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Pix Plus and Mepiquat Chloride Effects on Physiology, Growth, and Yield of Field-Grown Cotton

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ABSTRACT

An important objective for using plant growth regulators in cotton (*Gossypium hirsutum* L.) is to balance vegetative and reproductive growth as well as to improve lint yield and fiber quality. Field studies were conducted at two locations (Clarkedale and Fayetteville) in Arkansas in 1997 and 1998 to determine physiologic and yield responses of cotton to foliar applications of mepiquat chloride [*N,N*-dimethylpiperidinium chloride and inert ingredients] (MC) and Pix Plus [MC + *Bacillus cereus*]. Compared with the untreated control, application of Pix Plus and MC efficiently reduced plant height, improved leaf CO_2-exchange rate, and increased leaf starch content. Neither Pix Plus nor MC affected photoassimilate translocation from leaves to 10- to 15-day-old bolls. Pix Plus and MC had very similar effects on plant growth and most physiologic parameters investigated in our studies. There was no difference in lint yield between Pix Plus and mepiquat chloride. However, Pix Plus increased the fraction of fruit dry matter in total dry matter.

Key words: *Gossypium hirsutum*; Mepiquat chloride; Pix Plus; CO_2 exchange rate; Photoassimilate translocation; Lint yield

INTRODUCTION

Cotton is a major economic crop with an indeterminate growth habit, and it is very responsive to environmental changes and management. Excessive vegetative growth results in shade within the plant canopy, increased fruit abscission, and reduced yield (Guinn 1974). Consequently, producers and researchers have long been interested in the use of plant growth regulators (PGRs) for adjusting plant vegetative and reproductive growth, improving cotton yield (Oosterhuis and Egilla 1996) and facilitating harvest. The PGR mepiquat chloride (MC) consists of 4.2% of mepiquat chloride [*N, N*-dimethylpiperidinium chloride] and 95.8% of inert ingredients. MC has been the most successful and widely used PGR to control cotton plant size in cotton production in the United States. However, cotton yield responses to MC have been inconsistent. MC increased yields in some tests (Armstrong and

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others 1982; Oosterhuis and Egilla 1996; York 1983 a, b), had no effect in others (Heilman 1981, 1985; Stuart and others 1984), and reduced yield in some other tests (Crawford 1981; Thomas 1975).

Pix Plus, formerly MepPlus, is a new PGR first tested in 1994 and registered in 1997 by Micro-Flo Company (Memphis, TN) and now marketed by BASF Corp. (Research Triangle Park, NC). It consists of MC (4.2%), the bacteria *Bacillus cereus* (0.05%), and inert ingredients (95.75%). The *Bacillus cereus* component was reported to have a tolerance exemption on all crops. Recent studies (Oosterhuis and others 1998; Parvin and Atkins 1997) have indicated that Pix Plus had a similar effect on plant height control as MC. In addition, Pix Plus has been reported to improve leaf photosynthesis, dry matter partitioning (Oosterhuis and others 1998), and lint yield (Parvin and Atkins 1997) of field-grown cotton compared with both untreated control and MC-treated plants. However, the physiologic mechanisms of Pix Plus affecting plant growth and yield are not well understood. The hypothesis of our studies was that application of Pix Plus may improve translocation of photoassimilate from leaves to fruits and partitioning of dry matter among plant tissues and result in an increase in yield. Field studies were conducted at two locations in Arkansas in 1997 and 1998 to compare Pix Plus with MC for effects on growth and yield of cotton and to investigate the physiologic effect of Pix Plus compared with MC on plant growth.

Materials and Methods

Plant Culture

Field trials were conducted at the Delta Branch Experimental Station at Clarkedale in northeast Arkansas and the Arkansas Agricultural Research and Extension Center at Fayetteville in northwest Arkansas in 1997 and 1998. In Clarkedale, the cotton cultivar Suregrow 125 was seeded on 7 May 1997 and 7 May 1998. The cotton cultivar Deltapine 20 was planted on 19 May 1997, and Suregrow 125 was planted 15 May 1998 in Fayetteville. Plots consisted of four rows spaced 1 m apart by 15 m at Clarkedale or 5 m at Fayetteville. Cotton was hand-thinned to 10 plants per meter of row when seedlings had approximately three true leaves. Weed and insect control, fertilizer management, and furrow irrigation were according to Arkansas cotton production recommendations.

