Pistil and Style Elongations Beyond the Anthers: Results From 2005 Field Experimentation

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Abstract

A rapid heat increase from the high 80s-low 90s to high temperatures of 110^{o} F on May 21 and 22, 2005, was recorded in the Blythe, CA, area, resulting in abnormal cotton flowers in mid-June, being especially characterized by elongated styles and stigmas being beyond the anthers.

Two different cotton variety trials conducted in the Palo Verde Valley allowed data to be collected semi-weekly beginning June 17-20, with 100+ flowers per plot examined in each plot (four replications) of each of the 14 cotton variety entries. Every cotton variety had an average of 90+% of flowers expressing heat stress abnormalities (elongated styles) at the beginning of data collection even though the trials varied by planting date and location. Abnormal flowers were noted for several weeks, with some stigmas 21 mm beyond the anthers. Varieties differed in their responses to heat stress as measured by elongation later in the summer. Limited data were also collected for fruit retention and correlated with length of stigma extension beyond anthers. Retention percentages decreased as distance between anthers/stigmas increased, however boll size increased with less retention, possibly through nutrient partitioning.

Various foliar fertilizers containing calcium were also evaluated for their effect on stigma elongations of DPL 449BR cotton. Significant differences existed for stigma elongations, with 2.5 qts./acre of CalMax resulting in statistical reduction of elongation when compared with the untreated check at 3 weeks after application. Statistical differences did not exist at four weeks although statistical differences did exist at this date for the percentage of flowers affected, with the highest percentage (81.7%) noted in untreated cotton.

Introduction

Heat stress of cotton during the summer in the low desert has been noted to result in elongated styles, reduced pollen viability, reduced retention, hook-beaked bolls and associated reduction in yield (Brown and Zeiher, 1997). Spring heat is not unusual in the low desert, but is not thought to affect flowers and subsequent fruiting structures.

The spring of 2005 was very abnormal however, in that it was abnormally cool early in the season followed by a very rapid increase in both daytime and night temperatures shortly after mid-May. This resulted in daytime high temperatures reaching 110^{o}F and low temperatures of only 70^{o}F (Fig. 1), about a month previous to first bloom of many later planted fields in the area. This early heat stress was thought to

have affected pinhead and developing embryonic reproductive structures, with this damage becoming quite obvious some time later. This unique situation allowed the rare opportunity to document the longevity and severity of early season heat events on cotton flowers and reproductive structures.

Methods and Materials

Varieties

Two fields with replicated variety trials were selected. Both fields had seven varieties with four replications, with plots being 12 rows wide by approximately 1,200 foot long. The first field, located near Blythe, CA, was an early to mid-season variety trial planted March 29, 2005, with the following varieties: DPL 444BR, DPL 445BR, DPL 449BR, PHY 470WR, PHY 480WR, STV 4575BR and STR 5599BR. The second field, located near Ripley, CA, consisted of the following mid to late season varieties: DPL 449BR, DPL 454BR, DPL 455BR, DPL 555BR, PHY 710R, STV 5599BR, and STV 6636BR. This field was planted April 6, 2005. Both fields were irrigated for germination/enhanced moisture levels a few days after planting, as well as containing two varieties that could serve as comparative references (DPL 449BR and STV 5599BR).

Open flowers were examined on a semi-weekly basis (as field conditions allowed) beginning after first open flower when enough flowers were open to adequately count and measure. On the first few sample dates in each field, flowers were recorded as being either normal (stigmas not beyond anthers) or abnormal (minimum of one mm between the anthers and stigma), with 100-150 flowers so examined per plot on each sample date. In late June, records of the distance between anthers and stigmas were also recorded of 25 flowers per plot to further document the extent of varietal differences for this floral aspect.

