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PHYSIOLOGY

Continuous Whole Plant Carbon Dioxide Exchange Rates in Cotton Treated with Pyrithiobac

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INTERPRETIVE SUMMARY

Until recently, no selective post-emergence, over-the-top herbicides were available for annual broadleaf-weed control in nontransgenic cotton. Fluometuron and MSMA are registered for post-emergence, over-the-top use in cotton, but applications may result in delayed crop maturity and reduced yields. In 1992 a new herbicide, Staple® (common name pyrithiobac; DuPont Agricultural Products, Wilmington, DE), was introduced for post-emergence, over-the-top use in cotton. Cotton has exhibited tolerance to Staple® applied post-emergence over-the-top with no adverse effect on yield. Degree of tolerance, however, may depend on cotton variety and crop-growing conditions.

One injury symptom that may be observed with Staple® is leaf chlorosis. While this chlorosis may be transient in nature, it may result in an equally ephemeral reduction in carbon exchange rates, which could result in reduced crop growth and yield. Therefore, the objective of this study was to determine if Staple® alters carbon exchange rates in cotton and, if so, to what magnitude and duration.

Three-week-old cotton plants, "SureGrow 125" were placed inside transparent chambers after foliar application of tap water (control) or one of three Staple® treatments: (i) 0.6 oz of formulated product/acre (0.5 X rate), (ii) 1.2 oz of formulated product/acre (1.0 X rate), and (iii) 2.4 oz of formulated product/acre (2.0 X rate). The transparent chambers were then placed inside growth chambers and carbon exchange rate was measured every 20 minutes for 14 days. Daily averages of net photosynthesis, dark respiration, daily carbon gain, gross photosynthesis, and carbon use efficiency were determined from the gas exchange data.

What effects does Staple® have on cotton plant photosynthesis and its associated parameters?

Reductions in net photosynthesis, daily carbon gain, carbon use efficiency, and cumulative carbon gain were consistently observed in the 2.0 X rate of Staple® during the study period. However, these were transient reductions and by 12 days after treatment established carbon exchange rates were not different from the untreated plants.

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ABSTRACT

Injury symptoms that may be observed with pyrithiobac (the active ingredient in Staple®) are leaf chlorosis and plant stunting. While leaf chlorosis may be transient in nature, it could result in reduced carbon exchange rates, which could result in reduced crop growth and lint yield. This study was conducted to determine if pyrithiobac alters carbon exchange rates in cotton (*Gossypium hirsutum* L.) and, if so, to what magnitude and duration. Three-week-old cotton plants "SureGrow 125" were placed inside transparent chambers after foliar application of tap water (control) or one of three pyrithiobac treatments. The transparent chambers were then placed inside growth chambers and crop carbon exchange rates were measured every 20 minutes for 14 days. Daily averages of net photosynthesis, dark respiration, daily carbon gain, gross photosynthesis, and carbon use efficiency were determined from the gas exchange data. Significant reductions in net photosynthesis, daily carbon gain, carbon use efficiency, and cumulative carbon gain were consistently observed with the highest rate of pyrithiobac. However, these were transient reductions and by 12 days after treatment established carbon exchange rates were not different from the untreated plants. The 10 days of reduced carbon

exchange rates from the highest rate of pyrithiobac would represent a 0.65% reduction in aboveground dry mass at cutout, which is considered minimal. However, the severity and duration of post-emergence, over-the-top applications of pyrithiobac under adverse field conditions could be extended.

Until recently, no selective post-emergence, over-the-top herbicides were available for broadleaf weed control in nontransgenic cotton. Fluometuron {*N*,*N*-dimethyl-*N*'-[3-(trifluoromethyl)phenyl]urea} and monosodium acid methanearsonate (MSMA) are registered for post-emergence, over-the-top use in cotton, but applications may delay crop maturity and reduce lint yield (Baker et al., 1969; Byrd and York, 1987; Guthrie and York, 1989; Shankle et al., 1996; Snipes and Byrd, 1994). In 1992 a new herbicide, Staple® (common name: pyrithiobac sodium; sodium 2-chloro-6-[(4,6-dimethoxy pyrimidin-2-yl)thio]benzoate; DuPont Agricultural Products, Wilmington, DE), was introduced for post-emergence, over-the-top use in cotton. Preliminary research indicated cotton exhibits excellent post-emergence tolerance to this annual broadleaf herbicide with no yield reductions or maturity delays (Mitchell et al., 1992).

