selected for cold tolerance by planting experimental genotypes early in the season and applying selection pressure for early uniform emergence. While this method of selection has worked for many years, it would benefit cotton breeders to have an objective method for selecting for cold tolerance.

Table 9. Correlation of Imbibition tests (4 hours) with field emergence and establishment.

Laboratory Test		(R^2)	Field Data	References
Cool germination test		0.20	Emergence rate index [a]	(Schulze et al., 1996)
		0.26	Establishment – 4 weeks [b]	(Schulze et al., 1996)
		0.57	Emergence – 1 week [a]	(Bolek, 2006)
		0.70	Establishment – 4 weeks [b]	(Duesterhaus, 2000)
		0.20	Establishment – 4 weeks [b]	(Duesterhaus, 2000)
Cool warm vigour index		0.21	Emergence rate index [a]	(Schulze et al., 1996)
		0.16	Establishment – 4 weeks [b]	(Schulze et al., 1996)
		0.80	Establishment – 4 weeks [b]	(Duesterhaus, 2000)
		0.22	Establishment – 4 weeks [b]	(Duesterhaus, 2000)
Imbibition test	4 hours	-0.64	Establishment – 6 weeks [c]	(Hopper et al., 1994)
	4 hours	-0.62	Establishment – 6 weeks [c]	(Duesterhaus, 2000)
	4 hours	-0.08	Establishment – 6 weeks [c]	(Duesterhaus, 2000)

[a] A measure of rate and total emergence.

[b] The percent of total seedlings resulting in established plants 4 weeks after planting.

[c] The percent of total seedlings resulting in established plants 6 weeks after planting.

(a) Warm germination test

The warm germination test uses 50 seeds rolled in paper towel and placed in a temperature controlled environment of 30°C for 8 hours then 20°C for 16 hours (Duesterhaus *et al.* 1999; Duesterhaus *et al.* 2000; Schulze *et al.* 1996; Schulze *et al.* 1997; Smith and Varvil 1984). The germinated seeds with a radicle length of 3.8 cm are counted after 4 days and again after 7 days. The 4 day count is used in determining the cool warm vigour index while the 7 day count establishes the final germination percentages. The warm germination test is a poor indicator of emergence if cooler temperatures are experienced during planting (Bourland, 1992).

The warm germination test is commonly used in conjunction with the cool germination test to determine the cool warm vigour index. The warm germination test is also used to correct other tests and indicate the maximum number of seeds that would germinate if grown under optimum germinating conditions. This is rarely achieved as optimum conditions are uncommon during germination in the field.

(b) Cool germination test

The cool germination test uses 50 seeds rolled in paper towel and placed in a temperature controlled environment at a constant temperature of 18°C (Hopper et al., 1994; Schulze et al., 1996; Schulze et al., 1997). As with the warm germination test, the germinated seeds with a radicle length of 3.8 cm are counted after 7 days. The 7 day count is used in determining the cool warm vigour index and establishes the final germination percentages at 18°C. The importance of maintaining the temperature within 0.5°C was examined by Tollivar, Savoy & Drummond (1997) who found that results could be variable if the temperature was not maintained constant. The cool germination test is often referred to as the "Texas Cool Test" originally developed by Bird and Reyes (1967). Several studies reported good correlations with seedling vigour using the "Texas Cool Test" method (Glat, Taylor & Williams, 1982; Gregory, Hopper & Jividen, 1986).

(c) Cool warm vigour index

The cool warm vigour index is calculated using both the warm and cool germination tests. The germination percentage of the warm germination test after 4 days is added to the germination percentage of the cool germination test after 7 days and they are averaged to produce a cool warm vigour index. Buxton et al. (1977) found that the correlation of the average of the warm germination test (25°C) and the cool germination test (15°C) gave the best prediction for field emergence. Vigorous cotton cultivars with good seed quality have warm germination test (30°C) values greater then 80% and cool germination test (18°C) values greater then 60% (Smith & Varvil, 1984). Cool germination test (18°C) probabilities below 0.50 indicate that a variety should not be planted when sub-optimal temperatures are expected (Smith & Varvil, 1984).

