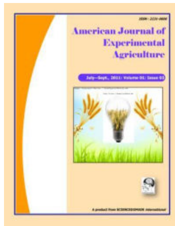

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Seedling Root Growth Response to Cool Environmental Conditions for Diverse Cotton Cultivars

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Research Article

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ABSTRACT

Cotton (*Gossypium hirsutum* L.) grown in the Mississippi River Delta is generally planted in cool conditions that can lead to slow germination, uneven emergence, and poor root growth. A study was conducted to determine the effects of low temperature on four genetic and geographically diverse cotton genotypes. The cultivars chosen were: Acala Maxxa, grown primarily in California; Stoneville 4892BR, grown primarily in the Mississippi River Delta; Tamcot Sphinx, from the plains of Texas; and FiberMax 966, with a genetic makeup from Australia. The cultivars were grown for 10 days in six temperature regimes: 15/20°C, 15/25°C, 15/30°C, 15/35°C, 15/40°C, and 15/45°C (night/day). The temperature regimes resulted in different root growth patterns for each cultivar. Tamcot Sphinx was statistically greater than all other cultivars in root dry matter, while the statistical least amount of cotyledon dry matter was produced in the 15/20°C temperature regime. The highest lateral root length, lateral root numbers, and root branch intensity were produced at 15/35°C. Tamcot Sphinx was numerically higher than all other cultivars in mean taproot length and total lateral root number over all temperature regimes. In the cool temperature regime of 15/20°C, Stoneville 4892BR had the greatest taproot length, showing tolerance for cooler temperatures. The results from the study on taproot and lateral root growth showed the existence of genetic diversity for root growth among cotton genotypes which can be exploited to find a cultivar able to tolerate cool temperatures and produce a more vigorous cotton seedling.

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Keywords: Gossypium hirsutum; cotton; roots; temperature; early planting; cold tolerance; genetic variability.

1. INTRODUCTION

Cotton (*Gossypium hirsutum* L.*)* is a warm climate plant growing under the 200 day frost-free isotherm, but is often planted during cool conditions that slow germination, emergence, and seedling growth. Burke et al. (1988) found the thermal kinetic window where the optimal temperature for cotton plants to function is between 23.5 and 32°C and that the cotton plant is in this window only 25 percent of the season. Genetic differences have been noted to exist in and between species for root response to soil conditions (Mitchell and Bourland, 1986; Kaspar and Bland, 1992), but this has not been explored in cotton germplasm development. Some genetic diversity has also been shown to exist in root systems (McMichael and Burke, 1998: Kennedy et al., 1987). Roots function to provide plant stability, as well as water and nutrient absorption. Low temperatures can reduce water and nutrient uptake in the roots (Nielsen, 1974; Hund et al., 2007), thereby reducing the overall health of the plant (Jiang et al., 2010). As the temperature of the roots decrease, hydraulic conductance can slow due to reduced membrane permeability (Bolger et al., 1992; Alline et al., 2009), and enzymatic activity decreases (Nielsen, 1974; Cruz et al., 1983). McMichael and Burke (1998) demonstrated improved seedling growth when temperatures are favorable for mobilization of seed reserves. Jennings et al. (1999) found that cotton seedling shoot dry weight was reduced under cool temperatures.

Bradow (1990) found the optimal temperature for growth of shoots and roots differed for cotton seedlings at 10 days. In the cooler temperature of 15°C Bradow (1990) reported the shoot fresh weight to be higher than the root weight and the opposite to occur in the higher temperature of 35°C. McMichael and Burke (1994) discovered that cotton roots grew larger at a constant day-night temperature of 28°C. Quisenberry et al. (1981) documented that the optimal temperature for taproot length and lateral roots varied according to genotype, Furthermore, Forbes et al. (1997) found that in ryegrass (*Lolium perenne*), root length increased as temperatures increased to 27°C. In Monterey pine (*Pinus radiata*) seedlings, Bowen (1970) showed that the taproot length and the number of lateral roots increased with temperature. Huang et al. (1991) recorded fewer numbers of lateral roots and reduced lateral root length in winter wheat (*Triticum aestivum* L.) when grown in cooler temperatures. Balisky and Burton (1997) had similar findings with Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) and Lodgepole pine (*Pinus contorta* Dougl. ex Loud.) seedlings. Xu and Huang (2001) revealed that a creeping bent grass cultivar (*Agrostis palustris* Huds.) possessed a higher tolerance to stress that was associated with a more extensive root system for water uptake and transpirational cooling. Bolger et al. (1992) reported greater cotton seedling branching ability facilitated higher conductivity, and suggested this could be a source for better adaptation to cooler soil temperatures. If a cotton cultivar could be found that was more tolerant to cooler planting temperatures, some insects and pathogens may be avoided (Munro, 1987), as well as late-season harsh climate events such as drought and hurricanes. Although root length and dry matter have been studied in relation to cool temperatures, there have been no quantification of the number and length of branch roots in modern cultivars from major geographical regions of cotton production. It was hypothesized that genotypic differences exist in cotton genotypes for tolerance to cool temperatures. The objective of this study was to screen geographically diverse cotton cultivars for morphological root differences under cooler temperatures in controlled environmental conditions.

2. MATERIALS AND METHODS

A diverse set of cotton (*Gossypium hirsutum* L.) cultivars: Acala Maxxa, Stoneville 4892BR, Tamcot Sphinx, and FiberMax 966 (Table 1) were germinated and grown in transparent 16.5-cm wide by 17.7-cm high CYG germination pouches (Mega International, West St Paul, MN) for 10 days in Conviron Growth Chambers (Controlled Environments Limited, Winnipeg, Manitoba, Canada). A Conviron CMP 3023 growth chamber was used for the first run and multiple Conviron growth chambers, models CMP 3023 and CMP 4030, were used in the second run. Each growth pouch contained 50-ml of half-strength Hoagland's solution (Hoagland and Arnon, 1950). Additional nutrient solution was stored in the growth chamber with the pouches, so that when extra nutrient solution was needed in the growth pouches, it would be at the same temperature as the seedlings.