Pyrithiobac rapidly enters plants through the foliage and roots (Mitchell et al., 1992) and inhibits the enzyme acetolactate synthase (ALS; E.C. 4.1.3.18) in susceptible plants. Although not a sulfonylurea or imidazolinone herbicide, pyrithiobac inhibits growth and cell division similarly to these herbicides, resulting in chlorosis, necrosis, and death (Allen et al., 1997). Cotton has exhibited tolerance to pyrithiobac applied post-emergence over-the-top with no adverse effect on yield (Allen et al., 1997). Degree of tolerance, however, may depend on cotton variety (Baldwin et al., 1997) and crop growing conditions (Harrison et al., 1996; Shankle et al., 1996).

One injury symptom that may be observed with pyrithiobac, and ALS inhibitors in general, is leaf chlorosis (Allen et al., 1997). Although the chlorosis may be transient in nature (Allen et al., 1997), it could result in a reduction in carbon exchange rates, which may result in reduced crop growth and ultimately lead to a reduction in lint yield. Therefore, the objective of this study was to determine if pyrithiobac alters whole plant carbon exchange rates in cotton and, if so, to what magnitude and duration.

Carbon exchange rates are commonly measured on individual leaves, but often there is a poor correlation between leaf carbon exchange rate and dry matter production or yield (Evans, 1993). Whole plant carbon exchange measurements are a direct measure of plant growth and are therefore better suited to studies of growth responses than are single-leaf measurements. This report describes the effects of pyrithiobac on whole plant carbon exchange rates in cotton.

MATERIALS AND METHODS

Cotton, "SureGrow 125" was planted in 850 cm^3 pots filled with soil-less growing medium in a glasshouse at the University of Georgia, Coastal Plain Experiment Station in Tifton in April 1998. Each pot contained one cotton plant and a total of 112 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 where continuous measurements of gas exchange were made following the principles described by Bugbee (1992).

Eight sealed, transparent acrylic chambers (DuPont Lucite; 50 cm long by 32 cm wide by 60 cm high, 96 L) containing 14 cotton plants each were placed inside two growth chambers (Conviron model number E-15, Asheville, NC). Carbon exchange rates of the eight groups of plants were measured with an open CO_2 exchange system. Ambient air was enriched with an additional 50 mg L^{-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 system and affecting the measurements.

An infrared gas analyzer (SBA-1, PP Systems, Haverhill, 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

infrared 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, Logan, UT) operated by a datalogger (CR10T, Campbell Scientific). The mass flow meters and CO_2 analyzers were also 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 s for 90 s during the 14 d of the experiment.

Errors in the CO_2 measurements due to water vapor in the air were minimized by cooling the air to 2°C and draining the water condensate from the air stream. Whole chamber CO_2 exchange (mmol s^{-1}) was calculated as the product of mass flow (mol s^{-1}) and the difference in CO_2 concentration (mmol mol^{-1}). Carbon dioxide exchange rates were subsequently expressed on a per plant basis (mmol $plant^{-1}$ s^{-1}). Fluorescent lighting was used in the growth chambers and photosynthetic photon flux density (at the canopy level inside the acrylic chambers) was 410 µmol m^{-2} s^{-1} in one growth chamber and 450 µmol 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 h.

Cotton plants were placed inside the transparent chambers for 2 h during the light period before treatment to generate a baseline CO_2 exchange rate for each group of plants. There were no significant differences in initial CO_2 exchange rates among the treatments (P = 0.05). The plants were then removed from the chambers and the pyrithiobac treatments were applied. Three pyrithiobac treatments [0.036 kg a.i. ha^{-1} (0.5 X recommended rate), 0.071 kg a.i. ha^{-1} (1.0 X recommended rate), and 0.143 kg a.i. ha^{-1} (2.0 X recommended rate)] and a control (tap water) were applied with a CO_2 backpack sprayer with 80° nozzles in a delivery volume of 187 L ha^{-1}.

