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## Impact of Growth Retardants on Corn Leaf Morphology and Gas Exchange Traits

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### ABSTRACT

Growth retardant (GR) application often produces compact plants with dark green foliage and reduced leaf size. These morphological alterations can affect leaf gas exchange and biochemical traits associated with photosynthesis. We conducted greenhouse experiments by growing corn (*Zea mays* L.) plants under optimum water and nutrient conditions to evaluate the impact of two GR treatments, a seed-applied treatment of BAS110..W [1-(2,4-dichlorophenyl)-2-methoxy-1-methyl-2-(1H-1,2,4-triazol-1-yl) ethanol] at 250 mg kg<sup>-1</sup> of seed and an ethephon (2-chloroethyl phosphonic acid) treatment (a foliar-applied solution containing 100 µg a.i ethephon L<sup>-1</sup> of water applied at three-leaf stage), on leaf gas exchange characteristics (leaf stomatal conductance to H<sub>2</sub>O vapor (g<sub>s</sub>), leaf intercellular CO<sub>2</sub> concentration (C<sub>i</sub>), and leaf CO<sub>2</sub> exchange rate (CER)) and water use efficiency, and other plant morphological traits. A CER vs. C<sub>i</sub> response curve was also determined to assess the carboxylation efficiency for control and treated plants. Both GR reduced total plant leaf area and dry weight by about 21 to 31% relative to the control but increased specific leaf weight and weighted stomatal density by 7 to 19%. Leaf protein and chlorophyll contents were nearly doubled in GR-treated plants. Leaf g<sub>s</sub>, C<sub>i</sub>, and CER were significantly higher in GR-treated vs. control plants while CER/g<sub>s</sub> was significantly lower in GR-treated plants. The CER vs. C<sub>i</sub> response curve exhibited higher initial slope in GR-treated plants compared to control plants, indicating a higher carboxylation efficiency. Our data indicate that GR application reduced leaf size, concentrated photosynthetic pigments and enzymes, and enhanced CER of corn.

**P**LANT GROWTH RETARDANTS are widely used to control lodging in intensively managed cereal grain crops by reducing plant height, and therefore enhancing yield. These effects have been observed in barley (Waddington

and Cartwright, 1986), wheat (Wiersma et al., 1986), and corn (Cox and Andrade, 1988; Gaska and Oplinger, 1988; Langan and Oplinger, 1987; Norberg et al., 1988). Additionally, other researchers have observed suppression of leaf expansion and production of more compact plants with darker green foliage following GR application (Lee et al., 1985; Shanahan and Nielsen, 1987). These changes in plant growth also appear to improve productivity under water stress conditions (Appleby et al., 1966; Shanahan and Nielsen, 1987).

The commonly used GR are categorized as gibberellin biosynthesis inhibitors (GBI) or ethylene generators. The GBI inhibit the biosynthesis of gibberellin in the plant (Izumi et al., 1984) and limit cell expansion and division in the subapical meristematic zone of the stem (Sachs et al., 1960). Classes of compounds that promote the GBI response are quaternary ammonium compounds, pyrimidines, triazoles, and norbornenodietines (Jung, 1984). Ethylene, a naturally occurring plant hormone, is responsible for shortening the internode regions by impeding cell elongation and division (Burg et al., 1971). One ethylene generating chemical is ethephon, or 2-chloroethyl phosphonic acid (Warner and Leopold, 1969). Ethephon is a synthetic plant growth regulator that undergoes chemical biodegradation at a pH greater than 4.1 resulting in a release of ethylene (Warner and Leopold, 1969). Since plant cells usually have a pH greater than 4.1, an aqueous solution of ethephon can enter plant tissue, undergo degradation, release ethylene, and thus affect plants in a manner similar to ethylene.

While the impacts of GR on corn stem elongation, plant height, and lodging control have been well studied, their effects on leaf gas exchange and biochemical traits

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**Abbreviations:** GR, growth retardant; CER, leaf CO<sub>2</sub> exchange rate; g<sub>s</sub>, leaf stomatal conductance to H<sub>2</sub>O vapor; C<sub>i</sub>, ambient CO<sub>2</sub> concentration; C<sub>i</sub>, leaf intercellular CO<sub>2</sub> concentration; CER/g<sub>s</sub>, gas exchange water use efficiency; PPFD, photosynthetic photon flux density; WSD, weighted stomatal density; SLW, specific leaf weight.

associated with photosynthesis (chlorophyll and protein contents) have been less intensively investigated. Some researchers (Appleby et al., 1966; Humphries, 1968) have reported in other crop species a reduced leaf size and increased leaf chlorophyll and nitrogen concentrations with GR application. Mooney (1980) suggested that these traits are desirable attributes by which plants optimize productivity under limited water supply environments. We postulated that these alterations in leaf morphology and composition may affect leaf photosynthesis, leaf gas exchange and water use efficiency. The objective of this study was, therefore, to assess the influence of ethephon and GBI treatments on corn biomass production, leaf area, leaf stomatal density, chlorophyll and soluble protein contents, and gas exchange characteristics.

