The *Journal of Cotton Science* 2:136-142 (1998)
http://journal.cotton.org, © The Cotton Foundation 1998

PHYSIOLOGY

Semi-Continuous Carbon Dioxide Exchange Rates in Cotton Treated with Commercially Available Plant Growth Regulators

C.W. Bednarz* and M.W. van Iersel

INTERPRETIVE SUMMARY

Foliar-applied growth-enhancing compounds are some of the many inputs used in cotton (*Gossypium hirsutum* L.) production systems across the U.S. Cotton Belt. Their usefulness, however, in producing high-yielding, high-quality cotton remains to be resolved. Foliar-applied growth-enhancing compounds generally contain fertilizers, plant growth regulators, or combinations of the two. These products have in common the possibility of increased profit for the grower. Plant growth regulators have been shown to increase lint yield in cotton (Clark et al., 1992; Guo and Oosterhius, 1995; Oosterhuis, 1995; Oosterhuis et al., 1995; Oosterhuis and Zhao, 1994a; Oosterhuis and Zhao, 1994b; Oosterhuis and Zhao, 1993). Increased lint yield may occur through changes in carbon partitioning, increased fruit set, and/or increased dry weight accumulation. Carbon partitioning and fruit set are highly dependent on dry weight accumulation. The physiological mechanism responsible for dry weight accumulation is photosynthesis. Increasing photosynthesis, however, is unlikely without further improvements in traditional and molecular plant breeding (Evans, 1993). Therefore, the objective of this investigation was to determine if commercially available plant growth regulators influence photosynthetic rates in cotton.

Three-week-old cotton plants, 'SureGrow 404' were placed inside transparent chambers after foliar application of a commercially available plant growth regulator. The transparent chambers were then placed inside growth chambers and photosynthesis was measured every 20 minutes for 14 days. Daily averages of net photosynthesis, respiration, daily carbon gain, gross photosynthesis, and carbon use efficiency were determined from the gas exchange data.

What effects do commercially available plant growth regulators have on cotton plant photosynthesis and its associated parameters?

Significant differences in net photosynthesis, dark respiration, daily carbon gain, carbon use efficiency, and cumulative carbon gain were not consistently detected during the study period. Also, significant increases above the untreated plants were not detected in leaf area, shoot dry weight, and leaf area ratio at the completion of the study.

Conclusions/Recommendations.

We conclude the plant growth regulators used in this study did not influence photosynthesis or any associated parameters. Perhaps the most important consideration in crop production is maintenance of photosynthesis at reasonably high rates throughout the season through stress-avoidance management. The avoidance of stress-induced decreases in photosynthesis would be more feasible in achieving yield potential than are short-term increases in photosynthesis with the use of plant growth regulators.

ABSTRACT

The usefulness of foliar-applied growth-enhancing compounds in producing high-yielding, high-quality cotton (*Gossypium hirsutum* L.) is unresolved. Since plant growth regulators may increase dry-weight accumulation through increased net carbon assimilation, a study was designed to determine if commercially available plant growth regulators influence CO_2 exchange rates in cotton.

C.W. Bednarz, Univ. of Georgia, Dep. of Crop and Soil Sciences, Coastal Plain Exp. Stn., P.O. Box 748, Tifton, GA 31794; and M.W. van Iersel, Univ. of Georgia, Dep. of Horticulture, Georgia Stn., 1109 Exp. Stn., Griffin, GA 30223.
Received 3 Mar. 1998. *Corresponding author (cbednarz@tifton.cpes.peachnet.edu).

Fifteen 3-week-old cotton plants, 'SureGrow 404' were placed inside transparent chambers after foliar application of PGR IV, Stimulate, RyzUp, Early Harvest, or tap water (control). The transparent chambers were then placed inside two growth chambers. Gas exchange was measured for 90 seconds in each transparent chamber every 20 minutes for 14 days. Daily averages of net photosynthesis during the light period and respiration during the dark period were calculated, and daily C gain, gross photosynthesis, and C use efficiency were determined from the gas exchange data. Significant differences in net photosynthesis, dark respiration, daily C gain, C use efficiency, and cumulative C gain were not detected during the study. Also, significant increases above the untreated control in leaf area, shoot dry weight, and leaf area ratio were not detected at the completion of the study. We conclude that the growth regulators used in this study did not influence C exchange rate or any associated parameters.

