Physiological Basis of Differential Sensitivity of Selected Graminaceous Species to Aminocyclopyrachlor

by

Ethan Trent Parker

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Approved by

J. Scott McElroy, Chair, Professor of Crop, Soil and Environmental Sciences
Glenn R. Wehtje, Professor of Crop, Soil and Environmental Sciences
Andrew J. Price, Affiliate Assistant Professor of Crop, Soil and Environmental Sciences

Abstract

Synthetic auxin herbicides are widely used in many grass systems due to their selective control of broadleaf weeds and safety to grass species. Aminocyclopyrachlor (ACPC) is a new synthetic auxin herbicide used for broadleaf weed control in pasture and rangeland systems. The fate of ACPC within treated grasses is not well understood. Research was conducted to establish the tolerance of four graminaceous species to ACPC application, observe the absorption, translocation, and metabolism of ACPC within treated grasses, and to determine if ACPC is exuded from the roots of treated grass species. Results indicate that tall fescue is the most tolerant of ACPC at rates that provide acceptable weed control. Bahiagrass and bermudagrass are marginally tolerant of ACPC and cogongrass is the most sensitive species. Tall fescue and bahiagrass absorbed more ACPC than bermudagrass and cogongrass, but cogongrass absorption is the most rapid and complete within 2 days after treatment (DAT). Cogongrass and bermudagrass moved the least herbicide out of the target area while bahiagrass and tall fescue translocated the most from the target area. No metabolism of ACPC was detected in any grass species out to 42 DAT. ACPC does exude through grass roots after foliar treatment into the surrounding environment in all species evaluated but the effect is minor and less than dicamba.

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List of Abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid
ABA	abscisic acid
ACPC	aminocyclopyrachlor
ae	acid equivalent
ai	active ingredient
AL	Alabama
ANOVA	analysis of variance
^{14}C	carbon 14
C	celsius
cm	centimeter
DAI	days after insertion
DAT	days after treatment
Dicot	dicotyledonous
DT_{50}	50% dissipation time
g	grams
h	hours
ha	hectare
HAT	hours after treatment
IAA	indole-3-yl-acetic acid

kBq	kilobecquerel
kg	kilogram
L	liter
LSD	least significant difference
LSS	liquid scintillation spectroscopy
MCPA	2-methyl-4-chlorophenoxyacetic acid
m	meter
mg	milligram
min	minute
ml	milliliter
mm	millimeter
Monocot	monocotyledonous
MOA	mode of action
NIS	nonionic surfactant
ppb	parts per billion
R_f	retardation factor
RI	radioisotope imaging
s	second
SAS	statistical analysis system
TLC	thin layer chromatography
µL	microliter
µm	micrometer
µmol	micromole

U.S.	United States
v	volume
WAT	weeks after treatment
wk	week
wks	weeks

Literature Review

Synthetic Auxin Herbicides

In terms of vegetation management, herbicides are an important tool employed to control undesirable species. This is especially true along roadsides, in turf and cereal crops and in electrical distribution systems (Luken et al. 1994; Slaughter et al. 1999). The class of herbicides known as the synthetic auxins, also called auxinic herbicides or auxin-mimics, is widely used in grain crops, pastures, right of ways, industrial areas, and also in turfgrass. The synthetic auxin herbicides are a group of compounds that were initially developed in the mid-1940s (Grossmann 2010; Sciumbato et al. 2004). They were the first selective herbicides used for control of dicotyledonous (dicot) weeds in monocotyledonous (monocot) cropping systems (Anderson 1996; Devine et al. 1993; Sterling and Hall 1997). Some of first synthetic auxin products such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) are still widely used today (Cobb and Reade 2010). This class of herbicides is among the most successful in all of agriculture with nearly a tenth of all herbicide sales in 2000 being synthetic auxin products (Grossmann 2003; Sterling and Hall 1997). Their wide usage in these areas is primarily due to the aforementioned exemplary control of broadleaf weeds and safety in many desirable grass species (Bell et al. 2000; Curtis et al. 2009; Mansue and Hart 2010; McElroy et al. 2005). This selectivity is common in the synthetic auxin class with the exception of the quinolinecarboxylic acid family and other select compounds which are also phytotoxic to certain monocot species (Grossmann 2003; Kaufman 1955). These auxin-mimics are typically applied post-emergence and can be absorbed rapidly into plant foliage and roots (Bovey et al. 1983; Ross and Lembi

1999; Senseman 2007). Synthetic auxin compounds function by mimicking the naturally occurring ‘master hormone’ indole-3-yl-acetic acid (IAA), which stimulates cell division, differentiation, and ultimately plant growth at meristematic plant tissues (Grossmann 2010; Ross et al. 2002). Symptoms of these herbicides include leaf epinasty, uncontrolled root growth, changes in nucleic acid levels, and increased biosynthesis of ethylene (Anderson 1996; Deshpande and Hall 2000; Devine et al. 1993). Synthetic auxin herbicides typically kill sensitive plants slowly, often taking up to 5 wks for complete control. This is contrasted by how quickly some symptoms of these herbicides can appear, e.g. as early as 2 days after treatment (Senseman 2007; Sterling and Hall 1997). Physiological binding sites of this herbicide class have not been clearly identified, and the true phytotoxic mechanism of synthetic auxins is not well understood (Senseman 2007). Early research of 2,4-D in dandelion (*Taraxicum sp.* L.) noted that the loss of carbohydrates due to increased plant respiration could be a contributing factor in plant death (Rasmussen 1947). More current studies indicate the disruption of a number of different plant biochemical pathways could account for phytotoxicity and the related physiological, anatomical and genetic effects (Badescu and Napier 2006; Grossmann 2010; Quint and Gray 2006). A leading theory of activity involves the stimulation of specific auxin-binding proteins. In turn, ethylene and abscisic acid (ABA) are overproduced. ABA is then distributed within the plant and facilitates stomatal closure, which limits transpiration and carbon assimilation, as well as an overproduction of reactive oxygen species. In addition, ABA inhibits cell division and expansion and along with ethylene, promotes foliar senescence with chloroplast damage and destruction of membrane and vascular system integrity (Grossmann 2010).

Some synthetic auxin herbicides such as clopyralid and aminopyralid have been utilized for effective weed control at low use rates compared to other standard treatments (Bukun et al. 2009; Senseman 2007). The impact of these low use rates however can result in injury to non-target plants if they persist in the environment or if off-site movement occurs via volatility, soil movement or if herbicide residues remain inside spray tanks (Andersen et al. 2004; Arle 1954; Bovey and Meyer 1981; Strachan et al. 2010). Undesired injury to sensitive species like tomato (*Solanum lycopersicum* L.) and lettuce (*Lactuca sativa* L.) can occur at rates as low as 0.001% of the labeled rate with 2,4-D butyl (2,4-DB), an auxin mimic herbicide (van Rensburg and Breeze 1990). Off-site injury via synthetic auxins is very easy to identify because of the distinct leaf epinasty, tissue swelling, and stem curling (Anderson 1996; Grossmann 2010; Sciumbato et al. 2004). Sciumbato et al. (2004) even developed a numerical system of visual ratings for use in determining the severity of identifiable synthetic auxin injury. Though discernable injury occurs at miniscule rates, complete herbicidal control typically requires significantly higher doses (Devine et al. 1993; Sterling and Hall 1997). In recent years, weeds resistant to some synthetic auxin herbicides have become a growing concern. Many herbicides including 2,4-D have become less effective due to repeated application and the resulting selection pressure (Holt and Lebaron 1990; Whitehead and Switzer 1963), spurning the need for new herbicide compounds and rotations.

Absorption and Translocation. The amount of herbicide absorption and the extent of translocation within treated plants can greatly influence control of both annual and perennial target weeds (Lym and Moxness 1989; Radosevich and Bayer 1979). With synthetic auxin compounds, absorption into the plant can occur via either foliar, root, or both mechanisms; however, root absorption is typically less effective (Ross and Lembi 1999). Once inside a treated plant, auxinic herbicides translocate quickly and systemically. This movement is followed by accumulation of the applied compounds in the meristems (Anderson 1996; Bovey et al. 1983). The rapid absorption and translocation of synthetic auxin herbicides throughout plants often occurs in as little as 48 hours after treatment (HAT) (Bovey and Mayeux Jr. 1980; Bovey et al. 1983; Chang and VandenBorn 1968; O'Sullivan and Kossatz 1984). Root absorption and subsequent movement of auxins tends to be slightly slower and less pronounced (Bovey et al. 1967; Ross and Lembi 1999). Synthetic auxin methyl ester herbicide derivatives facilitate penetration through the lipophilic leaf layer and increase absorption (Gershater et al. 2006; Hassall 1990). Besides relying on alternative formulations, pairing synthetic auxins with surfactants can also result in an increase in both the amount and rate of absorption (Bukun et al. 2009, 2010; Jansen et al. 1961) Previous research supports these claims and indicates that foliar absorption of ^{14}C-clopyralid in Canada thistle proceeded very rapidly, with approximately 85 and 95% of the applied dose being absorbed after 24 and 72 hours, respectively. The same study found that ^{14}C-clopyralid was readily exported from the treated leaf into the remainder of treated plants. This movement was complete at 24 HAT (Devine and VandenBorn 1985). Other studies indicate no difference between the amount of absorption of dicamba (3,6-dichloro-2-methoxybenzoic acid) was detected when comparing samples from 3 and 9 days after treatment (DAT), demonstrating rapid plant uptake (Chang and VandenBorn 1968). Dicamba has been shown to translocate from treated foliage to the roots of bean plants as rapid as within 24 HAT (Linder et al. 1964).

Studies involving the herbicide picloram by Lym and Moxness (1989) reported that 14% of foliar-applied picloram was absorbed by leafy spurge (*Euphorbia esula* L.) at 3 DAT while only 10% of the applied picloram was present when combined with 2,4-D. While the addition of 2,4-D to picloram inhibited absorption, it was found to significantly increase the amount of picloram translocated throughout treated plants. When picloram was applied alone, only 28% of the herbicide was translocated compared to 48% of the herbicide translocated when applied together with 2,4-D. Similarly, picloram was absorbed in a sufficient concentration to kill 90% of huisache (*Acacia farnesiana* (L.) Willd.) foliage within 3 DAT. This study also showed that the concentration of foliar-applied picloram translocated to plant roots was no more than 0.17 µg (micrograms) of picloram applied at 0.28 kilograms per hectare (kg ha^{-1}) per gram of plant material. Comparing this to 100 µg g^{-1} of picloram recovered in plant leaves, it was assessed that picloram is not very well translocated into roots but is more mobile than other synthetic auxins such as 2,4-D and dicamba (Bovey et al. 1967; Scott and Morris 1970).

Beside the characteristic rapid absorption of synthetic auxin compounds, their uptake often coincides with plant susceptibility, that is, the most sensitive plants to a herbicide application will absorb more of the active compound (Davis et al. 1968). Many tolerant species have also been shown absorb the herbicide, but at a much slower rate than most sensitive species (Fang and Butts 1953). Translocation within tolerant species is typically slower and also less complete than in susceptible ones (Fang and Butts 1953; Lym and Moxness 1989). Studies show that ^{14}C-dicamba accumulates in young, actively growing tissues in susceptible plants species, while remaining more evenly distributed within tolerant species. The treated leaf of dicamba tolerant plants typically retained the majority of the radiolabeled dicamba, exhibiting little translocation to meristematic tissues (Chang and VandenBorn 1971). Conditions that favor photosynthate production, transport and growth at meristematic tissues frequently enhance the phytotoxic effect of synthetic auxin herbicides by increasing mobility within treated plants (Anderson 1996; Radosevich and Bayer 1979). These conditions do not however, always increase plant uptake, translocation or efficacy of synthetic auxin herbicides (Davis et al. 1968). While this general behavior occurs in many cases, a number of exceptions do exist. Bukun et al. (2009) showed that aminopyralid was absorbed more slowly and translocated less than clopyralid while offering greater control at lower rates. Therefore, it needs to be noted that the above information is highly variable in regard to synthetic auxin herbicides and can differ depending on species, age of treated plants, surfactant used, the synthetic auxin applied, the formulation of the herbicide, and the dosage of the herbicide applied (Bukun et al. 2009; Chang and VandenBorn 1968; Sharma and VandenBorn 1973).

Metabolism and Fate. Though first discovered many years ago, synthetic auxin herbicides are seeing increased publicity due to newly developed compounds, the possibility of use in herbicide resistance management, and the persistence of some herbicides in this class. Synthetic auxin herbicides are known to persist within plant tissues as well as in the environment (Bovey and Mayeux Jr. 1980; Harmoney et al. 2012; Scott and Morris 1970; Sharma and VandenBorn 1973). Bovey et al. (1967) showed that defoliated leaves containing picloram were able to control huisache out to at least one month after placement. Miltner et al. (2003) found that clopyralid could persist in turfgrass clippings up to two years. Evidence suggests that a minimum of one year should be allowed before clopyralid-treated clippings are utilized as mulch in sensitive cropping systems. Other studies have also shown that using synthetic auxin treated plant material as compost can result in unwanted injury to non-target species out to 14 DAT (Blewett et al. 2007, Branham and Lickfeldt 1997; Patton et al. 2013). Triclopyr, picloram and 2, 4, 5-T have also been found in significant amounts within plants out to 30 days after treatment (DAT) (Bovey and Mayeux Jr. 1980; Bovey et al. 1967).

Previous research indicates that major synthetic auxin herbicides such as 2,4-D and dicamba can be metabolized by tolerant species (Broadhurst et al. 1966; Chang and VandenBorn 1968, 1971; Fang and Butts 1953; Jaworski et al. 1955). Chang and Vandenborn (1971) noted that dicamba was metabolized in both monocot and dicot plants and that the ability to detoxify dicamba in all species studied correlated well with the degree of tolerance of each species to dicamba. The ability of the species to absorb and translocate dicamba, on the other hand, showed an inverse relationship to tolerance. Jaworski et al. (1955) found that 2,4-D was metabolized in both healthy and stressed bean plants, indicating little to no relationship between metabolism and herbicide efficacy.

Differing results have been reported amongst many other synthetic auxin herbicides. Many studies have found that most ^{14}C recovered following treatment with ^{14}C-2,4-D, ^{14}C-triclopyr, ^{14}C-2,4,5-T or ^{14}C-picloram exists as the parent compound. It needs to be noted however, that conjugation of triclopyr may be prominent in some species, especially grasses (Bovey et al. 1967; Radosevich and Bayer 1979; Scott and Morris 1970). Overall, some water-soluble metabolites as well as herbicide conjugates have been detected in both tolerant and susceptible species indicating that synthetic auxin fate does not always correlate well with herbicide activity (Bovey et al. 1967; Lym and

Moxness 1989; Radosevich and Bayer 1979; Scott and Morris 1970; Sharma and VandenBorn 1973). It is believed, however, that metabolism of synthetic auxins can play a role in the selectivity differences among some plant species (Grossmann 2003; Sterling and Hall 1997).