Calcium Foliar Fertilizers

Calcium has been shown in laboratory studies to serve as an anti-oxidant in stressed cotton (Banks et al., 2001), and therefore could potentially be beneficial for low desert cotton during heat stress conditions. Previous limited evaluation of foliar fertilizers containing calcium in low desert cotton associated with heat stress has shown mixed results. Calcium applied to SureGro521BR cotton grown under a very short season production window resulted in increased retention from stressed areas of the field when Calcium Metalosate® was used but not other products (Rethwisch et al., 2003). All three calcium containing foliar fertilizer treatments resulted in lower lint yields but also increased lint quality and value per acre over the untreated check (Rethwisch et al., 2003).

Evaluation of two foliar fertilizers containing calcium applied to DPL 555BR cotton shortly after first bloom resulted in a small numeric increase in yield but slight decreases in quality (Rethwisch et al., 2004). This experiment involved long season (top crop) production, and was characterized by a very hot growing season, little retention and few bolls during the first fruiting cycle, and a vast majority of crop being set as top crop.

No existing data are known for the subject of foliar fertilizers containing calcium and if they affect stigma positioning in relation to anthers on heat stressed cotton.

Treatments were applied to DPL 449BR cotton the morning of June 24, 2005, immediately following three successive days of Level 1 heat stress. A tractor mounted sprayer calibrated to deliver 16 gpa with a single nozzle T-Jet 8008 nozzle positioned over each row was used for the application. Plots were eight rows wide (40" spacing) by field length (approximately 1,200 ft.), with four replications using a randomized complete block design. Plants were thought to have had begun blooming about 2 weeks prior to application date, and were 19 inches tall and 24-25 inches wide on June 24.

Seven foliar fertilizers containing calcium were applied to the field. Treatments were:
a) 2 qt./acre of Calcium Metalosate®, an amino acid chelated calcium (2-0-0, 6% Ca, 10.0 lbs./gal, Albion Laboratories, Clearfield, Utah).

b) 1 qt/acre CalBit® + 1 qt/acre MegaFol®. Both of these products are manufactured by Valagro, and marketed by Nutrecology. The CalBit® formulation is 4-0-0, with 12% calcium and 0.4% boron (10.96 lbs./gallon). MegaFol® is a 4-0-2 formulated product, with the label stating that it is specially formulated to improve the quality and vigor of fruits, vegetables and field crops. These special formulations may be due to the large number of amino acids (aspartic acid, glutamic acid, alanine, arginine, cystine, glycine, histidine, isoleucine, leucine, lysine, methionine, praline, phenylalanine, serine, threonine, tryptophan, tyrosine, and valine) contained in the product, which weighs 12.08 lbs/gallon.

c) CalMax® at 2.5 qt./acre. CalMax® is a foliar fertilizer marketed by Western Farm Services consisting of 10% nitrogen, 11% calcium, 1.2% magnesium, 0.1% iron, 0.1% manganese, 0.05% boron, 0.05% copper, 0.05% zinc, and 0.001% molybdenum. 12.52 lbs./gal.

d) Foli-Gro® Aminofol® Maximize at 2 qts/acre (Wilbur-Ellis Company). This product consists of 5% calcium, 1.25% zinc, and also contains 0.25% N Acetyl-thiazolidin-4-Carboxylic Acid (ATCA). 10.17 lbs./gallon. The product label notes that this product helps to supplement calcium during periods of rapid plant growth when "localized calcium deficiency" occurs while the zinc helps to promote auxin production.

e) 1 lb./acre of Calcium Chelate (Miller Chemical), 9.5% chelated calcium, chelated with EDTA (ethylenediaminetetra acetic acid)

f) 1.6 pts./acre Calexin (Miller Chemical). This product contains 2% calcium, in addition to amino acids, carbohydrates and a plant vitamin package.

g) 1.5 qts./acre of EleMax Phos-Cal LC, + 1 pt./acre of Trafix® + 1 pt./acre of MegaFol®. Ele-Max Phos-Cal LC 3-23-0, also contains 3% Ca ((Helena Chemical). Each gallon weighs 10.95 lbs. Trafix® consists of 0.01% humic acids derived from leonardite, and weighs 8.69 lbs./gal (Helena Chemical).