## MATERIALS AND METHODS

### Experimental Design and Treatments

These studies were conducted under greenhouse conditions during the summer period from June to September of 1990. The incident photosynthetic photon flux density (PPFD) during the growing period ranged from 600 to 1300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and the daily temperature from 25 to 40°C. Seeds of the corn hybrid Pioneer brand 3732 were sown in plastic pots (0.23-m diam. by 0.23-m height) containing approximately 2.5 kg of a mixture (1:1 v/v) of soil and vermiculite. Seedling number was reduced to two per pot at the two-leaf stage. Plants were kept well-watered and received 100 mL of nutrient solution (modified Hoagland solution) twice per week. Supplemental light was supplied by incandescent bulbs to extend the photoperiod to approximately 16 h.

The treatments included a control (no GR), an ethephon treatment, and a GBI treatment arranged in a completely randomized design with four replications. The GBI treatment consisted of seed treatment with BAS110..W [1-(2,4-dichlorophenyl)-2-methoxy-1-methyl-2-(1H-1,2,4-triazol-1-yl)ethanol; a proprietary triazole produced by BASF Wyandotte Corp., Research Triangle Park, NC] at a rate of 250 mg a.i.  $\text{kg}^{-1}$  of seed. The ethephon treatment was accomplished by drenching the plants at the 3-leaf growth stage with a solution containing 100  $\mu\text{g a.i. L}^{-1}$  of ethephon (commercial product Cerone from Rhône-Poulenc Agricultural Co., Research Triangle Park, NC) and 1 mL  $\text{L}^{-1}$  of a surfactant. Preliminary experiments using various concentrations of ethephon indicated that this concentration provided a desired level of growth retardation.

### Experimental Observations

Prior to gas exchange measurements, plants were transferred from a greenhouse bench to an acrylic plexiglass acclimation chamber (1.2 by 1.2 by 1.2 m) on an adjacent bench. Supplemental light was provided by a 1000-W metal halide lamp positioned above the chamber so that the incident PPFD on the upper leaves of the plants in the chamber would be near 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The heat load generated by the light source on the leaves was minimized by placing a plexiglass water reservoir between the light source and the chamber. The chamber was constantly supplied with air from outside the greenhouse through a flexible tubing (0.15-m diam) fitted with an electric fan. This provided a slight positive air pressure inside the chamber, and maintained ambient  $\text{CO}_2$  levels ( $\approx 345 \mu\text{L L}^{-1}$ ). Chamber temperature and relative humidity (20-70%)

varied according to ambient conditions. Plants were placed in the chamber for a minimum of 1 h prior to gas exchange measurements.

Instantaneous gas exchange measurements ( $g_s$ ,  $C_i$ , and CER) were made about 30 d after planting, which was about 14 to 16 d after ethephon application for the ethephon treatment, during a period between 1100 and 1300 h with a portable photosynthesis system (LI-6200, LI-COR, Lincoln, NE) with a 1.0-L leaf cuvette. Measurements were made by enclosing the midsection (38 mm) of the 6th leaf (collar visible) in the cuvette while maintaining the system in the open mode, adjusting air flow through the desiccant to maintain steady-state humidity conditions in the cuvette, then switching the system to closed mode. When  $\text{CO}_2$  levels began dropping rapidly a measurement was logged, normally requiring about 20 to 60 s from time of leaf enclosure to measurement. Cuvette air temperatures increased less than 2°C during measurement. These measurements were repeated on five different sets of plants grown across five different calendar dates. Stomatal conductance and  $C_i$  were calculated according to von Caemmerer and Farquhar (1981), where  $C_i = C_a - 1.6(\text{CER}/g_s)$ . To standardize  $C_i$  determinations, the  $C_a$  value used in calculating  $C_i$  was assumed to be 345  $\mu\text{L L}^{-1}$  (ambient conditions). A measure of water use efficiency, the CER/ $g_s$  ratio, was also calculated according to Morgan et al. (1993) and Hall et al. (1992).

