Warm-Season Turfgrass Response to Aminocyclopyrachlor

by

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Abstract

Synthetic auxin herbicides are widely used in the turfgrass industry due to their selective control of broadleaf weeds and safety to turfgrass species. Aminocyclopyrachlor (AMCP) is a new synthetic auxin herbicide currently pursuing registration that effectively controls broadleaf weeds. However, injury to warm-season turfgrasses has been reported. Research was conducted to investigate this phenomenon in three areas: 1) quantification of AMCP rates that lead to unacceptable turfgrass injury, 2) safening of turfgrass injury, and 3) the anatomical and morphological response of St. Augustinegrass to AMCP. Results indicate that zoysiagrass is the most tolerant of AMCP. Bermudagrass and centipedegrass have marginal tolerance relative to AMCP rates that effectively control weeds. St. Augustinegrass is the most sensitive of AMCP. Attempts to safen or reduce turfgrass injury through tank-mixing AMCP with various agrochemicals were unsuccessful. The anatomical response of St. Augustinegrass to AMCP is similar to the response of eudicot weeds to other synthetic auxin herbicides and is characterized by vascular inhibition and deleterious growth stimulation.

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2,4-D	2,4-dichlorophenoxyacetic acid
ABP	auxin binding proteins
ACCase	acetyl-CoA carboxylase
ae	acid equivalent
ai	active ingredient
AL	Alabama
AMCP	aminocyclopyrachlor
ANOVA	analysis of variance
C	Celsius
Ca^{2+}	calcium cation
cm	centimeter
cv	cultivar
DNA	deoxyribonucleic acid
EPTC	S-ethyl dipropyl(thiocarbamate)
FAA	formalin-acetic-acid-alcohol
GSTs	glutathione S-transferases
H^{+}	hydrogen cation or proton
ha	hectare

IAA	indole-3-acetic acid
kg	kilogram
L	liter
LS	least squares
m	meter
mm	millimeter
MCPA	2-methyl-4-chlorophenoxyacetic acid
mRNA	messenger ribonucleic acid
MSM	metsulfuron-methyl
MSMA	monosodium methylarsonate
MSO	methylated seed oil
µ	micrometer
NA	naphthalic anhydride
PGC	percent green cover
RNA	ribonucleic acid
s	second
SAS	Statistical Analysis System
VTQ	visual turfgrass quality
v v^{-1}	volume to volume
WAT	weeks after treatment
wk	week
wks	weeks

Literature Review

Synthetic Auxin Herbicides.

Herbicides that act similarly to the endogenous plant hormone auxin (indole-3-acetic acid; IAA) are classified as synthetic auxin herbicides. These herbicides were discovered in the 1940s and became the first herbicides to be widely used due to their selective control of broadleaf weeds in cereal crops (Anderson 1996; Devine et al. 1993; Sanders and Pallett 1987; Sterling and Hall 1997). Also called auxinic or auxin mimicking herbicides, these compounds are different than other herbicide groups in that auxinic herbicides do not have a specific binding site or protein conjugate that inhibits a vital function of the plant. Rather, auxinic herbicides disrupt the hormone balance of the plant leading to a number of responses including increased cell wall plasticity, nucleic acid metabolism, and uncontrolled growth particularly in meristematic regions (Anderson 1996; Beal 1951; Devine et al. 1993). These herbicides are applied post emergence and are rapidly absorbed by plants (Bovey et al. 1983). Absorption into the plant can occur via foliar, root or both mechanisms; however, root absorption is generally less effective (Ross and Lembi 1999). Auxinic herbicides translocate quickly and systemically throughout treated plants and accumulate in the meristems (Anderson 1996; Bovey et al. 1983). Although, treatment with low very rates of auxinic herbicides can lead to growth regulation of treated plants, herbicidal activity occurs with high rates (Devine et al. 1993; Sterling and Hall 1997).

Symptomology. Symptoms of susceptible plants include epinasty of leaves and stems, cupping of new leaf tissue, and uncontrolled and undifferentiated growth including the

elongation and swelling of cells (Sterling and Hall 1997). Early work with growth regulating substances revealed that treated stems of *Coleus* (Lour.) responded with enlarged parenchyma cells, proliferations of cells surrounding and including the cambium, new vascular bundles, and adventitious root formation (Beal 1951). Later, other authors concluded that these responses may contribute to the disruption of vascular function (Anderson 1996; Senseman 2007; Scott 1938; Sterling and Hall 1997). Anderson (1999) summarizes the vascular disruption, "Eventually, the xylem and phloem conduits become disorientated and the phloem conduits become clogged. Disorientation of the transport conduits leads to disruption of the movement of transpiration and photosynthate streams, depriving the plant of assimilates needed for continued growth and development, which in turn results in the collapse and death of the plant."

Additional symptoms include prolific formation of adventitious roots and general loss of cellular function (Callahan and Engel 1965; Gorrell et al. 1988; Kaufman 1955; Ross and Lembi 1999; Scott 1938). Berghaus and Wuerzer (1987) found inhibition of root formation by quinmerac in cleaver (*Galium aparine* L.). Gorrell et al. (1988) reported accumulation of triclopyr in the roots of horsenettle (*Solanum carolinense* L.), which may account for adventitious root formation seen from synthetic auxin treatment. Scott (1938) reported lateral root formation from auxinic substances. Kaufman (1955) found stimulation of meristems, adventitious roots, malformed shoot apices and other highly differentiated tissues in rice (*Oryza* sativa L.). Complete death of susceptible plants occurs slowly (3 to 5 wks) but symptoms appear quickly (2 to 4 days) (Senseman 2007; Sterling and Hall 1997). Conditions that favor photosynthate production, transport, and meristematic activity, such as warm temperatures and long day length, enhance the

phytotoxicity of auxinic herbicides by increasing their mobility within the plant (Anderson 1996; Radosevich and Bayer 1979).

Induction of responses to auxinic herbicides. Once absorbed and translocated, plant tissue responses (noted above) are induced by a number of factors. Due to the complex nature of responses to auxinic herbicide treatment and the incomplete understanding thereof, there is great debate in regards to the specific mechanisms involved and what processes are responsible for precisely what response.

The development of responses occurs in two stages (Devine et al. 1993; Fedtke 1982; Sanders and Pallett 1987). The first stage occurs within 10 minutes of treatment and includes cell wall acidification and cell growth by elongation. The second stage begins about 30 to 45 minutes after treatment and continues until death and includes DNA and RNA transcription activation, leading to mRNA synthesis and protein synthesis (Beal 1951; Fedtke 1982; Sanders and Pallett 1987). There is evidence, however, that transcription occurs at or before cell elongation (Walker and Key 1982; Wei et al. 2000). Clearly, increased transcription leads to a deluge of other responses. Additionally, it should be noted that different plant tissues respond quite dissimilarly to auxin and auxinic herbicides; even similar plant tissues with different stages of physiological development respond differently to treatment (Beal 1951; Devine et al. 1993; Gorrell et al. 1988; Sterling and Hall 1997).

The immediate growth response is thought to be triggered when auxin binds to the plasmalemma, which leads to secondary events with the calcium cation (Ca^{2+}) as the messenger (Devine et al. 1993). The direct secondary event is an auxin-induced proton (H^{+}) excretion into the cell wall which lowers cell wall pH and causes cell wall plasticity

(Devine et al. 1993; Sterling and Hall 1997). Proton excretion also causes increased turgor pressure that leads to cell elongation, which is aided by the increased plasticity of the cell walls (Devine et al. 1993; Fedtke 1982).

The secondary, long term growth response is also thought to be initiated by the binding of auxin to release Ca^{2+} as a secondary messenger, suggesting that Ca^{2+} release could be a single trigger mechanism for auxinic herbicide response (Devine et al. 1993). The increase in Ca^{2+} leads to increased transcription (of RNA polymerases and other enzymes) and gene activation. These two events lead to increased protein production, which are responsible for many tissue responses (Devine et al. 1993; Fedtke 1982; Walker and Key 1982). Transcription causes the production of many enzymes, including ACC synthase (1-aminocyclopropane-1-carboxylic acid) which stimulates ethylene production via the ethylene biosynthesis pathway (Bradford and Yang 1980; Grossmann 2000). Ethylene contributes to morphological changes often observed after treatment including cell elongation. (Grossmann 2000; Hall et al. 1985; Walker and Key 1982; Wei et al. 2000). In nontreated plants, ethylene production is thought to be regulated by the concentration of free auxin (Abeles et al. 1992). Due to this fact, plants are thought to respond to auxinic treatment by attempting to reestablish the delicate hormone balance between auxin and ethylene (Sterling and Hall 1997). Grossmann and Kwiatkowski (1993) proposed evidence that quinclorac (an auxin type herbicide) is phytotoxic due to the production of cyanide from ethylene production by promoting ACC synthesis. Sunohara et al. (2003) reported that light is a factor in the phytotoxic action in maize (*Zea mays* L.) of quinclorac via the enhancement of ethylene biosynthesis. Ethylene biosynthesis leading to the accumulation of cyanide has been observed in monocots

(Grossmann and Kwiatkowski 1993) and eudicots from 2,4-D treatment (Grossmann and Kwiatkowski 1993; Tittle et al. 1990; Senseman 2007). Accumulation of cyanide is thought to contribute to the phytotoxicity of 2,4-D (Tittle et al. 1990). Cyanide may contribute to foliar chlorosis and necrosis (generally observed after cessation of growth and epinasty of stems and leaves) but is not believed to contribute to cessation of growth typically observed within 24 hours after treatment with auxinic herbicides (Scheltrup and Grossmann 1995).

Another major, characteristic response of many plants following treatment with auxinic herbicides is epinasty, or the twisting bending of the foliage and sometimes stem tissues (Senseman 2007). Epinasty is mainly associated with ethylene evolution; however, Scheltrup and Grossmann (1995) attribute leaf epinasty to ethylene activity and stem epinasty to auxin activity (Senseman 2007). Epinasty may also involve the splitting of the stem and tissue proliferation (Sterling and Hall 1997).

Abscisic acid (a plant hormone that promotes leaf senescence, controls growth via stomatal closure, cell division, and expansion) has also been reported to be produced as a response to auxinic herbicide treatment (Grossmann 2000). Absicic acid may be the main contributor to growth retardation, but this information is only speculative (Grossmann et al. 1996; Scheltrup and Grossmann 1995). Notably, abscisic acid accumulation and growth retardation are significantly less in tolerant crops, such as wheat when compared to more susceptible species (Grossmann et al. 1996).

Auxin binding proteins (ABPs) play a critical role in all the responses noted, although their role is not completely understood. Elucidating ABPs role in physiological responses is complicated by the fact that ABPs are known to be localized within several

intercellular and membranous locations (Fedtke 1982; Sterling and Hall 1997). Different ABPs are known to trigger different physiological responses (Sterling and Hall 1997).

Detoxification of auxinic herbicides. Metabolism of auxinic herbicides is similar to that of endogenous auxin. There are two methods of metabolism: the detoxification reactions of oxidation and hydrolation and conjugation reactions with amino acids (Bovey et al. 1983; Devine et al. 1993; Sterling and Hall 1997). The concentration of conjugates is typically 10 to 100 times greater than the concentration of free auxin (Devine et al. 1993; Sterling and Hall 1997). The formation of conjugates acts as a slow release mechanism of free auxin and is therefore not a detoxification mechanism. Detoxification metabolism occurs through oxidation, hydroxylation, and possibly lignin binding of the herbicide (Devine et al. 1993; Scheel and Sandermann 1981).

Selectivity. Selectivity of auxinic herbicides between monocotyledons and eudicotyledons is a subject of debate; confounding the matter is the large number of variables and inability to study each variable individually (Sterling and Hall 1997). Differences in metabolism, morphology, growth physiology, anatomy, auxin responsiveness, uptake, translocation, and even nuclear volume have been studied between monocotyledons and eudicotyledons (Devine et al. 1993; Lewer and Owen 1990; Sterling and Hall 1997).

There is evidence that metabolism in tolerant species (generally monocotyledons) occurs primarily via the irreversible, detoxification mechanisms, and the metabolism in susceptible species (eudicotyledons) is primarily the reversible conjugation formation

(Devine et al. 1993; Schell and Sandermann 1981). Providing support for this hypothesis is the fact that the two different metabolites of the herbicide are transported to different sub-cellular spaces. The detoxified metabolites are transported to the vacuole and the reversible amino acid conjugates are excreted into the cell wall space. The study of tolerant eudicotyledons also supports the hypothesis that differential metabolism leads to the herbicide's selectivity. Studies suggest that 2,4-D (a synthetic auxin herbicide) is detoxified by hydroxylation, glycosylation, and insoluble residue formation in the eudicotyledon birdsfoot trefoil (*Lotus corniculatus* L.) (Davies and Linscott 1986).

Some data that shows tolerance to auxinic herbicides are mainly correlated with the plant's ability to metabolize the herbicide. Many studies have not been able to correlate metabolism with reduced injury and therefore with tolerance (Devine et al. 1993; Lewer and Owen 1989; Sterling and Hall 1997). There is some evidence that differential interaction with auxin leading to increased ethylene biosynthesis in susceptible species results in selectivity (Hall et al. 1985). Hall et al. (1985) reported a direct correlation between injury and increased ethylene production. This correlation was also found by Grossmann and Kwiatkoski (1993) while working with monocotyledons. Clearly, the combined effects from ethylene and auxin could also result in selectivity (Sterling and Hall 1997; Wei et al. 2000). Further evidence of combined effects from ethylene and auxin come from Wei et al. (2000), who found two separate mechanisms that caused transcriptional activation: one from auxin herbicide-induced ethylene and one not mediated by ethylene.

Anatomical considerations. Certain authors believe monocotyledonous plants are tolerant to auxinic herbicides due to the phloem scattered in bundles and surrounded by protective sclerenchyma tissue (Berghaus and Wuerzer 1987; Sterling and Hall 1997). The lack of protective tissue in eudicotyledonous plants contributes to their susceptibility to auxinic herbicides. The cambium (if present) and pericycle also seem to be less sensitive to auxin in monocotyledons (Sterling and Hall 1997). Sensitive plants respond with proliferation in the cambium, phloem, and cortical tissues (Eames 1949a, b; Kaufman 1955; Morey et al. 1976; Scott 1938). Additionally, ethylene stimulation may result in a number of responses including cell division, cell elongation, hypertrophy in the cortical and periderm tissues, and cell wall thickening (Wei et al. 2000; Sanders and Pallett 1987). The vascular elements are affected via abnormal xylem and phloem fiber formation (Eames 1949a; Robnett and Morey 1974; Scott 1938). It seems that anatomical differences may have a major role in tolerance of grasses and other monocots.

All of these responses lead to the overall effect of the entire plant response. Callahan and Engel (1965) reported several anatomical responses of turfgrasses to treatment: abnormal formation of root hairs, adventitious roots, cortical cells, and pericycle cells; accelerated and prolific formation of roots and root hairs; cortical cells 24 times the size of nontreated cells; pericycle cells large in size, with nucleus swollen; lateral roots form abnormally close to root tip. Of course, these responses are noted when compared to a nontreated plant, with overall a very orderly and uniform arrangement; xylem and phloem lack prominence. These disruptions appear to obstruct normal nutrient flow through conductive tissue and destroy the function of many cells (Callahan and Engel 1965)

Triclopyr. Triclopyr is an auxinic, pyridine herbicide that is used in non-crop, pasture, rangeland, and cool-season turfgrass for the control of many annual and some perennial broadleaf weeds (Senseman 2007). However, triclopyr causes severe injury to warm-season turfgrasses, especially to bermudagrass (common- *Cynodon dactylon* Burtt-Davey; hybrid- *C. dactylon* Burtt-Davey X *C. transvaalensis* L. Pers. 'Tifway') (Bell et al. 2000; Cudney et al. 1997; Wehtje 2008). The reason for this intolerance is not fully understood. In tolerant species such as cereals, triclopyr uptake and metabolism are rapid (Bovey et al. 1983; Lewer and Owen 1990; Senseman 2007). Triclopyr is rapidly translocated, primarily in the xylem and phloem (Senseman 2007). Studies conducted by Lewer and Owen (1990) indicated that uptake by eudicotyledons is much slower, but this difference was not found to be the reason for the selectivity of triclopyr. Triclopyr was found to accumulate in the upper leaves and stem of chickweed [*Stellaria media* (L.) Cyrillo; eudicot] compared to no particular accumulation point in cereals (monocot). This difference, however, was not attributed to the selectivity of triclopyr and may be the result of differences in metabolism (Devine et al. 1993). Metabolism of triclopyr was found to be faster in monocotyledons than eudicotyledons. It is important to note that even though absorption and uptake were less for eudicotyledons, the ability of the monocotyledons to quickly metabolize the herbicide results in their tolerance (Lewer and Owen 1990). Metabolism of triclopyr leads to many polar metabolites, which in eudicotyledonous plants are primarily aspirate and glutamate conjugates (Lewer and Owen 1989). The polarity of these metabolites is believed to impair their ability to be transported across membranes (Lewer and Owen 1990). Tolerance to triclopyr is

attributed to this impairment and the ability to metabolize the parent herbicide (Devine et al. 1993). Intolerance is attributed to slower metabolism, which leads to fewer polar metabolites. This reduction results in the swelling and disruption of the chloroplasts, plasmalemma, and tonoplast and accelerated senescence in addition to the common symptoms of synthetic auxin herbicides (Ayling 1976; Sanders and Pallett 1987). Since triclopyr acts as a plant hormone, it is unclear whether all observations are a direct result of triclopyr or if some effects may be attributed to natural plant defenses or attempts to reestablish hormone balance in the plant. Ethylene biosynthesis may be the result of the plant attempting to reestablish hormone balance but lead to other responses observed following auxinic herbicide treatment (Berghaus and Wuerzer 1987; Hall et al. 1985; Sterling and Hall 1997).