Many chemical compounds are found to leave plant tissues rather than be degraded within them. All plant roots have the capability of exuding (discharging) both low and high molecular weight molecules into the rhizosphere in response to biotic and abiotic stresses. These exudates include water, ions and oxygen, but mostly consist of carbon-containing compounds (Bertin et al. 2003). The amount of root exudates produced varies with the plant species, cultivar, age and stress factors (Uren 2000). Plants in the Poaceae or grass family often exude a special class of siderophores called phytosiderophores in order to aid in the acquisition of iron and zinc under stress conditions (Bertin et al. 2003; Romheld 1991). Other low molecular weight compounds exuded from plant roots include sugars and simple polysaccharides, amino acids, organic acids (such as acetic, ascorbic, benzoic, malic acids), and phenolic compounds. In addition, higher molecular weight compounds such as flavonoids, enzymes, fatty acids, nucleotides, tannins, carbohydrates, steroids, terpenoids, alkaloids, vitamins and growth regulators are released in large quantities as well (Curl and Truelove 1986; Fan et al. 1997; Rovira 1969; Uren 2000). It comes as no surprise then, that a number of carbon-containing synthetic auxin herbicides have been documented to exude from plant roots. Linder et al. (1964) as well as Chang and VandenBorn (1968) showed that dicamba and other related benzoic acid auxins were exuded from plant roots in amounts high enough to affect nearby untreated plants. 2, 3, 6-trichlorobenzoic acid was found to exude at a rate of approximately 1 percent of applied per day out to 2 DAT. This herbicide persisted as the original applied compound, rendering it available for secondary uptake (Linder et al. 1964).

Aminocyclopyrachlor

Aminocyclopyrachlor (formerly DPX-MAT28 and hence referred as ACPC) is the first and only member of the pyrimidine carboxylic acid herbicide family to be commercialized. It is structurally similar to the pyridine carboxylic acid herbicides picloram, clopyralid, and aminopyralid (Senseman 2007). It exhibits the synthetic auxin herbicide mode of action (MOA), which disrupts gene expression resulting in undifferentiated cell division and elongation, but ACPC is unique at the molecular level (USEPA 2010; Claus et al. 2009). This distinctiveness translates into a more potent herbicide activity with most sensitive perennial weeds being controlled by ACPC at 0.07-0.14 kg acid equivalent per hectare (kg ae ha^{-1}) (Claus et al. 2009; Westra et al. 2009). The lure of ACPC lies in its ability to selectively control many acetolactate synthase, protoporphyrinogen oxidase, triazine, and glyphosate resistant weeds, while functioning in a manner consistent with the other auxins mentioned above (Claus et al. 2009; Montgomery et al. 2009). ACPC is proven to control kochia [*Bassia scoparia* (L.) A.J. Scott] in right-of-ways, yellow toadflax (*Linaria vulgaris* P. Mill.) in rangeland, and cogongrass [*Imperata cylindrica* (L.) P. Beauv.] in right-of-ways while remaining less injurious to desired grass species (Belcher and Walker 2010; Curtis et al. 2009; Flessner et al. 2011b; Jenks and Walter 2012; Mansue and Hart 2010; Montgomery et al. 2009). Some grasses however can be injured and exhibit differing levels of tolerance to ACPC

(Brecke et al. 2010; Flessner et al. 2011c). For example, while tall fescue [*Schedonorous arundinaceus* (Schreb.) Dumort, nom. cons.], buffalograss [*Bouteloua dactyloides* (Nutt.) J.T. Columbus], and Zoysiagrass (*Zoysia japonica* Steud.) are tolerant (Anonymous 2010; Curtis et al. 2009; Flessner et al. 2011b, 2011c; Harmoney et al. 2012; Reed et al. 2013), cogongrass, winter wheat (*Triticum aestivum* L.), smooth crabgrass [*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.], and St. Augustinegrass are susceptible to ACPC (Belcher and Walker 2010; Flessner et al. 2011a; Kniss and Lyon 2011; Reed et al. 2013). These types of differing sensitivities are not a new phenomenon as many synthetic auxin herbicides show differential injury across families, species, and even among different cultivars (Brian 1958; Fang and Butts 1953; Johnson 1978, 1983; Koo et al. 1997; McElroy et al. 2005; van Rensburg and Breeze 1990). Whitehead & Switzer (1963) noted that many factors such as differences in leaf surface, leaf arrangement, absorption, translocation, adsorption to inactive sites, and metabolism could regulate the degree of plant susceptibility to 2,4-D. These factors, then, likely hold true for many of the recently developed synthetic auxin herbicides as well (Bukun et al. 2009; Flessner et al. 2011c; Mullison 1950; Rasmussen 1947). Along with other synthetic auxins, ACPC also features low use rates, low mammalian toxicity, and low volatility (USEPA 2010, 2010b; Claus et al. 2009; Strachan et al. 2010; Turner et al. 2008). Between fall 2010 and August 2011, ACPC was marketed as Imprelis® by E.I. du Pont de Nemours and Company (DuPont) for the selective control of broadleaf weeds in cool-season and some warm-season turfgrasses on lawns, golf courses, sod farms, and athletic fields. The use of Imprelis® for select established warm-season grasses was labeled at 52.5-79.0 g ae ha^{-1} while established cool-season grasses such as tall fescue were labeled as high as 105.0 g ae ha^{-1}

(Anonymous 2010). Initial reports also indicate that ACPC shares characteristics with clopyralid and aminopyralid such as persistence within treated soil and plant material, systemic movement in plants, and high seedling emergence toxicity. These features are believed to contribute to herbicide carryover in manure, compost, and plant clippings (Claus et al. 2009; Patton et al. 2013). ACPC is currently being explored for use in pastures, roadsides, and forestry situations alone and in combination with other active ingredients (Ezell and Self 2013; Isreal et al. 2013; Meredith et al. 2013; Sellers et al. 2013).

Absorption and Translocation. ACPC absorption can occur via foliar and root mechanisms (Anonymous 2010; Lewis et al. 2013b; Westra et al. 2009). Similar to other synthetic auxin herbicides, the absorption of ACPC is rapid (Bukun et al. 2010; Lewis et al. 2013b; Lindenmayer et al. 2013). In tall fescue, ACPC absorption was complete at only 48 HAT, with the majority of the absorbed ACPC remaining in the above ground foliage (Lewis et al. 2013b). In Canada thistle, ACPC reached peak absorption at only 24 HAT. This rapid absorption of ACPC corresponds well with the rapid absorption of other similar synthetic auxin herbicides such as aminopyralid and clopyralid (Bukun et al. 2009, 2010). Lewis et al. (2013a) reported that ACPC absorption and efficacy increased in the presence of ambient moisture on plant foliage. Analogous to other synthetic auxins, the amount of absorption is highly dependent on the ACPC formulation (methyl vs. free acid) and the surfactant used (Bukun et al. 2010).

Translocation of ACPC likewise resembles other synthetic auxins such as aminopyralid and clopyralid in both Canada thistle and tall fescue as the majority of each of these herbicides was detected in either the treated leaf or above ground foliage (Bell et al. 2011; Bukun et al. 2009, 2010; Lewis et al. 2013b). Although ACPC was found to translocate from the treated leaf to the remainder of foliage, limited ACPC was detected in tall fescue crowns (Lewis et al. 2013b). This finding is contrary to that of broadleaf plants, where ACPC travelled to meristematic sink tissues (Bell et al. 2011) and then into the roots, which could explain their increased sensitivity compared to monocot species (Lindenmayer et al. 2013). Lewis et al. (2013) speculated that this behavior could be attributed to rapid phloem mobility of the compound within the crown region of tall fescue plants. Minimal ACPC was detected in tall fescue roots. This limited movement into below ground tissue is consistent with other synthetic auxin herbicides (Bukun et al. 2009, 2010; Lewis et al. 2013b; Lym and Moxness 1989). Also similar to other synthetic auxins, the amount of absorption and subsequent movement of ACPC seems to be dependent upon the sensitivity of the treated species (Bell et al. 2011; Bukun et al. 2009; Davis et al. 1968).

Metabolism and Fate. Consistent with other synthetic auxin herbicides, ACPC can persist within plants and the soil for more than 100 DAT (USEPA 2010; Conklin and Lym 2013; Patton et al. 2013; Strachan et al. 2011). Concentrations of ACPC in tree branches of Norway spruce [*Picea abies* (L.) Karst.] and honey locust (*Gleditsia triacanthos* L.) ranged from 1.7 to 14.7 ppb while concentrations in the soil were much lower at 0.1 ppb (Patton et al. 2013). Oliveira et al. (2011) and USEPA (2010) suggest that based on sorption coefficients, ACPC is very mobile once in the soil profile. Oliveira et al. (2011) reported that adsorption of ACPC to the soil is minimal, rendering it available for plant uptake. These small amounts are significant as sensitive species can exhibit synthetic auxin symptoms at very low concentrations (Patton et al. 2013; van Rensburg and Breeze 1990; Strachan et al. 2011). ACPC has a 50% dissipation time (DT_{50}) ranging from 3 to more than 112 days. This wide range is due to a number of factors such as soil type, moisture content, and temperature. Due to the possibility of prolonged persistence, planting or seeding into areas previously treated with ACPC should be done with caution (Conklin and Lym 2013).

This prolonged persistence carries over within plants as metabolism of ACPC has yet to be documented either in dicot or monocot species suggesting that it moves to sink tissues and then is incorporated slowly into the plant. This stability of the free acid herbicide has been observed in both monocot and dicot species (Bell et al. 2011; Lewis et al. 2013b; Lindenmayer et al. 2013). This correlates with the lack of metabolism of similar pyridine carboxylic acid herbicides (Bukun et al. 2009; Lym and Moxness 1989) and many other synthetic auxins (Scott and Morris 1970).

Comparable to other synthetic auxins such as dicamba, ACPC has been reported to exude from plant roots (Lewis et al. 2013b; Linder et al. 1958, 1964). ACPC root exudation from tall fescue roots was greatest at 48 HAT with 8% of applied ACPC detected in below ground media (Lewis et al. 2013b). This small amount, however, could be enough to elicit damage to sensitive species (Patton et al. 2013; van Rensburg and Breeze 1990). ACPC was shown to exude from wood chips under irrigated conditions (Patton et al. 2011, 2013), but limited research exists on the ability of ACPC to exude from clippings and plant compost. Significant exudation of other synthetic auxins has been observed; therefore it is not beyond reason to suspect that ACPC could behave in a similar manner (Blewett et al. 2007; Bovey et al. 1967; Branham and Lickfeldt 1997; Chang and VandenBorn 1968; Linder et al. 1964; Miltner et al. 2003).

Significant Poaceae Species

Bahiagrass (*Paspalum notatum* Flueggé) is a major sub-tropical forage species grown from the southeastern United States (U.S.) to Argentina (Burton 1946). The Bureau of Plant Industry originally introduced it from Brazil into the U.S. in 1913, at the Florida Agricultural Experiment Station in Gainesville, FL. It has long been used in roadsides, pastures, forages, and lawns because of its drought tolerance, low-fertility requirements and ability to stabilize many soils. Several cultivars have been developed since the introduction of bahiagrass into the U.S., with over 2.5 million ha of improved bahiagrass pastures planted (Bunnell et al. 2003; Smith et al. 2002). Bahiagrass has excellent seeding habits, aggressive growth, and persistence that have helped it become not only an extensively used forage grass, but a bothersome weed as well (Burton 1967).

Bermudagrass [*Cynodon dactylon* (L.) Pers.], a tropical grass native to Africa, is a desired turf and pasture grass that is adapted to the northern borders of the subtropical climate zone of the United States (Bell et al. 2000; Kneebone 1966). Bermudagrass is desired for its ability to grow on acid or alkaline soils, withstand flooding or drought and high salt concentrations. It can also tolerate variable fertility soils and be grazed more closely than most grass species can endure. In turfgrass systems, it provides a dense and wear resistant surface for golf course fairways (Kneebone 1966). A number of bermudagrass cultivars are frequently used in lawns, on golf courses, athletic fields, tennis and bowling greens, parks and playgrounds, highway medians and roadsides, and private grounds (Caetano-Annolles et al. 1997). One particular variety, coastal bermudagrass, is currently being grown on over 1.6 million ha in the southeastern U.S. alone (Kneebone 1966). Bermudagrass is also a weedy species in many cropping systems, and has been designated one of the world's worst weeds (Holm et al. 1977).

Cogongrass is a rhizomatous weedy grass native to tropical and subtropical areas of the eastern hemisphere. It was introduced into southern Alabama in the early 1900s where it continues to present major problems in agronomic and horticultural crops when intense cultivation is not practiced (Dickens 1974; Halliedozier et al. 1998). It also thrives on roadways and in pastures and mining areas, pine forests, parks, and other recreational areas due to limited disturbance (Halliedozier et al. 1998; Willard et al. 1990). The unpalatability and low nutrient quality of cogongrass also make it a threat for pasture and forage production. Growth from rhizomes is rapid, especially under favorable conditions (Bryson and Carter 1993; Dickens 1974). Cogongrass is relatively easily and rapidly dispersed by seed, and can quickly become established in most subtropical areas. The strong growing rootstocks enable it to spread deep beneath the surface, and to remain vigorous through long, hot and dry seasons. Moreover, cogongrass produces a significant amount of heat when burned. Thus, once cogongrass becomes established in forest clearings or in plantations of tree crops, it is extremely difficult to exterminate via prescribed burning. If unmanaged, it also becomes a serious fire hazard (Bryson and Carter 1993; Halliedozier et al. 1998; Pendleton 1948). Cogongrass is considered one of the greatest invasive plant threats in the southeastern U.S. and was rated one of the top 10 most serious weeds in the world (Belcher and Walker 2010; Holm 1969).

Tall fescue is a perennial cool-season bunch grass native to Europe. It gained popularity beginning in 1931 in Kentucky as it was found to be a productive forage grass (Ball et al. 2007). It has more recently been adapted for use in home lawns, athletic fields and roadsides throughout the U.S. (Ball et al. 2007; Beard 1973; Turgeon 1980). It is one of the most prevalent cool-season grasses with over 14 million ha planted in the U.S. alone. While it grows best in the transition zone, it can be found growing in acid or alkaline soils from Florida to Canada (Ball et al. 1993; Buckner et al. 1979). Tall fescue is the most heat tolerant of the major cool-season grasses owing to its deep root system (Ball et al. 1993). This adaptability makes tall fescue a popular choice for conservation work in wetlands, roadsides, eroded pastures, and other disturbed areas. It can also function as an unwanted weed species in home lawns, pastures, and wildlife restoration efforts (Buckner et al. 1979; Dernoeden 1990; Washburn et al. 2000).