During treatment application, all plants from each treatment were placed on the floor in two rows at a density of nine plants per meter to simulate field conditions. After each treatment application the plants were returned to the same transparent chamber and growth chamber 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 carbon gain (mmol $plant^{-1}$ d^{-1}, a measure of plant growth rate), gross photosynthesis (µmol $plant^{-1}$ s^{-1}), and carbon use efficiency (mol mol^{-1}, the ratio of C stored in biomass to total C fixed in photosynthesis), were determined from the gas exchange data as follows (Yamaguchi, 1978; Amthor, 1989):

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

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

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

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

Cumulative carbon gain (mol $plant^{-1}$) was calculated as the integral of daily carbon gain over time and is directly proportional to dry mass increase, assuming a constant C content of the plants. In the calculations of carbon 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 bottom of each chamber was covered with three layers of capillary matting and plants were watered by wetting the mats. On day 10 this method did not water all plants thoroughly and the plants were watered from the top of each chamber with a garden hose equipped with a water breaker.

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 a known amount of $NaHCO_3$ with acid and measuring the evolved CO_2. The CO_2 recovery for the system was 98.4%.

At the end of the experiment (14 d later), leaf area was determined using a LI-3100 area meter (Li-Cor, Lincoln, NE). Shoot dry mass was determined after drying the plant material to uniform weight at 60°C.

Data were analyzed using the General Linear Model procedure (SAS, 1997). The experimental design was a randomized complete block with two replications (one replication per growth chamber) and a group of 14 plants as the experimental unit. One replication of each treatment was placed in each growth chamber. Thus, growth chambers were used as an experimental block and potential differences between the two growth chambers are accounted for as a block effect in the analysis of variance.

RESULTS AND DISCUSSION

Figure 1 illustrates the effect of pyrithiobac on cotton net photosynthesis and dark respiration. Net photosynthesis trended upward during the 14 days 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. Pyrithiobac treatments significantly affected net photosysnthesis from 2 to 11 days after treatment

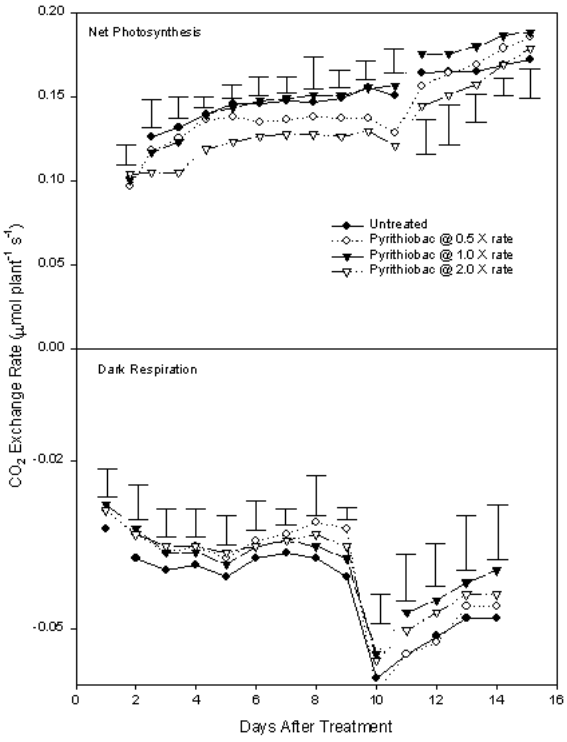

Fig. 1. The effect of pyrithiobac on net photosynthesis and dark respiration in cotton. Data represent the mean of two gas-exchange chambers with 14 plants each. Error bars represent $LSD_{0.05}$'s

application with the exception of day 7 (Table 1). On most of these days, P_{net} in the 2.0 X rate of pyrithiobac was significantly lower than the other treatments. Dark respiration was not affected by pyrithiobac (Table 1).

There was a sharp increase in R_{dark} (higher absolute value) in all treatments on day 10. This increase is probably an artifact of the hand watering

Table 1. Results from the analysis of variance procedure. Values indicate the probability that observed treatment differences were due to experimental error, rather than treatment effects. (NS = nonsignificant, *P* > 0.10)

