ABSTRACT

BURKE, IAN CRISTOFER. Influence of Environmental Factors on Broadleaf Signalgrass (*Brachiaria platyphylla*) and Crowfootgrass (*Dactyloctenium aegyptium*) Germination and Antagonism of Clethodim by CGA 362622 and Imazapic. (Under the direction of Dr. John W. Wilcut)

Laboratory and greenhouse studies were conducted to determine the effect of temperature, solution pH, water stress, and planting depth on broadleaf signalgrass and crowfootgrass germination. Onset, rate, and total broadleaf signalgrass germination (87%) was greatest in alternating 20/30 C temperature regime, while onset, rate, and total crowfootgrass germination (95%) was greatest in an alternating 20/35 C temperature regime. For both species, germination decreased as solution pH increased, with greatest germination occurring at pH 4 and 5. Germination of both species decreased with increasing water potential, and no germination occurred below -0.8 mPa. Germination decreased with burial depth. No seed of either species emerged from 10 cm. Field and greenhouse experiments were conducted to evaluate clethodim applied alone, in mixture and sequential treatments of clethodim plus CGA 362622 or imazapic for control of broadleaf signalgrass, fall panicum, goosegrass, and large crabgrass. Clethodim alone controlled broadleaf signalgrass, goosegrass, fall panicum and large crabgrass >93%, regardless of rate, while CGA 362622 did not control grasses in greenhouse or field experiments. Imazapic provided <82% control of grass weeds. When CGA 362622 or imazapic were applied in mixture with clethodim the effectiveness of the graminicide was reduced for all annual grasses. Clethodim applied 7 d before or after CGA 362622 or imazapic controlled the four grass species as well as clethodim applied alone. Therefore,
while antagonism was variable, it usually occurred when ≤3 d separated applications of clethodim and CGA 362622 or imazapic. CGA 362622 or imazapic did not affect absorption, translocation, or metabolism of 14C-clethodim by goosegrass. When CGA 362622 was applied to goosegrass, fresh weight accumulation stopped for a period of 4 d compared to nontreated plants. Growth resumed 4 d after application of CGA 362622. One day after treatment, the photosynthetic rate in plants treated with CGA 362622 or imazapic was less than non-treated goosegrass. Photosynthetic rate of goosegrass was less for a period of 6 d after treatment with CGA 362622. Goosegrass photosynthetic rate did not recover when treated with imazapic. These data suggest that the antagonism of clethodim by CGA 362622 and imazapic may result from CGA 362622 or imazapic altering the sensitivity of ACCase to clethodim.
INFLUENCE OF ENVIRONMENTAL FACTORS ON BROADLEAF SIGNALGRASS (*Brachiaria platyphylla*) AND CROWFOOTGRASS (*Dactyloctenium aegyptium*) GERMINATION AND ANTAGONISM OF CLETHODIM BY CGA 362622 AND IMAZAPIC

by

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A thesis submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Master of Science

DEPARTMENT OF CROP SCIENCE

Raleigh

2002

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This thesis is dedicated to my parents,

and to the memory of my father,

whose dedication to me, my education, and my development

have provided me with the ability and drive necessary

to succeed in any endeavor I choose.

Susan Canal Burke

in memory of Donald Ray Burke
BIOGRAPHY

Ian Cristofer Burke was born on May 12, 1973, on Hill Air Force Base in Ogden, Utah. His birthday was two days following the launching of Skylab by NASA and three days before the resignation of Richard Nixon. Since his father, Donald R. Burke, held a commission in the United States Air Force, Ian had the opportunity to do a great deal of traveling. After several fitful years in Utah, he endured successive moves to Kansas City, Missouri, Belleville, Illinois, Washington, D. C., Tampa, Florida, a dust mote of an Air Force Base called Hahn in the then Federal Republic of Germany, and Hampton, Virginia. As both his parents were from Louisiana, Ian spent many days mucking around in Yellow Bayou and the swamps of Lake Pontchartrain. He graduated from Poquoson High School in June 1991 and immediately began searching for himself.

That search led him in the fall of that year to the University of South Florida, where three years were wasted in the pursuit of an engineering degree. He was thankful he failed in that pursuit in later years, but at the time it was quite discouraging. His experience of working on golf courses, notably Tampa Palms in Tampa, Florida, influenced his decision to pursue a degree in botany. Ian transferred to Old Dominion University, Virginia, where he met and studied botany under Drs. Lytton Musselman and Rebecca Bray. Dr. Musselman, as an eminent scholar, proved a great inspiration for Ian’s education, and it was at this time, in 1995, that Ian finally began to succeed in his education and endeavors. Ian received a Bachelor’s of Science Degree from Old Dominion University in 1996.
Ian accepted a position with the North Carolina Employment Security Commission in 1997 as grounds keeper for the Commission’s 17 acre campus in Raleigh, North Carolina. His intention was to pursue a higher degree at North Carolina State University. It was in this pursuit that he met Dr. John Wilcut, professor of Weed Science and who eventually became Ian’s graduate sponsor and Master’s advisory chair.

Ian met his soul mate in early 2000 in the person of Adrienne Dawn Bryan, to which he is happily engaged and intends to marry as soon he has an opportunity. Ian has way too many hobbies and interests for his own good, and they include racing and tinkering on his toy, a 1993 Ford Probe GT, cooking, and especially eating.
ACKNOWLEDGEMENTS

I would like to thank my major advisor, Dr. John Wilcut, for his guidance. I would also like to express my sincere thanks to my committee members, Dr. Janet Spears, Dr. Randy Wells, and Dr. David Monks. In addition, I would like to acknowledge the friendships and encouragement of Shawn Askew, Scott Clewis, Andy Price, Wendy Pline, Walter Thomas, and Shawn Troxler.

For their support and encouragement, I express my deepest gratitude to my parents, Donald and Susan Burke. Thanks also to my sister Sara Burke (keep it up!) and my Aunts and Uncles Julie and Buddy Engert, and Dick Canal and Rena Stoplebein. Thanks especially to Adrienne Bryan, whose support and advice in times thick and thin has been and will forever be priceless.
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CHAPTER 1

Literature Review

Studies of germination requirements yield basic ecological information for soil emergence of many weed species (Bhowmik 1997). Such information can be used to characterize the competitiveness and the potential infestation range of the weed as well as enhance management practices, allowing biological, chemical, or mechanical control options to be properly timed (Bhowmik 1997; Dyer 1995; Potter et al. 1984; Wilson 1988). Broadleaf signalgrass [*Brachiaria platyphylla* (Griseb.) Nash] is a serious weed in the southeastern United States (Dowler 1998) where it is widespread and tolerant to some commonly used herbicides (Chamblee et al. 1982a; Gallaher et al. 1999). Yet, despite its importance very little information could be found or is apparently known of its germination and seedling establishment requirements.

In 1973, broadleaf signalgrass was reported in only one county in North Carolina (Radford et al. 1973). By 1978, broadleaf signalgrass was reported in many Coastal Plain counties including all peanut (*Arachis hypogaea* L.) producing counties in North Carolina (Chamblee et al. 1982b). Its infestation now occurs throughout North Carolina especially when corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), peanut, and soybean (*Glycine max* (L.) Merr.) are part of a crop rotation (Johnson and Coble 1986a). Broadleaf signalgrass is the second most common weed in North Carolina cotton, infesting 94,000 hectares (Byrd 2000; Dowler 1998) as well as 46,000 hectares of peanut (Bridges et al. 1994). Broadleaf signalgrass has been listed among the top 10 common and troublesome weeds in broadleaf crops and corn in Alabama, Florida, Kentucky, Louisiana, ...
Mississippi, North Carolina, South Carolina, Tennessee, and Texas (Dowler 1998; Webster 2000). Less than four broadleaf signalgrass plants per 10 m row were predicted to reduced peanut yield (Chamblee et al. 1982b). McGregor et al. (1988) observed that each broadleaf signalgrass plant m⁻² reduced rough rice (Oryza sativa L.) yield 18 kg ha⁻¹.

Broadleaf signalgrass, a native summer annual grass of North America, has reclining 30 to 60 cm long stems that root at the lower nodes. The leaf blades are flat and thick, ranging from 5 to 10 mm wide and 3 to 10 cm long. The inflorescence is a small panicle (hence Brachiaria, Latin brachium for arm) with two to six short racemes, each 4-8 cm long with winged rachis. Spikelets are in two rows on one side of the winged axis. Seeds are generally 3 mm long and finely roughened (Hitchcock and Agnes 1971; Radford et al. 1973). Flowering of broadleaf signalgrass occurs from June to October (Hitchcock and Agnes 1971; Lorenzi and Jeffery 1987; Radford et al. 1973), with seed production reported as a high as 32,140 seed m⁻² and as low as 5,460 seed m⁻², depending on crop rotation – about two percent that of large crabgrass [Digitaria sanguinalis (L.) Scop.] (Johnson and Coble 1986b).

The accession of broadleaf signalgrass as a serious weed competitor was primarily due to its tolerance to widely used herbicides in the late 1970’s and 1980’s, especially alachlor in peanut and later primisulfuron in corn (Chamblee et al. 1982a; Gallaher et. al. 1999). Broadleaf signalgrass competes with a wide variety of successful weedy grasses – johnsongrass [Sorghum halepense (L.) Pers.] in corn, fall panicum [Panicum dichotomiflorum (L.) Michx.] and large crabgrass in cotton, peanut and soybean. A shift in species composition toward broadleaf signalgrass has been recorded in these crops.
under standard herbicide regimes (Johnson and Coble 1986b). Numerically lower densities of broadleaf signalgrass have been noted in crop rotations that included no-till or strip-till production practices (Mueller and Hayes 1997).

Taylorson and Brown (1977) examined the effects of accelerated after-ripening for overcoming the seed dormancy of broadleaf signalgrass and found that accelerated after ripening or light exposure treatments had no effect on broadleaf signalgrass germination. No other seed germination or growth requirement studies have been reported on broadleaf signalgrass. Dormancy in the genus *Brachiaria* is typically overcome by removing the husk, which is made up of the overlapping lemma and palea of the fertile floret (Hopkinson et al. 1996; Renard and Capelle 1976; Whiteman and Mendra 1982).

The genus *Dactyloctenium* is widespread, and member species occur mostly in dry, sandy soils ranging in habitat from meso- to xerophytic (Holm et al. 1979; Watson and Dallwitz 1992). Crowfootgrass [*Dactyloctenium aegyptium* (L.) Willd] is a native of the old world tropics. It occurs as both a weed and an important pasture species (Sharma and Chivinge 1982), and it is the only representative species of the genus in the southeastern United States (Hitchcock and Agnes 1971; Radford et al. 1973). Information about the germination and seedling establishment requirements is available for populations in Africa and India (Kumar et al. 1971; Okusanya and Sonaike 1991; Sharma and Chivinge 1982), but the species has received little attention in the Americas. Crowfootgrass is among the 20 most globally widespread weeds (Holm et al. 1979; Simpson 1990). Crowfootgrass responded to constant temperatures in India, where an optimal constant temperature of 30 C for germination was observed (Gupta 1973; Kumar et al. 1971).
Adaptation to low fertility acid soils is typical of the chloridoid grasses, of which crowfootgrass is a member (Surrey 1986). Interestingly, Buchanan et al. (1975) observed that growth of crowfootgrass was less when grown in soil with pH 5.4 than at pH 6.3. In terms of yield relative to the highest yielding treatment, however, crowfootgrass produced more growth at lower pH than other species observed (Buchanan et al. 1975). Others have noted decreasing germination of crowfootgrass seed with depth in populations from Nigeria and India, and the preponderance of germination occurred at depths of 1.5 cm or less (Kumar et al. 1971; Sharma and Chivinge 1982).

Broadleaf signalgrass, fall panicum, goosegrass [Eleusine indica (L.) Gaertn.], and large crabgrass are among the most common and troublesome grass weeds in U. S. cotton, and are among the most troublesome annual grass weeds in agriculture (Byrd 2000; Dowler 1998). Inadequate control of these weed infestations can reduce cotton yields and cotton fiber quality (Byrd 2000). Typically, these and other grass and broadleaf weeds are present together in cotton fields. For this reason, optimum application timings for selective herbicides having either grass or broadleaf weed activity often coincide.

Clethodim is a graminicide registered on cotton, peanut, and soybean (Anonymous 2001). Clethodim inhibits acetyl-CoA carboxylase (ACCase), thereby blocking fatty acid synthesis and the production of phospholipids used in building new membranes required for cell growth (Ahrens 1994, Burton et al. 1987). CGA 362622 and imazapic inhibit acetolactate synthase (ALS). Plant death results from events occurring in response to
ALS inhibition, which is a key enzyme in the biosynthesis of the branched-chain amino acids isoleucine, leucine, and valine (Ahrens 1994).

CGA 362622 is a sulfonylurea herbicide under development for use in cotton for postemergence (POST) control of broadleaf weeds, particularly sicklepod [Senna obtusifolia (L.) Irwin and Barneby] and common ragweed (Ambrosia artemisiifolia L.) (Hudetz et al. 2000; Wilcut et al. 2000). The effectiveness of clethodim on annual and perennial grass weeds and CGA 362622 on numerous broadleaf weeds make the use of these herbicides applied POST either sequentially or in tank mixtures a likely option for broad spectrum weed control in cotton.

A complex of grass and broadleaf weeds is often prevalent in peanut fields as well. As with cotton, optimum application timings for peanut herbicides having either grass or broadleaf herbicide activity often coincide. The effectiveness of imazapic on grasses, broadleaf weeds, and perennial sedges, and clethodim on annual and perennial grass weeds make the use of these herbicides applied postemergence (POST) either sequentially or in tank mixtures a good option for broad spectrum weed control in peanut. Imazapic is a postemergence herbicide registered in peanut used to control many annual broadleaf weeds and has some activity on small annual and perennial grasses (Wilcut 1996).

However, acetolactate synthesis (ALS) inhibitors such as imidazolinone, pyrimidylbenzoate, and sulfonylurea herbicides including chlorimuron, imazethapyr, pyrithiobac, and thifensulfuron have antagonized cyclohexanedione herbicides (clethodim and sethoxydim) causing a reduction in grass control (Culpepper et al. 1999a;
Chlorimuron, when mixed with clethodim or sethoxydim, reduced control of johnsongrass \([\textit{Sorghum halapense} (\text{L.}) \text{Pers}]\) and barnyardgrass \([\textit{Echinocloa crus-galli} (\text{L.}) \text{P. Beauv.}]\) compared to clethodim or sethoxydim alone, although the reduction in control was inconsistent (Jordan 1995; Vidrine et al. 1995). Chlorimuron did not antagonize broadleaf signalgrass control with clethodim (Jordan 1995; Myers and Coble 1992). Thifensulfuron mixed with sethoxydim reduced control of large crabgrass and giant foxtail \((\textit{Setaria faber} \text{R. Herrm})\) by sethoxydim alone from 95% to 70% (Foy and Witt 1992).

Broadleaf signalgrass control with clethodim was not antagonized by imazethapyr (Myers and Coble 1992) when the two herbicides were applied in mixture. The lack of antagonism with imazethapyr may be the result of imazethapyr also controlling broadleaf signalgrass (87%) when applied alone (Myers and Coble 1992). Large crabgrass control was between 67 and 83% using a mixture of imazethapyr and clethodim, whereas clethodim alone provided >90% control. Control of fall panicum by sethoxydim or clethodim was also antagonized by imazethapyr (Myers and Coble 1992). Myers and Coble reported that control of large crabgrass (50 to 83%) and fall panicum (53 to 80%) was lowest when clethodim was applied 1 d after imazethapyr (1992), although the reduction of control of grass weeds by imazethapyr-clethodim systems was not as great as that for imazapic-clethodim systems.
Several mechanisms have been proposed for graminicide antagonism. Both absorption and translocation have been suggested as possible mechanisms for reduction in grass control by graminicides when applied with broadleaf herbicides (Chow 1975; Culpepper et al. 1999b; Culpepper et al. 1999c; Ferreira et al. 1995; Myers and Coble 1992; Olson and Nalewaja 1982). The metabolic inactivation of herbicides could also be a basis for selectivity, and this mechanism accounts for the resistance of wheat (*Triticum aestivum* L.) to the graminicide diclofop (Shimabukuro et al. 1979). It could also be possible that CGA 362622 stimulates the activity of enzymes involved with herbicide metabolism and therefore increases the detoxification of clethodim (Dean et al. 1990).

