

# Physiological basis for antagonism of clethodim by CGA 362622

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Greenhouse and laboratory experiments were conducted to determine the effect of CGA 362622 on the herbicidal activity of clethodim on goosegrass. CGA 362622 did not affect absorption and translocation of  $^{14}\text{C}$ -clethodim by goosegrass. Averaged across the two treatments of clethodim alone and clethodim plus CGA 362622, absorption was 27 and 85% of the applied  $^{14}\text{C}$ -clethodim at 0.5 and 96 h, respectively. By 96 HAT, only 0.8% of applied  $^{14}\text{C}$  had translocated to the shoot below the treated leaf. Metabolism of clethodim was not affected by the presence of CGA 362622. Three metabolites of clethodim were detected in treated tissue at all harvest intervals. By 96 HAT, 56% of absorbed  $^{14}\text{C}$  converted to a relatively polar form when clethodim was applied alone or in the presence of CGA 362622. One day after treatment, the photosynthetic rate in plants treated with CGA 362622 had decreased below the rate in the nontreated check and remained lower until 6 d after treatment. These data suggest that the antagonism of clethodim by CGA 362622 may result from CGA 362622 altering the photosynthetic rate of goosegrass and therefore the sensitivity of acetyl-coenzyme A carboxylase to clethodim.

**Nomenclature:** CGA 362622, *N*-([4,6-dimethoxy-2-pyrimidinyl]carbamoyl)-3-(2,2,2-trifluoroethoxy)-pyridin-2-sulfonamide sodium salt; clethodim; goosegrass, *Eleusine indica* (L.) Gaertn. ELEIN.

**Key words:** Absorption, acetyl-coenzyme A carboxylase, metabolism, site of action, translocation.

CGA 362622 is a sulfonylurea herbicide under development, for use in cotton (*Gossypium hirsutum* L.), for post-emergence (POST) control of broadleaf weeds, particularly sicklepod [*Senna obtusifolia* (L.) Irwin and Barneby] and common ragweed (*Ambrosia artemisiifolia* L.) (Hudetz et al. 2000; Porterfield et al. 2003). Cotton tolerance to CGA 362622 is based on limited absorption and rapid metabolism (Askew and Wilcut 2003). Clethodim is a graminicide registered for use in cotton, peanut (*Arachis hypogaea* L.), and soybean (*Glycine max* L.) (Anonymous 2001).

As cotton fields are typically infested with both grass and broadleaf weeds, it may be desirable to apply CGA 362622, an acetolactate synthase [4.1.3.18] (ALS) inhibitor with clethodim, an acetyl-coenzyme A carboxylase [6.4.1.2] (ACCase) inhibitor to control both types of weeds. Mixing the two herbicides would not only increase the spectrum of control but also reduce the cost associated with separate applications (Ickeringill 1995). Unfortunately, CGA 362622 has been reported to cause severe antagonism with clethodim and other graminicides (Burke et al. 2002; Crooks et al. 2001).

Several mechanisms have been proposed for antagonism of graminicides by other herbicides. Both absorption and translocation have been suggested as possible mechanisms for reduced grass control by graminicides when applied with broadleaf herbicides (Chow 1988; Culpepper et al. 1999a, 1999c; Ferreira et al. 1995; Myers and Coble 1992; Olson and Nalewaja 1982). The metabolic inactivation of cyclohexanedione herbicides also may be a basis for selectivity. Metabolism of the graminicide diclofop to a nontoxic metabolite confers selectivity to wheat (*Triticum aestivum* L.) (Shimabukuro et al. 1979). It is possible that CGA 362622 stimulates the activity of enzymes involved with herbicide

metabolism, such as herbicide antidotes that induce glutathione-S-transferase enzymes in sorghum (*Sorghum bicolor* L.) (Dean et al. 1990) and, therefore, increase the detoxification of clethodim.

Graminicides require actively growing meristematic regions for inhibition of ACCase (Devine et al. 1993). ALS-inhibiting herbicides such as CGA 362622 cause a wide variety of physiological responses in plants. One of the first responses to inhibition of ALS is a cessation of mitosis (Reynolds 1986; Rost 1984). Another symptom of the activity of ALS-inhibiting herbicides in plants is the inhibition of photosynthate transport. Shortly after an application of an ALS-inhibiting herbicide, neutral sugars accumulate in treated leaves because photosynthetic transport is inhibited (Bestman et al. 1990). Although ALS-inhibiting herbicides do not directly affect photosynthesis, a decrease in respiration has been reported within 24 h of application (Shaner and Reider 1986). The decrease in respiration could be a secondary response to a decrease in total metabolism (Shaner and Reider 1986; Shaner and Singh 1997). Therefore, research was conducted to determine the basis of the antagonistic interaction between CGA 362622 and clethodim. The objectives of this research were to determine the effect of CGA 362622 on the absorption, translocation, and metabolism of clethodim in goosegrass and to examine the effect of CGA 362622 on the photosynthetic rate of actively growing goosegrass.

## Materials and Methods

### Plant Material

Seeds of goosegrass were planted in a 1:1 mixture of pure sand and Norfolk loamy sand (fine-loamy, siliceous, thermic,

Typic Paleodults) in 10- by 10-cm square plastic pots. Upon emergence, plants were thinned to one per pot. Plants were maintained in a glasshouse with daily minimum and maximum temperatures of approximately 20 to 32 C. A 14-h photoperiod of natural and supplemental metal halide lighting with an average midday photosynthetic photon flux density of 700 to 1,400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  was provided. All pots received 10 ml of a 25-g  $\text{L}^{-1}$  commercial fertilizer<sup>1</sup> dissolved in deionized water at emergence and at 11 d after emergence.