Objectives of Research

Research included three principal objectives for determining the physiology of ACPC in four graminaceous species. First, the response of bahiagrass, bermudagrass, cogongrass, and tall fescue to ACPC was used to determine the tolerance of each species to the herbicide. Secondly, ^{14}C-ACPC was used to track the amount of absorption, translocation and metabolism of the herbicide within each grass species. This information allowed conclusions to be drawn concerning the role that absorption and the ultimate fate of ACPC play in the overall susceptibility of each grass species. Lastly, root exudation of ACPC was tested against dicamba utilizing bioassay studies to determine the probability of ACPC leaking from plant roots. These studies will determine if ACPC is being lost via root leakage and assist in accounting for ^{14}C loss in alternative physiology studies.

Response of Four Graminaceous Species to Aminocyclopyrachlor

Introduction

Synthetic auxin herbicides are widely used in turfgrass, pasture, roadside, and grass cropping systems for control of broadleaf weed species while remaining safe to grass species. While this exclusion of injury to grass species by synthetic auxins is a general rule, a number of exceptions exist, chiefly among warm-season grasses (Bell et al. 2000; Cudney et al. 1997; Doroh et al. 2009; Grossmann 2003; Johnson 1995; Kaufman 1955; McElroy et al. 2005; Patton et al. 2010).

Currently, aminocyclopyrachlor (ACPC) is the first and only member of the pyrimidine carboxylic acid herbicide family to be registered and commercialized. It is structurally similar to the pyridine carboxylic acid herbicides, which include picloram, clopyralid, and aminopyralid (Senseman 2007). ACPC exhibits the synthetic auxin herbicide mode of action (MOA), which disrupts gene expression resulting in undifferentiated cell division and tissue elongation (Grossmann 2003; Woodward and Bartel 2005). ACPC is however, unique from the aforementioned compounds in some respects (USEPA 2010; Claus et al. 2009). Compared to most synthetic auxin herbicides, ACPC features low use rates, low mammalian toxicity, and low volatility (USEPA 2010, 2010b; Claus et al. 2009; Strachan et al. 2010; Turner et al. 2008). Between fall 2010 and August 2011, the potassium salt formulation of ACPC was marketed as Imprelis® by E.I. du Pont de Nemours and Company (DuPont) for the selective control of broadleaf weeds in cool-season and some warm-season turfgrasses on lawns, golf courses, sod farms, and athletic fields (Anonymous 2010). This formulation has since been removed from the market for more extensive testing as it is reported to injure or kill some trees and shrubs

(Patton et al. 2011, 2013). Imprelis® was labeled for select established warm-season grasses at 52.5-79.0 g ae ha^{-1}, while established cool-season grasses such as tall fescue were labeled as high as 105.0 g ae ha^{-1} (Anonymous 2010). ACPC controls a number of troublesome weeds while remaining less injurious to desired grass species (Belcher and Walker 2010; Curtis et al. 2009; Flessner et al. 2011b; Gannon et al. 2009; Jenks and Walter 2012; Mansue and Hart 2010; Montgomery et al. 2009). Some grasses however, can be injured and exhibit differing levels of tolerance to ACPC application (Anonymous 2010; Belcher and Walker 2010; Brecke et al. 2010; Curtis et al. 2009; Flessner et al. 2011b, 2011c; Harmoney et al. 2012; Kniss and Lyon 2011; Reed et al. 2013). ACPC is currently being explored for use in pastures, roadsides, and forestry situations alone and in combination with other active ingredients (Ezell and Self 2013; Isreal et al. 2013; Meredith et al. 2013; Sellers et al. 2013). In order to effectively implement ACPC in future weed control systems; the tolerance level of widely used and weedy graminaceous species must be considered.

Four economically significant graminaceous species were selected for rate response tests. Bahiagrass (*Paspalum notatum* Flueggé) is a major sub-tropical forage species native to Brazil grown from the southeastern U.S. to Argentina (Burton 1946). Bahiagrass has long been used in roadsides, pastures, forages, and lawns because of its drought tolerance, low-fertility requirements and ability to stabilize soil. Bahiagrass has excellent seeding habits, aggressive growth, and persistence that have helped it become not only an extensively used forage grass, but a bothersome weed as well (Burton 1967). Currently bahiagrass is listed as ‘tolerant’ of ACPC application provided it is established (Anonymous 2010).

Bermudagrass [*Cynodon dactylon* (L.) Pers.], a tropical grass native to Africa, is a desired turf and pasture grass that is adapted to the northern borders of the subtropical climate zone of the United States (Bell et al. 2000; Kneebone 1966). Bermudagrass is desired for its ability to grow on either acid or alkaline soils, withstand flooding or drought and high salt concentrations. It can also tolerate variable fertility soils and be grazed more closely than most grass species can endure (Kneebone 1966). Bermudagrass is also a weedy species in many cropping systems, and has been designated one of the world's worst weeds (Holm et al. 1977). Previous research has shown bermudagrass to be sensitive to some synthetic auxin herbicides while tolerant of others (Bell et al. 2000; Flessner et al. 2011c).

Cogongrass [*Imperata cylindrica* (L.) P. Beauv.] is a rhizomatous weedy grass native to tropical and subtropical areas of the eastern hemisphere. It was introduced into southern Alabama in the early 1900s where it continues to present major problems in agronomic and horticultural crops when intense cultivation is not practiced (Dickens 1974; Halliedozier et al. 1998). Cogongrass also thrives along roadsides, in pastures, mining areas, pine forests, parks, and other recreational lands due to limited disturbance (Halliedozier et al. 1998; Willard et al. 1990). The unpalatability and low nutrient quality of cogongrass also make it a threat for pasture and forage production (Bryson and Carter 1993; Dickens 1974). Cogongrass is considered one of the greatest invasive plant threats in the southeastern U.S. and was rated one of the top 10 most serious weeds in the world (Belcher and Walker 2010; Holm 1969). Cogongrass has been reported to be sensitive to ACPC, which provides short-term vegetative control and seedhead suppression (Belcher and Walker 2010; Enloe et al. 2012).

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] is a perennial cool-season bunch grass native to Europe. It was imported to the United States in the late 19th century and rose to prominence in the 1930s in Kentucky when found to be a productive forage grass (Ball et al. 2007). Tall fescue has more recently been adapted for use in home lawns, athletic fields and roadsides throughout the U.S. (Ball et al. 2007; Beard 1973; Turgeon 1980). It can also function as an unwanted weed species in home lawns, pastures and wildlife restoration efforts (Buckner et al. 1979; Dernoeden 1990; Washburn et al. 2000). Tall fescue has been reported to be tolerant of ACPC application (Anonymous 2010; Lewis et al. 2013b; Reed et al. 2013).

A number of studies exist on graminaceous tolerance level to ACPC, however none of these studies directly compare species that are reported to be tolerant or susceptible (Flessner et al. 2011b, 2011c; Kniss and Lyon 2011; Lewis et al. 2013b). The objective of this study was to observe the injury sustained by four graminaceous species (bahiagrass, bermudagrass, cogongrass and tall fescue) to ACPC at previously labeled rates. This data in conjunction with physiological data can then be used to determine possible causes of these differing tolerances. Our hypothesis is that the four aforementioned species will rank from most tolerant to least tolerant in the following order: tall fescue, bermudagrass, bahiagrass and cogongrass.

Materials and Methods

Plant and Chemical Materials. Grass species selected for evaluation were ‘Pensacola’ bahiagrass (Pennington® Seed), common bermudagrass, cogongrass, and ‘Rebel III’ tall fescue (Pennington® Seed). The herbicide used was the free-acid formulation of ACPC

(Aminocyclopyrachlor, DPX-MAT28, E. I. du Pont de Nemours and Company). All experiments were conducted twice (repeated) during 2014. Experiments used a randomized complete block design with four replications per treatment. Two experiments each were conducted for bahiagrass, bermudagrass, cogongrass, and tall fescue for a total of eight experiments. All herbicide-containing treatments also included a non-ionic surfactant (NIS) (Activator 90 Non-Ionic Surfactant, Loveland Products Inc.) at 0.25% v v^{-1}. This adjuvant was included because previous research involving ACPC and other related synthetic auxins indicate it substantially increases herbicide absorption (Bell et al. 2011; Bukun et al. 2009; Lewis et al. 2013b).

Greenhouse Studies. The greenhouse was located in Auburn, AL. Greenhouse temperatures were maintained between 23 and 26 C for the duration of the experiments. Normal sunlight was supplemented with sodium-halide growth lamps producing 150-µmol m^{-2} s^{-1} at the turf canopy. Total peak irradiance was <800-µmol m^{-2} s^{-1} throughout the experiment. Grasses were grown from seed in plastic flats containing a potting substrate (Scott's Miracle-Gro® Potting Mix, The Scott's Company, LLC). Grasses were then prepared for treatment by transplanting 5 individual plants into 700 cm^3 pots using Marvyn loamy sand (native) soil. Grasses were allowed to recover for 2 wks prior to treatments being applied. Grasses were maintained with weekly fertilizer applications (Miracle-Gro® All Purpose Plant Food, The Scott's Company, LLC), daily irrigation, and monthly insecticide applications. Treatments were based on the lowest and highest labeled rates of ACPC for effective weed control (Anonymous 2010). Rates were 52.5 and 105.0 g ae ha^{-1} with a nontreated check. Treatments were applied in a spray volume of 280 L ha^{-1} using an enclosed spray chamber with a single nozzle (TeeJet TP8002EVS, Spraying Systems Co.®). All experiments were repeated in time and applied on February 3 and March 17, 2014.

Data Analysis. Visual ratings of each grass species were taken at 10 and 20 days after treatment (DAT) and were chosen to bracket a typical synthetic auxin rating date of 2 weeks after treatment (WAT) (Sciumbato et al. 2004). Visual quality ratings were based on a scale of 0 to 100 with 0 corresponding to dead plants and 100 indicating healthy plants. All plants were clipped to a uniform height of approximately 7.5 cm following the 10 DAT rating date to simulate typical grass systems and allow for measurement of regrowth. Plant heights in cm and both fresh and dry weights in g were then taken at 20 DAT to quantify any physical effects of the herbicide treatments. Fresh plant material was then dried for 1 wk and weighed. This process was to remove any water retention bias from plant weight data. All species were analyzed together. Experimental units consisted of a single pot. Data were subjected to ANOVA in SAS (SAS® Institute Inc., v. 9.2) using PROC GLM to test for significance of replication-in-time, herbicide rate, grass species, and allow for lack-of-fit testing. Experimental run and replication were considered random effects with treatment, species, and harvest interval being considered fixed effects. Means were separated using Fisher's Protected LSD and considered significant if $P < 0.05$.

Results and Discussion

Visual Injury. ANOVA revealed that repetition in time and replications within the experiment were not significant. Therefore data were pooled across repetitions and replications within experiments. A three-way interaction of DAT, treatment, and species was significant ($P < 0.005$) indicating that injury to most species increases with time. At 10 DAT, cogongrass and bahiagrass were the most sensitive species, particularly at the high rates (Table 1). Bermudagrass and tall fescue are much more tolerant of ACPC with injury to both species negligible at both the low and high rate. At 20 DAT, cogongrass exhibited significant injury at both rates. Visual symptoms of cogongrass injury included stunting of growth, reddening and weakening of crown tissues, followed by necrosis of plant foliage (Figure 1). Bahiagrass was significantly more sensitive at the high rate than at the low rate, and was injured at an unacceptable level for use in either turfgrass or forage systems (Johnson and Murphy 1995; Johnson 1995). Symptoms of bahiagrass injury were brittle crowns, reddening of stems, moderate leaf epinasty, and in some cases complete plant necrosis (Figure 2). Like bahiagrass, bermudagrass was more sensitive to the high rate, while the low rate had little effect. In time, ACPC had an affect on bermudagrass growth resulting in significant swelling at nodes and moderate leaf epinasty (Figure 3). These symptoms however, did not prove fatal to treated plants or compromise grass quality (Figure 4). Tall fescue was tolerant of both rates with only temporary leaf epinasty. The tolerance of tall fescue is in agreement with previous research (Anonymous 2010; Lewis et al. 2013b).

Foliar Weight Reduction. ANOVA revealed that treatment levels and replications within each experiment were not significant. Therefore, data were pooled across repetitions and treatments within experiments. The run by species interaction for this study was significant ($P < 0.01$) and is presented in the most concise way. Data indicate that tall fescue is the most tolerant grass species to ACPC treatment at both rates evaluated (Table 2). Compared to the non-treated, tall fescue plants had no significant gain or loss of mass. Cogongrass is again the most sensitive species as treated plants experienced a loss of over 80% mass compared to the non-treated. Studies indicate that bermudagrass and bahiagrass also lose a significant amount of mass compared to nontreated checks. In one study, bahiagrass appeared to lose much more weight compared to the nontreated. This is most likely because plants in the February study showed leaf necrosis several days earlier than those in March studies. Dry weights were also taken for all treatments and species though the data is not presented here. Due to the nature of synthetic auxin herbicides, that is, their tendency to cause uncontrolled cell division and growth, it was deemed that the best way to account for weight changes was by avoiding the use of this data (no significances were found). The idea is that desiccated plant tissues heavily affected by auxins will weigh the same when as dried non-treated plants if the only symptoms were uncontrolled growth, leaf epinasty, and swelling.

Foliar Height Reduction. Contrary to previous studies, no significant interactions were detected. Therefore data were pooled across repetitions and replications. Both the treatment and species main affect were significant in regard to percent reduction of plant height. Tall fescue height was minimally impacted by ACPC treatment (Table 3). It has been documented that tall fescue is tolerant of ACPC application. Our results agree with those studies, as well as the general trend that warm-season grasses are more sensitive to synthetic auxin herbicides than cool-season grasses (Bell et al. 2000; Lewis et al. 2013b; Senseman 2007; Wehtje 2008). Bermudagrass and bahiagrass heights were moderately reduced compared to the nontreated, indicating that ACPC at both rates slowed the rate of growth following a mowing event. Cogongrass height was significantly reduced regardless of treatment due to plant necrosis. Though the species by treatment interaction was not significant, bahiagrass plants treated with the higher rate had greater height reductions than the lower rate. Treatment data indicate the expected outcome, that greater rates of ACPC application result in impaired plant growth across all species (Table 4).