Graminicides require actively growing meristematic regions for inhibition of ACCase (Devine et al. 1993). Sethoxydim, a closely related compound to clethodim, rapidly inhibits $^{14}$C-acetate incorporation into lipids in corn root tips, but no inhibition was found in the less metabolically active root regions (Hosaka 1987). Visible symptoms of herbicidal activity are most rapidly and most strongly observed in meristematic regions, and on an ultrastructural level, in the chloroplast (Brezeanu et al. 1976; Chandrasena and Sagar 1987). ALS-inhibiting herbicides like 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 effect of ALS inhibitors on plants is the inhibition of photosynthate transport. Soon after application of an ALS inhibitor, there is an accumulation of neutral sugars in the treated leaves (Bestman et al. 1990), although the herbicides do not affect photosynthesis directly (Shaner and Singh 1997).
Literature Cited


Chapter 2

Influence of environmental factors on broadleaf signalgrass (*Brachiaria platyphylla*) germination.

**Abstract.** Laboratory and greenhouse studies were conducted to determine the effect of temperature, solution pH, water stress, and planting depth on broadleaf signalgrass germination. Broadleaf signalgrass seed required removal of the husk for germination. When treated with constant temperature, broadleaf signalgrass germinated over a range of 20 to 35 C, with the optimum germination occurring at 30 and 35 C. Onset, rate and total germination (87%) was greatest in alternating 20/30 C temperature regime. Germination decreased as solution pH increased, with greatest germination occurring at pH 4 and 5. Germination decreased with increasing water potential, and no germination occurred below -0.8 mPa. Emergence was above 42% when seed were placed on the soil surface or buried at a depth of 0.5 cm. Germination decreased with burial depth, but 10% of broadleaf signalgrass seed emerged from 6.0 cm. No seed emerged from 10 cm. These data suggest that broadleaf signalgrass may emerge later in the season after a precipitation event, and could germinate rapidly and in high numbers. These attributes could contribute to poor control later in the season by soil-applied herbicides, or allow broadleaf signalgrass to emerge after final postemergence treatments were made.

**Nomenclature:** *Brachiaria platyphylla* (Griseb.) Nash #BRAPP, broadleaf signalgrass.

**Key Words:** scarification, solution pH, water stress.
Introduction

Studies of germination requirements yield basic ecological information for soil emergence of many weed species (Bhowmik 1997). Such information can be used to characterize the competitiveness and the potential infestation range of the weed as well as enhance management practices, allowing biological, chemical, or mechanical control options to be properly timed (Bhowmik 1997; Dyer 1995; Potter 1984; Wilson 1988). Broadleaf signalgrass [Brachiaria platyphylla (Griseb.) Nash] is a serious weed in the southeastern United States (Dowler 1998) where it is widespread and tolerant to some commonly used herbicides (Chamblee et al. 1982a; Gallaher et al. 1999;). Yet, despite its importance very little information could be found or is apparently known of its germination and seedling establishment requirements.

In 1973, broadleaf signalgrass was reported in only one county in North Carolina (Radford et al. 1973). By 1978, broadleaf signalgrass was reported in many Coastal Plain counties including all peanut (Arachis hypogaea L.) producing counties in North Carolina (Chamblee et al. 1982b). Its infestation now occurs throughout North Carolina especially when corn (Zea mays L.), cotton (Gossypium hirsutum L.), peanut, and soybean [(Glycine max (L.) Merr.)] are part of a crop rotation (Johnson and Coble 1986a). Broadleaf signalgrass is the second most common weed in North Carolina cotton, infesting 94,000 hectares (Byrd 1999; Dowler 1998) as well as 46,000 hectares of peanut (Bridges et al. 1994). Broadleaf signalgrass has been listed among the top 10 common and troublesome weeds in broadleaf crops and corn in Alabama, Florida, Kentucky, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, and Texas (Dowler 1998;
Webster 2000). Less than four broadleaf signalgrass plants per 10 m row were predicted to reduced peanut yield (Chamblee et al. 1982b). McGregor et al. (1988) observed that each broadleaf signalgrass plant m⁻² reduced rough rice (*Oryza sativa* L.) yield 18 kg ha⁻¹.

Broadleaf signalgrass, a native summer annual grass of North America, has reclining 30-60 cm long stems that root at the lower nodes. The leaf blades are flat and thick, ranging from 5-10 mm wide and 3-10 cm long. The inflorescence is a small panicle (hence *Brachiaria*, Latin *brachium* for arm) with two to six short racemes, each 4-8 cm long with winged rachis. Spikelets are in two rows on one side of the winged axis. Seeds are generally 3 mm long and finely roughened (Hitchcock and Agnes 1971; Radford et al. 1973). Flowering of broadleaf signalgrass occurs from June to October (Hitchcock and Agnes 1971; Lorenzi and Jeffery 1987; Radford et al. 1973), with seed production reported as a high as 32,140 seed m⁻² and as low as 5,460 seed m⁻², depending on crop rotation – about two percent that of large crabgrass [*Digitaria sanguinalis* (L.) Scop.] (Johnson and Coble 1986b).

The emergence of broadleaf signalgrass as a serious weed competitor was primarily due to tolerance of widely used herbicides in the late 1970’s and 1980’s, especially alachlor in peanut and later primisulfuron in corn (Chamblee et al. 1982a; Gallaher et. al. 1999), allowing it to escape herbicide treatments. It competes with a wide variety of successful weedy grasses – johnsongrass [*Sorghum halepense* (L.) Pers.] in corn, fall panicum [*Panicum dichotomiflorum* (L.) Michx.] and large crabgrass in cotton, peanut and soybean. A shift in species composition toward broadleaf signalgrass has been recorded in these crops under standard herbicide regimes (Johnson and Coble 1986b).
Taylorson and Brown (1977) examined the effects of accelerated after-ripening for overcoming the seed dormancy of broadleaf signalgrass and found that accelerated after ripening or light exposure treatments had no effect on broadleaf signalgrass germination. No other seed germination or growth requirement studies have been reported on broadleaf signalgrass.

Therefore, research was initiated to gain an understanding of the germination requirements of this problematic annual grass. The objectives of this research were to determine broadleaf signalgrass germination response to (1) temperature, (2) solution pH, (3) water stress, and (4) planting depth.

**Materials and Methods**

Broadleaf signalgrass seed were harvested from fallow fields near Rocky Mount, NC on August 13, 2000. The seed were allowed to dry to 9% moisture and stored at room temperature until their use in experiments. The seed were sieved to remove any extraneous plant or floral material. The sieved seed were divided in an air column separator\(^1\) and separated into light and heavy fractions. The heavy fraction, the majority of which were fully developed seed, was used in germination and emergence experiments. Seed were tested for viability using 1% tetrazoleum chloride solution prior to each study (Peters 2000).

A randomized complete block design was used for experiments in seed germination chambers. Experiments performed on the gradient table precluded randomization as the zones of temperature were fixed in position (Larson 1971). There were six flasks per
temperature zone on the gradient table and each flask represented one replication.

Studies in seed germination chambers had four replications of treatments, each of which was arranged on a different shelf within the respective seed germination chamber.

Preliminary experiments indicated broadleaf signalgrass germinated independent of light in experiments in growth chambers. Therefore, light was provided for 8 h to coincide with the length of the high temperature component of the temperature regime for all studies conducted in growth chambers. Observations were made during the 8 h light period.

**Effect of Temperature**

The effect of constant temperature was evaluated by evenly spacing twenty broadleaf signalgrass seed in 50 ml erlenmeyer flasks containing three pieces of filter paper and 8 ml of deionized water. The flasks were arranged on a gradient table (Larson 1971) in six lanes corresponding to a constant temperature of 15, 20, 25, 30, 35, and 40 C, with six flasks per lane. Flasks were sealed using parafilm to hold in moisture. Light was provided by florescent overhead bulbs set for a 8 h light 16 h dark regime. Daily germination counts were made for the first 7 d, then every 3 d until no seed germination was observed for 7 continuous days. Each seedling was considered germinated when a visible radicle could be discerned, and was removed from the petri dish (Baskin and Baskin, 1998). The study was conducted twice.

A further study was conducted in growth chambers to determine broadleaf signalgrass response to diurnal temperature. Fifty broadleaf signalgrass seed were evenly spaced in 110 mm diameter by 20 mm petri dishes containing 2 pieces of germination paper and
10 ml of deionized water. Four temperature regimes were selected to reflect typical seasonal variation in North Carolina. The regimes, 10/25, 15/30, 20/30, and 20/35 C, correspond to mean daily low and high temperatures for the months of May, June, July, and August, respectively, in Goldsboro, NC (Owenby and Ezell 1992). These regimes also correspond to a range of effective day and night temperatures for June, July, and August for diverse locations throughout the U. S. (Patterson 1990). The high temperature component of the regime was maintained for 8 h. Daily germination counts were made until no seed germination was observed for 7 continuous days. Each seedling was removed upon germination as previously mentioned. The study was conducted twice.

**Effect of solution pH**

Buffered pH solutions were prepared according to the method described by Gortner (1949), using potassium hydrogen phthalate in combination with either 0.1 M HCl or 0.1 M NaOH to obtain solution pH levels of 4, 5, and 6. A 25 mM borax solution was used in combination with 0.1 M HCl or 0.1 M HCl to prepare solutions with pH levels of 7, 8, or 9. Sets of 25 broadleaf signalgrass seed were placed in petri dishes containing 10 ml of the appropriate pH solution and the petri dishes placed in 10/25, 15/30, 20/30, and 20/35 C germination chambers. Germination was determined by as previously mentioned. The study had four replications of treatments and was conducted twice.

**Effect of Water Stress**

Solutions with water potentials of 0.0, -0.3, -0.4, -0.6, -0.9, and -1.2 mPa were prepared by dissolving 0, 154, 191, 230, 297, or 350 g of polyethylene glycol5 (PEG) in 1 L of deionized water (Michel 1983). Twenty-five broadleaf signalgrass seed were placed
in petri dishes containing 10 ml of PEG solution and the petri dishes placed in 10/25, 15/30, 20/30, and 20/35 C germination chambers. Germination was determined as previously mentioned. The study had four replications of treatments and was conducted twice.

**Depth of Emergence**

A depth of emergence study was conducted in a glasshouse at an average daily temperature of 33 ± 5 C and a nightly temperature of 23 ± 5 C. Natural light supplemented with fluorescent lamps at a light intensity of 300 ± 20 µEm²s⁻¹ were used to extend light duration to 14 h in glasshouse studies and simulate field conditions. The depth of emergence study included four replications of treatments in a randomized complete block design. The study was conducted three times.

Containers were filled to a depth of 10 cm with a sandy loam soil sifted with a #306 sieve. Using sieved soil facilitated the recovery of any non-germinated seed. Twenty broadleaf signalgrass seed were placed on the soil surface or covered to depths of 0.5, 1.0, 2.0, 4.0, 6.0, and 10.0 cm with the same soil. Pots were sub-irrigated initially to field capacity, and then surface irrigated daily to field capacity. Emergence counts were recorded daily for the first 7 d, then every 3 d until no seed germination was observed for 7 continuous days. Plants were considered emerged when a cotyledon could be visibly discerned. The soil was placed in a root washer on a #30 sieve and the soil removed to recover and quantify any non-germinated seed.
**Statistical Analysis**

Germination resulting from constant temperature was described by a parabolic model of the form:

\[ y = \beta_0 + \beta_1 \text{temp} + \beta_2 \text{temp}^2 \]  \[1\]

where \( \beta_0, \beta_1, \) and \( \beta_2 \) are the intercept, first and second order regression coefficients, respectively, and \( y \) is the cumulative germination at temperature \( \text{temp} \).

Germination resulting from alternating temperatures was modeled using the logistic function:

\[ y = M \left[ 1 + \exp(-K(t - L)) \right]^{-1} \]  \[2\]

where \( y \) is the cumulative percentage germination at time \( t \), \( M \) is the asymptote or theoretical maximum for \( y \), \( L \) is the time scale constant or lag to onset of germination, and \( K \) is the rate of increase (Rochd et al. 1997). Estimation used the Gauss-Newton algorithm, a nonlinear least squares technique (SAS 1998).

Depth of emergence data were subjected to an analysis of variance using the general linear models procedure provided with SAS (SAS 1998). Sums of squares were partitioned to evaluate planting depth and trial repetition. Emergence was described by a model of the form:

\[ y = mx + b \]  \[3\]

where \( m \) is the decrease in percent emergence with unit increase in depth, \( b \) is the intercept, and \( y \) is the cumulative emergence at burial depth \( x \).
Results and Discussion

Broadleaf signalgrass seed tested 92% viable by tetrazolem chloride (TZ) tests (Peters 2000) before each study was conducted (data not shown). The seed exhibited innate dormancy and initial experiments on seed with the husk in place resulted in maximum germination of 9% (data not shown). Dormancy in the genus Brachiaria is typically overcome by removing the husk, which is made up of the overlapping lemma and palea of the fertile floret (Hopkinson et al. 1996; Renard and Capelle 1976; Whiteman and Mendra 1982). Removal of the husk resulted in increased germination, and this procedure was used in all subsequent experiments. Others have noted numerically lower densities of broadleaf signalgrass in crop rotations that included no-till or strip-till production practices (Mueller and Hayes 1997), which suggests that broadleaf signalgrass seed may require mechanical action to break or remove the husk for germination to occur in the field.

Temperature

Broadleaf signalgrass germination was influenced by constant temperature. When exposed to constant temperature, broadleaf signalgrass seed germinated over a temperature range of 20 to 35 C (Figure 1). Broadleaf signalgrass seed did not germinate when placed in a constant temperature of 15 C. Constant temperature resulted in a maximum germination of 35% at 30 and 35 C.

Onset, rate, and maximum germination varied with alternating temperature regimes (Figure 2), and analysis of variance indicated a significant temperature regime by germination interaction, so broadleaf signalgrass germination is presented for each
temperature regime. Maximum cumulative germination (87%) of broadleaf signalgrass occurred when seeds were exposed to 20/30 C. The germination rate (parameter $K$) produced by the 20/30 C regime was lower than the rates at 15/30 and 20/35 C regimes. However, these differences are most likely related to differences in final germination percentages. If one considers how many seed germinated in the first 48 h, there were considerably more seedlings started at 20/30 C than any other temperature regime. The time to 50% cumulative seed germination was fastest in the 20/30 C regime at 1.4 d, and could be caused by the 10 degree difference in the temperature components of the 20/30 C regime. Time to 50% germination in the 15/30 and 20/35 C regimes were 1.7 and 1.5 d, respectively, and both regimes had a 15 degree difference between their respective temperature components. Total % germination was lowest in the 10/25 C regime. The lower cumulative germination at low temperature could indicate that broadleaf signalgrass germinates later in the warmer portion of the growing season which would include June, July and August.

It is interesting to note that when germinated at constant temperature, the seeds used in this experiment germinated equally as well at 30 and 35 C (Figure 1). However, there was a substantial difference in maximum germination when seeds were exposed to 20/30 or 20/35 alternating temperatures (Figure 2). The preference for alternating temperatures, therefore, could be associated with the spread between maximum and minimum temperatures, 10 verses 15 C for 20/30 and 20/35 C respectively, in addition to the absolute maximum and minimum temperature.
**Response to solution pH**

Analysis of variance indicated a significant temperature regime by solution pH interaction, so broadleaf signalgrass germination is presented for each solution pH within each temperature regime. The 20/30 C regime produced the greatest amount of cumulative germination at each solution pH level (Figure 5). No germination of seed was observed when exposed to a solution pH of 8 or 9 in the 10/25 and 15/30 C temperature regimes (Figure 3,4), or a solution pH of 9 in the 20/30 or 20/35 C regimes (Figure 5,6). The order of cumulative germination for each pH solution, from highest to lowest, was similar in the 15/30, 20/30, and 20/35 C temperature regimes (Figure 4,5,6). In the 10/25 C regime, more broadleaf signalgrass seed germinated at solution pH 5 than solution pH 4 (Figure 3). Cumulative broadleaf signalgrass seed germination was greater and time to 50% germination lower at solution pH 4 and 5 than at all other solution pHs in all temperature regimes. Maximum cumulative germination decreased as solution pH increased, indicating that broadleaf signalgrass germination is sensitive to changes in solution pH. These data suggest that broadleaf signalgrass prefers acidic soil conditions, which are common throughout the major crop production regions of the North Carolina Piedmont and Coastal Plain (Tucker et al. 1997). Adaptation to acid soils is typical of the genus *Brachiaria*, which has its center of diversity in Africa (Rao et al. 1996; Parsons 1972).

**Response to Water Stress**

Analysis of variance indicated a significant temperature regime by water stress treatment interaction, so broadleaf signalgrass germination is presented for each water
stress treatment within each temperature regime. As water stress increased, cumulative broadleaf signalgrass seed germination decreased (Figures 7,8,9,10). No germination occurred when the water potential was -1.2, regardless of the germination temperature (data not shown). Broadleaf signalgrass seed germinated in a water potential of –0.8 mPa only in the 15/30 regime (Figure 8). When the water potential was 0.0 (seed in deionized water), maximum germination (87%) occurred in the 20/30 C temperature regime (Figure 9). Placing seeds in water stress situations delayed the onset of germination in all temperature regimes, causing the time to 50% germination (L) to increase for -0.3 mPa and -0.4 mPa compared to 0.0 mPa (Figure 7,8,9,10). Although imbibition is a complicated process, most likely the osmotic gradient was overcome by a greater concentration of solutes within the seed than without, and consequently water was able to move into the seed (Baskin and Baskin 1998). The requirement for low water stress also suggests that broadleaf signalgrass is dependent upon irrigation or a precipitation event for germination in the field.