Immediately after gas exchange measurements, leaf epidermal impressions of the adaxial and abaxial surfaces of the leaf were obtained with nail polish from the area where gas exchange measurements were taken. These impressions were mounted on microscope slides and used to determine stomatal density with a light microscope. Stomata were counted for five microscope fields per impression and averaged for a single impression. Since both sides of the leaf were involved in gas exchange measurements, a weighted stomatal density (WSD) value was calculated ( $\text{WSD} = \text{ADSD}^2 + \text{ABSD}^2 / (\text{ADSD} + \text{ABSD})$ ) for each leaf according to El-Sharkawy et al. (1985). Where  $\text{ADSD} = \text{adaxial stomatal density (stomata mm}^{-2}\text{)}$  and  $\text{ABSD} = \text{abaxial stomatal density (stomata mm}^{-2}\text{)}$ .

Leaves used for gas exchange measurements were immediately removed from the plants at the leaf collar and placed in an insulated container with dry ice and brought to the laboratory for area measurement with a LI-COR model 3100 leaf area meter then stored in a refrigerator for later determination of chlorophyll and soluble protein contents. Chlorophyll determination was done as described by Arnon (1949) using 0.8 L  $\text{L}^{-1}$  acetone solution for extraction. Soluble protein was determined according to the procedures of Bradford (1976) with a Bio-Rad (BIO-RAD, Inc. Richmond, CA) dye-binding protein assay kit, using a 50 mM sodium phosphate buffer (pH 7.5) for extraction. Chlorophyll and soluble protein contents were expressed on a per unit leaf area basis. The remainder of the plant was harvested, separated into stems (including leaf sheaths) and leaves, and leaf area was determined. Plant material was oven dried (70°C for 48 h) and weighed separately to determine DW and SLW calculated.

The CER vs.  $C_i$  response curves for control and ethephon-treated plants were determined using procedures of McDermitt et al. (1989). Plants were grown and treated with ethephon as previously described. Leaves from control or ethephon-treated plants were placed in the cuvette, allowing the leaf to deplete  $\text{CO}_2$  to near the compensation point (approximately 10  $\mu\text{L L}^{-1}$  for corn) while making seven measurements of CER and  $C_i$ . This required 5-10 min to complete measurements on one plant. The  $C_a$  value used for calculating  $C_i$  was the measured  $\text{CO}_2$  concentration in the cuvette at the time of individual CER determinations. Using the four plants associated with treatment

Table 1. Plant leaf area (LA) and dry weight (DW), specific leaf weight (SLW), weighted stomatal density (WSD), leaf chlorophyll (Chl) and protein (Prt) contents, leaf conductance to water vapor ( $g_s$ ), intercellular  $CO_2$  concentration ( $C_i$ ), carbon dioxide exchange rate (CER), and CER/ $g_s$  ratio as affected by growth retardant (GR) treatments.

GR trt.†	Plant traits measured										
	LA	DW	SLW	WSD	Chl	Prt	$g_s$	$C_i$	CER	CER/ $g_s$	
	$m^2\ pl^{-1}$	$g\ pl^{-1}$	$g\ m^{-2}$	no. $mm^{-2}$	$—\ g\ m^{-2}\ —$		$mol\ m^{-2}\ s^{-1}$	$\mu mol\ mol^{-1}\ CO_2$	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$	$\mu mol\ mol^{-1}$	
Control	0.19	18.6	35.5	55	0.17	1.57	0.23	175	23.5	106.4	
Ethephon	0.15	12.9	42.0	68	0.44	2.82	0.39	192	31.0	95.6	
GBI	0.14	13.7	39.9	61	0.41	2.60	0.40	203	32.5	88.5	
LSD(0.05)	0.02	1.5	1.6	5	0.04	0.45	0.07	12	2.6	10.0	

† Ethephon was foliarly applied at the three leaf stage by drenching plants with solution containing  $100\ \mu g$  ethephon  $L^{-1}$ . The GBI treatment (gibberellin biosynthesis inhibitor) was done by treating seeds with  $250\ mg\ BAS110..W.\ kg^{-1}$  of seed.

replications, 28 data points were generated for response curves of control and ethephon-treated plants. These data were taken on two separate sets of plants grown across two calendar dates.