## Absorption and Translocation

A study was conducted as a randomized complete block design with a split-split-plot treatment arrangement and four replications of treatments to evaluate absorption and translocation of clethodim alone and in the presence of CGA 362622. Main plots were harvest timings, subplots were plant portions, and sub-subplots were the two herbicide treatments of clethodim alone or clethodim plus CGA 362622. The study was repeated in time. At the four-leaf growth stage, the leaf to which  $^{14}\text{C}$ -clethodim was to be applied was covered with aluminum foil, and formulated clethodim at 140 g ai  $\text{ha}^{-1}$  was applied to uncovered plant portions either alone or with CGA 362622 at 5 g ai  $\text{ha}^{-1}$ . This rate of CGA 362622 is within the anticipated use rate in cotton (Hudetz et al. 2000). Applications were made using a spray chamber equipped with a single 8001E flat fan nozzle<sup>2</sup> calibrated to deliver 160 L  $\text{ha}^{-1}$  at 200 kPa. Crop oil concentrate<sup>3</sup> at 1.0% (v/v) was included in both mixtures. Immediately after application, five 1- $\mu\text{l}$  droplets of  $^{14}\text{C}$ -clethodim solution, containing approximately 1.7 kBq of radioactivity, were placed and spread on 1  $\text{cm}^2$  of the adaxial surface of the second fully expanded leaf of four-leaf goosegrass using a microliter syringe.<sup>4</sup> The solution contained  $^{14}\text{C}$ -clethodim (dissolved in acetonitrile) alone or with CGA 362622 to correspond with the nonradiolabeled mixtures. These solutions were prepared by diluting clethodim, labeled with  $^{14}\text{C}$  in the phenyl ring [Ring-4,6- $^{14}\text{C}$ ] and with a specific activity of 2.1 kBq  $\mu\text{mol}^{-1}$ , with either high-performance liquid chromatography (HPLC)-grade water and the commercial formulation of clethodim (Select<sup>™</sup>) or HPLC-grade water, Select<sup>™</sup>, and CGA 362622 at 0.1  $\mu\text{g ml}^{-1}$ . Select<sup>™</sup> was used to bring the total amount of clethodim applied to the treated leaf to 140 g  $\text{ha}^{-1}$ . Crop oil concentrate<sup>3</sup> was included in both mixtures at 1% (v/v). The rates of clethodim and CGA 362622 in the spotting solution were the same as in the solution applied using the spray chamber. Five microliters of solution were added to liquid scintillation cocktail<sup>5</sup> at the beginning of the  $^{14}\text{C}$ -label application for each treatment. These samples were used to calculate the amount of  $^{14}\text{C}$  applied to each plant by liquid scintillation spectrometry (LSS).<sup>6</sup>

Plants were removed from soil 0.5, 1, 2, 8, 24, 48, or 96 h after treatment (HAT) and were divided into treated leaf, roots, and aerial portions above and below the treated leaf. The treated leaves were rinsed for 20 s with 10 ml methanol-water (1:1, v/v) and 0.25% (v/v) nonionic surfactant<sup>7</sup> to remove nonabsorbed clethodim. A 1-ml aliquot of the rinse was added to 20 ml scintillation fluid,<sup>5</sup> and radioactivity was quantified via LSS. All plant parts, including washed roots, were dried for 48 h at 40 C, weighed, and

combusted with a biological sample oxidizer.<sup>8</sup> Radioactivity in the oxidized samples was quantified by LSS.

## Metabolism

The metabolism study was conducted as a randomized complete block design with a split-split-plot treatment arrangement and four replications of treatments to evaluate metabolism of clethodim alone and in the presence of CGA 362622. Treatment design was the same as in the absorption and translocation study. The study was repeated in time. Plants used for the metabolism study were grown, treated, and sectioned as described for the absorption and translocation experiments, with two exceptions. The amount of radioactivity applied to each leaf was 4.2 kBq, and the harvest intervals were 4, 8, 24, or 96 h. At harvest, plants were sectioned as described previously and placed immediately in a freezer and stored at  $-30$  C until further analysis. Based on the results of the absorption and translocation study, only the treated leaf contained sufficient radioactivity for evaluation. Treated leaf sections were homogenized in 2 to 4 ml acetonitrile using a tissue grinder.<sup>9</sup> The homogenate was then rinsed through a vacuum filtration apparatus with an additional 6 to 8 ml of acetonitrile. The residue and filter paper<sup>10</sup> were air-dried, wrapped in aluminum foil to retain any dry matter recovered during the filtration process, and stored at room temperature. The homogenate was concentrated to 1.0 ml under a stream of air and stored at  $-30$  C until further analysis (Valent USA Corp., personal communication). To evaluate the potential effects of the extraction process on herbicide degradation, fresh plant leaves were harvested, spotted with 5  $\mu\text{l}$  of the  $^{14}\text{C}$  herbicide solutions, and immediately processed in conjunction with the study samples. All herbicide extraction techniques were conducted on these freshly spotted leaves, so that effects of extraction could be elucidated later by comparing pure  $^{14}\text{C}$ -clethodim standard with the fresh-leaf extraction.

A 200- $\mu\text{l}$  aliquot of each concentrated sample was fractionated by reversed-phase HPLC and quantified with inline  $^{14}\text{C}$  detection. To determine the efficiency of both the detection and the extraction process using LSS, each injection, mobile phase solution, and scintillation cocktail<sup>11</sup> was collected in its entirety and an aliquot taken, and the percent  $^{14}\text{C}$ -clethodim and metabolites were determined by the ratio of each peak to the total  $^{14}\text{C}$  of the injection.