Calcium Chelate had the surfactant NuFilm 17 (Miller Chemical) added at the rate of 4 oz./acre, and Aminofol Maximize had the surfactant R-56 (Wilbur Ellis Co.) added at the rate of 1 qt/100 gal.

Several aspects of plant growth parameters were examined to determine if treatments affected plant growth, which included chlorophyll content, plant mapping and stigma/anther distances. Chlorophyll data were collected from plants on approximately a weekly interval for five weeks beginning July 7 with a Minolta 502 SPAD meter. Data were collected from the fifth terminal leaf of 15-25 plants per plot.

Open flowers were examined on July 13 and 21, and flowers were recorded as being either normal (stigmas not beyond anthers) or abnormal (minimum of one mm between the anthers and stigma), with 100-150 flowers so examined per plot on each sample date. Distance between anthers and stigmas were also recorded of 25 flowers per plot to determine if treatments affected this floral aspect.

Relationship of Stigma-Anther Distance to Boll Sizes and Retentions

Flowers of DPL444BR cotton were used in for this experiment, which commenced on June 23, 2005. Distances between stigmas and anthers were measured, and flowers were placed into one of three classes of elongation: 0-2.5 mm, 2.5-6.0 mm and greater than 6 mm. Each of the 50 flowers in each elongation class was marked with ribbon to assure that later examination would be the same flower. Each class of elongation length was marked with a different color of ribbon.

Flowers were re-examined on July 6 and 21 for retention. Developing bolls from previously marked flowers were recorded as present or missing on both sample dates. On the July 21 sample date, diameters of bolls were also obtained using an electronic calipers.

Bolls were allowed to complete development and were hand harvested in December. Bolls were placed in plastic bags (bags contained bolls only from one elongation class) and returned to the laboratory. Complete bolls (not missing any lint or locks) were then used for further data collection. These data included numbers of locks and seeds per boll. After cotton lint was hand separated from seeds, seed weights were also obtained.

Results

Varieties

Greater than 90% of the flowers for each variety were classified as abnormal (stigma being at least 1 mm beyond the anthers) in both variety trials on each of the first three sample dates (Tables 1, 2). Percentage of abnormal flowers peaked on June 23 in trial #1, with a slight reduction in percentage of affected flowers by June 27 (Table 1). A similar peak was noted on June 21 for most varieties in trial #2 with the exception of Phytogen 710R, in which the percentage of abnormal flowers continued to increase (Table 2). These initial June peaks are thought to be in response to the late May temperatures, rather than the heat noted June 21-23.

A second peak was noted in both variety trials on July 5, as reductions in percentage abnormal flowers were noted from the immediate subsequent sample. This peak was thought to be in response to the June 21-23 high temperatures. Although no statistical differences were noted on July 5 in trial #1, such differences were evident in trial #2. DPL 454BR had the lowest percentage of abnormal flowers in this trial on July 5 (78.1%), while Phytogen 710R had the highest percentage of such flowers (97.9%)

Two other peaks were noted in the trials (July 15 in trial #1; and July 28 in trial #2). The latter peak was thought to be a response to high night temperatures exceeding 80^{o}F shortly after July 15, while that in trial #1 may be a response to temperatures reaching over 110^{o}F on June 30. Of the varieties in trial #1, DPL 449BR and PHY 480WR generally had the fewest percentage of abnormal flowers on most sample dates, while highest percentage of such flowers was noted in the varieties STV4575BR and DPL 444BR. In trial #2 Phytogen 710R generally had the highest percentage of abnormal flowers on each sample date, while DPL 454BR and DPL 455BR had the lowest percentage of such flowers.

Stigma distance beyond the anthers were somewhat similar to that of abnormal flowers, although much more variable. In trial #1, least distance between stigmas and anthers was usually noted in DPL 449BR cotton (Table 3, fig. 2). Measured distance for this parameter was variable in STV 5599BR, with greatest distance of any variety in trial #1 noted on June 23 and July 29.