Plant growth regulators for cotton have received much attention in recent years. Some studies with plant growth regulators have reported yield increases (Oosterhuis et al., 1995; Oosterhuis and Zhao, 1994a; Weir et al., 1995). Some authors, however, reported that yield increase was inconsistent across multiple locations (Weir et al., 1995). In other studies, plant growth regulators did not affect yield (Bednarz, 1998a; Locke et al., 1994; Robertson and Cothren, 1995). Decreases in lint yield (Abaye et al., 1995; Bednarz, 1998a; Weir et al., 1995) and lint quality (Bednarz, 1998a) have also been reported.

The effects of plant growth regulators on cotton root growth (Oosterhuis and Zhao, 1994a; Oosterhuis and Zhao, 1994b) and shoot growth and development (Guo and Oosterhius, 1995; Oosterhuis, 1995; Oosterhuis et al., 1995; Oosterhuis and Zhao, 1993) have generally been positive. Nutrient uptake (Guo et al., 1994; Guo and Oosterhuis, 1995); membrane leakage (Guo and Oosterhuis, 1995); and net C uptake (Cadena and Cothren, 1995; Cadena et al., 1994; Guo and Oosterhuis, 1995) were also positively influenced by foliar applied plant growth regulators. Beneficial effects of plant growth regulators under stressed conditions have also been reported (Cadena and Cothren, 1995; Cadena et al., 1994; Zhao and Oosterhuis, 1995; Zhao and Oosterhuis, 1997).

The basis of all crop productivity is photosynthesis (Evans, 1993). Therefore, increases in productivity would require increases in radiation use efficiency, which would require increases in whole plant photosynthesis (Sinclair, 1993). However, very large increases in leaf photosynthesis are required to achieve even modest increases in radiation use efficiency. Also, increasing photosynthesis is unlikely without further improvements in traditional and molecular plant breeding (Evans, 1993). Therefore, when the crop is not predisposed to stress (i.e., water or nutrient) increases in photosynthesis and crop productivity through the use of plant growth regulators would appear to occur in violation of basic plant physiology and crop production principles. This investigation was undertaken to determine the short-term effects of commercially available plant growth regulators on C exchange rates in cotton under no environmental stress.

MATERIALS AND METHODS

Cotton, 'SureGrow 404' was planted in 850 cm^3 pots filled with potting soil in a glasshouse at the University of Georgia Coastal Plain Experiment Station in Tifton in May 1997. Each pot contained one cotton plant and a total of 150 plants were used for the study. At 22 days after planting (four-leaf stage), the cotton plants were transferred to a calibrated, semi-continuous multi-chamber photosynthesis system at the University of Georgia, Georgia Station in Griffin.

Continuous measurements of gas exchange were made following the principles described by Bugbee (1992). Ten sealed, transparent acrylic chambers (DuPont Lucite; 50 cm long by 32 cm wide by 60 cm high, 96 L; Dupont, Wilmington, DE) containing 15 cotton plants each were placed inside two growth chambers (Conviron model number E-15, Conviron, Asheville, NC). Carbon exchange rate of the 10 groups of plants was measured with an open CO_2 exchange system. Ambient air was enriched with an additional 50 $\mu mol\ mol^{-1}$ CO_2 and blown into the acrylic chambers. This enrichment assured that the CO_2 concentration of the air remained close to ambient during the light period. The blower produced a positive pressure in the system, which prevented surrounding air from leaking into the CO_2 exchange

Table 1. The product name, manufacturer, active ingredients, and application rate of the commercially available plant growth regulators used in this study.