Table 1. Origin and parent lines of cotton cultivars screened

Cultivar	Origin	Parent line
Acala Maxxa Adapted to dry, mild conditions	Western U.S.	T7538 crossed with S4959
Stoneville 4892BR Planted into cool, moist conditions	Mississippi River Delta	Stacked gene addition to Stoneville 474
Tamcot Sphinx Grown in hot and cool extremes	Southwestern U.S.	MAR-CDP3HPIH 1-1-86 crossed with Paymaster 145
FiberMax 966 Grown throughout the Southern U.S.	Australian Continent	Not Public

Individual CYG germination pouches contained a germination paper with a "v" fold at the top of the paper to hold the seed. Perforations were torn on each side of the germination paper to allow the cotton root to penetrate to the nutrient solution. Each growth pouch was clipped with two mini-binder clips on either side to a cardboard backing measuring 21-cm wide by 20-cm high. The growth pouch and backing were placed upright on a metal tray separated by metal arched dividers. Ten growth pouches with two seeds each were placed in each tray with two trays per temperature regime. This allowed for ten cotton seedlings per cultivar with an experimental unit consisting of two seedlings per pouch and five replications per cultivar per temperature regime. The experiment was repeated (Studies A and B) for confirmation of data and the statistical model used was a split plot. SAS 9.1 (SAS Institute Inc., Cary, N.C.) was used for the statistical evaluation of data using analysis of variance and the least significant difference (LSD) test according to the general linear model (GLM) procedures of SAS. No statistical difference was found between the two trials as a main effect.

The night temperature used for all six temperature regimes was 15℃. This temperature was chosen because it is the threshold temperature for enzymatic activity in cotton plants (Bradow, 1990). Treatments used a 10 hour night temperature gradually increasing to a four hour peak day temperature. The six temperature regimes were: 15/20℃, 15/25℃, 15/30℃, 15/35℃, 15/40℃, and 15/45℃ (night/day temperature). The temperature of 45℃ was used because it was the highest possible temperature the growth chambers were able to produce. No lights were used at any time in the growth chambers and the cotton seedlings were only exposed to light during daily measurements for no longer than 10 minutes. Each day the trays were placed at a different location in the growth chamber to ensure uniform temperature regimes for the pouches.

Each day at approximately 4:00 p.m., taproot length was marked on the front of each growth pouch with a line of alternating color and a date to indicate the amount of growth for that day. Also, if lateral roots appeared, the pouch was marked in front of the lateral root tip with an alternating color indicating which day the lateral root emerged from the tap root. At day 10, the cotton seedlings were carefully extracted from the growth pouch by splitting all perforations and carefully removing the seedlings through the top of the pouch for measurement.

Seedlings were dissected on the laboratory bench using a scalpel with a surgical blade to separate the cotyledons from the hypocotyl, and the hypocotyl from the roots. The cotyledons were determined to end where the hypocotyl connected to the cotyledon. The hypocotyl was determined to end and the taproot began where the hypocotyl's green coloration whitened and the gossypol gland numbers diminished. Due to the variation in size of the cotyledons, from none to fully extended, the size was not recorded throughout the temperature regimes. The length of the taproot was measured daily, including the tenth day, during which the height of the hypocotyl was measured. After drying for a period of 24 hours at 60℃, the dry weight of the roots, shoots, and cotyledons were recorded.

3. RESULTS AND DISCUSSION

3.1 Root Dry Weight

The highest root dry matter weight(0.05 g) occurred in the 15/25℃ temperature regime (Fig. 1A).The temperature of 25℃ is similar to what McMichael and Burke (1994) found to be the optimal temperature for cotton root growth, which is 28/28℃ (night/day). This is numerically similar to the finding of Reddy et al. (1997) who observed that cotton root dry weight in the top 40-cm of soil was greatest at the temperatures of 30℃ and 35℃.

The cotton root dry weight was lower when the plants were exposed to temperatures above and below these regimes. These findings agree with the sunflower (*Helianthus annuus*) study of Allinne et al. (2009) where root dry weights were lower in cool conditions. Examining the root dry matter in the study by cultivar over all temperature regimes, Tamcot Sphinx was statistically different ($P \leq 0.05$) than all cultivars at 0.048g root dry matter (Fig. 1B).Stoneville 4892BR and FiberMax 966 were not statistically different than each other but were statistically different than Acala Maxxa. Acala Maxxa had the lowest dry root matter at 0.035g and was statistically lower than the other three cultivars. Differences in root dry weight by cultivar were also found to exist in beans (*Phaseolus coccineus* L.) by Rodiño et al. (2007).

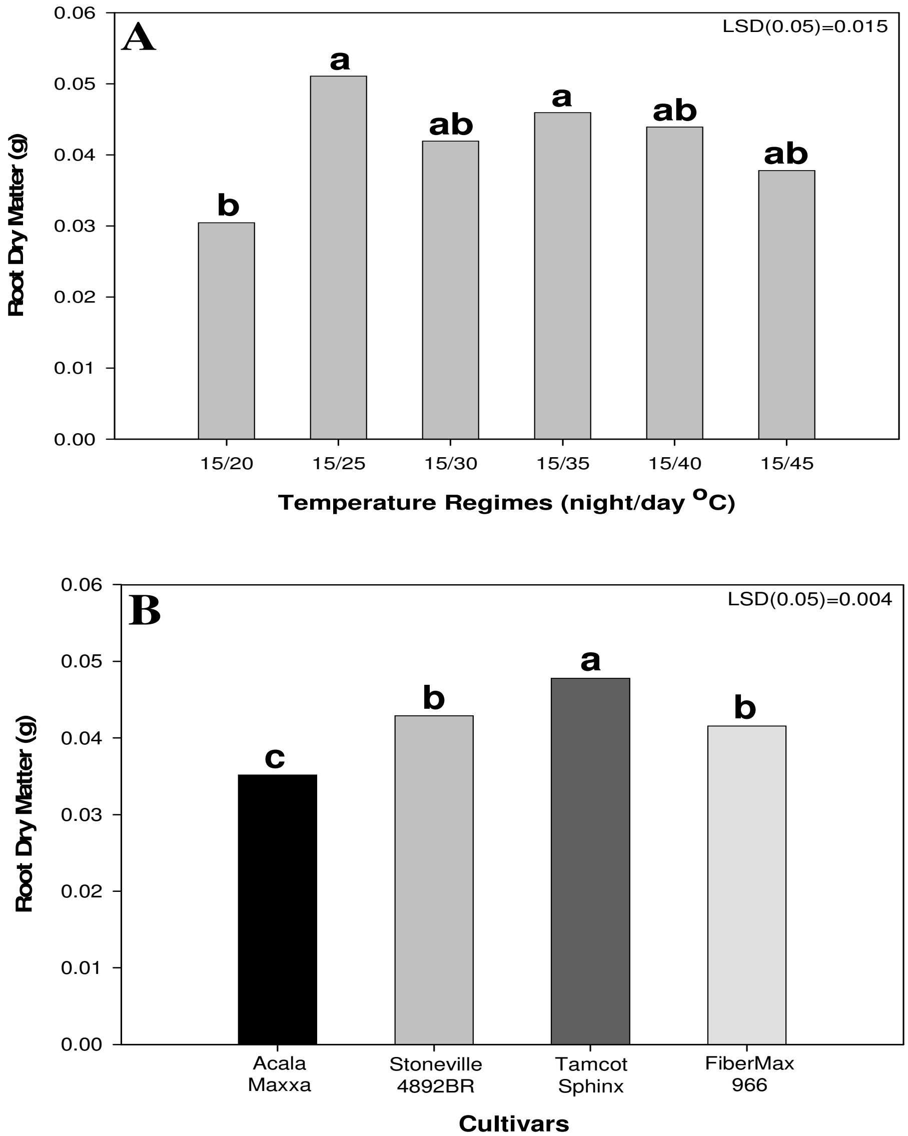

Fig. 1. Effect of temperature on root dry matter in the growth pouch experiment (A) averaged across all temperature regimes for each cultivar, and (B) averaged across the four cultivars and pooled into each temperature regime.
Columns superseded by the same letter are not significantly different (P≤0.05).