The liquid chromatographic system consisted of an autosampler<sup>12</sup> equipped with a 200- $\mu\text{l}$  sampling loop, two chromatographic pumps,<sup>13</sup> and a flow-through liquid scintillation spectrophotometer<sup>14</sup> with a 100- $\mu\text{l}$  flow cell. Gradients were controlled with an automated gradient controller.<sup>15</sup> An HPLC column<sup>16</sup> with a 1.5-cm guard column of the same material was used with a mobile-phase gradient consisting of HPLC-grade water acidified with 1.0% acetic acid and HPLC-grade acetonitrile (Valent USA Corp., personal communication).

## Photosynthetic Rate

To evaluate the response of goosegrass photosynthetic rate to CGA 362622, a study was conducted as a randomized complete block with three replications of treatments. The study was repeated in time. At the four-leaf growth stage, CGA 362622 was applied at 5 g  $\text{ha}^{-1}$ , using a spray cham-

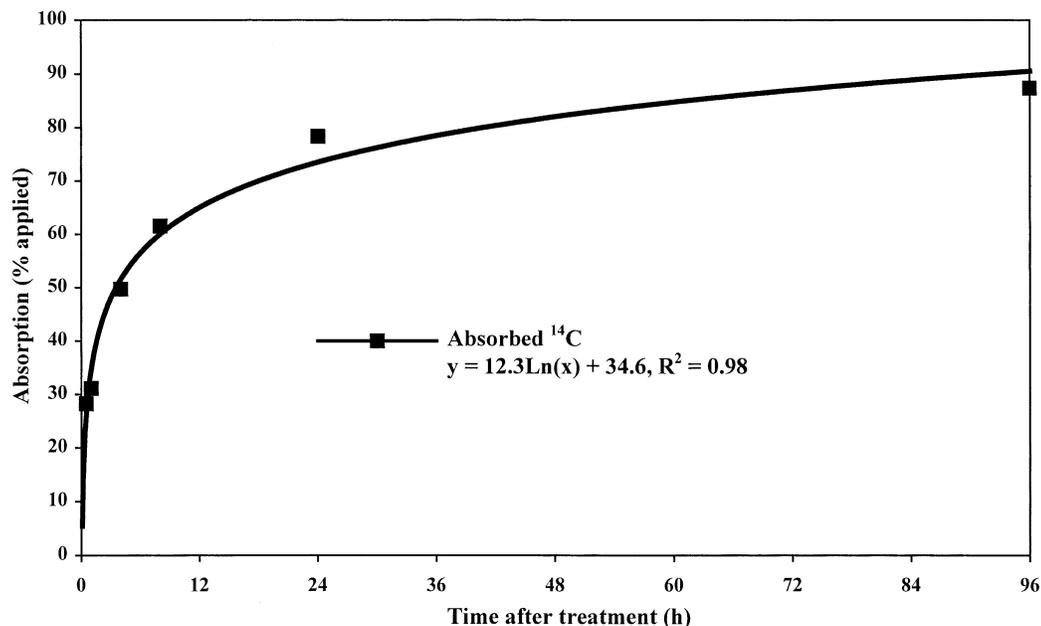


FIGURE 1. Foliar absorption of  $^{14}\text{C}$ -clethodim over time by goosegrass [*Eleusine indica* (L.) Gaertn.] averaged over herbicide treatments ( $^{14}\text{C}$ -clethodim or  $^{14}\text{C}$ -clethodim plus CGA 362622) ( $n = 16$  for each data point).

ber equipped with a single 8001E flat fan nozzle<sup>2</sup> calibrated to deliver  $160 \text{ L ha}^{-1}$  at 200 kPa. Nonionic surfactant<sup>4</sup> at 0.25% (v/v) was used with CGA 362622 as will be specified on the proposed herbicide label (Hudetz et al. 2000).

Single-leaf net photosynthetic rates were measured with a portable photosynthesis system.<sup>17</sup> To ensure light saturation, photosynthetic rate was measured between 11:00 A.M. and 1:00 P.M. immediately before treatment and 1, 2, 6, and 8 d after treatment (DAT) with CGA 362622. A 0.25-L chamber was used to enclose the middle portion of the second uppermost fully expanded leaf, and each measurement was made from the same leaf for the duration of the experiment. For each measurement, the leaf area was calculated by measuring the dimensions of the sample area using a ruler. The gas exchange system was operated as a closed system to measure photosynthetic rate as a function of time to depletion of 3 ppm  $\text{CO}_2$  (Peng and Krieg 1991). The measurement was repeated three times per leaf each day. The area of leaf enclosed by the chamber was determined after each set of measurements.

### Statistical Analysis

Data were tested for homogeneity of variance before statistical analysis. Analysis of variance (ANOVA) was performed on absorption as percentage of applied  $^{14}\text{C}$  over time. Linear, quadratic, and higher-order polynomial equations were fit to absorption as percentage of applied  $^{14}\text{C}$  over time by partitioning sums of squares (Draper and Smith 1981). Regression analysis was performed when significant ( $P \leq 0.05$ ) absorption into the treated leaf of goosegrass was observed over time. Nonlinear models used as ANOVA indicated that higher-order polynomial effects of absorption were significant ( $P \leq 0.05$ ). Estimation used the Gauss-Newton algorithm, a nonlinear least-squares technique (SAS 1998).

For the translocation study, data were subjected to AN-

OVA with sums of squares partitioned to reflect a split-split-plot treatment structure and trial effects using the general linear models procedure SAS (SAS 1998). The harvest timings were considered main plots, the plant sections were considered subplots, and the two spray mixtures (clethodim with or without CGA 362622) were considered sub-subplots. Regression analysis was performed when significant ( $P \leq 0.05$ ) translocation into the treated leaf of goosegrass was observed over time. Nonlinear models used as ANOVA indicated that higher-order polynomial effects of translocation were significant ( $P \leq 0.05$ ). Estimation used the Gauss-Newton algorithm, a nonlinear least-squares technique (SAS 1998). Statistical procedures for the metabolism study were similar to those of the translocation study.