**Depth of Emergence**

Emergence of broadleaf signalgrass decreased with increased planting depth, with the numerical maximum of 48% occurring at 14 DAP from the 0.5 cm planting depth (Figure 11). There was no difference in germination of seeds planted on the surface, at 0.5 or 1.0 cm depth. No viable seed were recovered from the soil after 14 d. Emergence in soil systems depends, in part, on seed size. Larger seed with greater carbohydrate reserves can emerge from greater depths of burial (Baskin and Baskin 1998). Broadleaf signalgrass possesses a similar seed size, at 3 mm in length, compared with other grasses
such as giant foxtail (*Setaria faberi* Herrm.) (2 to 2.5 mm long) or fall panicum (2 to 2.5 mm long) (Hitchcock and Agnes 1971; Radford et al. 1973). Broadleaf signalgrass percent emergence was similar to fall panicum and giant foxtail at depths of 0 and 1.0 cm and less than either deeper than 1.0 cm (Fausey and Renner 1997). Both fall panicum and giant foxtail germinated from a depth of 7.5 cm (Fausey and Renner 1997). Broadleaf signalgrass germinated to a depth of 6 cm, but no germination was observed from a depth of 10 cm (Figure 11). Emergence from depths of up to 6 cm could allow broadleaf signalgrass to escape control from preplant-incorporated herbicides.

Broadleaf signalgrass seed did not tolerate water stress and required warm alternating temperatures, burial depths 2.0 cm or less, and an acidic solution pH for maximum germination. These data suggest that broadleaf signalgrass may emerge throughout the season from mid-May through mid-September and germination is likely triggered by a precipitation event. Furthermore, if conditions are favorable, broadleaf signalgrass will germinate rapidly and in high numbers. These attributes could contribute to poor control later in the season by soil-applied herbicides as they degrade in the soil. Additionally, high weed densities have been shown to decrease herbicide efficacy (Doub et al. 1988; Hartzler and Roth 1993). Emergence after final postemergence herbicide applications or from greater depths could also contribute to a lack of season-long control in many weed management programs (Chamblee et al. 1982a; Mueller and Hayes 1997).

Since the seed require scarification for germination, soil disturbance in conventional tillage systems could increase broadleaf signalgrass emergence and therefore deplete the seedbank. Other grass weeds with similar attributes (rapid high percentage germination),
such as woolly cupgrass *Eriochloa villosa* (Thunb.) Kunth) and giant foxtail have been shown to decline rapidly from the seedbank (Buhler and Hartzler 2001). A weed control system that took advantage of the seed depletion and controlled late season weed escapes might deplete the soil of broadleaf signalgrass seed over several seasons. Use of a no-tillage cropping system also could reduce broadleaf signalgrass germination. These attributes, coupled with reported tolerance to commonly used herbicides in corn, cotton, and peanut such as alachlor, pendimethalin and primisulfuron (Chamblee et al. 1982a; Gallaher 1999; Mueller and Hayes 1997), should be taken into account when managing for broadleaf signalgrass.

*Sources of Materials*

1. Seed Blower, Seedburo Equipment Company. 1022 W. Jackson Blvd., Chicago, IL 60607.
2. 9.0 cm germination paper, Anchor Paper Company, 480 Broadway, St. Paul, MN 55165-0648.
5. PEG 8000, Sigma Chemicals, P. O. Box 14508, St. Louis, MO 63178.
Literature Cited


Agronomy, and Improvement. Cali, Colombia: International Center for Tropical Agriculture.


Figure 1. Influence of constant temperature on cumulative germination of broadleaf signalgrass at 14 d as described by the equation $y = \beta_0 + \beta_1\text{temp} + \beta_2\text{temp}^2$. 

\[ y = -249.5 + 18.5(\text{temp}) - 0.3(\text{temp})^2 \]

$R^2 = 0.93$
Figure 2. Influence of four temperature regimes on broadleaf signalgrass germination, modeled using the equation $y = M \left[1 + \exp(-K(t - L))\right]^{-1}$. Light was provided for 8 h and coincided with the high temperature portion of the regime, which also had an 8 h duration.
Figure 3. Influence of solution pH on broadleaf signalgrass germination under a 10–25 C temperature regime, modeled using the equation $y = M \left[ 1 + \exp(-K(t - L)) \right]^{-1}$. Light was provided for 8 h and coincided with the high temperature portion of the regime, which also had an 8 h duration.
Influence of solution pH on broadleaf signalgrass germination under a 15 – 30 C temperature regime, modeled using the equation $y = M \left[1 + \exp(-K(t - L))\right]^{-1}$.

Figure 4. Influence of solution pH on broadleaf signalgrass germination under a 15 – 30 C temperature regime, modeled using the equation $y = M \left[1 + \exp(-K(t - L))\right]^{-1}$. 

- pH 4: $M=55.6, L=3.3, K=1.97$
- pH 5: $M=46.7, L=3.1, K=2.47$
- pH 6: $M=35.9, L=3.6, K=2.81$
- pH 7: $M=19.9, L=5.2, K=3.50$
Figure 5. Influence of solution pH on broadleaf signalgrass germination under a 20 – 30 C temperature regime, modeled using the equation $y = M \left[ 1 + \exp(-K(t - L)) \right]^{-1}$. 

20/30 C Regime
- pH 4
  - $M=56.4, L=2.5, K=1.96$
- pH 5
  - $M=48.9, L=2.8, K=1.61$
- pH 6
  - $M=29.8, L=3.3, K=1.40$
- pH 7
  - $M=21.7, L=3.6, K=2.16$
- pH 8
  - $M=14.7, L=2.9, K=2.96$
Figure 6. Influence of solution pH on broadleaf signalgrass germination under a 20 – 35 C temperature regime, modeled using the equation $y = M \left[1 + \exp(-K(t - L))\right]^{-1}$. 
Figure 7. Influence of water stress on broadleaf signalgrass germination under a 10 – 25 C temperature regime, modeled using the equation $y = M \left[1 + \exp(-K(t - L))\right]^{-1}$. Light was provided for 8 h and coincided with the high temperature portion of the regime, which also had an 8 h duration.
Figure 8. Influence of water stress on broadleaf signalgrass germination under a 15 – 30 C temperature regime, modeled using the equation $y = M \frac{1 + \exp(-K(t - L))}{1}$.
Figure 9. Influence of water stress on broadleaf signalgrass germination under a 20 – 30 C temperature regime, modeled using the equation $y = M \left[ 1 + \exp(-K(t - L)) \right]^{-1}$. 

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\text{Time (d)} & \text{Germination (%)} & \text{-0.3 mPa} & \text{-0.4 mPa} \\
\hline
0 & 0 & M=33.0, L=3.7, K=3.85 & M=7.9, L=4.1, K=3.15 \\
1 & 0 &蓝色 M=87.1, L=2.3, K=3.07 & \\
2 & & & \\
3 & & & \\
4 & & & \\
5 & & & \\
6 & & & \\
7 & & & \\
\hline
\end{tabular}
\end{table}
Figure 10. Influence of water stress on broadleaf signalgrass germination under a 20 – 35 C temperature regime, modeled using the equation $y = M \left[1 + \exp\left(-K(t - L)\right)\right]^{-1}$. 

Table: 

<table>
<thead>
<tr>
<th>Regime</th>
<th>$M$</th>
<th>$L$</th>
<th>$K$</th>
</tr>
</thead>
<tbody>
<tr>
<td>20/35 C Regime</td>
<td>0.0 mPa</td>
<td>70.6</td>
<td>1.5</td>
</tr>
<tr>
<td>-0.4 mPa</td>
<td>10.1</td>
<td>2.7</td>
<td>2.97</td>
</tr>
<tr>
<td>-0.3 mPa</td>
<td>25.6</td>
<td>2.1</td>
<td>5.22</td>
</tr>
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Germination (%) | Time (d)
Figure 11. Cumulative emergence of broadleaf signalgrass seed buried 0, 0.5, 1, 2, 4, and 6 cm 7 and 14 days after planting (DAP).

14 DAP: $y = -5.84x + 45.1$
$R^2 = 0.93$

7 DAP: $y = -1.6x + 7.6$
$R^2 = 0.56$
CHAPTER 3

Influence of environmental factors on crowfootgrass (*Dactylolctenium aegyptium*) germination.

**Abstract.** Laboratory and greenhouse studies were conducted to determine the effect of temperature, pH, water stress, and planting depth on crowfootgrass germination. When treated with constant temperature, crowfootgrass germinated over a range of 15 to 40 C, with the optimum germination occurring at 30 C. Onset, rate, and total germination (93%) were greatest in an alternating 20/35 C temperature regime. Germination decreased as pH increased, with greatest germination occurring at pH 4 and 5. Germination was reduced when seed was subjected to water stress, and no germination occurred below -0.8 mPa. Emergence was similar when seed were placed on the soil surface or buried at depths of 0.5 or 1.0 cm. Germination decreased with burial depth and no seed emerged from 10 cm. These data suggest that crowfootgrass may emerge later in the season with warmer temperatures and after a precipitation event, and may germinate rapidly and in high numbers. These attributes could contribute to poor control later in the season by soil-applied herbicides, or allow crowfootgrass to emerge after final postemergence treatments are made.

*Nomenclature: Dactylolctenium aegyptium* (L.) Willd. # DTTAE, crowfootgrass.

*Key Words: pH, scarification, water stress, weed biology.*
Introduction

The genus *Dactyloctenium* is widespread, and member species occur mostly in dry, sandy soils ranging in habitat from meso- to xerophytic (Holm et al. 1979; Watson and Dallwitz 1992). Crowfootgrass [*Dactyloctenium aegyptium* (L.) Willd] is a native of the old world tropics, and is among the 20 most globally widespread weeds (Holm et al. 1979; Simpson 1990). It occurs as both a weed and an important pasture species (Sharma and Chivinge 1982), and it is the only widespread representative species of the genus in the southeastern United States (Hitchcock and Agnes 1971; Radford et al. 1973). Information about the germination and seedling establishment requirements is available for populations in Africa and India (Kumar et al. 1971; Okusanya and Sonaike 1991; Sharma and Chivinge 1982). The species has received little attention in the Americas apart from its inclusion in after-ripening studies (Taylorson and Brown 1977).

Studies of germination and seedling establishment requirements yield basic ecological information for soil emergence (Bhowmik 1997). Such information can be used to characterize the competitiveness and the potential infestation range of the weed as well as enhance management practices, allowing biological, chemical, or mechanical control options to be properly timed (Bhowmik 1997; Dyer 1995; Potter et al. 1984; Wilson 1988). Therefore, research was initiated to gain an understanding of the germination requirements of this problematic annual grass. The objectives of this research were to determine crowfootgrass germination response to temperature, solution pH, water stress, and planting depth.
Methods and Materials

Crowfootgrass seed was harvested from fallow fields near Clayton, NC on September 3, 2000. The seed were allowed to dry to 11% moisture and stored at room temperature until their use in experiments. The seed were sieved to remove any extraneous plant or floral material. The sieved seed were further cleaned in an air column separator and tested for viability using 1% tetrazoleum chloride solution prior to each trial (Peters 2000). Crowfootgrass seed tested 93 ± 4% viable by tetrazoleum chloride tests before each study was conducted.

A randomized complete block design was used for experiments in seed germination chambers. Experiments performed on the gradient table precluded randomization as the zones of temperature were fixed in position (Larson 1971). There were six flasks per temperature zone on the gradient table, and each flask represented one replication. Studies in seed germination chambers had four replications of treatments, each of which was arranged on a different shelf within the respective germination chamber.

Preliminary studies and previous reports (Kumar et al. 1971; Taylorson and Brown 1977) indicated crowfootgrass germination was dependent on light in studies in germination chambers. Therefore, light was provided for 8 h to coincide with the length of the high temperature component of the temperature regime for all studies conducted in germination chambers. Observations were made during the 8 h light period.

Crowfootgrass seed reportedly required a considerable period of storage or after-ripening before dormancy was relieved (Gupta 1973). Consequently, seed were stored at room temperature (28 C) for a period of 6 months before experimentation was begun. It
was also necessary to remove the pericarp prior to germination experiments as greater germination was observed without it in place (Gupta 1973).

**Effect of Temperature**

The effect of constant temperature was evaluated by evenly spacing twenty crowfootgrass seed in 25 ml erlenmeyer flasks containing three pieces of filter paper and 8 ml of deionized water. The flasks were arranged on a gradient table (Larson 1971) in six lanes corresponding to a constant temperature of 15, 20, 25, 30, 35, and 40 C, with six flasks per lane. Flasks were sealed using parafilm to hold in moisture. Light was provided by florescent overhead bulbs set for an 8 h light 16 h dark regime. Daily germination counts were made for the first 7 d, then every 3 d until no seed germination was observed for two observations. Each seedling was removed when a visible radicle could be discerned (Baskin and Baskin 1998). The study was conducted twice.

A further study was conducted in growth chambers to determine crowfootgrass response to diurnal temperature. Twenty-five crowfootgrass seed were evenly spaced in 110 mm diameter by 20 mm petri dishes containing 2 pieces of germination paper and 10 ml of deionized water. Four temperature regimes were selected to reflect typical seasonal variation in North Carolina. The regimes, 10/25, 15/30, 20/30, and 20/35 C, correspond to mean daily low and high temperatures for the months of May, June, July, and August, respectively, in Goldsboro, NC (Owenby and Ezell 1992). These regimes also correspond to a range of effective day and night temperatures for June, July, and August for diverse locations throughout the U. S. (Patterson 1990). The high temperature component of the regime was maintained for 8 h. Daily germination counts were made
for 7 d, then every 3 d until no seed germination was observed for 7 continuous days. Each seedling was removed upon germination as previously mentioned. The study was conducted twice.

**Effect of Solution pH**

Buffered pH solutions were prepared according to the method described by Gortner (1949), using potassium hydrogen phthalate in combination with either 0.1 M HCl or 0.1 M NaOH to obtain solution pH levels of 4, 5, and 6. A 25 mM borax solution was used in combination with 0.1 M HCl or 0.1 M HCl to prepare solutions with pH levels of 7, 8, or 9. Sets of 25 crowfootgrass seed were placed in petri dishes containing 10 ml of the appropriate pH solution and the petri dishes placed in 10/25, 15/30, 20/30, and 20/35 C germination chambers. Germination was determined as previously mentioned. The study had four replications of treatments and was conducted twice.

**Effect of Water Stress**

Solutions with water potentials of 0.0, -0.3, -0.4, -0.6, -0.9, and -1.2 mPa were prepared by dissolving 0, 154, 191, 230, 297, or 350 g of polyethylene glycol (PEG) in 1 L of deionized water (Michel 1983). Twenty-five crowfootgrass seed were placed in petri dishes containing 10 ml of PEG solution and the petri dishes placed in 10/25, 15/30, 20/30, and 20/35 C germination chambers. Germination was determined as previously mentioned. The study had four replications of treatments and was conducted twice.

**Depth of Emergence**

A depth of emergence study was conducted in a glasshouse at an average daily temperature of 33 ± 5 C and a nightly temperature of 23 ± 5 C. Natural light
supplemented with fluorescent lamps at a light intensity of 300 ± 20 µEm⁻²s⁻¹ was used to extend the daylength to 14 h in the glasshouse study and to simulate field conditions. The experimental design was a randomized complete block with treatments replicated four times. The study was conducted three times.

Containers were filled to a depth of 10 cm with a Norfolk loamy sand soil (fine-loamy, siliceous, thermic, Typic Paleudults). Twenty crowfootgrass seed were placed on the soil surface or covered to depths of 0.5, 1.0, 2.0, 4.0, 6.0, and 10.0 cm with the same soil. Pots were sub-irrigated initially to field capacity, and then surface irrigated daily to field capacity. Emergence counts were recorded daily for the first 7 d, then every 3 d until no seed germination was observed for 7 continuous days. Plants were considered emerged when a cotyledon could be visibly discerned. The study was conducted three times.

**Statistical Analysis**

Germination resulting from constant temperature treatments was described by a parabolic model of the form:

\[
y = \beta_0 + \beta_1 \text{temp} + \beta_2 \text{temp}^2 \tag{1}
\]

where \( \beta_0, \beta_1, \) and \( \beta_2 \) are the intercept, first and second order regression coefficients, respectively, and \( y \) is the cumulative germination at temperature \( \text{temp} \).