### Data Analysis

The ANOVA procedure was used to determine GR treatment effects on variables measured and treatment means were compared using LSD values. Polynomial regression analysis was used to develop the CER vs.  $C_i$  response curves for each of the treatments, and correlation analysis was used to evaluate the associations among plant traits.

## RESULTS AND DISCUSSION

The GR treatments had similar effects on plant response variables for all five experiments; therefore, the data are shown as averages for all five experiments (Table 1). The application of both ethephon and GBI reduced LA and DW by 21 to 31% relative to the control, with little difference between GR treatments. Specific leaf weight and WSD were increased by 7 to 19% in treated plants. Mean chlorophyll and soluble protein contents were more than doubled in plants treated with both GR. The GR also had striking effects on gas exchange characteristics. Mean CER,  $C_i$ , and  $g_s$  were considerably higher in plants subjected to GR treatments as compared to the control. The increase ranged from a minimum of 16% for  $C_i$  to a maximum of 74% for  $g_s$ . However, GR application reduced CER/ $g_s$  by 10 to 17% relative to the control. In general, our data indicate that both GR used in this study altered the morphology and vegetative growth of corn, producing plants with smaller and thicker leaves (greater SLW), with increased stomatal density, enhanced chlorophyll and protein contents, and increased CER.

Khan and Tsunoda (1970) and Hesketh et al. (1981)

used the dilution hypothesis to explain higher CER in small leaves. This hypothesis suggests that the content of photosynthetic pigments (chlorophyll) and enzymes per unit leaf area are reduced as leaf area increases, leading to lower CER on a leaf area basis for large leaves compared to small ones. Data in our study appear to confirm this hypothesis, as shown by negative correlations between LA and CER, protein, and chlorophyll and the positive correlations between CER and protein and chlorophyll (Table 2). The negative correlation of LA with CER reported here also agrees with observations by Bhagsari and Brown (1986).

The results shown in Fig. 1 (data for the second replication of this experiment are not shown but were similar to those shown in Fig. 1) indicate that internal leaf composition factors (chlorophyll and protein content) were also probably associated with differences in CER between control and GR treatments. Collatz et al. (1992) indicated that the capacity (or activity) of photosynthetic enzymes of  $C_4$  leaves to fix  $CO_2$  can be estimated using the slope of the CER vs.  $C_i$  response curve. The greater slope (linear term significantly different at  $P \leq 0.01$ ) of the CER vs.  $C_i$  response curve for GR treated leaves indicates that these leaves maintained comparatively greater carboxylation efficiency than the control leaves. Since in  $C_4$  leaves, ribulose-1, 5-bisphosphate carboxylase accounts for 10 to 25% of soluble protein (Schmitt and Edwards, 1981) and phosphoenolpyruvate carboxylase accounts for another 10% of the soluble protein fraction (Brown, 1978), in many cases, leaf soluble protein content per unit area may reflect the leaf potential for  $CO_2$  assimilation per unit area. The higher concentration of soluble protein in GR-treated leaves observed in our work supports this assumption. The  $C_i$ -saturation of CER for the control treatment was  $90\ \mu L\ L^{-1}$  (maximum CER value on regression curve), which is typical for  $C_4$  plants

Table 2. Linear correlation coefficients for the relationships among leaf area (LA), specific leaf weight (SLW), weighted stomatal density (WSD), soluble leaf protein concentration (Prt), chlorophyll concentration (Chl), intercellular  $CO_2$  concentration ( $C_i$ ), leaf conductance to water vapor ( $g_s$ ),  $CO_2$  exchange rate (CER), and CER/ $g_s$  ratio.

	SLW	WSD	Prt	Chl	$C_i$	$g_s$	CER	CER/ $g_s$
LA	-0.65**	-0.53*	-0.75**	-0.86**	-0.59*	-0.65**	-0.72**	0.59*
SLW		0.81**	0.69**	0.79**	0.53*	0.72**	0.83**	-0.52*
WSD			0.62*	0.69**	0.59*	0.60*	0.48*	-0.59*
Prt				0.87**	0.82**	0.85**	0.73**	-0.82**
Chl					0.83**	0.91**	0.86**	-0.83**
$C_i$						0.93**	0.67**	-1.00**
$g_s$							0.88**	-0.93**
CER								-0.66**

\*, \*\*, Correlation values (n = 15) significant at 0.10, 0.05 and 0.01 levels, respectively.