Bolek (2006) claimed that the best predictor of field emergence at 7, 14 and 21 days after planting was achieved by subtracting 18°C germination percentages (after 7 days) from 30°C germination percentages (after 7 days). The difference between 18°C and 30°C germination percentages accounted for 93%, 81% and 45% of the variation in field emergence for 7, 14, and 21 days after planting, respectively. This test has a similar concept to the cool warm vigour index

(d) Metabolic chilling test

The metabolic activity for cotton is most efficient in the thermal window of between 23°C and 32°C (Burke, Mahan & Hatfield, 1988). The metabolic chilling test establishes the response of cotton cultivars to temperatures below the efficient thermal window for metabolic activity. Fifty seeds of each cultivar are germinated in sand, wet to field capacity, at a depth of 25mm. The different cultivars are subjected to constant suboptimal temperatures of 18°C and stand counts are taken daily for 21 days (Duesterhaus et al., 2000; Hall & Gannaway, 2005).

Table 10. Overall Cold Tolerance Rating (Duesterhaus et al., 2000)

Cold Tolerance Rating	Emergence Percentage
Excellent	80 – 100
Good	65 – 80
Fair	50 – 65
Poor	0 – 50

The Cold Tolerance Rating (CTR) reported by Duesterhaus et al. (2000) combined both the metabolic chilling test and the imbibition chilling test to provide different cultivars with a rating (Table 9) of their overall cold tolerance. If a cultivar has differing ratings for each test then it is classified as the lower rating of cold tolerance.

(e) Imbibitional chilling test

Cotton seed can be killed when it imbibes water for 12 hours at 5°C. Initial injury stems from the imbibition of cold water (Duesterhaus et al., 2000) while secondary injury can occur 18 to 24 hours after the initiation of germination if temperatures remain below 18°C (Christiansen, 1967). The imbibitional chilling test determines how cultivars respond after being subjected to extreme chilling temperatures during the seed's water imbibition, a critical period when damage from chilling occurs (Schulze et al., 1996). Temperatures of 5°C to 10°C during imbibition kill the embryo or cause abnormal development of the seedling, such as sloughing of the root cortex or formation of nub-roots symptoms, depending upon the time of chilling (Christiansen, 1963).

This test imbibes 200 seeds of each cotton cultivar in water at a constant temperature of 5°C for 6 hours. The imbibed seeds are germinated in sand, wet to field capacity, at a depth of

25mm. The different cultivars are subjected to constant optimal temperatures of 30°C with stand counts taken daily for 21 days (Duesterhaus et al., 2000; Hall & Gannaway, 2005; Hopper et al., 1994; Schulze et al., 1996). Table 9 demonstrates the Cold Tolerance Rating (CTR) and Figure 11 indicates how each cultivar performs in the cold tolerance screening test (Duesterhaus et al., 2000).