Days after treatment	Net photosynthesis	Dark respiration	C use efficiency	Daily C gain	Cumulative C gain
1	NS	NS	NS	NS	NS
2	0.0256	NS	0.0380	0.0170	0.0495
3	0.0047	NS	0.0940	0.0017	0.0216
4	0.0110	NS	NS	0.0305	0.0169
5	0.0344	NS	NS	0.0153	0.0110
6	0.0188	NS	NS	0.0092	0.0091
7	NS	NS	0.0601	0.0872	0.0052
8	0.0276	NS	0.0963	0.0170	0.0034
9	0.0139	NS	0.0999	0.0125	0.0031
10	0.0091	NS	0.0245	0.0130	0.0019
11	0.0766	NS	0.0916	0.0545	0.0022
12	NS	NS	NS	NS	0.0040
13	NS	NS	NS	0.0435	0.0056
14	NS	NS	0.0920	0.0316	0.0071

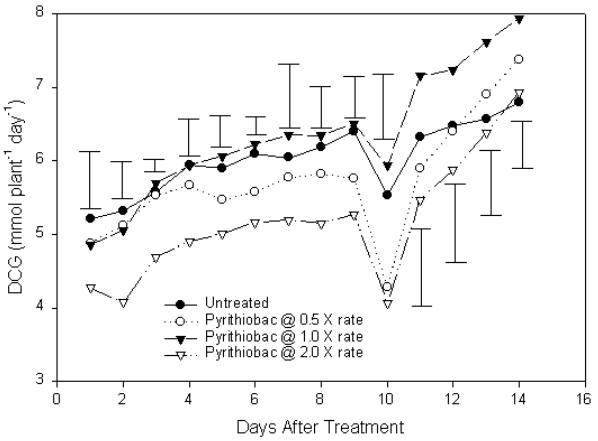

Fig. 2. The effect of pyrithiobac on daily carbon gain (DCG) in cotton. Data represent the mean of two gas-exchange chambers with 14 plants each. Error bars represent $LSD_{0.05}$'s.

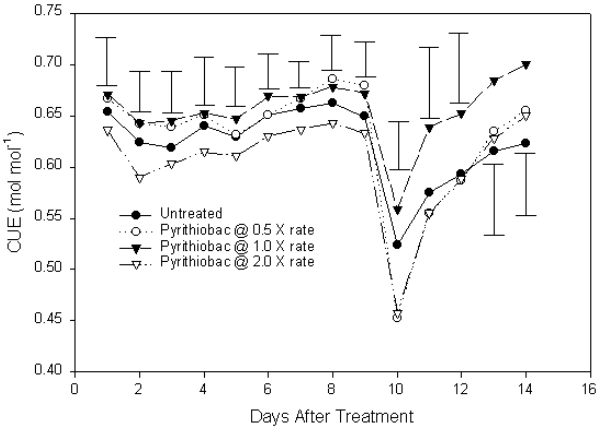

Fig. 3. Effect of pyrithiobac on carbon use efficiency (CUE), the ratio of carbon stored in biomass to total carbon fixed in photosynthesis, in cotton. Data represent the mean of two gas-exchange chambers with 14 plants each. Error bars represent $LSD_{0.05}$'s.

that occurred on the previous day. On this day, plants were watered from the top of the chambers instead of the bottom, which may have dissolved some of the lime that is present in soilless growing media and resulted in a CO_2 efflux from the pots. This type of CO_2 would appear in the data as an increase in the respiration rate of the plants, since the measurements cannot distinguish between CO_2 coming from the growing medium and that coming from the plant. It is unlikely that the measured increase in dark respiration was a true response of the plants to the watering. Also, the apparent increase in dark respiration on day 10 was not a response to water deficit stress. If water stress were involved in the decline in dark respiration stomatal closure would have also resulted in a significant reduction in net photosynthesis on this day, which did not occur (Fig. 1).

Daily carbon gain also increased during the 14-day study period (Fig. 2), an effect that is again considered normal during development. Daily carbon gain was also significantly lower than the control treatment in the 2.0 X rate of pyrithiobac from 2 to 14 days after treatment establishment with the exception of day 11 (Table 1). The 0.5 X rate of pyrithiobac also resulted in a significantly lower daily carbon gain than the control treatment at 6 and 10 days after treatment establishment. A sharp decline in daily carbon gain was also observed on day 10 in all treatments, which is attributed to the decline in dark respiration on this day (Fig. 1).