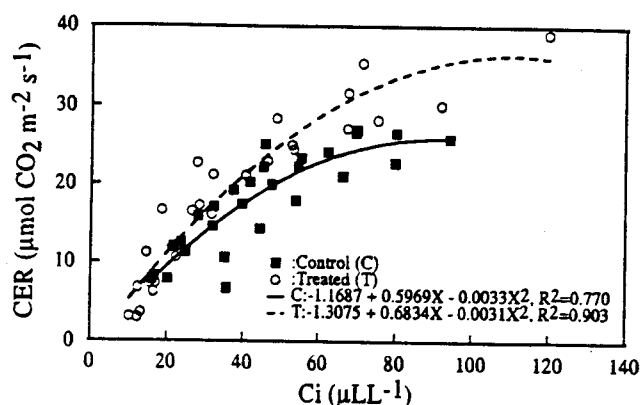


Fig. 1 The relationship between internal leaf  $[CO_2]$  ( $C_i$ ) and carbon dioxide exchange rate (CER) for control and ethephon treated plants. Measurements were taken between 1100 and 1300 h when the photosynthetic photon flux density was at least  $1900 \mu mol m^{-2} s^{-1}$  and air temperature in the cuvette  $30^\circ C$ .

(Collatz et al., 1992; Pearcy and Ehleringer, 1984). At this  $C_i$ , phosphoenolpyruvate carboxylase becomes saturated with substrate ( $CO_2$ ), and additional substrate does not enhance CER (Usuda et al., 1987). However, saturation of CER occurred at a lower  $C_i$  value for the control vs. GR treatment (90 vs.  $110 \mu L L^{-1}$ ), again indicating that  $V_{max}$  of carbon fixing enzymes imposed a greater constraint on CER of control leaves than GR-treated leaves (Collatz et al., 1992).

At high  $C_i$ , CER is also thought to be limited by light reaction and electron transport factors (Farquhar and Sharkey, 1982; Farquhar et al., 1980). Thus, the higher chlorophyll concentration of GR-treated plants vs. the control was also probably beneficial in increasing CER. LeCain et al. (1989) also concluded that the higher chlorophyll contents of smaller semidwarf wheat leaves, relative to large leaves of isogenic tall plants, was associated with enhanced CER.

The concentration of photosynthetic pigments and enzymes in smaller leaves is hypothesized to be due to a secondary effect of smaller leaves having smaller mesophyll cells (Turrell, 1942). Morgan et al. (1990), LeCain et al., (1989) and Pyke and Leech (1985) found that the  $Rht_2$  dwarfing gene in wheat produced smaller leaves with smaller mesophyll cells and higher carboxylation capacity. We did not investigate mesophyll cell size in our studies. However, Appleby et al. (1966) concluded that GR application reduced leaf size in wheat by reducing cell size. Thus, it is likely that the GR used in our study produced smaller leaves by reducing cell size.

Stomata should also exert control over CER. Leaf area and WSD were negatively associated while WSD and  $g_s$  were positively associated, indicating smaller leaves (GR-treated plants) had higher WSD, resulting in higher  $g_s$  values. These observations are consistent with observations of El-Sharkawy et al. (1984) and Austin et al. (1982). Higher  $g_s$  values were also associated with higher  $C_i$  values (Tables 1 and 2), which should theoretically increase CER (Farquhar and Sharkey, 1982), and our data support this conclusion as  $C_i$  was positively associated with CER.

Our initial hypothesis was that GR would increase

photosynthesis by concentrating photosynthetic pigments and improving carboxylation efficiency, and according to Mooney (1980) these are desirable attributes by which plants optimize productivity under limited water supply. While CER was increased by GR application, water use efficiency (expressed as CER/ $g_s$ ) was decreased by GR application (Table 1). Our data also showed that CER/ $g_s$  was negatively correlated with all of the traits enhanced by GR application (SLW, WSD, protein and chlorophyll concentrations,  $C_i$ ,  $g_s$ , and CER). Thus, it might be concluded, based only on gas exchange measurements, that GR would have no potential for improving corn water use efficiency. However, this appears to be true only at the single leaf level since results from two field studies we have conducted using GBI (Shanahan and Nielsen, 1987) and ethephon treatments (Kasele et al., 1994) showed that GR enhanced grain yields of corn grown under water stress. Increased grain yields were attributed to decreased early season crop canopy growth and water use, which reduced crop water stress during reproductive growth and grain filling. Apparently, the GR mediated effects on crop canopy development and seasonal water use are of greater importance to field performance under drought than potential reductions in intrinsic single leaf water use efficiency.

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