Treatments

At both locations, three treatments were used consisting of (i) an untreated control, (ii) Pix Plus, and (iii) mepiquat chloride (MC). The specific rates in different years and growth stages were determined according to plant height and the number of main-stem nodes using a chart for cotton production recommendations (Micro-Flo, Memphis, TN). Details of timing and rate of application of Pix Plus or MC are given in Table 1. The same rate of Pix Plus or MC was used each time as a foliar application with a CO_2-pressurized backpack sprayer in 94 L water per hectare.

Measurements

In Clarkedale, five consecutive plants from one of the middle two rows of each plot were marked 1 day before initiating the treatments. Plant height and number of main-stem nodes were measured four times from these marked plants during plant growth. Plants were harvested at the boll development stage (90 days after planting) in 1997 from a 1-m section of row of each plot within the first three replicates. Plant height, numbers of main-stem nodes, fruiting branches, bolls, and squares (floral buds with bracts) were recorded. Thereafter, plants were separated into leaves, fruits (bolls + flowers + squares), petioles, and stems. Leaf area was measured using an LI-3100 area meter (Li-Cor, Lincoln, NE). Samples of plant components were dried at 60°C and weighed. Specific leaf weight (SLW) was calculated using leaf dry weight divided by leaf area.

When about 10% of the bolls were open, 2 m of the two middle rows of each plot were marked, and the numbers of total bolls, green bolls, and open bolls within the 2-m section were counted three times weekly to record maturity. Before harvesting, distributions of bolls in the plant canopy were recorded using a plant-mapping computer program (Bourland and Watson 1990). Fifty bolls were harvested from the middle two rows of each plot, weighed, and ginned to determine seed cotton weight per boll (boll weight) and lint percentage. Finally, a mechanical picker was used to harvest the middle two rows of each plot. Lint yield was calculated according to the seed cotton weight and lint percentage.

In Fayetteville, seed cotton samples were harvested by hand from all plants in 2 m of the middle two rows of each plot. The number of bolls and seed cotton weight were recorded, and the seed cotton was ginned to determine average boll weight, lint percentage, and lint yield. In addition, the CO_2 exchange rate, stomatal conductance, transpiration, and intercellular CO_2 concentration of uppermost fully expanded main-stem leaves were determined using a portable photosynthesis system (model LI-

Table 1. The timing and rate of Pix Plus and mepiquat chloride (MC) treatments at Clarkedale and Fayetteville, Arkansas in 1997 and 1998

Treatment	Rate and timing 1997	1998
Clarkedale		
Control	—[a]	—
Pix Plus	293 mL ha^{-1} at ES[b], ES+9 d, FF, and FF+9 d	220 mL at ES and 440 mL ha^{-1} at FF
MC	293 mL ha^{-1} at ES, ES+9 d, FF, and FF+9 d	220 mL at ES and 440 mL ha^{-1} at FF
Fayetteville		
Control	—	—
Pix Plus	586 mL ha^{-1} at ES and FF	293 mL at ES and 586 mL ha^{-1} at FF
MC	586 mL ha^{-1} at ES and FF	293 mL at ES and 586 mL ha^{-1} at FF

[a]*Without Pix Plus and MC.*
[b]*ES, early square stage; FF, first flower stage. In this study, the ES and FF stages are defined as 50% of plants in the field having a square or a white flower, respectively.*

6200, Li-Cor Inc., Lincoln, NE). The measurements were made from three plants of each plot between 1100 and 1200 h at 5 and 10 days after spraying Pix Plus and MC at the first flower stage. After measuring the leaf CO_2 exchange rate, concentrations of adenosine triphosphate (ATP) in the leaves were determined using the method of Bednarz (1995) and nonstructural carbohydrates determined using the methods of Hendrix (1993).