3.2 Cotyledon Dry Weight

A significant effect (P≤0.05) of temperature treatment was only shown for cotyledon dry matter at day 10 for the15/20°C temperature regime as compared to the other temperatures (Fig. 2A).There were more differences in cultivar response to temperature (Fig. 2B) with

Stoneville 4892BR being significantly lower in cotyledon dry matter weight than Acala Maxxa and FiberMax 966, with a mean of 0.37g for cotyledon dry matter (Fig. 2B).

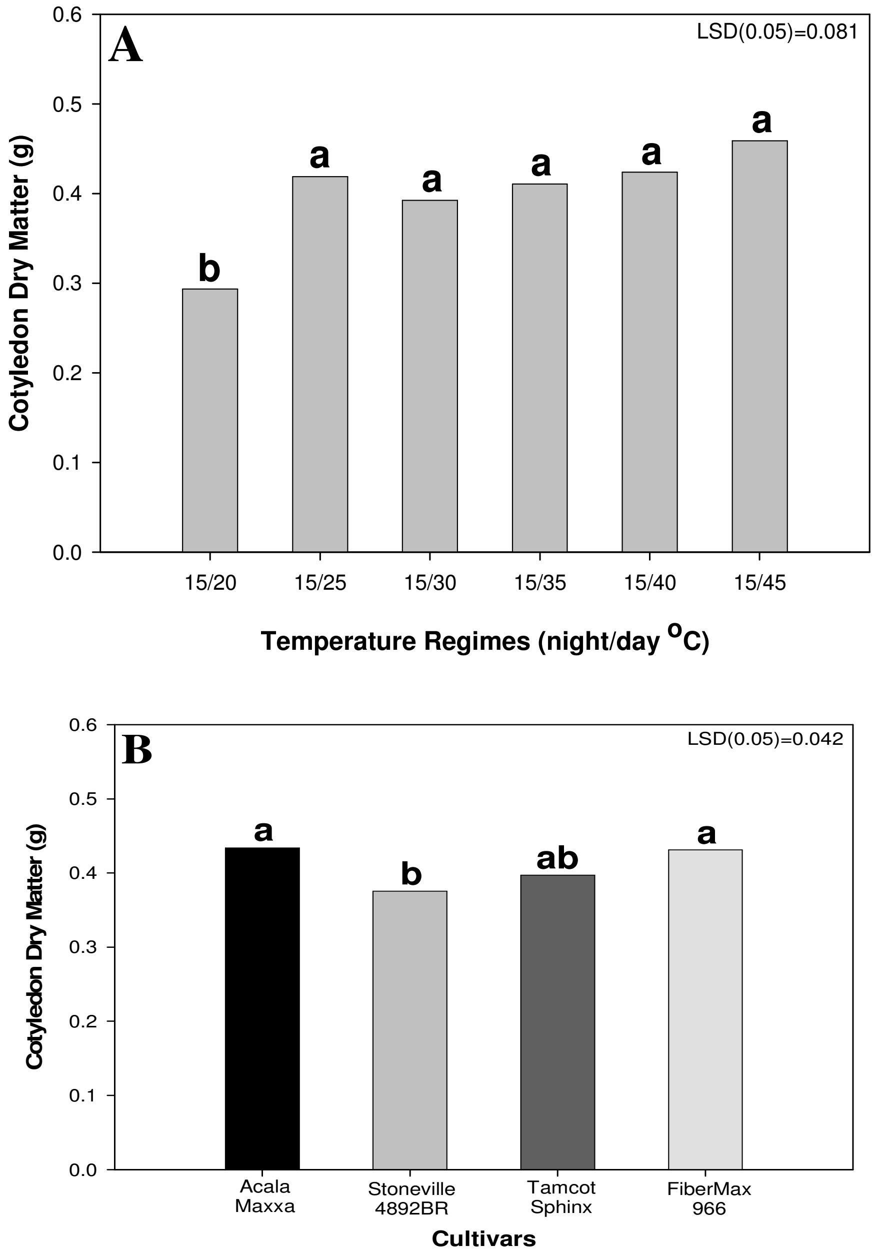

Fig. 2. Effect of temperature on cotyledon dry matter (A) averaged across the four cultivars and pooled into each temperature regime for the growth pouch experiment, and (B) across all temperature regimes in each cultivar for the growth pouch experiment.

Columns superseded by the same letter are not significantly different (P≤0.05).

A reduction in dry matter accumulation between genotypes during low temperatures was noticed by Allino et al.(2009) and for shoot dry matter of runner beans in cool growth conditions (Rodiño et al., 2007).

Measurements of cotyledon dry matter shown in Figure2B revealed information on cotton seedling root and shoot growth. In more favorable warm temperatures, cotton seedlings can mobilize seed reserves better for seedling growth (McMichael and Burke, 1998). Figure 2B shows Tamcot Sphinx and Stoneville 4892BR mean cotyledon dry matter at day 10 over all temperature regimes to be lower than Acala Maxxa and FiberMax 966, while Figure 1B shows mean root dry matter at day 10 was higher in Tamcot Sphinx and Stoneville 4892BR as compared to Acala Maxxa and FiberMax 966. This reveals that perhaps these two cultivars were superior at mobilizing sugars to produce a larger, more vigorous root system.

3.3 Taproot Length

The effect of temperature on daily taproot length is presented in Figures 3 and 4A, B, C, and D. In daily taproot growth of the first pouch study (A), Stoneville 4892BR exhibited the greatest overall taproot growth for all temperatures, that culminated around 50 mm in length at day 10, showing its stability over a wide range of temperature regimes (Fig. 3B). The second run of the growth pouch study (B) revealed similar trends in taproot growth where taproot growth for all temperature regimes of Stoneville 4892BR seemed to converge on one point again at day 10, except the temperature regime of 15/30°C (Fig. 4B). This is possibly due to the day time temperature being in the thermal kinetic window for the cotton plant resulting in greater growth of the seedlings.