Aminocyclopyrachlor (active ingredient in DPX-KJM44 and DPX-MAT28). Aminocyclopyrachlor (AMCP) is a new herbicide active ingredient under development by the E. I. du Pont de Nemours and Company (Wilmington, Delaware) with the experimental formulations DPX-KJM44 and DPX-MAT28. DPX-KJM44 is the methyl formulation and DPX-MAT28 is the free acid formulation. Aminocyclopyrachlor is to be labeled in rights-of-ways, bareground, roadsides, and invasive weed management (Turner et al. 2009). Literature about this compound is limited but several properties are known. AMCP is a pyrimidine carboxylic acid type synthetic auxin herbicide with both foliar and soil activity on susceptible species (Armel et al. 2009; Blair and Lowe 2009; Turner et al. 2009). Typical of synthetic auxin herbicides, AMCP is systemically translocated (Bukun et al. 2009). The herbicide has some soil residual activity, however further research is

necessary in this area (Bukun et al. 2009, Gannon et al. 2009; Westra et al. 2009). Research indicates that this compound may provide excellent control of some difficult weeds such as Virginia buttonweed (*Diodia virginiana* L.) among many other broadleaf weeds (Bukun et al. 2009; Turner et al. 2009). Weeds in the mustard family, however, are not well controlled with AMCP (Westra et al. 2009). AMCP is known to be phytotoxic to warm-season turfgrasses (Brecke et al. 2010). Methylated seed oil is the most effective adjuvant for foliar aminocyclopyrachlor uptake (Bukun et al. 2009; Westra et al. 2009). Initial soil characteristics indicate that soil organic matter and soil pH are the most critical factors influencing adsorption of the compound. Furthermore, the DPX-KJM44 formulation may convert to the DPX-MAT28 formulation in soil. Data indicate that AMCP is metabolized by soil microbes (Lindenmayer and Westra 2009).

Safening of Herbicides

Herbicide safening is a means of improving selectivity by reducing the toxicity of the herbicide to the desirable species without reducing control of weed species (Abu-Qare and Duncan 2002). Safening is accomplished through use of a chemical safener, although in some respects, genetic engineering of plants can also be considered safening (Davies and Caseley 1999; Davies 2001; Parker 1983). Timing of application(s) of some chemicals relative to subsequent application(s) can also lead to safening (Hall and Soni 1989). Hoffmann (1960) reported the first practical application of a safener. Hoffmann found that 4'chloro-2-hydroxyiminoacetanilide and other compounds used as seed treatments safened the seedlings to barban injury. The first commercial herbicide safener was naphthalic anhydride (NA), which was used as a seed-coating to protect maize and

other crops against EPTC or other thiocarbamate herbicide injury (Abu-Qare and Duncan 2002; Parker 1983; Senseman 2007). Since then, many compounds have been screened for potential use as safeners; most have been abandoned due to the result of safening of the herbicide to weed species (control reduction) as well as the desirable species (Phatak and Varvina 1989).

Chemical safening is achieved by combining chemicals or by pretreating plants with one compound followed by another chemical after a certain time period. When combining chemicals, interaction could be synergistic, additive, antagonistic, or no effect. It is important to recognize that any interaction observed could be the result of the inert ingredient(s) of one herbicide formulation reacting to the active ingredient(s) of another compound or vice versa. Furthermore, not all chemical interactions lead to safening. For example, many organic solvents (inert ingredients in a product formulation) increase the postemergence activity of certain herbicides (Putnam and Penner 1974).

Uses of safeners. Safeners have many benefits and uses. Successful safening agents may, depending on the situation, allow a broader range of herbicides to be used or allow the use of herbicides that control a broader spectrum of weeds. By allowing the use of multiple herbicides, the risks of developing herbicide resistant weeds is reduced. There is also the possibility that safeners could allow a cheaper herbicide to be used thus reducing costs to the user (Parker 1983). Safeners could replace some herbicides that are ecologically risky by allowing another herbicide to be used (Caseley and Davies 1999). Greater freedom of crop rotation by protecting crops against injurious herbicide residuals and by controlling volunteer crops from the previous year could also be made possible

through the use of safeners (Davies and Caseley 1999; Phatak and Varvina 1989). Furthermore, safeners could make possible the use of higher rates of herbicides which could increase weed control. Safeners have also shown potential for the control of weeds that are very similar to the desirable crop such as a grass weeds in a turfgrass crop; a difficult challenge even for selective herbicides (Lewis et al. 2007). An interesting application of safeners could be as research tools to investigate a herbicide's mode-of-action which makes up a herbicide's selectivity and resistance (Davies and Caseley 1999).

Mode-of-action of safeners. There are many different mechanisms and interactions between herbicide and safener that can constitute the mode-of-action of a safener, however, the mode-of-action for many chemical safeners is not fully understood (Davies and Caseley 1999). Historically, many hypotheses were proposed for explaining how safeners worked. Safeners were thought to act by enhancing herbicide metabolism, either directly or indirectly. Other hypotheses included competition at the site-of-action, interference with metabolites of the herbicide, and degradation of the epicuticular wax, which leads to increased transpiration and excretion of toxic compounds (Abu-Qare and Duncan 2002; Davies and Caseley 1999). Safeners could also act by reducing or enhancing the absorption and translocation of the herbicide. That is that a safener may act by disruption or competition at the site(s) of entry of the herbicide. Disruptions of the translocation of the herbicide, such as inhibition of the vascular system, could lead to a decreased amount of herbicide reaching the site-of-action within the plant (Davies and Caseley 1999). Hoffman (1960) proposed this antagonism (one chemical interfering with

another chemical) type mode-of-action when working with 2,4-D and barban. Hoffman hypothesized that the increased growth rate caused by 2,4-D antagonized the decreasing growth rate of effect of barban, thus reducing barban injury. A combination of the aforementioned mechanisms was also thought to be responsible for safening action (Vavrina 1987).

The prevailing hypothesis for the mode-of-action of safeners is that safeners function by regulating the expression of genes involved in herbicide metabolism (Davies 2001). This hypothesis includes a wide range of mode-of-actions by proposing that a safener stimulates, inhibits, or by other means influences genes in the plant that regulate safening. Other mechanisms could be inducing degradation, elimination, or stimulation of metabolism. Burton et al. (1994) proposed that safeners work with plant enzymes to detoxify a given herbicide. This would explain why many safener/herbicide combinations are species dependent. That is, the safener may work by combining or conjugating with a specific enzyme that may only be produced by certain species. This new combination of safener + enzyme may subsequently detoxify the herbicide. Also under the hypothesis of gene regulation via xenobiotics, is the potential for safeners to act by increasing herbicide degradation via their own metabolism, which safens the herbicide by essentially keeping the plants metabolic pathways busy with the safener. So called "inducible metabolic activities" or, more accurately "inducible gene expression systems," allow safeners to act by "turning on" a gene that induces the metabolism of an otherwise unsafe herbicide (Yenne et al. 1990). Metabolic detoxification is the mechanism of sulfonylurea resistance in most crops and resistant weed species. Therefore, if metabolism and/or gene expression can be enhanced by a safener, tolerance could be achieved (Burton et al 1994).

Del Buono (2007) found such enhanced gene expression. Studies found the chemical safener benoxacor increased the glutathione S-transferases (GSTs) in tall fescue (*Festuca arundinacea* Shereb.), which act by catalyzing the conjugation of glutathione with many herbicides. FaGST I and FaGST II were found to be particularly inducible from benoxacor treatment (Senseman 2007; Del Buono 2007). Furthermore, Brazier-Hicks et al. (2008) found the safener fenclorim to increase expression of GSTs as well as glycosyltransferases and ATP-binding cassette proteins in *Arabidopsis* [*Arabidopsis thaliana* (L.) Heynh.] (Senseman 2007). Their studies concluded that fenclorim enhances the expression of GSTs which induces the safener's own metabolism. Brazier-Hicks et al. (2008) also stated the enhanced expression of GSTs may result in both detoxification and bioactivation depending upon species. Scarponi et al. (2006) reported that while benoxacor, cloquintocet-mexyl, fenchlorazole-ethyl, fenclorim, and fluxofenim all increased GST activity in wheat wheat (*Triticum aestivum* L.) and maize, only cloquintocet-mexyl, fenchlorazole-ethyl, and fluxofenim enhanced GST activity against butachlor and only benoxacor and fenchlorazole-ethyl enhanced GST activity against terbuthylazine in maize (Senseman 2007). These studies provide evidence that enhanced gene expression can lead to safening through the safeners own metabolism and through enhanced GST expression.

Cytochrome P450 is another large enzyme family that serves in the metabolism of secondary plant metabolites and xenobiotics in plants. While a complete understanding of their role in herbicide detoxification has not been achieved, it is believed that metabolism is achieved by an excellent binding site on the enzyme for many herbicides. Similar to GSTs, stimulation of cytochrome P450s would lead to greater herbicide tolerance in

many species. Many factors contribute to P450 induction: light, herbicides, herbicide safeners, ethanol, heavy metals, and wounding. Many herbicides are known to be metabolized by P450s including certain sulfonylreas, imidazolinones, and triazolopyrimidines. Furthermore, there have been reports of resistant weed populations owing their resistance to enhanced P450 genes; however, due to the many factors that induce P450s, proof of resistance has been elusive (Barrett 2000).

Gene regulation by chemical safeners can be incurred in a variety of ways in addition to herbicide metabolism stimulation stated above. A safener may bind to the target site of the herbicide, thus blocking the activity of the herbicide. Additionally, a safener may bind to the herbicide itself and render the herbicide non-phytotoxic, bind to a promoter of herbicide degrading enzymes, or by other means inhibit herbicide action (Davies and Caseley 1999).

Growth regulators can also be used to increase or decrease metabolism on the whole plant level and lead to safening. For example, water and nutrient flow can be altered, chlorophyll production may be slowed, and herbicide pooling location may be altered via vascular system inhibition or interruption. All these effects can lead to safening (Phatak and Varvina 1989).

Safeners can disrupt the metabolism of endogenous plant compounds, specifically phenolics. Cloquinocet-mexyl applied to wheat seedlings was shown to enhance phenolic metabolism without causing any identifiable stress to the plant (Cummins et al. 2006). This disruption occurs in a similar manner as herbicide and safener activity. The metabolism is influenced by the safener or herbicide binding to sites within the plant that influence metabolism of endogenous compounds. Whatever the mode-of-action, it is

important to remember that for a safener to be successful, it must improve the tolerance of the desirable species, without reducing the control of weed species.

Safening of herbicides related to research. MSMA (monosodium methylarsonate) + triclopry + clopyralid is reported to suppress seashore paspalum (*Paspalum vaginatum*) and produce injury to bermudagrass (*Cynodon* spp.) (Johnson and Duncan 2001; Senseman 2007). If bermudagrass injury occurs, recovery is reported to occur within 1 to 2 weeks following treatment (Johnson and Duncan 2001).

Pretreatment of rapeseed (*Brassica napus* L. cv Altex) with clopyralid rendered picloram less injurious (Hall and Soni 1989). Clopyralid and picloram are both pyridines with auxin-like activity. Picloram was found to increase ethylene evolution in both rapeseed and sunflower (He*lianthus annuus* L.); clopyralid only increased ethylene production in sunflower.

Fluroxypyr (an auxinic herbicide) in combination with diflufenzopyr (herbicide synergist/auxin transport inhibitor) allowed 40% more control of Virginia buttonweed (*Diodia virginiana* L.) while not increasing turfgrass injury. Turfgrasses tested included St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze. 'Raleigh'], hybrid zoysiagrass (*Zoysia japonica* Steud. × *Z. tenufolia* Willd. Ex Trin 'Emerald'), hybrid bermudagrass, and centipedegrass [*Eremochloa ophiuroides* (Munro.) Hack. 'Common'] (Ni et al. 2006).

Diflufenzopyr is thought to make auxinic herbicides more effective by inhibiting the vascular systems which in turn concentrates the herbicide in the meristematic regions (Wehtje 2008). Reportedly, diflufenzopyr reduced the foliar absorption of dicamba in St.

Augustinegrass by 18% and in hybrid zoysiagrass by 16%. In doing so, 20 to 25% less dicamba was needed for control of common lespedeza [*Kummerowia striata* (Thunb.) Schindl.] and purple cudweed (*Gnaphalium purpureum* L.) (Wehtje 2008).

Objectives of Research

Research included three primary objectives to investigate warm-season turfgrass response to AMCP treatment. First, quantification of warm-season turfgrass injury from triclopyr and AMCP was conducted to determine the rate of herbicide at which excessive injury results. This information allowed for the comparison of AMCP to an established herbicide (triclopyr) as well as established an AMCP rate that is tolerable by warm-season turfgrasses. Secondly, a screening of various agro-chemicals admixed with AMCP was conducted to evaluate their safening potential. This objective was an attempt to reduce AMCP induced turfgrass injury. If safening was achieved, it could have allowed the use of AMCP in warm-season turfgrasses for weed control. Thirdly, microscopy was used to observe the anatomical response of warm-season turfgrasses to AMCP treatment to determine if anatomical structure plays a role in susceptibility. For this objective, St. Augustinegrass was be used. Therefore the goal of this research is to gain insight into warm-season turfgrass response to AMCP treatment.

Quantification of Warm-Season Turfgrass Injury to Triclopyr and Aminocyclopyrachlor

Introduction

Synthetic auxin herbicides are widely used in the turfgrass industry due to their effective control of broadleaf weeds and safety in many turfgrass species. Despite the trend of turfgrass safety from auxinic herbicides, injury has been reported from certain herbicides, especially to warm-season turfgrasses. 2,4-D, dicamba, triclopyr, and fluroxypyr have all been reported to be injurious to certain warm-season turfgrasses (Bell et al. 2000; Cudney et al. 1997; Doroh et al. 2009, Johnson 1978, 1995; Johnson and Duncan 2001; Kelly and Coats 2000; Lewis et al. 2008, 2009; McCalla et al. 2004; McElroy et al. 2005; Patton et al. 2010).

Triclopyr is a pyridine carboxylic acid-type synthetic auxin herbicide that exhibits both foliar and soil activity on susceptible species (Senseman 2007). Triclopyr is among the most injurious auxinic herbicides to warm-season turfgrasses. Turflon® Ester, the only turfgrass registration of triclopyr, specifically states in the label, "Do not use on… bermudagrass [*Cynodon dactylon* (L.) Pers.], centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.], St. Augustinegrass [*Stenoaphrum secondatum* (Walter) Kuntze], or zoysiagrass [*Zoysia japonica* Steud.] unless turfgrass injury can be tolerated" (Anonymous 2008). Injury has been reported to centipedegrass, St. Augustinegrass, bermudagrass, and zoysiagrass (Anonymous 2008; Bell et al. 2000; Cudney et al. 1997; Doroh et al. 2009; Lewis et al. 2008, 2009).

Aminocyclopyrachlor (AMCP) is the first in the pyrimidine carboxylic acid class to be utilized for weed control and has structural similarities to triclopyr (Senseman 2007;

Turner et al. 2009). AMCP is a synthetic auxin herbicide under development by the E. I. du Pont de Nemours and Company for use in turfgrass and other markets (Turner et al. 2009). Thus in the future, AMCP may have a similar use pattern to triclopyr. Like triclopyr, reports indicate that AMCP may be injurious to warm-season turfgrasses (Brecke et al. 2010). Also similar to triclopyr, AMCP is effective at low use-rates, has a safe mammalian toxicity, and has a favorable environmental profile (Senseman 2007; Turner et al. 2009). AMCP effectively controls many broadleaf weeds common in turfgrass via foliar and root absorption (Armel et al. 2009; Blair and Lowe 2009; Brecke et al. 2010; Gannon et al. 2009; Turner et al. 2009). However unlike triclopyr, AMCP also provides some residual weed control due to a prolonged soil-life (Gannon et al. 2009; Lindenmayer et al. 2009; Westra et al. 2009). Initial research indicates that AMCP is injurious to certain warm-season turfgrasses such as bermudagrass and St. Augustinegrass (Brecke et al. 2010; Montgomery et al. 2009). Brecke et al. (2010) reported that zoysiagrass was slightly injured from AMCP treatment. Centipedegrass was found to be the most tolerant of AMCP of the warm-season turfgrasses evaluated (Brecke et al. 2010).

We speculate that AMCP may be comparable to triclopyr in respect to warm-season turfgrass tolerance. However, precise quantification of warm-season turfgrass tolerance to triclopyr and AMCP is not available. The objective of this research was to quantify and compare the tolerance of selected warm-season turfgrasses from triclopyr and AMCP.

Materials and Methods

General Information. Warm-season turfgrasses selected for evaluation included: 'Tifsport' bermudagrass [*C. dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy], 'Palmetto' St. Augustinegrass, common centipedegrass, and 'Emerald,' 'Meyer,' and 'Zenith' zoysiagrass [*Z. japonica* Steud. x *Z. tenuifolia* L. var. *matrella* (Willd. ex Thield) Sasaki]. The two herbicides used were the butoxyethyl ester formulation of triclopyr[1] and the methyl ester formulation of AMCP[2] (DPX-KJM44). All experiments were conducted twice (repeated) during 2009. All experiments used a randomized complete block design with four replications per treatment. A single experiment was conducted for bermudagrass, St. Augustinegrass, zoysiagrass, and centipedegrass at each location (either greenhouse or field) and repeated in time for a total of 16 experiments (4 turfgrasses × 2 locations × 2 herbicides). Both field experimental plots and greenhouse pots were mowed twice weekly at a height of 4.5 cm using a rotary style mower. All herbicide-containing treatments also included a methylated seed oil[3] (MSO) adjuvant at 1% v v^{-1}. This adjuvant was included because previous research indicated that AMCP achieves maximum weed control with MSO (Bukun et al. 2009; Gannon et al. 2009; Westra et al. 2009).

Greenhouse Experiments. The greenhouse was located in Auburn, AL. Greenhouse temperatures were maintained between 23 and 26 C, optimal for warm-season turfgrass growth (McCarty 2005). Normal day-time irradiance was supplemented with overhead sodium-halide lights producing 150 µmol m^{-2} s^{-1} at the turf canopy. Total peak irradiance was <800 µmol m^{-2} s^{-1} throughout the experiment. Turfgrasses were prepared for treatment by harvesting 5 cm diameter plugs from mature turfgrass stands from the

Auburn University Turfgrass Research Unit in Auburn, AL. Plugs were potted in 700 cm^3 pots using Wickham sandy loam (fine-loamy, siliceous, subactive, thermic Typic Hapludult) soil. Turfgrasses were allowed to recover and adjust to the greenhouse environment for 4 wks before treatments were applied. Turfgrasses were maintained with weekly fertilizer applications and daily irrigation. 'Emerald' zoysiagrass was used for this experiment. Treatments were spaced on a logarithmic scale, centered around the labeled or projected labeled rate of each respective herbicide as this rate where effective weed control occurs in the field. Treatments included triclopyr applied at 0.001, 0.007, 0.034, 0.21, 1.2, 6.1, and 39 kg ha^{-1}, AMCP applied at 0.001, 0.005, 0.024, 0.11, 0.52, 2.4, and 11 kg ha^{-1}, and a nontreated check. Treatments were applied in a spray volume of 280 L ha^{-1} using an enclosed spray cabinet with a single TeeJet TP8002EVS nozzle[7]. Each experiment was repeated in time and applied on February 17 and March 3, 2009.

Field Experiments. Field experiments on centipedegrass, St. Augustinegrass, and zoysiagrass experiments were conducted at the Auburn University Turfgrass Research Unit, Auburn, AL, and were irrigated. Bermudagrass experiments were conducted at the Auburn University Intramural Fields, Auburn, AL and received only rainfall. Soil type was a Marvyn sandy loam (fine-loamy, kaolinitic, thermic Typic Kanhapludult) for both locations. Plot sizes of 1 m^2 were utilized. Zoysiagrass cultivars evaluated included one experiment on 'Meyer', and 'Zenith' zoysiagrass (both *Z. japonica* species). Treatments were selected in the same manner as greenhouse experiments. Treatments included triclopyr applied at 0.037, 1.2, and 6.8 kg ha^{-1}, AMCP applied at 0.005, 0.111, and 0.52 kg ha^{-1}, and a nontreated check. Treatments were applied in a spray volume of

280 L ha^{-1} using a backpack sprayer with three TeeJet TP8002VS nozzles[7] spaced at 25 cm. Each experiment was repeated in time and applied on May 1 and July 7, 2009.

Data Analysis. Data were collected at 1, 2, 3, 4, 6, and 8 wks after treatment (WAT) and included visual turfgrass quality (VTQ) and percent green cover (PGC) ratings. Visual turf quality ratings were based on a scale of 1 to 9 with 1 corresponding to dead turfgrass, 7 to optimal, healthy turfgrass, and 9 to superior turfgrass. Percent green cover was determined using digital image analysis as described by Richardson et al. (2001), where digital images are taken of each experimental unit and subjected to software to determine PGC. A portable box with an internal light source was affixed to the camera to provide a standard, controlled lighting environment for all images. A Canon Powershot G9[4] camera was used. All camera settings were fixed; all images taken were subjected to identical camera settings. White balance (Tone) was calibrated under lightbox conditions using a standard 18% photographic gray card. Other camera settings included a shutter speed of 1/30 s, ISO 100, and an aperature of F2.8. SigmaScan Pro 5[5] software was used to evaluate each image for green color. Within the software, the saturation range was set from 30 to 100% for bermudagrass, 28 to 100% for zoysiagrass, and 32 to 100% for both centipedegrass and St. Augustinegrass. The hue range was set to 47 to 100% for bermudagrass, 45 to 113% for centipedegrass, and 43 to 113% for both St. Augustinegrass and zoysiagrass. This technique has been successfully used by other authors to evaluate turfgrass green cover in response to herbicide treatment (McCalla et al. 2004; McElroy and Walker 2009).

Data were analyzed separately by rating date (WAT), field and greenhouse experiments, and herbicide (triclopyr or AMCP), respectively. Experimental units consisted of one pot or plot. The nontreated check data were artificially assigned a herbicide rate of 0.0001 kg ai ha^{-1} triclopyr or AMCP for data analysis. Data were subjected to ANOVA in SAS[6] using PROC GLM to test for significance of replication-in-time, herbicide rate, turfgrass species, and allow for lack-of-fit testing. Subsequent analysis was performed with PROC NLIN in SAS using a log-logistic regression technique with the model:

$$y = C + \{[D - C]/[1 + (x/I_{50})^b]\} \quad [1]$$

where y is the response (e.g. VTQ or PGC) at herbicide dose x, D is the upper limit for y (e.g. nontreated turfgrass), C is the lower limit for y (e.g. dead turfgrass), b is the slope of the line at the I_{50}, and I_{50} is rate of herbicide giving a 50% response (50% reduction in VTQ or in PGC). This model has been reported as appropriate for defining dose-response curves by Seefeldt et al. (1995). Best-fit parameters were determined by allowing the four parameters (D, C, b, and I_{50}) to differ for each turfgrass. Best-fit parameters were compared between turfgrasses using an F-test with a significance level of 0.05 (Seefeldt et al. 1995). I_{50} values were used to compare the response of each turfgrass to AMCP and triclopyr, respectively, as I_{50} values are the most accurate estimate possible of turfgrass sensitivity to a herbicide (Seefeldt et al. 1995). True r-squared values cannot be calculated in a log-logistic regression technique. However, pseudo r-squared values were calculated using the formula:

$$\text{Pseudo } R^2 = 1 - SS_{error}/SS_{corrected\ total} \quad [2]$$

where SS_{error} is the sum of squares for the error and $SS_{corrected\ total}$ is the sum of squares for the corrected total (Chism et al. 1992; Draper and Smith 1968). These values were obtained from the output of PROC NLIN in SAS.

Results and Discussion

ANOVA revealed that repetition in time and replications within each experiment were not significant for either field or greenhouse experiments. Therefore data were pooled across repetitions and replications within field and greenhouse experiments, respectively. For all turfgrasses VTQ and PGC decreased as herbicide rate increased.

Turfgrass managers and home owners are generally willing to accept a small amount of turfgrass injury in order to control weeds. Turfgrass injury above this minimally-acceptable-injury threshold is unacceptable, making this threshold the emphasis of this research. For the purposes of this paper, turfgrass injury is characterized by a reduction in VTQ and/or PGC and turfgrass injury is inversely proportional to turfgrass tolerance. Turfgrass injury from herbicide application is dynamic over time. Immediately following herbicide application, no injury is evident. However, as the herbicide enters the plant and takes effect, injury begins to occur and to increase with time. At some time after treatment, maximum injury will result and subsequent recovery from injury will occur if the plant death did not occur from the treatment. The minimally-acceptable-injury level must be determined at the time when maximum turfgrass injury results, as this time is when a herbicide dose has reached its maximum injury potential. Maximum injury across turfgrasses occurred at 4 to 8 WAT (data not shown). Therefore a regression line for each turfgrass by herbicide and data type (VTQ or PGC) was

determined at 6 WAT for both field and greenhouse experiments (Figures 1 and 2). Discussion is based upon the turfgrass response to triclopyr or AMCP at 6 WAT.

Greenhouse Experiment. Lack-of-fit tests were not significant for any curve tested, so the log-logistic model was a proper choice to describe the data (Seefeldt et al. 1995). Corresponding best-fit regression parameters with pseudo r-squared values by turfgrass are listed in Tables 1 to 4. Notably, I_{50} and slope values, respectively, were the same between the two data types (VTQ and PGC) and herbicides when accounting for the standard errors (Tables 1 to 4). Similar estimates from both data types yield accuracy to the slope and I_{50} estimates.

The tolerance of warm-season turfgrasses to triclopyr is minimal as evaluated by VTQ, compared to the use-rate required for adequate weed control (Figures 1b and d). Zoysiagrass is the most tolerant of triclopyr of the turfgrasses tested, with an I_{50} value of 5.9 kg ha^{-1} as determined by VTQ (Table 2). Bermudagrass is the most susceptible to triclopyr, with an I_{50} value of 0.68 kg ha^{-1}. Centipedegrass and St. Augustinegrass are equal in tolerance to triclopyr and median to both zoysiagrass and bermudagrass. Centipedegrass has an I_{50} value of 2.22 kg ha^{-1}, and St. Augustinegrass has an I_{50} value of 2.36 kg ha^{-1}.

As evaluated by PGC, the relative tolerance of the turfgrasses tested was statistically similar to the VTQ evaluation. Zoysiagrass is the most tolerant of triclopyr with an I_{50} value of 5.06 kg ha^{-1} (Table 4). Bermudagrass is the least tolerant with an I_{50} value of 0.39 kg ha^{-1}. Centipedegrass and St. Augustinegrass were intermediate with I_{50} values of 1.31 and 1.80 kg ha^{-1}, respectively. These data indicate that zoysiagrass is about

two times more tolerant of triclopyr than centipedegrass or St. Augustinegrass, and about three times more tolerant than bermudagrass. When comparing the triclopyr tolerance of these turfgrasses to the registered rate range of triclopyr in turfgrass (0.56 to 1.12 kg ha^{-1}), it becomes evident that zoysiagrass is the only turfgrass where triclopyr can be effectively utilized for weed control without injury.

As a whole, the warm-season turfgrasses evaluated exhibited minimal tolerance to AMCP, relative to the use-rate required for adequate weed control (Figures 1a and c). As evaluated by VTQ, zoysiagrass is the most tolerant of the warm-season turfgrasses tested, with an I_{50} value of 0.52 kg ha^{-1} (Table 1). Data indicate that bermudagrass, centipedegrass, and St. Augustinegrass are equally susceptible to AMCP, having I_{50} values of 0.10, 0.07 and 0.09 kg ha^{-1}, respectively.

As evaluated by PGC, a similar trend in herbicide tolerance occurred, i.e. zoysiagrass is the most tolerant of AMCP, with an I_{50} value of 0.56 kg ha^{-1} (Table 3). Bermudagrass, centipedegrass, and St. Augustinegrass are again equal in tolerance to AMCP, with I_{50} values of 0.07, 0.05, and 0.10 kg ha^{-1}, respectively. This trend can be observed in Figure 1c. VTQ and PGC data indicate that zoysiagrass is about five times more tolerant of AMCP than the other warm-season turfgrasses evaluated. The projected labeled rate range for AMCP in turfgrass is 0.05 to 0.09 kg ha^{-1}. However, the labeled rate for AMCP use in warm-season turfgrass is expected to be limited to 0.05 kg ha^{-1}. With this projected use-rate in mind, it becomes evident that zoysiagrass is the only turfgrass where AMCP can be effectively utilized for weed control without the risk of injury (Figure 1a and b). Other turfgrasses may be injured, especially if an exact rate application is not made.

Notably, no differences in the slope (*b*) were detected between turfgrasses for both herbicides and data types (VTQ and PGC), indicating there is equal variation in sensitivity between turfgrass populations to triclopyr and AMCP, respectively (Seefeldt et al. 1995).

In summary, greenhouse research indicates that the tolerance of warm-season turfgrasses to triclopyr and AMCP at labeled or projected labeled rates, respectively, are comparable. Zoysiagrass is the most tolerant of triclopyr and AMCP. Centipedegrass and St. Augustinegrass have similar tolerance to triclopyr and AMCP and are less tolerant than zoysiagrass to triclopyr and AMCP. Bermudagrass is the most sensitive to triclopyr but equal to centipedegrass and St. Augustinegrass in sensitivity to AMCP.

Field Experiment. Similar to the greenhouse experiment, maximum injury across the turfgrasses occurred 4 to 8 WAT (data not shown). However, PROC NLIN did not converge on the 6 WAT field data (most likely due to too few herbicide rates) so no log-logistic regression lines could be plotted. Therefore LS Means with standard errors were plotted and connected with lines to show trends (Figure 2).

Relative triclopyr tolerance of the turfgrass species was comparable between field and greenhouse experiments as evaluated by VTQ and PGC. Zoysiagrass is the most tolerant of the turfgrasses and St. Augustinegrass is the least (Figures 2b and d). Reductions in VTQ and PGC were not as sharp as in the greenhouse but nevertheless present in the field data. Overall, field triclopyr tolerance data support the findings in the greenhouse experiments.

Relative AMCP tolerance of the turfgrass species is comparable between field and greenhouse experiments as determined by both VTQ and PGC—zoysiagrass is clearly the most tolerant to AMCP (Figures 2a and c). St. Augustinegrass is the least tolerant to AMCP, and bermudagrass and centipedegrass are median to zoysiagrass and St. Augustinegrass in tolerance. However, bermudagrass and centipedegrass appear to be tolerant of slightly higher AMCP rates under field conditions compared to greenhouse conditions. Notably, there is no reduction in VTQ or PGC for zoysiagrass, bermudagrass, and centipedegrass from AMCP rates less than or equal to the probable registered rate range, indicating AMCP may be a viable option for weed control in these turfgrasses.

Differences between the field and greenhouse experiments are likely due to environmental and soil characteristic differences. Both triclopyr and AMCP are root absorbed (Gannon et al. 2009; Lindenmayer et al. 2009; Senseman 2007; Westra et al. 2009). The small and limited soil contained in each pot in the greenhouse likely does not accurately mimic field conditions and likely contributed to differences observed between greenhouse and field conditions. Furthermore, greenhouse conditions were kept ideal for St. Augustinegrass growth throughout the experiment. Pots received regular fertilizer application and irrigation, and were not subjected to weed competition. However, field studies were likely not as ideal for St. Augustinegrass growth.

Research Implications. Overall, warm-season turfgrass response to triclopyr data essentially agrees with the Turflon Ester product label, i.e. "Do not use on… bermudagrass, centipedegrass, St. Augustinegrass, or zoysiagrass unless turfgrass injury can be tolerated" (Anonymous 2008). Zoysiagrass may be tolerant enough of triclopyr for

use as a weed control agent, but this tolerance may not be robust enough to allow unlimited use on zoysiagrass across all cultivar and environmental conditions. The ability of bermudagrass to recover from triclopyr injury was also observed in this study, which agrees with many authors (Bell et al. 2000; Cudney et al. 1997; Doroh et al. 2009; Lewis et al. 2009; Patton et al. 2009). Therefore, if temporary bermudagrass injury can be tolerated, then triclopyr may be utilized for weed control in bermudagrass.

When taking the probable registered rate into account for AMCP, zoysiagrass is the only warm-season turfgrass tested to display enough tolerance for the use of AMCP as a weed control agent at the current possible use rate of 0.09 kg ha^{-1} (Figure 1). AMCP may be a viable option for broadleaf weed control in bermudagrass if temporary injury can be tolerated, due to the strong recuperative ability of bermudagrass (observed under field conditions). Bermudagrass injury from AMCP with subsequent recovery has been previously reported (Brecke et al. 2010; Montgomery et al. 2009). Centipedegrass has been reported to be more tolerant of AMCP than either zoysiagrass or bermudagrass (Brecke et al. 2010). However our data do not support this conclusion. Differences in findings may be due to cultivar, environmental, and/or soil characteristic differences. Centipedegrass displayed more tolerance to AMCP under field conditions than greenhouse in this research but was less tolerant than zoysiagrass under both circumstances. Our research also indicates that AMCP should not be used in St. Augustinegrass due to a lack of tolerance, which agrees with previous research (Brecke et al. 2010). The use of AMCP at lower rates will result in less turfgrass injury, however less effective weed control will likely result. Differential cultivar response to herbicides has been previously reported (Bell et al. 2000; Johnson 1978, 1995; McCalla et al. 2004;

McElroy et al. 2005; Turgeon et al. 1974). Thus, future research on cultivars should be conducted.

Results indicate that triclopyr and AMCP use in warm-season turfgrass at high labeled rate ranges may result in turfgrass injury, characterized by a reduction in VTQ and PGC. Sensitivity to either triclopyr and AMCP will vary between turfgrass species. Zoysiagrass has sufficient tolerance of either triclopyr or AMCP to allow their use as weed control agents. Conversely, St. Augustinegrass lacks tolerance to either herbicide. Warm-season turfgrass tolerance of both triclopyr and AMCP is comparable between the two herbicides, with respect to labeled use-rates.

Sources of Materials

[1] Turflon® Ester herbicide, Dow AgroSciences LLC, Indianapolis, IN 46268.

[2] Aminocyclopyrachlor, DPX-KJM44, E. I. du Pont de Nemours and Company, Wilmington, DE 19805.

[3] Methylated seed oil source, Alligare, LLC, Opelika, AL 36801.

[4] Canon® Powershot G9, Canon USA, Inc., Lake Success, NY 11042.

[5] SigmaScan® Pro 5, Systat Software Inc., San Jose, CA 95110.

[6] SAS® Institute v. 9.1, Cary, NC 27513.

[7] TeeJet® TP8002VS and TP8002EVS nozzles, Spraying Systems Co., Wheaton, IL 60187.

Table 1. Best fit regression parameters and their standard errors for each turfgrass species describing visual turfgrass quality response to aminocyclopyrachlor at 6 wks after treatment.

Species	*D** (maximum)	*C** (minimum)	*b** (slope)	I_{50} [a]	Pseudo R^2
	----------1 to 9----------			---kg ha^{-1}---	
Bermudagrass	6.45 ± 0.24	0.91 ± 0.29	1.78 ± 0.74	0.10 ± 0.02	0.82
Centipede	6.45 ± 0.25	0.96 ± 0.28	1.50 ± 0.45	0.07 ± 0.02	0.83
St. Augustinegrass	6.58 ± 0.18	1.10 ± 0.23	1.30 ± 0.28	0.09 ± 0.02	0.90
Zoysiagrass	6.84 ± 0.15	1.63 ± 0.32	1.79 ± 0.63	0.52 ± 0.09	0.86

[a] Aminocyclopyrachlor rate giving a 50% reduction in turfgrass quality.
* Not signficantly different between species.

Table 2. Best fit regression parameters and their standard errors for each turfgrass species describing visual turfgrass quality response to triclopyr at 6 wks after treatment.

Species	*D* (maximum)	*C** (minimum)	*b** (slope)	I_{50} [a]	Pseudo R^2
	----------1 to 9----------			---kg ha^{-1}---	
Bermudagrass	6.31 ± 0.25	1.33 ± 0.37	2.06 ± 1.06	0.68 ± 0.21	0.72
Centipede	5.60 ± 0.27	0.98 ± 0.64	1.84 ± 0.96	2.22 ± 1.05	0.56
St. Augustinegrass	5.52 ± 0.23	1.02 ± 0.56	1.91 ± 0.86	2.36 ± 0.99	0.62
Zoysiagrass	6.65 ± 0.16	1.35 ± 0.47	3.12 ± 8.66	5.90 ± 0.65	0.80

[a] Triclopyr rate giving a 50% reduction in turfgrass quality.
* Not signficantly different between species.

Table 3. Best fit regression parameters and their standard errors for each turfgrass species describing green cover response to aminocyclopyrachlor at 6 wks after treatment.

Species	*D* (maximum)	*C** (minimum)	*b** (slope)	I_{50} [a]	Pseudo R^2
	----------%	----------		---kg ha^{-1}---	
Bermudagrass	52.0 ± 4.15	0.78 ± 4.40	1.56 ± 0.81	0.07 ± 0.03	0.61
Centipede	67.1 ± 3.23	3.62 ± 3.23	1.98 ± 0.58	0.05 ± 0.01	0.80
St. Augustinegrass	82.5 ± 2.67	2.42 ± 3.48	1.16 ± 0.24	0.10 ± 0.02	0.90
Zoysiagrass	81.0 ± 2.65	8.36 ± 6.23	1.44 ± 0.50	0.56 ± 0.14	0.80

[a] Aminocyclopyrachlor rate giving a 50% reduction in green cover.
* Not signficantly different between species.

Table 4. Best fit regression parameters and their standard errors for each turfgrass species describing green cover response to triclopyr at 6 wks after treatment.

Species	*D* (maximum)	*C** (minimum)	*b** (slope)	I_{50} [a]	Pseudo R^2
	----------%----------			---kg ha^{-1}---	
Bermudagrass	44.5 ± 3.19	5.98 ± 4.76	1.41 ± 0.80	0.39 ± 0.21	0.51
Centipede	55.1 ± 3.12	5.72 ± 6.65	2.25 ± 3.35	1.31 ± 0.58	0.55
St. Augustinegrass	65.4 ± 3.45	1.22 ± 9.66	1.16 ± 0.56	1.80 ± 0.91	0.62
Zoysiagrass	81.3 ± 2.78	1.24 ± 2.58	1.95 ± 1.37	5.06 ± 1.24	0.66

[a] Triclopyr rate giving a 50% reduction in green cover.

* Not signficantly different between species.

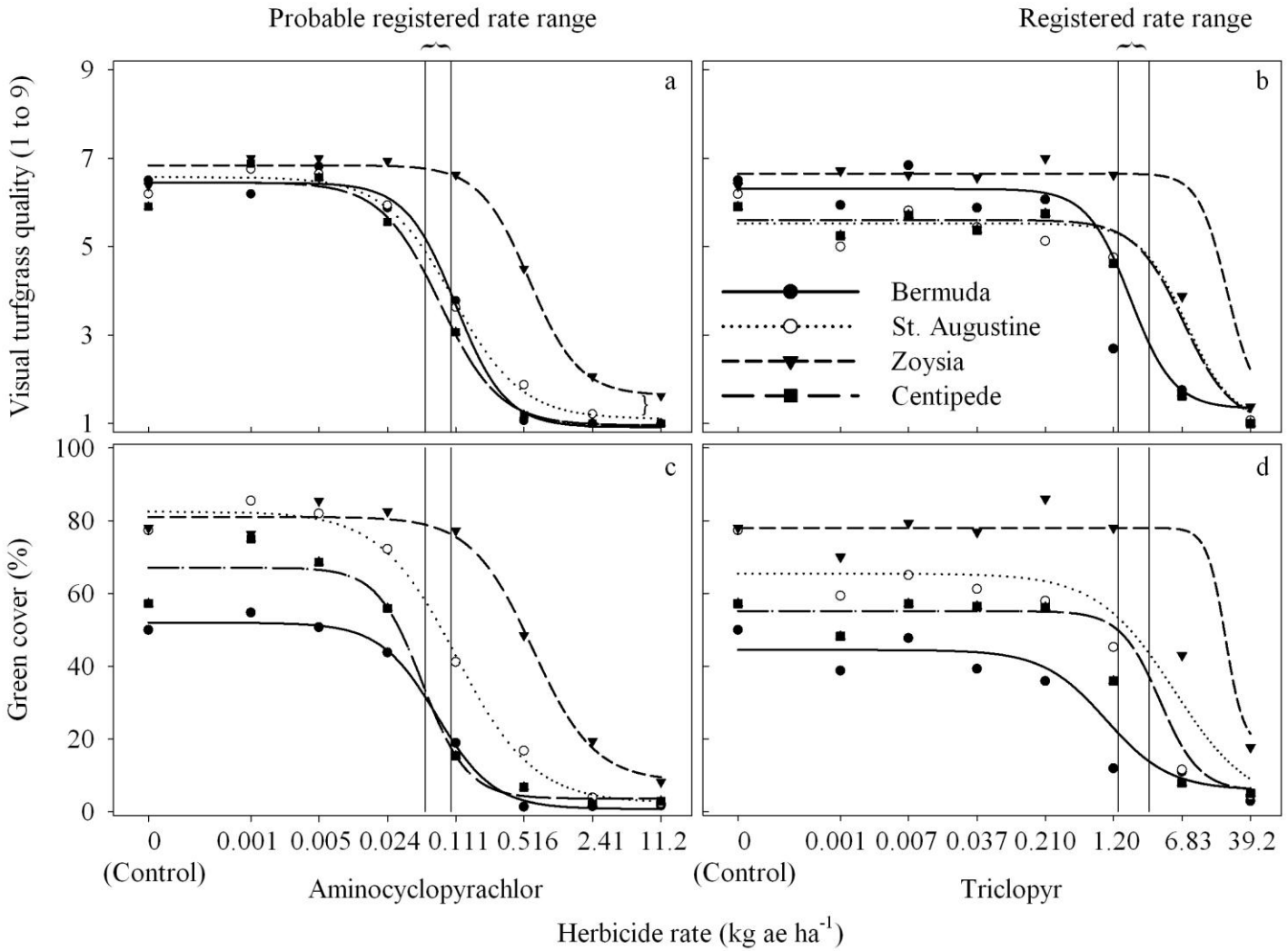

Figure 1. Effect of herbicides on warm-season turfgrasses in the greenhouse 6 weeks after treatment. Points represent LS Means and lines represent the predicted response as determined by log-logistic analysis. Regression parameters with standard errors are listed in Tables 1-4.

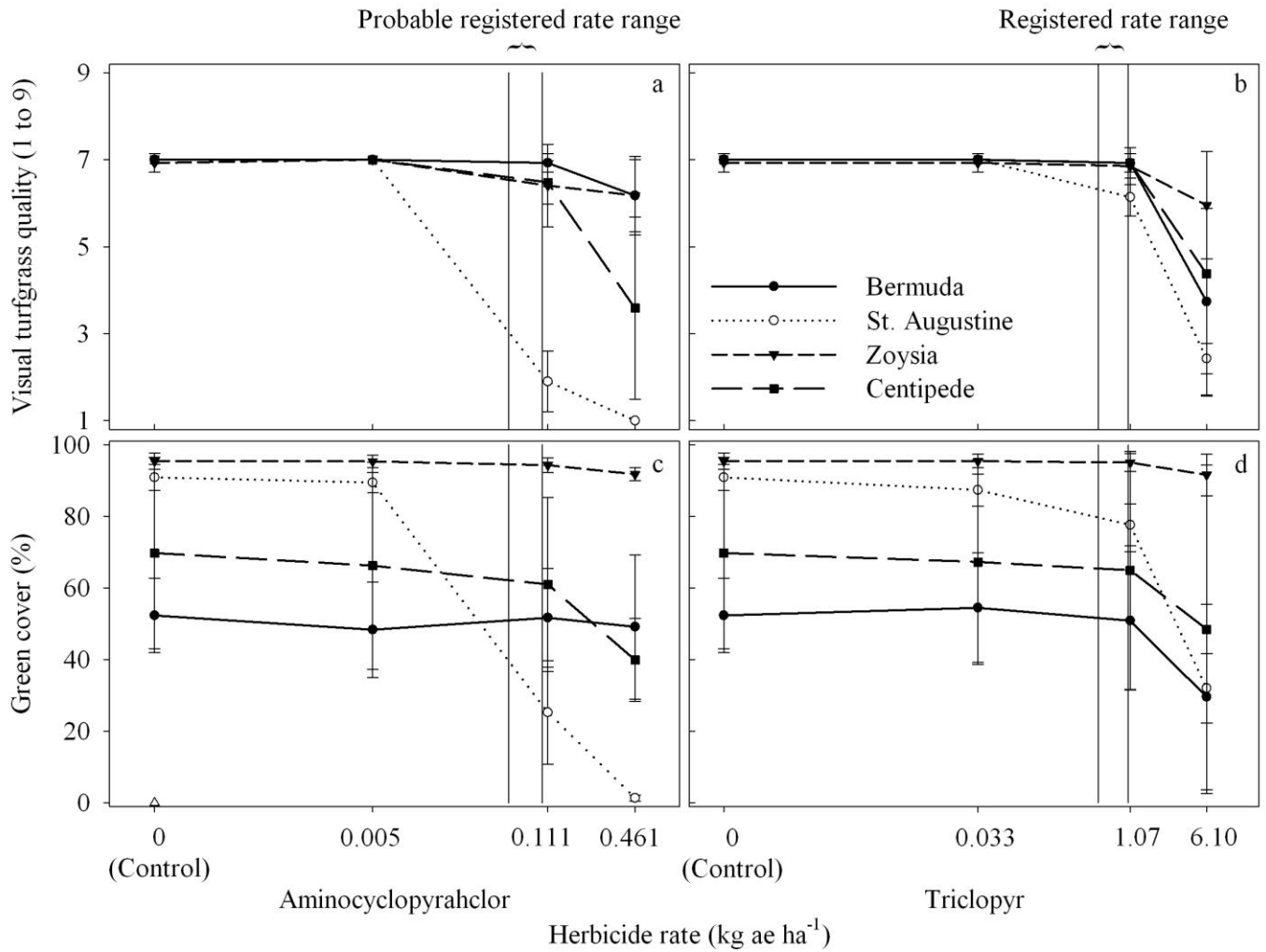

Figure 2. Effect of herbicides on warm-season turfgrasses in the field 6 weeks after treatment. Points represent LS Means with standard error bars. (a) Visual turfgrass quality as effected by aminocyclopyrachlor; (b) visual turfgrass quality as effected by triclopyr; (c) percent green cover as effected by aminocyclopyrachlor; (d) percent green cover as effected by triclopyr.

Safening of Aminocyclopyrachlor to St. Augustinegrass

Introduction

Synthetic auxin herbicides are widely used in turfgrass due to their ability to selectively control broadleaf weeds and safety to turfgrass species (Sterling and Hall 1997). However, the new auxinic herbicide aminocyclopyrachlor (AMCP) is known to be injurious to certain warm-season turfgrasses. Previous greenhouse and field research conducted at Auburn University indicates that AMCP is injurious to centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.], bermudagrass [*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy 'Tifsport'], seashore paspalum (*Paspalum vaginatum* Sw. 'Sea Spray'), St. Augustinegrass [*Stenotaphrum secundatum* (Walter) Kuntze 'Palmetto'], and zoysiagrass [*Zoysia japonica* Steud. x *Z. tenuifolia* L. var. *matrella* (Willd. ex Thield) Sasaki 'Emerald'] (Flessner et al. 2009). Of these five turfgrasses, St. Augustinegrass was the most susceptible of AMCP. This intolerance is especially evident at AMCP rates that provide acceptable weed control (≥0.07 kg ai ha^{-1}). Therefore if AMCP is to be used for weed control in St. Augustinegrass and other warm-season turfgrasses, injury must be reduced.

St. Augustinegrass use in the United States is limited to the south eastern states, due to poor cold tolerance (McCarty 2005). However, St. Augustinegrass is an important and widely used turfgrass in these areas. In Florida, St. Augustinegrass accounts for 84% of the harvestable sod production with a value of over $262 million (Haydu 2003).

Safening is a reduction in herbicide induced turfgrass injury through the addition of another agrochemical. Generally, safening is extremely difficult to achieve. Many factors can influence herbicide safening—herbicide rate, plant species, mode of action,

formulations and adjuvants, growth stage, chemical interactions between two agrochemicals, and time interval between applications (Abu-Qare and Duncan 2002; Davies and Caseley 1999; Green 1989). Herbicide rate has obvious consequences in relation to herbicide safening. Olson and Nalewaja (1981) observed that as MCPA (an auxinic herbicide) rate increased in combination with diclofop, injury to wild oat (*Avena fatua* L.) was reduced. Another factor that can influence safening is plant species. Similar plant species generally respond likewise to herbicide mixtures; however, sometimes species do respond differently (Green 1989). An ideal scenario is when an agrochemical combination shows safening on the desired species and increased activity on the weed species. While exceedingly rare, this interaction has been observed in warm-season turfgrass by Lewis et al. (2007) who reported triclopyr and fluazifop combinations increased bermudagrass control and increased safety to zoysiagrass. Ni et al. (2006) also reported increased Virginia buttonweed (*Diodia virginiana* L.) control and increased turfgrass safety with a fluoxypyr and diflufenzopyr combination. Herbicides with different modes of action may lead to physiological antagonisms and safening effects (Green 1989). Combinations of diclofop and auxinic herbicides have been shown to reduce oat (*Avena sativa* L. and *A. fatua* L.) and barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] injury. Researchers attributed this effect to an interaction at the apical meristem—where auxinic compounds are known to be active (Deschamps et al. 1990; Fletcher and Drexler 1980; Olson and Nalewaja 1981). Indeed, Todd and Stobbe (1980) confirmed a decrease in translocation of diclofop-methyl to the apical meristem when applied with an auxinic herbicide, resulting in insufficient toxicant to permanently disrupt meristematic activity. Growth regulation may serve to safen the herbicide combination by

slowing the metabolism at the whole-plant level, thereby slowing the metabolism of the herbicide (Abu-Qare and Duncan 2002; Davies and Caseley 1999; Green 1989).

Particular to auxinic herbicides such as AMCP, different processes may lead to safening. The use of auxin transport inhibitors may serve to safen the herbicide combination by slowing the translocation of the auxin-type herbicide within the plant (Ni et al. 2006). Auxinic herbicides are known stimulate ethylene biosynthesis; ethylene inhibitors may reduce some secondary auxinic herbicide injury. Other compounds that influence hormone balance within the plant may similarly reduce injury. The objective of this preliminary research was to screen various AMCP + agrochemical combinations for their safening ability (reduction in turfgrass injury) in St. Augustinegrass.

Materials and Methods

A series of both field and greenhouse studies were conducted to assess the safening potential of the treatments to St. Augustinegrass. In field studies, treatments were applied to 1 m^2 plots of mature 'Palmetto' ® St. Augustinegrass at the Auburn University Turfgrass Research Unit, Auburn, Alabama. In the greenhouse trials, treatments were applied to St. Augustinegrass that was transplanted as 5 cm diameter plugs harvested from a mature turfgrass stand. Plugs were placed in 700 cm^3 pots and allowed to acclimate to the greenhouse environment for at least 4 weeks before treatment. All research was conducted while St. Augustinegrass was actively growing.

Treatments in the field were applied using a handheld sprayer with four TeeJet TP8002VS[1] nozzles using CO_2 propellant and calibrated to deliver a 280 L ha^{-1} spray volume. In the greenhouse, treatments were applied in a stationary spray chamber using

TeeJet TP8002EVS nozzle[1], compressed air as propellant, and also calibrated to deliver a 280 L ha^{-1} spray volume. Agrochemical combinations (treatments) were combined with 0.11 kg ha^{-1} AMCP. Each experiment also included a nontreated and an AMCP alone control treatments. All treatments were applied with methylated seed oil (MSO) at 1% v v^{-1}. This adjuvant was included because previous research indicates that AMCP achieves maximum weed control with MSO (Bukun et al. 2009; Gannon et al. 2009; Westra et al. 2009). DPX-KJM44 (methyl-ester formulation of AMCP) or DPX-MAT28 (free-acid formulation of AMCP) was used as noted. Due to the fact that AMCP is an experimental compound, it was difficult to maintain a consistent supply of either formulation; therefore this research was conducted under the assumption that the response of St. Augustinegrass was similar to both formulations. This assumption was found to be valid in Field Experiment Two (below), which was the only experiment where both formulations of AMCP were used in the same experiment. Plots or pots were arranged in a randomized complete block design with 3 or 4 replications as noted. Sources of chemicals are listed in Table 11.

In all experiments, visual injury assessment was used to assess turfgrass response to the herbicide treatment. Visual ratings were based on a 0 to 100 scale where 0 represents no injury and 100 corresponds to complete plant death. Some studies also included percent green cover assessment. Percent green cover was determined via digital image analysis in Sigma Scan Pro 5[2] as described by Richardson et al. (2001). A portable box with an internal light source was affixed to the camera to provide a standard, controlled lighting environment for all images. Digital images were taken to determine percent cover of the treated turfgrass. To quantify green cover from the images with the

software, the saturation range was set from 32 to 100 and the hue range was set to 43 to 113. Both greenhouse and field measurements were taken 1, 2, 3, 4, and 6 weeks after treatment (WAT). Data were subjected to PROC MIXED in SAS®[3] and the differences of least square means were separated by individually comparing each treatment to AMCP alone or the nontreated check, respectively. The alpha level was adjusted to control the type I error according to 0.05/n where n is the number of pairwise comparisons (Littell et al. 2006; Onofri et al. 2010).

An experimental timeline is shown in Figure 3. Generally, treatments that showed potential for safening were repeated in a following study to verify the results. Also, each experiment introduced new treatments to further evaluate agrochemical combinations for safening potential. Agrochemicals evaluated were selected based upon various hypotheses previously discussed.

Two initial greenhouse trials (Greenhouse Experiments One and Two) were conducted in the winter and early spring of 2008-09 (Figure 3). Treatments are listed in Table 5 and 6. These experiments used 3 replications and the DPX-KJM44 formulation of AMCP. Data collected was limited to visual injury assessment.

A field study (Field Experiment One) was conducted following initial greenhouse trials (Figure 3). Treatments were selected based upon greenhouse treatments that showed potential safening (Table 7). This study utilized 4 replications and the DPX-KJM44 formulation of AMCP. This field study was repeated in the greenhouse (Greenhouse Experiment Three; Table 8). Visual injury assessment and percent green cover analysis was conducted.

An additional field study (Field Experiment Two) was conducted to evaluate the substituted urea herbicides diuron and siduron in combination with the DPX-MAT28 (free acid formulation) and DPX-KJM44 (methyl ester formulation) formulations of AMCP (Figure 3; Table 9). This study was conducted as these treatments possessed the greatest potential for safening, as observed in both the field and greenhouse studies. This additional field study was evaluated with percent cover analysis and visual injury assessment. This study used 4 replications.

Due to a lack of safening observed by previous studies, a final greenhouse (Greenhouse Experiment Four) study was initiated involving compounds that are known to influence plant hormones (Figure 3). Some additional treatments were included to further explore existing agro-chemical combinations. Treatments are shown in Table 10. This experiment used 4 replications and the DPX-MAT28 formulation of AMCP. Data collected included visual injury assessment.

Results and Discussion

Generally, the most injury to St. Augustinegrass occurred 4 to 6 WAT. With the exception of the nontreated check, injury increased as time after treatment increased; no recovery from injury was observed by any treatment in any experiment. Results presented were based upon WAT as a parameter in the model; estimates are indirectly related to time after treatment and represent an overall representation of injury observed through the duration of the study. For the purposes of this discussion, a reduction in turfgrass injury or an increase in green cover is equivalent to safening. Injury estimates are given relative to the AMCP alone treatment and the nontreated check treatment.

Greenhouse Experiment One. While a large range of herbicides and growth regulator combinations were tested, none proved to be safer (less injurious) than AMCP applied alone (Table 5). AMCP alone injured St. Augustinegrass 19 % more than the nontreated check. Ethofumesate at 0.42 kg ha^{-1}, glypohsate at 0.14 kg ha^{-1}, clopyralid, and paclobutrazol did exhibit a slight safening effect. While this trend was not statistically significant, it may indicate combinations that could be applied at different rates, thus resulting in a safening effect. Fenoxaprop and clodinafop + AMCP combinations increased injury to St. Augustinegrass compared to AMCP applied alone. Fenoxaprop + AMPC greatly increased injury compared to AMCP alone and may be a viable combination for burn-down weed control, although further research is necessary. Lastly, no herbicide combination was similar to the nontreated check.

Greenhouse Experiment Two. Similar to greenhouse experiment one, most combinations did not result in differences compared to AMCP alone (Table 6). Trinexapac-ethyl + AMCP was the only treatment to reduce injury (safen) compared to AMCP alone and was similar to the nontreated check. Trinexapac-ethyl + AMCP reduced St. Augustinegrass injury by 30% compared to AMCP alone. Diuron, glyphosate (at 0.02 and 0.14 kg ha^{-1}), clopyralid, siduron, and diflufenzypyr (at 0.03 kg ha^{-1}) in combination with AMCP all resulted in slightly less injury compared to AMCP alone but results were not statistically significant. No treatments were similar to the nontreated check in this experiment.

Field Experiment One and Greenhouse Experiment Three. This experiment was conducted in the field and greenhouse. Statistical analysis indicated that location (greenhouse and field) was significant and data could not be combined. Therefore data are presented separately (Table 7 and 8).

The field experiment identified three safened treatment combinations—diuron, metsulfuron-methyl (MSM), and chelated iron + AMCP respectively (Table 7). Diuron + AMCP resulted in 20% less injury and 19% more green cover than AMCP alone. However, diuron + AMCP was not similar to the nontreated check. Metsulfuron-methyl and chelated iron + AMCP combinations only marginally reduced injury compared to AMCP alone. Injury was reduced by 5 and 5%, respectively, and green cover was increased 9 and 8%, respectively. Three treatments resulted in safening as evaluated by green cover, but not as evaluated by visual injury-- trinexapac-ethyl at all rates, glyphosate at 0.02 kg ha^{-1} and clopyralid in combination with AMCP all increased green cover compared to AMCP alone but did not reduce injury compared to AMCP alone. AMCP alone resulted in 71% more injury and 61% less green cover compared to the nontreated check.

The greenhouse experiment resulted in no safened treatments compared to AMCP alone (Table 8). Diuron, trinexapac-ethyl, and clopyralid in combination with AMCP all increased visual injury compared to AMCP alone. These results are in contrast to the results of greenhouse experiments one and two, where these treatments were similar to AMCP alone. Diuron + AMPC caused the most injury of any treatment in this experiment, resulting in 52% more injury and 41% less green cover than AMCP alone. This result is in contrast to Field Experiment One, where diuron + AMCP reduced St.

Augustinegrass injury. The difference in the result of the diuron + AMCP combination may be due to differences in irrigation between the greenhouse and the field. Diuron is almost exclusively root absorbed and must be moved into the soil profile before entering the soil and available for uptake by the plant (Senseman 2007). The field experiment did not receive irrigation for more than 48 hours after treatment while the greenhouse experiment received irrigation 24 hours after treatment. No treatments resulted in more green cover compared to AMCP alone. AMCP alone resulted in 45% more injury and 37% less green cover than the nontreated check.

Field Experiment Two. Statistical analysis did not detect differences between the DPX-KJM44 and DPX-MAT28 formulations of AMCP. Therefore data were pooled across formulations and presented accordingly.

No treatments reduced injury compared to AMCP alone (Table 9). Diuron + AMCP increased visual injury 67% and decreased green cover 72% compared to AMCP alone. Siduron at all rates tested + AMCP combinations did not change injury compared to AMCP alone, indicating that siduron may be combined with AMCP to control a larger weed spectrum in tolerant crops. The nontreated check was 23% less injured and had 8% more green cover.

Greenhouse Experiment Four. Since no combinations tested to this point resulted in a significant safening of injury to St. Augustinegrass, compounds that are known hormone disruptors were tested, with the addition of other agrochemicals not previously tested (Table 10).

St. Augustinegrass visual injury was similar with most agrochemical combinations. However, two combinations did result in safening. Simazine and aminoethoxyvinylglycine in combination with AMCP reduced injury 22 and 14%, respectively. Furthermore, these two combinations were similar to the nontreated check. Therefore these agrochemical combinations need further research to fully evaluate their safening potential. Notably, imazapyr + AMCP resulted in 26% more injury compared to AMCP alone. AMCP alone resulted in 24% more injury compared to the nontreated check in this experiment. This treatment may be utilized for burn-down type weed control.

Summary. While thirty six agrochemcials were screened at various rates, no chemical + AMCP combinations were identified that consistently safened (reduced) St. Augustinegrass injury compared to AMCP alone. Diuron, MSM, chelated iron, and trinexapac-ethyl were the only treatments to safen the injury from any experiment. However, safening was <5% or highly inconsistent. The variability may be due to differences in environmental conditions when treated (Abu-Qare and Duncan 2002; Davies and Caseley 1999; Green 1989). However, further research is also necessary to elucidate the variability in the responses observed. AMCP + simazine did result in safening but was only tested in the final experiment (Greenhouse Experiment Four). Therefore further research is necessary before a sound conclusion as to this combination's safening potential can be made.

Overall, this research agrees with previous reports that safened herbicide combinations are difficult to achieve and generally very unique to the combination of

herbicides used and the species treated (Green 1989; Davies and Caseley 1999). Previous reports of synthetic auxin herbicide safening through the addition of an aryloxyphenoxypropionate herbicide (Fops) were not confirmed in this research (Lewis et al. 2007; Olson and Nalewaja 1981; Scherder et al. 2005). In fact, fenoxaprop, clodinafop, and fluazifop all resulted in >28% more St. Augustinegrass injury. This difference is most likely due to species differences between current and previous research. Research also suggested acetyl-CoA carboxylase (ACCase)-inhibiting herbicides combined with auxinic herbicides may result in safening (Scherder et al. 2005). However, no AMCP + ACCase combinations tested resulted in safening. Ni et al. (2006) reported that the synthetic auxin herbicide fluoxypyr was safened with the addition of the auxin transport inhibitor diflufenzopyr. However, an AMCP + diflufenzopyr combination did not result in safening. Other auxin transport inhibitors showed similar results-- a lack of safening. Finally, previous research suggests herbicide combinations with plant growth regulators may result in safening (Abu-Qare and Duncan 2002; Davies and Caseley 1999; Green 1989). Again, combinations of the plant growth regulators tested and AMCP did not result in safening.

Although safening was not observed, certain AMCP + agrochemcial combinations may have utility. Combinations that resulted in significantly more injury may be utilized for burn-down weed control. These combinations include AMCP + fenoxaprop, clodinafop, fluazifop, imazapyr, or MSMA, which resulted in 67, 50, 28, 26, and 25 % more injury than AMCP alone, respectively (Table 5). Other AMCP + agrochemcial combinations did not result in safening, but did not increase injury either. Potentially, these combinations may be utilized in AMCP tolerant species to control a

broader spectrum or weeds or to increase activity on marginally controlled weeds, compared to AMCP alone. These combinations include AMCP + melfluidide, flucarbazone, imazapic, clopyralid, siduron, 2,4-D, mesotrione, metribuzin, or fluoxypyr. Finally, many plant growth regulators combined with AMCP did not increase St. Augustinegrass injury. These combinations may be utilized to treated tolerant turfgrasses for weed control and plant growth with a single application, as opposed to multiple applications. These combinations include AMCP + ethofumesate, trinexapac-ethyl, paclobutrazol, or low rates of glyphosate. Further research is necessary in each of these scenarios before utilization of these combinations in the field.

Sources of Materials

[1] TeeJet TP8002VS and TP8002EVS nozzles, Spraying Systems Co., Wheaton, IL 60187.

[2] Sigma Scan Pro 5, Systat Software Inc., San Jose, CA 95110.

[3] SAS Institute v. 9.1, Cary, NC 27513.

Table 5. Greenhouse Experiment One. Change in aminocyclopyrachlor induced St. Augustinegrass visual injury due to tank mixing with selected agrochemicals. Positive numbers indicate an increase in St. Augustinegrass injury whereas negative numbers indicate a decrease in injury realtive to aminocyclopyrachlor alone or the nontreated check respectively.

Tank-mixed additive to AMCP[a]			% Visual injury	
Trade name	Active ingredient	Rate	AMCP	Nontreated
		kg ha^{-1}	----relative change[b]----	
none (AMCP alone)	---	---	---	**19**
Acclaim Extra	fenoxaprop	0.14	**67**	**87**
Cutless	flurprimidol	1.12	**12**	**31**
Discover	clodinafop	0.09	**50**	**70**
Embark	melfluidide	0.28	3	**23**
Everest	flucarbazone	0.67	1	**20**
Fusillade II	fluazifop	0.11	**28**	**47**
Plateau	imazapic	0.03	3	**22**
Primo Maxx	trinexapac-ethyl	0.44[c]	5	**24**
Prograss	ethofumesate	0.42	0	**19**
Prograss	ethofumesate	1.68	8	**27**
RoundUp Pro	glyphosate	0.02	6	**26**
RoundUp Pro	glyphosate	0.04	2	**22**
RoundUp Pro	glyphosate	0.14	-1	**18**
Target 6 Plus	MSMA	2.2	**25**	**45**
Target 6 Plus + Transline	MSMA + clopyralid	2.2 + 0.10	**13**	**33**
Target 6 Plus + Turflon Ester	MSMA +	2.2 + 0.31	**28**	**48**
Transline	clopyralid	0.5	-5	**15**
Trimmit	paclobutrazol	1.12	-2	**17**
Turflon Ester	triclopyr	0.14	**17**	**37**
Turflon Ester	triclopyr	0.28	**19**	**39**
nontreated	---	---	**-19**	---

[a] All treatments combined with 1 % v v^{-1} methylated seed oil; DPX-KJM44 formulation of AMCP used at 0.11 kg ha^{-1}.

[b] Bold bumbers indicate significance at the 0.002 alpha level (adjusted to control type I error according to 0.05/n where n is the number of pairwise comparisons).

[c] Rate in L ha^{-1}.

[d] Abbreviations: AMCP, aminocyclopyrachlor.

Table 6. Greenhouse Experiment Two. Change in aminocyclopyrachlor induced St. Augustinegrass visual injury due to tank mixing with selected agrochemicals. Positive numbers indicate an increase in St. Augustinegrass injury whereas negative numbers indicate a decrease in injury realtive to aminocyclopyrachlor alone or the nontreated check respectively.

Tank-mixed additive to AMCP[a]			% Visual injury	
Trade name	Active ingredient	Rate	AMCP	Nontreated
		kg ha^{-1}	---relative change[b]---	
none (AMCP alone)	---	---	---	**32**
Cotoran	flumeturon	2.24	**19**	**51**
Diuron 4L	diuron	2.24	-13	**19**
Primo Maxx	trinexapac-ethyl	0.44[c]	**-30**	3
Prograss	ethofumesate	0.42	-5	**27**
RoundUp Pro	glyphosate	0.02	-6	**27**
RoundUp Pro	glyphosate	0.04	4	**36**
RoundUp Pro	glyphosate	0.14	-10	**22**
Transline	clopyralid	0.50	-11	**22**
Trimmit	paclobutrazol	1.12	5	**37**
Tupersan	siduron	4.48	-11	**22**
Vista	fluoxypyr	0.42	2	**34**
---	diflufenzypyr	0.03	-2	**31**
---	diflufenzypyr	0.06	2	**34**
nontreated	---	---	**-32**	---

[a] All treatments combined with 1 % v v^{-1} methylated seed oil; DPX-KJM44 formulation of AMCP used at 0.11 kg ha^{-1}.

[b] Bold bumbers indicate significance at the 0.004 alpha level (adjusted to control type I error according to 0.05/n where n is the number of pairwise comparisons).

[c] Rate in L ha^{-1}.

[d] Abbreviations: AMCP, aminocyclopyrachlor.

Table 7. Change in aminocyclopyrachlor induced St. Augustinegrass visual injury or green cover due to tank mixing with selected agrochemicals. Positive numbers indicate a relative increase in St. Augustinegrass visual injury or green cover whereas negative numbers indicate a relative decrease in visual injury or green cover.

Tank-mixed additive to AMCP[a]			% Visual injury		% Green cover	
Trade name	Active ingredient	Rate	AMCP	Nontreated check	AMCP	Nontreated check
		---kg ha^{-1}---	----------relative change[b]----------			
DPX-KJM44	AMCP	0.11	---	**71**	---	**-62**
Diuron 4L	diuron	2.24	**-20**	**50**	**19**	**-43**
MSM	MSM	0.04	**-5**	**65**	**9**	**-53**
Primo Maxx	trinexapac-ethyl	0.06[c]	-3	**68**	**5**	**-57**
Primo Maxx	trinexapac-ethyl	0.33[c]	-3	**68**	**4**	**-58**
Primo Maxx	trinexapac-ethyl	0.47[c]	-2	**69**	**7**	**-55**
RoundUp Pro	glyphosate	0.02	-2	**69**	**3**	**-59**
RoundUp Pro	glyphosate	0.14	2	**72**	0	**-62**
Sprint 330	chelated iron	814[c]	**-5**	**66**	**8**	**-55**
Transline	clopyralid	0.5	-3	1	**6**	**-56**
nontreated	---	---	**-71**	---	**62**	---

[a] All treatments combined with 1 % v v^{-1} methylated seed oil; DPX-KJM44 formulation of AMCP used at 0.11 kg ha^{-}

[b] Bold bumbers indicate significance at the 0.004 alpha level (adjusted to control type I error according to 0.05/n where n is the number of pairwise comparisons).

[c] Rate in L ha^{-1}.

[d] Abbreviations: AMCP, aminocyclopyrachlor.

Table 8. Greenhouse Experiment Three. Change in aminocyclopyrachlor induced St. Augustinegrass visual injury or green cover due to tank mixing with selected agrochemicals. Positive numbers indicate an increase in St. Augustinegrass injury or green cover whereas negative numbers indicate a decrease in injury or green cover realtive to aminocyclopyrachlor alone or the nontreated check respectively.

Tank-mixed additive to AMCP[a]			% Visual injury		% Green cover	
Trade name	Active ingredient	Rate	AMCP	Nontreated	AMCP	Nontreated
		kg ha^{-1}	----------relative change[b]----------			
none (AMCP alone)	---	---	---	**40**	---	**-38**
Diuron 4L	diuron	2.24	**53**	**93**	**-41**	**-79**
MSM	MSM	0.04	5	**46**	1	**-37**
Primo Maxx	trinexapac-ethyl	0.06[c]	**7**	**47**	0	**-38**
Primo Maxx	trinexapac-ethyl	0.33[c]	5	**45**	1	**-36**
Primo Maxx	trinexapac-ethyl	0.47[c]	-1	**39**	7	**-31**
RoundUp Pro	glyphosate	0.02	1	**41**	5	**-33**
RoundUp Pro	glyphosate	0.14	1	**41**	5	**-33**
Sprint 330	chelated iron	814[c]	5	**46**	-1	**-39**
Transline	clopyralid	0.5	**8**	**48**	-1	**-39**
nontreated	---	---	**-40**	---	**38**	---

[a] All treatments combined with 1 % v v^{-1} methylated seed oil; DPX-KJM44 formulation of AMCP used at 0.11 kg ha^{-1}.

[b] Bold bumbers indicate significance at the 0.004 alpha level (adjusted to control type I error according to 0.05/n where n is the number of pairwise comparisons).

[c] Rate in L ha^{-1}.

[d] Abbreviations: AMCP, aminocyclopyrachlor.

Table 9. Field Experiment Two. Change in aminocyclopyrachlor induced St. Augustinegrass visual injury or green cover due to tank mixing with selected agrochemicals. Positive numbers indicate an increase in St. Augustinegrass injury or green cover whereas negative numbers indicate a decrease in injury or green cover realtive to aminocyclopyrachlor alone or the nontreated check respectively.

Tank-mixed additive to AMCP[a]			% Visual injury		% Green cover	
Trade name	Active ingredient	Rate	AMCP	Nontreated	AMCP	Nontreated
		kg ha^{-1}	----------relative change[b]----------			
none (AMCP alone)	---	---	---	**23**	---	-8
Diuron 4L	diuron	2.24	**67**	**90**	**-72**	**-80**
Tupersan	siduron	2.24	0	**22**	0	-8
Tupersan	siduron	4.48	-3	**19**	-5	-12
Tupersan	siduron	13.5	-2	**20**	-4	-12
nontreated	---	---	**-23**	---	8	---

[a] All treatments combined with 1 % v v^{-1} methylated seed oil; DPX-KJM44 formulation of AMCP used at 0.11 kg ha^{-1}.

[b] Bold bumbers indicate significance at the 0.001 alpha level (adjusted to control type I error according to 0.05/n where n is the number of pairwise comparisons).

[c] Abbreviations: AMCP, aminocyclopyrachlor.

Table 10. Greenhouse Experiment Four. Change in aminocyclopyrachlor induced St. Augustinegrass visual injury due to tank mixing with selected agrochemicals. Positive numbers indicate a relative increase in St. Augustinegrass visual injury whereas negative numbers indicate a relative decrease in visual injury.

Tank-mixed additive to AMCP[a]			% Visual injury	
Trade name	Active ingredient	Rate[b]	AMCP	Nontreated
		---kg ha^{-1}---	------relative change[b]------	
none (AMCP alone)	AMCP	0.11	---	**24**
---	1,8-naphthalic anhydride	0.12	1	**25**
---	2,3,5-trichlorobenzoic acid	7.0[c]	-2	**22**
---	2,3,5-triiodobenzoic	7.0[c]	2	**26**
2,4-D LV6	2,4-D	1.12	9	**33**
Callisto	mesotrione	70	-8	**16**
Illoxan	diclofop	1.0	8	**32**
MCPA	MCPA	1.24	**11**	**35**
Prinicep 4L	simazine	3.0	**-22**	2
ReTain	aminoethoxyvinylglycine	0.82	**-14**	10
Sencor 75DF	metribuzin	0.56	-3	**21**
---	cobalt chloride	1.01[c]	-5	**19**
---	dimethylamine salt	25[c]	-3	**21**
Imazapyr 2SL	imazapyr	0.7	**26**	**50**
---	salicylic acid	0.63[c]	-7	**17**
---	silver nitrate	0.77[c]	4	**28**
nontreated	---	---	**-24**	---

[a] All treatments combined with 1 % v v^{-1} methylated seed oil; DPX-KJM44 formulation of AMCP used at 0.11 kg ha^{-1}.

[b] Bold bumbers indicate significance at the 0.003 alpha level (adjusted to control type I error according to 0.05/n where n is the number of pairwise comparisons).

[c] Rate in L ha^{-1}.

[d] Abbreviations: AMCP, aminocyclopyrachlor.

Table 11. Sources of chemicals for safening experiments.

Agrochemcial		Manufacturer	Address
Trade name	Active ingredient		
---	1,8-Naphthalic anhydride	Arcos Organics	Geel, Belgium
---	2,3,5-trichlorobenzoic acid	Arcos Organics	Geel, Belgium
---	2,3,5-triiodobenzoic acid	Arcos Organics	Geel, Belgium
---	cobalt hexahydrate	MP Biomedicals LLC	Solon, OH 44139
---	diflufenzypyr	Arcos Organics	Geel, Belgium
---	dimethylamine salt	Arcos Organics	Geel, Belgium
---	silver nitrate	Arcos Organics	Geel, Belgium
---	salicylic acid	Arcos Organics	Geel, Belgium
2,4-D LV 6	2,4-D	Agri Star by Albaugh Inc.	Ankeny, IA 50021
Acclaim Extra	fenoxaprop	Bayer Enviromental Science	Research Triangle Park, MC 27709
Callisto	mesotrione	Sygenta Group Company	Greensboro, NC 27419
Cotoran	flumeturon	DuPont Company	Wilmington, DE 19805
Cutless	flurprimidol	SePRO Corporation	Carmel, IN 46032
Discover	clodinafop	PBI/Gordon Corp.	Kansas City, MO 64101
Diuron 4L	diuron	Agrisolutions	St. Paul, MN 55164
DPX-KJM44	aminocyclopyrachlor	DuPont Company	Wilmington, DE 19805
DPX-MAT28	aminocyclopyrachlor	DuPont Company	Wilmington, DE 19805
Embark	melfluidide	PBI/Gordon Corp.	Kansas City, MO 64101
Everest	flucarbazone	Arysta Lifescience North America, LLC	Cary, NC 27513
Fusillade II	fluazifop	Sygenta Group Company	Greensboro, NC 27419
Illoxan	diclofop	Bayer Enviromental Science	Research Triangle Park, NC 27709
Imazapyr 2SL	imazapyr	Vegetation Management, LLC	Raleigh, NC 27609
MCPA	MCPA ester	Rhône-Poulenc Ag Company	Research Triangle Park, NC 27709
MSM	MSM	FarmSaver.com, LLC	Raleigh, NC 27609
MSO	methylated seed oil	Alligare, LLC	Opelika, AL 36801
Plateau	imazapic	Bayer Enviromental Science	Research Triangle Park, NC 27709
Primo Maxx	trinexapac-ethyl	Sygenta Group Company	Greensboro, NC 27419
Prinicep 4L	simazine	Sygenta Group Company	Greensboro, NC 27419
Prograss	ethofumesate	Bayer Enviromental Science	Research Triangle Park, NC 27709
ReTain	aminoethoxyvinylglycine	Valent BioSciences Corporation	Libertyville, IL 60048
RoundUp Pro	glyphosate	Monsanto Company	St. Louis, MO 63167
Sencor 75DF	metribuzin	Bayer Enviromental Science	Research Triangle Park, NC 27709
Sprint 330	chelated iron	Becker Underwood, Inc.	Ames, IA 50010
Target 6 Plus	MSMA	Luxembourg-Pamol, Inc.	Memphis, TN 38137
Transline	clopyralid	Dow AgroSciences LLC	Indianapolis, IN 46268
Trimmit	paclobutrazol	Sygenta Group Company	Greensboro, NC 27419
Tupersan	siduron	PBI/Gordon Corp.	Kansas City, MO 64101
Turflon Ester	triclopyr	Dow AgroSciences LLC	Indianapolis, IN 46268
Vista	fluoxypyr	Dow AgroSciences LLC	Indianapolis, IN 46268

[a] Abbreviations: MCPA, (4-chloro-2-methylphenoxy)acetic acid ; MSM, metsulfuron-methyl; MSMA, monosodium methylarsonate.

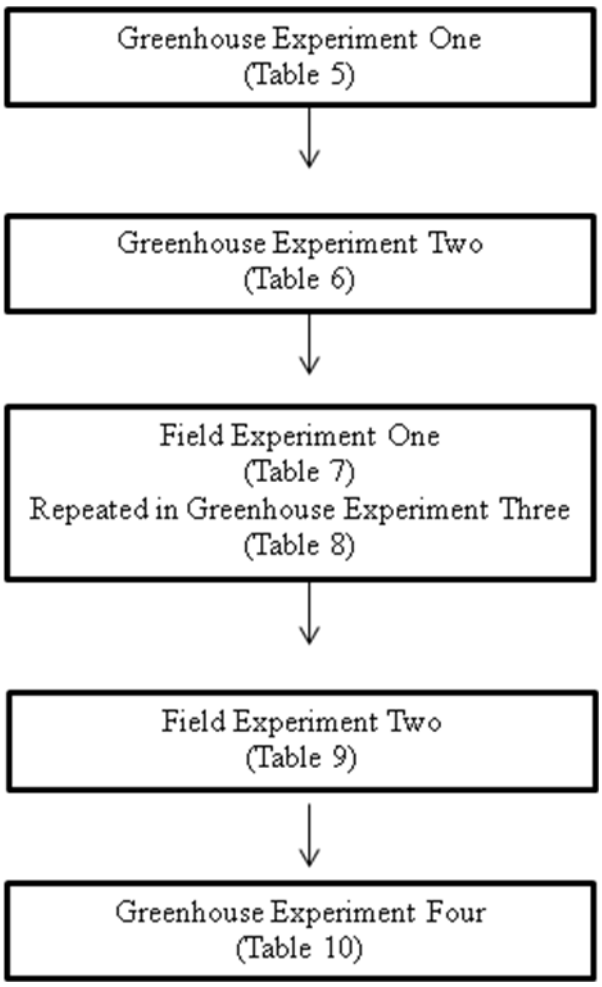

Figure 3. Timeline of experiments conducted to evaluate agrochemical combinations with AMCP for safening potential. Each box corresponds to an experiment. Experiments progressed in the order indicated by the arrows. Treatments that resulted in safening potential were included in the following experiment; new treatments were also added. The screening assessment proceeded in this fashion.

Anatomical Response of St. Augustinegrass to Aminocyclopyrachlor Treatment

Introduction

Synthetic auxin herbicides are known to act similar to the endogenous plant hormone auxin, which regulates growth in plants (Senseman 2007). Auxinic herbicides disrupt growth regulation leading to epinasty, proliferation in the cambium, phloem, and cortical tissues, vascular disruption and loss of function, and subsequent death of susceptible plants (Beal 1951; Eames 1949a, b; Kaufman 1955; Scott 1938; Swanson 1946). As a general rule, synthetic auxin herbicides are nonphytotoxic to grass plants but extremely phytotoxic to broadleaf plants (Struckmeyer 1951; Watson 1950). This selectivity enables the discriminatory removal of broadleaf weeds in grass crops. For this reason, auxinic herbicides are widely used in the turfgrass industry (Sterling and Hall 1997).

The reason for the selectivity of auxinic herbicides between grasses and broadleaf plants is a subject of debate. Differences in morphology, growth physiology, auxin responsiveness, and herbicide uptake and translocation have been studied between grasses and broadleaf plants (Lewer and Owen 1990; Devine et al. 1993; Sterling and Hall 1997). However, most attribute the selectivity to differences in either metabolic ability and/or anatomical structure (Sterling and Hall 1997). There is evidence that metabolism (a plant's ability to deactivate a herbicide) in tolerant species (generally moncotyledons) occurs primarily via true detoxification mechanisms, and metabolism in susceptible species (typically broadleaf plants) is primarily through reversible conjugate formation. However, many studies have not been able to correlate metabolism with

reduced injury and therefore with tolerance (Lewer and Owen 1990; Devine et al. 1993; Sterling and Hall 1997).

Anatomical differences exist between grass and broadleaf plants that may lead to the selectivity of auxinic herbicides. These differences involve the vascular and meristematic tissues. Grasses have vascular bundles surrounded by protective sclerenchyma tissue that may prevent auxinic herbicides from readily entering the vascular tissue and translocating throughout the plant (Berghaus and Wuerzer 1987; Sterling and Hall 1997). The lack of this protective tissue in eudicotyledons (eudicots) or broadleaf plants may contribute to their susceptibility through the increased translocation of the herbicide to more sensitive tissues within the plant. The presence of an auxin-sensitive cambium and pericycle in eudicots also contributes to eudicots' susceptibility (Sterling and Hall 1997). The ring arrangement of vascular bundles in eudicots may serve to concentrate auxinic herbicides around these more sensitive cambium and pericycle tissues. The resulting proliferation in these tissues is known to crush the phloem and may also inhibit the function of the xylem (Eames 1950; Swanson 1946). Conversely, the scattered arrangement of vascular bundles in grass plants (combined with the protective sclerenchyma tissue) prevents this concentration of toxin and subsequent destruction of vascular tissue (Devine et al. 1993). Struckmeyer (1951) attributed the selectivity of auxinic compounds to the presence of immature cells, which are more responsive to auxinic compounds and more readily revert to a meristematic state than mature cells (Eames 1950; Swanson 1946). These immature cells are generally more abundant in eudicots compared to monocots (Struckmeyer 1951). Lastly, differences in the overall structure may mask injury in a grass plant. In the monocot, the shoot apical meristem and

leaf primordia are concealed by more mature leaves, so even if injury occurs, the symptoms may be inconspicuous (Watson 1950).