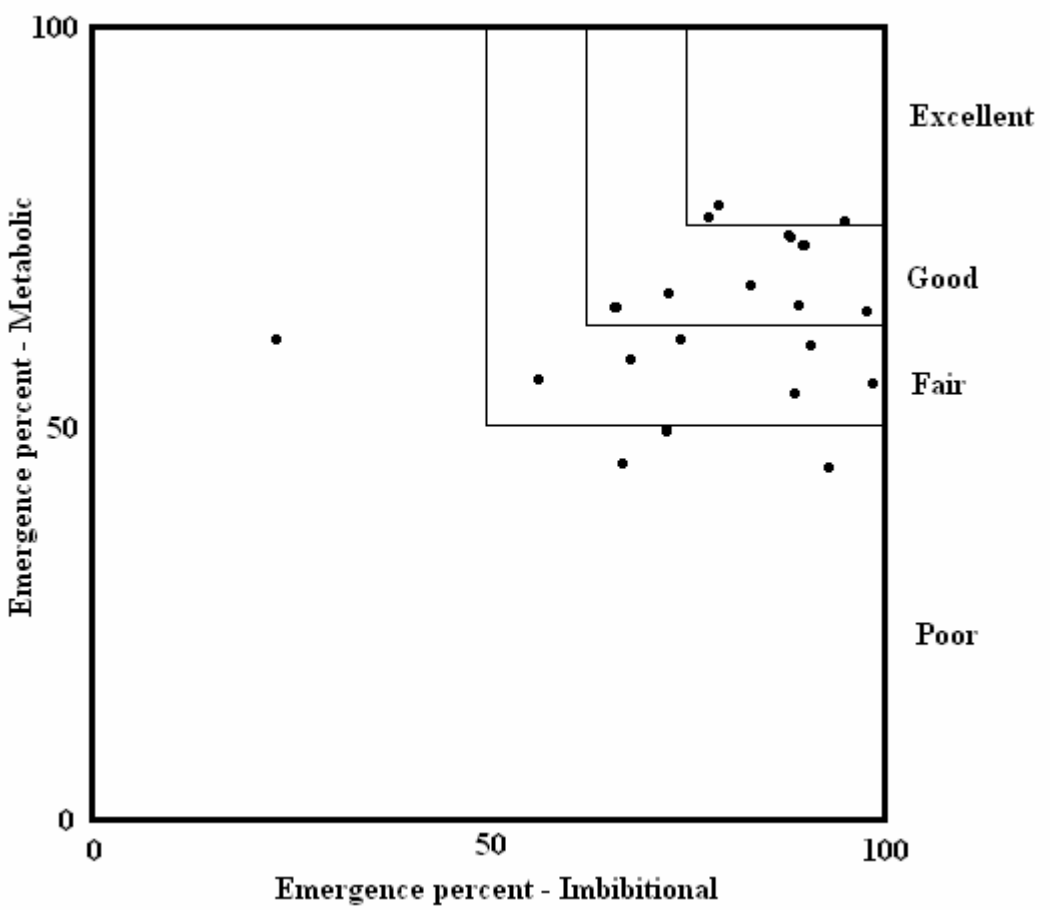

Figure 11. Cold tolerance screening test (Duesterhaus, 2000; Duesterhaus et al., 1999)

(f) Imbibition test

Imbibition is an important part of germination as it is responsible for activating the metabolic processes of germination due to the number of hydrolytic enzymes involved (Cothren, 1999). Therefore when chilling temperatures cause cellular damage during seed imbibition (Schulze et al., 1997), it can cause problems with germination and stand establishment. Hence, before the radicle emerges to 3 mm, the majority of accumulated seed water is the result of imbibition with the remaining due to metabolic activity of the emerging seed (Wanjura and Buxton 1972). In previous studies by Duesterhaus et al. (1999) and Duesterhaus et al. (2000),

cotton seeds where imbibed at 5C for six hours. The seeds were then touch dried and weighed to determine the increase in seed weight from water imbibition. Duesterhaus et al. (2000) suggested that at least 40% of a variety's imbibitional chilling tolerance is explained by the imbibition rate. In another study, Duesterhaus (2000) argued that a negative relationship between imbibitions rate and stand establishment suggested that faster imbibition rates at chilling temperatures may be detrimental to field stand establishment.

(g) Electrolyte leakage tests

Several important enzymes in photosynthesis and respiration are set in membranes and require a fluid membrane for proper function. Damage to these membranes due to chilling or freezing is often indicated by leakage of solutes and water from cells into intercellular spaces (Knox et al., 2005). During the imbibition of water by the seed, there is also leakage of substances. The substances released are mostly sugars with various organic and amino acids (Simon & Harun, 1972). Damage caused by cotton seed being imbibed at chilling temperatures can be measured by determining the electrical conductivity of the leachate (Schulze et al., 1996). One hundred cotton seeds are placed in 50 ml of deionised water at 5°C and placed in an incubator for 24 hours at 5°C (Schulze et al., 1997). The seeds are removed after the 24 hours and the electrical conductivity measured.

(2) Techniques in other summer crops

Klos and Brummer (2000) found that seedling height was a better trait than germination time to predict field performance when the traits are measured in a laboratory or greenhouse. Yaklich, Kulik and Anderson (1979) reported that field emergence and vigour in soybeans were negatively related to electrolyte leakage.