When both growth pouch studies for the temperature regime of 15/20°C were averaged together for statistical analysis, Stoneville 4892BR was significantly greater than Tamcot Sphinx with a mean taproot length of 37.6 mm (Fig. 5). Tamcot Sphinx had the lowest taproot length of the four cultivars at 16.6 mm in the 15/20°C temperature regime. This may be due to Tamcot Sphinx being selected for slow radicle growth at cool temperatures.

When the taproot lengths of all cultivars were averaged together over both growth pouch studies for all temperature regimes (Fig. 7), Stoneville 4892BR had the largest mean taproot length at slightly less than 50 mm and was significantly different than the two lowest cultivars, Acala Maxxa and FiberMax 966. Tamcot Sphinx had the second longest mean taproot length and was slightly below Stoneville 4892BR. FiberMax 966 had inconsistent growth, revealed by being statistically lowest in taproot length (Fig. 7).

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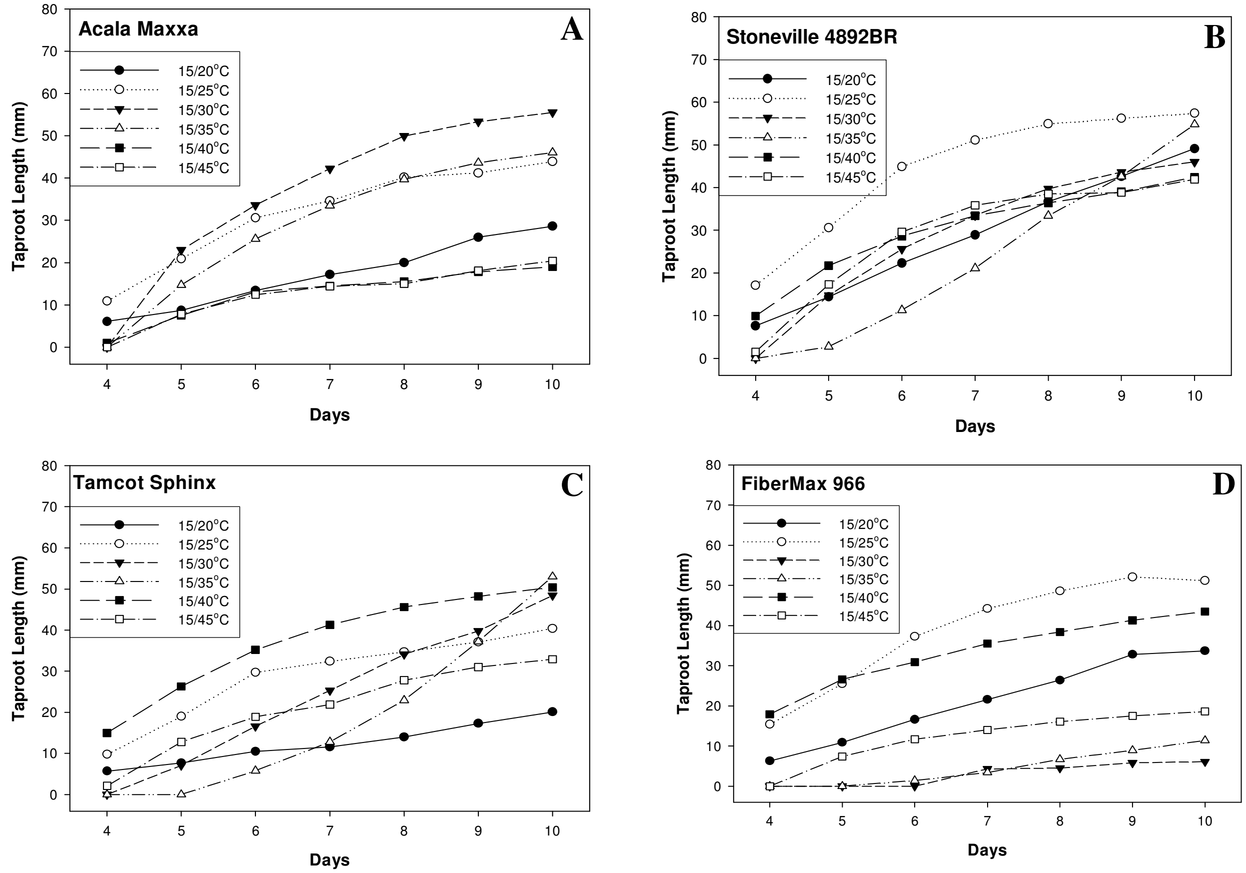


Fig. 3. Daily taproot growth of the first growth pouch study A for (A) Acala Maxxa, (B) Stoneville 4892BR, (C) Tamcot Sphinx, and (D) FiberMax 966 over six temperature regimes for 10 days.