Young plants respire 25 to 35% of their daily assimilate to support growth and 1.5 to 3.0% for maintenance processes (Hay and Walker, 1989). Thus, carbon use efficiency appears normal (Fig. 3). Carbon use efficiency was also significantly lower than the control treatment in the 2.0 X rate of pyrithiobac for several days throughout the study period (Table 1). These reductions in carbon use efficiency are directly related to the reductions in net photosynthesis observed throughout the study period in this treatment (Fig. 1 and Table 1). Again, the sharp decline in carbon use effeciency observed on day 10 in all treatments is attributed to the decline in R_{dark} on this day (Fig. 1).

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 0.5 X and 2.0 X rates of pyrithiobac. Differences in leaf area ratio were not detected.

All treatments accumulated between 68 and 83 mmol C $plant^{-1}$ during the 14 days (Fig. 4) with the 2.0 X rate of pyrithiobac resulting in significantly less cumulative carbon gain than the control treatment from 2 to 14 days after treatment establishment. Assuming dry matter is approximately 40% carbon (Radin and Eidenbock, 1986), there was a gain of 33.2 g biomass per chamber in the untreated plants and 28.6 g biomass per chamber in the 2.0 X rate of pyrithiobac treatment. This represents a loss of 4.6 g biomass per chamber or (assuming 100 000 plants ha^{-1}) 32.9 kg ha^{-1}. Considering a crop at cutout contains about

Table 2. Effect of pyrithiobac on shoot dry mass and leaf area in cotton.

Treatment	Shoot dry mass (g plant^{-1})	Leaf area ratio (cm^2 g^{-1})	Leaf area (cm^2 plant^{-1})
control	2.43	129.8	313.7
Pyrithiobac @ 0.5 X rate	2.29	129.4	295.3
Pyrithiobac @ 1.0 X rate	2.63	121.1	318.5
Pyrithiobac @ 2.0 X rate	2.27	122.7	278.3
$LSD_{(0.05)}$	0.20	NS	NS

5090 kg ha^{-1} of aboveground dry mass (Bednarz, 1998), this represents a 0.65% reduction in aboveground dry mass from an off-label, 2 X application of pyrithiobac, which is not likely to be of much practical significance.

Net photosynthesis in the 2.0 X rate of pyrithiobac treatment was not significantly lower than the untreated controls at 12, 13, and 14 days after treatment establishment. Therefore, carbon exchange rates were affected for approximately 10 days after treatment establishment beginning with day 2. It should be noted, however, leaf chlorosis was not observed after pyrithiobac application in this study, as was the case in other work (Allen et al., 1997). Also, suboptimal growing conditions could result in more pronounced effects of pyrithiobac (Harrison et al., 1996, Shankle et al., 1996). Finally, the cotton variety used in this study "SureGrow 125" was not found to be susceptible to pyrithiobac injury (Baldwin, et al., 1997). Therefore, the severity and duration of post-emergence over-the-top applications of pyrithiobac under field conditions could be greater.

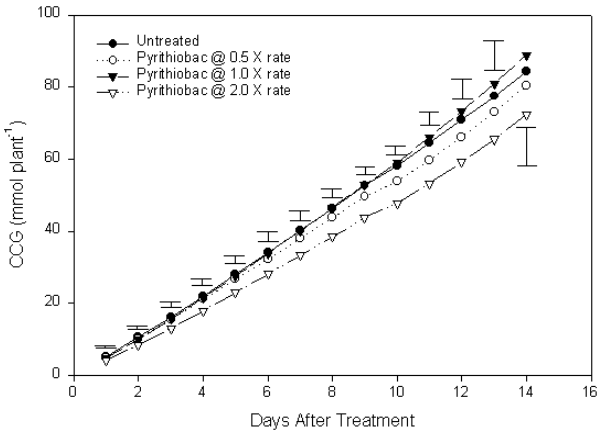

Fig. 4. The effect of pyrithiobac on cumulative carbon gain (CCG) in cotton. Data represent the mean of two gas-exchange chambers with 14 plants each. Error bars represent $LSD_{0.05}$'s.

CONCLUSIONS

The 10 days of reduced carbon exchange rates in the 2.0 X rate of Staple® would result in a 0.65% reduction in aboveground dry mass at cutout, which is considered minimal. It should be noted, however, that leaf chlorosis was not observed in this study after Staple® application, as was the case in other studies. Also, suboptimal growing conditions could result in more pronounced effects of Staple®. Therefore, the severity and duration of post-emergence, over-the-top applications of Staple® could be greater under field conditions.

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