Product name	Manufacturer	Active ingredient(s)	Application rate†
PGR IV[a]	MicroFlo Company Lakeland,FL	Indolebutyric acid (0.0028%) Gibberellic acid (0.0030%)	146
Stimulate[a]	Stoller Enterprises, Inc. Houston, TX	Cytokinin, as Kinetin (0.009%) Gibberellic acid (0.005%) Indole-3-butyric acid (0.005%)	292
RyzUp[a]	Abbott Laboratories North Chicago, IL	Gibberellic acid (4.0% w/w)	219
Early Harvest[a]	Griffin Corporation Valdosta, GA	Cytokinin, as Kinetin (0.09%) Gibberellic acid (0.03%) Indolebutyric acid (0.045%)	146

† Milliliter per hectare equivalency applied on a 30 cm band.

system and affecting the measurements. An infra-red gas analyzer (SBA-1, PP Systems, Haverhillm, MA) was used to measure the CO_2 concentration of the incoming air. Airflow through the gas exchange chambers was measured with mass flow meters (GFM37-32, Aalborg Instruments and Controls, Monsey, NY). The difference in the CO_2 concentration of the air entering and exiting the chamber was measured with a differential infra-red gas analyzer (Li-6251, Li-Cor, Lincoln, NE). The air for the differential CO_2 measurements was sampled from the incoming air (before it reached the flow meters) and the acrylic gas exchange chambers. Air in the chambers was sampled using plastic tubing connected to solenoid valves, which were opened and closed using a relay driver (SDM-CD16AC, Campbell Scientific) operated by a datalogger (CR10T, Campbell Scientific). The mass flow meters and CO_2 analyzers also were connected to the datalogger, which took all measurements and calculated CO_2 exchange rates. This setup allowed for the fully automated measurement of CO_2 exchange throughout the experiment. Gas exchange was measured in each transparent chamber once every 1200 seconds for 90 seconds during the 14 days of the experiment. To minimize errors in the CO_2 measurements due to water vapor in the air, the air was cooled to 2°C and the water condensate was drained from the air stream.Whole chamber CO_2 exchange (mol s^{-1}) was calculated as the product of mass flow (mol s^{-1}) and the difference in CO_2 concentration (mol mol^{-1}). Photosynthetic photon flux density (at the canopy level inside the acrylic chambers) was 410 mmol m^{-2} s^{-1} in one growth chamber and 450 mmol m^{-2} s^{-1} in the other.These differences in photosynthetic photon flux density resulted in differences in CO_2 exchange between the two growth chambers, and treatments were blocked within a growth chamber. Temperature and relative humidity inside the growth chambers were maintained at 20/26°C dark period/light period and 100/75% dark period/light period. The photoperiod was 14 hours.

Cotton plants were placed inside the transparent chambers for 2 hours during the light period before treatment to allow a baseline CO_2 exchange rate for each group of plants to be generated. This indicated that the initial CO_2 exchange rates within replications were uniform. The plants were then removed from the chambers and the foliar treatments were applied. Four commercially available plant growth regulators (Table 1) and a control consisting of tap water were applied with a CO_2 backpack sprayer. The application rate for each plant growth regulator was in accordance with the manufacturer's recommendations. During plant growth regulator application, all plants for each treatment were placed on the floor in two rows at a density of nine plants $meter^{-1}$. After each plant growth regulator application the plants were returned to the same transparent chambers from which they were removed.

Daily averages of net photosynthesis during the light (net photosynthesis) and respiration during the dark period (dark respiration) were calculated from the CO_2 exchange data. Because net photosynthesis and dark respiration were calculated as the net CO_2 exchange rate of the plants, net photosynthesis is positive, and dark respiration has a negative value.

Daily C gain (mmol $plant^{-1}$ day^{-1}), gross photosynthesis (mmol $plant^{-1}$ s^{-1}), and C use efficiency (mol mol^{-1}), the ratio of C stored in biomass to total C fixed in photosynthesis

(Yamaguchi, 1978; Amthor, 1989), were determined from the gas exchange data as follows:

$$DCG = (LP \; X \; P_{net} + DP \; X \; R_{dark}) \; x \; 10^{-3} \quad [1]$$

$$P_{gross} = P_{net} - R_{dark} \quad [2]$$

$$CUE = DCG / (LP \; x \; P_{gross} \; x \; 10^{-3}) \quad [3]$$

where

LP = light period (s) and *DP* = dark period (s).