Germination resulting from alternating temperature treatments, solution pH treatments, and water potential treatments were modeled using the logistic function:

\[
y = M \left[ 1 + \exp(-K(t - L)) \right]^{-1} \tag{2}
\]

where \( y \) is the cumulative percentage germination at time \( t \), \( M \) is the asymptote or theoretical maximum for \( y \), \( L \) is the time scale constant or lag to onset of germination, and
$K$ is the rate of increase (Roché et al. 1997). Estimation used the Gauss-Newton algorithm, a nonlinear least squares technique (SAS 1998).

Depth of emergence data were subjected to an analysis of variance using the general linear models procedure provided with SAS (SAS 1998). Sums of squares were partitioned to evaluate planting depth and trial repetition. Emergence was described by a model of the form:

$$y = mx + b$$ [3]

where $m$ is the decrease in percent emergence with unit increase in depth, $b$ is the intercept, and $y$ is the cumulative emergence at burial depth $x$.

**Results and Discussion**

**Effect of Temperature**

Crowfootgrass germination was influenced by temperature. When exposed to constant temperature, crowfootgrass seed germinated over a temperature range of 15 to 40 C (Figure 1). Constant temperature resulted in a maximum germination of 43% at 30 C. Our data supports that reported by Gupta (1973) and Kumar et al. (1971), who reported an optimal constant temperature of 30 C for crowfootgrass seed germination (Gupta 1973; Kumar et al. 1971).

Analysis of variance indicated a significant temperature regime by germination interaction, so crowfootgrass germination is presented for each temperature regime (Figure 2). Maximum cumulative germination (94%) of crowfootgrass occurred when seed were exposed to a 20/35 C regime and was lowest at 10/25 C. The germination rate
(parameter $K$) produced by the 20/35 C regime was similar to the rates at 15/30 and 20/35 C regimes and the time to 50% germination in 15/30, 20/30, and 20/35 C temperature regimes was 2.0 d. The lower and slower germination at reduced temperatures would indicate that crowfootgrass germinates in the warmer portion of the growing season which would include June, July and August. Goosegrass $[Eleusine indica \text{ (L.) Gaertn.}]$, a closely related species, also requires warm fluctuating temperatures for maximum germination (Nishimoto and McCarty 1997). The preference for warm fluctuating temperatures may be the cause of both crowfootgrass and goosegrass emergence from depths of 1.0 cm or less (Adu et al. 1994; Gupta 1973; Nishimoto and McCarty 1997; Sharma and Chivinge 1982), where the greatest diurnal fluctuations and also the warmest temperatures would be expected.

**Response to Solution pH**

Analysis of variance indicated a significant main effect of solution pH treatment, so crowfootgrass germination is presented by solution pH treatment averaged over temperature regimes. Crowfootgrass seed had the highest cumulative germination at solution pH of 4, and cumulative germination decreased with increasing pH (Figure 3). Cumulative seed germination and rate ($K$) was greater at solution pH 4 and 5 than at all other solution pHs, indicating that crowfootgrass germination is sensitive to changes in solution pH. Germination for each solution pH began within 2 to 6.3 d of exposure of seed to the treatment solution. These data suggest that crowfootgrass seed germinate more readily in acidic soil conditions, which are common throughout the major crop production regions of the North Carolina Piedmont and Coastal Plain (Tucker et al. 1994; Nishimoto and McCarty 1997; Adu et al. 1994; Sharma and Chivinge 1982).
Adaptation to acid soils is typical of the chloridoid grasses, of which crowfootgrass is a member (Surrey 1986). Interestingly, Buchanan et al. (1975) observed that growth of crowfootgrass was less when grown in soil with pH 5.4 than at pH 6.3. In terms of yield relative to the treatment yielding the highest biomass, however, the decrease in biomass at lower pH was less for crowfootgrass than for other species studied (Buchanan et al. 1975).

**Response to Water Stress**

Analysis of variance indicated a significant main effect of water stress treatment, thus crowfootgrass germination is presented by water stress treatment averaged over temperature regime. As water stress increased, cumulative crowfootgrass seed germination decreased (Figure 4). No germination occurred when the water potential was −0.8 or -1.2, regardless of the germination temperature. When the water potential was 0.0 (seed in deionized water), maximum germination was 85% averaged across the four temperature regimes. Placing seeds in water stress situations delayed the onset of germination, causing the time to 50% germination \( (L) \) to increase for -0.3 mPa, -0.4 mPa, and -0.6 mPa compared to 0.0 mPa (Figure 4). Although imbibition is a complicated process, most likely the osmotic gradient was overcome by a greater concentration of solutes within the seed than without, and consequently water was able to move into the seed (Baskin and Baskin 1998). The requirement for low water stress suggests that crowfootgrass is dependent upon a precipitation or an irrigation event for germination in the field.
**Depth of Emergence**

Emergence of crowfootgrass decreased with increased planting depth, with the numerical maximum of 40% occurring at 14 DAP from the 1.0 cm planting depth (Figure 5). Seven days after planting crowfootgrass emergence was greater from burial depths of 0.5 and 1.0 cm. Emergence was similar when seeds were planted on the surface, at 0.5 or 1.0 cm depths 14 d after planting. Emergence on the surface or from a depth of 4.0 cm increased from 7 to 14 d after planting. The increasing seed emergence over time for seed sown on the surface may be due to the duration of time water was available for imbibition after an irrigation event. Delayed emergence from a depth of 4.0 cm may be due to the larger distance to extend the coleoptile to the soil surface. Others have noted decreasing emergence of crowfootgrass seed with increasing burial depth in populations from Nigeria and India, and the preponderance of emergence occurred at burial depths of 1.5 cm or less (Kumar et al. 1971; Sharma and Chivinge 1982).

Larger seed with greater carbohydrate reserves can emerge from greater depths of burial (Baskin and Baskin 1998). Crowfootgrass seed is 0.7-1.0 mm in diameter. Broadleaf signalgrass seed are relatively larger, at 3 mm in length, as do other grasses such as giant foxtail (*Setaria faber* Herrm.) (2 to 2.5 mm long) or fall panicum (*Panicum dichotomiflorum* (L.) Michx.) (2 to 2.5 mm long) (Hitchcock and Agnes 1971; Radford et al. 1973). Crowfootgrass percent emergence was similar to broadleaf signalgrass, fall panicum, and giant foxtail at depths of 0 and 1.0 cm and less than all three when buried deeper than 2.0 cm (Fausey and Renner 1997). Both fall panicum and giant foxtail germinated from a depth of 7.5 cm (Fausey and Renner 1997), while broadleaf
signalgrass germinated to a depth of 6 cm, but no germination was observed from a depth of 10 cm (Burke et al. 2002). Only 3% of crowfootgrass seed emerged from a planting depth of 6 cm 14 d after planting (Figure 5).

Crowfootgrass did not tolerate water stress and required warm alternating temperatures, burial depths of 0.0 - 1.0 cm, and an acidic solution pH for maximum germination. These data suggest that crowfootgrass may emerge throughout the season in the months of June, July, and August and germination is likely triggered by a precipitation event. Furthermore, if conditions are right, crowfootgrass will germinate rapidly and in high numbers. The preference for fluctuating temperatures and shallow burial depths may indicate a preference for crowfootgrass to emerge on disturbed bare ground (Adu et al. 1994; Gupta 1973; Nishimoto and McCarty 1997; Sharma and Chivinge 1982), where the greatest diurnal fluctuations would be expected. Shallow cultivation for weed control could potentially stimulate germination by placing crowfootgrass seed at the optimum depth for germination. The aforementioned attributes may also contribute to poor control later in the season by soil-applied herbicides as they degrade in the soil. Additionally, high weed densities have been shown to decrease herbicide efficacy (Doub et al. 1988; Hartzler and Roth 1993). Emergence after final postemergence herbicide applications may also contribute to a lack of season-long control in many weed management programs (Prostko et al. 2001).

Other grass weeds with similar attributes (rapid high percentage germination), such as woolly cupgrass [Eriochloa villosa (Thunb.) Kunth] and giant foxtail have been shown to decline rapidly from the seedbank (Buhler and Hartzler 2001). A weed control system
that took advantage of the seed depletion and controlled late season weed escapes might
deplete the soil of crowfootgrass seed in several seasons. These attributes should be
taken into account when managing for crowfootgrass.

Sources of Materials

1. Seed Blower, Seedburo Equipment Company. 1022 W. Jackson Blvd., Chicago, IL 60607.

2. 9.0 cm germination paper, Anchor Paper Company, 480 Broadway, St. Paul, MN 55165-0648.


5. PEG 8000, Sigma Chemicals, P. O. Box 14508, St. Louis, MO 63178.
Literature Cited


Figure 1. Influence of constant temperature on cumulative germination of crowfootgrass at 8 d as described by the equation

\[ y = -61.3 + 6.9(\text{temp}) - 0.12(\text{temp})^2 \]

\[ R^2 = 0.81 \]
Figure 2. Influence of four temperature regimes on crowfootgrass germination, modeled using equation $y = M \left[ 1 + \exp(-K(t - L)) \right]^{-1}$. 

- 20/30 C: $M=86.3$, $L=2.0$, $K=20.40$
- 10/25 C: $M=67.8$, $L=3.9$, $K=1.32$
- 15/30 C: $M=86.3$, $L=2.0$, $K=20.40$
- 20/35 C: $M=94.7$, $L=2.0$, $K=19.51$
- 20/30 C: $M=86.6$, $L=2.0$, $K=19.65$
Figure 3. Influence of solution pH on crowfootgrass germination averaged across temperature regimes, modeled using the equation $y = M \left[ 1 + \exp(-K(t - L)) \right]^{-1}$. 
Figure 4. Influence of water stress on crowfootgrass germination averaged across temperature regimes, modeled using the equation $y = M \left[ 1 + \exp(-K(t - L)) \right]^{-1}$. 

Graph shows germination percentage (%) against time (d) for different water stress levels: 0.0 mPa (solid line, M=81.3, L=2.0, K=19.8), -0.3 mPa (dashed line, M=57.3, L=2.0, K=19.6), -0.4 mPa (dotted line, M=16.9, L=4.1, K=1.3), -0.6 mPa (dashed-dotted line, M=2.5, L=5.5, K=0.67).
Figure 5. Cumulative emergence of crowfootgrass seed buried 0, 0.5, 1, 2, 4, and 6 cm 7 and 14 days after planting (DAP).
CHAPTER 4

**CGA 362622 antagonizes annual grass control with clethodim.**

**Abstract.** Field and greenhouse experiments were conducted to evaluate clethodim, CGA 362622, mixtures thereof, and sequential treatments for control of broadleaf signalgrass, fall panicum, goosegrass, and large crabgrass. In greenhouse experiments, clethodim alone provided 93 and 100% control of 3 to 4 leaf goosegrass at the low (105 g ai/ha) and high (140 g/ha) rates, respectively, while CGA 362622 did not control grasses in greenhouse or field experiments. Control of 6 to 8 leaf goosegrass in the greenhouse with clethodim was 75% for the low rate and 89% for the high rate. Control of goosegrass in greenhouse studies was reduced at least 43 percentage points with CGA 362622 and clethodim at the high rate in mixture compared to control provided by clethodim at the high rate alone. When CGA 362622 and clethodim were applied in mixture in field studies, the effectiveness of the graminicide was decreased from >97% to <57% control for all annual grasses. Antagonism of clethodim activity was greater than that of the tank mixture when clethodim was applied 1 d after CGA 362622 on large crabgrass, goosegrass, and fall panicum. Clethodim applied 7 d before or after CGA 362622 controlled the four grass spp. as well as clethodim applied alone. When CGA 362622 was applied to goosegrass alone, fresh weight accumulation stopped for a period of 4 d compared to nontreated plants. Normal growth resumed after 4 d.

**Nomenclature:** CGA 362622, N-[4,6-dimethoxy-2-pyrimidinyl)carbamoyl]-3-(2,2,2-trifluoroethoxy)-pyridin-2-sulfonamide sodium salt; clethodim; broadleaf signalgrass
Brachiaria platyphylla (Griseb.) Nash # BRAPP; goosegrass, [Eleusine indica (L.) Gaertn.] # ELEIN; fall panicum [Panicum dichotomiflorum (L.)] # PANDI; large crabgrass [Digitaria sanguinalis (L.) Scop.] # DIGSA.

Additional index words: Antagonism, growth analysis, orthogonal contrasts.

Abbreviations used: ALS, acetolactase synthase; DAT, days after treatment; POST, postemergence.

Introduction

Broadleaf signalgrass, fall panicum, goosegrass, and large crabgrass are among the most common and troublesome grass weeds in U. S. cotton (Gossypium hirsutum L.), and are among the most troublesome annual grass weeds in agriculture (Byrd 2000; Dowler 1998). Inadequate control of these weed infestations can reduce cotton yields and cotton fiber quality (Byrd 2000). These and other grass and broadleaf weeds are prevalent together in cotton fields. For this reason, optimum application timings for selective herbicides having either grass or broadleaf weed activity often coincide.

CGA 362622 is a sulfonylurea herbicide under development for use in cotton for postemergence (POST) control of broadleaf weeds, particularly sicklepod [Senna obtusifolia (L.) Irwin and Barneby] and common ragweed (Ambrosia artemisiifolia L.) (Hudetz et al. 2000; Wilcut et al. 2000). Clethodim is a graminicide registered on cotton, peanut (Arachis hypogaea L.), and soybean (Glycine max L.) (Anonymous 2001). The

1Letters following this symbol are WSSA-approved computer code from Composite List of Weeds, Revised 1989. Available from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.
effectiveness of clethodim on annual and perennial grass weeds and CGA 362622 on numerous broadleaf weeds make the use of these herbicides applied POST either sequentially or in tank mixtures a likely option for broad spectrum weed control in cotton.

However, acetolactate synthesis (ALS) inhibitors such as imidazolinone, pyrimidylbenzoate, and sulfonyleurea herbicides including chlorimuron, imazethapyr, pyrithiobac, and thifensulfuron have antagonized cyclohexanedione herbicides (clethodim and sethoxydim) causing a reduction in grass control (Culpepper et al. 1999; Ferreira and Coble 1994; Ferreira et al. 1995; Foy and Witt 1992; Holshouser and Coble 1990; Meyers and Coble 1992; Minton et al. 1989; Snipes and Allen 1996; Vidrine et al. 1995). Therefore the objectives of this study were to determine the potential for antagonism on four annual grasses with mixtures of CGA 362622 and clethodim, to determine if antagonism in mixtures can be avoided by applying CGA 362622 and clethodim separately, and to evaluate goosegrass growth as influenced by CGA 362622.

**Materials and Methods**

*Methods common for Field and Greenhouse Experiments.*

The experiments were a randomized complete block design with three replications of treatments. Clethodim and CGA 362622 were applied alone, in mixture, and sequentially at 1, 3, 7, or 14 d intervals. All initial herbicide applications were made on the first day of the experiment. Sequential treatments followed at the specified intervals for a total application interval of 14 d. Each experiment included a non-treated control for comparative purposes.
Two rates of clethodim were used in greenhouse experiments, 105 and 140 g ai/ha. Clethodim was applied at 140 g/ha in field experiments. CGA 362622 was applied at 5 g ai/ha in both greenhouse and field experiments. Crop oil concentrate\(^2\) at 1.0\% (v/v) was included with herbicide treatments containing clethodim. Applications of CGA 362622 alone included a non-ionic surfactant\(^3\) at 0.25\% (v/v). Visual estimates of grass control were recorded based on a scale of 0\% (no control) to 100\% (plant death) 17 to 23 d after the final herbicide application (Frans et al. 1986).

**Greenhouse Experiments.**

Ten to twelve goosegrass seed were planted in 500 mL pots filled with a commercial potting mix\(^4\). Upon emergence, plants were thinned to three plants per pot. Plants were grown in approximate day/night temperatures of 30/17 C and were surface irrigated daily to field capacity. All pots received 10 ml of a 13 g/L solution containing commercial greenhouse fertilizer\(^5\) dissolved in deionized water at emergence and 14 d later. Experiments were performed on goosegrass at 3 to 4 and 6 to 8 leaf stages. The experiment

\(^2\) Crop oil concentrate, Agri-Dex (83\% paraffin-base petroleum oil and 17\% surfactant blend). Helena Chemical Co., 5100 Poplar Avenue, Memphis TN 38137.

\(^3\) Non-ionic surfactant, Induce (90\% alkyl aryl polyoxykane ether and free fatty acids). Helena Chemical Co., 5100 Poplar Avenue, Memphis TN 38137.

\(^4\) Potting media, Metro-Mix 220, Scotts-Sierra Horticultural Products Co., 14111 Scottslawn Rd., Marysville, OH 43041.

was repeated for each goosegrass size. Applications of herbicides were made in a spray chamber with a single 8001EVS flat fan nozzle\textsuperscript{6} calibrated to deliver 140 L/ha at 297 kPa.