VII. CONCLUSION

(1.) Germination and seedling establishment in the field is a complex process influenced by many interacting factors. Temperature is the most influential factor in cotton production. During emergence and establishment, low temperatures cause a reduction in growth and development, which emphasises the importance of planting into warm soil to encourage rapid, uniform emergence.

(2.) Cotton seed with low vigour can cause slower seedling emergence, delayed stand establishment and reduced competitiveness. Farmers should select cotton with high vigour as seeds are often planted at suboptimal temperatures.

(3.) There are two time periods when cotton seeds can incur chilling injury. The first is during imbibition of water into the seed, which is essential for initiation of the metabolic processes germination. The second is 18 to 30 hours after imbibition when the metabolic processes begin. If the seed is damaged at either time, they are referred to as having imbibitional chilling injury and metabolic chilling injury, respectively.

(4.) Breeders currently screen new lines in field experiments early in the season to determine their chilling tolerance. However, environmental conditions do not always allow for proper evaluation. The development of a quick and simple test for chilling tolerance would allow breeders to screen many breeding lines and begin to select for chilling tolerance in future cultivars. Also, agronomists can recommend to producers chilling tolerant cultivars for planting early in the season to take advantage of a longer growing season.

(5.) Areas for further research in germination and emergence of cotton are to refine the germination chill protocols so that breeders can screen for chilling tolerance. It will also be important to determine if germination chill tests can provide an indication of cultivar differences in field chilling tolerance

VIII. REFERENCES

ABARE. (2007). Australian Commodity Statistics. Australian Bureau of Agricultural and Resource Economics, Canberra.

ALI, M. G., NAYLOR, R. E. L. & MATTHEWS, S. (2006). Distinguishing the effects of genotype and seed physiological age on low temperature tolerance of rice (Oryza sativa L.). *Experimental Agriculture* 42, 337-349.

BANGE, M. P. & MILROY, S. P. (2001). Effect of temperature on the rate of early fruiting developmental processes of cotton. In *Proceedings of the 10th Australian Agronomy Conference*. Australian Society of Agronomy., Hobart, Australia.

BANGE, M. P. & MILROY, S. P. (2004). Impact of short-term exposure to cold night temperatures on early development of cotton (*Gossypium hirsutum* L.). *Australian Journal of Agricultural Research* 55, 655-664.

BATTEN, G. & BLACK, S. (2003). Crop nutrition. In *Principles of field crop production* (ed. J. Pratley). Oxford University Press, South Melbourne, Victoria, Australia.

BENNETT, M. A. (2004). Seed and agronomic factors associated with germination under temperature and water stress. In *Handbook of seed physiology: applications to agriculture* (ed. R. L. Benech-Arnold and R. A. Sanchez), pp. 97-124. The Haworth Press, Inc., Binghamton, New York.

BIRD, L. S. & REYES, A. A. (1967). Effects of cottonseed quality on seed and seedling characteristics. In *Proceedings of the Beltwide Cotton Production Research Conference*, pp. 199-206.

BOLEK, Y. (2006). Predicting cotton seedling emergence for cold tolerance: Gossypium barbadense. *Journal of Agronomy* 5, 461-465.

BOM. (2008). Climate statistics for Australian sites. Bureau of Meteorology.

BORTH, T. D., KRIEG, D. R. & JIVIDEN, G. M. (1997). Genetic and environmental factors affecting the fatty acid composition of polar and non-polar lipids of cottonseed. In *Proceeding of the Beltwide Cotton Conference*, pp. 1455-1458.

BOURLAND, F. M. (1992). Characterization and improvement of seed and seedling vigor in cotton. In *Proceeding of the Beltwide Cotton Conference*, pp. 1326-1329. National Cotton Council, Memphis, Tennessee.

BRADOW, J. M. (1990). Chilling sensitivity of photosynthetic oil-seedlings .1. cotton and sunflower. *Journal of Experimental Botany* 41, 1585-1593.

BRADOW, J. M. (1991). Cotton cultivar responses to suboptimal postemergent temperatures. *Crop Science* 31, 1595-1599.