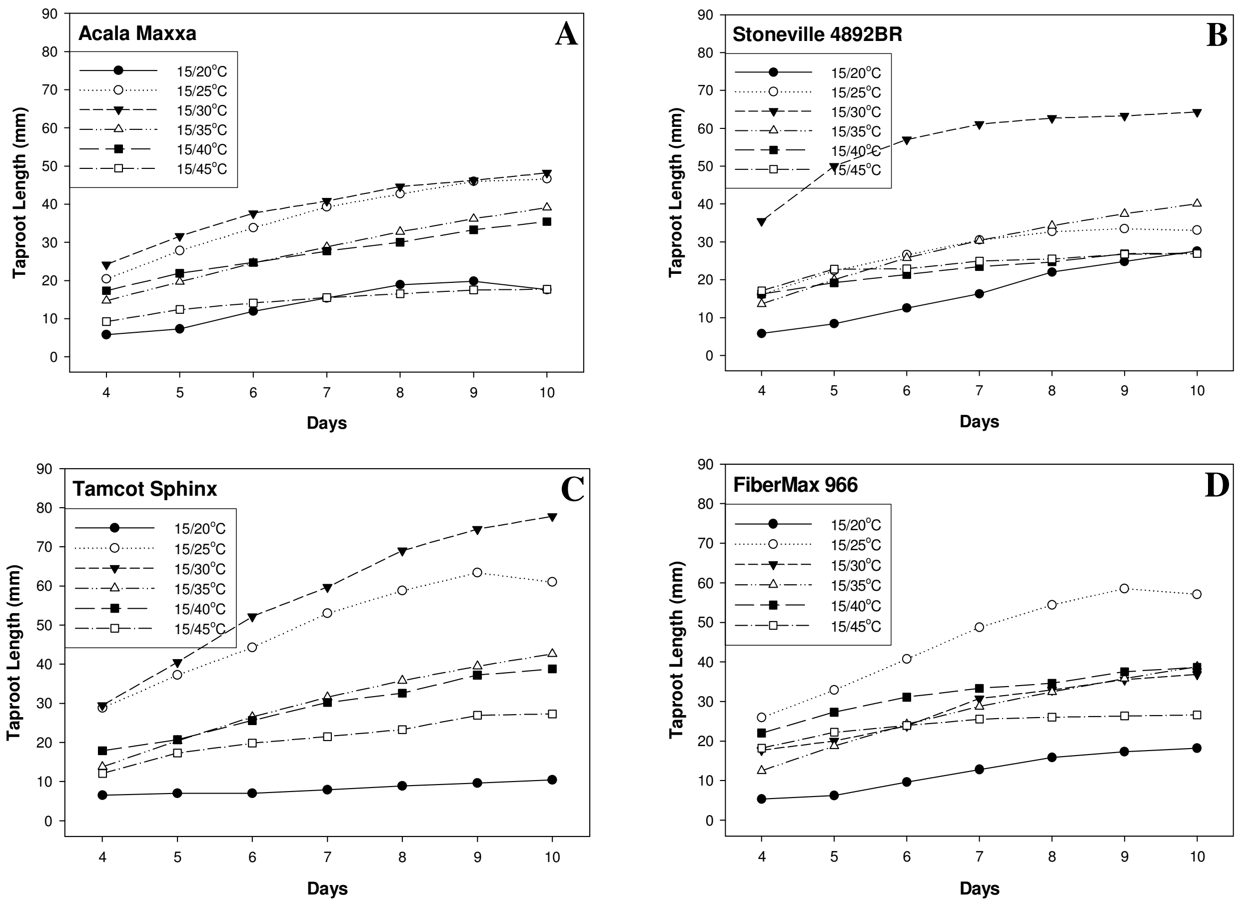


Fig. 4. Daily taproot growth of the second growth pouch study B for (A) Acala Maxxa, (B) Stoneville 4892BR, (C) Tamcot Sphinx, and (D) FiberMax 966 over six temperature regimes for 10 days.

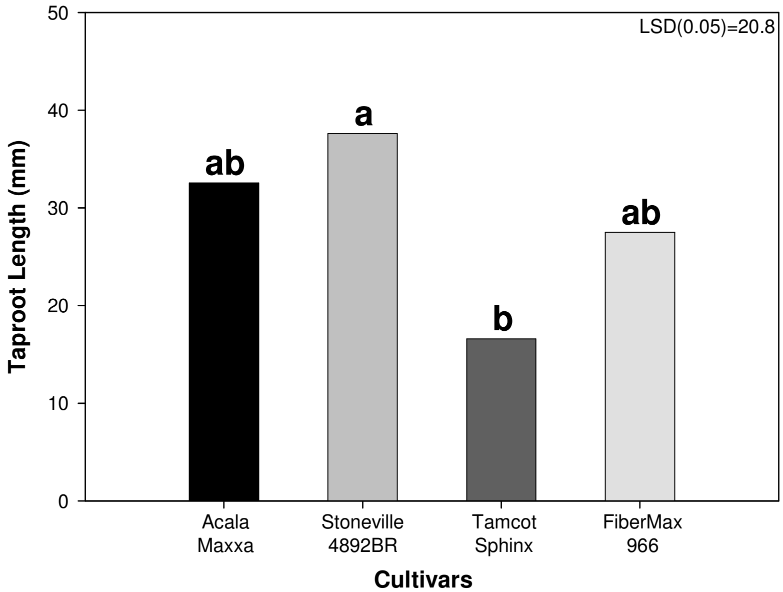


Fig. 5. Taproot length at 15/20° C temperature regimes averaged over growth pouch studies A and B.

Columns with the same letter are not significantly different (P≤0.05).

Stoneville 4892BR had the highest mean taproot length of 37.6 mm in the temperature regime of 15/20℃ (Fig. 5), which was significantly higher than Tamcot Sphinx. This indicates that Stoneville 4892BR was more tolerant to cooler temperatures while Tamcot Sphinx was able to produce a longer taproot if conditions were more favorable for taproot growth, as in the 15/35℃ temperature regime (Fig. 6C). This concurs with Kaspar and Bland (1992) who reported that similar genotypic differences exist among plant species with respect to root response to soil temperatures, although their report focused on a model predicting that root depth may follow the downward progression of increasing temperatures. They reported similar results for corn (*Zea mays* L.) and pecans (*Carya illinoinensis*) with root growth peaking around 30℃ and then declining until 40℃ or 45℃.

Comparing the effect of temperature regimes on taproot length across cultivars, the longest taproot length of most cultivars occurred at the 15/35℃ temperature regime (Figs. 6A, B, C, and D). This agrees with Bradow (1990) who reported smaller root growth than shoot growth at 15℃ temperatures, even growth at 30℃, and larger root growth at 35℃ in 10 day old cotton seedlings. Gay et al. (1991) found that root length of sunflowers (*Helianthus annuus* L.) increased until optimal temperature was reached, then declined. Stoneville 4892BR showed resilience at the extreme temperature regimes of 15/20℃, 15/40℃, and 15/45℃ (Fig. 6B). The mean taproot length for Stoneville 4892BR at all temperature regimes was around 40 mm, which was longer than the other cultivars. Stoneville 4892BR was statistically longer than two other cultivars in mean overall taproot growth at48.9 mm (Fig. 7), with both pouch studies averaged together.