Complete Response. Our hypothesis concerning the ranking of tolerance of each grass species was proven accurate. ACPC appears to follow the general trend of cool-season grass tolerance while injuring warm-season grasses (Flessner et al. 2009, 2011b). Tall fescue was the most tolerant species with negligible loss of height, weight and overall appearance regardless of the rate. Bermudagrass and bahiagrass were much more sensitive. This was especially evident in the reduction of plant heights and weights. Visually, bermudagrass at both rates appeared unaffected with the exception of swollen nodes. Bahiagrass plants suffered from brittle and reddening crowns, indicating the susceptibility of this grass is near unacceptable levels for use at the tested rates in most grass systems, especially in the presence of regular mowing events. Cogongrass was the most susceptible species. Treated plants suffered from stunting of growth, loss of weight, and an overall loss of plant vigor. This result offers the possibility that repeated applications of ACPC may offer selective control of cogongrass or other weedy grasses in some cropping systems. The variance in the tolerance of these grass species offers hope of future grass-in-grass control. The differing tolerance of the species studied may be due to dissimilar absorption, translocation or metabolism within each grass species.

Table 1. Visual injury[a] of select grass species in response to two rates of foliar-applied aminocyclopyrachlor from two combined experiments in 2014 in Auburn, AL.[b]

Grass	ACPC[c] Rate	10 DAT[d]		20 DAT	
Species	g ae ha^{-1}	-------------------------%		-------------------------	
	52.5	12.5	DE	21.9	C
cogongrass	105.0	71.9	A	72.5	A
	52.5	6.9	EF	20.6	CD
bahiagrass	105.0	20.0	CD	55.6	B
	52.5	2.5	F	0.6	F
bermudagrass	105.0	6.3	EF	16.9	CD
	52.5	0.0	F	0.0	F
tall fescue	105.0	3.1	F	2.5	F
$LSD_{(0.05)}$				16.9	

[a] Visual injury rated on a scale of 0 to 100 with 0 equivalent to the control and 100 corresponding to complete plant death.

[b] Data were pooled across studies as a significant treatment by study interaction was not detected. A three-way interaction of species, treatment and DAT was significant ($P < 0.001$).

[c] Abbreviations: DAT, days after treatment; ACPC, aminocyclopyrachlor

[d] Means sharing a letter are not different according to Fisher's protected $LSD_{(0.05)}$.

Table 2. Foliar weight reduction of selected grass species in (g) in response to foliar-applied aminocyclopyrachlor in 2014 in Auburn, AL.[a]

Species	Feb. 3 2014[b]	Mar. 17 2014
	--------------------------------Percent Reduction[c]--------------------------------	
cogongrass	-82 C	-88 C
bahiagrass	-82 C	-42 B
bermudagrass	-35 B	-49 B
tall fescue	-2 A	2 A
$LSD_{(0.05)}$	23	24

[a] Data are presented by run as a significant species by study interaction was detected. Treatment by species interaction was not significant.

[b] Means sharing a letter within column are not different according to Fisher's protected $LSD_{(0.05)}$.

[c] Reduction relative to appropriate nontreated control.

Table 3. Foliar height reduction of selected grass species in (cm) in response to foliar-applied aminocyclopyrachlor.[a]

	Percent Reduction[bc]
Species	-------------%-------------
cogongrass	-67 C
bahiagrass	-31 B
bermudagrass	-27 B
tall fescue	-10 A
$LSD_{(0.05)}$	13

[a] Data are presented by species as a significant species by treatment interaction was not detected (P < 0.0001). Species by run interaction was not significant.

[b] Means sharing a letter are not different according to Fisher's protected $LSD_{(0.05)}$.

[c] Reduction relative to appropriate nontreated control.

Table 4. Foliar height reduction of selected grass species in (cm) in response to foliar-applied aminocyclopyrachlor at two rates.[a]

ACPC Rate[b]	Percent Reduction[c]
g ae ha^{-1}	-------------%-------------
52.5	-27 A
105.0	-40 B
$LSD_{(0.05)}$	9

[a] Data are presented by treatment as a significant species by treatment interaction was not detected. Treatment by run interaction was not significant.

[b] Abbreviations: ACPC, aminocyclopyrachlor

[c] Means sharing a letter are not different according to Fisher's protected $LSD_{(0.05)}$.

Figure 1. Aminocyclopyrachlor-induced injury on cogongrass. Aminocyclopyrachlor was foliar-applied at 52.5 g ha^{-1}, photograph taken 10 days after treatment. Note reddening of stem (A) and leaf necrosis (B).

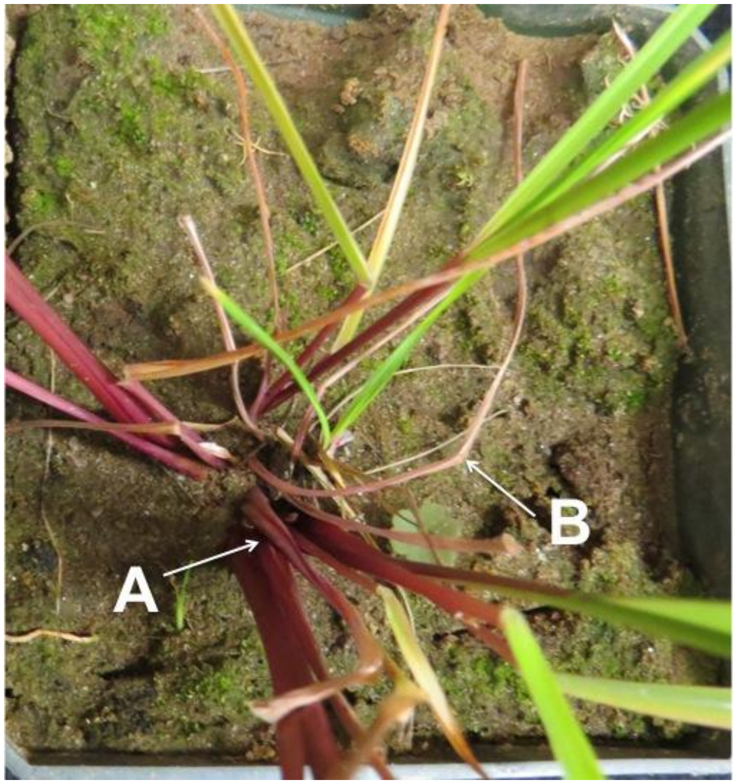

Figure 2. Aminocyclopyrachlor-induced injury on bahiagrass. Aminocyclopyrachlor was foliar-applied at 52.5 g ha^{-1}, photograph taken 10 days after treatment. Note reddening of stem (A), and leaf necrosis (B).

Figure 3. Aminocyclopyrachlor-induced injury on bermudagrass. Aminocyclopyrachlor was foliar-applied at 105.0 g ha^{-1}, photograph taken 20 days after treatment. Note swelling of stem and nodes (A).

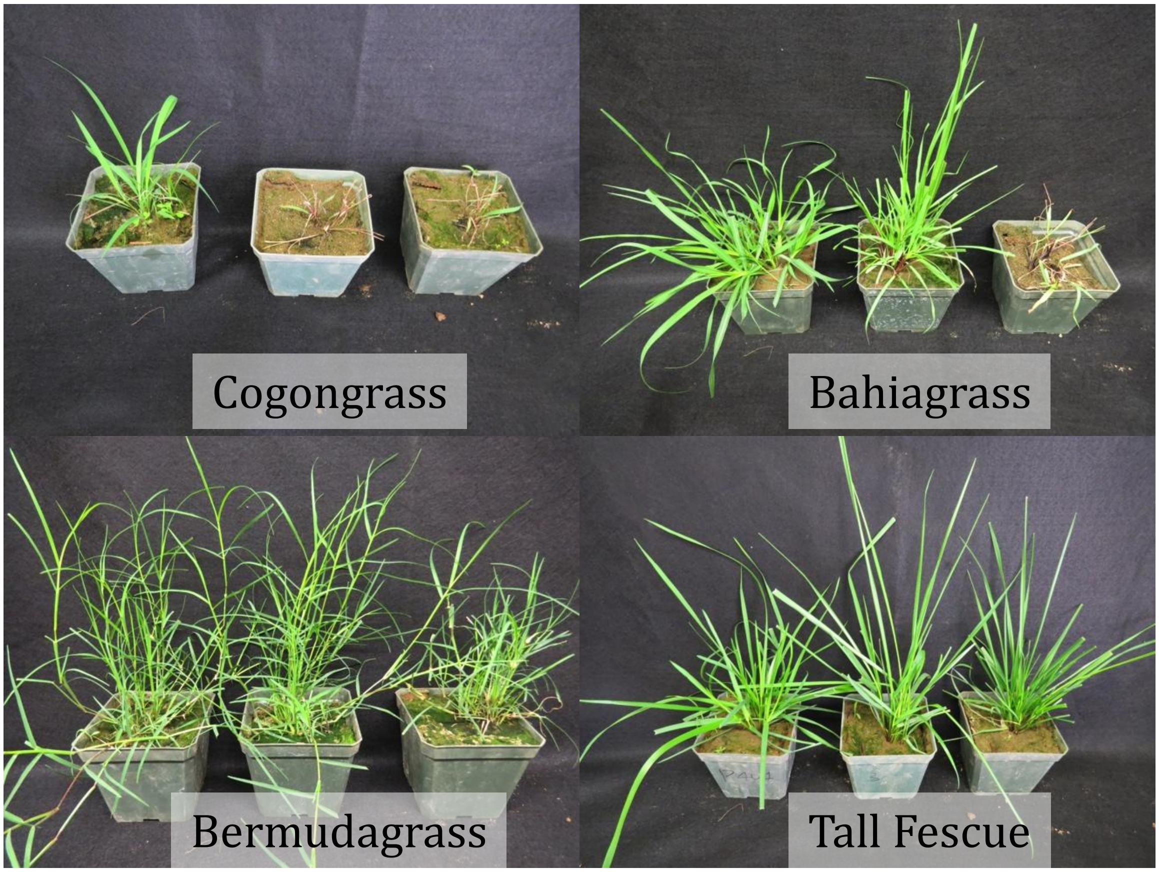

Figure 4. Response of four grasses to aminocyclopyrachlor at 20 days after treatment.

From left to right treatments are nontreated, 52.5 g ae ha^{-1}, and 105.0 g ae ha^{-1}

Absorption and Fate of Aminocyclopyrachlor in Select Graminaceous Species

Introduction

Synthetic auxin herbicides have long been used in turfgrass, pasture, roadside, and grass crops due to their exemplary control of broadleaf weed species while remaining safe to grass species (Bell et al. 2000; Curtis et al. 2009; Mansue and Hart 2010; McElroy et al. 2005). These herbicides are among the most successful in all of agriculture with nearly a tenth of all herbicide sales in 2000 being synthetic auxin products (Grossmann 2003; Sterling and Hall 1997). Their wide usage in these areas is primarily due to the aforementioned control of broadleaf weeds and safety in many desirable grass species (Bell et al. 2000; Curtis et al. 2009; Mansue and Hart 2010; McElroy et al. 2005). Synthetic auxins are typically applied post-emergence and subsequently absorbed rapidly, primarily into the plant foliage, and in some cases the roots also (Bovey et al. 1983; Ross and Lembi 1999; Senseman 2007). Synthetic auxin compounds function by mimicking the naturally occurring 'master hormone' indole-3-yl-acetic acid (IAA), which stimulates cell division, differentiation, and ultimately plant growth at meristematic plant tissues (Grossmann 2010; Ross et al. 2002). Symptoms of these herbicides include leaf epinasty, uncontrolled root growth, changes in nucleic acid levels and increased biosynthesis of ethylene (Anderson 1996; Deshpande and Hall 2000; Devine et al. 1993).

Aminocyclopyrachlor (ACPC), is a new synthetic auxin characterized by comparatively low use rates and low mammalian toxicity (USEPA 2010, 2010b; Claus et al. 2009; Strachan et al. 2010; Turner et al. 2008). ACPC controls kochia [*Bassia scoparia* (L.) A.J. Scott] and cogongrass [*Imperata cylindrica* (L.) P. Beauv.] in right-of-ways, as well as yellow toadflax (*Linaria vulgaris* P. Mill.) in rangeland while remaining less injurious to desired grass species (Belcher and Walker 2010; Curtis et al. 2009; Flessner et al. 2011b; Jenks and Walter 2012; Mansue and Hart 2010; Montgomery et al. 2009). As in the case of cogongrass, some grass species can be injured and exhibit differing levels of tolerance to ACPC (Brecke et al. 2010; Flessner et al. 2011b; Reed et al. 2013). Due to their related structures and behaviors, and based on previous research, it is reasonable to assess that ACPC will behave similarly to other synthetic auxins within plants (Curtis et al. 2009; Mansue and Hart 2010).

Previous research has revealed that ACPC is absorbed into both tolerant and susceptible plant species (Bell et al. 2011; Bukun et al. 2010; Lewis et al. 2013b; Lindenmayer et al. 2013). Absorption of ACPC in broadleaf species has been classified as rapid, with complete absorption occurring within 24 to 48 hours after treatment (HAT) (Bell et al. 2011; Bukun et al. 2010; Lindenmayer et al. 2013). Lewis et al. (2013) found that absorption of ACPC into tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.], a tolerant poaceae species, was also rapid with maximum absorption at 48 HAT. This study also reported that maximum translocation away from the treated leaf occurred within only 96 HAT, indicating limited but rapid movement throughout aboveground tissues. Studies indicate that ACPC movement in broadleaf plants reached a peak between 24 and 192 HAT depending on the species (Bell et al. 2011; Bukun et al. 2010; Lindenmayer et al. 2013). Pooling results from these studies, it appears that both susceptible and tolerant as well as monocot and dicot species exhibit similar absorption characteristics in regard to ACPC. Absorption and translocation did not directly relate to the specific level of susceptibility to ACPC across species (Bell et al. 2011). There is no documentation of ACPC metabolism in either susceptible or tolerant species out to 192

HAT (Bell et al. 2011; Bukun et al. 2010; Lewis et al. 2013b; Lindenmayer et al. 2013) This correlates well with the lack of metabolism found with similar pyridine carboxylic acid herbicides such as aminopyralid, clopyralid and picloram (Bukun et al. 2009; Lym and Moxness 1989).

Following its use in turfgrass in 2010 and early 2011 as Imprelis®, ACPC was removed from the turfgrass market in the summer of 2011 after a stop sale, use, or removal order was issued to DuPont (Ferdas 2011; Patton et al. 2011). This removal of Imprelis® came after widespread reports of off-target damage to trees and ornamentals in the landscape where applications were made. The Office of Indiana State Chemist (OISC) reported that damage observed could not be attributed to misapplications as the herbicide was applied to lawns per label instructions (Patton et al. 2011). Despite these previous issues, ACPC has the potential to be widely used for broadleaf weed control in various grass crops as an alternative to currently labeled phenoxyacetic and pyridine herbicides (Anonymous 2012, 2014; Lewis et al. 2013b). ACPC also has the possibility of being used to control troublesome grass species within grass crops (Enloe et al. 2012; Reed et al. 2013). There is limited information available about the absorption, translocation, and metabolism of ACPC in both tolerant and susceptible graminaceous species. In light of the damage to off-target species and to ensure the success of ACPC in the future, its fate must be examined not only in dicot species, but in tolerant and susceptible graminaceous species as well. If ACPC is moderately absorbed by grasses and remains as the active form, there is a possibility that it can be returned to the soil through mowing, grazing, or root exudation (Lewis et al. 2013b). Provided ACPC remains as the active form within the plant, protected from soil degradation by microbes and from photolysis (USEPA 2010; Finkelstein et al. 2008), it is likely that activity in applied grass systems will be prolonged.