For photosynthetic rate measurements, data were subjected to ANOVA with sums of squares partitioned to reflect trial, day of measurement, and treatment. Trial effects were not significant, so data were combined over trials ( $P > 0.05$ ). For all analyses, trial effects were considered random, and mean squares were tested appropriately based on the treatment design (McIntosh 1983).

## Results and Discussion

### Absorption and Translocation

The average recovery of radioactivity for the absorption and translocation experiments was  $89.3 \pm 1.1\%$ .

ANOVA indicated that CGA 362622 did not influence  $^{14}\text{C}$ -clethodim absorption or translocation ( $P > 0.05$ ); thus, data were pooled over the two herbicide treatments of clethodim and clethodim plus CGA 362622. Averaged across the two herbicide treatments, absorption was 27 and 85% of the applied clethodim at 0.5 and 96 h, respectively (Figure 1). Clethodim exhibited a biphasic mode of absorption, with 61% of the  $^{14}\text{C}$ -clethodim absorbed in the first 8 h and a further 24-percentage point increase in absorption in

TABLE 1. Influence of harvest timings of 0.5, 1, 4, 8, 24, and 96 h after treatment on the distribution of absorbed  $^{14}\text{C}$  in goosegrass [*Eleusine indica* (L.)] averaged over herbicide treatments of  $^{14}\text{C}$ -clethodim and  $^{14}\text{C}$ -clethodim plus CGA 362622, and the corresponding regression equations.

Plant portion	Absorbed $^{14}\text{C}$ over time <sup>a</sup> (SEM)						Regression equations $y = m \ln(x) + b$ ( $R^2$ )
	0.5 h	1 h	4 h	8 h	24 h	96 h	
	————— % <sup>b</sup> —————						
Treated leaf	96.1 (0.5)	96.5 (0.6)	97.8 (0.7)	98.7 (0.3)	98.2 (0.2)	97.6 (0.6)	$y = 0.35 \ln(x) + 96.7$ (0.46)
Shoot above	1.7 (0.42)	1.7 (0.59)	0.7 (0.14)	0.4 (0.10)	0.6 (0.17)	1.2 (0.30)	$y = -0.17 \ln(x) + 1.35$ (0.33)
Shoot below	1.3 (0.23)	1.1 (0.14)	1.0 (0.67)	0.6 (0.18)	0.9 (0.17)	0.9 (0.30)	$y = -0.08 \ln(x) + 1.11$ (0.32)
Root	0.9 (0.16)	0.7 (0.07)	0.5 (0.06)	0.3 (0.04)	0.3 (0.03)	0.3 (0.06)	$y = -0.12 \ln(x) + 0.67$ (0.80)

<sup>a</sup> Clethodim was applied at 140 g ha<sup>-1</sup> alone or in mixture with CGA 362622 at 5 g ha<sup>-1</sup>.

<sup>b</sup> For each plant portion and harvest interval,  $n = 16$ .

the following 88 h. Clethodim and other cyclohexanedione herbicides in general are rapidly absorbed and have a biphasic absorption pattern (Culpepper et al. 1999a; Wanamarta and Penner 1989). Although absorption increased over time, little  $^{14}\text{C}$  translocated from the treated leaf to other plant portions at any harvest interval (Table 1). By 96 HAT, only 0.9% of absorbed  $^{14}\text{C}$  had moved into the portion of the plant below the treated leaf. The plant portion below the treated leaf includes the intercalary meristem (Esau 1977), the site of action of ACCase inhibitors (Burton et al. 1987; Rendina and Felts 1988). Other researchers also have reported that cyclohexanedione herbicides are readily absorbed into leaf tissue but are generally not translocated out of the treated leaf (Campbell and Penner 1987; Culpepper et al. 1999a).

Although our data suggest that CGA 362622 does not affect absorption or translocation of clethodim out of the treated leaf, others have noted a difference in translocation of graminicides when mixed with an ALS-inhibiting herbicide (Chow 1988; Croon et al. 1989; Ferreira et al. 1995). It has been suggested that ALS-inhibiting herbicides affect transport processes and may therefore affect the movement of the graminicides or the corresponding bioactivated metabolite to the site of action. It should be noted that the amount of cyclohexanedione herbicide required for ACCase inhibition is very low, with a calculated 50% inhibition ( $I_{50}$ ) value for sethoxydim of 2.9  $\mu\text{M}$  and greater than 90% inhibition at 100  $\mu\text{M}$  (Burton et al. 1987; Focke and Lichtenhaler 1987; Rendina and Felts 1988). Therefore, the small differences in translocation reported in other studies may not account for the magnitude of herbicide antagonism resulting in a lack of control. A mechanism of antagonism different from, or in addition to, translocation may account for the reduction in grass control observed in management studies (Burke et al. 2002; Crooks et al. 2001).

## Metabolism

ANOVA indicated that CGA 362622 did not influence  $^{14}\text{C}$ -clethodim metabolism ( $P > 0.05$ ); thus, data were pooled over the two herbicide treatments of clethodim and clethodim plus CGA 362622. Three major metabolites of clethodim were detected in treated tissue at all harvest intervals, whereas no  $^{14}\text{C}$ -clethodim (retention time of 35.5 min) was recovered at any harvest interval (data not shown). Of the three metabolites, the greatest percentage of total metabolite at the 4-h harvest consisted of Metabolite C (retention time of 27 min) (Figure 2). From the 4-h to the

96-h harvest, Metabolite C decreased from 50 to 3% of total recovered  $^{14}\text{C}$ . Metabolite B (retention time of 14 min) also decreased as percentage of total recovered  $^{14}\text{C}$  from the 24-h harvest to the 96-h harvest. Metabolite A (retention time of 4.0 min) increased from 7% of total metabolite at the 4-h harvest to 56% of total metabolite at the 96-h harvest.