BURKE, J. J., MAHAN, J. R. & HATFIELD, J. L. (1988). Crop-specific thermal kinetic windows in relation to wheat and cotton biomass production. *Agronomy Journal* 80, 553-556.

BUXTON, D. R., MELICK, P. J., PATTERSON, L. L. & PEGELOW, E. J. (1977). Relationships among cottonseed vigor and emergence. *Agronomy Journal* 69, 677-681.

BUXTON, D. R. & SPRENGER, P. J. (1976). Genetic variability for cottonseed germination at favourable and low temperatures. *Crop Science* 16, 243-246.

CHRISTIANSEN, M. N. (1963). Influence of chilling upon seedling development of cotton. *Plant Physiology* 38, 520-522.

CHRISTIANSEN, M. N. (1967). Periods of sensitivity to chilling in germinating cotton. *Plant Physiology* 42, 431-433.

CHRISTIANSEN, M. N. (1968). Induction and prevention of chilling injury to radicle tips of imbibing cottonseed. *Plant Physiology* 43, 743-746.

CHRISTIANSEN, M. N. & ROWLAND, R. (1981). Cotton physiology – Seed and germination. In *Proceedings of the Beltwide Cotton Conference*, vol. 3, pp. 315-318. National Cotton Council of America, New Orleans, LA.

CHRISTIANSEN, M. N. & THOMAS, R. O. (1969). Season-long effects of chilling treatments applied to germinating cottonseed. *Crop Science* 9, 672-673.

COLE, D. F. & WHEELER, J. E. (1974). Effect of pregermination treatments on germination and growth of cottonseed at suboptimal temperatures. *Crop Science* 14, 451-454.

CONSTABLE, G. A. (1976). Temperature effects on the early field development of cotton. *Australian Journal of Experimental Agriculture and Animal Husbandry* 16, 19-24.

CONSTABLE, G. A., HARRIS, N. V. & PAULL, R. E. (1976). The effect of planting date on the yield and some fibre properties of cotton in the Namoi Valley. *Australian Journal of Experimental Agriculture and Animal Husbandry* 16, 265-271.

CONSTABLE, G. A., ROCHESTER, I. J. & COOK, J. B. (1988). Zinc, copper, iron, manganese and boron uptake by cotton on cracking clay soils of high pH. *Australian Journal of Experimental Agriculture* 28, 351-356.

CONSTABLE, G. A. & SHAW, A. J. (1988). Temperature requirements for cotton. In *Agfacts - Division of Plant Industries*, pp. 1-4. New South Wales Department of Agriculture and Fisheries P5.3.5.

COTHREN, J. T. (1999). Physiology of the cotton plant. In *Cotton: Origin, History, Technology, and Production. The Wiley Series in Crop Science* (ed. C. W. Smith and J. T. Cothren), pp. 207-268. John Wiley & Sons, New York.

COTTEE, N. S., TAN, D. K. Y., COTHREN, J. T., BANGE, M. P. & CAMPBELL, L. C. (2007). Screening cotton cultivars for thermotolerance under field conditions In *World Cotton Research Conference 4*, Lubbock, TX, USA.

COTTON AUSTRALIA. (2008). Cotton in Australia, vol. 2008. Cotton Australia.

DOUGLAS, A. G., FLORES, J. A. & ANDREWS, C. H. (1974). Effects of seed vigor and competition on performance of cotton. In *Proceeding of the Beltwide Cotton Production Research Conference*, pp. 75-77. National Cotton Council, Memphis, Tennesse.

DUESTERHAUS, B. (2000). Laboratory screening test for the evaluation of cold tolerance in cotton, Texas Tech University.

DUESTERHAUS, B., HOPPER, N. W., GANNAWAY, J. R. & JIVIDEN, G. M. (1999). Development of a laboratory screening test for the evaluation of cold tolerance in cotton seed germination. In *Proceeding of the Beltwide Cotton Conference*, vol. 1, pp. 621-623. National Cotton Council, Memphis, Tennessee.