When plants reached the first flower (FF) stage, 10 white flowers at the first fruiting position in each plot were tagged with paper jewelers' tags, and the PGRs were applied at the same day (1997) or 5 days after tagging (1998). Ten days after PGR application at the FF stage (at that time, the tagged bolls were 10 [1997] or 15 [1998] days post flowering), the carbon fixation and translocation of the subtending leaves of tagged bolls were determined by monitoring the ^{14}C radioactivity in various plant organs after exposure of the subtending leaves to $^{14}CO_2$. Each treatment included six plants from three replications. The ^{14}C-labeling technique used was similar to that of Wullschleger and Oosterhuis (1990). Individual sympodial leaves at the same first fruiting position of the tagged bolls were individually exposed to $^{14}CO_2$ for 15 min starting at 1130 h CDST. Tissues of the petiole and blade of the source leaf and the peduncle, bracts, and the boll of the tagged fruits were harvested 6 and 24 h after $^{14}CO_2$-feeding. Three tagged bolls were harvested each time for each experimental unit. Individual samples were dried at 70°C and weighed. Subsamples of individual tissues were subsequently combusted in a sample oxidizer, and the ^{14}C radioactivity counted in a Packard Tri-Carb 4530 liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, IL). Details of the ^{14}C-trace technique have been described by Zhao and Oosterhuis (1999).

Experiments were arranged in a randomized complete block design with six replications. Most results are presented by individual year and location because of differences in growth environments and in rates of PGRs applied. Data were subjected to ANOVA, and means were separated using Fisher's Protected LSD test at $p = 0.05$.

Results and Discussion

Plant Growth

Plants at Clarkedale receiving Pix Plus and MC were significantly shorter than untreated control plants 3 (1997) or 6 weeks (1998) after the PGRs were applied (Table 2). There were no differences between Pix Plus and MC treatments in plant height, and the number of main-stem nodes did not differ among treatments. Therefore, the height/node ratios were similar for plants treated with both PGRs and significantly smaller (17% averaged over the 2 years) than the untreated control. This indicated that decreased plant height was mainly due to shorter internode length as has been previously reported for MC (Kerby, 1985).

Accumulation and Partitioning of Dry Matter

Plant growth analysis at 90 days after planting indicated there were no statistical differences in the number of bolls and leaf area index among treatments, although both PGR treatments had a numerically smaller leaf area index compared with the control (data not shown). However, Pix Plus- and

Table 2. Effect of Pix Plus and mepiquat chloride (MC) on plant height, the number of main-stem nodes and height-to-node ratio of field-grown cotton in 1997 and 1998 (Clarkedale)[a]

Treatment	Plant height (cm)	Main-stem nodes (no. plant^{-1})	Height/ node
1997			
Control	93.2 a[b]	20.7 a	4.5 a
Pix Plus	68.6 b	20.3 a	3.4 b
MC	67.1 b	19.9 a	3.4 b
1998			
Control	87.4 a	20.4 a	4.3 a
Pix Plus	78.5 b	20.0 a	3.9 b
MC	74.2 b	19.5 a	3.8 b

[a] *Measured 3 (1997) or 6 (1998) weeks after first PGR application.*
[b] *Means within a column and a year followed by the same letter are not different* ($p > 0.05$).

MC-treated plants exhibited significantly higher SLW (11–25%) than untreated control plants. The SLW of the control, Pix Plus, and MC was 6.17, 7.19, and 7.35 mg cm^{-2}, respectively.

Among the three treatments, no significant differences were observed in total dry weight and fruit dry weight, although the dry weights of stems, leaves, and petioles for Pix Plus–treated plants were lower than the untreated control (Figure 1A). The fraction of fruit dry weight in total dry matter of the Pix Plus treatment (41%) was significantly higher than that of both the control (33%) and the MC treatments (34%) (Figure 1B). Applying Pix Plus seemed to improve partitioning of dry matter in plants compared with MC and the untreated control because a greater proportion of assimilate was partitioned into the fruits of Pix Plus-treated plants.