Cumulative C gain (mol plant^{-1}) was calculated as the integral of daily C gain over time and is directly proportional to dry mass increase. In the calculations of C use efficiency and gross photosynthesis, it is assumed that dark respiration and respiration during the light period were equivalent (Amthor, 1989). Although this is not necessarily true, this assumption will affect all treatments similarly, and therefore allows for meaningful comparisons among treatments.

The gas-exchange system performance accuracy was determined by measuring the CO_2 exchange rate in empty chambers, which was practically zero and by reacting to a known amount of $NaHCO_3$ with acid and measuring the evolved CO_2. The CO_2 recovery for the system was 98.4 ± 3.0% (mean ± standard deviation, n = 8) and did not differ among the acrylic chambers.

At the end of the experiment (13 days later), the plants were transferred back to Tifton, GA. Leaf area was determined using a LiCor LI-3100 area meter. Leaf and shoot dry mass was also determined after drying the plant material to uniformity at 60°C. Data were analyzed using the General Linear Model procedure (SAS Institute, 1997).The experimental design was a randomized complete block with two replications (one replication per growth chamber) and a group of 15 plants as the experimental unit.

RESULTS AND DISCUSSION

Figure 1 illustrates the effect of the various plant growth regulators on net photosynthesis and dark respiration. Net photosynthesis trended upward during the two-week period while dark respiration trended downward. These trends are considered normal for crop communities in early developmental stages (Hay and Walker, 1989). Net photosynthesis normally increases because the increasing leaf area intercepts more of the incident radiation. Dark respiration increases because growth and maintenance respiration increases as growth rate and plant mass increase. Significant plant growth regulator effects were not found.

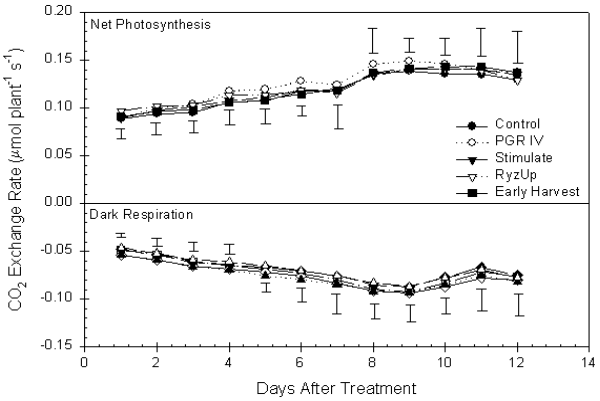

Figure 1. The effect of commercially available plant growth regulators on net photosynthesis and dark respiration in cotton. Data represent the mean of two gas-exchange chambers with 15 plants each. Error bars represent LSD's (0.05).

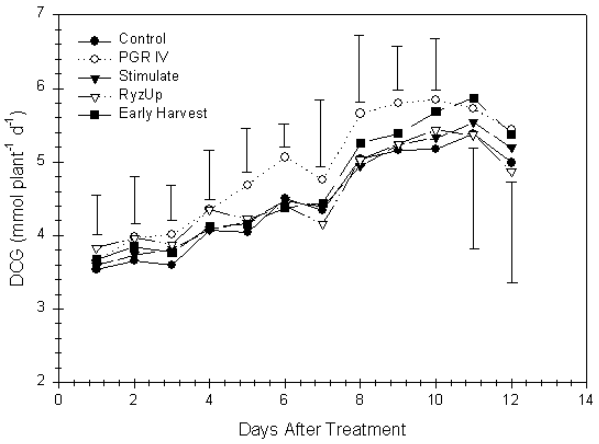

Figure 2. The effect of commercially available plant growth regulators on daily C gain in cotton. Data represent the mean of two gas-exchange chambers with 15 plants each. Error bars represent LSD's (0.05).

Daily C gain also increased during the 2-week study period (Fig. 2), an effect that is again considered normal during development. At 6 days after treatment establishment, PGR IV showed a significantly higher daily C gain than all other growth regulators. This increase was not observed at any other time during the study.