DUESTERHAUS, B., HOPPER, N. W., GANNAWAY, J. R. & VALCO, T. D. (2000). A screening test for the evaluation of cold tolerance in cottonseed germination and emergence. In *Proceedings of the Beltwide Cotton Conference*, vol. 1, pp. 596-599. National Cotton Council, Memphis, Tennessee.

FAROOQ, M., AZIZ, T., BASRA, S. M. A., CHEEMA, M. A. & REHMAN, H. (2008). Chilling tolerance in hybrid maize induced by seed priming with salicylic acid. *Journal of Agronomy and Crop Science* 194, 161-168.

FARRELL, T., SERAFIN, L. & KNEIPP, J. (2007). Summer crop production guide 2007. NSW Department of Primary Industries.

FARRELL, T. C., FOX, K. M., WILLIAMS, R. L. & FUKAI, S. (2006). Genotypic variation for cold tolerance during reproductive development in rice: Screening with cold air and cold water. *Field Crops Research* 98, 178-194.

GLAT, D., TAYLOR, B. B. & WILLIAMS, M. D. (1982). The relationship of predictive tests for planting seed quality and field performance. In *Proceeding of the Beltwide Cotton Production Research Conference*, pp. 65-67, Memphis, Tennessee.

GREEN, J. M. & ROBERTS, F. L. (2001). Cold tolerant cotton for ultra early planting. In *Proceeding of the Beltwide Cotton Conference*, vol. 1, pp. 30-32. National Cotton Council, Memphis, Tennessee.

GREGORY, J., HOPPER, N. W. & JIVIDEN, G. M. (1986). Evaluation of several laboratory tests for determining cottonseed quality. In *Proceeding of the Beltwide Cotton Production Research Conference*, pp. 62-63, Memphis, Tennessee.

GUINN, G. (1971). Changes in sugars, starch, RNA, protein, and lipid-soluble phosphate in leaves of cotton plants at low temperatures. *Crop Science* 11, 262-265.

HALL, A. J. & GANNAWAY, J. R. (2005). Evaluation of fatty acid composition of cotton germplasm and association with cold tolerance. In *Proceeding of the Beltwide Cotton Conference*, pp. 923-931, New Orleans, Louisiana.

HEARN, A. B. & CONSTABLE, G. A. (1984). Cotton. In *The Physiology of Tropical Field Crops* (ed. P. R. Goldsworthy and N. M. Fisher), pp. 495-527. John Wiley & Sons Ltd, Chinchester.

HODGES, H. F., REDDY, K. R., MCKINION, J. M. & REDDY, V. R. (1993). Temperature effects on cotton, pp. 1-15. Mississippi Agricultural & Forestry Experiment Station, Mississippi State.

HODGES, S. C. (1992). Nutrient Deficiency Disorders. In *Cotton Diseases* (ed. R. J. Hillocks). CAB International.

HOPPER, N. W., GANNAWAY, J. R., JIVIDEN, G. M. & STRUVE, T. H. (1994). Selection of cold tolerant lines of cotton based on seedling response. In *Proceeding of the Beltwide Cotton Conference*, pp. 1375. National Cotton Council, Memphis, Tennesse.

KANEMASU, E. T., BARK, D. L. & CHOY, E. C. (1975). Effect of soil temperature on sorghum emergence. *Plant and Soil* 43, 411-417.

KELLEHER. (2003). Crop Adaption. In *Principles of field crop production* (ed. J. Pratley), pp. 78-158. Oxford University Press, South Melbourne, Victoria, Australia.

KERBY, T. A., KEELEY, M. & JOHNSON, S. (1989). Weather and seed quality variables to predict cotton seedling emergence. *Agronomy Journal* 81, 415-419.

KITTOCK, D. L., TAYLOR, B. B. & HOFMANN, W. C. (1987). Partitioning yield reduction from early cotton planting. *Crop Science* 27, 1011-1015.

KLOS, K. L. E. & BRUMMER, E. C. (2000). Field response to selection in alfalfa for germination rate and seedling vigor at low temperatures. *Crop Science* 40, 1227-1232.