**Field Experiments.**

Field experiments were conducted in two separate fields 4.5 km apart at both the Central Crops Research Station near Clayton, NC, and the Upper Coastal Plain Research Station near Rocky Mount, NC, in 2000. Soils were a Norfolk loamy sand (fine-loamy, siliceous, thermic Typic Paleudults) with 0.8% organic matter and pH 5.5 to 5.7 at Clayton, and a Conetoe loamy sand (loamy, mixed, thermic Arenic Hapludults) with 1.1% organic matter and pH 5.6 to 5.8 at Rocky Mount. Each study was placed in field areas where grass populations were >20 plants m$^2$. Grass heights were 20 to 25 cm at time of initial herbicide applications. Grasses were 30 to 60 cm tall at the 14 d treatments. Plots were 3 m wide by 6.1 m long. Herbicides were applied using a CO\textsubscript{2} backpack sprayer calibrated to deliver a volume of 190 L/ha at 140 kPa through XR-11002VS spray nozzles.

**Growth Analysis.**

To evaluate goosegrass growth as influenced by CGA 362622, ten to twelve goosegrass seed were planted in 500 mL pots filled with a commercial potting mix. Upon emergence, plants were thinned to one plant per pot. Plants were grown in approximate day/night temperatures of 30/17 C and were surface irrigated as needed. All pots received 10 ml of a 13 g/L solution containing commercial greenhouse fertilizer dissolved in deionized water at emergence and 14 d later. Plants were blocked according

\textsuperscript{6}Nozzles, TeeJet Spray Nozzles, Spraying Systems Co., P.O. Box 7900, Wheaton, IL. 60189.
to leaf number, which ranged from 4 to 8 leaves. The experiment was a randomized complete block with goosegrass treated with CGA 362622 and nontreated goosegrass as main plots and harvest timings as subplots in a split plot design. The experiment had four replications. CGA 362622 was applied at 5 g/ha with non-ionic surfactant\textsuperscript{7} at 0.25% (v/v) using a spray chamber with a single 8001EVS flat fan nozzle calibrated to deliver 140 L/ha at 297 kPa. Plants were harvested upon treatment and at 2, 4, 6, and 8 days after treatment (DAT) and fresh weight in g were recorded. The experiment was repeated once.

\textbf{Statistical Analysis.}

Field and greenhouse data, analyzed separately, were tested for homogeneity of variance by plotting residuals. The non-treated control was removed from both field and greenhouse data to stabilize variance. An arcsine square-root transformation did not improve variance homogeneity, so non-transformed data were used in analysis and presentation for clarity.

Greenhouse data, separated by goosegrass growth stage, and field data were subjected to an analysis of variance using the general linear models procedure SAS\textsuperscript{8}. Sums of squares were partitioned to evaluate, in the case of greenhouse trials, CGA 362622 and clethodim mixtures, sequential applications thereof, clethodim rate, and trial replication.

\textsuperscript{7} Non-ionic surfactant, Induce (90\% alkyl aryl polyoxykane ether and free fatty acids). Helena Chemical Co., 5100 Poplar Avenue, Memphis TN 38137.

Field trials were partitioned to evaluate the effect of CGA 362622 and clethodim mixtures, sequential applications thereof, and location. Both experiment replication and location or trials were considered random variables and main effects and interactions were tested by the appropriate mean square associated with the random variable (McIntosh 1983). Mean separations were performed using Fisher’s Protected Least Significant Difference (LSD) test at $P = 0.05$.

The expected response for herbicide mixtures and sequential treatments was calculated according to Colby (1967). Expected and observed values were compared using the appropriate LSD value at the 5% level. If the observed response for the herbicide mixture or sequential application was either significantly less than or greater than the expected value, the combination was declared either antagonistic or synergistic, respectively. Mixtures or sequential applications were considered additive (i.e., no interaction) when differences between observed and expected responses were not significant (Hicks et al. 1998).

Growth analysis data was subjected to an analysis of variance using the general linear models procedure in SAS$^9$ and sums of squares were partitioned to evaluate the effect of treatment and harvest timing. Data was log-transformed (base $n$) to compensate for the increasing variance with time. Study repetition was considered a random variable and main effects and interactions were tested by the appropriate mean square associated with the random variable (McIntosh 1983). Regression analysis was used to describe the response of both nontreated goosegrass and goosegrass treated with CGA 362622, and
then orthogonal polynomial contrasts were used to compare the growth rate of treated and nontreated plants over the intervals of 0 to 4 and 4 to 8 DAT.

Results and Discussion

Greenhouse Studies.

CGA 362622 alone did not control goosegrass in greenhouse studies (Table 1). Clethodim alone controlled 93 and 100% of 3 to 4 leaf goosegrass at the low (105 g/ha) and high (140 g/ha) rates, respectively. Control of 6 to 8 leaf goosegrass with clethodim was 75% for the low rate and 89% for the high rate. Others have noted a decrease in grass control with clethodim as grass size increased or rate decreased (Askew et al. 2000; Culpepper et al. 1999).

Control of goosegrass in greenhouse studies was reduced at least 43 percentage points with CGA 362622 and clethodim at the high rate in mixture (57% control for 3 to 4 leaf goosegrass, 27% control for 6 to 8 leaf goosegrass) compared to control provided by clethodim alone at the high rate. Control of both sizes of goosegrass with mixtures of the two herbicides decreased with the reduction in the clethodim rate.

For sequential applications, the greatest reduction in grass control at both growth stages occurred when CGA 362622 was applied first followed by clethodim 1 d later (Table 1). Furthermore, reduced grass control was observed when clethodim was applied within 3 d of CGA 362622 treatment, regardless of growth stage. Antagonism also occurred when CGA 362622 was applied first to the larger goosegrass, followed by clethodim 7 d later, although no other 7 d sequential application reduced goosegrass control. No antagonism was
observed in greenhouse studies when clethodim was applied first followed by CGA 362622 7 or 14 d later, or when CGA 362622 was applied first, followed by clethodim 14 d later.

**Field Studies.**

CGA 362622 did not control broadleaf signalgrass, fall panicum, goosegrass, or large crabgrass in field studies (Table 2). Clethodim alone controlled broadleaf signalgrass, fall panicum, goosegrass, and large crabgrass at 100, 99, 97, and 98%, respectively (Table 2). Clethodim at 140 g/ha provided >90% control of broadleaf signalgrass, fall panicum, goosegrass, and large crabgrass (Jordan 1995; Myers and Coble 1992; Vidrine et al. 1995; York and Culpepper 2000).

Broadleaf signalgrass, fall panicum, goosegrass, and large crabgrass control in the field was less (30%, 31%, 29%, and 37% control, respectively) when CGA 362622 and clethodim were applied in mixture than when clethodim was applied alone (Table 2). There was a difference between the predicted and observed control values for all grass weeds, indicating antagonism (Colby 1967). CGA 362622 has antagonized other graminicides in a similar manner (Crooks et al. 2001).

Reduced grass control was observed in field studies when clethodim was applied within 3 d of CGA 362622 treatment. For sequential applications, the greatest reduction in grass control occurred when CGA 362622 was applied first followed by clethodim 1 d later. When CGA 362622 was applied first followed by clethodim 1 d later, broadleaf signalgrass, fall panicum, goosegrass, and large crabgrass control was 31, 17, 22, and 23%, respectively (Table 2). Antagonism of clethodim by CGA 362622 also occurred when clethodim was applied first followed 3 d later by CGA 362622, or when CGA 362622 was applied first,
followed by clethodim 3 d later. No antagonism was observed when clethodim was applied first followed by CGA 362622 7 or 14 d later, or when CGA 362622 was applied first, followed by clethodim 7 d later. Reduced control of fall panicum, goosegrass, and large crabgrass was observed, however, when CGA 362622 was applied first followed by clethodim 14 d later (Table 2). Rather than antagonism, the 14 d reduction in control most likely was caused by reduced effectiveness of clethodim on larger grasses as the 14 d treatments were made onto grasses exceeding the recommended maximum treatment size (20 to 25 cm) for clethodim (Anonymous 2001). The reduction in grass control by clethodim in the field corresponds with the reduction in grass control observed in the greenhouse studies.

**Growth Analysis.**

Data was pooled over trials as there was not a trial main effect. CGA 362622 reduced goosegrass biomass accumulation compared to non-treated goosegrass from 0 to 4 DAT (Figure 1). Thereafter, the increase of biomass was similar for both CGA 362622 treated and non-treated goosegrass. Orthogonal polynomial contrasts were used to quantify the significance between the growth rates. From 4 to 8 DAT, the growth rates for treated and non-treated goosegrass were similar. However, the rate of biomass increase of treated goosegrass was less than the non-treated goosegrass from 0 to 4 DAT (Table 3). These data suggest that CGA 362622 affects one or more physiological processes within goosegrass. Others have found that when ALS-inhibiting herbicides were applied 1 or 2 d before an application of a graminicide there was a reduction in translocation of the graminicide (Croon et al. 1989; Ferreira et al. 1995). Furthermore, other ALS-inhibiting herbicides have been
found to inhibit a number of physiological processes including photosynthate transport and mitosis (Shaner and Singh 1997).

The reduction in grass control by CGA 362622 and clethodim in mixture was greater than that reported for other ALS inhibitors such as chlorimuron or thifensulfuron, or imazethapyr when applied in mixture with cyclohexanedione herbicides (Foy and Witt 1992; Jordan 1995; Myers and Coble 1992, Vidrine et al. 1995). Chlorimuron, when mixed with clethodim or sethoxydim, reduced control of johnsongrass [*Sorghum halapense* (L.) Pers] and barnyardgrass [*Echinocloa crus-galli* (L.) P. Beauv.] compared to clethodim or sethoxydim alone, although the reduction in control was inconsistent (Jordan 1995; Vidrine et al. 1995). Chlorimuron did not antagonize broadleaf signalgrass control with clethodim (Jordan 1995; Myers and Coble 1992). Thifensulfuron mixed with sethoxydim reduced control of large crabgrass and giant foxtail (*Setaria faberi* R. Herrm.) by sethoxydim alone from 95% to 70% (Foy and Witt 1992). Broadleaf signalgrass control with clethodim was not antagonized by imazethapyr (Myers and Coble 1992) when the two herbicides were applied in mixture, although the lack of antagonism with imazethapyr may be the result of imazethapyr also controlling broadleaf signalgrass (87%) when applied alone (Myers and Coble 1992).

CGA 362622 antagonized clethodim control of broadleaf signalgrass, fall panicum, goosegrass, and large crabgrass when applied in mixture with clethodim. Sequential applications with a minimum of a 7 d interval between treatments were required to overcome this antagonism. This study indicates that clethodim should be applied before CGA 362622 for greatest control of broadleaf signalgrass, fall panicum, goosegrass, and
large crabgrass. However, growers should base their decision of which herbicide to apply in part on the sizes (and consequent time period for optimum herbicide efficacy at those sizes), densities, and competitive indices of the weeds present in their fields.
Literature Cited


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Table 1. Interaction of CGA 362622 and application sequences of clethodim for control of goosegrass in greenhouse trials. Data are presented separately by plant growth stage and averaged over greenhouse trials.

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<thead>
<tr>
<th>Clethodim rate (g ai/ha)</th>
<th>CGA 362622 rate (g ai/ha)</th>
<th>Goosegrass growth stage</th>
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<th>6 to 8 leaf (%)</th>
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<td>54*</td>
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</tr>
<tr>
<td>LSD</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Crop oil concentrate at 1.0% (v/v) was included with all clethodim treatments.

\(^b\) These values indicate the length of time (d) between sequential applications of CGA 362622 or clethodim.

\(^c\) Means within a column separated according to Fisher’s protected LSD (p = 0.05). A ‘*’ denotes antagonism and no marking indicates an additive effect. Interactions were considered significant only if the differences between the observed and computed expected values (Colby 1967) exceeded the appropriate LSD.
Table 2. Broadleaf signalgrass, fall panicum, goosegrass, and large crabgrass control in field studies with CGA 362622 and clethodim applied alone, in mixture, and in sequential applications\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Application sequence of clethodim\textsuperscript{b}</th>
<th>Broadleaf signalgrass</th>
<th>Fall panicum</th>
<th>Goosegrass</th>
<th>Large crabgrass</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGA 362622 alone\textsuperscript{d}</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14 d before</td>
<td>100</td>
<td>97</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>7 d before</td>
<td>99</td>
<td>99</td>
<td>97</td>
<td>98</td>
</tr>
<tr>
<td>3 d before</td>
<td>75*</td>
<td>74*</td>
<td>71*</td>
<td>75*</td>
</tr>
<tr>
<td>1 d before</td>
<td>48*</td>
<td>41*</td>
<td>32*</td>
<td>40*</td>
</tr>
<tr>
<td>Mixture</td>
<td>30*</td>
<td>31*</td>
<td>29*</td>
<td>37*</td>
</tr>
<tr>
<td>1 d after</td>
<td>31*</td>
<td>17*</td>
<td>22*</td>
<td>23*</td>
</tr>
<tr>
<td>3 d after</td>
<td>48*</td>
<td>54*</td>
<td>43*</td>
<td>47*</td>
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<td>7 d after</td>
<td>96</td>
<td>95</td>
<td>93</td>
<td>94</td>
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<tr>
<td>14 d after</td>
<td>96</td>
<td>91*</td>
<td>86*</td>
<td>89*</td>
</tr>
<tr>
<td>Clethodim alone</td>
<td>100</td>
<td>99</td>
<td>97</td>
<td>98</td>
</tr>
<tr>
<td>LSD</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Data averaged over locations. Means within a column are separated according to Fisher’s Protected LSD (p = 0.05).

\textsuperscript{b} Application sequence of clethodim relative to the application of CGA 362622.

\textsuperscript{c} A ‘*’ denotes antagonism and no marking indicates an additive effect. Interactions were significant only if the differences between the observed and computed expected values (Colby 1967) exceeded the appropriate LSD.

\textsuperscript{d} CGA 362622 was applied at 5 g ai/ha, and clethodim was applied at 140 g ai/ha. All herbicide mixtures included crop oil concentrate at 1.0% (v/v).
Table 3. Orthogonal contrasts of the growth rate of CGA 362622 treated and non-treated goosegrass from 0 to 4 DAT\textsuperscript{a} and 4 to 8 DAT.

<table>
<thead>
<tr>
<th></th>
<th>0 to 4 DAT</th>
<th>4 to 8 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>—— Δ fresh weight (ln g) ——</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated\textsuperscript{b}</td>
<td>-0.0025</td>
<td>0.48</td>
</tr>
<tr>
<td>Non-treated</td>
<td>0.19</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Statistical analysis

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Square</td>
<td>2.30</td>
<td>0.03</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0053</td>
<td>0.76</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Days after treatment.

\textsuperscript{b}CGA 362622 was applied at 5 g ai/ha with 0.25% (v/v) non-ionic surfactant.
Figure 1. Log-transformed fresh weight (g) accumulation of non-treated goosegrass [*Eleusine indica* (L.) Gaertn.] and goosegrass treated with CGA 262622 at 5 g ai/ha over time.
CHAPTER 5

Physiological basis for antagonism of clethodim by CGA 362622.

Abstract. 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 $^{14}$C-clethodim by goosegrass. Averaged across the two treatments of clethodim alone and clethodim plus CGA 362622, absorption was 27% and 85% of applied $^{14}$C-clethodim at 0.5 h and 96 h, respectively. By 96 h after treatment, only 0.8% of applied $^{14}$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 h after treatment, 56% of absorbed $^{14}$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 ACCase to clethodim.


Key Words: absorption, acetyl-coenzyme A carboxylase, metabolism, site of action, translocation.
**Introduction**

CGA 362622 is a sulfonylurea herbicide under development for use in cotton (*Gossypium hirsutum* L.) for postemergence (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. 2002). Cotton tolerance to CGA 362622 is based on limited absorption and rapid metabolism (Askew and Wilcut 2002). 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 the separate applications (Ickeringill 1995). Unfortunately, CGA 362622 has been reported to cause severe antagonism with clethodim and other graminicides (Burke et al. 2001; 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. 1999b, 1999c; Ferreira et al. 1995; Myers and Coble 1992; Olson and Nalewaja 1982). The metabolic inactivation of herbicides may be a basis for selectivity. Metabolism of the graminicide diclofop to a non-toxic metabolite confers selectivity to wheat (*Triticum aestivum* L.) (Shimabukuro et al. 1979). Further, it
may also be possible that CGA 362622 stimulates the activity of enzymes involved with herbicide metabolism, such as herbicide antidotes induce glutathione-S-transferases enzymes in sorghum, and therefore increases the detoxification of clethodim (Dean et al. 1990).

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 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). However, ALS-inhibiting herbicides do not directly affect photosynthesis (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 of the effect of CGA 362622 on photosynthetic rate of actively growing goosegrass.

Methods and Materials

Plant Material

Seeds of goosegrass were planted in a 1:1 mixture of pure sand and Norfolk loamy sand (fine-loamy, siliceous, thermic, Typic Paleudults) in 10-cm by 10-cm square plastic
pots. Upon emergence, plants were thinned to one per pot. Plants were maintained in a glasshouse with approximate daily minimum and maximum temperatures of 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 1400 µmol m⁻² s⁻¹ was provided. All pots received 10 ml of a 25 g L⁻¹ commercial fertilizer¹ 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, sub-plots were plant portions, and sub-sub-plots were the two herbicide treatments of clethodim alone or clethodim plus CGA 362622. The study was repeated in time. At the 4-leaf growth stage, the leaf to which ¹⁴C-clethodim was to be applied was covered with aluminum foil and formulated clethodim at 140 g ai ha⁻¹ was applied to uncovered plant portions either alone or with CGA 362622 at 5 g ai 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 nozzle² calibrated to deliver 160 L ha⁻¹ at 200 kPa. Crop oil concentrate³ at 1.0% (v/v) was included in both mixtures. Immediately after application, 5 1-µL droplets of ¹⁴C-clethodim solution, containing approximately 1.7 kBq of radioactivity, were placed on the adaxial surface of the second fully expanded leaf of 4-leaf goosegrass. The solution, which contained either ¹⁴C-clethodim (dissolved in acetonitrile) alone or with CGA
362622 to correspond with the nonradiolabeled mixtures, was applied to the adaxial side of the second fully expanded leaf with a microliter syringe. These solutions were prepared by diluting clethodim, labeled uniformly with $^{14}$C in the phenyl ring [Ring-4,6-$^{14}$C] and a specific activity of 2.1 kBq µmole$^{-1}$, with either HPLC-grade water and the commercial formulation of clethodim (Select™), or HPLC-grade water, Select™, and CGA 362622 at 0.1 µg ml$^{-1}$. Select™ was used to bring the total amount of clethodim applied to the treated leaf to 140 g ha$^{-1}$. Crop oil concentrate$^3$ 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 µL of solution were added to liquid scintillation cocktail at the beginning of the $^{14}$C-label application for each treatment. These samples were used to calculate the amount of $^{14}$C applied to each plant as determined by liquid scintillation spectrometry (LSS)$^6$.

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$^4$ to remove non-absorbed clethodim. A 1 mL aliquot of the rinse was added to 20 ml scintillation fluid$^5$ 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$^7$. 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 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 previously described and were immediately placed 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. The homogenate was then rinsed through a vacuum filtration apparatus with an additional 6 to 8 ml of acetonitrile. The residue and filter paper 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 µL of the 14C 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 by later comparing pure $^{14}$C-clethodim standard to the fresh-leaf extraction.

A 200 $\mu$L aliquot of each concentrated sample was fractionated by reversed-phase HPLC and quantified with in-line $^{14}$C detection. To determine efficiency of both detection and extraction process using LSS, each injection, mobile phase solution, and scintillation cocktail$^{10}$ was collected in its entirety and an aliquot taken, and the percent $^{14}$C-clethodim and metabolites were determined by the ratio of each peak to the total $^{14}$C of the injection.

The liquid chromatographic system consisted of an autosampler$^{11}$ equipped with a 200 $\mu$L sampling loop, two chromatographic pumps$^{12}$, and a flow-through liquid scintillation spectrophotometer$^{13}$ with a 100 $\mu$L flow cell. Gradients were controlled with an automated gradient controller$^{14}$. A HPLC column$^{15}$ with a 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, personal communication).

**Photosynthetic rate**

To evaluate 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 4-leaf growth stage, CGA 362622 was applied at 5 g ha$^{-1}$ using a spray chamber equipped with a single 8001E flat fan nozzle$^{2}$ calibrated to deliver 160 L ha$^{-1}$ at 200 kPa. Non-ionic surfactant$^{4}$ at 0.25% (v/v) was used with CGA 362622.
Single leaf net photosynthetic rates were measured with a portable photosynthesis system. To ensure light saturation, photosynthetic rate was measured between 1100 and 1300 h immediately before treatment and 1, 2, 6, and 8 d after treatment (DAT) with CGA 362622. A 1.0 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. The gas exchange system was operated as a closed system to measure photosynthetic rate as a function of time to depletion of 3 ppm CO₂ (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 measurement.

**Statistical Analysis**

Data were tested for homogeneity of variance prior to statistical analysis. Analysis of variance (ANOVA) was performed on absorption as percent of applied ¹⁴C over time. Linear, quadratic, and higher order polynomial equations were fit to absorption as percent of applied ¹⁴C over time by partitioning sums of squares (Draper and Smith 1981). Regression analysis was performed when significant absorption into the treated leaf of goosegrass was observed over time. Nonlinear models were used if ANOVA indicated that higher order polynomial effects of absorption were more significant than linear or quadratic estimates. Estimation used the Gauss-Newton algorithm, a nonlinear least squares technique (SAS 1998).

For the translocation study, data were subjected to ANOVA 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-sub-plots. Statistical procedures for the metabolism study were similar to 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. In each experiment data was combined over trials. For all analyses, trial effects were considered random and mean squares were tested appropriately based on the treatment design (McIntosh 1983). Translocation, metabolism, and photosynthetic rate were separated by Fisher's Protected LSD test at P = 0.05.

Results and Discussion

Absorption and Translocation

Analysis of variance indicated that CGA 362622 did not influence $^{14}$C-clethodim absorption or translocation, 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 applied clethodim at 0.5 h and 96 h, respectively (Figure 1). Clethodim exhibited a biphasic mode of absorption, with 61% of the $^{14}$C-clethodim absorbed in the first 8 h with a further 24 percentage point increase in absorption in the following 88 h. Clethodim and other cyclohexandione herbicides in general are rapidly absorbed, and have a biphasic absorption pattern (Culpepper et al. 1999c, Wanamarta and Penner 1989). While absorption increased over time, little $^{14}$C translocated from the treated leaf to other plant portions at any harvest interval (Table 1).
By 96 h after treatment, only 0.8% of applied $^{14}$C had moved into the portion of the shoot below the treated leaf. The shoot below the treated leaf includes the intercalary meristem (Esau 1977). Other researchers have also 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 1999c).

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 movement of the graminicides or the corresponding bio-activated 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 $I_{50}$ value for sethoxydim of 2.9 $\mu$mol and greater than 90% inhibition at 100 $\mu$mol (Burton et al. 1987; Focke and Lichtenthaler 1987; Rendina and Felts 1988). Therefore, 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 efficacy studies (Burke et al. 2001; Crooks et al. 2001).

**Metabolism**

Analysis of variance indicated that CGA 362622 did not influence $^{14}$C-clethodim metabolism, 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, while no $^{14}$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) (Table 2). From the 4 h to the 96 h harvest, metabolite ‘C’ decreased from 50% to 3% of total recovered $^{14}$C. Metabolite ‘B’ (retention time of 14 min) also decreased as percentage of total recovered $^{14}$C from the 48 h harvest to the 96 h harvest. Metabolite ‘A’ (retention time 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, allyoxidim, 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 correspond to the sulfone and sulfoxide, respectively, of clethodim. 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 to the other two metabolites and clethodim as determined by its retention time. After oxidation, herbicide metabolites are typically conjugated to a more polar product in preparation for sequestration (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 (Culpepper et al. 1999b; Culpepper et al 1999c; Ferreira et al. 1995; Wanamarta and Penner 1986).

**Photosynthetic rate**

Immediately before an application of CGA 362622, rates of photosynthesis were similar for all goosegrass plants (Figure 2). One DAT, the single leaf photosynthetic rate in plants treated with CGA 362622 had decreased by 5.7 µmol CO$_2$ m$^{-2}$ s$^{-1}$ compared to 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. The reduction in photosynthesis and growth of CGA 362622-treated goosegrass compared with nontreated goosegrass may have implications for ACCase inhibition.

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 $^{14}$C-acetate incorporation into lipids in corn root tips, but not in the less metabolically active root regions (Hosaka 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 et al. 1987). Chlorimuron and pyrithiobac did not specifically affect ACCase activity in vitro (Bjelk and Monaco 1992;
Ferreira et al. (1995), but chlorsulfuron reduced lipid synthesis in isolated soybean leaf cells were reduced after 30 min (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 (Table 2). Graminicides require actively growing meristematic regions for inhibition of ACCase (Devine et 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 for an actively growing plant for herbicidal activity upon ACCase inhibition may be compromised by the reduction of plant growth and photosynthesis caused by ALS-inhibiiton. This growth suppression would 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. 1999a; 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 [Digitaria sanguinalis (L.) Scop.] when mixed with bromoxynil were 220, 220, and 290% greater, respectively, than rates required when these graminicides were applied alone (Culpepper et al. 1999a). At 2-3 times the registered rates, cyclohexanedione and aryloxyphenoxypropionate
herbicides may interfere with plant membranes, thus the observed control by increasing rate may not be due inhibition of ACCase, but rather 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/or growth rate of goosegrass and therefore the herbicidal consequence of ACCase inhibition. 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 and photosynthesis (8 DAT) essentially no active herbicide remained in the plant. Therefore, CGA 362622 may prevent the herbicidal activity of ACCase-inhibiting herbicide clethodim, thus causing the observed antagonism. Further studies are needed to examine whether sensitivity to ACCase inhibiting herbicides can be influenced by environmental factors that slow or inhibit photosynthesis and growth, as was demonstrated with CGA 362622 treatments.

Sources of Materials


2 TeeJet Spray Nozzles, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189.

3 Crop oil concentrate, Agri-Dex (83% paraffin-base petroleum oil and 17% surfactant blend). Manufactured by Helena Chemical Company, Suite 500, 6075 Poplar Avenue, Memphis, TN 38137.
Induce® nonionic low foam wetter/spreader adjuvant, 90% nonionic surfactant (alkylarylpolyoxyalkane ether and isopropanol), free fatty acids, and 10% water. Helena Chemical Company, Suite 500, 6075 Poplar Avenue, Memphis, TN 38137.


Packard TRI-CARB 2100TR Liquid Scintillation Spectrometer, Packard Instrument Company, 800 Research Parkway, Meriden, CT 06450.

Model OX-500 Biological Material Oxidizer, R. J. Harvey Instrument Corp., 123 Patterson Street, Hillsdale, NJ 07642.

Pyrex® Tissue Homogenizer No. 7727-40, Corning Inc., Corning, NY 14831.

Watman #3 filter paper, Fisher Scientific, P. O. 4829, Norcross, GA 30091.

Ultima-Flo™ M Flow Liquid Scintillation Cocktail, Packard Instrument Company, 800 Research Parkway, Meriden, CT 06450.

Model 715 Waters ULTRA WISP Sample Processor, Waters, 34 Maple St. Milford, MA 01757.

Model 6000 Waters Chromatography Pump, Waters, 34 Maple St. Milford, MA 01757.

Model 500 Radiomatic Flo-One Liquid Scintillation Spectrometer, Packard Instrument Company, 800 Research Parkway, Meriden, CT 06450.

Model 680 Waters Automated Gradient Controller, Waters, 34 Maple St. Milford, MA 01757.
15 Allsphere ODS-1 5 μm 250 x 4.6 mm reversed phase column. Alltech Associates, Inc., 2051 Waukegan Rd., Deerfield, IL 60015.

16 Model LI-6200 Portable Photosynthesis System, LI-COR, P.O. Box 4425, Lincoln, NE 68504.
**Literature Cited**


Table 1. Influence of harvest timings of 0.5, 1, 4, 8, 24, and 96 h after treatment (HAT) on the distribution of absorbed $^{14}$C in goosegrass averaged over herbicide treatments of $^{14}$C-clethodim alone or $^{14}$C-clethodim plus CGA 362622.

<table>
<thead>
<tr>
<th>Harvest timing (h)</th>
<th>Absorbed $^{14}$C over time$^a$</th>
<th>Plant portion$^b$</th>
<th>% of applied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated leaf</td>
<td>Shoot above</td>
<td>Shoot below</td>
</tr>
<tr>
<td>0.5</td>
<td>27</td>
<td>0.8</td>
<td>0.4</td>
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<td>1</td>
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<td>0.3</td>
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<td>0.7</td>
</tr>
<tr>
<td>96</td>
<td>85</td>
<td>1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>8</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^a$ Alone, clethodim applied at 140 g ha$^{-1}$; the mixture is CGA 362622 at 5 g ha$^{-1}$ plus clethodim at 140 g ha$^{-1}$.

$^b$ Means within a column separated according to Fisher’s protected LSD (p = 0.05).
Table 2. Influence of harvest timings of 4, 8, 24, and 48 HAT on the proportion of absorbed $^{14}$C-label metabolites in treated leaves of goosegrass averaged over herbicide treatments of $^{14}$C-clethodim alone or $^{14}$C-clethodim plus CGA 362622.

<table>
<thead>
<tr>
<th>Metabolite (retention time in min)</th>
<th>Time</th>
<th>A (4.0)</th>
<th>B (14.0)</th>
<th>C (26.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-h-</td>
<td>% of total detected metabolites</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>7</td>
<td>39</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>16</td>
<td>43</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>33</td>
<td>45</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>56</td>
<td>34</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>LSD (0.05)</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

a Alone, clethodim applied at 140 g ha$^{-1}$; the mixture is CGA 362622 at 5 g ha$^{-1}$ plus clethodim at 140 g ha$^{-1}$.

b Means within a column separated according to Fisher’s protected LSD (p = 0.05).
Figure 1. Foliar absorption of $^{14}\text{C}$-clethodim over time by goosegrass averaged over herbicide treatments ($^{14}\text{C}$-clethodim or $^{14}\text{C}$-clethodim plus CGA 362622).

Absorbed $^{14}\text{C}$

$y = 12.3\ln(x) + 34.6$, $R^2 = 0.98$
Figure 2. Photosynthetic rate ($A_{max}$) response of single non-treated light saturated goosegrass leaves and single light saturated goosegrass leaves treated with CGA 362622. Error bars indicate standard error of the mean.
CHAPTER 6

Annual grass control in peanut (Arachis hypogaea) with clethodim and imazapic.

Abstract. Field experiments were conducted to evaluate clethodim, imazapic, mixtures thereof, and sequential treatments for control of fall panicum, goosegrass, and large crabgrass. Clethodim was applied at 140 g ai/ha, while imazapic was applied at 70 g ai/ha. Imazapic alone provided 95% and 79% control of fall panicum and large crabgrass, respectively. Goosegrass control with imazapic alone was ≤62%. Reduced fall panicum, goosegrass, and large crabgrass control was observed when the two herbicides were applied in mixture. Antagonism of clethodim activity on fall panicum and large crabgrass occurred when clethodim was applied 1 d before or 1 and 3 d after an application of imazapic. Goosegrass control and antagonism varied by location. Goosegrass control was reduced when clethodim was applied 1 and 3 d before or 1, 3, and 7 d after an application of imazapic at Clayton, NC. At Lewiston, NC, goosegrass control was variable. Antagonism of goosegrass control at Rocky Mount, NC, occurred when clethodim was applied 1 d after imazapic.

Nomenclature: Clethodim; imazapic; goosegrass, Eleusine indica (L.) Gaertn. # ELEIN; fall panicum [Panicum dichotomiflorum (L.)] # PANDI; large crabgrass [Digitaria sanguinalis (L.) Scop.] # DIGSA; peanut, Arachis hypogaea L., 'NC 10C'.

Additional index words: antagonism.

Abbreviations used: acetolactate synthase, ALS; acetyl-CoA carboxylase, ACCase.
Introduction

Imazapic (AC 263,222) is a postemergence herbicide registered in peanut (*Arachis hypogaea* L.) used to control many annual broadleaf weeds such as cocklebur (*Xanthium strumarium* L.) and sicklepod [*Senna obtusifolia* (L.) Irwin and Barneby] as well as purple (*Cyprus rotundus* L.) and yellow (*C. esculentus* L.) nutsedge (Richburg et al. 1994; Warren and Coble 1999; Wilcut et al. 1996). Imazapic also controls annual and perennial grasses such as broadleaf signalgrass [*Brachiaria platyphylla* (Greisb.) Nash], rhizome and seedling johnsongrass (*Sorghum halepense* L.), and crabgrass species (*Digitaria* spp.) (Anonymous 2001b; Jennings et al. 1996; Wilcut et al. 1999). Imazapic inhibits acetolactate synthase [4.1.3.18] (ALS), and plant death results from events occurring in response to ALS inhibition, which is a key enzyme in the biosynthesis of the branched-chain amino acids isoleucine, leucine, and valine (Ahrens 1994). Clethodim is a graminicide registered on cotton (*Gossypium hirsutum* L.), peanut, and soybean (*Glycine max* L.) (Anonymous 2001a). Clethodim inhibits acetyl-CoA carboxylase [6.4.1.2] (ACCase), thereby blocking fatty acid synthesis and the production of phospholipids used in building new membranes required for cell growth (Ahrens 1994, Burton et al. 1987). Clethodim provides control of many annual and perennial grasses (Brewster and Spinney 1989; Grichar and Boswell 1993; Leys et al. 1988; Johnson and Frans 1991).

As complexes of grass and broadleaf weeds exist together in agricultural fields, optimum application timings for clethodim and imazapic may coincide. However, members of the imidazolinone herbicide family, such as imazethapyr or imazaquin, have
agonized graminicides, including clethodim, causing a reduction in grass control (Holshouser and Coble 1989; Minton et al. 1989, Myers and Coble 1992). Furthermore, reduced grass control is often observed when broadleaf herbicides are mixed with graminicides (Burke et al. 2001; Crooks 2001; Culpepper et al. 1999; Ferreira and Coble 1994; Ferreira et al. 1995; Foy and Witt 1992; Gerwick et al. 1988; Holshouser and Coble 1990; Rhodes and Coble 1984; Snipes and Allen 1996; Vidrine et al. 1995). Antagonism may occur when clethodim and imazapic are applied in mixture. Therefore the objectives of this study were to examine grass control obtained with imazapic alone, the potential for antagonism on four annual grasses with mixtures of imazapic and clethodim, and the application timing necessary to avoid antagonism should it occur using sequential applications of clethodim and imazapic.

**Materials and Methods**

A study was conducted in two separate fields at both the Central Crops Research Station near Clayton, NC, and the Upper Coastal Plains Research Station near Rocky Mount, NC, in 2000, while a single study was performed at the Peanut Research Station in Lewiston-Woodville in 2000. Soils were a Norfolk loamy sand (fine-loamy, siliceous, thermic Typic Paleudults) with 1.8% organic matter and a pH of 5.5 to 5.7 at Clayton, a Norfolk loamy sand (fine-loamy, siliceous, thermic Typic Kandiudults) with 1.0% organic matter and a pH of 5.9 at Lewiston-Woodville, and a Conetoe loamy sand (loamy, mixed, thermic Arenic Hapludults) with 1.1% organic matter and a pH of 5.6 to 5.8 at Rocky Mount. The study had a randomized complete block design with three replications of treatments. Clethodim
and imazapic were applied alone, in mixture, and sequentially at 1, 3, 7, or 14 d intervals. All initial herbicide applications were made at the initiation of the study. Sequential treatments followed at the specified intervals for a total application interval of 14 d. Each study included a non-treated control for comparative purposes. Clethodim was applied at 140 g ai/ha and imazapic was applied at 70 g ai/ha. Crop oil concentrate\textsuperscript{9} at 1.0\% (v/v) was included with treatments containing clethodim. Applications of imazapic alone included a non-ionic surfactant\textsuperscript{10} at 0.25\% (v/v). Visual estimates of grass control were recorded based on a scale of 0\% (no control) to 100\% (plant death) 17 to 35 d after the final herbicide application (Frans et al. 1986). Each study was placed in field areas where grass populations were >20 plants m\textsuperscript{2}. Grass heights were 20 to 25 cm at time of initial herbicide applications. Grasses were 30 to 60 cm tall at the 14 d treatments. Plots were 3 m wide by 6.1 m long. Herbicides were applied using a CO\textsubscript{2} backpack sprayer calibrated to deliver a volume of 190 L/ha at 140 kPa through XR-11002VS spray nozzles\textsuperscript{11}.

Data were tested for homogeneity of variance by plotting residuals. The non-treated control was removed from the data to stabilize variance. An arcsine square-root transformation did not improve variance homogeneity, so non-transformed data were used in analysis and presentation. Field data was subjected to an analysis of variance using the

\textsuperscript{9} Crop oil concentrate, Agri-Dex (83\% paraffin-base petroleum oil and 17\% surfactant blend). Helena Chemical Co., 5100 Poplar Avenue, Memphis TN 38137.

\textsuperscript{10} Non-ionic surfactant, Induce (90\% alkyl aryl polyoxylkane ether and free fatty acids). Helena Chemical Co., 5100 Poplar Avenue, Memphis TN 38137.

\textsuperscript{11} Nozzles, TeeJet Spray Nozzles, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189.
general linear models procedure in SAS\textsuperscript{12} and sums of squares were partitioned to evaluate the effect of imazapic and clethodim mixtures, sequential applications thereof, and location. Both experiment replication and location or trials were considered a random variable and main effects and interactions were tested by the appropriate mean square associated with the random variable (McIntosh 1983). Means separations were performed using Fisher’s Protected Least Significant Difference (LSD) test at $P = 0.05$.

The expected response for herbicide mixtures and sequential treatments was calculated according to Colby (1967). Expected and observed values were compared using the appropriate LSD value at the 5\% level. If the observed response for the herbicide mixture or sequential application was either significantly less than or greater than the expected value, the combination was declared either antagonistic or synergistic, respectively. Mixtures or sequential applications were considered additive (i.e., no interaction) when differences between observed and expected responses were not significant (Hicks et al. 1998).

**Results and Discussion**

**Fall Panicum Control.**

Analysis of variance indicated a treatment main effect, therefore fall panicum response to treatments were pooled over location. Imazapic alone provided 95\% control of fall panicum, while clethodim alone provided 100\% control (Table 1). Fall panicum control

was reduced to 82% when imazapic and clethodim were applied in mixture (Table 1). Antagonism was also observed when clethodim was applied 1 d before or 1 d after an application of imazapic, although control of fall panicum with these treatments were excellent (≥91%). Control of fall panicum was ≥96% when clethodim was applied 3 or more d before or after imazapic.

Others have observed antagonism or a reduction in control of fall panicum between graminicides and imidazolinone herbicides. Control of fall panicum by sethoxydim or clethodim was antagonized by imazethapyr (Myers and Coble 1992). Myers and Coble (1992) reported that control fall panicum (53 to 80%) was lowest when clethodim was applied 1 d after imazethapyr, although the reduction of control of grass weeds by imazapic-clethodim systems was not as great as that of imazethapyr-clethodim systems. Imazaquin, however, severely antagonized control of fall panicum by sethoxydim, reducing control by 88 percentage points compared to control obtained by sethoxydim alone (Holshouser and Coble 1990).

**Goosegrass Control.**

There was a location by treatment interaction for goosegrass, so goosegrass control is presented for each location. Goosegrass control with imazapic alone was variable and ranged from 37% at Clayton to 62% at Rocky Mount (Table 2), and is only labeled for suppression of goosegrass (Anonymous 2001a). Clethodim provided ≥99% control of goosegrass at Clayton and Rocky Mount. At Lewiston, however, control of goosegrass obtained from an application of clethodim was 82%. Goosegrass control in field tests at
Clayton and Rocky Mount was reduced (from 99% with clethodim alone to 54 and 79%, respectively) when imazapic and clethodim were applied in mixture.

Control of goosegrass at Clayton was reduced when imazapic was applied first followed by clethodim 1 d (51%), 3 d (65%), and 7 d (88%) later. Goosegrass control at Clayton was also reduced when clethodim was applied 1 d (63%) and 3 d (83%) before an application of imazapic. No antagonism was observed at this location when clethodim was applied 14 or 7 d before or 14 d after an application of imazapic.

Goosegrass control at Rocky Mount was reduced (87%) when imazapic was applied first followed by clethodim 1 d later. At Rocky Mount, no antagonism was observed when clethodim was applied 1 or more d before or 3 or more d after an application of imazapic. There was no difference in goosegrass control between clethodim applied alone, in mixture, or in sequential applications with imazapic at Lewiston, although goosegrass control was numerically less when the two herbicides were applied in mixture than the control obtained from clethodim alone. Bentazon and imazaquin both antagonized control of goosegrass by sethoxydim, reducing control by 50 and 64 percentage points, respectively, compared to control obtained by sethoxydim alone (Holshouser and Coble 1990).

Large Crabgrass Control.

Analysis of variance indicated a treatment main effect, therefore goosegrass control is presented averaged over location. Imazapic alone controlled large crabgrass 79% (Table 1). Clethodim alone provided excellent (100%) control of large crabgrass. Large crabgrass control was reduced (77%) when imazapic and clethodim were applied in mixture. When clethodim was applied 1 d before or after an application of imazapic large crabgrass control
was also reduced, at 86 and 87%, respectively, as it was when clethodim was applied 3 d after an application of imazapic (90%). No antagonism of large crabgrass control was observed when clethodim was applied 3 or more d before or 7 or more d after an application of imazapic. Large crabgrass control was between 67 and 83% using a mixture of imazethapyr and clethodim, whereas clethodim alone provided >90% control (Myers and Coble 1992). Myers and Coble (1992) also reported that control of large crabgrass was lowest when clethodim was applied 1 d after imazethapyr (50 to 83%). In this study greater antagonism occurred when the two herbicides were applied in mixture.

The reduction in grass control by imazapic and clethodim in mixture similar to antagonism reported for other ALS inhibitors as well. Herbicides such as chlorimuron or thifensulfuron have antagonized the activity of cyclohexanedione herbicides (Jordan 1995; Myers and Coble 1992, Vidrine et al. 1995). Chlorimuron, when mixed with clethodim or sethoxydim, reduced control of johnsongrass and barnyardgrass [Echinocloa crus-galli (L.) P. Beauv.] compared to clethodim or sethoxydim alone, although the reduction in control was inconsistent (Jordan 1995; Vidrine et al. 1995). Chlorimuron did not antagonize broadleaf signalgrass control with clethodim (Jordan 1995; Myers and Coble 1992). Thifensulfuron mixed with sethoxydim reduced control of large crabgrass and giant foxtail [Setaria faberi (R.) Herrm] by sethoxydim alone from 95% to 70% (Foy and Witt 1992).

Imazapic reduced clethodim control of fall panicum, goosegrass, and large crabgrass when applied in mixture with clethodim. Sequential applications with >3 d interval between treatments were required to overcome antagonism of fall panicum and large crabgrass. Goosegrass may require a greater time interval between applications of
clethodim and imazapic. Clethodim should be applied before imazapic for unhindered control of large crabgrass, goosegrass, and fall panicum. However, growers should base their decision of which herbicide to apply first in part on the sizes (and consequent time period for optimum herbicide efficacy at those sizes), densities, and competitive indices of the weeds present in their fields.
Literature Cited


120


(Sorghum halepense) and imidazolinone-resistant corn (Zea mays) to AC 263,222.


Cooperative Extension Service, College of Agriculture and Life Sciences. AG-417.
Table 1. Fall panicum and large crabgrass control with imazapic and clethodim applied alone, in mixture, and in sequential applications.

<table>
<thead>
<tr>
<th>Application sequence of clethodim&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Fall panicum</th>
<th>Large crabgrass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clethodim alone</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Imazapic alone</td>
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<td>79</td>
</tr>
<tr>
<td>14 d before imazapic</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>7 d before imazapic</td>
<td>98</td>
<td>100</td>
</tr>
<tr>
<td>3 d before imazapic</td>
<td>98</td>
<td>97</td>
</tr>
<tr>
<td>1 d before imazapic</td>
<td>91&lt;sup&gt;*&lt;/sup&gt;</td>
<td>86</td>
</tr>
<tr>
<td>Mixture</td>
<td>82&lt;sup&gt;*&lt;/sup&gt;</td>
<td>77</td>
</tr>
<tr>
<td>1 d after imazapic</td>
<td>93&lt;sup&gt;*&lt;/sup&gt;</td>
<td>87</td>
</tr>
<tr>
<td>3 d after imazapic</td>
<td>96</td>
<td>90&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>7 d after imazapic</td>
<td>98</td>
<td>96</td>
</tr>
<tr>
<td>14 d after imazapic</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>LSD</td>
<td>4</td>
<td>9</td>
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</table>

<sup>a</sup> Means within a column separated according to Fisher’s protected LSD (p = 0.05).

<sup>b</sup> Application sequence of clethodim relative to the application of imazapic.

<sup>c</sup> Interactions were evaluated by the method described by Colby (1967), a * denotes antagonism and no marking indicates an additive effect. Interactions were significant only if the differences between the observed and expected values exceeded the appropriate least significant difference (LSD) values.
**Table 2.** Goosegrass control with imazapic and clethodim applied alone, in mixture, and in sequential applications.

<table>
<thead>
<tr>
<th>Application sequence of clethodim&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Goosegrass</th>
<th>Clayton</th>
<th>Lewiston</th>
<th>Rocky Mount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clethodim alone</td>
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<td>99</td>
<td>82</td>
<td>100</td>
</tr>
<tr>
<td>Imazapic alone</td>
<td></td>
<td>37</td>
<td>55</td>
<td>62</td>
</tr>
<tr>
<td>14 d before imazapic</td>
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<td>96</td>
<td>93</td>
<td>100</td>
</tr>
<tr>
<td>7 d before imazapic</td>
<td></td>
<td>95</td>
<td>97</td>
<td>100</td>
</tr>
<tr>
<td>3 d before imazapic</td>
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<td>83*</td>
<td>68</td>
<td>100</td>
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<td>63*</td>
<td>83</td>
<td>97</td>
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<td>Mixture</td>
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<td>79*</td>
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<td>1 d after imazapic</td>
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<td>92</td>
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<td>7 d after imazapic</td>
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<td>94</td>
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<tr>
<td>14 d after imazapic</td>
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<td>80</td>
<td>99</td>
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<tr>
<td>LSD</td>
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<td>9</td>
<td>41</td>
<td>9</td>
</tr>
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</table>

<sup>a</sup> Means within a column separated according to Fisher’s protected LSD (p = 0.05).

<sup>b</sup> Application sequence of clethodim relative to the application of imazapic.

<sup>c</sup> Interactions were evaluated by the method described by Colby (1967), a * denotes antagonism and no marking indicates an additive effect. Interactions were significant only if the differences between the observed and expected values exceeded the appropriate least significant difference (LSD) values.
CHAPTER 7

Physiological basis for antagonism of clethodim by imazapic.

Abstract. Greenhouse and laboratory experiments were conducted to determine the effect of imazapic on the herbicidal activity of clethodim on goosegrass. Imazapic did not affect absorption of $^{14}$C-clethodim by goosegrass. Averaged across the two treatments of clethodim alone and clethodim plus imazapic, absorption was 36 and 89% of applied $^{14}$C-clethodim at 0.5 and 96 h, respectively. The majority of clethodim (79% of applied) was absorbed by 24 h. Translocation of $^{14}$C was not affected imazapic, and 3.6% of applied $^{14}$C had translocated into the portion of the shoot below the treated leaf at 96 h after treatment. Metabolism of clethodim was not affected by the presence of imazapic. Three major metabolites of clethodim were detected in treated tissue at all harvest intervals. The majority (58%) of $^{14}$C-clethodim was converted to a relatively polar form 96 h after treatment, whether clethodim was applied alone or in the presence of imazapic. One day after treatment, the photosynthetic rate in plants treated with imazapic decreased below the rate in the non-treated check, and was less for 8 d, the duration of the study. These data suggest that the antagonism of clethodim by imazapic may be caused by imazapic reducing the photosynthetic rate of goosegrass and therefore the sensitivity of ACCase to clethodim.

Nomenclature: imazapic; clethodim, *Eleusine indica* (L.) Gaertn. # ELEIN, goosegrass.

Key Words: absorption, acetyl-coenzyme A carboxylase, metabolism, translocation.
Introduction

Imazapic (AC 263,222) is an imidazolinone herbicide registered in peanut (*Arachis hypogaea* L.) for postemergence (POST) control of broadleaf weeds such as cocklebur (*Xanthium strumarium* L.) and sicklepod [*Senna obtusifolia* (L.) Irwin and Barneby] as well as purple (*Cyperus rotundus* L.) and yellow (*C. esculentus* L.) nutsedge (Richburg et al. 1994; Warren and Coble 1999; Wilcut et al. 1996). Clethodim, an acetyl-coenzyme A carboxylase [6.4.1.2] (ACCase) inhibitor, is a graminicide registered on cotton (*Gossypium hirsutum* L.), peanut, and soybean (*Glycine max* L.) (Anonymous 2001). Applying imazapic, an acetolactate synthase [4.1.3.18] (ALS) inhibitor, with clethodim would not only increase the spectrum of weed control but also reduce the cost associated with separate applications (Ickeringill 1995). Unfortunately, imazapic has been reported to cause antagonism of clethodim activity (Burke 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. 1999b, 1999c; Ferreira et al. 1995; Myers and Coble 1992; Olson and Nalewaja 1982). Metabolism of the graminicide diclofop to a non-toxic metabolite confers selectivity to wheat (*Triticum aestivum* L.) (Shimabukuro et al. 1979). Further, it may also be possible that imazapic stimulates the activity of enzymes involved with herbicide metabolism, such as herbicide antidotes induce glutathione-S-transferase enzymes in sorghum, and therefore increases the detoxification of clethodim (Dean et al. 1990).
Graminicides require actively growing meristematic regions for inhibition of ACCase (Devine et al. 1993). ALS-inhibiting herbicides such as imazapic 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 ALS inhibiting herbicides in plants is the inhibition of photosynthate transport. Shortly after application of an ALS-inhibiting herbicide, neutral sugars accumulate in treated leaves because photosynthetic transport is inhibited (Bestman et al. 1990). However, ALS-inhibiting herbicides do not affect photosynthesis directly (Shaner and Singh 1997). Therefore, this research was conducted to determine the basis of the antagonistic interaction between imazapic and clethodim. The objectives of this research were to determine the effect of imazapic on the absorption, translocation, and metabolism of clethodim in goosegrass, and to examine goosegrass photosynthetic rate in response to an application of imazapic.

**Methods and Materials**

**Plant Material**

Seeds of goosegrass were planted in a 1:1 mixture of pure sand and Norfolk loamy sand (fine-loamy, siliceous, thermic, Typic Paleudults) in 10-cm by 10-cm square plastic pots. Upon emergence, plants were thinned to one per pot. Plants were maintained in a glasshouse approximate daily minimum and maximum temperatures of 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 1400 µmol m$^{-2}$ s$^{-1}$ was provided. All pots
received 10 ml of a 25 g L\(^{-1}\) commercial fertilizer\(^1\) at emergence and at 11 d after emergence.

**Absorption and Translocation**

The study was conducted as a randomized complete block with a split-split-plot treatment design and four replications of treatments to evaluate absorption and translocation of clethodim alone and in the presence of imazapic. Main plots were harvest timings, sub-plots were plant portions, and sub-sub-plots were the two herbicide treatments of clethodim alone or clethodim plus imazapic. The study was repeated in time. At the 4-leaf growth stage, the leaf to which \(^{14}\)C-clethodim was to be applied was covered with aluminum foil and formulated clethodim at 140 g ai ha\(^{-1}\) was applied to uncovered plant portions either alone or with imazapic at 70 g ai ha\(^{-1}\). Applications were made using a spray chamber equipped with a single 8001E flat fan nozzle\(^2\) calibrated to deliver 160L ha\(^{-1}\) at 200 kPa. Crop oil concentrate\(^3\) at 1.0% (v/v) was included in both mixtures. Immediately after application, 5 1-\(\mu\)L droplets of \(^{14}\)C-clethodim solution, containing approximately 1.7 kBq of radioactivity, were placed on the adaxial surface of the second fully expanded leaf of 4-leaf goosegrass. The solution, which contained either \(^{14}\)C-clethodim (dissolved in acetonitrile) alone or with imazapic to correspond with the nonradiolabeled mixtures, was applied to the adaxial side of the second fully expanded leaf with a microliter syringe. These solutions were prepared by diluting clethodim, labeled uniformly with \(^{14}\)C in the phenyl ring [Ring-4,6-\(^{14}\)C] and a specific activity of 2.1 kBq \(\mu\)mole\(^{-1}\), with either HPLC-grade water and Select\(^\text{TM}\), or HPLC-grade water, Select\(^\text{TM}\), and imazapic at 0.1 \(\mu\)g ml\(^{-1}\). Select\(^\text{TM}\) was used to bring the total amount of clethodim
applied to the treated leaf to 140 g ha$^{-1}$. Crop oil concentrate$^3$ was included in both mixtures at 1% (v/v). The rates of clethodim and imazapic in the spotting solution were the same as in the solution applied using the spray chamber. Five $\mu$L of solution were added to liquid scintillation cocktail at the beginning of the $^{14}$C-label application for each treatment. These samples were used to calculate the amount of $^{14}$C applied to each plant as determined by liquid scintillation spectrometry (LSS)$^6$.

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$^4$ to remove non-absorbed clethodim. A 1 ml aliquot of the rinse was added to 20 ml scintillation fluid$^5$ 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$^7$. 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 imazapic. Treatment design was the same as the absorption and translocation study. The study was repeated in time. Plants used for the metabolism experiments were grown, treated, and partitioned 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 partitioned as previously described and were immediately placed in a freezer and stored at -30 C until further analysis. Based on absorption and translocation experiments, only the treated leaf contained sufficient radioactivity for evaluation. Treated leaf sections were homogenized in 2 to 4 mL acetonitrile using a tissue grinder⁸. The homogenate was then rinsed through a vacuum filtration apparatus with an additional 6 to 8 ml of acetonitrile. The residue and filter paper⁹ 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, personal communication). To evaluate the potential effects of the extraction process on herbicide degradation, fresh plant leaves were harvested, spotted with 5 µL of the ¹⁴C 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 by later comparing pure ¹⁴C-clethodim standard to the fresh-leaf extraction.

A 200 µL aliquot of each concentrated sample was fractionated by reversed-phase HPLC and quantified with in-line ¹⁴C detection. To determine efficiency of both detection and extraction process using LSS, each injection, mobile phase solution, and scintillation cocktail¹⁰ was collected in its entirety and an aliquot taken, and the percent ¹⁴C-clethodim and metabolites were determined by the ratio of each peak to the total ¹⁴C of the injection.
The liquid chromatographic system consisted of an autosampler equipped with a 200 µL sampling loop, two chromatographic pumps, and a flow-through liquid scintillation spectrophotometer with a 100 µL flow cell. Gradients were controlled with an automated gradient controller. A HPLC column with a guard column 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 response of goosegrass photosynthetic rate to imazapic, a study was conducted as a randomized complete block with three replications of treatments. The study was repeated in time. At the 4-leaf growth stage, imazapic was applied at 70 g ha\(^{-1}\) using a spray chamber equipped with a single 8001E flat fan nozzle calibrated to deliver 160 L ha\(^{-1}\) at 200 kPa. Non-ionic surfactant at 0.25% (v/v) was included in the spray solution.

Single leaf net photosynthetic rates were measured with a portable photosynthesis system. To ensure light saturation, photosynthetic rate was measured between 1100 and 1300 h immediately before treatment and 1, 2, 6, and 8 d after treatment (DAT) with imazapic. 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. The gas exchange system was operated as a closed system to measure photosynthetic rate as a function of time to depletion of 3 ppm 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 measurement and used in the calculation of the photosynthetic rate.

**Statistical Analysis**

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

For the translocation study, data were subjected to ANOVA with sums of squares partitioned to reflect a split-split-plot treatment structure and trial effects using the general linear models procedure in SAS (SAS 1998). The six harvest timings were considered main plots, the six plant portions were considered subplots, and the two spray mixtures (clethodim with or without imazapic) were considered sub-sub-plots. Statistical procedures for the metabolism study were similar to those used in 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. In each experiment data was combined over trials. For all analyses, trial effects were considered random and mean squares were tested appropriately based on the treatment design.
Results and Discussion

Absorption and Translocation

Analysis of variance indicated that imazapic did not influence $^{14}$C-clethodim absorption or translocation, thus data were pooled over the two herbicide treatments of clethodim and clethodim plus imazapic. Clethodim exhibited biphasic absorption, with 36% of the $^{14}$C-clethodim absorbed in the first 0.5 h and 79 percentage points in absorption in the following 23.5 h (Figure 1). By 96 HAT, 89% of the $^{14}$C-clethodim had been absorbed into the leaves of goosegrass. Clethodim and other cyclohexandione herbicides in general are rapidly absorbed, and have a similar biphasic absorption pattern (Culpepper et al. 1999c, Wanamarta and Penner 1989). While absorption into the treated leaf increased over time, little $^{14}$C translocated from the treated leaf to other plant portions (Table 1). By 96 h after treatment, 3.6% of applied $^{14}$C had moved into the portion of the shoot below the treated leaf, the location of the intercalary meristem (Esau 1977), while 4.7% of applied $^{14}$C had moved to the shoot above the treated leaf. Other researchers have also 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. 1999c).

Although our data suggest that imazapic does not affect translocation of clethodim out of the treated leaf, others have noted differences 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 photosynthate transport processes, and may therefore affect movement of the graminicides or the corresponding bio-activated 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 I$_{50}$ value for sethoxydim of 2.9 µmol and greater than 90% inhibition at 100 µmol (Burton et al. 1987; Focke and Lichtenthaler 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 lack of control. A mechanism of antagonism different from, or in addition to, translocation may account for the reduction in grass control observed in efficacy studies with clethodim and imazapic (Burke et al. 2001).

**Metabolism**

Analysis of variance indicated that imazapic did not influence $^{14}$C-clethodim metabolism, thus data were pooled over the two herbicide treatments of clethodim and clethodim plus imazapic. Three major metabolites of clethodim were detected in treated tissue at all harvest intervals, while no $^{14}$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) (Table 2). From the 4 h to the 96 h harvest, metabolite ‘C’ decreased from 36% to 3% of total detected metabolite. Metabolite ‘B’ (retention time of 14 min) also decreased as percentage of total metabolite from the 48 h harvest to the 96 h harvest.
Metabolite ‘A’ (retention time 4.0 min) increased from 14% of total metabolite at the 4 h harvest to 58% of total metabolite at the 96 h harvest.

No metabolites of clethodim have been previously described, however, the metabolites of a structurally related compound, allyoxidim, 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’ may correspond to the sulfone and sulfoxide, respectively, of clethodim. 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; Campbell and Penner 1987). Metabolite ‘A’ is relatively polar compared to the other two metabolites and clethodim as determined by its retention time. After oxidation, herbicide metabolites are typically conjugated to a more polar product in preparation for sequestration (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 (Culpepper et al. 1999b; Culpepper et al 1999c; Ferreira et al. 1995; Wanamarta and Penner 1986).

**Photosynthetic rate**

Immediately before an application of imazapic, rates of photosynthesis were similar for all goosegrass plants (Figure 2). One DAT, the photosynthetic rate in plants treated
with imazapic had decreased by 5.9 µmol CO₂ m⁻² s⁻¹, and was less at 2, 6, and 8 DAT. The photosynthetic rate in non-treated goosegrass continued to increase. Thus imazapic appears to reduce photosynthetic rate of goosegrass. The reduction in photosynthetic rate of imazapic-treated goosegrass compared with non-treated goosegrass may have implications for ACCase inhibition.

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 ¹⁴C-acetate incorporation into lipids in corn root tips, but not in the less metabolically active root regions (Hosaka 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 et al. 1987). Chlorimuron and pyrithiobac did not specifically affect ACCase activity in vitro (Bjelk and Monaco 1992; Ferreira et al. 1995), but chlorsulfuron reduced lipid synthesis in isolated soybean leaf cells were reduced after 30 min (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 (Table 2). Graminicides require actively growing meristematic regions for inhibition of ACCase (Devine et al. 1993). These data suggest that photosynthetic rate of goosegrass is reduced by treatment with imazapic. Therefore, the requirement for 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 would 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. 1999a; 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 [Digitaria sanguinalis (L.) Scop.] when mixed with bromoxynil were 220, 220, and 290% greater, respectively, than rates required when these graminicides were applied alone (Culpepper et al. 1999a). At 2-3 times registered rates, cyclohexanedione and aryloxyphenoxypropionate herbicides may interfere with plant membranes, thus the observed control by increasing rate may not be due inhibition of ACCase, but rather toxicity at another site of action (Devine and Shimabukuro 1994).

These data presented in the current study suggest that the antagonism of clethodim by imazapic may be influenced by imazapic altering the photosynthesis and/or growth rate of goosegrass and therefore the herbicidal consequences of ACCase inhibition. Clethodim was absorbed and translocated similarly to other cyclohexanedione herbicides, and metabolism of clethodim was not affected by the presence of imazapic. Photosynthetic rates of goosegrass, however, were reduced by imazapic treatment. As the plants were not growing, but did metabolize clethodim, essentially no active herbicide remained in the plant to inhibit any reactivated ACCase. Therefore, imazapic may
prevent the herbicidal activity of the ACCase-inhibiting herbicide clethodim, thus causing the observed antagonism. Further studies are needed to examine whether sensitivity to ACCase inhibiting herbicides can be influenced by environmental factors that slow or inhibit photosynthesis and growth, as was demonstrated with imazapic treatments.

Sources of Materials


2TeeJet Spray Nozzles, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189.

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

4Induce® nonionic low foam wetter/spreader adjuvant. 90% nonionic surfactant (alkylarylpolyoxyalkane ether and isopropanol), free fatty acids, and 10% water. Helena Chemical Company, Suite 500, 6075 Poplar Avenue, Memphis, TN 38137.


6Packard TRI-CARB 2100TR Liquid Scintillation Spectrometer, Packard Instrument Company, 800 Research Parkway, Meriden, CT 06450.

7Model OX-500 Biological Material Oxidizer, R. J. Harvey Instrument Corp., 123 Patterson Street, Hillsdale, NJ 07642.

8Pyrex® Tissue Homogenizer No. 7727-40, Corning Inc., Corning, NY 14831.

10Ultima-Flo™ M Flow Liquid Scintillation Cocktail, Packard Instrument Company, 800 Research Parkway, Meriden, CT 06450.

11Model 715 Waters ULTRA WISP Sample Processor, Waters, 34 Maple St. Milford, MA 01757.

12Model 6000 Waters Chromatography Pump, Waters, 34 Maple St. Milford, MA 01757.

13Model 500 Radiomatic Flo-One Liquid Scintillation Spectrometer, Packard Instrument Company, 800 Research Parkway, Meriden, CT 06450.

14Model 680 Waters Automated Gradient Controller, Waters, 34 Maple St. Milford, MA 01757.

15Allsphere ODS-1 5 μm 250 x 4.6 mm reversed phase column. Alltech Associates, Inc., 2051 Waukegan Rd., Deerfield, IL 60015.

16Model LI-6200 Portable Photosynthesis System, LI-COR, P.O. Box 4425, Lincoln, NE 68504.


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imazaquin, and chlorimuron on the absorption and translocation of the methyl ester of

Culpepper, A. S., A. C. York, and C. Brownie. 1999a. Influence of bromoxynil on

Basis for antagonism in mixtures of bromoxynil plus quizalofop-P applied to yellow

Influence of adjuvants and bromoxynil on absorption of clethodim. Weed Technol.
13: 536-541.

Dean, J. V., J. W. Gronwald, and C. V. Eberlein. 1990. Induction of glutathione S-


Table 1. Influence of harvest timings of 0.5, 1, 4, 8, 24, and 96 h after treatment (HAT) on the distribution of absorbed $^{14}$C in goosegrass averaged over herbicide treatments of $^{14}$C-clethodim and $^{14}$C-clethodim plus imazapic.

<table>
<thead>
<tr>
<th>Harvest timing (h)</th>
<th>Treated leaf</th>
<th>Shoot above</th>
<th>Shoot below</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>36</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>1.0</td>
<td>50</td>
<td>1.2</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>4.0</td>
<td>70</td>
<td>1.6</td>
<td>2.3</td>
<td>0.4</td>
</tr>
<tr>
<td>8.0</td>
<td>79</td>
<td>1.6</td>
<td>2.4</td>
<td>0.4</td>
</tr>
<tr>
<td>24</td>
<td>74</td>
<td>1.8</td>
<td>3.2</td>
<td>0.5</td>
</tr>
<tr>
<td>96</td>
<td>89</td>
<td>4.7</td>
<td>3.6</td>
<td>0.6</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>7</td>
<td>1.6</td>
<td>1.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*Alone, clethodim applied at 140 g ha$^{-1}$; the mixture is imazapic at 70 g ha$^{-1}$ plus clethodim at 140 g ha$^{-1}$.*

*Means within a column separated according to Fisher’s protected LSD (p = 0.05).*
Table 2. Influence of harvest timings of 4, 8, 24, and 48 HAT on the proportion of absorbed \(^{14}\)C-label metabolites in treated leaves of goosegrass averaged over herbicide treatments of \(^{14}\)C-clethodim alone and \(^{14}\)C-clethodim plus imazapic.

<table>
<thead>
<tr>
<th>Metabolite (retention time in min)</th>
<th>A (4.0)</th>
<th>B (14.0)</th>
<th>C (26.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-h- % of total detected metabolites</td>
<td>---------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>42</td>
<td>36</td>
</tr>
<tr>
<td>24</td>
<td>26</td>
<td>46</td>
<td>19</td>
</tr>
<tr>
<td>48</td>
<td>32</td>
<td>42</td>
<td>14</td>
</tr>
<tr>
<td>96</td>
<td>58</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>7</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

\(^a\) Alone, clethodim applied at 140 g ha\(^{-1}\); the mixture is imazapic at 70 g ha\(^{-1}\) plus clethodim at 140 g ha\(^{-1}\).

\(^b\) Means within a column separated according to Fisher’s protected LSD (\(p = 0.05\)).
Figure 1. Foliar absorption of $^{14}$C-clethodim over time by goosegrass averaged over herbicide treatments ($^{14}$C-clethodim or $^{14}$C-clethodim plus imazeic).
Figure 2. Photosynthetic rate ($A_{max}$) response of single non-treated light saturated goosegrass leaves and single light saturated goosegrass leaves treated with imazapic. Error bars indicate standard error of the mean.