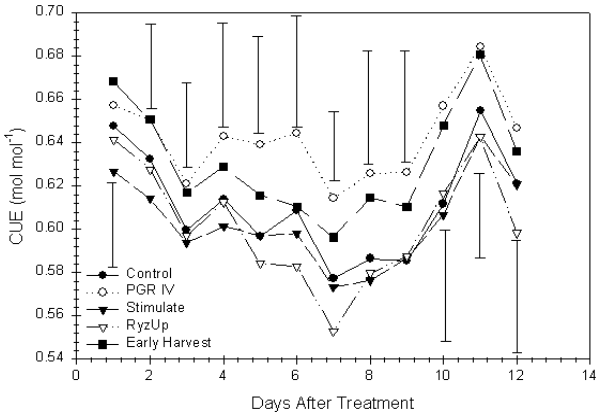

Figure 3. The effect of commercially available plant growth regulators on C use efficiency in cotton. Data represent the mean of two gas-exchange chambers with 15 plants each. Error bars represent LSD's (0.05).

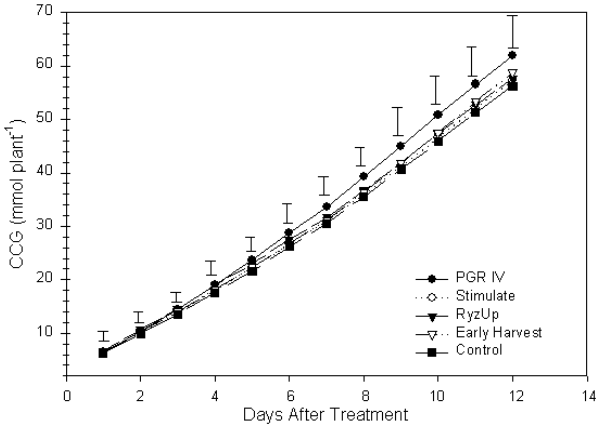

Figure 4. The effect of commercially available plant growth regulators on cumulative C gain in cotton. Data represent the mean of two gas-exchange chambers with 15 plants each. Error bars represent LSD's (0.05).

Young plants use 25 to 35% of their daily assimilate to support growth and 1.5 to 3.0% for maintenance processes (Hay and Walker, 1989). Thus, the C use efficiencies generated in our study appear normal (Fig. 3.). At 5 and 6 days after treatment, C use efficiency in the PGR IV-treated plants were higher than RyzUp-treated plants. At 7 days after treatment, the PGR IV-treated plants again exhibited a higher C use efficiency than the untreated control and plants treated with Stimulate and RyzUp. Significant effects in C use efficiency were not observed at any other time in the study.

Leaf area was not significantly different among the treatments at the conclusion of this study (Table 2). Shoot dry mass was significantly lower in the stimulate-treated plants compared to the RyzUp-treated plants and untreated plants. Application of plant growth regulators in this study did not increase shoot dry mass compared to the untreated control. Differences in leaf area ratio were not detected.

CONCLUSIONS

The total amount of photosynthetically active radiation available to the plants during this study was approximately 40% of what could be expected during a sunny 14-day period in May. However, all treatments accumulated between 50 and 60 mmol C $plant^{-1}$ during the 14 days (Fig. 4). Assuming dry matter is approximately 40% C (Radin and Eidenbock, 1986), there was a gain of 25 g biomass per chamber. If we assume a root/shoot ratio of 0.4 for cotton plants growing in small containers (Zhao and Oosterhuis, 1997), this would represent more than a 200% increase in total plant dry mass for the 14-day period. Bednarz (1998b) observed a 260% increase in shoot dry mass of young cotton plants during an identical time period under field conditions. We conclude plant growth and development in all treatments during this study was acceptable.

The gas exchange performance accuracy of our system was 98.4% at the initiation of this study. The sensitivity of the CO_2 exchange system produced observable developmental trends in net photosynthesis, dark respiration, and daily C gain over the 14 days. We therefore conclude with confidence that consistent changes in CO_2 exchange did not occur after the application of commercially

Table 2. The effects of different commercially available plant growth regulators on leaf area, shoot dry weight, and leaf area ratio of cotton. Data represent the mean of two gas exchange chambers with 15 plants each.