KNOX, R. B., LADIGES, P., EVANS, B. & SAINT, R. (2005). *Biology: an Australian focus*, 3 edition. McGraw Hill Australia Pty Limited, Sydney, Australia.

LAUTERBACH, B., KRIEG, D. R. & JIVIDEN, G. M. (1999). Fatty acid composition of lipid fractions in germinating cotton as affected by temperature. In *Proceedings of the*

beltwide cotton conference, vol. 1, pp. 564-565. National Cotton Council of America, Memphis, Tennessee.

LAWRENCE, C. & HOLADAY, A. S. (2000). Effects of mild night chilling on respiration of expanding cotton leaves. *Plant Science* 157, 233-244.

LYONS, J. M. (1973). Chilling injury in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 24, 445-466.

LYONS, J. M. & RAISON, J. K. (1970). Oxidative activity of mitochondria isolated from plant tissues sensitive and resistant to chilling injury. *Plant Physiology* 45, 386-389.

MAHAN, J. R. & GITZ, D. C., III. (2007). A dynamic model of cotton emergence based on the thermal dependence of malate synthase. *Agronomy Journal* 99, 1668-1674.

MAUNEY, J. R. (1986). Vegetative growth and development of fruiting sites. In *Cotton Physiology* (ed. J. R. Mauney and J. M. Stewart), pp. 11-28. The Cotton Foundation, Memphis, Tennesse.

MCDOWELL, A. J., BANGE, M. P. & TAN, D. K. Y. (2007). Cold temperature exposure at 10^{o}C for 10 and 20 nights does not reduce tissue viability in vegetative and early flowering cotton plants. *Australian Journal of Experimental Agriculture* 47, 198-207.

MCMAHON, J. & LOW, A. (1972). Growing degree days as a measure of temperature effects on cotton. *Cotton Growing Review* 49, 39-49.

MCMICHAEL, B. L. & BURKE, J. J. (1994). Metabolic activity of cotton roots in response to temperature. *Environmental and Experimental Botany* 34, 201-206.

MCMICHAEL, B. L., UPCHURCH, D. R. & BURKE, J. J. (1996). Soil temperature derived prediction of root density in cotton. *Environmental and Experimental Botany* 36, 303-312.

OOSTERHUIS, D. M. (1990). Growth and development of a cotton plant. In *Nitrogen Nutrition in Cotton: Practical Issues*. Cooperative Extension Service, University of Arkansas.

RADIN, J. W., LU, Z., PERCY, R. G. & ZEIGER, A. (1994). Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation. *Proclimations of the National Academy of Science. USA* 91, 7217-7221.

RAHMAN, H. U. (2005). Genetic analysis of stomatal conductance in upland cotton (*Gossypium hirsutum* L.) under contrasting temperature regimes. *The Journal of Agricultural Science* 143, 161-168.

REDDY, V. R., BAKER, D. N. & HODGES, H. F. (1991). Temperature effects on cotton canopy growth, photosynthesis and respiration. *Agronomy Journal* 83, 699-704.

RIKIN, A. (1991). Temperature-induced phase shifting of circadian rhythms in cotton seedlings as related to variations in chilling resistance. *Planta* 185, 407-414.

ROCHESTER, I. J. & LARSON, D. (2001). *NUTRIpak: a practical guide to cotton nutrition*. Cotton Research and Development Corporation, Narrabri, New South Wales, Australia.

ROUSSOPOULOS, D., LIAKATAS, A. & WHITTINGTON, W. J. (1998). Cotton responses to different light-temperature regimes. *Journal of Agricultural Science* 131, 277.

SCHULZE, D. H., HOPPER, N. W., GANNAWAY, J. R. & JIVIDEN, G. M. (1996). Laboratory tests used to screen for chilling tolerance in cotton genotypes. In *Proceedings of the Beltwide Cotton Conference*, vol. 2, pp. 1240-1243. National Cotton Council, Memphis, Tennessee.

SCHULZE, D. H., HOPPER, N. W., GANNAWAY, J. R. & JIVIDEN, G. M. (1997). Evaluation of chilling tolerance in cotton genotypes. In *Proceedings of the Beltwide Cotton Conference*, vol. 2, pp. 1383-1385. National Cotton Council, Memphis, Tennessee.