No metabolites of clethodim have been described; however, the metabolites of a structurally related compound, alloxymid, have been elucidated (Hashimoto et al. 1979; Soeda et al. 1979). Clethodim could be transformed similarly in plant tissue. The sulfur in clethodim is available for oxidation to the corresponding sulfoxide and sulfone. Sulfur is readily oxidized in other pesticidal molecules (Ashton and Crafts 1981), and Metabolites B and C could possibly correspond to the sulfone and sulfoxide of clethodim, respectively. In this study, the metabolism of clethodim proceeded rapidly, as has been reported for sethoxydim as well. Within 24 h, 98% of sethoxydim was degraded in tolerant as well as in sensitive species (Campbell and Penner 1985, 1987). Metabolite A is relatively polar compared with the other two metabolites and clethodim as determined by its retention time. After oxidation, herbicide metabolites are typically conjugated to more polar products (Devine et al. 1993). Both major families of graminicides, the cyclohexanedione and aryloxyphenoxypropionate herbicides, are metabolized at similar rates alone or when applied in the presence of other herbicides, including ALS-inhibiting herbicides, in large crabgrass [*Digitaria sanguinalis* (L.) Scop.] and yellow foxtail [*Setaria glauca* (L.) Beauv.] (Culpepper et al. 1999c; Ferreira et al. 1995).

## Photosynthetic Rate

The interaction of treatment by sampling time was significant ( $P = 0.012$ ). Immediately before an application of CGA 362622, rates of photosynthesis were similar for all goosegrass plants (Figure 3). One DAT, the single-leaf photosynthetic rate in plants treated with CGA 362622 had decreased by 5.7  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  compared with the nontreated goosegrass single-leaf photosynthetic rate and remained lower at 2 and 6 DAT. In previous work, the amount of fresh weight of biomass remained unchanged for CGA 362622-treated goosegrass while continuing to increase for nontreated goosegrass for 4 DAT (Burke et al. 2002). Thus, CGA 362622 appears to reduce both photosynthetic and growth rates of goosegrass. Cyclohexanedione herbicides do not initially affect photosynthesis, and photosynthetic inhibition was not observed until 2 DAT (Gealy

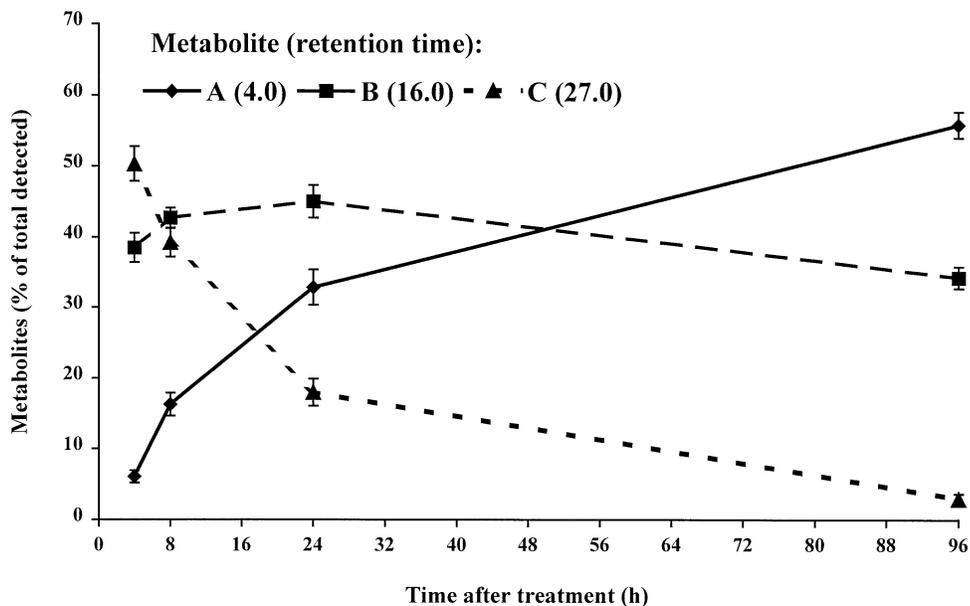


FIGURE 2. Influence of harvest timings of 4, 8, 24, and 96 HAT on the proportion of  $^{14}\text{C}$ -labeled metabolites in treated leaves of goosegrass [*Eleusine indica* (L.) Gaertn.] averaged over herbicide treatments of  $^{14}\text{C}$ -clethodim alone and  $^{14}\text{C}$ -clethodim plus CGA 362622. Error bars represent the standard error of the mean ( $n = 16$  for each data point).

and Slife 1983). Therefore, the reduction in photosynthesis and growth of CGA 362622-treated goosegrass compared with nontreated goosegrass may have implications for the effect of ACCase inhibition on plant growth.

Target ACCase is present in rapidly dividing cells and in active chloroplasts (Burton et al. 1987). Sethoxydim, a closely related compound to clethodim, rapidly inhibits  $^{14}\text{C}$ -acetate incorporation into lipids in corn root tips but not in the less metabolically active root regions (Hosaka and Tagaki 1987). Visible symptoms of ACCase herbicidal activity are most rapidly and strongly observed in meristematic regions and on an ultrastructural level in the chloroplast

(Brezeanu et al. 1976; Chandrasena and Sagar 1987). Chlorimuron and pyrithiobac did not specifically affect ACCase activity in vitro (Bjelk and Monaco 1992; Ferreira et al. 1995), but chloresulfuron reduced lipid synthesis in isolated soybean leaf cells 30 min after treatment (Hatzios and Howe 1982). In the current study, metabolism of clethodim was not affected by the presence of CGA 362622. By 4 DAT, when goosegrass resumed growth (Burke et al. 2002), and therefore ACCase activity, the active species of clethodim was no longer present in sufficient quantity to inhibit the enzyme (Figure 2). Graminicides require actively growing meristematic regions for inhibition of ACCase (Devine et