Treatment	Leaf area (cm^2 $plant^{-1}$)		Shoot dry wt. (g $plant^{-1}$)		Leaf area ratio (cm^2 g^{-1})	
RyzUp	**415.7**	**a***	**2.34**	**a**	**177.62**	**a**
Control	**436.3**	**a**	**2.24**	**ab**	**194.59**	**a**
PGR IV	**412.6**	**a**	**2.20**	**abc**	**187.31**	**a**
Early Harvest	**420.4**	**a**	**2.18**	**bc**	**192.83**	**a**
Stimulate	**431.8**	**a**	**2.06**	**c**	**210.21**	**a**

*** Means followed by the same letter within a column are not different ($P \geq 0.05$).**

available plant growth regulators in this investigation. The lack of a significant increase in leaf area, leaf area ratio, or shoot dry weight compared to the untreated control support this conclusion.

Perhaps the most important consideration in crop production is the maintenance of leaf C exchange rates at reasonably high rates throughout the season (Sinclair, 1993) through stress-avoidance management. The avoidance of stress-induced decreases in leaf C exchange rate would be more feasible in achieving yield potential than short-term increases in leaf C exchange rate with the use of plant growth regulators.

REFERENCES

Abaye, A.O., D.M. Oosterhuis, and J.A. Hickey. 1995. Field evaluations of PGR-IV. p. 1164. *In* D.A. Richter and J. Armour (ed.) Proc. Beltwide Cotton Conf., San Antonio, TX. 4–7 Jan. 1995. Natl. Cotton Council Am., Memphis, TN.

Amthor, J.S. 1989. Respiration and crop productivity. Springer-Verlag New York, New York.

Bednarz, C.W. 1998a. Evaluation of cotton yield enhancing products. p.217–220. *In* C.W. Bednarz (ed.) 1997 Georgia Cotton Res. Ext. Rep. UGA/CPES Research-Extension Publ. 4.

Bednarz, C.W. 1998b. Growth and development of cotton grown under several population densities. p. 200–216. *In* C.W. Bednarz (ed.) 1997 Georgia Cotton Res. Ext. Report. UGA/CPES Research-Extension Publ. 4.

Bugbee, B. 1992. Steady-state canopy gas exchange: System design and operation. HortScience 27(7):770–776.

Cadena, J., and J.T. Cothren. 1995. Yield response of cotton to nitrogen, irrigation, and PGR IV regimes. p. 1142–1150. *In* D.A. Richter and J Armour (ed.) Proc. Beltwide Cotton Conf. 4–7 Jan. 1995. Natl. Cotton Council Am., Memphis, TN.

Cadena, J., J.T. Cothren, and C.J. Fernandez. 1994. Carbon balance of PGR IV treated cotton plants at two temperaure regimes. p. 1309–1313. *In* D.J. Herber and D.A. Richter (eds.) Proc. Beltwide Cotton Conf., San Diego, CA. 5–8 Jan. 1995. Natl. Cotton Council Am., Memphis, TN.

Clark, T.H., C.A. Stutte, R.K. Ball, and C. Guo. 1992. Root activity in cotton as affected by stress and bioregulants. p. 1028. *In* D.J. Herber and D.A. Richter (eds.) Proc. Beltwide Cotton Conf. Natl. Cotton Council Am., Memphis, TN.

Evans, L.T. 1993. Processes, genes, and yield potential. p. 687–696. *In* D.R. Buxton et al. (ed.) International crop science. CSSA, Madison, WI.

Guo, C., and D.M. Oosterhuis. 1995. Atonik: A new plant growth regulator to enhance yield in cotton. p. 1086–1088. *In* D.A. Richter and J Armour (ed.) Proc. Beltwide Cotton Conf., San Antonio, TX. 4–7 Jan. 1995. Natl. Cotton Council Am., Memphis, TN.

Guo, C., D.M. Oosterhuis, and D. Zhao. 1994. Enhancing mineral nutrient uptake of cotton plants with plant growth regulators. p. 244–251. *In* T.J. Tworkoski (ed.) Proc. 21st Annual Meet. Plant Growth Regulator Soc. Am., Portland, OR. 3–6 Aug. 1994.