SHAW, A. J. (1991). Sunflower Variety and sowing guide 1990-91, vol. Agnote Reg 2/002. New South Wales Agriculture and Fisheries.

SIMON, E. W. & HARUN, R. M. R. (1972). Leakage during seed imbibition. *Journal of Experimental Botany* 23, 1076-1085.

SINGH, R. P., PRASAD, P. V. V., SUNITA, K., GIRI, S. N. & REDDY, K. R. (2007). Influence of High Temperature and Breeding for Heat Tolerance in Cotton: A Review. In *Advances in Agronomy*, vol. Volume 93, pp. 313-385. Academic Press.

SMITH, C. W. & VARVIL, J. J. (1984). Standard and cool germination tests compared with field emergence in upland cotton. *Agronomy Journal* 76, 587-589.

STAMP, P. (1984). Chilling tolerance of young plants demonstrated on the example of maize (Zea mays L.). *Advances in Agronomy and Crop Science 7*, 1-83.

STEINER, J. J. & JACOBSON, T. A. (1992). Time of planting and diurnal soil temperature effects on cotton seedling field emergence and rate of development. *Crop Science* 32, 238-244.

STEWART, J. M. D. & GUINN, G. (1971). Response of cotton mitochondria to chilling temperatures. *Crop Science* 11, 908-910.

TAYLOR, H. M. & RATLIFF, L. F. (1969). Root elongation rates of cotton and peanuts as a function of soil strength and soil water content. *Soil Science* 108, 113-119.

THOMSON, N. J., REID, P. E., CONSTABLE, G. A., ROCHESTER, I., PURCELL, J., HULME, P., APPERSON, P. & BUSTER, S. (2001). *Applied cotton production. Resource book*. University of New England, Armidale.

TOLLIVAR, J., SAVOY, B. R. & DRUMMOND, E. A. (1997). Cool germination test on cotton - Variability between seed testing laboratories. In *Proceeding of the Beltwide Cotton Conference*, pp. 442-443, Memphis, Tennessee.

TYAGI, S. K. & TRIPATHI, R. P. (1983). Effect of temperature on soybean germination. *Plant and Soil* 74, 273-280.

WANG, C. Y. (1990). *Chilling injury of horticultural crops*. CRC Press, Inc, Boca Ration, Florida.

WANJURA, D. F. (1981). Germination and strand establishment. In *Proceeding of the Beltwide Cotton Conference*, pp. 321. National Cotton Council Memphis, Tennessee.

WANJURA, D. F. & BUXTON, D. R. (1972a). Hypocotyl and radicle elongation of cotton as affected by soil environment. *Agronomy Journal* 64, 431 - 434.

WANJURA, D. F. & BUXTON, D. R. (1972b). Water uptake and radicle emergence of cottonseed as affected by soil moisture and temperature. *Agronomy Journal* 46, 427 - 431.

WANJURA, D. F., BUXTON, D. R. & STAPLETO, H. N. (1970). Temperature Model for Predicting Initial Cotton Emergence. *Agronomy Journal* 62, 741-743.

WANJURA, D. F., HUDSPETH, E. B. J. & BILBRO, J. D. J. (1969). Temperature effect on emergence rate of cotton *(Gossypium hirsutum* L.) under field situations. *Agronomy Journal* 61, 387-389.

WOOD, A. W., TAN, D. K. Y., MAMUN, E. A. & SUTTON, B. G. (2006). Sorghum can compensate for chilling-induced grain loss. *Journal of Agronomy and Crop Science* 192, 445-451.

YAKLICH, R. W., KULIK, M. M. & ANDERSON, J. D. (1979). Evaluation of vigor tests in soybean seeds: relationship of ATP, conductivity, and radioactive tracer multiple criteria laboratory tests to field performance. *Crop Science* 19, 806-810.

YU, J. M. & TUINSTRA, M. R. (2001). Genetic analysis of seedling growth under cold temperature stress in grain sorghum. *Crop Science* 41, 1438-1443.