Four economically significant grass species were used as model plants for absorption and fate studies. They were chosen for their differing tolerances to ACPC application and for their economic importance or for their prolific, weedy nature (Anonymous 2010; Belcher and Walker 2010; Burton 1967; Flessner et al. 2011c). Bahiagrass (*Paspalum notatum* Flueggé) has long been used in roadsides, pastures, forages, and lawns because of its drought tolerance, low-fertility requirements, and ability to stabilize most soils. Several cultivars have been developed since the introduction of bahiagrass into the United States in 1913, with over 2.5 million ha of improved pasture planted (Bunnell et al. 2003; Smith et al. 2002). It has excellent seeding habits, aggressive growth characteristics, and persistence that have helped it become not only an extensively used forage grass, but a bothersome weed as well (Burton 1967).

Bermudagrass [*Cynodon dactylon* (L.) Pers.] is a desired turf and pasture grass that is adapted to the northern borders of the subtropical climate zone of the United States (Bell et al. 2000; Kneebone 1966). It can tolerate low or high fertility soils and be grazed more closely than most other grasses can endure. As a turfgrass, it provides a dense, wear resistant turf for golf course fairways (Kneebone 1966). A number of bermudagrass cultivars are frequently used in lawns, on golf courses, athletic fields, tennis and bowling greens, parks and playgrounds, highway medians and roadsides, and private grounds because of these attributes (Caetano-Annolles et al. 1997). Bermudagrass is also a weedy species in many cropping systems, and has been designated one of the world’s worst weeds (Holm et al. 1977). Previous research has shown bermudagrass to be sensitive to some synthetic auxin herbicide applications while remaining tolerant of others (Bell et al. 2000; Flessner et al. 2011c).

Cogongrass is a rhizomatous weedy grass that presents a problem in agronomic and horticultural crops where intense cultivation is not practiced. Its unpalatability and low nutrient quality also make it a threat for pasture and forage production (Dozier et al. 1998). Growth from rhizomes is rapid, especially under favorable conditions (Bryson and Carter 1993; Dickens 1974; Yager et al. 2010). Cogongrass is considered one of the greatest invasive plant threats in the southeastern U.S. and was rated one of the top 10 most serious weeds in the world (Belcher and Walker 2010; Holm 1969). Studies have revealed that ACPC is able to injure cogongrass and also exhibit seedhead suppression at lower rates than comparable treatments (Belcher and Walker 2010; Enloe et al. 2012).

Tall fescue is a cool-season grass commonly utilized for home lawns, athletic fields, pastures and roadsides throughout the U.S. (Ball et al. 2007; Beard 1973; Turgeon 1980). It is one of the most prevalent cool-season grasses with over 14 million ha in the U.S. alone. While it grows best in the transition zone, it can be grown in acid or alkaline soils from Florida to Canada (Ball et al. 1993; Buckner et al. 1979). This adaptability makes tall fescue a popular choice for conservation work in wetlands, roadsides, eroded pastures and other disturbed areas. It can also function as an unwanted weed species in home lawns, pastures and wildlife restoration efforts (Buckner et al. 1979; Dernoeden 1990; Washburn et al. 2000). Previous research reports that tall fescue is tolerant of ACPC application (Anonymous 2010; Lewis et al. 2013b; Reed et al. 2013).

Because several graminaceous species exhibit differential tolerances to ACPC (Flessner et al. 2011a, 2011b; Reed et al. 2013) and (ET Parker, unpublished data), an attempt was made to determine the role of absorption, translocation and metabolism as possible mechanisms of the observed variance in plant tolerance. The objective of this study was to utilize reliable radiotracing techniques to explore ACPC absorption, translocation, and metabolism within four graminaceous species (Bell et al. 2011; Bukun et al. 2010; Fang and Butts 1953; Jaworski et al. 1955; Lewis et al. 2013b; Lindenmayer et al. 2013; Mitchell and Linder 1950).

Materials and Methods

Plant and Chemical Materials. Two experiments each were conducted for bahiagrass, bermudagrass, cogongrass, and tall fescue for a total of 8 experiments in Auburn, AL to determine the absorption, translocation and metabolism of ACPC within these species. Each experiment was repeated in time and applied on February 3 and March 17, 2014. A randomized complete block design in a five by four factorial arrangement (five plant parts across four harvest intervals) was used for absorption and translocation studies while a three by four factorial (three plant parts across four harvest intervals) was used for metabolism studies. All studies had four replications and two experimental runs. 'Pensacola' bahiagrass (Pennington® Seed), common bermudagrass, cogongrass, and 'Kentucky 31' tall fescue (Pennington® Seed) were used in all studies. The free-acid formulation of ^{14}C-ACPC (Aminocyclopyrachlor DPX-MAT28, E. I. du Pont de Nemours and Company) with a specific activity of 1522 kilobecquerels (kBq) mg^{-1} was applied in both experiments. Greenhouse temperatures were maintained between 23 and 26 C. Normal sunlight was supplemented with sodium-halide growth lights producing 150-µmol m^{-2} s^{-1} at the turf canopy. Total peak irradiance was 150 to 800-µmol m^{-2} s^{-1}

throughout the experiment. Grasses were grown from seed in plastic flats containing a potting substrate (Scott's Miracle-Gro® Potting Mix, The Scott's Company, LLC). Plants were prepared for treatment by transplanting single plants into 66 cm^3 cell pack pots using Marvyn loamy sand (native) soil. Grasses were allowed to recover for 2 wks prior to treatments being applied. Grasses were maintained by sub-irrigation to prevent removal of any unabsorbed herbicide from the leaf surface. A labeled rate of ACPC at 52.5 g ae ha^{-1} was used for all studies (Anonymous 2010). All herbicide-containing treatments included a non-ionic surfactant (NIS) (Activator 90 Non-Ionic Surfactant, Loveland Products Inc.) at 0.25% v v^{-1}. This adjuvant was included because previous research involving ACPC and other related synthetic auxins indicate it substantially increases herbicide absorption (Bell et al. 2011; Bukun et al. 2009; Lewis et al. 2013b).

Absorption and Translocation. For absorption studies, a single 10-µl drop of ^{14}C-ACPC was applied to the adaxial side of a fully mature leaf for a total of 1.67 kBq $plant^{-1}$. Plants were harvested at intervals of 1, 2, 4, and 8 days after treatment (DAT) then divided into the following sections: leaf wash, target area (a 2 cm leaf section to which the herbicide drop had been directly applied), treated leaf, remainder foliage, and crown plus roots. The leaf wash was obtained by placing the target area in a 20-ml plastic vial containing 1 ml of a 90:10 v v^{-1} methanol : deionized water solution and agitating it for 30 seconds to remove unabsorbed ^{14}C-ACPC. The leaf wash was combined with 15 ml of Universol™ fluid (MP Biomedicals, LLC) after harvest. Radioactivity of the leaf wash fluid was quantified via liquid scintillation spectroscopy (LSS) using a liquid scintillation spectrometer (Beckman Coulter™ LS-6500 Multipurpose Scintillation Counter, Beckman Coulter, Inc.). This allowed the determination of the amount of unabsorbed herbicide relative to the amount applied. Upon removing the washed target area from the scintillation vial, the remainder of the plant was dissected as indicated above. Plant material was oven dried at a temperature of 85 C, combusted using a Harvey biological oxidizer (OX501 Biological Oxidizer, R.J. Harvey Instrument Corporation), and was quantified via LSS.

Metabolism. For metabolism studies, two drops of ^{14}C-ACPC totaling 3.34 kBq plant^{-1} were applied to separate leaves in order to increase the likelihood of detecting metabolism. Only the target areas and remainder of above ground foliage were analyzed for metabolism of ^{14}C-ACPC. Plants were harvested at intervals of 7, 14, 21, and 42 DAT to examine the possibility that persistence of the parent compound could lend itself to gradual metabolism by select grass species. Immediately after harvest, plant sections were placed in 7 by 12 cm plastic bags and stored at -20 C until processing. Root material was placed in aluminum trays for oxidizing. Plant parts were ground using a mortar and pestle with liquid nitrogen. Ground material was next moved into a glass grinding tube with 3 ml of methanol in order to improve extraction. Ground material was then placed into 14-ml glass tubes and centrifuged at 2000 × g for 10 minutes. Supernatants were decanted into 20-ml scintillation vials and concentrated using a nitrogen evaporator (N-EVAP™122, OA-SYS™, Organomation Associates, Inc.). The concentrated solution was immediately re-eluted in 1 ml of methanol for spotting onto silica chromatography plates (Silica gel G 20 × 20 cm TLC plates, Analtech, Inc.). The resulting pellet from centrifuging was oxidized along with harvested roots and the dried remainder of the 1 ml concentrated solution for recovery purposes.

Analysis of herbicide metabolites was done by normal-phase thin layer chromatography (TLC). Silica gel plates were used as the stationary phase, and a solvent system of methanol, isopropanol, ethyl acetate and acetic acid (7:1:1:1 v v^{-1}) was used as the mobile phase (Bell et al. 2011). Plates were manually scored into 19 individual 1 cm lanes, and a 100-µL aliquot of concentrated solution was applied 3 cm above the bottom of the plate, leaving a blank lane between each replicate. The area of application was maintained to ~10 mm by successive spotting and drying. The first lane of each plate was left blank to use as a background. A 10-µL drop of ^{14}C-ACPC was spotted on the next lane for metabolite comparison. Plates were developed for approximately 75 min or until the mobile phase had moved 15 cm above the origin, then allowed to air dry prior to scanning.

Radioisotope Imaging. To visually confirm the final position of radiolabeled material within plants, radioisotope images (RI) were taken using a multipurpose image analyzer (Fujifilm FLA-5100, Fujifilm Holdings Corporation). Plants used for imaging were treated identically to those in absorption and translocation experiments prior to harvest. Upon harvest, plant roots were washed and entire intact plants were placed in between layers of newspaper. These paper layers were pressed between two wooden boards held by fiber straps with metal clasps. After two weeks time, plants were removed from pressed boards and placed onto 8 by 10 cm cardstock mounts. These mounts were covered in plastic wrap then placed in cassettes (Amersham biosciences exposure cassette) and pressed against phosphor imaging plates in order to produce an image. Phosphor plates are desired over standard X-ray film because phosphor plates are proven to be more sensitive and less radiation is needed for a quality image. Phosphor plates also develop significantly faster than X-ray film and are not limited to an optimum range of radioactivity (Wehtje et al. 2007). Phosphor plates were exposed to plant material for approximately 24 hrs then scanned using the image analyzer.

Data Analysis. Percentage of ^{14}C-ACPC absorption was calculated by summing the total amount of radioactivity in kBq of all harvested plant parts minus the leaf wash. Translocation was calculated by dividing the radioactivity recovered in each plant part by the total radioactivity absorbed. Percent recovery was calculated as total radiation recovered across all plant parts in kBq divided by total radiation applied. Percent recovery for absorption and translocation studies averaged 89% across all four species and harvest intervals. Percent recovery in metabolism studies averaged 86% across species and harvest intervals. The lower recovery is likely due to extra processing steps associated with recovering material in metabolism studies compared to the direct harvest methods used in absorption and translocation studies. Loss could also occur in the form of ^{14}C-ACPC root exudation, which has previously been reported by Lewis et al. (2013) in tall fescue. This is within an acceptable range considering losses due to sample harvesting and processing and is consistent with previous research involving synthetic auxin absorption and fate (Bukun et al. 2009, 2010; Lewis et al. 2013b). Radiation in the form of percent recovered of applied was the response variable for both absorption and translocation studies.

For metabolism studies, the radioactive positions, percentages, and corresponding retardation factor (R_f) value of the parent compound and possible metabolites were determined by scanning TLC plates using radio-chromatogram scanner (AR-2000 imaging scanner, Bioscan, Inc.). Radioactive trace peaks were manually integrated. Peaks below 5% of total radioactivity were rejected. Peaks were identified by comparing their R_f values with those from the corresponding ^{14}C-ACPC standard. Data collected consisted of the percentage of the parent herbicide, the percentage of all metabolites detected that were more polar than the parent herbicide and the percentage of all metabolites that were less polar than the parent herbicide (Bell et al. 2011). Data from both studies were subjected to ANOVA in SAS (SAS® Institute Inc., v. 9.2) using PROC GLIMMIX to test for significance of replication-in-time, DAT, plant part, grass species, and allow for lack-of-fit testing. Means were separated using Fisher's Protected LSD ($P \leq 0.05$).

Results and Discussion

Absorption and Translocation. ANOVA revealed that repetition in time and replications within the experiment were not significant. Therefore data were pooled across repetitions and replications within experiments. Cogongrass absorption of ^{14}C-ACPC was more rapid than other species and reached a maximum within 2 DAT (Table 5). Bahiagrass and tall fescue both reached maximum absorption by 4 DAT, averaging 46 and 52% respectively. Bermudagrass reached maximum absorption slower than other species with 37% absorption at 8 DAT. All species absorbed greater than 30% of the total uptake within only 24 HAT. This rapid absorption is consistent with past research involving ACPC (Bell et al. 2011; Bukun et al. 2010; Lewis et al. 2013b). The most sensitive of all species tested, cogongrass (ET Parker, unpublished data), likely absorbed the least ^{14}C-ACPC due to rapid uptake and desiccation of plant tissues, hindering further absorption from taking place. Across all species, no more than half of the applied ACPC was absorbed. This indicates that the majority of all ACPC applied to grass species, whether tolerant or susceptible, remains on the leaf surface or within aboveground tissues. Herbicide remaining within aboveground tissues may be released if mowing events or grazing occurs after ACPC application. This comes as no surprise as previous reports involving many other synthetic auxin herbicides indicate that these herbicides may be released from treated tissues in quantities large enough to solicit plant injury (Branham and Lickfeldt 1997; Miltner et al. 2003).