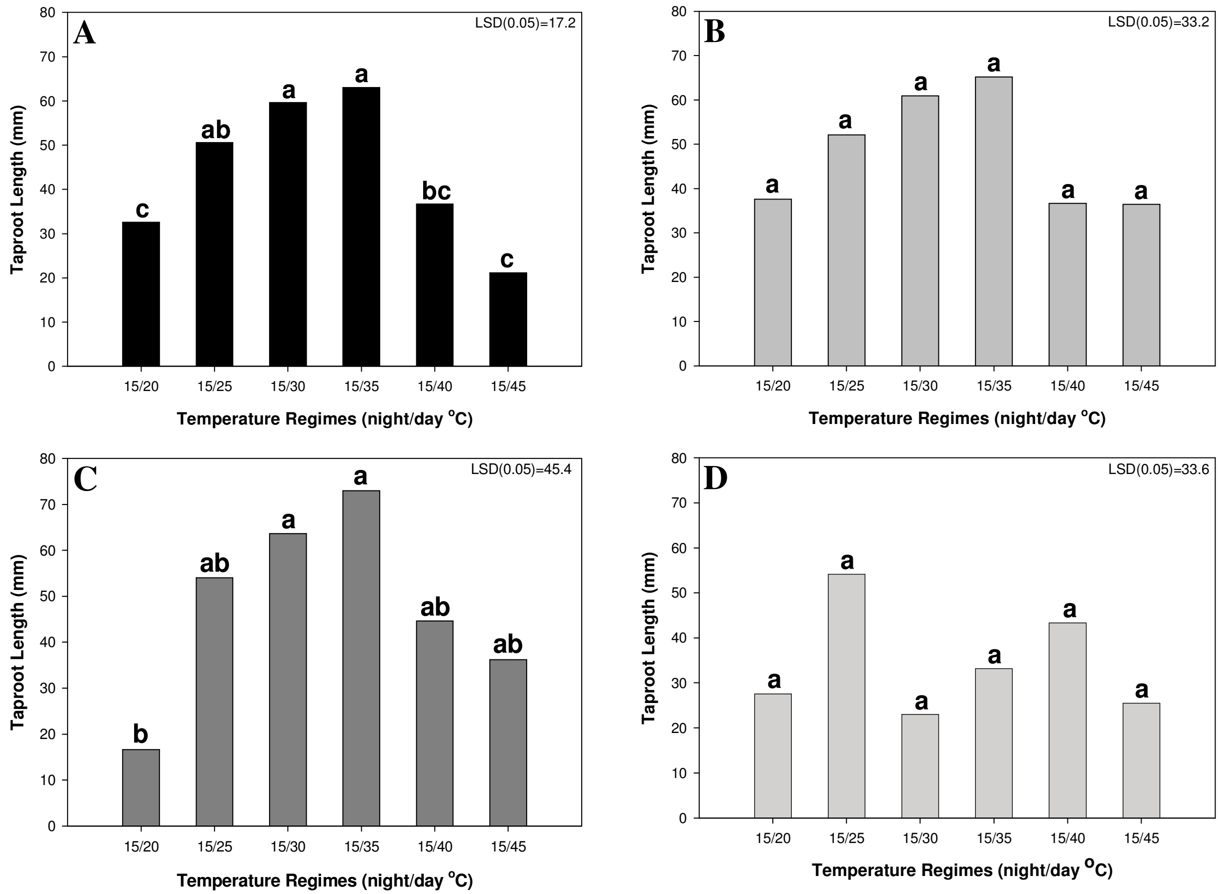


Fig. 6. Effect of temperature on taproot length of (A) Acala Maxxa, (B) Stoneville 4892BR, (C) Tamcot Sphinx, and (D) FiberMax 966 in all temperature regimes averaged over growth pouch studies A and B.
Columns with the same letter are not significantly different (P≤0.05).

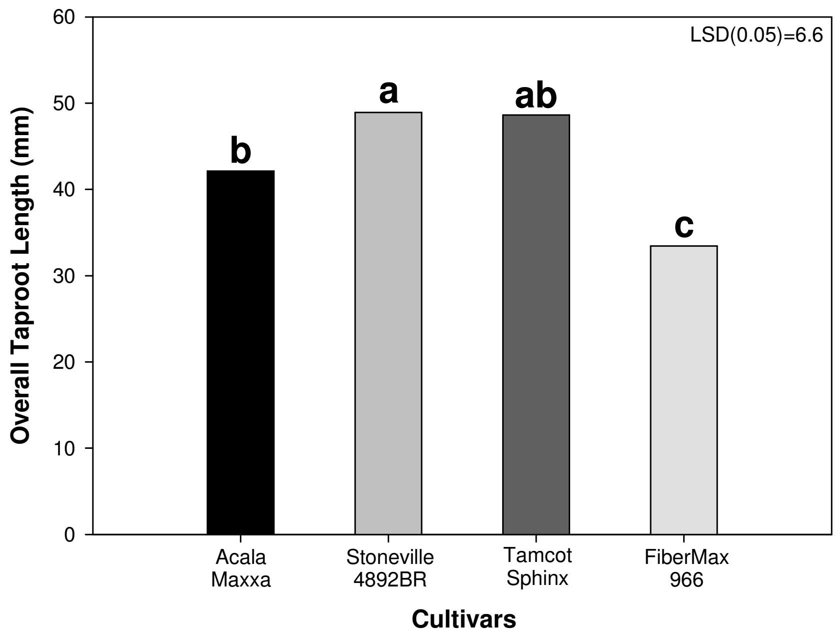


Fig. 7. Taproot length for the four cultivars averaged over all temperature regimes in growth pouch studies A and B.

Columns with the same letter are not significantly different (P≤0.05).

3.4 Lateral Root Number

The temperature regimes that produced lateral roots were 15/30℃, 15/35℃, and15/40℃ (Fig. 8A), which agrees with Balisky and Burton (1997) and Bowen (1970) who found an increasing number of lateral roots on conifer trees with increasing soil temperature. Lateral root growth was reduced at the lower temperature regimes which coincides with Huang et al. (1991) findings that wheat (*Triticum aestivum* L.) lateral root numbers decreased at cooler temperatures. When temperatures increased, lateral root growth increased with the most lateral roots occurring in the 15/35℃ temperature regime (Fig. 8A).Between all cultivars over all temperature regimes, Tamcot Sphinx had the largest mean lateral root number, 48.2 (Fig. 8B). This was not significantly different from Stoneville 4892BR or the other two cultivars. The mean lateral root number of Stoneville 4892BR was 37.0.