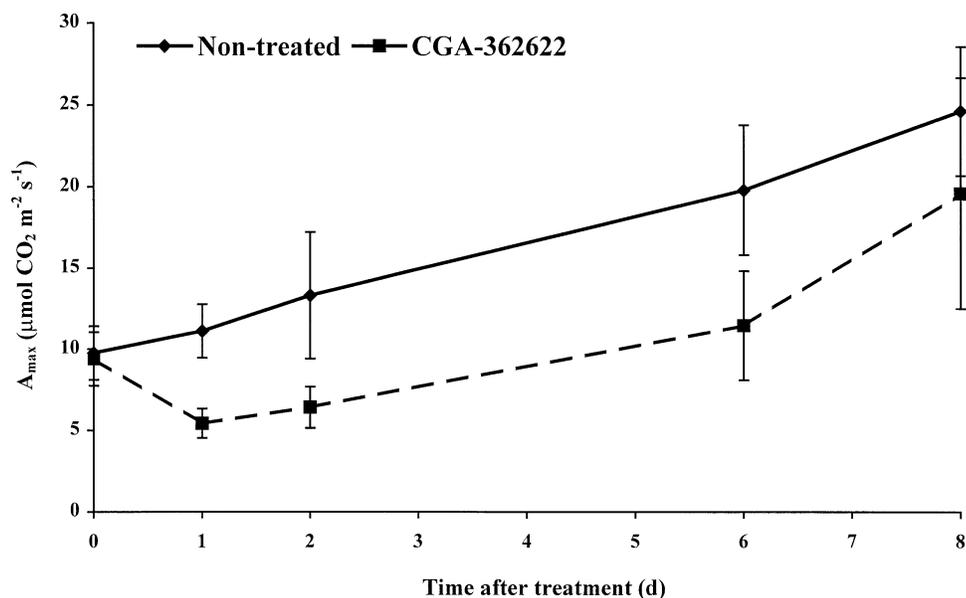


FIGURE 3. Single-leaf photosynthetic rate ( $A_{\max}$ ) response of nontreated light-saturated goosegrass [*Eleusine indica* (L.) Gaertn.] and single light-saturated goosegrass treated with CGA 362622. Error bars indicate standard error of the mean ( $n = 16$  for each data point).

al. 1993). These data suggest that the growth rate of goosegrass is reduced for approximately 6 DAT by treatment with CGA 362622. Therefore, the requirement of an actively growing plant for herbicidal activity upon ACCase inhibition may be compromised by the reduction of plant growth and photosynthesis caused by ALS inhibition. This growth suppression may reduce plant demand for lipid biosynthesis by ACCase, thus reducing the efficacy of ACCase-inhibiting herbicides.

Several authors have noted that increasing the rate of the graminicide reverses antagonism (Byrd and York 1987; Culpepper et al. 1999b; Ferreira et al. 1995; Minton et al. 1989; Myers and Coble 1992). Ferreira et al. (1995) reported a need for 2 to 2.5 times the registered rate of fluazifop-P to achieve greater than 90% control when applied with DPX-PE350 (pyrithiobac). Rates of fluazifop-P, fluazifop-P plus fenoxaprop-P, and quizalofop-P required for 80% control of large crabgrass when mixed with bromoxynil were 220, 220, and 290% greater, respectively, than rates required when these graminicides were applied alone (Culpepper et al. 1999b). At 2 to 3 times the registered rates, cyclohexanedione and aryloxyphenoxypropionate herbicides may interfere with plant membranes; thus, the observed control by increasing rate may not be due to inhibition of ACCase but rather to toxicity at another site of action (Devine and Shimabukuro 1994).

The data presented in the current study suggest that the antagonism of clethodim by CGA 362622 may be influenced by CGA 362622 altering the photosynthesis and growth rate of goosegrass (Burke et al. 2002) and, therefore, the herbicidal inhibition of ACCase. Clethodim was absorbed and translocated similarly to other cyclohexanedione herbicides, and metabolism of clethodim was not affected by the presence of CGA 362622. Photosynthetic rates of goosegrass, however, were reduced by CGA 362622 treatment. By the time plants had recovered to normal growth (Burke et al. 2002) and photosynthesis (8 DAT), essentially no active herbicide remained in the plant. Therefore, CGA 362622 may prevent the herbicidal activity of the ACCase-inhibiting herbicide clethodim, causing the observed antagonism. Further studies are needed to examine whether sensitivity to ACCase-inhibiting herbicides can be influenced by environmental factors that slow down or inhibit photosynthesis and growth, as was demonstrated with CGA 362622 treatments.

### Sources of Materials

<sup>1</sup> Fertilizer, Peters Professional 20-20-20, Scotts-Sierra Horticultural Products Co., 14111 Scottslawn Road, Marysville, OH 43041.

<sup>2</sup> TeeJet spray nozzles, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189.

<sup>3</sup> Crop oil concentrate, Agri-Dex (83% paraffin-base petroleum oil and 17% surfactant blend), Helena Chemical Company, Suite 500, 6075 Poplar Avenue, Memphis, TN 38137.

<sup>4</sup> Microliter syringe, Hamilton705N 50 µl syringe, Hamilton Company, P.O. Box 10030, Reno, NV 89520.

<sup>5</sup> ScintiVerse® SX18-4 universal liquid scintillation cocktail, Fisher Scientific, 2000 Park Lane Drive, Pittsburgh, PA 15275.

<sup>6</sup> Packard TRI-CARB 2100TR liquid scintillation spectrometer, Packard Instrument Company, 800 Research Parkway, Meriden, CT 06450.