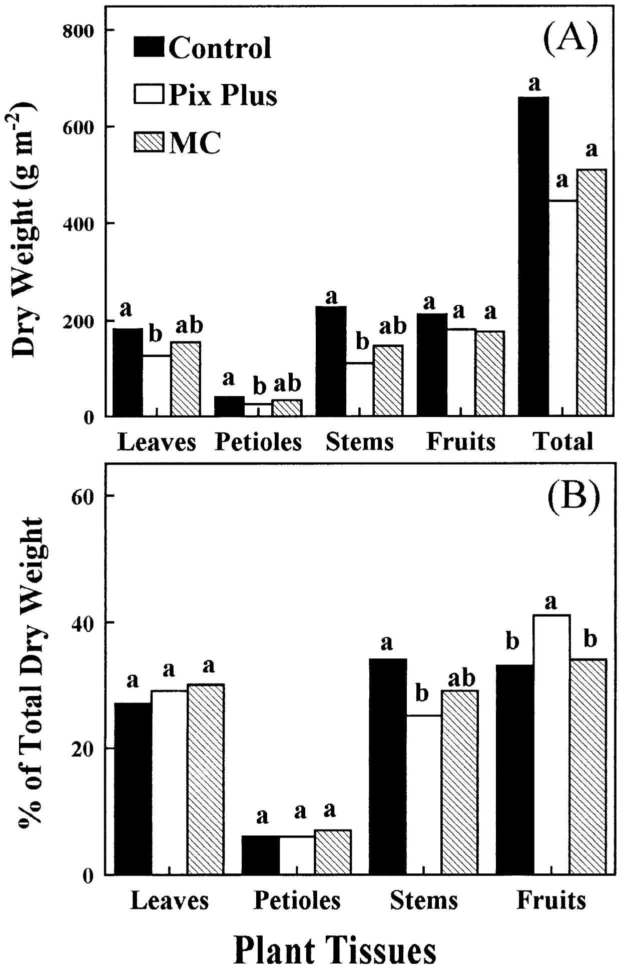

Figure 1. Effects of Pix Plus and mepiquat chloride (MC) on (A) dry matter accumulation and (B) partitioning of field-grown cotton at Clarkedale in 1997. Means within a plant tissue followed by the same letter are not different at the 0.05 probability level (sampled 90 days after planting).

Leaf CO_2 Exchange Rate

At 5 days and 10 days after foliar application of Pix Plus or MC, both Pix Plus- and MC-treated plants exhibited significantly higher single leaf CO_2 exchange rates than untreated control plants (Table 3). Increased leaf CO_2 exchange rates from Pix Plus and MC was related to increased stomatal conductance (for Pix Plus) and SLW because the SLW of the leaves selected for photosynthesis measurements increased by 14% for Pix Plus and by 12% for MC compared with the untreated control. Pix Plus treatment also resulted in a higher leaf transpiration rate than both the control and MC treatments, whereas intercellular CO_2 concentration was similar among treatments. No statistical difference was observed in leaf photosynthesis between Pix Plus and MC treatments (Table 3).

Leaf Nonstructural Carbohydrate and ATP Concentrations

Under field conditions in 1998, the Pix Plus and MC did not affect hexose and sucrose concentrations in the uppermost fully-expanded main-stem leaves (Figure 2). However, leaves of plants treated with both PGRs had a significantly higher starch concentration than untreated control plants. Landivar and Marur (1996) reported that MC application did not influence the starch and reducing sugar of cotton leaves. Our results indicated that MC and Pix Plus increased the leaf starch concentration of field-

Table 3. Effects of Pix Plus and mepiquat chloride (MC) application on leaf CO_2 exchange rate (Pn), intercellular CO_2 concentration (c_i), stomatal conductance (g_s), transpiration rate (E), and specific leaf weight (SLW) of cotton (Fayetteville)[a]

Treatment	Pn ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	c_i (ppm)	g_s ($cm\ s^{-1}$)	E ($mol\ m^{-2}\ s^{-1}$)	SLW ($mg\ cm^{-2}$)
Control	24.7 b[b]	299 a	3.80 b	0.017 b	6.60 b
Pix Plus	29.4 a	297 a	4.87 a	0.019 a	7.52 a
MC	28.1 a	294 a	3.90 b	0.017 b	7.39 a