Hay, R.M., and A.J. Walker. 1989. An introduction to the physiology of crop yield. John Wiley & Sons, New York.

Locke, D.H., J.A. Landivar, and D. Moseley. 1994. The effect of PGR-IV and soil insecticides on early-season growth, square retention, and lint yield. p. 1272–1273. *In* D.J. Herber and D.A. Richter (ed.) Proc. Beltwide Cotton Conf., San Diego, CA. 5–8 Jan. 1995. Natl. Cotton Council Am., Memphis, TN.

Oosterhuis, D.M. 1995. Effects of PGR IV on the growth and yield of cotton: A review. p. 29–39. *In* G.A. Constable and N.W. Forrester (ed.) Proc. First World Cotton Research Conf., Brisbane, Australia. 14–17 Feb. 1994. CSIRO, Canberra.

Oosterhuis, D.M., L.D. Janes, and B.R. Bondada. 1995. Research on plant growth regulators in cotton: Summary of 1994 results. p. 1077–1079. *In* D.A. Richter and J Armour (ed.) Proc. Beltwide Cotton Conf., San Antonio, TX. 4–7 Jan. 1995. Natl. Cotton Council Am., Memphis, TN.

Oosterhuis, D.M., and D. Zhao. 1993. Effects of rates and timing of PGR IV application on cotton growth and development. p. 1284 . *In* D.J. Herber and D.A. Richter (ed.) Proc. Beltwide Cotton Conf., New Orleans, LA. 10–14 Jan. 1995. Natl. Cotton Council Am., Memphis, TN.

Oosterhuis, D.M., and D. Zhao. 1994a. Enhanced root growth with PGR-IV. p. 1348–1350. *In* D.J. Herber and D.A. Richter (ed.) Proc. Beltwide Cotton Conf., San Diego, CA. 5–8 Jan. 1995. Natl. Cotton Council Am., Memphis, TN.

Oosterhuis, D.M., and D. Zhao. 1994b. Increased root length and branching in cotton by soil application of the plant growth regulator PGR-IV. Plant Soil 167:51–56.

Radin, J.W., and M.P. Eidenbock. 1986. Carbon accumulation during photosynthesis in leaves of nitrogen- and phosphorous-stressed cotton. Plant Physiol. 82:869–871.

Robertson, W.C., and J.T. Cothren. 1995. A plant growth regulator program for cotton using mepiquat chloride and PGR-IV. p. 1150. *In* D.A. Richter and J. Armour (ed.) Proc. Beltwide Cotton Conf. Natl. Cotton Council Am., Memphis, TN.

SAS Institute. 1997. Users guide: Statistics. 5th ed. SAS Inst., Cary, NC.

Sinclair, T.R. 1993. Crop yield potential and fairy tails. p. 707–711. *In* D.R. Buxton et al. (ed.) International crop science. CSSA, Madison, WI.

Weir, B., D. Munk, R. Vargas, and S. Wright. 1995. PGR-IV responses to upland and pima cottons in the San Joaquin Valley of California. p. 577–579. *In* D.A. Richter and J Armour (ed.) Proc. Beltwide Cotton Conf., San Antonio, TX. 4–7 Jan. 1995. Natl. Cotton Council Am., Memphis, TN.

Yamaguchi, J. 1978. Respiration and the growth efficiency in relation to crop productivity. J. Faculty Agric., Hokkaido Univ. 59:59–129.

Zhao, D., and D.M. Oosterhuis. 1995. Effect of PGR IV on the growth and yield of environmentally stressed cotton. p. 1150. *In* D.A. Richter and J Armour (ed.) Proc. Beltwide Cotton Conf., San Antonio, TX. 4–7 Jan. 1995. Natl. Cotton Council Am., Memphis, TN.

Zhao, D., and D.M. Oosterhuis. 1997. Physiological response of growth-chamber grown cotton plants to the plant growth regulator PGR IV under water-deficit stress. Environ. Exp. Bot. 38:7–14.