<sup>7</sup> Induce nonionic low foam wetter-spreader adjuvant contain-

ing 90% nonionic surfactant (alkylaryl polyoxyalkane ether and isopropanol), free fatty acids, and 10% water, Helena Chemical Company, Suite 500, 6075 Poplar Avenue, Memphis, TN 38137.

<sup>8</sup> Model OX-500 biological material oxidizer, R. J. Harvey Instrument Corp., 123 Patterson Street, Hillsdale, NJ 07642.

<sup>9</sup> Pyrex® tissue homogenizer Nos. 7727-40, Corning Inc., 45 Nagog Park, Acton, MA 01720.

<sup>10</sup> Whatman #3 filter paper, Fisher Scientific, P.O. Box 4829, Norcross, GA 30091.

<sup>11</sup> Ultima-Flo® M flow liquid scintillation cocktail, Packard Instrument Company, 800 Research Parkway, Meriden, CT 06450.

<sup>12</sup> Model 715 waters ULTRA WISP sample processor, Waters, 34 Maple Street, Milford, MA 01757.

<sup>13</sup> Model 6000 waters chromatography pump, Waters, 34 Maple Street, Milford, MA 01757.

<sup>14</sup> Model 500 radiomatic flo-one liquid scintillation spectrometer, Packard Instrument Company, 800 Research Parkway, Meriden, CT 06450.

<sup>15</sup> Model 680 waters automated gradient controller, Waters, 34 Maple Street, Milford, MA 01757.

<sup>16</sup> Allsphere ODS-1 5 µm 250 by 4.6 mm reversed phase column, Alltech Associates, Inc., 2051 Waukegan Road, Deerfield, IL 60015.

<sup>17</sup> Model LI-6200 portable photosynthesis system, LI-COR, P.O. Box 4425, Lincoln, NE 68504.

### Acknowledgments

We thank Dr. Cavell Brownie, Department of Statistics, North Carolina State University, for her expert statistical assistance, Wendy Pline and Andy Price for lab assistance, and Shawn Troxler for assistance in data collection. We also thank the Cotton Growers Association of North Carolina, Cotton Incorporated, and Valent USA Corp. for partial funding of this research.

### Literature Cited

- Anonymous. 2001. Select 2EC®. Pages 2606-2614 in Crop Protection Reference. 17th ed. New York: C & P Press.
- Ashton, F. M. and A. S. Crafts. 1981. Mode of Action of Herbicides. 2nd ed. New York: J. Wiley. pp. 221-222.
- Askew, S. D. and J. W. Wilcut. 2002. Absorption, translocation, and metabolism of foliar-applied CGA-362622 in cotton, peanut, and selected weeds. *Weed Sci.* 50:293-298.
- Bestman, H. D., M. D. Devine, and W. H. Vanden Born. 1990. Herbicide chlorsulfuron decreases assimilate transport out of treated leaves of field pennycress (*Thalspi arvense* L.) seedlings. *Plant Physiol.* 93:1441-1448.
- Bjellk, L. A. and T. J. Monaco. 1992. Effect of chlorimuron and quizalofop on fatty acid biosynthesis. *Weed Sci.* 40:1-6.
- Brezeanu, A. G., D. G. Davis, and R. H. Shimabukuro. 1976. Ultrastructural effects and translocation of methyl 2-[4-(2,4-dichlorophenoxy)phenoxy]propionate in wheat (*Triticum aestivum*) and wild oat (*Avena fatua*). *Can. J. Bot.* 54:2038-2048.
- Burke, I. C., J. W. Wilcut, and D. Porterfield. 2002. CGA 362622 antagonizes annual grass control with clethodim. *Weed Technol.* 16:749-754.
- Burton, J. D., J. W. Gronwald, D. A. Somers, J. A. Connelly, B. G. Gengenbach, and D. L. Wyse. 1987. Inhibition of acetyl-CoA carboxylase by the herbicides sethoxydim and haloxyfop. *Biochem. Biophys. Res. Commun.* 148:1039-1044.
- Byrd, J. D., Jr. and A. C. York. 1987. Interaction of fluometuron and MSMA with sethoxydim and fluazifop. *Weed Sci.* 35:270-276.
- Campbell, J. R. and D. Penner. 1985. Sethoxydim metabolism in monocotyledonous and dicotyledonous plants. *Weed Sci.* 33:771-773.
- Campbell, J. R. and D. Penner. 1987. Retention, absorption, translocation, and distribution of sethoxydim in monocotyledonous and dicotyledonous plants. *Weed Res.* 27:179-186.
- Chandrasena, J.P.N.R. and G. R. Sagar. 1987. Effect of fluazifop-butyl on the chlorophyll content, fluorescence and chloroplast ultrastructure of *Elymus repens* (L.) Gould. leaves. *Weed Res.* 27:103-112.