[a]*Means of data measured at 5 and 10 days after application of Pix Plus and MC at the FF stage in 1997 and 1998.*
[b]*Means within a column followed by the same letter are not different* ($p > 0.05$).

grown cotton plants, although they did not affect the soluble sugar content. Leaf starch content did not differ between Pix Plus and MC treatments. Higher leaf starch concentrations for both PGR treatments were associated with a higher leaf CO_2 exchange rate (Table 3) because starch accumulation in chloroplasts was primarily a mechanism for storing carbon when the rate of photosynthesis exceeded the capacity of the leaf to export saccharides (Stitt 1984).

Pix Plus- and MC-treated plants had significantly lower leaf ATP concentrations at 5 and 10 days after PGR application at the FF stage (Figure 3). The correlation analysis among leaf CO_2 exchange rate, transpiration, ATP, and nonstructural carbohydrate concentrations indicated that no correlation existed between leaf ATP content and leaf CO_2 exchange rate or nonstructural carbohydrate concentration (data not shown). The ATP content in plant leaves mainly depends on both ATP production and consumption. Leaves produce ATP from two metabolic pathways: photosynthesis and respiration. The ATP was probably used for plant growth and other metabolic processes. Therefore, the ATP is involved in many complex plant metabolisms, and leaf ATP concentration cannot be used as an indicator for cotton leaf photosynthetic capacity.

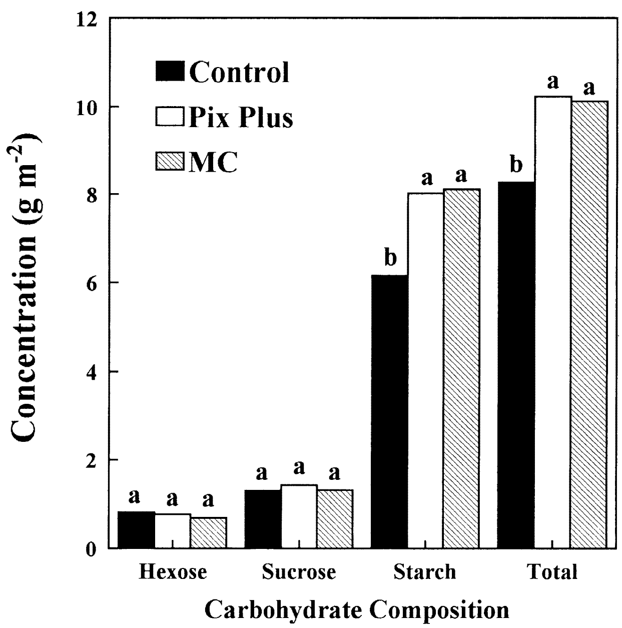

Figure 2. Effects of Pix Plus and mepiquat chloride (MC) on leaf nonstructural carbohydrate concentrations of field-grown cotton. Means of measurements 5 and 10 days after application of Pix Plus and MC at the first flower stage. Means within a carbohydrate followed by the same letter are not different at the 0.05 probability level at Fayetteville in 1998.

Leaf $^{14}CO_2$ Fixation and ^{14}C-Assimilate Translocation

Application of Pix Plus and MC improved leaf CO_2 exchange rate (Table 3), and Pix Plus increased the fraction of dry matter partitioning in fruits (Figure 1B), suggesting that the benefits from Pix Plus were probably associated with enhancement of photoassimilate translocation into fruits from leaves. However, the results of our ^{14}C trace measurements did not support the hypothesis of both PGRs improving assimilate translocation.