In trial #2, least distance between stigmas and anthers was noted in DPL 454BR on June 28 as well as July 14 and 28 (Table 4, fig. 3). Greatest distance was generally noted in Phytogen 710R cotton. At two points during the study (July 1, 28) distances for STV 6636 greatly increased from the previous sample date while those of PHY 710R decreased. This may indicate that these varieties differ in the immediate response and/or longevity of response to heat initiated style elongations resulting in stigmas beyond the anthers.

Calcium Foliar Fertilizers

Distances between stigmas and anthers were not as great as noted in varietal trials. Statistical differences were noted on July 13, but only CalMax® had significantly less elongation distance (0.2 mm) than the check (0.64 mm) on this date (Table 5). Several of the treatments had numerically larger distances between anthers and stigmas when compared with untreated cotton on July 13.

Elongation distance differences increased for all cotton when measured on July 21, but no statistical differences existed. Shortest distance was noted from cotton receiving the CalBit® + MegaFol® application (1.07 mm). This was the only treatment resulting in less distance between anthers and stigmas than the untreated check.

Greatest increase was between the two sample dates was noted from cotton treated with CalMax® (1.12 mm increase), followed by Calcium Metalosate® (0.86 mm). Treatments resulting in the least amount of increased mean stigma distance beyond anthers were the untreated check (0.50 mm), Trafix® + PhosCal + MegaFol® (0.52 mm), and Calcium Chelate (0.54 mm).

No treatment resulted in a significantly different percentage of abnormal flowers on July 13 than the untreated check (36.2) with the exception of Aminofol® Maximize (48.9). It may be that flowers opened on this date were not as affected by the heat of June 21-23 as percentages of abnormal flowers increased

greatly between the two sample dates. Calcium treatments may have affected percentage of abnormal flowers on July 21, as all foliar fertilizers had a smaller percentage of such flowers than the untreated check. Quantity of calcium may be the reason for this as treatments in which 2+ quarts were applied (Aminofol® Maximize, CalMax® and Calcium Metalosate®) had the smallest percentage of abnormal flowers (Table 5).

Chlorophyll levels

All treatments resulted in increased levels of leaf chlorophyll when measured with a Minolta 502 SPAD meter on July 7, with greatest increase (21.3%) noted from Calcium Metalosate® (Table 6). No statistical differences were noted for this parameter on July 13. On July 21 highest mean leaf chlorophyll content during any of the first four sample dates was noted from untreated cotton (57.25), which had significantly more chlorophyll than four treatments on this date. Leaf chlorophyll was lowest in untreated cotton (51.48) on July 25 however (Table 6), which was significantly less than the FoliGro® Aminofol® Maximize (55.45) on this date. The Aminofol® Maximize treatment was the only treatment which resulted in increasing chlorophyll levels on each subsequent sampling date from July 13-Aug. 4. Chlorophyll levels of cotton treated with Calcium Metalosate® remained very constant during this same period (52.9-53.6) and had the lowest chlorophyll level of chlorophyll on August 4, although it had the highest levels of leaf chlorophyll on the first sample date after application.

Relationship of Stigma-Anther Distance to Boll Sizes and Retentions

As stigma distance from anthers increased, retention rates decreased (Table 7). Diameters of bolls were significantly increased in the two longer separation distances when compared with that of the shortest separation distance. Number of seeds per boll or lock were not affected (Table 8), but seeds weights and size were much larger in the bolls associated with the reduced retention rates. These results are thought due to nutrient partitioning. Less boll retention (and therefore fewer bolls) would have resulted in more available nutrition per boll, hence larger bolls due to larger and heavier seeds, assuming that available nutrition is a limiting factor.