- Chow, P.N.P. 1988. Effect of chlorsulfuron on four graminicides for weed control and wheat yield. *Weed Res.* 28:145–150.
- Crooks, H. L., A. C. York, and A. S. Culpepper. 2001. Interaction of CGA 362622 and graminicides on annual grasses in cotton. *WSSA Abstr.* 41:59.
- Croon, K. A., M. L. Ketchersid, and M. G. Merkle. 1989. Effect of bentazon, imazaquin, and chlorimuron on the absorption and translocation of the methyl ester of haloxyfop. *Weed Sci.* 37:645–650.
- Culpepper, A. S., D. L. Jordan, A. C. York, F. T. Corbin, and Y. Sheldon. 1999a. Influence of adjuvants and bromoxynil on absorption of clethodim. *Weed Technol.* 13:536–541.
- Culpepper, A. S., A. C. York, and C. Brownie. 1999b. Influence of bromoxynil on annual grass control by graminicides. *Weed Sci.* 47:123–128.
- Culpepper, A. S., A. C. York, D. L. Jordan, F. T. Corbin, and Y. S. Sheldon. 1999c. Basis for antagonism in mixtures of bromoxynil plus quizalofop-P applied to yellow foxtail (*Setaria glauca*). *Weed Technol.* 13:515–519.
- Dean, J. V., J. W. Gronwald, and C. V. Eberlein. 1990. Induction of glutathione S-transferase isozymes in sorghum by herbicide antidotes. *Plant Physiol.* 92:467–473.
- Devine, M. D. and R. H. Shimakukuro. 1994. Resistance to acetyl coenzyme A carboxylase inhibiting herbicides. Pages 141–169 in S. B. Powles and J.A.M. Holtum, eds. *Herbicide Resistance in Plants: Biology and Biochemistry*. Boca Raton, FL: Lewis Publishers.
- Devine, M. D., S. O. Duke, and C. Fedtke. 1993. *Physiology of Herbicide Action*. Englewood Cliffs, NJ: PTR Prentice Hall. 101 p.
- Draper, N. R. and H. Smith. 1981. *Applied Regression Analysis*. New York: J. Wiley. pp. 33–42, 511.
- Esau, K. 1977. *Anatomy of Seed Plants*. New York: J. Wiley. 286 p.
- Ferreira, K. L., J. D. Burton, and H. D. Coble. 1995. Physiological basis for antagonism of fluazifop-P by DPX-PE350. *Weed Sci.* 43:184–191.
- Focke, M. N. and H. K. Lichtenthaler. 1987. Inhibition of acetyl-CoA carboxylase of barley chloroplasts by cycloxydim and sethoxydim. *Z. Naturforsch.* 42c:1361–1363.
- Gealy, D. R. and F. W. Slife. 1983. BAS 9052 effects on leaf photosynthesis and growth. *Weed Sci.* 31:457–461.
- Hashimoto, Y. K., I. Iwataki, and Y. Soeda. 1979. Fate of alloxidim-sodium on or in soybean plants. *Pestic. Sci.* 4:299–304.
- Hatzios, K. K. and C. M. Howe. 1982. Influence of the herbicides hexazinone and chlorsulfuron on the metabolism of isolated soybean leaf cells. *Pestic. Biochem. Physiol.* 17:207–214.
- Hosaka, H. and M. Takagi. 1987. Biochemical effects of sethoxydim in excised root tips of corn (*Zea mays*). *Weed Sci.* 35:612–618.
- Hudetz, M., W. Foery, J. Wells, and J. E. Soares. 2000. CGA 362622, a new low rate Novartis post-emergent herbicide for cotton and sugarcane. *Proc. South. Weed. Sci. Soc.* 53:162.
- Ickeringill, D. 1995. Tank mixing—its development—past, present and future. *Proc. Asp. Appl. Biol.* 41:33–39.
- McIntosh, M. S. 1983. Analysis of combined experiments. *Agron. J.* 75:153–155.
- Minton, B. W., M. W. Kurtz, and D. R. Shaw. 1989. Barnyardgrass (*Echinochloa crus-galli*) control with grass and broadleaf herbicide combinations. *Weed Sci.* 37:223–227.
- Myers, P. F. and H. D. Coble. 1992. Antagonism of graminicide activity on annual grass species by imazethapyr. *Weed Technol.* 6:333–338.
- Olson, W. and J. D. Nalewaja. 1982. Effect of MCPA on <sup>14</sup>C-diclofop uptake and translocation. *Weed Sci.* 30:59–63.
- Peng, S. and D. R. Krieg. 1991. Single leaf and canopy photosynthetic response to plant age in cotton. *Agron. J.* 83:704–708.
- Porterfield, D., J. W. Wilcut, and S. D. Askew. 2002. Weed management with CGA-362622, fluometuron, and prometryn. *Weed Sci.* 50:642–647.
- Rendina, A. R. and J. M. Felts. 1988. Cyclohexanedione herbicides are selective and potent inhibitors of acetyl-CoA carboxylase from grasses. *Plant Physiol.* 86:983–986.
- Reynolds, T. L. 1986. Effects of chlorsulfuron, valine, isoleucine on division and tracheary element differentiation in cell suspension cultures of *Solanum carolinense* L. *J. Plant Physiol.* 125:179–184.
- Rost, T. L. 1984. The comparative cell cycle and metabolic effects of chemical treatments on root tip meristems. III. Chlorsulfuron. *J. Plant Growth Regul.* 3:51–63.
- [SAS] Statistical Analysis Systems. 1998. *SAS/STAT User's Guide*. Release 7.00. Cary, NC: Statistical Analysis Systems Institute. 1028 p.
- Shaner, D. L. and M. L. Reider. 1986. Physiological responses of corn (*Zea mays*) to AC 243,997 in combination with valine, leucine, and isoleucine. *Pestic. Biochem. Physiol.* 25:248–257.
- Shaner, D. L. and B. K. Singh. 1997. Acetohydroxyacid synthase inhibitors. Pages 69–109 in R. M. Roe, J. D. Burton, and R. J. Kuhr, eds. *Herbicide Activity: Toxicology, Biochemistry and Molecular Biology*. Burke, VA: IOS Press.
- Shimabukuro, R. H., W. C. Walsh, and R. A. Hoerauf. 1979. Metabolism and selectivity of diclofop-methyl in wild oat and wheat. *J. Agric. Food Chem.* 27:615–623.
- Soeda, V., K. Ishihara, I. Iwataki, and H. Kamimura. 1979. Fate of a herbicide <sup>14</sup>C-alloxydim-sodium in sugar beet. *Pestic. Sci.* 4:121–128.
- Wanamarta, G. and D. Penner. 1989. Identification of efficacious adjuvants for sethoxydim and bentazon. *Weed Technol.* 3:60–66.

Received May 1, 2002, and approved March 31, 2003.