In the 1997 study, MC-treated plants had significantly higher leaf ^{14}C fixation than untreated control plants, but MC did not affect the ^{14}C-carbon translocation from the subtending sympodial leaf to a 10-day-old boll (Table 4). In 1998, the $^{14}CO_2$ fixation of the subtending leaf of a 15-day-old boll for the Pix Plus–treated plants was higher than that of the control or the MC-treated plants. However, no statistical differences were observed in ^{14}C-assimilate translocation from the leaf to the subtended boll among the treatments. These results fur-

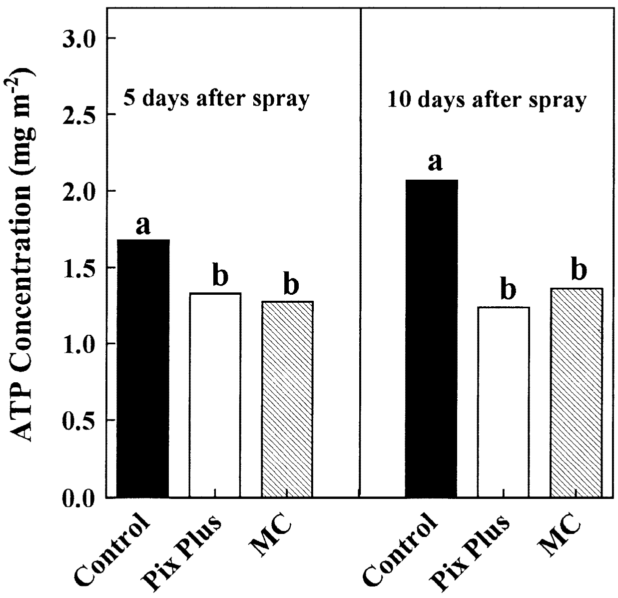

Figure 3. Effect of Pix Plus and mepiquat chloride (MC) application at the first flower stage on leaf ATP concentrations of field-grown cotton. Means within a sampling date followed by the same letter are not different at the 0.05 probability level at Fayetteville in 1998.

Table 4. Effects of Pix Plus and mepiquat chloride (MC) application on $^{14}CO_2$ fixation and translocation from the leaf to the boll (Fayetteville)[a]

Treatment	$^{14}CO_2$ fixation (dpm mg^{-1} DW)	^{14}C translocation to the boll (%) 6 h	24 h
1997			
Control	2156 b[b]	14.3 a	33.3 a
MC	2718 a	13.5 a	31.0 a
1998			
Control	2630 b	33.6 a	60.7 a
Pix Plus	3498 a	26.8 a	76.7 a
MC	2606 b	27.9 a	70.4 a

[a] *The subtending sympodial leaf at the first position of MSN 10 was labeled with $^{14}CO_2$ when the boll at this position was 10 (1997) or 15 (1998) days old (10 days after PGR application).*
[b] *Means within a column and a year followed by the same letter are not different (p > 0.05).*

ther indicated that application of Pix Plus and MC increased cotton leaf photosynthetic capacity from both a leaf area basis, and leaf dry matter basis but did not improve assimilate translocation rate from the leaf to a specific young fruit.

Lint Yield and Yield Components

In 1997 at Clarkedale, lint yield of the MC treatment was significantly lower than that of the untreated control but did not differ from that of the Pix Plus treatment (Table 5). In 1998, lint yields of Pix Plus and MC treatments were not different from the control treatment. Decreased lint yields for MC treatment compared with the control in 1997 might be associated with the extended growing season because plants receiving growth retardants (Pix Plus and MC) usually cutout earlier than the untreated control plants (Oosterhuis and others 1991), which may, therefore, have been able to continue to mature more late-season bolls in the extended favorable season than the MC-treated plants.

In 1997 at Fayetteville, there were also no differences among the treatments in lint yield (Table 5). In 1998, Pix Plus increased yield 18% compared with the control. Increased lint yield from Pix Plus at this location was mainly associated with an improved boll size. Of the three yield components, Pix Plus application mainly increased the average boll weight in 1997 at Clarkedale and in both years at Fayetteville compared with the control. Pix Plus did not affect the number of bolls and lint percentage, except for 1997 at Clarkedale in which Pix Plus treatment had lower lint percentage than the control. The MC treatment had significantly lower lint percentage than the control at both locations in 1997. Therefore, the responses of cotton yield and yield components to Pix Plus and MC depended on year and location. The maturity based on open boll percentage did not differ among treatments (data not shown).