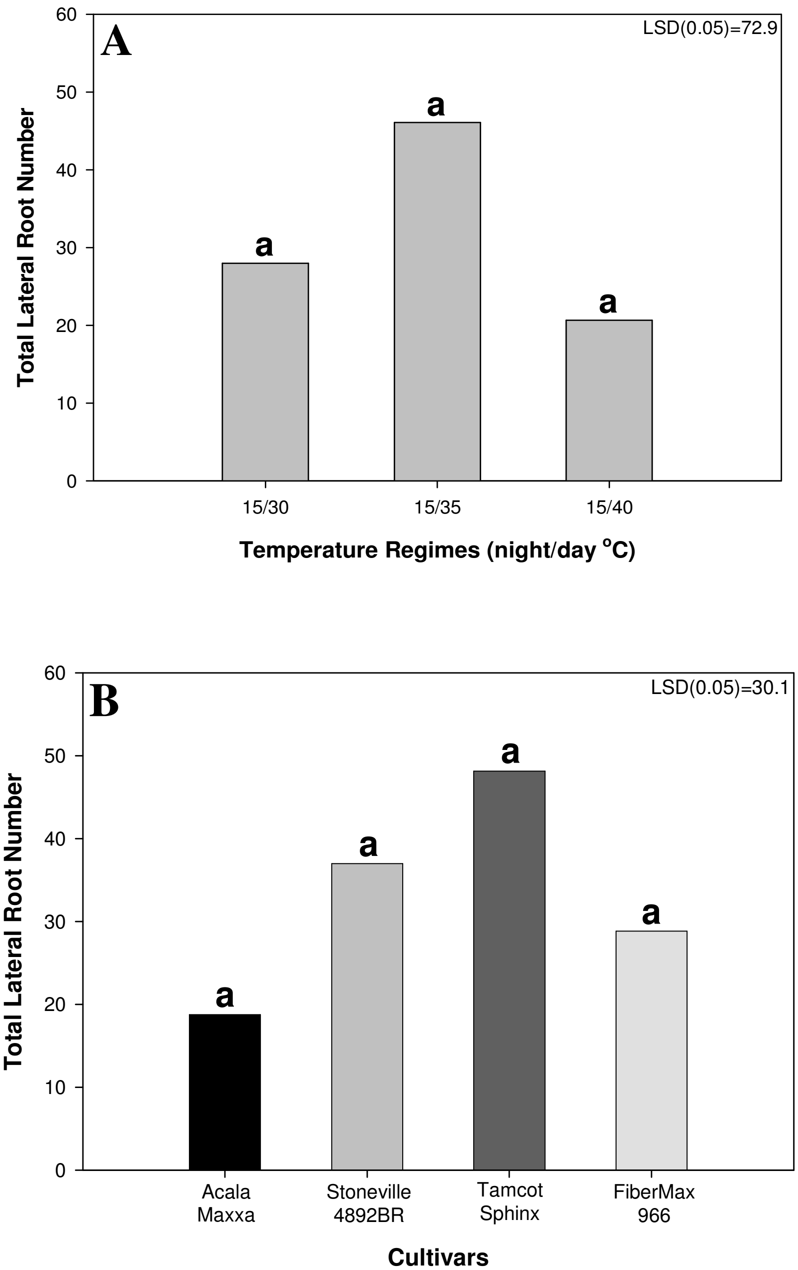


Fig. 8. Effect of temperature on mean total lateral root number with each cultivar averaged together by (A) temperature regime from both growth pouch studies, and (B) with all temperature regimes averaged together in each cultivar.

Temperature regimes not shown indicate no lateral root formation. Columns with the same letter are not significantly different (P≤0.05).

3.5 Total Lateral Root Length

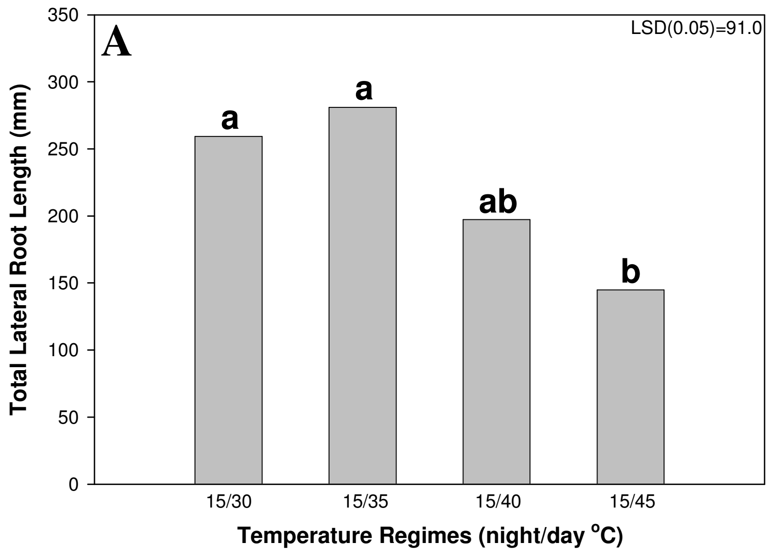
The total lateral root length was measured on the second growth pouch study. When all cultivars were averaged together according to temperature regimes, significant differences were discovered. The temperature regimes of 15/30℃ and 15/35℃ were statistically different than the lowest lateral root length in the temperature regime of 15/45℃ (Fig. 9A). Over all the temperature regimes, Tamcot Sphinx had the longest lateral root growth with the overall mean total lateral root length of 272.0 mm (Fig. 9B). Stoneville 4892BR had the second largest overall lateral root length with a mean total of 236.6 mm. Both cultivars were statistically different from FiberMax 966 which had an overall mean lateral root length of 152.3mm.

The temperature regime of 15/35℃ again produced the longest total lateral root length (Fig. 9A). Stone and Taylor (1983) found that soybean (*Glycine max* L.) lateral root length had varying optimal temperatures that differed from the ones for taproot elongation. Soybean taproots grew more favorably in slightly lower temperatures than the lateral roots, which differs from the current findings where the greatest lateral root number and taproot length were in the same temperature regime of 15/35℃.

3.6 Root Branch Intensity (Lateral Root Number Divided by the Taproot Length)

Neither 15/35℃, 15/30℃, nor 15/40℃ was significantly different from each other in the measurement of root branch intensity (Fig. 10A). The temperature regime of 15/35℃ was numerically superior again to the other temperature regimes in root branch intensity and this temperature regime is near the thermal kinetic window for cotton (Fig. 10A). This finding agreed with Huang et al. (1991) who found the greatest number of winter wheat (*Triticum aestivum* L.) lateral roots occurred at the optimal temperature regime.

When examined by cultivar, FiberMax 966 had the largest mean root branch intensity at 0.22 (Fig. 10B). FiberMax 966 had abnormal growth with stunted taproots that appeared to be swollen due to the inability to move stored reserves (Fig. 7). A number of lateral roots developed on the swollen, stunted taproot (Fig. 8B), which caused FiberMax 966 to appear to have a larger root branch intensity than other cultivars. Tamcot Sphinx and Stoneville 4892BR were intermediate and not significantly different from one another. Tan et al. (2002) found that increasing the number of root tips increased the total root surface area, which caused plants to have increased productivity. Weak plant performance was recorded by Hund et al. (2008) when poor root growth occurred. This confirms the need for vigorous lateral root growth in seedlings to increase plant productivity.