Out to 2 DAT, cogongrass moved more ACPC out of the target area than other species at 23 and 27% respectively (Table 6). At 4 DAT, bahiagrass and tall fescue had accumulated the most radioactive material outside the target area with 42 and 38% respectively. By 8 DAT, bahiagrass had moved the most radioactivity (38%) out of the treated leaf and into the remaining foliage and roots. Translocation within cogongrass peaked at 2 DAT with 27% ^{14}C-ACPC leaving the target area. All other species reached maximum translocation by 4 DAT. Bermudagrass, tall fescue, and bahiagrass moved 32, 38, and 44% of ^{14}C-ACPC respectively out of the target area. It is clear that all species absorb and translocate ^{14}C-ACPC in amounts sufficient to prompt injury (van Rensburg and Breeze 1990; Strachan et al. 2011) and (ET Parker, unpublished data). This absorption and movement data however, is not consistent with ACPC response data for these species (ET Parker, unpublished data). Data indicates that absorption and translocation are not the definitive reason for varying tolerances of grass species to ACPC. Although ACPC is moved to roots of susceptible species, it is unlikely to move in quantities large enough to offer prolonged control of either cogongrass (Enloe et al. 2012) or bahiagrass (Anonymous 2010) in turf, pasture, or forestry systems.

Metabolism. TLC analysis revealed no metabolites of C^{14}-ACPC out to 42 DAT across all four species tested. Comparisons of means determined that all peaks detected from treated plants had an R_f value not significantly different from the ACPC-only check. Across all fate studies conducted, results are insufficient to explain the varying tolerances of grass species. In the future, the role of auxin binding proteins and their possible mutations in plant selectivity should be explored (Grossmann 2010). Lack of ACPC metabolism paired with a potentially long soil half-life and low soil sorption (Conklin and Lym 2013; Oliveira et al. 2011) is a double-edged sword. Prolonged exposure to ACPC may offer extended control of hard to eliminate weeds, but there is also risk involved when a chemical remains in the soil profile for extended periods of time. Because the majority of ^{14}C-ACPC remains in above ground foliage, and because it remains as the parent compound, it is likely that ACPC will be returned to the soil profile in a “cyclic” manner as proposed by (Lewis et al. 2013b). It has also been reported that ACPC exudes from grass roots (Lewis et al. 2013b) and (ET Parker, unpublished data). Compounding these observations, future ACPC use will be limited in some weed control systems but highly effective in others.

Radioisotope Imaging. Radioisotope images (Figures 5-8) correlate well with absorption and translocation data. Both sets of data indicate that the majority of applied ^{14}C-ACPC remained in the target area and treated leaf for the duration of the experiment. It is interesting to note that tall fescue and bahiagrass appear to be sequestering the radiolabeled material in leaf tips. This same sequestering is not evident in cogongrass or bermudagrass. This could be a possible method that tolerant species, namely some grasses, use to protect vital meristematic tissues such as the crown and roots. The collection of ^{14}C-ACPC in leaf tips supports the argument that ACPC will likely experience "cyclic" movement in grass systems as treated material is mowed or grazed, returned to the soil profile, and then reabsorbed by plants. Future research may be in order to explore the role this sequestration of ACPC plays in plant tolerance and the ultimate fate of ACPC within grass weed control systems.

Table 5. Absorption of foliar-applied ^{14}C-aminocyclopyrachlor as influenced by grass species and time.[ab]

Grass Species	1 DAT[cd]	2 DAT	4 DAT	8 DAT
	--------------------------% of applied--------------------------			
cogongrass	29 C-G	34 B-E	21 EFG	24 E-G
bahiagrass	16 G	28 E-G	46 AB	45 ABC
bermudagrass	21 EFG	18 FG	29 C-G	37 A-D
tall fescue	25 E-G	33 B-F	52 A	48 AB
$LSD_{(0.05)}$				15

[a] Absorption calculated as sum of applied ^{14}C-ACPC recovered in target area, treated leaf, remainder foliage, and crown plus roots.

[b] Run effect was not significant, therefore data are pooled across studies. A two-way interaction of species and DAT was significant ($P < 0.001$).

[c] Abbreviations: DAT, days after treatment

[d] Means sharing a letter are not different according to Fisher's protected $LSD_{(0.05)}$.

Table 6. Fate of foliar-applied ^{14}C-aminocyclopyrachlor as influenced by grass species and time.[a]

Grass Species	Part	1 DAT[bc]		2 DAT		4 DAT		8 DAT	
		---% of applied---							
cogongrass	LW	71	B	66	B	81	A	76	A
	TA	5	CDE	7	DE	7	D-G	6	FGH
	TL	11	CD	9	CD	5	EFG	9	E-H
	RF	6	CDE	9	CD	4	EFG	6	FGH
	RT	6	CDE	9	CD	3	FG	4	FGH
bahiagrass	LW	84	A	72	B	54	B	55	BC
	TA	7	CDE	6	DE	5	EFG	7	FGH
	TL	6	CDE	8	DE	16	CDE	10	EF
	RF	3	CDE	9	CDE	18	CD	16	E
	RT	1	DE	6	DE	8	D-G	12	EF
bermudagrass	LW	79	AB	82	A	71	A	63	B
	TA	12	C	4	DE	6	D-G	5	FGH
	TL	2	DE	5	DE	13	C-G	25	D
	RF	6	CDE	1	DE	7	D-G	6	FGH
	RT	0	E	0	E	2	FG	1	GH
tall fescue	LW	75	AB	66	B	48	B	52	C
	TA	10	CDE	10	CD	14	C-F	11	EF
	TL	9	CDE	17	C	22	C	27	D
	RF	5	CDE	6	DE	14	C-F	10	EFG
	RT	1	DE	1	DE	2	G	1	H
$LSD_{(0.05)}$		10		9		12		9	

[a] Run effect was not significant, therefore data are pooled across studies. A three-way interaction of species, DAT, and sample was significant ($P < 0.001$). Data are presented in the most meaningful format.

[b] Abbreviations: DAT, days after treatment; LW, leaf wash; TA, target area; TL, treated leaf; RF, remainder foliage; RT, crown plus roots

[c] Means sharing a letter within columns are not different according to Fisher's protected $LSD_{(0.05)}$.

Table 7. Summary of absorption and fate of ^{14}C-aminocyclopyrachlor in select grass species.[a]

Grass Species	Absorption	Translocation	Metabolism
	--------------------	% of applied	--------------------
cogongrass	27	20	0
bahiagrass	34	28	0
bermudagrass	26	17	0
tall fescue	40	29	0

[a] Data presented are averaged across all DAT for each species.

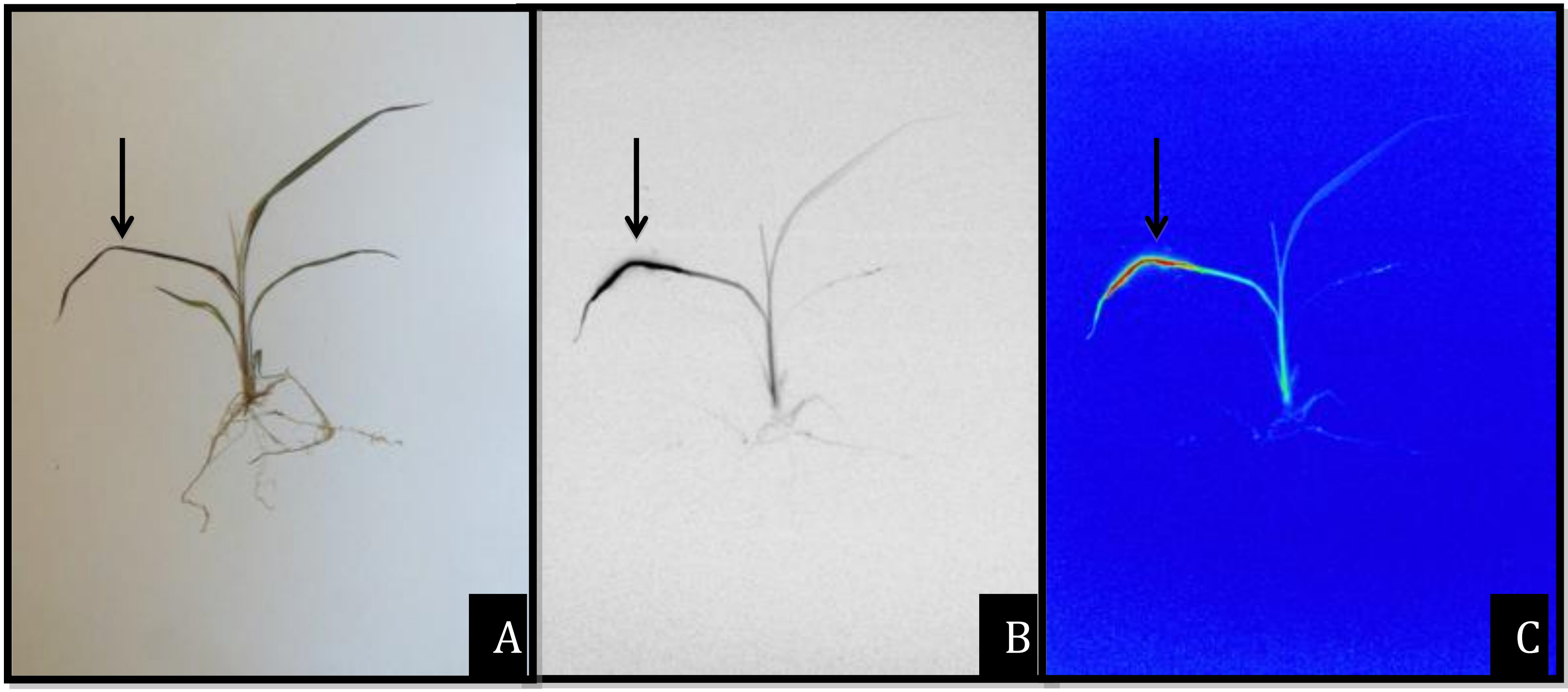

Figure 5. Images of cogongrass treated with ^{14}C-aminocyclopyrachlor at 8 days after treatment. Arrows indicate the location at which the herbicide drop was applied. Images from left to right: photograph of treated plant (A), a gray-scale radioisotope image (B), and a full color radioisotope image (C).

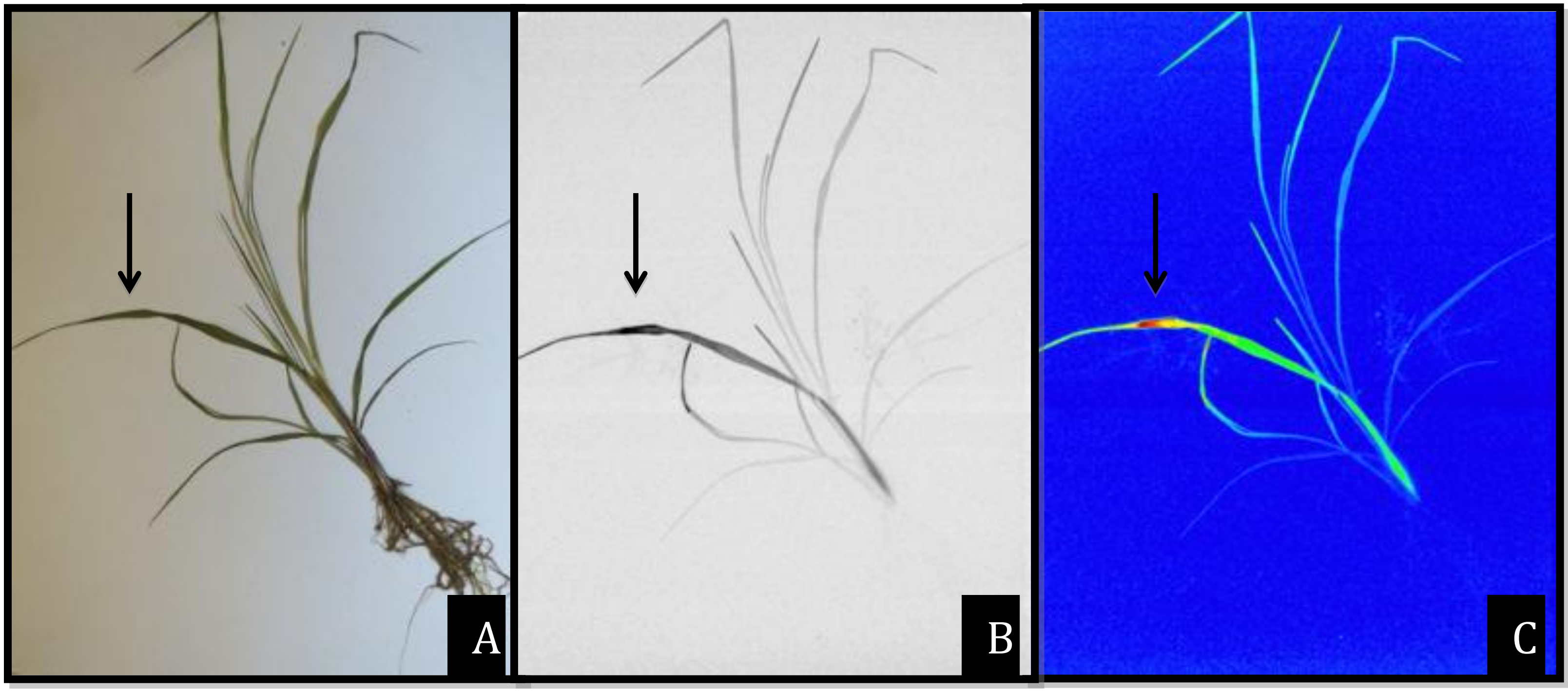

Figure 6. Images of bahiagrass treated with ^{14}C-aminocyclopyrachlor at 8 days after treatment. Arrows indicate the location at which the herbicide drop was applied. Images from left to right: photograph of treated plant (A), a gray-scale radioisotope image (B), and a full color radioisotope image (C).