Previous research indicates that auxinic herbicides do influence monocot plants, especially at certain growth stages and in meristematic regions (Eames 1951; Kaufman 1953; Struckmeyer 1951; Wilde 1951). However, typical monocot response is less injurious than a typical eudicot response. Anatomical responses of monocots are similar to eudicots and include: the formation of new lateral and adventitious roots, swelling of roots and stems, curling of the stem, proliferation of parenchyma tissue, and abnormal leaf and inflorescence development (Carlton 1943; Eames 1949b; Kaufman 1953; Struckmeyer 1951; Watson 1950, Wilde 1951). Despite these responses, most agree that grasses are more tolerant of auxinic compounds than broadleaf plants (Struckmeyer 1951; Wilde 1950).

The auxinic herbicide aminocyclopyrachlor (AMCP) is a new compound that effectively controls many broadleaf weed species (Armel et al. 2009; Blair and Lowe 2009; Turner et al. 2009). AMCP is effective at much smaller rates that other auxinic herbicides such as 2,4-D (Turner et al. 2009). However, AMCP is known to be phytotoxic to certain warm-season turfgrasses, especially St. Augustinegrass [*Stenoaphrum secondatum* (Walter) Kuntze] (Brecke et al. 2010; Flessner et al. 2009). In light of the fact that synthetic auxin herbicides are usually safe when applied to monocots, the mechanism for this injury is not well understood.

The objective of this research was to gain insight into how AMCP injures warm-season turfgrasses through the investigation of the morphological and anatomical responses of St. Augustinegrass to AMCP treatment at the cellular level with the

hypothesis that St. Augustinegrass injury occurs in a similar manner as sensitive eudicot injury from other auxinic herbicides.

Materials and Methods

'Palmetto' ® St. Augustinegrass was used for this research. Treatments were made in field and greenhouse settings during the summer of 2009. St. Augustinegrass was actively growing throughout the duration of treatments. Field treatments were applied to a mature St. Augustinegrass stand at the Auburn University Turfgrass Research Unit, Auburn, Alabama. St. Augustinegrass was also grown in greenhouse conditions. The greenhouse was also located in Auburn, Alabama. For greenhouse experiments, 5 cm diameter plugs were taken from the field location prior to treatment. Plugs were placed in 700 cm^3 pots with native soil in the greenhouse and allowed to adjust and acclimate to the greenhouse environment for 4 weeks before treatment. Field and greenhouse turfgrass stands were irrigated daily and mowed twice weekly with a rotary style mower to a height of 4.5 cm. Treatments included AMCP[1] at 0.01 and 0.11 kg ae ha^{-1} in addition to a nontreated control. These rates were chosen because symptoms at the cellular level were clear and severe injury of root and shoot tissue occurred, respectively. Treatments were applied with a CO_2 pressurized sprayer calibrated at 280 L ha^{-1} at 4.8 km hr^{-1}. St. Augustinegrass tissue samples were collected 2 and 4 weeks after treatment (WAT). Tissues collected were selected on the basis of visually representing the injury within each treated turfgrass stand. Fresh tissue samples were used for dissecting microscope observations. For compound light microscopy, samples were placed in formalin-acetic-acid-alcohol (FAA) for killing and preservation then

dehydrated in a graded alcohol series and embedded in paraffin wax. Samples were sectioned at 10 µm with a rotary microtome and stained with 0.5% buffered toluidine blue O, a non-specific, metachromatic stain (Lillie 1977). The sectioned material was viewed and photographed with a Nikon ® Biophot[2] equipped with a Nikon ® D70™ camera[3]. Responses presented were consistently observed among samples compared to the nontreated control.

Results and Discussion

The response of St. Augustinegrass to treatment was identical in the field and greenhouse settings. Therefore no subsequent distinction is made. St. Augustinegrass death occurred 4 to 6 WAT from 0.11 kg ha^{-1} AMCP in most plants. Macroscopic, visual symptoms included necrosis at the leaf base, swelling of the nodes, and unregulated growth of stem tissue in the node resulting in leaf sheath rupture (Figure 4a). Leaves readily fell from the node when handled. This condition was also observed by Eames (1949a) as well as leaf sheath rupture in nutsedge (*Cyperus rotundus* L.) treated with 2,4-D. Treatment also resulted in the stimulation of adventitious and lateral roots. AMCP at 0.01 kg ha^{-1} resulted in minimal visual symptoms, limited to slight necrosis of immature adventitious root tips. It is important to note that the responses from treatment are dynamic with time. As time increased, development of responses also increased. The overall response was similar to previous work in monocots by Kaufman (1953) in rice (*Oryza sativa* L.) and Watson (1950) in bentgrass (*Agrostis canina* L.).

Apical Node Response. For the purpose of this paper, the apical node is the node containing the shoot apical meristem; it is the node at the end of a chain of nodes and internodes, where no further internode exists. Subtending nodes include all nodes other than the apical node and are discussed separately below. Apical node response was similar at 2 and 4 WAT, with the only difference being the further development of responses at 4 WAT. There was not sufficient growth during the experiment for an apical node to become a subtending node.

The apical node responded with many symptoms of auxinic herbicide treatment. A proliferation of undifferentiated, thin-walled, parenchyma tissue, or callus tissue formed in a half-dome shape over the apex of the node (Figure 4b). Swanson (1946) referred to a formation similar to this in kidney bean (*Phaseolus vulgaris* L.) as a gall. Indeed, *Agrobacterium tumefaciens* is known to insert genetic material into the host plant that up-regulates or increases the endogenous auxin production in the plant resulting in the proliferation of callus tissue. This proliferation is a symptom of crown gall disease (Krikorian et al. 1987). The callus tissue in St. Augustinegrass is nearly identical in appearance to callus tissue formed in tissue culture plant propagation, at the microscopic level. This result is to be expected as auxinic compounds are used to produce and maintain a tissue callus in tissue culture plant propagation (Murashige 1974). Callus tissue formation in response to auxinic compounds is well documented. Similar proliferations of parenchyma were previously observed in the monocots nutsedge (Eames 1949a), rice (Kaufman 1953, 1955), and quackgrass [*Elymus repens* (L.) Gould] (Struckmeyer 1951) following treatment with auxinic compounds. Parenchyma proliferation has also been observed in bean plants (Eames 1950), pea (*Pisum sativum* L.;

Scott 1938), and other broadleaves (Struckmeyer 1951). Parenchyma tissue is characterized as being capable of reverting to a meristematic state, especially in the presence of an auxinic compound (Grossmann 2000). Therefore, this parenchyma proliferation is a direct response of St. Augustinegrass to AMCP treatment.

The apical meristem also responded to treatment with abnormal growth and was disorganized (Figure 5a). The apical meristem was frequently located on the periphery of the node as opposed to an organized, central location above the node that is found in nontreated grass apical nodes. It is unclear if callus tissue development influenced the apical meristem itself through physical displacement. Abnormalities included multiple apical domes and leaf primordia, indicating a hormonal imbalance within the plant. Malformed shoot meristems were previously observed in rice by Kaufman (1955) and in bentgrass by Watson (1950). More commonly, disruption of normal meristematic activity was previously observed through cambium stimulation in eudicot plants (Scott 1938; Struckmeyer 1951; Swanson 1946).

Natural auxin is produced almost exclusively in the developing leaf primordia, which are located directly adjacent to the apical meristem (Ljung et al. 2001). Meristem tissue is known to be highly sensitive to auxinic compounds (Ljung et al. 2001; Sterling and Hall 1997). Therefore exogenous auxinic compounds such as AMCP may disrupt auxin levels at the apical meristem and result in the disorder observed at the apical meristem.

The vascular system at the apical node responded with vessel occlusions in the xylem vessel members in response to treatment (Figure 5b). The degree of blockage varied, but was consistently present in all nodal tissue examined. These blockages appear

to be gum deposits and not tyloses. Tyloses are ingrowths of surrounding parenchyma tissue into the xylem vessels and were not observed (Bonsen and Kučera 1990). However, tyloses are sometimes surrounded by gum deposits. Gum consists of polysaccharides interlinked by structural proteins and are secreted from xylem parenchyma cells (Soukup and Votrubová 2005). Gum deposits and tyloses are known to form in response to wounding or embolism (Dute et al. 2002). Peterson et al. (1974) noted similar xylem blockages while working with picloram (a synthetic auxin herbicide) treated red maple (*Acer rubrum* L.). In their study, blockages near the stem apex were the first anatomical sign of treatment to develop. Subsequently, the apex became desiccated and necrotic, which Peterson et al. (1974) attributed to the lack of xylem-conducted materials to the region. St. Augustinegrass undoubtedly suffers from a similar lack of conduction due to these blockages.

A possible explanation for the production of the blockages could be found in ethylene synthesis. Ethylene is known to play a role in gum production (Bonsen and Kučera 1990) and is stimulated by auxinic herbicide treatment (Sterling and Hall 1997) suggesting that AMCP treatment stimulated ethylene production that in turn stimulated gum formation in the xylem. Dute et al. (2002) proposed a similar hypothesis while working with *Cercis canadensis* L. Confoundingly, gum and ethylene production can both be triggered by wounding (Bonsen and Kučera 1990), which may be manifested by AMCP treatment in the necrosis observed at leaf bases (Figure 4a). Whichever the case, xylem vessel gum blockages were a result of AMCP treatment.

Vascular parenchyma proliferation was also observed in the apical node (Figure 5c). These proliferations appeared to be differentiating into root primordia. Subtending

nodes did not respond to treatment in this manner. Struckmeyer (1951) observed similar proliferations in quackgrass– parenchyma cells adjacent to developing vascular bundles proliferated and were responsible for formation of lateral roots. Eames (1950) reported crushing of phloem tissue by proliferating vascular parenchyma in young bean plants. Other authors have made similar observations in pea (Scott 1938) and kidney bean (Swanson 1946). Again, parenchyma cells are characterized as having the capability to revert to a meristematic state; auxinic compounds may stimulate this reversion (Grossmann 2000). Auxinic herbicides translocate primarily in the phloem but also in the xylem. Therefore, the close proximity of the vascular parenchyma to the translocating herbicide may also play a role in proliferation.

The vascular system at the apical node responded with necrosis in and around the developing vascular tissue (Figure 4b). This response only occurred in the apical node. This necrosis is attributed to the following: natural auxin travels from the apical meristem source to the root tip. Auxin moves in the vascular parenchyma, as opposed to the xylem or phloem conduits. It seems that vascular parenchyma cells have a special ability to move auxin molecules out of their basal ends only, resulting in a net basipetal flow of auxin from the shoot apical meristem toward the base of the plant. This movement of auxin results in an auxin concentration gradient with higher concentrations at the apical meristem and lesser concentrations at the base of the plant (Aloni 1987). Shoot vascular tissue formation occurs toward this source of auxin and uses the concentration gradient essentially as a directional bearing (Aloni 1987, 2001). Therefore, when the gradient is disrupted through AMCP treatment, vascular tissue development may become disoriented with subsequent necrosis the result. Necrosis of the developing vascular tissue has not

been observed before as a result of auxinic herbicide treatment; however, Peterson et al. (1974) and Struckmeyer (1951) both noted abnormal activity at the developing vascular tissue region as a result of auxinic compound treatment. This evidence further suggests that cellular activity is occurring in response to auxinic compounds. Responses to auxinic compounds are known to vary by species, by tissue within species, and even by relative maturity of a given tissue (Beal 1951). Therefore, it seems likely that necrosis may be one response manifested in the developing vascular bundles in St. Augustinegrass as a response to AMCP.

Vascular parenchyma proliferation and vascular necrosis may indicate a higher concentration of AMCP at the apical node compared to subtending nodes. However, these responses may also indicate an interaction of AMCP with the higher concentration of naturally occurring auxin at the apical node (Ljung et al. 2001).

Overall, apical node response to AMCP treatment is indicative of a loss of hormone balance within the plant. Treatment resulted in various growth responses and vascular blockages. Synthetic auxin herbicides are known to accumulate in meristems or growing points and have their most profound influences there (Anderson 1996; Sterling and Hall 1997). Therefore, these data provide evidence of AMCP accumulation in the apical node thus disrupting hormone balance and leading to vascular inhibition and a loss of growth regulation.

Subtending Node Response. Subtending nodes also responded to AMCP treatment through growth responses and vascular blockages. Subtending nodes were also swollen with necrotic tissue at the leaf base. Vascular inhibition was limited to xylem occlusions

identical to those observed in the apical nodes. Occlusions appeared to be gum blockages and were observed at 2 and 4 WAT. No occlusions were observed in any nontreated tissue.

Growth responses involved meristematic activity in the form of adventitious root formation and new shoot formation (Figure 6). The shoot formation was limited to new bud development at the time of observation. However, if allowed to grow, these buds would subsequently develop into new shoot tissue. In grasses, this action is known as tillering. Apical dominance is equivalent to tillering in grasses and is subject to the same auxin connection as lateral bud growth in eudicots (Leopold 1949). Apical dominance is the inhibition of lateral growth by the terminal bud that is controlled by auxin diffusion from the shoot tip (Thimann and Skoog 1934). Therefore, when the auxin gradient is disrupted through AMCP treatment, apical dominance is also disrupted. Subsequent tillering occurs. Beal (1951) also reported a stimulation of bud formation in Easter lily (*Lilium longiflorum* Thunb.). However, Kaufman (1953) reported the inhibition of tillering in rice as a response to 2,4-D treatment.

Adventitious root formation is also a response to increased levels of auxinic compounds within the plant (Sterling and Hall 1997). Adventitious root formation has been observed previously in response to auxinic compounds by several authors in both monocotyledons and eudicots (Beal 1951; Eames 1949b; Kaufman 1953; Swanson 1946). These responses were only observed at 4 WAT indicating that long-term growth responses occur from AMCP treatment.

Root Tip Response. Overall, the response of St. Augustinegrass root tips to AMCP treatment was characterized by a loss of organization (Figure 7a). The root tip itself was not well formed. Lateral root meristems were observed and occurred abnormally close to the root tip, compared to nontreated roots. Lateral root formation, similar to adventitious root formation, is stimulated by auxin (Torrey 1957). Therefore, AMCP treatment may have resulted in an increase in auxinic levels in the roots and subsequently stimulated the formation of lateral root primordia. Carlton (1943) observed lateral root formation in onion (*Allium cepa* L.), paper white (*Narcissus* L.), and tulips (*Tulipa* L.) (bulb-forming monocots) as well as tumor formation and temporary inhibition of root elongation in response to indole-3-acetic acid (auxin). Furthermore, Carlton (1943) indicated that the lateral root primordia were closer to the root tip than in nontreated plants. Watson (1950) and Callahan and Engel (1965) also observed lateral root stimulation in bentgrass. Others have observed this trend in various eudicots from treatment with auxinic compounds (Scott 1938; Struckmeyer 1951; Swanson 1946; Wilde 1951).

Vascular maturation was also distorted in the zone of maturation (Figure 7b). Xylem formation in particular was disrupted and was characterized by vessel members of various sizes and ontogenetic stages occurring adjacent to one another with many pits throughout the distorted zone. These vessel members are likely parenchyma cells that have redifferentiated into vessel members of abnormal size and shape after treatment. Swanson (1946) reported an almost identical finding while working with 2,4-D treated kidney bean-- “The cells laid down were not the usual elongated tracheal cells characteristic of the kidney bean but were short, with simple pits, and not very heavily

lignified." The abnormal xylem tissue in both cases is most likely functional but undoubtedly inhibited in efficiency.

The role of auxin in xylem differentiation is well established, especially in wound tissue; without a source of auxin, no new xylem is produced (Wetmore and Sorokin 1955). It seems reasonable that an auxin-like compound such as AMCP could also elicit similar responses. Fosket (1968) found in stem sections of *Coleus* that when new xylem is produced after wounding it forms a roughly isodiametric shape, which was also observed in the treated St. Augustinegrass roots (Figure 7b). This shape is in contrast to the columnar shape of normally formed xylem. Many times cell division precedes cell differentiation, although this is not a causal relationship (Fosket 1968). Obviously, cellular divisions lead to an increased number of cells. This increase may explain the abnormal number of cells observed in the treated St. Augustinegrass roots. Therefore the role of auxin in xylem differentiation after wounding may explain the abnormal number, orientation, and level of maturation observed in treated roots.

No xylem blockages, as previously seen in stem tissue, were observed in any root section. This finding agrees with Peterson et al. (1974), indicating that the blockages are shoot derived and not root derived.

Fungal hyphae (and to a very small extent nematodes) were noted in the root tissue of treated St. Augustinegrass (Figure 7c). Fungal hyphae were only present in the cortical parenchyma tissue and were not found in the vascular tissue or in the root apical meristem. No fungal hyphae were found in nontreated roots, suggesting that treatment resulted in less robust roots and declining plant vigor leading to subsequent invasion. The fungi observed did not appear to be influencing root growth and development or

otherwise contributing to anatomical modifications observed. Fungal hyphae and nematodes are known to preferentially attack less protected areas of roots such as the zone of elongation and where lateral root primordia break through the epidermis (Gunawardena and Hawes 2002). There were many lateral root primordia produced as a response to treatment, which would yield many potential sites of invasion. Interestingly, the apical root meristem overwhelmingly avoids infection in many plants, which was also the case in this research. Gunawardena and Hawes (2002) also noted that the zone of elongation is the primary site of root exudates that are thought to stimulate spore germination and therefore lead to increased infection in the zone of elongation. However, the role of AMCP and auxinic herbicides as related to root exudate production is largely unknown. It seems very likely that treatment caused abnormal proliferations in the root tissue making the treated roots more susceptible to fungal attack.

Research Implications. AMCP treatment to St. Augustinegrass results in harmful growth stimulation and concomitant vascular inhibition. The growth responses of callus tissue proliferation, lateral and adventitious root formation, stimulation of tillering, and vascular parenchyma proliferation all result in an increased demand for photosynthates, water, and other nutrients. However, the plant's ability to deliver these nutrients is greatly inhibited through the xylem gum blockages, abnormal development of vascular tissue in the shoots and roots, and vascular parenchyma proliferation, which characterized the vascular inhibition. These responses undeniably were injurious to the treated St. Augustinegrass.

Leaf tissue at the macroscopic level was necrotic at the leaf base, but the rest of the leaf remained green and healthy in appearance for some time after treatment, possibly indicating that these leaves were conducting photosynthesis (Figure 4a). In agreement with this notion, treated and nontreated leaves yielded no difference in Φ_{PSII} (chlorophyll fluorescence) as determined through the use of a chlorophyll fluorometer[4] (data not shown). However, it seems likely that the remainder of the plant was not privileged to this energy production due to the necrotic tissue blocking any translocation that could occur. Therefore the ability of the plant to produce its own energy was greatly reduced, which may account for the slow death and long-term injury (greater than 8 WAT) observed in surviving St. Augustinegrass plants.

Mature tissue was unaffected by AMCP treatment. This included fully mature internodes and leaf blades. Eames (1949a) also reported that mature tissue seemed unaffected while working with 2,4-D treated nutsedge. It is important to note that synthetic auxin herbicides are known to translocate in the xylem and phloem and accumulate in the meristems. Therefore, this action would exclude herbicide exposure to most of the mature tissues of a treated plant and further explain the lack of response observed there.

The response of St. Augustinegrass to AMCP taken as a whole is fundamentally the same as the response of other susceptible species to synthetic auxin herbicides. That is, St. Augustinegrass responds with typical symptoms of synthetic auxin herbicide treatment. Many authors have also concluded that susceptible monocot response to auxinic compounds is essentially the same as eudicot response (Eames 1949a; Kaufman 1953; Struckmeyer 1951; Wilde 1951). St. Augustinegrass injury and death at higher

rates of herbicide occur from harmful growth stimulation leading to non-functional tissues and concomitant vascular inhibition.

Sources of Materials

[1] DPX-MAT28 2SL, aminocyclopyrachlor, E. I. du Pont de Nemours and Company, Wilmington, DE 19805.

[2] Nikon ® Biophot, Nikon Inc., Melville, NY 11747.

[3] Nikon ® D70™ camera, Nikon, Inc., Melville, NY 11747.

[4] Opti-Sciences OS1-FL modulated fluorometer, OptiSciences, Inc., Hudson, NH 03051.

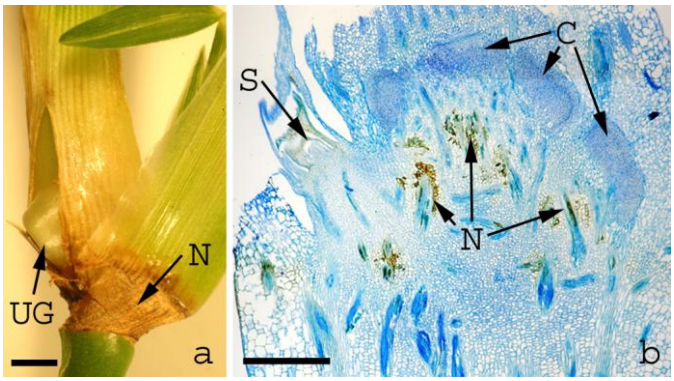

Figure 4. Symptoms of St. Augustinegrass nodes 2 weeks after 0.11 kg ha^{-1} aminocyclopyrachlor treatment. (a) Subtending node showing swelling, necrosis at the leaf base, and uncontrolled growth resulting in leaf sheath rupture (bar = 2 mm); (b) apical meristem containing node longitudinal section showing callus tissue, necrosis in and around the developing vascular tissue, and malformed shoot meristem (bar = 500 µm); C, callus tissue; N, necrosis; S, shoot meristem; UG, uncontrolled growth.

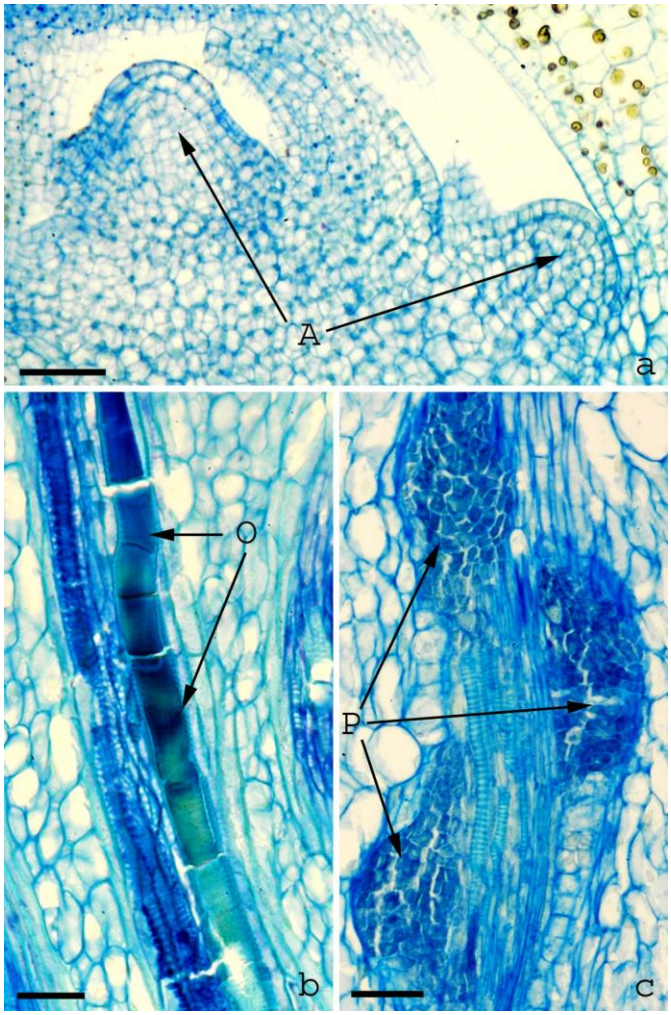

Figure 5. Longitudinal sections showing symptoms of St. Augustinegrass node tissue 2 weeks after 0.11 kg ha^{-1} aminocyclopyrachlor treatment. (a) Apical meristem with two apical domes (bar = 50 µm); (b) xylem vessel occlusions (bar = 20 µm); (c) vascular parenchyma proliferations that may be developing into root primordia (bar = 20 µm); A, apical dome; O, occlusion; P, proliferation of vascular parenchyma.

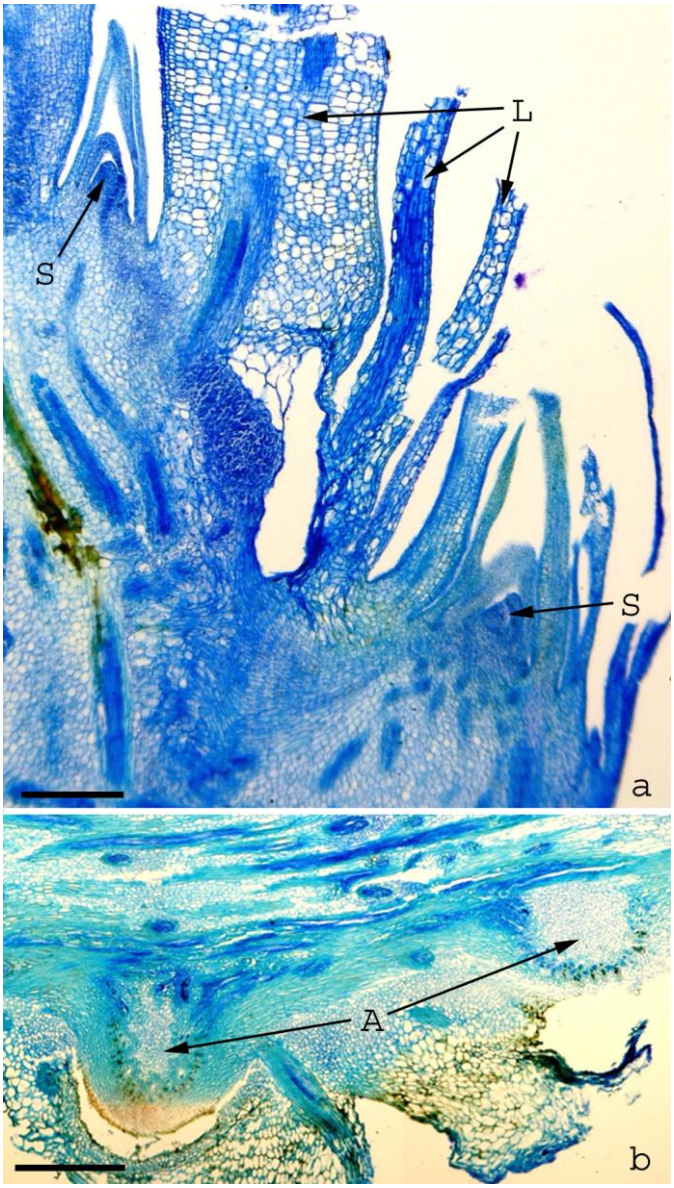

Figure 6. Shoot and adventitious root stimulation 4 weeks after 0.11 kg ha^{-1} aminocyclopyrachlor treatment longitudinal sections. (a) Multiple shoot meristems on periphery of node (bar = 100 µm); (b) multiple adventitious root primordia (bar = 200 µm); A, adventitious root primordia; L, mature leaf tissue; S, shoot meristem.

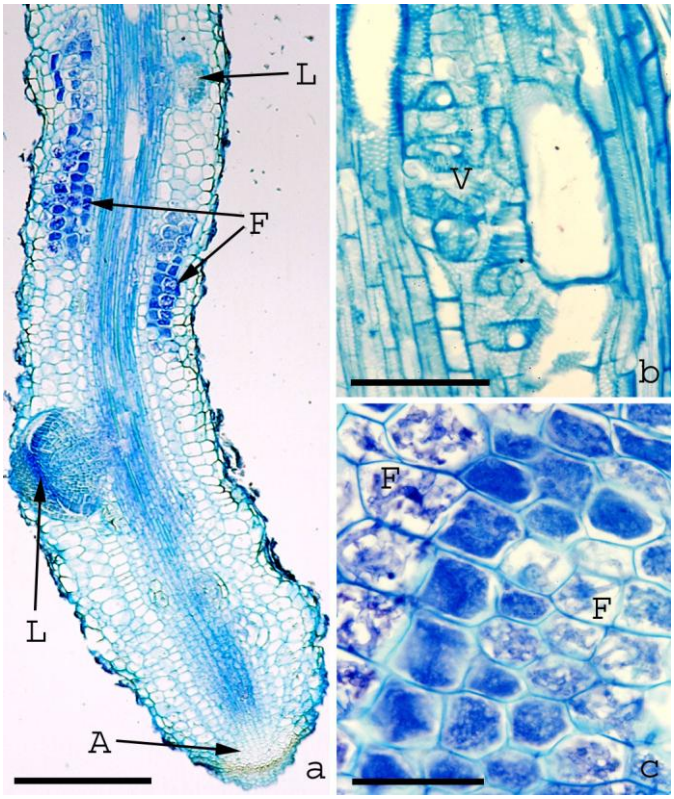

Figure 7. Root tip disorganization 4 weeks after 0.01 kg ha^{-1} aminocyclopyrachlor treatment and fungal infestation longitudinal sections. (a) Overview of root tip (bar = 200 µm); (b) malformed vessel elements (bar = 20 µm); (c) fungal hyphae in parenchyma tissue (bar = 20 µm); A, apical meristem; F, fungal hyphae; L, lateral root primordia; V, abnormal vessel elements.

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Appendix 1. *Weed Science Society of America* Approved Common and Chemical Nomenclature

2,4-D	(2,4-dichlorophenoxy)acetic acid
aminocyclopyrachlor	6-amino-5-chloro-2-cyclopropylpyrimidine-4-carboxylic acid
aminoethoxyvinylglycine	*S*-trans-2-amino-4-[2-aminoethoxy]-3-butenoic acid hydrochloride
barban	4'-chloro-2-butynyl *N*-(3-chlorophenyl)carbamate
benoxacor	4-(dichloroacetyl)-3,4-dihyro-3-methyl-2*H*-1,4-benzoxazine
butachlor	*N*-butoxymethyl-2-chloro-2',6'-diethylacetanilide
clodinafop	prop-2-ynyl(*R*(-2-[4-(5-chloro-3-fluoro-2-pryridyloxy)phenoxy]propionate
clopyralid	3,6-dichloropyridine-2-carboxylic acid
cloquintocet-mexyl	(*RS*)-1-methylhexyl [(5-chloro-8-quinolinyl)oxy]acetate
dicamba	3,6-dichloro-2-methoxybenzoic acid
diclofop	(*RS*)-2-[4-(2,4-dichlorophenoxy)phenoxy] propionoic acid
diflufenzopyr	(2-(1-[([3,5-difluorophenylamino]carbonyl)-hydrazono]ethyl-3-pyridinecarboxylic acid
diuron	3-(3,4-dichlorophenyl)-1,1-dimethylurea
EPTC	*S*-ethyl dipropyl(thiocarbamate)
ethofumesate	(*RS*)-2-ethoxy-2,3-dihydro-3,3dimethylbenzofuran-5-yl methanesulfonate

fenchlorazole-ethyl	ethyl 1-(2,4-dichlorophenyl)-5-trichloromethyl-1*H*-1,2,4-triazole-3-carboxylate
fenclorim	4,6-dichloro-2-phenylpyrimidine
fenoxaprop	(*R*)-2-[4[(6-chloro-1,3-benzoxazol-2-yloxy)phenoxy]propionic acid
fluazifop	(*R*)-2-{4-[5trifluoromethyl)-2-pyridyloxy]phenoxy}propionic acid
flucarbazone	sodium 4,5-dihydro-3-methoxy-4-methyl-5-oxo-*N*-{[2-(trifluoromethoxy)phenyl]sulfonyl}-1*H*-1,2,4-triazole-1-carboximidate
flumeturon	1,1-dimethyl-3-(, , -trifluoro-*m*-tolyl)urea
fluroxypyr	(butometyl ester)(*RS*)-2-butoxy-1-methylethyl 4-amino-3,5-dichloro-fluoro-2-pyridyloxyacetate
flurprimidol	(*RS*)-2-methyl-1-pyrimidin-5-yl-1-(4-trifluoromethoxyphenyl)propan-1-ol
fluxofenim	4'-chloro-2,2,2-trifluoroacetophenone (*EZ*)-*O*-1,3-dioxolan-2-ylmethyloxime
glyphosate	*N*-(phosphonomethyl)glycine
imazapic	2-[(*RS*)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]-5-methylnicotimic acid
imazapyr	2-[(*RS*)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)nicotinic acid
MCPA	4-chloro-o-toyl-oxy-acetic acid

melfluidide	5'-(1,1,1-trifluoromethanesulfonamido)acet-2',4'-xylidide
mesotrione	2-(4-mesyl-2-nitrobenzoyl)cyclohexane-1,3-dione
metribuzin	4-amino-6-*tert*-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one
MSM	methyl 2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)benzoic acid
MSMA	sodium hydrogen methylarsonate
naphthalic anhydride	naphthalene-1,8-dicarboxylicanhydride
paclobutrazol	(2*RS*,3*RS*)-1(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol)
picloram	4-amino-3,5,6-trichloropyridine-2-carboxylic acid
quinmerac	7-chloro-3-methyl-quinoline-8-carboxylic acid
simazine	6-chloro-N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine
terbuthylazine	N^2-tert-butyl-6-chloro-N^4-ethyl-1,3,5-triazine-2,4-diamine
triclopyr	[(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid
trinexapac-ethyl	ethyl (*RS*)-4-cyclopropyl(hydroxy)methylene-3,5-dioxocyclohexanecarboxylate
siduron	1-(2-methylcyclohexyl)-3-phenylurea