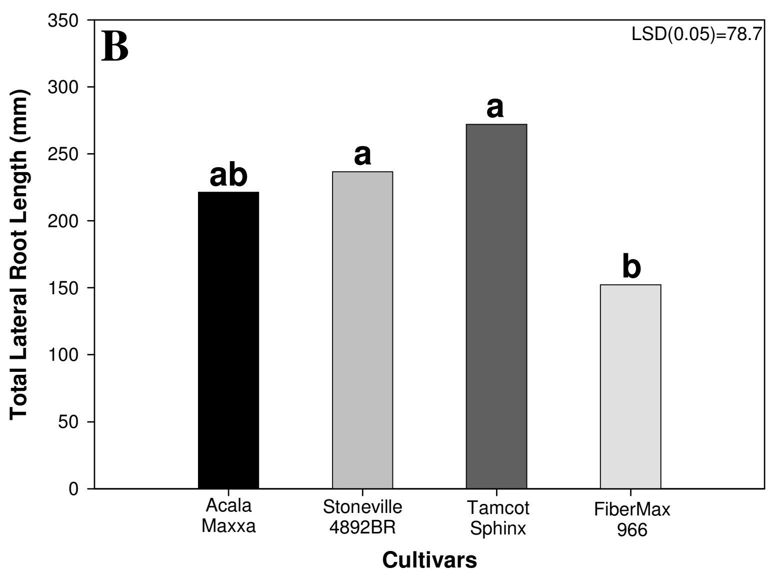


Fig. 9. Effect of temperature on mean total lateral root length (A) with all cultivars averaged into each temperature regime of growth pouch study B and (B) with all temperature regimes averaged into each cultivar of growth pouch study B.
Columns superseded by the same letter are not significantly different (P≤0.05).

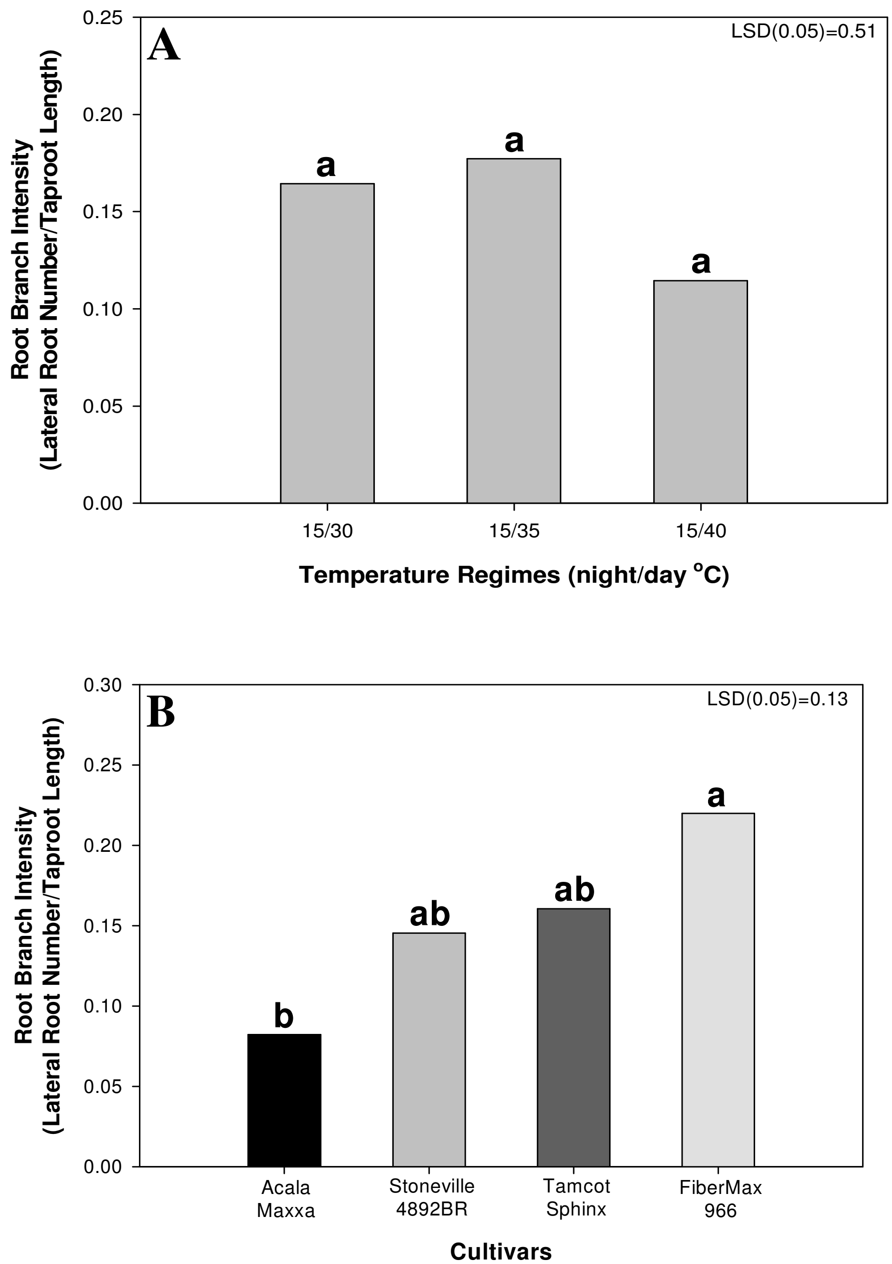


Fig. 10. Effect of temperature on mean root branch intensity of growth pouch studies A and B by all cultivars (A) averaged over in four temperature regimes and (B) all temperature regimes averaged into each cultivar.

Columns with the same letter are not significantly different (P≤0.05).

4. CONCLUSIONS

The paper focuses on four genetically diverse cotton cultivars representing all major cotton growing regions of the United States. A limited amount of studies have examined such a comprehensive range of root parameters relating to cotton seedling growth in cool temperatures. The results aid in verifying genetic differences among cultivars for improved root growth in cool conditions.

There were no significant trends for the effects of temperature on root dry matter production, but the 15/25°C temperature regime was numerically superior in root dry weight. Stoneville 4892BR was significantly longer than Tamcot Sphinx in taproot length at the temperature regime of 15/20°C. This showed resilience to cooler temperatures by Stoneville 4892BR and the inability of Tamcot Sphinx to adapt in cooler temperatures. Tamcot Sphinx had the longest taproot length of all the cultivars over all temperature regimes. Because of its longer root growth in the higher temperatures, it was statistically different than all cultivars in this measurement.

Tamcot Sphinx also had the largest number of lateral roots over all temperature regimes, but was not statistically different from the other cultivars. The largest lateral root number occurred near the thermal kinetic window for cotton at the temperature regime of 15/35°C.

The variations in cultivar root growth patterns are an indication of genotypic differences that exist in response to temperature. These results are unique in that a comprehensive range of root parameters were measured, including lateral root number, length, and branching to demonstrate substantial variation in response to temperatures in a wide range of geographically adapted cotton genotypes. Cotton genotypes in this study help to prove increased lateral roots hold promise for tolerance to cool growth conditions. These genotypes can be genetically explored for improved low temperature tolerance and exploited to produce a more vigorous, healthy cotton seedling.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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