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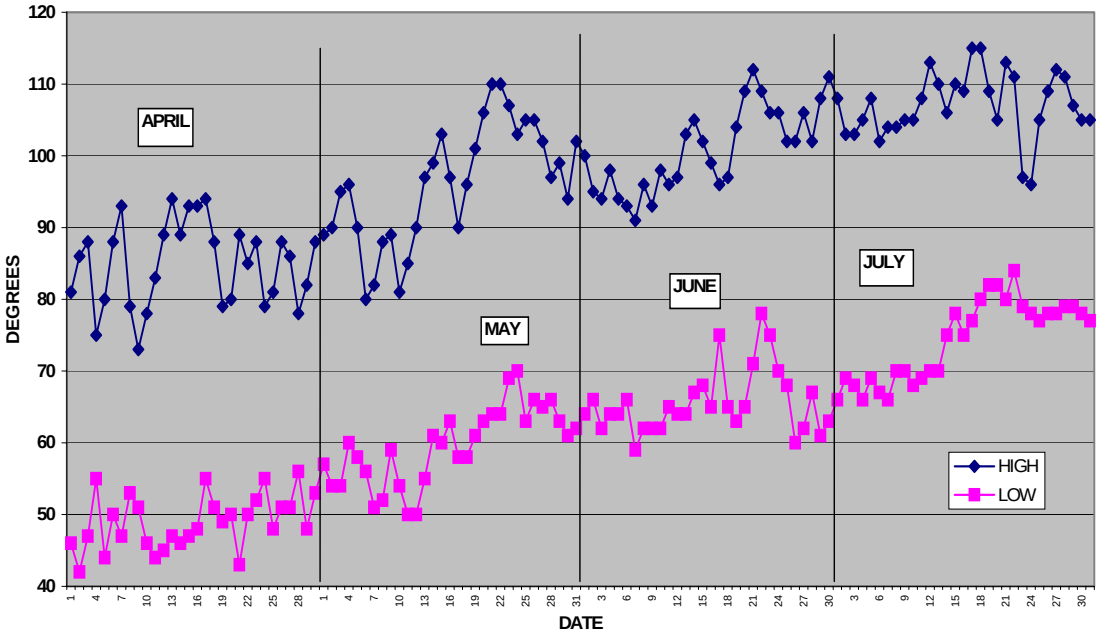

Fig. 1. Daily temperatures during April-July 2005, Blythe, CA.

Table 1. Percent abnormal flowers in variety trial #1, 2005, Blythe, CA.

	Sample Date							
Variety	Jun 20	Jun 23	Jun 27	July 5	Jul 8	Jul 12	Jul 15	Jul 29
DPL 444BR	94.5ab	99.0 b	97.8 c	96.7a	92.1cd	96.2b	98.3a	97.6a
DPL 445BR	95.6ab	96.0a	95.1 bc	100.0a	89.8cd	96.0b	98.1a	95.9a
DPL 449BR	92.0a	97.3ab	94.6 b	99.3a	80.2a	88.8a	97.1a	97.6a
PHYT 470WR	94.1ab	97.8ab	94.6 b	97.5a	86.4bc	94.1b	98.8a	97.4a
PHYT 480WR	92.2a	96.3ab	90.8a	98.4a	82.4ab	89.0a	97.9a	97.1a
STV 4575BR	97.1 b	97.8ab	96.8 bc	97.6a	92.6d	95.5b	98.6a	97.6a
STV 5599BR	95.6ab	97.5ab	94.6 b	98.8a	86.9bcd	92.6ab	98.3a	97.4a

Means in columns followed by the same letter are not statistically different at the $p \leq 0.05$ level (Fisher's LSD test).