Analysis of plant fruit mapping indicated that application of Pix Plus or MC increased the fraction of bolls located at fruiting branches 1 to 6 and decreased the fraction of bolls above fruiting branch 10 (1997) or 7–9 (1998) compared with the untreated control (Figure 4). This supports the explanation of higher than expected yields in the untreated control because of more late-season bolls being matured in the extended growing season in 1997.

Conclusions

Application of Pix Plus and MC effectively controlled cotton plant height and increased leaf CO_2 exchange rate of field-grown cotton, but did not improve photosynthetic assimilate translocation from the leaf to the young fruit. Increased leaf photosynthesis was associated with increases in stomatal conductance and in SLW. Both PGRs had very similar effects on most plant growth and physiologic parameters measured in our studies. Compared with MC, Pix Plus treatment had a higher proportion of fruit dry

Table 5. Effect of Pix Plus and mepiquat chloride (MC) application on lint yield and yield components of field-grown cotton at Clarkedale and Fayetteville

	Clarkedale				Fayetteville			
Treatment	Boll weight (g $boll^{-1}$)	Boll number (no. m^{-2})	Lint fraction (%)	Lint yield (kg ha^{-1})	Boll weight (g $boll^{-1}$)	Boll number (no. m^{-2})	Lint fraction (%)	Lint yield (kg ha^{-1})
1997								
Control	5.1 b[a]	78 a	39.8 a	1392 a	4.2 b	77 a	39.0 a	1244 a
Pix Plus	5.8 a	73 a	38.4 b	1300 ab	4.4 a	77 a	38.9 a	1270 a
MC	5.6 a	74 a	38.3 b	1261 b	4.1 b	79 a	37.3 b	1208 a
1998								
Control	4.2 a	76 a	37.5 a	1004 a	4.3 b	83 a	37.0 a	1134 b
Pix Plus	4.3 a	79 a	38.2 a	1059 a	4.8 a	82 a	38.9 a	1324 a
MC	4.1 a	77 a	37.9a	1017 a	4.6 a	80 a	38.0 a	1198ab

[a]*Means within a column and a year followed by the same letter are not different* ($p > 0.05$).

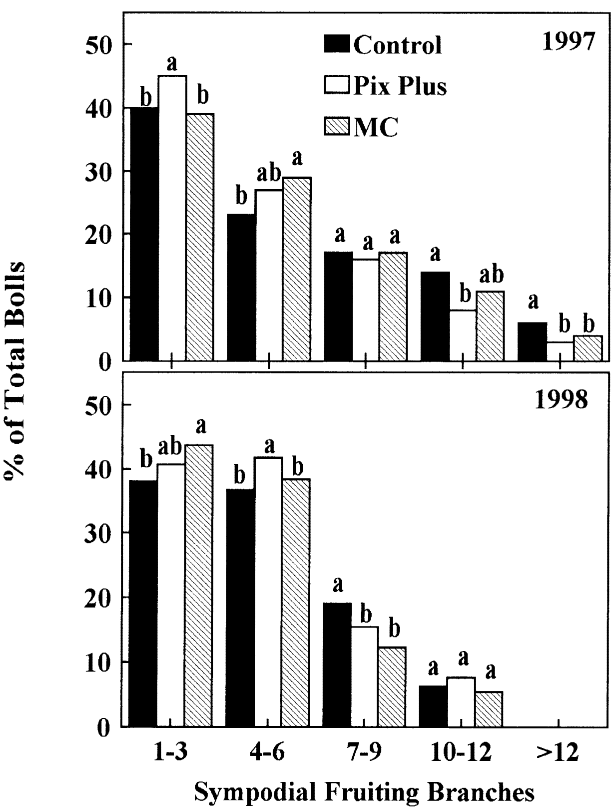

Figure 4. Effect of Pix Plus and mepiquat chloride (MC) application on boll distributions in the plant canopy. Measurements were taken before harvest at Clarkedale. Means within a fruiting branch group followed by the same letter are not different at the 0.05 probability level.

weight to total dry weight, although total dry matter accumulation did not differ between the two treatments. There was no significant difference in lint yield between Pix Plus and MC.

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