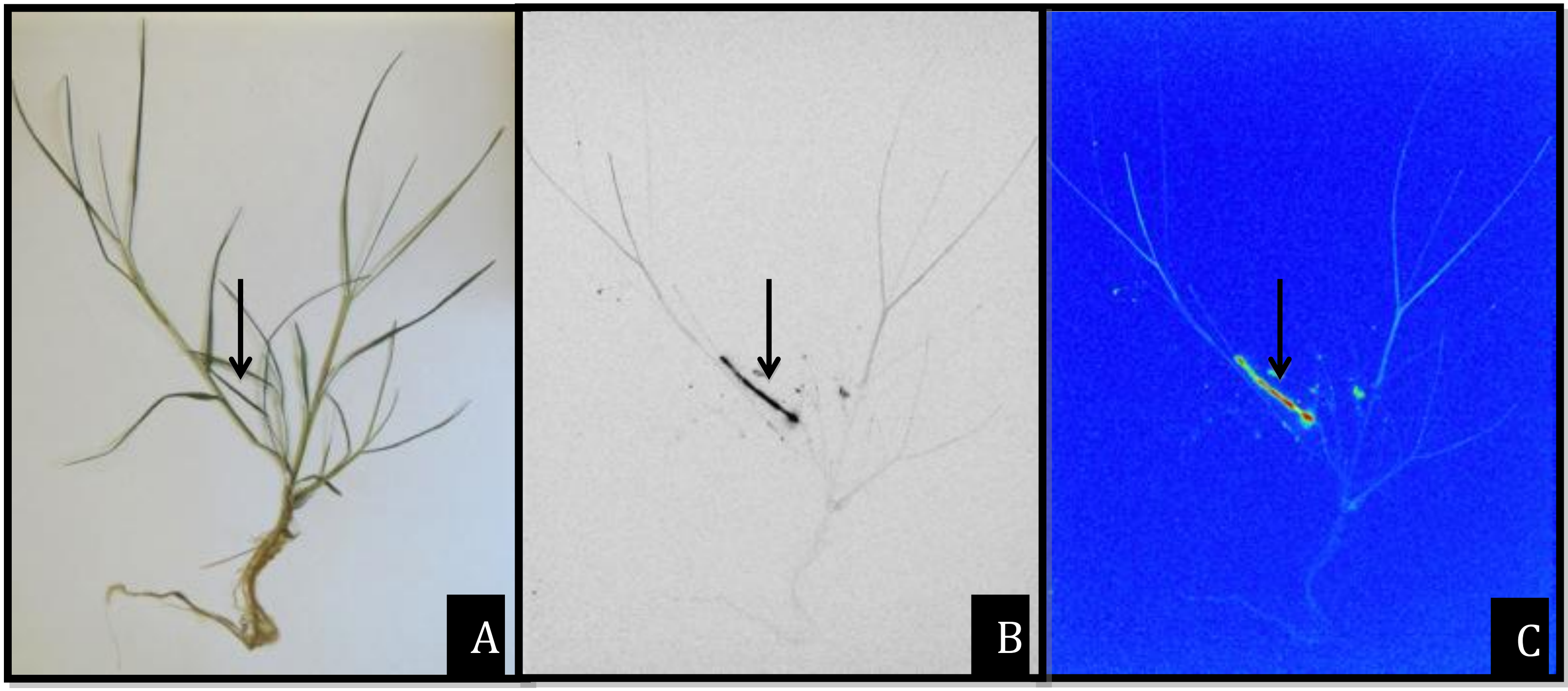

Figure 7. Images of bermudagrass treated with ^{14}C-aminocyclopyrachlor at 8 days after treatment. Arrows indicate the location at which the herbicide drop was applied. Images from left to right: photograph of treated plant (A), a gray-scale radioisotope image (B), and a full color radioisotope image (C).

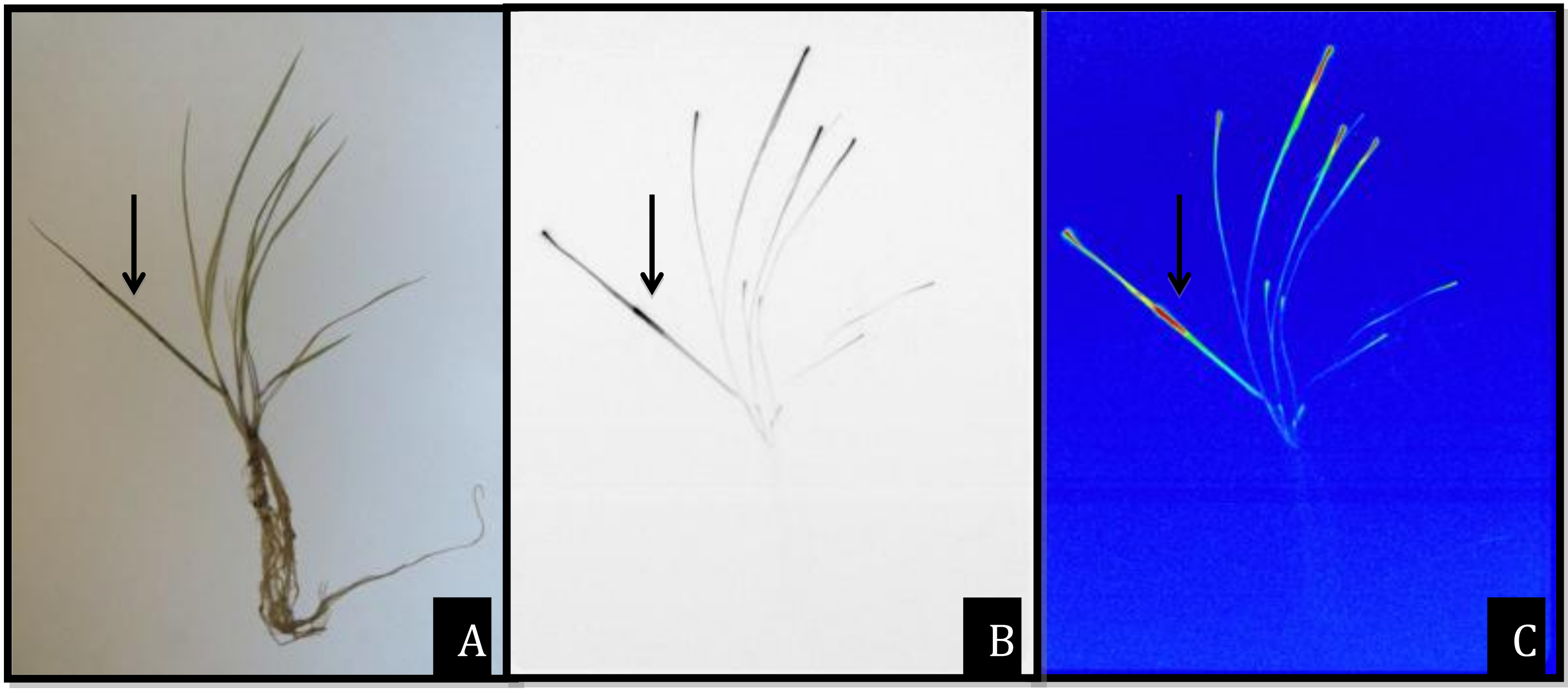

Figure 8. Images of tall fescue treated with ^{14}C-aminocyclopyrachlor at 8 days after treatment. Arrows indicate the location at which the herbicide drop was applied. Images from left to right are a photograph of treated plant (A), a gray-scale radioisotope image (B), and a full color radioisotope image (C).

Biological-Assay Techniques for Determining the Root Exudation of Aminocyclopyrachlor and Dicamba

Introduction

Following its use in turfgrass in 2010 and early 2011 as Imprelis®, the synthetic auxin herbicide aminocyclopyrachlor (ACPC) was removed from the turfgrass market in the summer of 2011 after a stop sale, use, or removal order was issued to DuPont (Ferdas 2011; Patton et al. 2011). This removal of Imprelis® came after widespread reports of off-target damage and death to trees and ornamentals in the landscape where applications were made. Symptoms included shoot dieback and epinastic twisting of shoots, leaves, and needles that were most noticeable in treetops and most severe on new growth (Patton et al. 2011, 2013). The Office of Indiana State Chemist (OISC) found that the widespread damage observed could not be attributed to misapplications as the herbicide was applied to lawns per label instructions (Patton et al. 2011). Despite its removal from the turfgrass market as Imprelis®, ACPC has the potential to be used for broadleaf weed control in various grass crops as an alternative to currently labeled phenoxyacetic and pyridine herbicides (Anonymous 2012, 2014; Lewis et al. 2013b). ACPC also has the possibility of being used to control troublesome grass weeds within grass crops (Enloe et al. 2012; Reed et al. 2013).

The first synthetic auxin herbicides were initially released in the mid 1940s (Grossmann 2010; Sciumbato et al. 2004). They were the first herbicides used for the selective control of dicot weeds in monocot crops (Anderson 1996; Devine et al. 1993; Sterling and Hall 1997). Some of the more recent synthetic auxin herbicides such as clopyralid, aminopyralid, and more recently aminocyclopyrachlor (ACPC) have been utilized for selective weed control at lower rates compared to other standard treatments (Bukun et al. 2009; Senseman 2007; Turner et al. 2008). However these low use rates can result in injury to non-target plants if they persist in the environment or if off-site movement occurs via volatility, soil movement, or if herbicide residues remain inside spray tanks (Andersen et al. 2004; Arle 1954; Bovey and Meyer 1981; Strachan et al. 2010). Undesired injury to sensitive species like tomato (*Solanum lycopersicum* L.) and lettuce (*Lactuca sativa* L.) occurs at rates as low as 0.001% of the labeled rate of both 2,4-D and 2,4-DB, commonly used synthetic auxin herbicides (van Rensburg and Breeze 1990). Off-site injury via synthetic auxin herbicides is very easy to identify because of the distinct leaf epinasty, tissue swelling and also stem curling (Anderson 1996; Grossmann 2010; Sciumbato et al. 2004). One way to take advantage of these characteristics and understand environmental fate is by using biological-assay (bioassay) test plants of various species along with several techniques of determining the presence and concentration of herbicide residues in the soil (Corbin and Upchurch 1967; Eshel and Warren 1967; Herr et al. 1966; Leasure 1964). The sensitivity of a test plant to a herbicide is measured by observed growth reactions. These reactions may vary from slightly abnormal growth characteristics to death (Dowler 1969). This practical approach of growing sensitive plant species in soils believed to be herbicide contaminated is a simple, straightforward and readily utilizable assay tool (Camper 1986). Losses due to undesired offsite movement can be reduced or eliminated when the fate of such herbicides within the environment are well understood and correct application practices are utilized.

Linder et al. (1964) as well as Chang and VandenBorn (1968) reported that dicamba and other related benzoic acid auxins were exuded from plant roots in sufficient amounts to affect nearby untreated plants. Several synthetic auxins, including ACPC, have been reported to persist within plant tissues as the active parent compound from one month to over two years (Bovey and Mayeux Jr. 1980; Bovey et al. 1967; Miltner et al. 2003; Patton et al. 2011). Therefore it is likely that as ACPC persists within plants, protected from photo-degradation, microbial degradation, and hydrolysis (USEPA 2010; Finkelstein et al. 2008), it will be exuded from the roots of treated plants deep into the soil. These characteristics could increase the active life of the herbicide within weed control systems and allow ACPC to reach the roots of sensitive trees and shrubs. It is important to better understand the fate of ACPC within grass plants in order to ensure successful use in pasture, rangeland and brush control systems (Meredith et al. 2013; Sellers et al. 2013). Previous research suggests that ACPC is absorbed and translocated throughout aboveground tissues of tall fescue (Lewis et al. 2013b). Lewis et al. (2013) also concluded that a small amount of ACPC exudes from treated tall fescue plants after foliar application. This correlates well with reports of other synthetic auxin herbicides that have been shown to exude from plants roots (Chang and VandenBorn 1968; Linder et al. 1964).

The objective of this study was to determine the presence of root-leaked ACPC in soil and hydroponic systems using bioassay techniques. ACPC was compared to dicamba, a synthetic auxin known to exude from plant roots in order to determine if foliar-applied ACPC is lost via exudation from treated grass roots using methods alternative to previous attempts (Chang and VandenBorn 1968; Lewis et al. 2013b; Linder et al. 1964).

Materials and Methods

Soil Studies. Experiments were conducted in a greenhouse located in Auburn, AL. Two experiments, one in 2012 and one in 2013 were conducted for each species to determine root exudation of foliar-applied ACPC compared to dicamba and a nontreated control. Grass species selected for evaluation were 'Pensacola' bahiagrass, common bermudagrass, cogongrass, and 'Rebel III' tall fescue. The herbicides used were the free-acid formulation of ACPC (Aminocyclopyrachlor, DPX-MAT28, E. I. du Pont de Nemours and Company) and the di-methyl amine salt formulation of dicamba (Agri Star®, Albaugh, Inc.). Experiments used a randomized complete block design with four replications per treatment. All herbicide-containing treatments included a non-ionic surfactant (NIS) (Activator 90 Non-Ionic Surfactant, Loveland Products Inc.) at 0.25% v v^{-1}. This adjuvant was included because previous research involving ACPC and other related synthetic auxin herbicides indicate it substantially increases herbicide absorption (Bell et al. 2011; Bukun et al. 2009; Lewis et al. 2013b).

Greenhouse temperatures were maintained between 23 and 26 C for the duration of the experiments. Normal sunlight was supplemented with sodium-halide growth lights producing 150-µmol m^{-2} s^{-1} at the turf canopy. Total peak irradiance was 150 to 800-µmol m^{-2} s^{-1} throughout the experiment. Grasses were grown from seed in plastic flats containing a potting substrate (Scott's Miracle-Gro® Potting Mix, The Scott's Company, LLC). Grasses were then prepared for treatment by transplanting single plants into 1480

cm^3 cylindrical pots containing Marvyn loamy sand (native) soil. Grasses were allowed to recover for 4 wks prior to herbicide applications with intermittent clipping to a height of approximately 7.5 cm. Grasses were maintained prior to treatment applications by trimming any lateral shoots or rhizomes to prevent foliar contact between treated species and bioassay species. At the time of treatment, bermudagrass was at a two to three tiller growth stage. All other grass species were treated at the four to six leaf stage. Cucumber (*Cucumis sativus* L.) was chosen as a bioassay species based upon previous research with synthetic auxin herbicides (Corbin and Upchurch 1967; Leasure 1964; Lynd et al. 1967; Ready and Grant 1947). Cucumber plants were grown from seed in plastic flats and transplanted upon emergence into the pots containing individual grasses. Two cucumber plants were placed on either side of a centrally located grass plant approximately 6 cm from the center of the pot to ensure detection of any possible herbicide exudation regardless of direction. Bioassay plants were allowed one wk of recovery prior to the application of treatments. Immediately prior to the application of treatments, 3 cm of perlite (Scott's Miracle-Gro® Perlite, The Scott's Company, LLC) was placed over the soil surface to absorb herbicide and prevent it from contacting the soil. Plastic cups were placed over cucumber seedlings to protect from herbicide contact (Figure 9). Treatments were based on previous labeled rates of ACPC (Anonymous 2010) and dicamba (Anonymous 2008). Rates were 52.5 g ae $ha^{-1}$of ACPC, 0.563 kg ae ha^{-1} of dicamba, and a nontreated check. Treatments were applied in a spray volume of 280 L ha^{-1} using an enclosed spray chamber with a single nozzle (TeeJet TP8002EVS, Spraying Systems Co.®). Perlite was removed 24 h after application with a vacuum, and plastic cups were discarded. Pots were irrigated using a glass funnel connected to a hose to aid in directing water away from the herbicide-treated foliage.