Table 2. Percent abnormal flowers in variety trial #2, 2005 Ripley, CA

		Sample Date								
Variety	Jun 18-19	Jun 21	Jun 24	Jun 28	July 1	Jul 5	Jul 11	Jul 14	Jul 19	Jul 28
DPL 449BR	94.4a	98.1 bc	96.2ab	92.0abc	85.6a	93.1cd	75.5a	82.5cd	52.1 b	89.0 b
DPL 454BR	91.3a	91.1a	90.4a	83.0a	83.1a	78.1a	77.9ab	67.4a	38.1a	82.9a
DPL 455BR	94.8a	97.1 bc	95.6ab	84.0ab	84.7a	84.7ab	77.6ab	74.6b	38.1a	95.2 c
DPL 555BR	93.8a	98.3 bc	94.8ab	96.0bc	81.0a	86.9 bc	83.6bcd	75.7bc	53.2 b	96.7 c
PHY 710R	94.8a	93.2a	99.0 b	98.0c	82.7a	97.9 d	86.4 cd	96.9e	95.0 d	97.6 c
STV 5599BR	93.8a	98.5 c	97.8 b	94.0abc	87.1a	88.6bc	89.5 d	87.1d	78.6 c	96.4 c
STV 6636BR	95.3a	96.1 b	95.8ab	95.0abc	81.4a	83.9ab	80.0abc	77.1bc	53.7 b	96.9 c

Means in columns followed by the same letter are not statistically different at the p≤0.05 level (Fisher's LSD test).

Table 3. Mean stigma elongations (mm) beyond anthers in flowers of variety trial #1, 2005, Blythe, CA.

	Sample Date						
Variety	Jun 23	Jun 27	July 5	Jul 8	Jul 12	Jul 15	Jul 29
DPL 444BR	4.61ab	3.25 bc	3.60a	4.43 b	3.41 b	3.75a	5.60 bc
DPL 445BR	4.08ab	2.68ab	3.56a	4.35 b	3.25 b	3.75a	4.42a
DPL 449BR	3.79a	2.44ab	2.71a	3.90 b	2.30a	2.87a	4.72ab
PHYT 470WR	4.80ab	2.92abc	2.90a	3.89 b	3.02ab	4.83a	4.76ab
PHYT 480WR	4.27ab	2.90abc	3.12a	2.75a	2.72ab	3.39a	5.41 bc
STV 4575BR	4.50ab	3.70 c	3.61a	4.04 b	3.47 b	4.46a	4.48a
STV 5599BR	5.07 b	2.36a	2.91a	3.75ab	2.81ab	4.14a	5.76 c

Means in columns followed by the same letter are not statistically different at the p≤0.05 level (Fisher's LSD test).

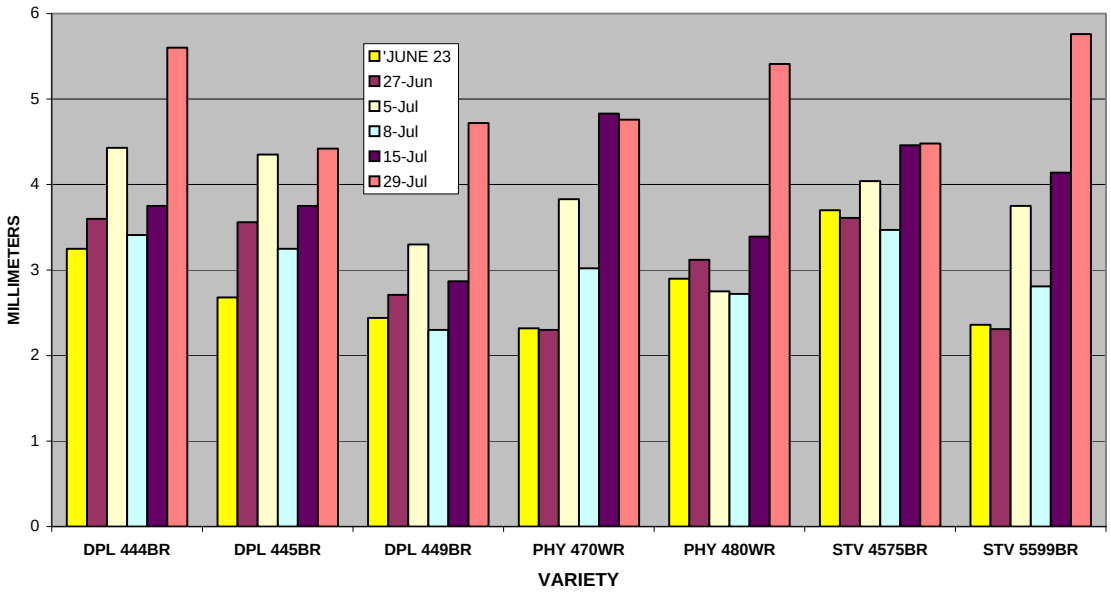

Fig. 2. Mean stigma elongation lengths beyond anthers, variety trial #1, Blythe, CA.

Table 4. Mean stigma elongations beyond anthers in flowers of variety trial #2, 2005 Ripley, CA

	Sample Date						
Variety	Jun 28	July 1	July 5	Jul 11	Jul 14	Jul 19	July 28
DPL 449BR	2.95bc	1.73a	2.48a	3.36a	2.24 b	1.65a	4.00a
DPL 454BR	1.91a	2.33a	1.54a	3.61a	0.76a	1.09a	3.73a
DPL 455BR	2.60b	1.88a	1.63a	4.03a	1.48ab	1.01a	3.96a
DPL 555BR	3.66cd	1.33a	1.52a	3.90a	1.43ab	1.48a	3.95a
PHY 710R	6.15e	1.88a	4.62b	4.61a	4.86 c	6.12 c	5.33 b
STV 5599BR	3.33cd	1.91a	2.30a	4.31a	1.95ab	3.33 b	3.87a
STV 6636BR	2.85bc	4.74 b	1.91a	3.17a	0.98a	1.27a	4.42ab

Means in columns followed by the same letter are not statistically different at the $p \leq 0.05$ level (Fisher's LSD test).

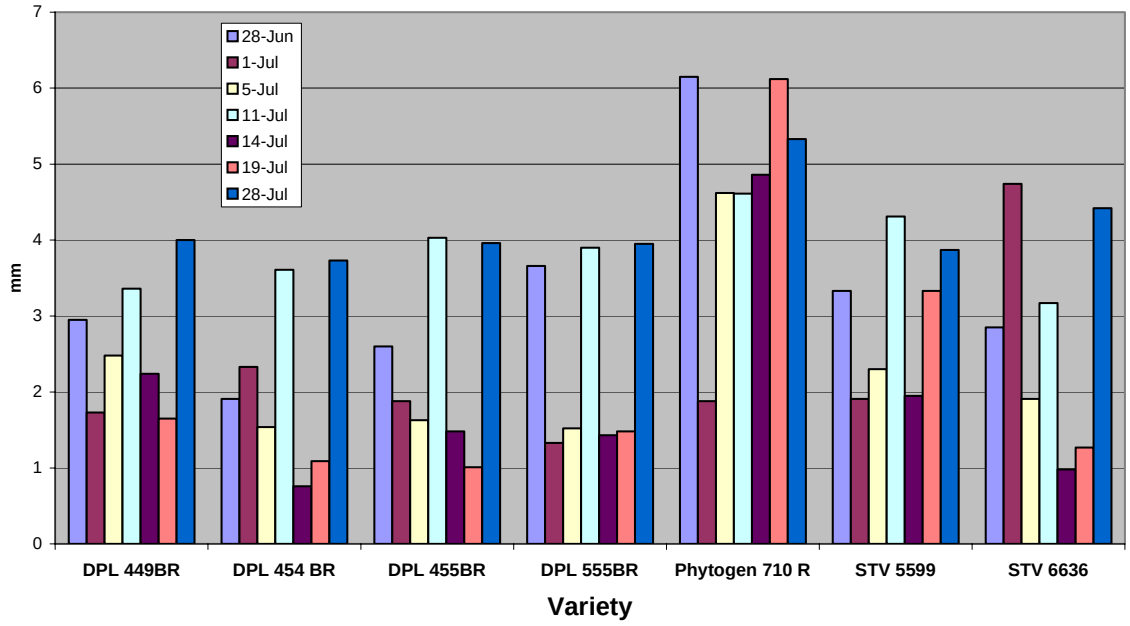

Fig. 3. Mean stigma elongation lengths beyond anthers, variety trial #2, Ripley, CA.

Table 5. Stigma elongations (mm) beyond anthers and percent abnormal flowers

Treatment	Rate/acre	Stigma Elongations July 13	Stigma Elongations July 21	Percent abnormal flowers July 13	Percent abnormal flowers July 21
Aminofol® Maximize	2 qts	0.84 c	1.47a	48.9 c	72.6ab
CalMax®	2.5 qt	0.20a	1.32a	28.8a	70.7a
Calcium Metalosate®	2 qts	0.40ab	1.26a	36.3ab	73.1abc
CalBit® + Megafol®	1 qt + 1 qt	0.40ab	1.07a	39.4abc	78.5abc
Calexin	1.6 pt	0.52abc	1.14a	44.9 bc	79.8 bc
Calcium Chelate	1 lb	0.82 bc	1.36a	45.2 bc	77.1abc
Trafix® + PhosCal + Megafol®	1 pt + 1.5 qts + 1 pt	0.83 c	1.35a	31.0a	77.4abc
Untreated Check	----	0.64 bc	1.14a	36.2ab	81.7 c

Means in columns followed by the same letter are not statistically different at the p≤0.05 level (Fisher's LSD test).

Table 6. Chlorophyll level (as measured with a SPAD 502) of 5th terminal leaf of DPL449BR cotton plants after application of materials on June 24, 2005, Ripley, CA

	Sample Date				
Treatment and rate/acre	July 7	July 13	July 21	July 25	Aug. 4
Calcium Metalosate® @ 2 qts	56.475a	53.6 a	53.25 b	52.93ab	53.58 b
CalBit® @ 1 qt + MegaFol® @ 1 qt	53.6 ab	53.65a	55.8 ab	54.93ab	56.98ab
Aminofol® Maximize @ 2 qts	51.6 b	49.93a	53.4 b	55.45a	57.05ab
Calexin @ 1.6 pts	51.25 b	49.5 a	54.25ab	53.2 ab	54.78ab
CalMax® @ 2.5 qts	51.25 b	53.7 a	52.68 b	52.5 ab	57.3a
Calcium Chelate @ 1 lb	50.9 b	52.9 a	55.55ab	52.88 ab	57.55a
Trafix® @ 1 pt + EleMax PhosCal LC @ 1.5 qts + MegaFol® @ 1 qt	50.025 bc	50.35a	53.05 b	52.73ab	56.5ab
Untreated check	46.55 c	50.3 a	57.25a	51.48 b	57.18ab

Means in columns followed by the same letter are not statistically different at the p≤0.05 level (Fisher's LSD test).

Table 7. Percent retention and boll diameters as a function of distance between anthers and stigmas.

	Percent Retention		Boll Diameter
Elongation Class	July 6	July 21	(mm)
0.0-2.5 mm	100	86	30.56 b
2.5-6.0 mm	96	82	32.06a
6.0+ mm	92	76	32.56a

Means in columns followed by the same letter are not statistically different at the p≤0.05 level (Fisher's LSD test).

Table 8. Mean seed and lock numbers and seed weights as a function of distance between stigma and anthers.

	Average/Boll		Seeds/	Wt (g)/
Elongation Class	Seeds	Locks	Lock	Seed
0.0-2.5 mm	28.6a	4.61a	6.2a	3.0
2.5-6.0 mm	27.1a	4.75a	5.7a	3.12
6.0+ mm	28.2a	4.60a	6.1a	3.235

Means in columns followed by the same letter are not statistically different at the p≤0.05 level (Fisher's LSD test).