Hydroponic Studies. An experiment for each species was conducted in 2013 and repeated in 2014. Experiments were conducted in the greenhouse mentioned previously and with the same objective in mind. Three replications of tall fescue and cogongrass were used for hydroponic studies. All herbicide treatments and greenhouse conditions were identical to those in the aforementioned soil studies. Plants were grown from seed as previously stated but transplanted after thorough root washing into individual 640 ml plastic containers with hydroponic solution (Grow Big® Hydroponic, FoxFarm Fertilizer, LLC). Supplies for hydroponic containers include: plastic aquarium tubing, air pumps, air regulators, air stones, and 9-cm^3 split bio-based foam pieces (POLY-FIL® TRU-FOAM™, Fairfield Processing Corp.) A 4 cm hole was drilled in the center of each lid for individual containers. A 0.75 cm hole was drilled in one corner of each lid to allow the air tubing to be inserted into the hydroponic solution (Figure 10). Plants were prepared for treatment by acclimating them to hydroponic containers over 7-day period. At the time of treatment, plants were removed from hydroponic solution and roots were sealed in plastic wrap to prevent contact with the applied herbicide. A half-rate of herbicide (26.25 g ae ha^{-1}) was then applied to one side of a prostrate grass plant. The grass was then rotated and a second half-rate was applied to the other side to ensure complete coverage (total herbicide applied was 52.5 g ae ha^{-1}). Plants were immediately placed into a 9-cm^3 piece of split foam with the crown wedged into the foam and after the plastic wrap was removed, roots were submerged in the hydroponic solution. At 21 DAT, grasses were carefully removed from hydroponic containers, and replaced with cucumber seedlings using new foam pieces to prevent contamination. Cucumber seedlings were allowed to grow in suspected contaminated solution for 14 days after insertion (DAI).

Data Analysis. Visual injury ratings of cucumber plants in soil studies were taken at 7, 14, and 21 days after treatment (DAT). Injury ratings were taken at 7 and 14 DAI for hydroponic studies. These rating intervals were chosen to bracket a typical synthetic auxin rating date of 2 weeks after treatment (WAT) (Sciumbato et al. 2004). Visual injury ratings were based on a scale of 0 to 100 with 0 corresponding to dead plants and 100 indicating healthy plants. Cucumber plant heights and dry weights were also taken in hydroponic studies at 14 DAT to quantify any physical effects of the herbicide treatments. All species were analyzed together for each study. Experimental units consisted of a single pot. Data were subjected to ANOVA in SAS (SAS® Institute Inc., v. 9.2) using PROC GLM to test for significance of replication-in-time, herbicide rate, grass species, and allow for lack-of-fit testing. Experimental run and replication were considered random effects with treatment, species, and harvest interval being considered fixed effects. Means were separated using Fisher's Protected LSD and considered significant if $P < 0.05$.

Results and Discussion

Soil Studies. ANOVA revealed that both repetition in time and replications within the experiment were not significant. Therefore data were pooled across repetitions and replications within experiments. A two-way interaction of treatment and species was significant (P < 0.0001) indicating that the amount of herbicide exudation from grass roots varies by grass species. Cucumber plants sharing pots with bermudagrass suffered the most injury when treated with foliar-applied ACPC, but the least injury when treated with foliar-applied dicamba (Table 5). Bioassay plants in bahiagrass and tall fescue pots fared similarly in their overall response, showing limited injury in ACPC treated pots and a high level of injury in dicamba treated pots (Figure 11). Bioassay plants in cogongrass pots treated with ACPC were relatively unaffected while pots treated with dicamba showed greater injury than all other species.

In addition to the first significant interaction, an interaction between treatment and DAT was also significant (P < 0.0001). Bioassay plants in dicamba treated pots show that the herbicide continues exuding from the roots, causing greater injury as time progresses from 7 to 21 DAT (Table 6). Our observation of dicamba root exudation is in agreement with previous research (Linder et al. 1964). ACPC also was exuded from grass roots, but injury to the bioassay species did not significantly increase with time. This indicates that either the amount of ACPC being exuded is small and constant, or that the ACPC initially exuded from grass roots persists within growth media, causing consistent injury across all three rating dates. Dicamba was exuded from roots of all species in amounts high enough to cause significant injury to sensitive cucumber bioassay. ACPC was also exuded from roots of all species but at a level much less than that of dicamba. However, this small amount of ACPC was still enough to elicit visual injury in sensitive cucumber plants. There appears to be no correlation between the amount of herbicide lost from plant roots and the apparent level of tolerance of these grass species (Anonymous 2010; Flessner et al. 2011c; Reed et al. 2013).

Hydroponic Studies. As in soil studies, ANOVA revealed that treatment levels and replications within each experiment were not significant. Significant treatment by species ($P < 0.0001$) and treatment by DAI ($P < 0.05$) interactions were detected. Similar to soil studies, dicamba exudation caused significantly more injury than ACPC across both species tested (Table 7). ACPC root exudation did not result in increasing injury over time indicating consistent leaking or herbicide persistence as mentioned previously (Table 8). Bioassay plants in dicamba treated containers did exhibit greater injury as time progressed, as visual injury nearly doubled from 7 to 14 DAI.

In order to quantify visual results, cucumber plant heights and dry weights were taken. Of these, only plant heights were significant ($P < 0.05$). This is likely because root mass and foliar mass are not accurate measures of synthetic auxin response at low rates. Low rates of these auxins cause cell elongation, uncontrolled cell division and growth of roots, meaning that treated plants may not actually lose weight compared to nontreated plants (Anderson 1996; Deshpande and Hall 2000; Devine et al. 1993; Senseman 2007). Relative to the nontreated, both species and herbicides tested resulted in a decrease of height to bioassay plants (Table 9). Containers with tall fescue did not vary significantly in bioassay injury. In containers with cogongrass, dicamba caused greater reduction in height than ACPC.

Based on these data from all studies, ACPC does exude through plant roots after foliar treatment into the surrounding environment in all four grass species evaluated but the effect is minor and less than dicamba. Similarly, Lewis et al. (2013) reported a small amount of ACPC exuding from grass roots and Linder et al. (1964) reported that dicamba exudes from treated plant roots. This research is in agreement with our findings and confirms that both soil and hydroponic methods are reliable and comparable when evaluating root exudation of foliar-applied synthetic auxins.

Table 8. Percent injury of cucumber bioassay plants in response to exuded synthetic auxins compared to a non-treated check in soil.[a]

Species	ACPC[bc]	dicamba
	---------------------------------%---------------------------------	
cogongrass	5 D	76 A
bahiagrass	11 D	73 A
bermudagrass	25 C	60 B
tall fescue	11 D	68 AB
$LSD_{(0.05)}$		9

[a]Data were pooled across studies as interactions involving experimental run were not significant. A two way interaction of species and treatment was significant ($P < 0.001$).

[b]Abbreviations: ACPC, aminocyclopyrachlor

[c]Means sharing a letter are not different according to Fisher's protected $LSD_{(0.05)}$.

Table 9. Percent injury[a] of cucumber bioassay plants in response to exuded synthetic auxins compared to a non-treated check in soil.[b]

Treatment	7 DAT[cd]		14 DAT		21 DAT	
	------------------------------------		%		------------------------------------	
ACPC	12	D	12	D	15	D
dicamba	57	C	70	B	81	A
$LSD_{(0.05)}$					8	

[a] Visual injury rated on a scale of 0 to 100 with 0 equivalent to the control and 100 corresponding to complete plant death.

[b] Data were pooled across studies, as interactions involving experimental run were not significant. A two-way interaction of treatment and DAT was significant ($P < 0.001$).

[c] Abbreviations: DAT, days after treatment; ACPC, aminocyclopyrachlor

[d] Means sharing a letter are not different according to Fisher's Protected $LSD_{(0.05)}$.

Table 10. Percent injury[a] of cucumber bioassay plants in response to exuded synthetic auxins compared to a non-treated check in hydroponic solution.[b]

Species	ACPC[cd]		dicamba	
	------------------------------------%		-------------------------------------	
cogongrass	14	B	58	A
tall fescue	8	B	68	A
$LSD_{(0.05)}$			8	

[a] Visual injury rated on a scale of 0 to 100 with 0 equivalent to the control and 100 corresponding to complete plant death.

[b] Data were pooled across studies, as interactions involving experimental run were not significant. A two-way interaction of species and treatment was significant ($P < 0.001$).

[c] Abbreviations: ACPC, aminocyclopyrachlor

[d] Means sharing a letter are not different according to Fisher's Protected $LSD_{(0.05)}$.

Table 11. Percent injury[a] of cucumber bioassay plants in response to exuded synthetic auxins compared to a non-treated check in hydroponic solution.[b]

Treatment	ACPC[cd]	dicamba
	------------------%------------------	
7 DAT	10 C	45 B
14 DAT	12 C	81 A
$LSD_{(0.05)}$		13

[a] Visual injury rated on a scale of 0 to 100 with 0 equivalent to the control and 100 corresponding to complete plant death.

[b] Data were pooled across studies, as interactions involving experimental run were not significant. A two-way interaction of treatment and DAT was significant ($P < 0.001$).

[c] Abbreviations: DAT, days after treatment; ACPC, aminocyclopyrachlor

[d] Means sharing a letter are not different according to Fisher's Protected $LSD_{(0.05)}$.

Table 12. Percent reduction of cucumber bioassay plant height in response to exuded synthetic auxins compared to a non-treated check in hydroponic solution.[a]

Species	ACPC[bc]	dicamba
	--------------------------------%--------------------------------	
cogongrass	-31 A	-48 B
tall fescue	-52 B	-45 AB
$LSD_{(0.05)}$		15

[a] Data were pooled across studies, as interactions involving experimental run were not significant. A two-way interaction of species and treatment was significant ($P < 0.001$).

[b] Abbreviations: ACPC, aminocyclopyrachlor

[c] Means sharing a letter are not different according to Fisher's Protected $LSD_{(0.05)}$.

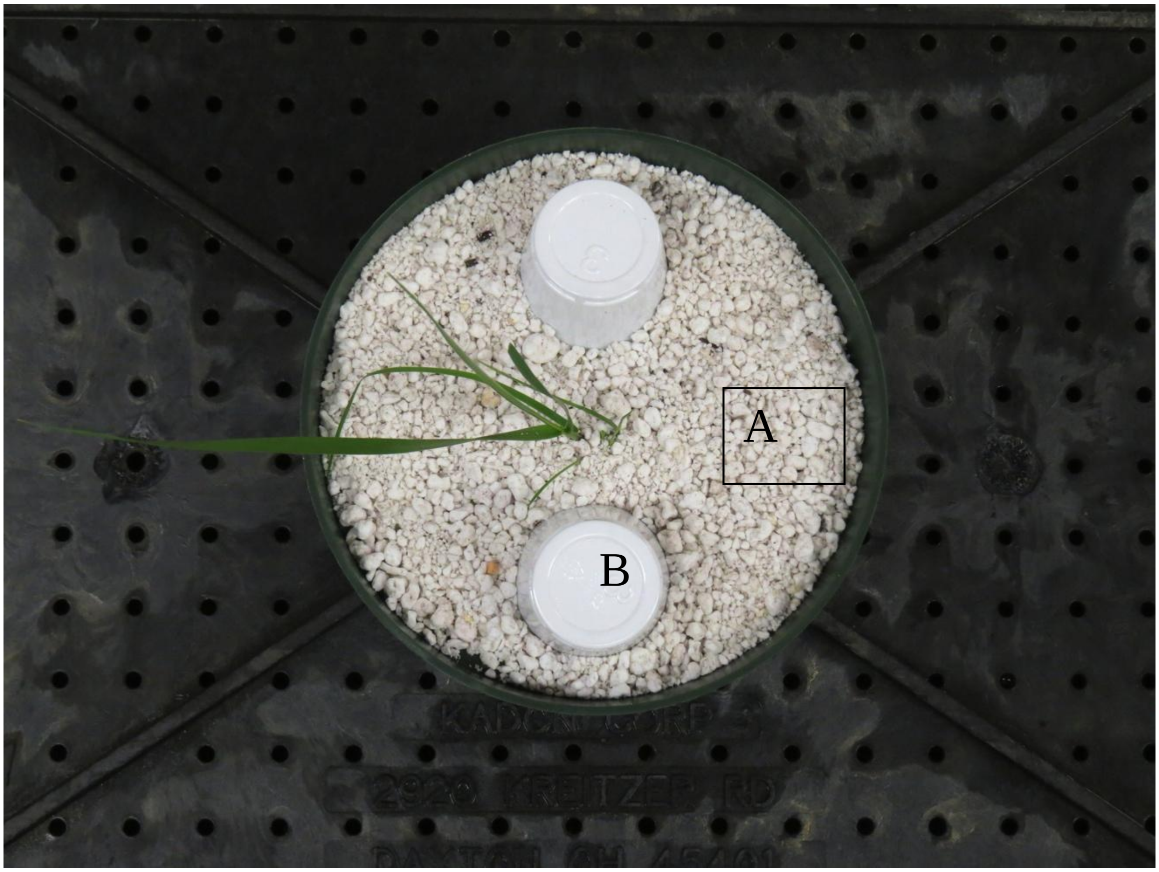

Figure 9. Pot prepared for foliar treatment of either aminocyclopyrachlor at 52.5 g ha^{-1} or dicamba at 0.563 kg ha^{-1}. Perlite (A) and plastic cups (B) were used to prevent herbicide contact with the soil surface and cucumber bioassays, respectively.

Figure 10. Assembled hydroponics materials utilized for exudation studies.

Figure 11. Cucumber injury sustained by root exudation of ACPC foliar-applied at 52.5 g ha^{-1} (B) and dicamba foliar-applied at 0.563 kg ha^{-1} (C) compared to the nontreated (A) at 14 DAT in cogongrass pots.

Figure 12. Cucumber injury sustained by root exudation of ACPC foliar-applied at 52.5 g ha^{-1} (B) and dicamba foliar-applied at 0.563 kg ha^{-1} (C) compared to the nontreated (A) at 14 DAT in bahiagrass pots.

Figure 13. Cucumber injury sustained by root exudation of ACPC foliar-applied at 52.5 g ha^{-1} (B) and dicamba foliar-applied at 0.563 kg ha^{-1} (C) compared to the nontreated (A) at 14 DAT in tall bermudagrass pots.

Figure 14. Cucumber injury sustained by root exudation of ACPC foliar-applied at 52.5 g ha^{-1} (B) and dicamba foliar-applied at 0.563 kg ha^{-1} (C) compared to the nontreated (A) at 14 DAT in tall fescue pots.

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

2,3,6-TBA	2,3,6-trichlorobenzoic acid
2,4,5-T	(2,4,5-trichlorophenoxy)acetic acid
2,4-D	(2,4-dichlorophenoxy)acetic acid
2,4-DB	4-(2,4-dichlorophenoxy)butanoic acid
aminocyclopyrachlor	6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid
aminopyralid	4-amino-3,6-dichloro-2-pyridinecarboxylic acid
clopyralid	3,6-dichloro-2-pyridinecarboxylic acid
dicamba	3,6-dichloro-2-methoxybenzoic acid
glyphosate	N-(phosphonomethyl)glycine
MCPA	(4-chloro-2-methylphenoxy)acetic acid
picloram	4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid
triclopyr	[(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid