Efficacy and Antagonism of Pinoxaden Alone and in Combination with Other Pesticides for Annual Grass Control

by

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Abstract

Pinoxaden is an acetyl coenzyme A carboxylase (ACCase) inhibiting herbicide in the phenylpyrazoline chemical family. Recently, pinoxaden was labelled for turfgrass use with a limited number of labelled susceptible weeds. Variable susceptibility to ACCase inhibiting herbicides between grassy weeds is common. For this reason, greenhouse trials were conducted in order to better understand grassy weed susceptibility to pinoxaden. A rate response screen was carried out using ten rates of pinoxaden on nine grassy weed species. Plant visual injury and above-ground biomass data were taken. Nonlinear regressions were run to estimate the rates of pinoxaden that injury and reduce the biomass of each species 50 and 90% (I_{50} and I_{90}). According to the data, the nine species tested can be ranked from most to least susceptible as such, perennial ryegrass (*Lolium perenne* L.) > yellow foxtail (*Setaria pumila* Poir.) > dallisgrass (*Paspalum dilatatum* Poir.) > southern sandbur (*Cenchrus echinatus* L.) > large crabgrass (*Digitaria sanguinalis* L.) > roughstalk bluegrass (*Poa trivialis* L.) > bahiagrass (*Paspalum notatum* Fleugg.) > goosegrass (*Eleusine indica* L. Gaertn.) > annual bluegrass (*Poa annua* L.). ACCase inhibiting herbicides are often called graminicides because they are active on grasses only. Because of this limited selectivity, graminicides are often tank mixed with herbicides targeting broadleaf weed species. Many of these tank mixtures have an antagonistic response on the ACCase inhibitor. Experiments were conducted in order to test different tank-mix combinations for antagonistic responses. According to the data, metsulfuron, halosulfuron, chlorothalonil and propiconazole antagonize pinoxaden injury of St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze). Metsulfuron, halosulfuron and chlorothalonil antagonize pinoxaden control of smooth crabgrass (*Digitaria ischaemum* Schreb.). The data

indicate that metsulfuron antagonizes pinoxaden to a greater extent than the other chemicals tested. Mixtures with chlorothalonil were also observed to induce unexpected injury similar to a contact herbicide.

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List of Abbreviations

ACCase	Acetyl Coenzyme A carboxylase
ALS	Acetolactate synthase
CI	Confidence interval
CT	Carboxyl transferase
DAT	Days after treatment
DAIT	Days after initial treatment
DEN	Pinoxaden
DIM	Cyclohexanedione
FOP	Aryloxyphenoxypropionate
g ai ha^{-1}	grams of active ingredient per hectare
kg ai ha^{-1}	kilograms of active ingredient per hectare
L ha^{-1}	Liters per hectare
NDVI	Normalized difference vegetative indices
NTSR	Nontarget-site resistance
%v/v	Percent volume per volume

I. Literature Review

Lipid Biosynthesis Lipids are water insoluble biomolecules that are an essential component of all plant cells. Within the plant cell, lipids serve as a structural component of the cell's membranes. They also form the sites where many of the plant's essential processes are carried out, such as light harvesting and electron transport. Externally, they form a protective covering, called the cuticle, on the surface of leaves. Lipids are a diverse class of compounds, and as a whole are made up of the products of several distinct biosynthetic pathways. However, the most abundant types of lipids are those that are derived from the glycerolipid and fatty acid biosynthesis pathways (Ohlrogge and Browse 1995).

The fatty acid biosynthesis pathway is considered a primary metabolic pathway because it is found in every plant cell and is also essential to growth. The fatty acids of plants generally have chain lengths of 16 to 18 carbons and will contain between one and three *cis* double bonds. Fatty acids make up roughly 90% of the acyl chains of structural lipids in most plant membranes (Ohlrogge and Browse 1995). For this reason, inhibition of the biosynthesis of these fatty acids typically results in plant death.

Acetyl-CoA is the product of respiration that occurs after pyruvic acid loses a carbon as CO_2. Acetyl-CoA can then either participate in the Krebs citric acid cycle or go towards the formation of other essential plant compounds, including fatty acids. The first step in the synthesis of fatty acids is the conversion of acetyl-CoA into malonyl-CoA. This conversion is catalyzed by the enzyme acetyl-CoA carboxylase (ACCase) in combination with ATP and CO_2. From there, malonyl-CoA undergoes numerous transformations to become a completed fatty acid (Burton et

al. 1989; Stoltenberg et al. 1989; Hopkins and Hunter 2004). There are two forms of ACCase, a prokaryotic and a eukaryote form. Both the eukaryotic and prokaryotic ACCase is made up of three catalytic domains, the biotin carboxyl carrier, biotin carboxylase, and carboxyl transferase (CT) domains (Guchhait et al. 1974; Tanabe et al. 1975). The ACCase enzyme is inhibited by three chemical families of herbicides (Shaner 2014) with the CT domain of the ACCase enzyme being the specific site of action for these herbicides (Collavo et al. 2011). Between the prokaryotic and eukaryotic forms, only the eukaryotic form is susceptible to current ACCase inhibiting herbicides (Konishi and Sasaki 1994).

ACCase Inhibiting Herbicides

ACCase inhibiting herbicides. There are three families of ACCase inhibiting herbicides. These are: the aryloxyphenoxypropionates (fops), cyclohexanediones (dims) and the phenylpyrazolins which consists only of pinoxaden (Shaner 2014). These herbicides are collectively known as the "graminicides" because all dicots and sedges are tolerant while grass species may be susceptible. It is for this reason that ACCase inhibiting herbicides are commonly used in broadleaf crops to selectively control annual and perennial grassy weed species.

ACCase inhibiting herbicides are absorbed through either the roots and foliage or just the foliage depending on the specific chemistry. They are rapidly translocated throughout the plant and they accumulate in the meristems, where they are primarily active. Inside the plant, the inhibition of fatty acid production will lead to cell membrane dysfunction, amino acid leakage, and eventually cell and plant death in susceptible species (McCarty et al. 2010). Externally, susceptible plants will usually stop growing within a few days of application. Leaf chlorosis will follow within one to three weeks with older leaves oftentimes turning purple, orange or red

before becoming necrotic. Leaf sheaths will become brown and flaccid near the point of attachment to the node and the plant eventually dies (Swisher and Corbin 1982).

Basis of selectivity. Grass species are generally susceptible to ACCase inhibiting herbicides while dicot species are all tolerant. This difference in susceptibility is due to the two different forms of the ACCase enzyme. Dicot species have both the prokaryotic and eukaryotic forms of ACCase. Dicot species tolerance stems from the *accD* gene that resides in the prokaryotic form of ACCase that is found in the cytoplasm (Konishi and Sasaki 1994). Grasses however lack the *accD* gene in their chloroplast genome. They only have the susceptible eukaryotic form that is nuclear encoded (Konishi et al. 1996). The eukaryotic form that grasses possess are the only form of ACCase that ACCase inhibiting herbicides bind to.

Oftentimes, a differential response is observed between two grassy species to the same ACCase inhibiting herbicide. This allows for selective control of grasses within another grass species with an ACCase inhibiting herbicide. McCarty et al. (1990) found that in centipede grass (*Eremochloa ophiuroides*), six hours after an application of sethoxydim, only trace amounts (<1%) of the herbicide was observed in the centipede grass tissue. In contrast, 81-98% of the sethoxydim was detected in goosegrass (*Eleusine indica*), a susceptible grass, tissue. From this study, it was determined that the basis of selectivity between the two grasses was a difference in metabolism. This type of resistance is considered inherent as opposed to plants that become resistant which is referred to as acquired resistance.

Acquired Resistance. The first case of acquired resistance to ACCase inhibiting herbicides was reported in 1982 in a rigid ryegrass (*Lolium rigidum*) population from an Australian wheat field (Heap and Knight 1982). Since then, 24 different genera across six different continents have developed resistance to ACCase inhibiting herbicides (Heap 2019). The most common weed

species to show resistance to ACCase herbicides is wild oat with 38 reported cases of resistance (Heap 2019). Currently, only two cases of ACCase resistance have been reported in turfgrass systems. Derr (2002) identified a population of smooth crabgrass (*Digitaria ischaemum*) on a southern New Jersey golf course that was resistant to fenoxaprop-p. This population did not show cross resistance to cyclohexanediones but did show lesser resistance to other aryloxyphenoxypropionates. The resistance mechanism for this population was an altered site of action (Derr 2002). McCullough et al. (2016) reported the first case of ACCase herbicide resistant goosegrass in the United States. This population was resistant to sethoxydim and was found in a centipedegrass sod field in Georgia. The proposed mechanism of resistance was also an insensitive ACCase enzyme (McCullough et al. 2016).

Nontarget-site resistance (NTSR) is recognized as being the predominant resistance mechanism for ACCase resistance (Delye et al. 2011). NTSR encompasses a range of diverse mechanisms that include, reduced penetration, translocation, and enhanced metabolism of herbicides (Powles and Yu 2010). In most cases of NTSR to ACCase herbicides, the mechanism of resistance is increased ability of the plant to degrade ACCase inhibiting compounds into nontoxic products (Powles and Yu 2010). Increased metabolic enzyme production can confer resistance not only to herbicides that have been frequently applied to a population, but also to chemical families and modes of action previously unintroduced to a resistant population. One example of this is a population of rigid ryegrass that evolved resistance towards ACCase inhibiting herbicides after several years of continued use. The same population was also found to have resistance to ALS inhibiting herbicides as well, despite the population never being previously exposed to ALS herbicides (Preston et al. 1996).

Cytochrome P450s. Cytochrome p450s are membrane bound enzyme systems that are often associated with herbicide insensitivity within crop species (Riviere and Cabanne 1987; Durst and Benveniste 1993). They have been shown to metabolize diclofop within wheat conferring natural tolerance (Zimmerlin and Durst 1990). Metabolic resistance has been inferred indirectly from the use of synergists that inhibit detoxifying cytochrome p450 enzymes that are known to be involved in ACCase resistance (Kaundun 2014). Two common cytochrome p450 inhibitors are the insecticides malathion and chlorpyrifos which have the ability to inhibit cytochrome p450 in plants (Kreuz and Fonne-Pfister 1992). Malathion plus chlorsulfuron controls a resistant ryegrass biotype suspected of NTSR (Christopher et al. 1994). When tank-mixed with pinoxaden, malathion enhanced pinoxaden efficacy on a resistant population of wild oats (Beckie et al. 2012). Chlropyrifos can also enhance injury to corn when tank-mixed with herbicides (Beckie et al. 2012).

Use in turfgrass systems. Since their commercial introduction in 1977, ACCase inhibiting herbicides have been used in a wide variety of crops for control of a broad range of grassy weeds. Virtually every broadleaf crop has an ACCase inhibitor that is labelled for use within that crop (Maneechote et al. 2005). However, ACCase inhibiting herbicides are also frequently used in turfgrass situations due to differential response between grasses and the relative difficulty of controlling a grass weed within another grass. In general, cool-season grasses (C3) are more tolerant to these herbicides than warm-season (C4) (Dernoeden 1987). However, there are several examples of differential susceptibility between grass species even amongst physiologically similar species.

Diclofop, an aryloxyphenoxypropionate, controls goosegrass within bermudagrass without injury the bermudagrass. It is safe to apply to putting greens as well. McCarty (1990)

found that the best goosegrass control with diclofop occurs when the goosegrass has been mowed to putting green heights (~1.3cm).

Sethoxydim, a cyclohexanedione, is very active against bentgrass (*Agrostis stolonifera*) and tall fescue (*Festuca arundinacea*) (Hosaka 1984; Hugh et al. 1986). Red fescue (*Festuca rubra*), and centipede grass have shown very high tolerance to sethoxydim, with tolerance being observed in rates as high as 1.12 kg ha^{-1} (McCarty et al. 1986; Hugh et al. 1986). Goosegrass control (>90%) with sethoxydim can be accomplished with a rate of 0.44 kg ha^{-1} (Chernicky et al. 1984).

Fenoxaprop is a member of the aryloxyphenoxypropionate chemical family. Although fenoxaprop is labelled for use in perennial ryegrass (*Lolium perenne*), tall fescue, fine fescue, Kentucky bluegrass (*Poa pratensis*) and creeping bentgrass, the relative tolerance between these species varies greatly. In turfgrass situations, it is effective at removing crabgrass (*Digitaria* spp.) and goosegrass from newly established perennial ryegrass and tall fescue (Dernoeden 1987). In Kentucky bluegrass, there have been differing reports on turfgrass injury. Neal et al. (1990) reported no reduction in turfgrass quality at rates between 0.20 and 0.40 kg ha^{-1}. However, Johnson (1994) and Bhowmik (1986) observed yellowing and stunting at rates of 0.14 and 0.28 kg ha^{-1}. Unacceptable levels of injury have been observed in creeping bentgrass mown at fairway heights at rates as low as 0.05 kg ha^{-1} (Carroll et al. 1992). However, velvet bentgrass (*Agrostis canina*) mown at putting green heights showed good tolerance at rates of 0.07 kg ha^{-1} (Henry and Hart 2004). Fenoxaprop has also been shown to control bermudagrass (*Cynodon dactylon*) in zoysiagrass (*Zoysia* spp.) and in cool-season turfgrass (Johnson 1992; Cudney et al. 1997). When fenoxaprop is applied alone, injury to zoysiagrass occurs and bermudagrass control requires multiple applications over the course of multiple years (Johnson 1992). However, when

fenoxaprop is tank mixed with triclopyr, zoysiagrass injury is reduced and bermudagrass control is increased (McElroy and Breeden 2006; Lewis et al. 2010).

Timing Considerations with ACCase Inhibitors. Timing of applications with ACCase inhibitors can be an important factor in control. Plowman et al. (1980) found that fluazifop control of barnyardgrass (*Echinochloa crus-galli*) and broadleaf signalgrass (*Brachiaria platyphylla*) was reduced when applications were made at the tillering stages versus when applications were made pre-tillering. Fluazifop control of large crabgrass (*Digitaria sanguinalis*) and giant foxtail (*Setaria faberi*) is significantly affected by application timing. Derr et al. (1985a) found that fluazifop controls large crabgrass 84% when an application is made pre-tillering. This is compared to 55, 40, and 7 percent control when applications were made at early tillering, mid-tillering and late tillering, respectively. Giant foxtail control was reduced from 79% pre-tillering to 58, 38, and 17 percent at early, mid and late tillering, respectively. Reduced control of large crabgrass at later growth stages with fluazifop has also been recorded by Ferreira and Coble (1994). Sethoxydim control was unaffected by application timing except for goosegrass and large crabgrass applied at the late tillering stage (Derr et al. 1985a). Derr et al. (1985b) proposed that the reason for reduced efficacy at later growth stages was a reduction in translocation of fluazifop within the plant at later growth stages. Askew et al. (2000) recorded clethodim efficacy on red rice (*Oryza sativa*) was reduced when applications were made at tillering stages versus pre-tillering stages. Reduction in clethodim efficacy as growth stage increased was also noted in goosegrass (Burke et al. 2002).

Antagonism of ACCase Inhibiting Herbicides

Tank mixtures. Simultaneous applications of two or more agrochemicals such as pesticides, adjuvants and fertilizers are often made. Oftentimes, herbicides are combined in a tank mix or prepackaged mixtures in order to: broaden the spectrum of weeds controlled, reduce production costs, or to reduce compaction by eliminating the number of times that a sprayer goes over an area (Anderson 1983). Generally, when two herbicides are applied at the same time, they act independently of each other. This interaction is generally referred to as the herbicide combination having and additive effect. On occasion, herbicide mixtures will be synergistic when the combination results in higher activity than expected based off of the rates of each herbicide in the combination. The opposite can also happen. When the net result of an herbicide combination is a reduction in activity of one or more of the herbicides in the mixture, that result is defined as being antagonistic (Akobundu et al. 1975).

Antagonism between various herbicides. Antagonism is a common type of interaction between herbicides (Cedergreen et al. 2007). Antagonism has been observed between a variety of different herbicides. In 1972, Beste and Schreiber reported antagonism between EPTC and 2,4-D. Glyphosate is antagonized by 2,4-D, dicamba and bromoxynil in wild oats (*Avena fatua* L.) and wheat (*Triticum aestivum* L.) (O'Sullivan and O'Donovan 1980) and also by atrazine and simazine (Appleby and Somabhi 1978). Diquat has been shown to antagonize penoxsulam in water hyacinth (*Eichhornia crassipes* [Mart.] Solms) (Wersal and Madsen, 2010). Diquat was also shown to antagonize the long-term efficacy of glyphosate (Whetje et al. 2008).

The basis for herbicide antagonism can vary between herbicide combinations. RNA synthesis disruption within the plant causes antagonism between EPTC and 2,4-D (Beste and Schreiber 1972). Simazine, atrazine, 2,4-D, dicamba and bromoxynil antagonism of glyphosate

is the result of a physical binding of the herbicides to glyphosate within the spray tank (Appleby and Somabhi 1978; O'Sullivan and O'Donovan 1980). Hamill et al. (1972) found that picloram antagonized 2,4-DB by reducing the translocation of the 2,4-DB. Diquat antagonizes glyphosate by inhibiting translocation as well (Wehtje et al. 2008).

Graminicide specific antagonism. Oftentimes, a mixture of grassy and broadleaf weeds is found in the same area. For economic reasons, producers/managers will try to control these weeds with as little input as possible. In situations where broadleaf and grassy weeds are present, it becomes advantageous to use a tank mixture of broadleaf herbicides and graminicides. Frequently, broadleaf herbicides will antagonize graminicides rendering many tank mixtures ineffective (Brommer et al. 2000).

Bentazon is a contact herbicide, used for the control of broadleaf weeds, that inhibits photosystem II (McCarty et al. 2010). It has been found to antagonize several different graminicides on several different target weeds. Bentazon antagonizes sethoxydim control of goosegrass, fall panicum (*Panicum dichotomiflorum*) and large crabgrass (Rhodes and Coble 1981; Holshouser and Coble 1990) by reducing goosegrass absorption of sethoxydim (Rhodes and Coble 1984; Holshouser and Coble 1990). Bentazon also prevented quackgrass (*Elymus* repens) absorption of quizalofop resulting in antagonism (Wilhm et al. 1986). Barnyardgrass control with clefoxydim was also antagonized by bentazon (Brommer et al. 2000). Bentazon also prevented the absorption and translocation of haloxyfop-methyl in sorghum (*Sorghum bicolor*) (Croon et al. 1989).

Bromoxynil is a photosystem II inhibitor that controls many broadleaf weeds with little to no grass activity (Culpepper et al. 1998; Gentsch 1986). Culpepper et al. (1998) found that bromoxynil reduced control of large crabgrass with fluazifop, fluazifop + fenoxaprop and

quizalofop 53, 55 and 69 percent respectively. Bromoxynil was less antagonistic when mixed with clethodim and sethoxydim (21 and 23% respectively). Bromoxynil induced antagonism is not consistent across grass species. Bromoxynil reduces the efficacy of fluazifop, fluazifop + fenoxaprop and quizalofop more on large crabgrass and yellow foxtail (*Setaria glauca*) than on Texas panicum (*Panicum texanum*) and goosegrass (Culpepper et al. 1999b). The basis of bromoxynil antagonism of quizalofop in yellow foxtail has been shown to be a reduction in quizalofop absorption into yellow foxtail leaf tissue (Culpepper et al. 1999a).

Acifluorfen is a protoporphyrinogen oxidase (Protox) inhibiting herbicide that is primarily used for postemergence selective broadleaf control (McCarty et al. 2010). Acifluorfen has been shown to antagonize fluazifop control of annual grass weeds (Dortenzio et al. 1984; Hopkins et al. 1984). Godley and Kitchen (1986) found that acifluorfen antagonized fluazifop control of large crabgrass. Wilhm et al. (1986) found that the basis for acifluorfen antagonism of graminicides was a reduction in translocation throughout the plant.

Acetolactate synthase (ALS) inhibition is a common herbicide mode of action. These herbicides inhibit the ALS enzyme which is required for the biosynthesis of the branched chain amino acids valine, leucine and isoleucine (McCarty et al. 2010). ALS inhibiting herbicides generally have a broad spectrum of broadleaf weed control activity making them an attractive tank mixture for graminicides. However, they are often associated with antagonism towards ACCase inhibiting herbicides. Imazaquin and chlorimuron antagonizes haloxyfop and fluazifop herbicidal activity on sorghum, reducing injury by 26% (Croon and Markle 1988). Croon et al. (1989) found that this antagonism was a result of decreased absorption and translocation of the graminicides within the sorghum plant. Chlorimuron and imazaquin have also been shown to antagonize sethoxydim control of fall panicum, large crabgrass and goosegrass. Chlorimuron

reduces sethoxydim control of fall panicum 68%, large crabgrass 29%, and goosegrass 30%. Imazaquin reduces sethoxydim control of fall panicum 88%, large crabgrass 29% and goosegrass 74% (Holshouser and Coble 1990). Ferreira and Coble (1994) found that pyrithiobac-sodium would antagonize fluazifop, sethoxydim and quizalofop, but that antagonism could be avoided if the pyrithiobac-sodium was applied at least three days before or three days after the graminicide application. This antagonism is due to a reduction of herbicide translocation and not a reduction in uptake (Ferreira et al. 1995). Chlorimuron is also shown to antagonize clefoxydim control of barnyardgrass reducing injury 22%. Pyrazolsulfuron-ethyl also antagonizes clefoxydim control of barnyardgrass reducing injury 16% (Brommer et al. 2000). Trifloxysulfuron antagonizes clethodim control of broadleaf signalgrass, fall panicum, large crabgrass and goosegrass (Burke et al. 2002). Imazapic antagonizes clethodim control of goosegrass, fall panicum and large crabgrass (Burke et al. 2001). Burke and Wilcut (2003) found that the basis for imazapic antagonism of goosegrass control was not a reduction in translocation and uptake, neither did it increase clethodim metabolism, but it appeared to be a reduction in goosegrass photosynthesis by imazapic that lead to decreased efficacy of clethodim. Cyhalofop control of barnyardgrass and broadleaf signalgrass is antagonized by halosulfuron, with a reduction of barnyardgrass and broadleaf signalgrass control 57 and 69 percent respectively (Scherder et al. 2005). Barnyardgrass control with fenoxaprop is also antagonized by halosulfuron and bensulfuron (Zhang et al. 2005). Quizalofop is antagonized when mixed with several different ALS inhibiting herbicides. Quizalofop tank mixed with bensulfuron, bispyribac, halosulfuron, orthosulfuron + halosulfuron, penoxsulam, and penoxsulam + triclopyr had reduced control of barnyard grass by 10, 63, 12, 14, 59, and 64 percent respectively (Rustom et al. 2018).

Synthetic auxin herbicides have also been shown to antagonize graminicide applications. Diclofop-methyl activity on wild oats is severely antagonized by 2,4-D, dicamba and MCPA (O'Sullivan et al. 1976; Todd and Stobe 1980). According to Qureshi and Vanden Born (1979), the basis of MCPA antagonism of diclofop is not occurring within the tank mixture, but within the plant. MCPA reduces the translocation of diclofop-methyl within wild oats resulting in antagonism (Olson and Nalewaja 1982). Todd and Stobe (1980) found that 2,4-D reduced translocation of diclofop-methyl to the meristematic sites of action within wild oats. Triclopyr antagonizes fenoxaprop control of barnyardgrass (Zhang et al. 2005) and also antagonizes cyhalofop activity on broadleaf signalgrass and barnyardgrass (Scherder et al. 2005). Occasionally, synthetic auxin herbicides will increase the efficacy of graminicides. For example, triclopyr antagonizes fenoxaprop activity on zoysiagrass species, but has a synergistic effect when applied to bermudagrass. This makes tank mix combinations of fenoxaprop and triclopyr a realistic bermudagrass control strategy within zoysiagrass turf (McElroy and Breeden 2006; Lewis et al. 2010).

Pinoxaden

Pinoxaden is an ACCase inhibiting herbicide that belongs to the phenylpyrazolin chemical family. Pinoxaden was introduced in 2006 to control annual and perennial grassy weeds postemergence within wheat and barley (*Hordeum* spp.) (Hofer et al. 2006; Muehlebach et al. 2011). Pinoxaden controls a wide variety of grassy weeds including ryegrass (*Lolium* spp.) and foxtails (*Setaria* spp.). Pinoxaden has a clean acute toxicological profile with rapid degradation in soil with no risk of persistence or accumulation. It requires a specialized adjuvant in order for optimal spread and translocation of the spray solution into grass weeds. Pinoxaden

generally has excellent rainfastness with performance not being negatively affected by rainfall in as little as 30 minutes after application. Pinoxaden is not considered an alternative to other ACCase herbicides when resistance to ACCase inhibition is a problem (Hofer et al. 2006).

Pinoxaden is formulated with the safener cloquintocet-mexyl in a 4:1 herbicide : safener ratio (Anonymous 2015). Cloquintocet-mexyl accelerates the metabolism of pinoxaden into inactive metabolites within wheat and barley, but not in susceptible ryegrass and wild oats (Muehlebach et al. 2011).

In turfgrass, pinoxaden was labelled for use in the United Kingdom until June 2019. It was labelled for removal of ryegrass species in maintained fine fescue (*Festuca* spp.) and annual bluegrass (*Poa annua*) turf (Anonymous 2015). Currently, pinoxaden is labelled in the United States for use on bermudagrass, zoysiagrass and St. Augustinegrass (*Stenotaphrum secundatum*). It is restricted to use only on bermudagrass and zoysiagrass fairways, roughs, tee boxes, athletic fields, sod farms and home lawns. Pinoxaden cannot be applied to putting greens and in St. Augustinegrass, it is restricted to sod farm use only. Pinoxaden has labelled control of tropical signalgrass (*Urochloa subquadripara*), tropical carpetgrass (*Axonopus compressus*), large and smooth crabgrass, bahiagrass (*Paspalum notatum*), dallisgrass (*Paspalum dilatatum*), and canoe grass (*Paspalum vaginatum*). Pinoxaden must be applied with Adigor® surfactant (methyl ester of fatty acids, alcohol ethoxylate, and petroleum distillates) (Anonymous 2018).

Thesis objectives

The objective of this thesis study is to better understand pinoxaden and how it can have a use in turfgrass situations. Differential response of grass species will be examined as well as potential antagonism concerns. The main objectives can be broken down into four parts as such:

1). Establish a greenhouse rate response screen in order to test the susceptibility of different grassy weed species. The current pinoxaden label has a limited number of weed species listed. Label expansion may be possible in order to more accurately display potential for pinoxaden use in turfgrass systems.

2). Explore potential herbicides or other agrochemicals that may increase pinoxaden safety for more effective and economical usage of pinoxaden in St. Augustinegrass sod production.

3). Identify possible antagonism issues pinoxaden may have when mixed with other agrochemicals in order to prevent ineffective applications.

4.) Exploration of possible herbicidal synergism with pinoxaden and tank mixtures to provide end users with better control methods and to help reduce the risk of ACCase inhibitor resistance.

Chapter 2. Rate Response of Select Grassy Weeds to Pinoxaden

Introduction

Pinoxaden is an ACCase inhibiting herbicide that belongs to the phenylpyrazolin chemical family. It was introduced commercially in 2006 to provide postemergence control of annual and perennial grassy weeds within barley and wheat (Hofer et al. 2006; Muehlebach et al. 2011). Until 2019, pinoxaden was registered in the United Kingdom for turfgrass use for the removal of ryegrass species (*Lolium* spp.) in maintained fine fescue (*Festuca* spp.) and annual bluegrass (*Poa annua* L.) turf (Anonymous 2015). In 2018, pinoxaden was labelled in the United States for use on bermudagrass (*Cynodon dactylon* L. Pers.), zoysiagrass (*Zoysia* spp.) and St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze). It is restricted to use only on bermudagrass and zoysiagrass fairways, roughs, tee boxes, athletic fields, sod farms and home lawns. Pinoxaden cannot be applied to putting greens and in St. Augustinegrass, it is restricted to sod farm use only because of injury concerns (Anonymous 2018a).

Acetyl coenzyme A (acetyl-CoA) is the product of respiration that occurs after pyruvic acid loses a carbon as CO_2. It is the first component of the lipid biosynthesis process where acetyl-CoA is converted into malonyl-CoA. This conversion is catalyzed by the enzyme acetyl-CoA carboxylase (ACCase) in combination with ATP and CO_2. From there, malonyl-CoA undergoes numerous transformations to become a completed fatty acid (Burton et al. 1989; Stoltenberg et al. 1989; Hopkins and Huner 2004). ACCase is inhibited by three chemical families of herbicides, the cyclohexanediones (DIMs), aryloxyphenoxypropionates (FOPs) and phenylpyrazolines (DEN), collectively referred to as “graminicides” (Shaner 2014). These herbicides are called graminicides because they only have activity against grasses and not sedges

or broadleaves. This difference in susceptibility is due to a differential ACCase between grasses and other plants (Konishi and Sasaki 1994).

ACCase inhibiting herbicides are frequently used in turfgrass situations due to differential response between grasses and the relative difficulty of controlling a grass weed within another grass. For example, diclofop, an aryloxyphenoxypropionate, controls goosegrass (*Eleuisine indica* L. Gaetrn.) within bermudagrass (McCarty 1990). Sethoxydim, a cyclohexanedione, is very active against bentgrass (*Agrostis stolonifera* L.) and tall fescue (*Lolium arundinaceum* (Schreb.) Darbysh) (Hosaka 1984; Hugh et al. 1986). However, red fescue (*Festuca rubra* L.), and centipede grass (*Eremochloa ophiuroides* (Munro.) Hack.) have shown very high tolerance to sethoxydim (McCarty et al. 1986; Hugh et al. 1986). Fenoxaprop, an aryloxyphenoxypropionate, is labelled for use in perennial ryegrass (*Lolium perenne* L.), tall fescue, fine fescue (*Festuca* spp.), Kentucky bluegrass (*Poa pratensis* L.) and creeping bentgrass but, the relative tolerance between these species varies greatly (Dernoeden 1987). Fenoxaprop significantly injures creeping bentgrass at rates as low as 0.05 kg ha^{-1} (Carroll et al. 1992) but does not injure velvet bentgrass (*Agrostis canina* L.) at rates as high as 0.07 kg ha^{-1} (Henry and Hart 2004). The wide selectivity of ACCase herbicides, even within the same chemical families, makes testing a variety of grass species necessary to understanding the efficacy and selectivity of a particular ACCase herbicide. In general, cool-season grasses (C3) are considered more tolerant to these herbicides than warm-season (C4) (Dernoeden 1987).

The relationship between herbicide rate and plant response is fundamentally important in understanding herbicide efficacy and selectivity (Seefeldt et al. 1995). Currently, grass selectivity of pinoxaden is not fully understood. There are many weeds that pinoxaden may potentially control but are not currently labelled. In turfgrass, pinoxaden is not labelled for

foxtail species (*Setaria* spp.) although foxtails are labelled in crop production. Several cool-season turf species were labelled safe under the turfgrass label in the United Kingdom. However, the turfgrass label in the United States does not allow for applications on any cool season turfgrasses.

The objectives of this research were to: (1) evaluate the rate-response of several grassy weeds to increasing rates of pinoxaden and (2) determine the I_{50} and I_{90} rates for control of each weed species evaluated using a nonlinear regression model. We hypothesize that C3 grasses will be more tolerant to pinoxaden while C4 grasses will be more susceptible. We also hypothesize that the C4 grasses tested will show differential response between individual species.

Materials and Methods

Greenhouse experiments were conducted between November 2018 and May 2019 at the Auburn University Weed Science Greenhouse located on the main campus of Auburn University in Auburn, Alabama (32.35°N, 85.29°W) to evaluate the rate response of select grassy weeds to pinoxaden. Nine different species of grassy weeds were evaluated: yellow foxtail (*Setaria pumila* Poir.), southern sandbur (*Cenchrus echinatus* L.), annual bluegrass, roughstalk bluegrass (*Poa trivialis* L.), large crabgrass (*Digitaria sanguinalis* L.), dallisgrass (*Paspalum dilatatum* Poir.), bahiagrass (*Paspalum notatum* Fleugg.), goosegrass and perennial ryegrass. Greenhouse day/night temperatures were maintained at 32/28 C (+/- 3C) throughout the study. Ambient lighting was used throughout the experiment with no supplemental light added. Relative humidity averaged ~70% throughout the experiment. All seeds were harvested from local populations in Auburn, Alabama and stored at 4 C until initiating this study. Seeds were planted in flats of potting medium and allowed to grow for four weeks. Individual seedlings were then

transplanted into separate 230 cm^3 pots filled with soil medium (Marvyn sandy loam). Seedlings were fertilized (Miracle-Gro Water-Soluble All-Purpose Plant Food, Scotts Miracle-Gro Products INC., Maryville, OH) (28-8-16; ~6 kg N ha^{-1}) upon transplanting. Plants were irrigated three times daily by an elevated misting system and by hand as needed throughout the experiment. Applications were made six weeks after germination for all species.

Foliar applications were delivered via a hand-held CO_2 pressurized sprayer equipped with TeeJet TP8002 flat fan nozzles (TeeJet Technologies, Glendale Heights, IL, USA) spaced 25 cm apart and calibrated to deliver 280 L ha^{-1}. The applied treatments were ten different rates of pinoxaden (Manuscript®, Syngenta Crop Protection LLC, Greensboro, NC, USA): 4, 10, 19, 39, 77, 156, 310, 621, 1240, and 2490 g ai ha^{-1} with surfactant (Adigor®, Syngenta Crop Protection LLC, Greensboro, NC, USA; methyl ester of fatty acids, alcohol ethoxylate, and petroleum distillates) at 0.5% v/v included in all treatments. One application was made of each treatment at the beginning of the trial. The treatments were arranged in a randomized complete block design with four replications and the trial was repeated in time. Treatments were compared to a nontreated control. Herbicide injury was visibly evaluated relative to the nontreated control on a 0 (no phytotoxic effect) to 100% (complete plant death) scale at 14, 28 and 35 days after treatment (DAT). Above-ground fresh plant material was recorded, in grams, at 35 DAT by clipping plants at soil level. Fresh weight was transformed into a percentage of the nontreated control for consistency in graphical presentations.

Data were subjected to ANOVA using the PROC GLM procedure using SAS 9.4 (SAS Institute Inc, Cary, NC) to test for significance ($P<0.05$) of species, pinoxaden rates and runs with the visual plant injury and fresh weight variables. No significant interaction between runs based on evaluation of the pinoxaden rate by species by run interaction ($P > 0.05$) was detected

so data were pooled over runs. A significant pinoxaden rate by species interaction ($P < 0.05$) was detected, thus individual species response to pinoxaden was analyzed further. Herbicide rate, including the nontreated control, was log-transformed to create equal spacing to facilitate nonlinear regressions. Nonlinear regressions were modelled using SigmaPlot 13.0 (Systat Software, San Jose, CA). Species were modelled with appropriate models that best described plant response. Plant visual injury rating data were fitted to a three-parameter sigmoidal model using the equation:

$$f= a/(1+exp(-(x-x_o)/b))$$

where *f* represents the percent visual injury relative to the nontreated control, *x* represents the log-transformed pinoxaden rate and *a*, *b* and x_o represent the regression parameters. This equation was used to calculate the rate at which the weed species were injured 50% (I_{50}) and 90% (I_{90}). Fresh weight data were fit to a two-parameter exponential decay model:

$$f= a*exp(-b*x)$$

where *f* represents weight as a percent of the nontreated, *x* represents the log transformed pinoxaden rate and *a* and *b* represent the regression parameters. This equation was used to calculate the weight at which fresh weight was reduced 50% (I_{50}) and 90% (I_{90}) for each species relative to that of the nontreated check.

Results and Discussion

Our initial hypothesis was that C3 grasses would have greater tolerance to pinoxaden than C4 grasses. However, such distinct differences based on grass physiology were not observed. Rather, seemingly random differences in grass response was observed between both C3 and C4 grasses. As will be demonstrated in the data, the grass species examined can be

ranked from most to least susceptible as such: perennial ryegrass > yellow foxtail > dallisgrass > large crabgrass > southern sandbur > roughstalk bluegrass > bahiagrass > goosegrass > annual bluegrass.

Based on visual control data, perennial ryegrass is the most susceptible species tested. All rates of pinoxaden tested injured the perennial ryegrass >95%. The I_{50} and I_{90} values determined for perennial ryegrass visual injury were 3.31 and 3.70 g ai ha^{-1} respectively. Pinoxaden injured yellow foxtail >95% at rates of 10 g ai ha^{-1} and higher. The I_{50} and I_{90} values determined for yellow foxtail visual injury were 3.42 and 4.76 g ai ha^{-1} respectively. Dallisgrass is similar to yellow foxtail in its susceptibility to pinoxaden. Pinoxaden injured dallisgrass >95% at rates of 10 g ai ha^{-1} and higher. The I_{50} and I_{90} values determined for dallisgrass visual injury were 3.96 and 8.39 g ai ha^{-1} respectively. Pinoxaden injured large crabgrass, southern sandbur and roughstalk bluegrass >90% at rates of 77 g ai ha^{-1} and higher. The I_{50} and I_{90} values determined for large crabgrass visual injury were 8.62 and 42.22 g ai ha^{-1} respectively. The I_{50} and I_{90} values determined for southern sandbur visual injury were 25.01 and 50.03 g ai ha^{-1} respectively and the I_{50} and I_{90} values determined for roughstalk bluegrass visual injury were 7.83 and 56.52 g ai ha^{-1} respectively. Pinoxaden injured bahiagrass > 90% at rates of 310 g ai ha^{-1} and higher. The I_{50} and I_{90} values determined for bahiagrass visual injury were 81.24 and 340.43 g ai ha^{-1} respectively. Both goosegrass and annual bluegrass are naturally resistant to pinoxaden applications. Pinoxaden did not injure (< 5%) either species at rates of 77 g ai ha^{-1} and lower. Pinoxaden only controlled goosegrass (>90%) at rates of 1240 g ai ha^{-1} and higher. No rate of pinoxaden injured annual bluegrass >90%. Pinoxaden at 2490 g ai ha^{-1} injured annual bluegrass 82%. The I_{50} and I_{90} values determined for goosegrass and annual bluegrass visual injury were 243.1 and 798.97 g ai ha^{-1} for goosegrass and 511.59 and >2490 g ai ha^{-1} for annual bluegrass.

Above ground biomass data supports perennial ryegrass being the most pinoxaden susceptible species examined. All rates of pinoxaden tested reduced perennial ryegrass biomass >95% in comparison to the nontreated. The I_{50} and I_{90} values determined for perennial ryegrass biomass reduction were 1.95 and 3.17 g ai ha^{-1} respectively. Pinoxaden reduced yellow foxtail biomass >90% at all rates tested. The I_{50} and I_{90} values determined for yellow foxtail biomass reduction were 2.05 and 3.75 g ai ha^{-1} respectively. Pinoxaden reduced dallisgrass biomass >95% at rates of 10 g ai ha^{-1} and higher. The I_{50} and I_{90} values determined for dallisgrass biomass reduction were 2.31 and 5.54 g ai ha^{-1} respectively. Pinoxaden reduced large crabgrass and roughstalk bluegrass biomass >90% at rates of 156 g ai ha^{-1} and higher. The I_{50} and I_{90} values determined for large crabgrass and roughstalk bluegrass biomass reduction were 4.92 and 64.03, and 4.17 and 48.73 g ai ha^{-1} and respectively. Low rates of pinoxaden caused an increase in plant biomass in southern sandbur, bahiagrass and goosegrass. This increase in plant biomass led to disproportionately high I_{90} values due to an inability of the nonlinear regression model to account for the initial increase in biomass at low rates. This is not uncommon in herbicides at low rates. Glyphosate applied at low doses has been shown to stimulate plant growth (Velini et al. 2008). Pinoxaden at 4 g ai ha^{-1} increased southern sandbur biomass 37%. Pinoxaden at rates of 156 g ai ha^{-1} and higher reduced southern sandbur biomass >85%. The I_{50} and I_{90} values determined for southern sandbur biomass reduction were 20.48 and 1470.82 g ai ha^{-1} respectively. Pinoxaden increased bahiagrass biomass by 5% at rates of 10 g ai ha^{-1} and lower. Pinoxaden reduced bahiagrass biomass >95% at rates of 621 g ai ha^{-1} and higher. The I_{50} and I_{90} values determined for bahiagrass biomass reduction were 74.32 and >2490 g ai ha^{-1} respectively. Pinoxaden increased goosegrass biomass by >15% at rates of 39 g ai ha^{-1} and lower. Rates of 1240 g ai ha^{-1} and higher were required to reduce goosegrass biomass >90%. The I_{50} and I_{90} values determined

for goosegrass biomass reduction were 247.51 and >2490 g ai ha^{-1} respectively. Pinoxaden had a growth regulatory effect on annual bluegrass without causing visual injury. All rates of pinoxaden reduced annual bluegrass biomass by >25%. However, rates of 621 g ai ha^{-1} and higher are were required to reduce annual bluegrass biomass >85%. No rates examined reduced annual bluegrass biomass >95%. The I_{50} and I_{90} values determined for annual bluegrass biomass reduction were 13.74 and >2490 g ai ha^{-1} respectively.

Based on these data, pinoxaden may be a viable post emergence control option for some of the weed species examined in this study. Yellow foxtail has a limited number of postemergence control options in warm season turfgrass. Topramezone and fenoxaprop are both labelled for postemergence foxtail control, but neither are labelled on bermudagrass turf (Anonymous 2018b; Anonymous 2019). Quinclorac has postemergence activity on both broadleaf weeds and grasses, including yellow foxtail, but has limited efficacy when applied to more mature plants (Curran et al. 2011; Anonymous 2018b). Dallisgrass is a difficult to control perennial grass that has very limited postemergence control options. Henry et al. (2007) observed that foramsulfuron can be used to suppress mature dallisgrass in warm season turf. However, full control of dallisgrass in warm season turf is difficult to obtain. Southern sandbur has limited control options in warm season turf as well. Imazapic is safe to use on bermudagrass and can be used for postemergence control of sandbur species (Grichar et al. 2008). Large crabgrass postemergence control in bermudagrass is limited to quinclorac and dithiopyr. Dithiopyr postemergence efficacy is directly related to the growth stage of the crabgrass (Rossi et al. 1988). Generally, dithiopyr must be applied pre tillering for effective postemergence crabgrass control (Keeley et al. 1997). Quinclorac is also more effective when postemergence applications are made earlier in the growth stage of the crabgrass plant (Enache and Ilnicki 1991).

Research Implications

Results from this study indicate that there is a differential response between grasses to pinoxaden. This is not uncommon amongst ACCase inhibiting herbicides and may be attributed to differential metabolism of the pinoxaden molecule between species. For example, McCarty et al. (1990) found that in centipede grass, six hours after an application of sethoxydim, only trace amounts (<1%) of the herbicide was observed in the centipede grass tissue. In contrast, 81-98% of the sethoxydim was detected in goosegrass, a susceptible grass, tissue. Based on this research, pinoxaden has the potential, at maximum labelled rates (156 g ai ha^{-1}), to effectively control (>80%) all grasses tested except goosegrass and annual bluegrass. Among the weed species tested, only large crabgrass, bahiagrass and dallisgrass are labelled for postemergence pinoxaden applications in turfgrass (Anonymous 2018). It must be noted, however, that dallisgrass and roughstalk bluegrass are both perennial weeds. This research was conducted on seedlings only and mature dallisgrass and roughstalk bluegrass plants will most likely be less susceptible to pinoxaden than seedlings are.

Pinoxaden is not considered a resistance breaker (Hofer et al. 2006) This means that weed biotypes that are already resistant to an ACCase inhibitor will most likely have developed cross resistance to pinoxaden. Therefore, the I_{50} and I_{90} values determined from this experiment may be used as a baseline for ACCase resistance screenings. Of the weed species examined, yellow foxtail, dallisgrass, southern sandbur, roughstalk bluegrass, bahiagrass and large crabgrass do not have an ACCase inhibiting herbicide labeled for bermudagrass turfgrass use. Pinoxaden may give turf managers an ACCase option to allow for herbicide mode of action rotation. This can help reduce the number of resistance cases observed in these weed species.

Table 1. Predictive model for percent visual injury, in response to increasing rates of pinoxaden, using a three parameter sigmoidal model. Parameter estimates and parameter estimate 95% confidence intervals (CI) are presented as a means of model comparison.

Species	Equation	R_2	Parameter estimates and confidence intervals					
	$f= a/(1+exp(-(x-x_o)/b))$		a	95% CI	b	95% CI	x_o	95% CI
Lolium perenne	$f= 100/(1+exp(-(x-0.52)/0.02))$	.99	100	99.7, 100.3	0.02	-25.3, 25.4	0.52	-92.4, 93.4
Poa trivialis	$f= 98.8/(1+exp(-(x-0.89)/0.37))$	.73	98.8	92.6, 105	0.37	0.25, 0.50	0.89	0.75, 1.02
Poa annua	$f= 79/(1+exp(-(x-2.65)/0.11))$	.76	79	69.9, 88.9	0.11	0.05, 0.17	2.65	2.56, 2.74
Paspalum notatum	$f= 105.27/(1+exp(-(x-1.94)/0.33))$	.91	105.27	98.1, 112.4	0.33	0.26, 0.41	1.94	1.85, 2.03
Cenchrus echinatus	$f= 98.82/(1+exp(-(x-1.4)/0.13))$	.99	98.82	97.4, 100.2	0.13	0.12, 0.14	1.4	1.38, 1.42
Paspalum dilatatum	$f= 99.04/(1+exp(-(x-0.6)/0.14))$	.80	99.04	95.3, 102.8	0.14	0.07, 0.22	0.6	0.53, 0.66
Eleusine indica	$f= 101.68/(1+exp(-(x-2.39)/0.25))$	.90	101.68	92.2, 111.2	0.25	0.17, 0.32	2.39	2.3, 2.49
Digitaria sanguinalis	$f= 99.23/(1+exp(-(x-0.93)/0.31))$	.77	99.23	97.4, 101	0.31	0.20, 0.41	0.93	0.82, 1.04
Setaria pumila	$f= 99.39/(1+exp(-(x-0.53)/0.06))$	.93	99.39	97.6, 101.2	0.06	-0.04, 0.17	0.53	0.42, 0.64

Table 2. Estimated rate of pinoxaden required to injure each species by 50% (I_{50}) and 90% (I_{90}) based on the injury ratings collected 35 days after treatment.

Species	Equation	R^2	Visual Injury	
			I_{50} (g ai ha^{-1})	I_{90} (g ai ha^{-1})
	$f= a/(1+exp(-(x-x_o)/b))$			
Lolium perenne	$f= 100/(1+exp(-(x-0.52)/0.02))$	.99	3.31	3.70
Poa trivialis	$f= 98.8/(1+exp(-(x-0.89)/0.37))$	.73	7.83	56.52
Poa annua	$f= 79/(1+exp(-(x-2.65)/0.11))$	.76	511.59	>2490
Paspalum notatum	$f= 105.27/(1+exp(-(x-1.94)/0.33))$	.91	81.24	340.43
Cenchrus echinatus	$f= 98.82/(1+exp(-(x-1.4)/0.13))$	.99	25.01	50.03
Paspalum dilatatum	$f= 99.04/(1+exp(-(x-0.6)/0.14))$	.80	3.96	8.39
Eleusine indica	$f= 101.68/(1+exp(-(x-2.39)/0.25))$	.90	243.1	798.97
Digitaria sanguinalis	$f= 99.23/(1+exp(-(x-0.93)/0.31))$	.77	8.62	42.22
Setaria pumila	$f= 99.39/(1+exp(-(x-0.53)/0.06))$	.93	3.42	4.76

Table 3. Predictive model for weed species weight presented as a percent of the nontreated, in response to increasing rates of pinoxaden, using a two parameter exponential decay model. Parameter estimates and parameter estimate 95% confidence intervals (CI) are presented as a means of model comparison.

Species	Equation	R_2	Parameter estimates and confidence intervals			
	*f= a*exp(-b*x)*		a	95% CI	b	95% CI
Lolium perenne	*f= 462.5*exp(-7.66*x)*	.99	462.5	376.60, 548.30	7.66	6.74, 8.58
Poa trivialis	*f= 127.9*exp(-1.51*x)*	.73	127.9	108.91, 146.95	1.51	1.22, 1.80
Poa annua	*f= 109.5*exp(-0.69*x)*	.46	109.5	87.74, 131.31	0.69	0.50, 0.88
Paspalum notatum	*f= 135.1*exp(-0.53*x)*	.60	135.1	115.70, 154.38	0.53	0.42, 0.65
Cenchrus echinatus	*f= 155.8*exp(-0.87*x)*	.54	155.8	123.88, 187.81	0.87	0.63, 1.10
Paspalum dilatatum	*f= 233.2*exp(-4.24*x)*	.95	233.2	202.22, 264.27	4.24	0.07, 0.22
Eleusine indica	*f= 150.4*exp(-0.46*x)*	.36	150.4	118.21, 182.52	0.46	0.42, 0.65
Digitaria sanguinalis	*f= 135.8*exp(-1.44*x)*	.57	135.8	106.26, 165.29	1.44	1.04, 1.85
Setaria pumila	*f= 341.9*exp(-6.15*x)*	.96	341.9	256.12, 427.71	6.15	4.93, 7.37

Table 4. Estimated rate of pinoxaden required to reduce the above ground biomass of each species by 50% (I_{50}) and 90% (I_{90}) based on weights collected 35 days after treatment.

Species	Equation	R^2	Weight Reduction	
			I_{50} (g ai ha^{-1})	I_{90} (g ai ha^{-1})
	*f= a*exp(-b*x)*			
Lolium perenne	*f= 462.5*exp(-7.66*x)*	.99	1.95	3.17
Poa trivialis	*f= 127.9*exp(-1.51*x)*	.73	4.17	48.26
Poa annua	*f= 109.5*exp(-0.69*x)*	.46	13.74	>2490
Paspalum notatum	*f= 135.1*exp(-0.53*x)*	.60	74.32	>2490
Cenchrus echinatus	*f= 155.8*exp(-0.87*x)*	.54	20.48	1470.82
Paspalum dilatatum	*f= 233.2*exp(-4.24*x)*	.95	2.31	5.54
Eleusine indica	*f= 150.4*exp(-0.46*x)*	.36	247.51	>2490
Digitaria sanguinalis	*f= 135.8*exp(-1.44*x)*	.57	4.92	64.03
Setaria pumila	*f= 341.9*exp(-6.15*x)*	.96	2.05	3.75

Figure 1. Percent visual injury response relative to the nontreated control of nine grassy weeds 35 days after treatment with increasing rates of pinoxaden. Response was modelled using a three-parameter sigmoidal model based on the log rate of pinoxaden to create equal spacing between rates. The equation used was: $f= a/(1+exp(-(x-x_o)/b))$. Non-log transformed rates are presented for reference. Means are expressed using differing symbols for each weed species and regression equation models are represented by differing line type foreach species. Vertical bars represent standard error (P=0.05).

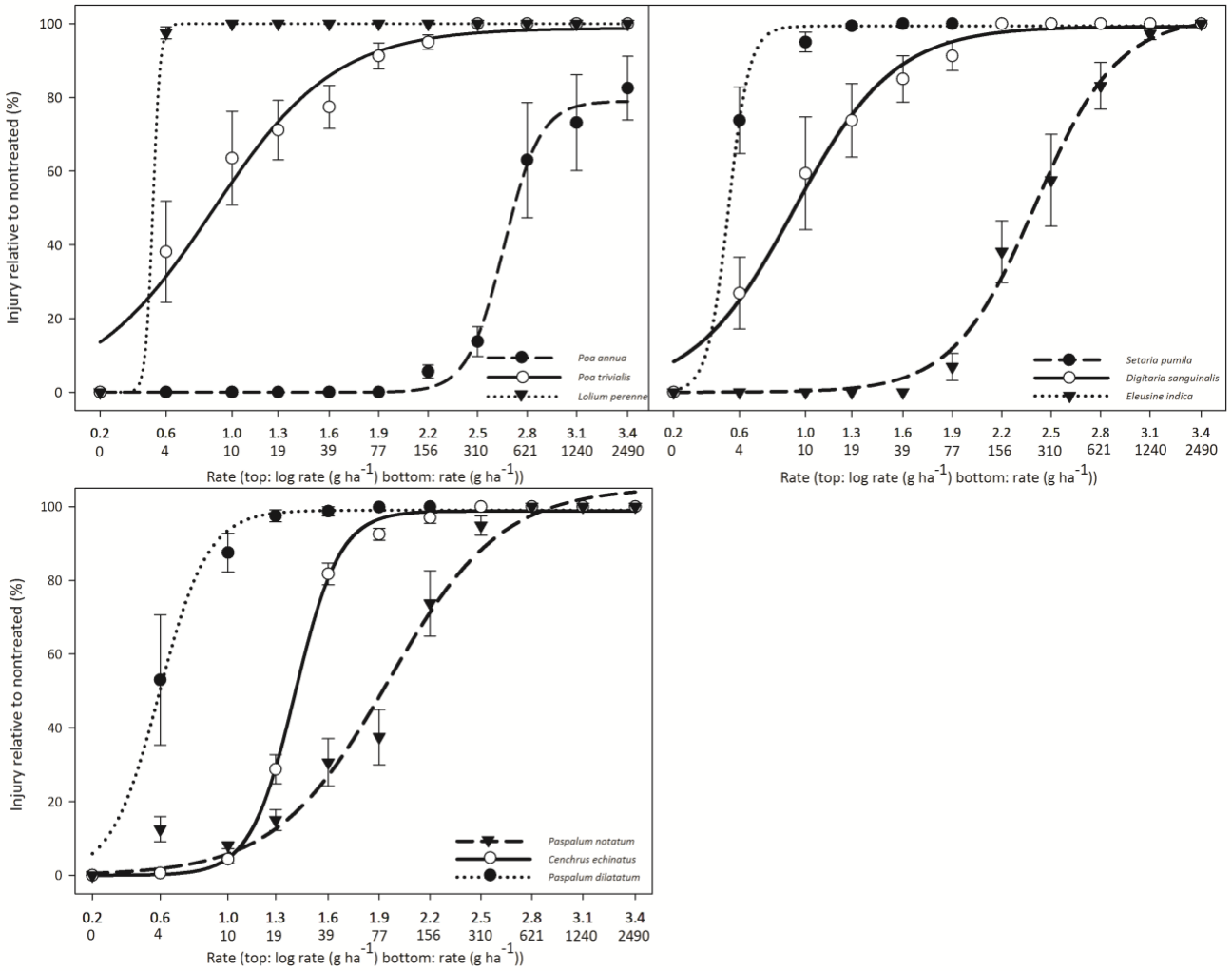
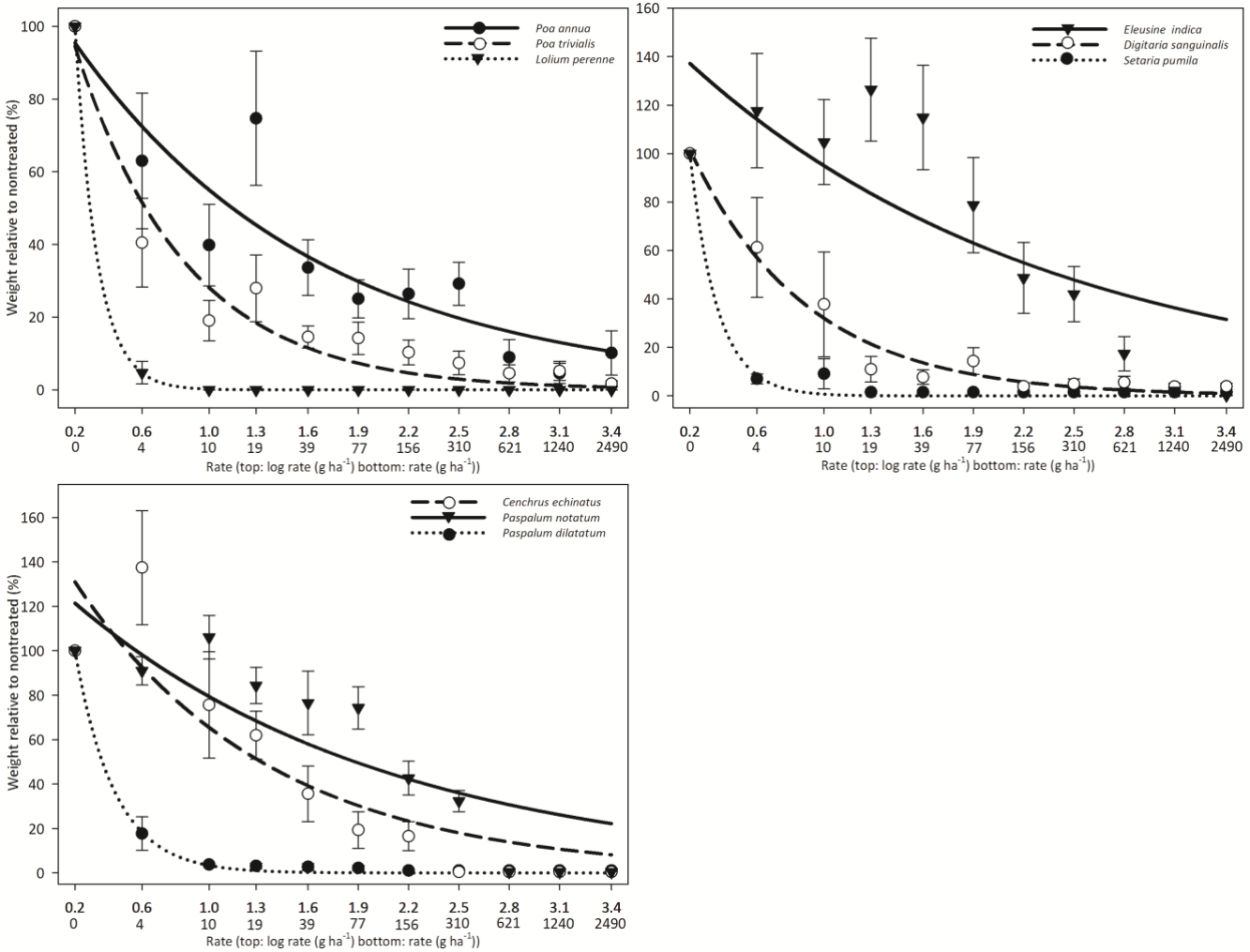


Fig 2. Above ground biomass, presented as a percent of the nontreated, 35 days after treatment with increasing rates of pinoxaden. All regressions were modelled based on the log rate of pinoxaden to create equal spacing between rates. All species were modelled with a two parameter exponential decay model using the equation: *f= a*exp(-b*x)*. Non-log transformed rates are presented for reference. Means are expressed using differing symbols for each weed species and regression equation models are represented by differing line type for each species. Vertical bars represent standard error (P=0.05).



Chapter 3. Tank Mixture Concepts for Turfgrass Injury Reduction

Introduction

Pinoxaden is an acetyl coenzyme A carboxylase (ACCase) inhibiting herbicide in the phenylpyrazolin chemical family. Pinoxaden was introduced in 2006 for postemergence control of annual and perennial grassy weed species in cereal crops (Hofer et al. 2006; Muehlebach et al. 2011). Until 2019, pinoxaden was registered in the United Kingdom for turfgrass use for the removal of ryegrass species (*Lolium* spp.) in maintained fine fescue (*Festuca* spp.) and annual bluegrass (*Poa annua* L.) turf (Anonymous 2015). In 2018, pinoxaden was labelled in the United States for use on bermudagrass (*Cynodon dactylon* L. Pers.), zoysiagrass (*Zoysia* spp.) and St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze). It is restricted to use only on bermudagrass and zoysiagrass fairways, roughs, tee boxes, athletic fields, sod farms and home lawns. Pinoxaden cannot be applied to putting greens, and in St. Augustinegrass it is restricted to sod farm use only due to injury concerns (Anonymous 2018c).

Simultaneous applications of two or more agrochemicals such as pesticides, adjuvants and fertilizers are often made. Oftentimes, herbicides are combined in a tank mix or prepackaged mixtures in order to: broaden the spectrum of weeds controlled, reduce production costs, or to reduce compaction by eliminating the number of times that a sprayer goes over an area (Anderson 1983). Generally, when two herbicides are applied at the same time, they act independently of each other. This interaction is referred to as the herbicide combination having and additive effect. On occasion, herbicide mixtures will be synergistic when the combination results in higher activity than expected based off of the rates of each herbicide in the combination. The opposite can also happen. When the net result of an herbicide combination is a

reduction in activity of one or more of the herbicides in the mixture, that result is defined as being antagonistic (Akobundu et al. 1975).

ACCase inhibiting herbicides, commonly known as graminicides, are frequently antagonized by herbicides that target broadleaf weeds or sedges (Brommer et al. 2000). Bentazon and bromoxynil frequently antagonize graminicide control of grass species (Rhodes and Coble 1981; Rhodes and Coble 1984; Gentsch 1986; Holshouser and Coble 1990; Culpepper et al. 1998; Culpepper et al. 1999b) by reducing graminicide absorption into the target weed species (Wilhm et al. 1986; Croon et al. 1989; Culpepper et al. 1999a). Acifluorfen antagonizes fluazifop control of annual grass weeds (Dortenzio et al. 1984; Hopkins et al. 1984; Godley and Kitchen 1986) by limiting the translocation of fluazifop throughout the plant (Wilhm et al. 1986).

Acetolactate synthase (ALS) inhibitors typically have a broad range of broadleaf weed control activity, making them an attractive tank mixture partner for graminicides. However, they are often associated with antagonism towards ACCase inhibiting herbicides. Imazaquin and chlorimuron antagonize haloxyfop and fluazifop herbicidal activity on sorghum (*Sorgum bicolor* L.), reducing injury up to 26%, (Croon and Markle 1988) by decreasing absorption and translocation of the graminicides within the plant (Croon et al. 1989). Chlorimuron and imazaquin have also been shown to antagonize sethoxydim control of fall panicum (*Panicum dichotomiflorum* Michx.), large crabgrass (*Digitaria sanguinalis* L.) and goosegrass (*Eleusine indica* L. Gaertn.) (Holshouser and Coble 1990). Ferreira and Coble (1994) found that pyrithiobac-sodium would antagonize fluazifop, sethoxydim and quizalofop by reducing herbicide translocation, but that antagonism could be avoided if the pyrithiobac-sodium was applied at least three days before or three days after the graminicide application (Ferreira and Coble 1994; Ferreira et al. 1995). Chlorimuron is also shown to antagonize clefoxydim control of

barnyardgrass (*Echinochloa crus-galli* L. P. Beauv.) (Brommer et al. 2000). Trifloxysulfuron antagonizes clethodim control of broadleaf signalgrass (*Urochloa platyphylla* Munro ex C. Wright R.D. Webster), fall panicum, large crabgrass and goosegrass (Burke et al. 2002). Imazapic antagonizes clethodim control of goosegrass by reducing overall goosegrass photosynthesis (Burke et al. 2001; Burke and Wilcut 2003). Cyhalofop and fenoxaprop control of barnyardgrass is antagonized by halosulfuron (Scherder et al. 2005; Zhang et al. 2005). Quizalofop is antagonized when mixed with several different ALS inhibiting herbicides. Quizalofop tank mixed with bensulfuron, bispyribac, halosulfuron, orthosulfuron + halosulfuron, penoxsulam, and penoxsulam + triclopyr reduced control of barnyardgrass by 10, 63, 12, 14, 59, and 64 percent respectively compared to quizalofop applied alone (Rustom et al. 2018).

Another, less documented, antagonist of graminicides are fungicides. Herbicide-fungicide tank mixtures are an attractive option to reduce application costs saves time and labor associated with pesticide applications (Lancaster et al. 2005). Chlorothalonil reduces clethodim control of large crabgrass, Texas panicum (*Panicum texanum* Buckl.), and goosegrass. However, clethodim efficacy on broadleaf signalgrass is not affected by chlorothalonil treatments (Jordan et al. 2003). Lancaster et al. (2005) observed that both clethodim and sethoxydim control of large crabgrass was antagonized by chlorothalonil as a result of reduced absorption of the graminicides.

The objectives of this research were to 1) determine if pinoxaden is antagonized by select ALS inhibitors 2) determine if fungicides may be used to reduce pinoxaden injury to St. Augustinegrass 3) determine if a difference in St. Augustinegrass cultivar tolerance is present.

Materials and Methods

Pinoxaden safening with fungicides. Field experiments were conducted in the summer of 2018 and 2019 to determine if there is a differential response to pinoxaden between four St. Augustinegrass cultivars. This study was located at the Auburn University Sports Surface Field Laboratory, located just off the main campus of Auburn University in Auburn, Alabama (32.35^{o}N, 85.29^{o}W). Four cultivars of St. Augustinegrass were examined: 'Classic', 'Palmetto', 'Raleigh' and 'Floratam'. The soil type for all four cultivars was a Marvyn sandy loam (fine-loamy, kaolinitic, thermic Typic Kanhapludults) with a pH of 6.5.

Foliar applications were delivered via a hand-held CO_2 pressurized sprayer equipped with TeeJet TP8002 flat fan nozzles (TeeJet Technologies, Glendale Heights, IL, USA) spaced 25 cm apart and calibrated to deliver 280 L ha^{-1}. Treatments were pinoxaden (0.156 kg ai ha^{-1}) alone, pinoxaden plus chlorothalonil (7.355 kg ai ha^{-1}) (Daconil Weatherstik®, Syngenta Crop Protection LLC, Greensboro, NC, USA) and pinoxaden plus propiconazole (0.501 kg ai ha^{-1}) (Banner Maxx II®, Syngenta Crop Protection LLC, Greensboro, NC, USA). All treatments included surfactant (Adigor®, Syngenta Crop Protection LLC, Greensboro, NC, USA; methyl ester of fatty acids, alcohol ethoxylate, and petroleum distillates) at 0.5% v/v. All treatments were reapplied at 28 days after the initial treatment (DAT). Treatments were arranged in a randomized complete block design with four replications and the trial was repeated in 2019. Plots were 5 feet long by 5 feet wide. Treatments were compared to a nontreated control. Herbicide injury was visually evaluated relative to the nontreated control on a 0 (no phytotoxic effect) to 100% (complete plant death) scale. Turfgrass color was visually rated on a 1 (completely brown with no active growth) to 9 (dark green, lush growth) scale. Visual ratings and measurements were obtained every seven days after initiation of the study until the

conclusion of the trial. Normalized difference vegetative indices (NDVI) measurements were also taken, but only in 2019. Research suggests that NDVI has a correlation with turfgrass density, color and percent live cover (Bell et al. 2002).

Data were subjected to ANOVA using the PROC GLM procedure using SAS 9.4 (SAS Institute Inc, Cary, NC) to test for significance ($P<0.05$) of cultivar, treatments and runs. Means were separated using Fishers protected LSD ($P=0.05$). No significant interaction between years based on evaluation of the treatment by year interaction ($P > 0.05$) was detected so visual data were pooled over years. A significant treatment by cultivar by days after treatment interaction ($P < 0.05$) was detected, therefore cultivar response to treatments by days after treatment were further examined.

Pinoxaden safening with ALS inhibiting herbicides. A field experiment was conducted in the summer of 2019 to determine if common ALS inhibiting herbicides may antagonize pinoxaden injury of St. Augustinegrass. This study was located at the Auburn University Sports Surface Field Laboratory, located just off the main campus of Auburn University in Auburn, Alabama. Treatments were applied to 'Floratam' St. Augustinegrass on a Marvyn sandy loam (fine-loamy, kaolinitic, thermic Typic Kanhapludults) with a pH of 6.5.

Foliar applications were delivered via a hand-held CO_2 pressurized sprayer equipped with TeeJet TP8002 flat fan nozzles (TeeJet Technologies, Glendale Heights, IL, USA) spaced 25 cm apart and calibrated to deliver 280 L ha^{-1}. Treatments were pinoxaden (156 g ai ha^{-1}) alone, pinoxaden plus halosulfuron (3.36 g ai ha^{-1}) (Sedgehammer®, Syngenta Crop Protection LLC, Greensboro, NC, USA) and pinoxaden plus metsulfuron (31.52 g ai ha^{-1}) (Manor®, Syngenta Crop Protection LLC, Greensboro, NC, USA). All treatments included surfactant (Adigor®, Syngenta Crop Protection LLC, Greensboro, NC, USA; methyl ester of fatty acids, alcohol

ethoxylate, and petroleum distillates) at 0.5% v/v. All treatments were reapplied at 21 days after the initial treatment (DAT). Treatments were arranged in a randomized complete block design with four replications. Plots were 5 feet long by 5 feet wide. Treatments were compared to a nontreated control. Herbicide injury was visually evaluated relative to the nontreated control on a 0 (no phytotoxic effect) to 100% (complete plant death) scale. Turfgrass color was visually rated on a 1 (completely brown with no active growth) to 9 (dark green, lush growth) scale. NDVI measurements were also taken, but only in 2019. All ratings and measurements in 2019 were obtained every seven days, beginning 14 DAT, until the conclusion of the trial.

Data were subjected to ANOVA using the PROC GLM procedure using SAS 9.4 (SAS Institute Inc, Cary, NC) to test for significance ($P<0.05$) of treatments. Means were separated using Fishers protected LSD ($P=0.05$). A significant treatment by days after treatment interaction ($P < 0.05$) was detected, therefore St. Augustinegrass response to treatments by days after treatment were examined further.

Smooth crabgrass response to pinoxaden tank mixtures. A field experiment was conducted in the summer of 2019 to determine if common ALS inhibiting herbicides and chlorothalonil may antagonize pinoxaden control of smooth crabgrass (*Digitaria ischaemum* Schreb.). This study was located at the Auburn University Sports Surface Field Laboratory, located just off the main campus of Auburn University in Auburn, Alabama. Treatments were applied to 'Tifway' bermudagrass that was heavily infested (>90% coverage) with smooth crabgrass. The soil was a Marvyn sandy loam (fine-loamy, kaolinitic, thermic Typic Kanhapludults) with a pH of 6.5.

Foliar applications were delivered via a hand-held CO_2 pressurized sprayer equipped with TeeJet TP8002 flat fan nozzles (TeeJet Technologies, Glendale Heights, IL, USA) spaced 25 cm apart and calibrated to deliver 280 L ha^{-1}. Treatments were pinoxaden (71.35 g ai ha^{-1}) alone,

pinoxaden plus halosulfuron (3.36 g ai ha^{-1}) (Sedgehammer®, Syngenta Crop Protection LLC, Greensboro, NC, USA), halosulfuron alone, pinoxaden plus metsulfuron (31.52 g ai ha^{-1}) (Manor®, Syngenta Crop Protection LLC, Greensboro, NC, USA), metsulfuron alone and pinoxaden plus chlorothalonil (7355 g ai ha^{-1}) (Daconil Weatherstik®, Syngenta Crop Protection LLC, Greensboro, NC, USA) (Table 11). All treatments included surfactant (Adigor®, Syngenta Crop Protection LLC, Greensboro, NC, USA; methyl ester of fatty acids, alcohol ethoxylate, and petroleum distillates) at 0.5% v/v. Initial applications were made on May 20, when the smooth crabgrass had 1-3 tillers. Reapplications were made on June 3, 14 days after initial treatment (DAT).

Treatments were arranged in a randomized complete block design with four replications. Plots were 5 feet long by 5 feet wide. Treatments were compared to a nontreated control. Smooth crabgrass control was visually evaluated relative to the nontreated control on a 0 (no phytotoxic effect) to 100% (complete plant death) scale. Smooth crabgrass coverage was visually rated on a 0 (no smooth crabgrass plants observed) to 100% (complete coverage of smooth crabgrass) scale. Data were collected at 14, 28, 42 and 56 DAT.

Data were subjected to ANOVA using the PROC GLM procedure using SAS 9.4 (SAS Institute Inc, Cary, NC) to test for significance ($P<0.05$) of treatments. Means were separated using Fishers protected LSD ($P=0.05$). A significant treatment by days after application interaction ($P < 0.05$) was detected. Therefore, data were examined separately for each rating date.

Greenhouse response to pinoxaden tank mixtures. Greenhouse studies were conducted between July and August of 2019 at the Auburn University Weed Science Greenhouse located on the main campus of Auburn University in Auburn, Alabama (32.35^{o}N, 85.29^{o}W) to evaluate

potential antagonism of pinoxaden injury to St. Augustine grass when tank mixed with different pesticides. 'Floratam' St. Augustinegrass plugs were planted in 230 cm^3 pots filled with soil medium (Marvyn sandy loam). Plants were fertilized (Miracle-Gro Water-Soluble All-Purpose Plant Food, Scotts Miracle-Gro Products INC., Maryville, OH) (28-8-16; ~6 kg N ha^{-1}) upon transplanting and as needed for complete establishment within each individual pot. Greenhouse day/night temperatures were maintained at 32/28 C (+/- 3C) throughout the study. Ambient lighting was used throughout the experiment with no supplemental light added. Relative humidity averaged ~70% throughout the experiment. Plants were irrigated four times daily by an elevated misting system and by hand as needed throughout the experiment.

Foliar applications were delivered via a hand-held CO_2 pressurized sprayer equipped with TeeJet TP8002 flat fan nozzles (TeeJet Technologies, Glendale Heights, IL, USA) spaced 25 cm apart and calibrated to deliver 280 L ha^{-1}. The applied treatments were three different rates of pinoxaden plus halosulfuron, metsulfuron and chlorothalonil (Table 14). All treatments included surfactant (Adigor®, Syngenta Crop Protection LLC, Greensboro, NC, USA; methyl ester of fatty acids, alcohol ethoxylate, and petroleum distillates) at 0.5% v/v. One application was made at the beginning of the experiment.

Above ground biomass (in grams) and visual injury (0-100% scale) data were collected 28 days after treatment. Data were subjected to ANOVA using the PROC GLM procedure using SAS 9.4 (SAS Institute Inc, Cary, NC) to test for significance ($P<0.05$) of treatments. Means were separated using Fishers protected LSD ($P=0.05$). A significant treatment interaction was detected ($P < 0.05$) so data were examined based on individual treatments.

Results and Discussion

Pinoxaden safening with fungicides. Variability in herbicide tolerance between turfgrass cultivars of the same species can exist. McElroy et al. (2005) and McCalla et al. (2004) reported that seedling Yukon common bermudagrass was more susceptible to broadleaf herbicides during seedling establishment than other seeded bermudagrass cultivars. This has been seen in ACCase inhibiting herbicides as well. Variability between Italian ryegrass (*Lolium multiflorum* Lam.) cultivar tolerance to fenoxaprop exists, with some cultivars having up to three times greater tolerance than others (Hassan et al. 2002). According to these data, variability in pinoxaden tolerance between cultivars of St. Augustinegrass exists.

Maximum visual injury, lowest color rating and lowest NDVI readings occurred at 42 days after initial treatment (DAIT) for all cultivars except Palmetto. The maximum visual injury and lowest color rating for Palmetto St. Augustinegrass occurred at 49 DAIT. However, the lowest NDVI reading was recorded at 42 DAIT. At the maximum visual injury date, pinoxaden alone injured Classic 83.75%, Raleigh 66.25%, Palmetto 48.75% and Floratam 50%. Pinoxaden alone reduced turfgrass color, at the maximum color reduction date, of Classic from 7 to 2.5, Raleigh from 7 to 4, Palmetto from 6.88 to 4.13, and Floratam from 7 to 4.75. At 42 DAIT, pinoxaden alone reduced the NDVI readings, in relation to the nontreated, for Classic 0.1111, Raleigh 0.0963, Palmetto 0.1152, and Floratam 0.0966. Based on the maximum visual injury ratings, lowest color ratings and lowest NDVI readings, the cultivars can be ranked from most to least susceptible as such Classic, Raleigh, Floratam, Palmetto.

Reduced turfgrass visual injury and increased relative color was observed when pinoxaden was tank mixed with either chlorothalonil or propiconazole. This was also supported

by higher relative NDVI readings. At the maximum visual injury date, pinoxaden plus chlorothalonil and pinoxaden plus propiconazole injured Classic 33.75 and 28.75% respectively, Raleigh 30 and 28.75% respectively, Floratam 20 and 22.5% respectively, and Palmetto 11.25% for both. At the maximum color reduction date, pinoxaden plus chlorothalonil and pinoxaden plus propiconazole reduced turfgrass color of Classic from 7 to 5 for both, Raleigh from 7 to 5 and 5.25 respectively, Floratam from 7 to 5 and 5.75 respectively, and Palmetto from 6.88 to 5.88 and 5 respectively. At 42 DAIT, pinoxaden plus chlorothalonil and pinoxaden plus propiconazole reduced the NDVI readings for Classic 0.0364 and 0.0330 respectively, Raleigh 0.0055 and 0.0331 respectively, Floratam 0.0497 and 0.0542 respectively and Palmetto 0.0639 and 0.0561 respectively.

These data suggest that chlorothalonil and propiconazole may have an antagonistic effect on pinoxaden injury to St. Augustinegrass regardless of cultivar. These results are similar to other studies conducted with chlorothalonil and ACCase inhibiting herbicides. Jordan et al. (2003) found chlorothalonil to be antagonistic to clethodim control of large crabgrass, Texas panicum and goosegrass. However, propiconazole has not been examined for potential antagonism with ACCase inhibiting herbicides. Also, studies that focus on potential antagonism between fungicides and herbicides are typically focused on crop production instead of turfgrass management.

Pinoxaden plus chlorothalonil treatments injured the St. Augustinegrass, regardless of cultivar, in a way that is atypical of ACCase inhibiting herbicides. Treated leaves would have a large bleached white area with a well defined necrotic contour where individual droplets landed on the leaf blade (Figure 10). Robinson et al. (2013) noted similar injury in winter wheat (*Triticum aestivum* L.) when broadleaf herbicides such as bromoxynil and MCPA were mixed

with fungicides such as azoxystrobin and propiconazole. This injury occurred 2-3 days after application and was mown off within 10 days with no residual effects.

Future research needs to be conducted in order to determine if pinoxaden antagonism by chlorothalonil and propiconazole occurs in other species, especially grassy weed species. Different formulations of each fungicide should also be examined in order to determine if the pinoxaden antagonism is a result of interaction between the two molecules or there is an interaction with an inert ingredient in the formulations examined and pinoxaden. Studies should also be conducted to determine the physiological basis for this antagonism. Lancaster et al. (2005) determined that chlorothalonil antagonism of sethoxydim and clethodim was a result of decreased absorption of the herbicide into the leaf tissue. More research can be conducted to determine if more contact fungicides across several chemical families are capable of restricting the uptake of herbicides into the plant's leaf tissue.

Pinoxaden safening with ALS inhibiting herbicides. ALS inhibiting herbicides often antagonize ACCase inhibiting herbicides (Croon and Markle 1988; Holshouser and Coble 1990; Ferreira and Coble 1994; Brommer et al. 2000; Burke et al. 2001; Burke et al. 2002; Scherder et al. 2005; Zhang et al. 2005; Rustom et al. 2018). This is problematic because ALS inhibiting herbicides generally have a broad range of weed control making them an attractive tank mix partner. According to these data, metsulfuron and halosulfuron antagonize pinoxaden injury of St. Augustinegrass.

Maximum visual injury occurred at 42 days after initial treatment (DAIT) for all treatments except pinoxaden plus metsulfuron. Plots treated with pinoxaden plus metsulfuron had begun to recover by 42 DAIT with maximum injury occurring at 35 DAIT. This trend was also seen in turfgrass color ratings and NDVI readings. At 42 DAIT, pinoxaden alone injured St.

Augustinegrass 77.5% compared to pinoxaden plus halosulfuron and pinoxaden plus metsulfuron which injured St. Augustinegrass 52.5 and 25% respectively. The only rating date that pinoxaden alone and pinoxaden plus halosulfuron injury was significantly different was 42 DAIT. All other rating dates did not have a statistically significant difference in visual injury. Pinoxaden plus metsulfuron visual injury was significantly lower than pinoxaden alone, statistically, for every rating date except 14 DAIT. At 42 DAIT, turfgrass color was reduced from 7 to 3 for pinoxaden alone. Pinoxaden plus halosulfuron reduced turfgrass color from 7 to 3.8 and pinoxaden plus metsulfuron reduced turfgrass color from 7 to 5. NDVI readings supported visual ratings with pinoxaden alone having the lowest NDVI reading at all rating dates. At 42 DAIT, the NDVI reading for pinoxaden alone was 0.2151 less than the nontreated. This is compared to a reduction in NDVI of 0.1409 and 0.076 from pinoxaden plus halosulfuron and pinoxaden plus metsulfuron respectively.

These data indicate that both metsulfuron and halosulfuron antagonize pinoxaden injury of St. Augustinegrass. However, metsulfuron reduced visual injury, maintained green color and had a consistently higher NDVI reading than halosulfuron. According to these data, metsulfuron is more antagonistic to pinoxaden than halosulfuron. The physiological mechanisms of antagonism between ALS and ACCase inhibiting herbicides can vary from reduced uptake (Croon et. al 1989) to reduced translocation (Ferreria et al. 1995) to reduced photosynthesis (Burke and Wilcut 2003). Further research is needed to determine the physiological basis for metsulfuron and halosulfuron antagonism of pinoxaden. Research also needs to be conducted to determine if this antagonism is limited to St. Augustinegrass or if different grassy weeds would not be controlled by these tank mixtures.

Smooth crabgrass response to pinoxaden tank mixtures. Metsulfuron and halosulfuron alone provided insignificant control (<10%) throughout the study as expected. However, antagonistic responses were observed for smooth crabgrass control at 14 DAIT when pinoxaden was mixed with metsulfuron and when pinoxaden was mixed with chlorothalonil. Pinoxaden plus metsulfuron reduced smooth crabgrass control from 65%, when pinoxaden was applied alone, to 20%. This was the highest percentage of crabgrass control obtained throughout the study with the mixture of pinoxaden plus metsulfuron. Pinoxaden plus metsulfuron control was insignificant (<10%) at all other rating dates throughout the trial. At 14 DAIT, chlorothalonil reduced pinoxaden control of smooth crabgrass by reducing control from 65% to 36.25%. At 28 DAIT, all tank mixtures, except pinoxaden plus metsulfuron, resulted in a statistically neutral response. At 42 DAIT, antagonistic responses were observed in tank mixtures of pinoxaden plus halosulfuron, metsulfuron and chlorothalonil. Pinoxaden plus halosulfuron, metsulfuron and chlorothalonil reduced smooth crabgrass control relative to pinoxaden alone from 87.5 to 63.75, 8.75 and 65% respectively. At 56 DAIT, pinoxaden plus halosulfuron, metsulfuron and chlorothalonil reduced smooth crabgrass control relative to pinoxaden alone from 72.5 to 45, 0 and 25% respectively.

These results indicate that metsulfuron, halosulfuron and chlorothalonil all antagonize pinoxaden control of smooth crabgrass. According to the data, metsulfuron is the most antagonistic of smooth crabgrass control with pinoxaden with chlorothalonil and halosulfuron being similar in antagonistic capability. Further research is needed to determine if these pesticides are antagonistic towards other grassy weed species that are normally controlled by pinoxaden. Occasionally, antagonism of ACCase inhibitors is only expressed in certain weed species and not others. Chlorothalonil antagonizes clethodim control of large crabgrass, Texas

panicum, and goosegrass. However, chlorothalonil does not antagonize clethodim control of broadleaf signalgrass (Jordan et al. 2003). Triclopyr reduces fenoxaprop injury to zoysiagrass but increases fenoxaprop injury to bermudagrass (McElroy and Breeden 2006).

Metsulfuron antagonism of pinoxaden is important to confirm because of the high use rate of metsulfuron in turfgrass. Because of its broad activity on weeds and its relatively low cost (< $10/oz) mixed with low product use rates (0.01 oz/1000ft$_2$), metsulfuron is often utilized in turfgrass situations (Goncalves et al. 2019). Further research is needed to both confirm metsulfuron antagonism of pinoxaden and also to determine the physiological basis for this antagonism.

Chlorothalonil, when mixed with pinoxaden, injured smooth crabgrass in a way that was atypical of ACCase injury. Large light brown to white spots were observed on the leaf blade, usually with a necrotic ring surrounding the whitened area (Figure 9). This is similar to injury observed on St. Augustinegrass in other trials of this study (Figure 10). Further research is needed to determine the cause of this injury and how it affects pinoxaden efficacy.

Greenhouse response to pinoxaden tank mixtures. At the conclusion of the experiment, pinoxaden at the low, medium and high rates injured St. Augustinegrass 30.83, 33.33 and 58.33% respectively. Both pinoxaden plus halosulfuron and pinoxaden plus metsulfuron injured St. Augustinegrass less than pinoxaden alone. Halosulfuron mixed with the low, medium and high rate of pinoxaden injured St. Augustinegrass 8.33, 25 and 45% respectively. Metsulfuron mixed with the low, medium and high rate of pinoxaden injured St. Augustinegrass 7.5, 19.17 and 20.83% respectively. Chlorothalonil reduced pinoxaden injury of St. Augustinegrass only at the lowest pinoxaden rate. Chlorothalonil mixed with the low, medium and high rate of pinoxaden injured St. Augustinegrass 19.17, 33.33 and 75% respectively.

The biomass of the nontreated control was 20.55 g. No treatment had a biomass as high as the nontreated even with little to no apparent injury. In field trials, growth regulation was observed even when herbicide visual injury was not observed. Pinoxaden at the low, medium and high rates reduced the biomass of St. Augustinegrass to 6.79, 7.60 and 4.36 g respectively. Halosulfuron mixed with the low, medium and high rate of pinoxaden reduced the biomass of St. Augustinegrass to 11.81, 10.17 and 7.3 g respectively. Metsulfuron mixed with the low, medium and high rate of pinoxaden reduced the biomass of St. Augustinegrass to 14.8, 10.37 and 8.36 g respectively. Chlorothalonil mixed with the low, medium and high rate of pinoxaden reduced the biomass of St. Augustinegrass to 11.7, 8.98 and 5.52 g respectively.

Chlorothalonil mixed with all three rates of pinoxaden treatments resulted in injury to the St. Augustinegrass plants that was not typical of ACCase inhibitor injury. Treated leaves would have a large bleached white area with a well defined necrotic contour where individual droplets landed on the leaf blade (Figure 10), similar to findings by Robinson et al. (2013) on winter wheat. This injury was worsened at the medium and high rates of pinoxaden. Further research is needed to understand why this injury is occurring and what can be done to mitigate it.

The results from this trial supports the results from field trials that indicate that halosulfuron, metsulfuron and chlorothalonil have the ability to antagonize pinoxaden activity on grass species. Further research is needed to determine at what rate of pinoxaden can antagonism be overcome, if at all. Antagonist application timing may also need to be examined further. Ferreira and Coble (1994) found that pyrithiobac-sodium would antagonize fluazifop, sethoxydim and quizalofop, but that antagonism could be avoided if the pyrithiobac-sodium was applied at least three days before or three days after the graminicide application.

Overall, the results from these studies indicate that pinoxaden has the potential to be antagonized in a similar way to other ACCase inhibiting herbicides in other chemical families. More research is needed to determine the extent of the antagonism issues that pinoxaden may face and the physiological basis for this antagonism.

Table 5. Turfgrass percent visual injury rating separated by cultivar, treatment and rating date.

Percent injury of 'Classic' St. Augustinegrass†								
Treatment	DAIT‡§							
	7	14	21	28	35	42	49	56
LSD	6.43	5.37	4.64	5.16	6.88	7.8	7.67	10.45
Nontreated	0 c	0 d	0 d	0 d	0 c	0 c	0 d	0 d
Pinoxaden alone	32.5 a	41.25 a	42.5 a	29.38 a	55 a	83.75 a	68.13 a	63.75 a
+ Chlorothalonil	25 b	23.75 c	12.5 c	6.25 c	23.75 b	33.75 b	18.75 c	12.5 c
+ Propiconazole	24.38 b	33.75 b	25 b	18.75 b	26.25 b	28.75 b	35 b	35 b

Percent injury of 'Floratam' St. Augustinegrass†								
Treatment	DAIT‡§							
	7	14	21	28	35	42	49	56
LSD	6.26	4.43	3.09	2.70	5.09	7.42	5.99	6.15
Nontreated	0 b	0 c	0 d	0 d	0 d	0 c	0 d	0 b
Pinoxaden alone	21.25 a	28.75 a	20.63 a	16.88 a	35.63 a	50 a	36.25 a	23.75 a
+ Chlorothalonil	16.25 a	16.25 b	5.63 c	3.75 c	13.13 c	20 b	9.38 c	4.38 b
+ Propiconazole	20.63 a	25 a	16.88 b	10 b	21.88 b	22.5 b	23.75 b	20.63 b

Percent injury of 'Palmetto' St. Augustinegrass†								
Treatment	DAIT‡§							
	7	14	21	28	35	42	49	56
LSD	6.80	5.80	3.38	5.32	9.02	10.17	8.31	6.92
Nontreated	0 b	0 c	0 d	0 d	0 c	0 c	0 d	0 c
Pinoxaden alone	18.75 a	28.75 a	30 a	26.25 a	38.13 a	33.75 a	48.75 a	41.88 a
+ Chlorothalonil	16.88 a	16.25 b	7.5 c	5.63 c	18.75 b	11.25 b	15 c	5.63 c
+ Propiconazole	15.63 a	20.63 b	21.25 b	17.5 b	23.75 b	11.25 b	32.5 b	24.38 b

Percent injury of 'Raleigh' St. Augustinegrass†								
Treatment	DAIT‡§							
	7	14	21	28	35	42	49	56
LSD	6.53	4.93	5.09	3.53	6.37	9.43	6.46	5.31
Nontreated	0 b	0 c	0 d	0 d	0 d	0 c	0 d	0 c
Pinoxaden alone	22.5 a	32.5 a	25 a	13.13 a	42.5 a	66.25 a	35 a	25 a
+ Chlorothalonil	19.38 a	20.63 b	8.13 c	4.38 c	16.25 c	30 b	6.88 c	5 c
+ Propiconazole	21.25 a	28.13 b	18.13 b	9.38 b	23.75 b	28.75 b	24.38 b	16.3 b

† A visual injury rating of 20% was considered minimally acceptable
‡ Days after initial treatment (DAIT)
§ Column means not sharing any letter are considered significantly different according to Fisher's protected LSD (α=0.05).

Table 6. Turfgrass color rating separated by cultivar, treatment and rating date.

Color of 'Classic' St. Augustinegrass†								
Treatment	DAIT‡§							
	7	14	21	28	35	42	49	56
LSD	0.33	0.42	0.37	0.39	0.65	0.46	0.45	0.69
Nontreated	7.38 a	7.38 a	7.5 a	7.38 a	6.75 a	7 a	6.88 a	7 a
Pinoxaden alone	5.5 c	5 c	5.25 d	5.88 c	4.25 c	2.5 c	3.5 d	4.25 c
+ Chlorothalonil	5.75 bc	6 b	6.75 b	7.13 a	5.75 b	5 b	5.75 b	6.63 a
+ Propiconazole	6 b	5.38 c	5.88 c	6.5 b	5.38 b	5 b	4.75 c	5.38 b

Color of 'Floratam' St. Augustinegrass†								
Treatment	DAIT‡§							
	7	14	21	28	35	42	49	56
LSD	0.35	0.28	0.25	0.01	0.33	0.65	2.02	0.40
Nontreated	6.88 a	6.88 a	7 a	7 a	6.88 a	7 a	5.25 a	7 a
Pinoxaden alone	6.13 b	5.5 c	6.13 b	6.5 b	5.5 d	4.75 c	5.13 a	6.38 b
+ Chlorothalonil	6.25 b	6.13 b	7 a	7 a	6.5 b	5.5 b	5.75 a	6.88 a
+ Propiconazole	6 b	5.5 c	6.38 b	6.5 b	6 c	5.75 b	5.5 a	6.38 b

Color of 'Palmetto' St. Augustinegrass†								
Treatment	DAIT‡§							
	7	14	21	28	35	42	49	56
LSD	0.47	0.53	0.34	0.36	0.54	0.40	0.57	0.69
Nontreated	6.88 a	6.88 a	6.88 a	6.88 a	6.88 a	7 a	6.88 a	7 a
Pinoxaden alone	6.25 b	5.38 c	5 c	5.38 c	5 c	4.75 c	4.13 d	4.75 c
+ Chlorothalonil	6.13 b	6.38 ab	6.63 a	6.75 a	6 b	6 b	5.88 b	6.88 a
+ Propiconazole	6.5 ab	6 b	5.5 b	6 b	5.63 b	6 b	5 c	6.13 b

Color of 'Raleigh' St. Augustinegrass†								
Treatment	DAIT‡§							
	7	14	21	28	35	42	49	56
LSD	0.33	0.35	0.33	0.01	0.53	0.40	0.51	0.54
Nontreated	6.88 a	6.88 a	7 a	7 a	6.88 a	7 a	6.88 a	7 a
Pinoxaden alone	6 b	5.25 c	5.63 c	6.5 b	4.75 c	4 c	5 c	6.1 b
+ Chlorothalonil	6.13 b	6.13 b	6.63 b	7 a	5.88 b	5 b	6.75 a	7 a
+ Propiconazole	6.13 b	5.5 c	5.88 c	6.5 b	5.5 b	5.25 b	5.63 b	6.4 b

† A color rating of 6 was considered minimally acceptable and 9 was considered optimal turfgrass

‡ Days after initial treatment (DAIT)

§ Column means not sharing any letter are considered significantly different according to Fisher's protected LSD (α=0.05).

Table 7. Turfgrass NDVI readings separated by cultivar, treatment and rating date.

NDVI of 'Classic' St. Augustinegrass								
Treatment	DAIT‡§							
	7	14	21	28	35	42	49	56
LSD	0.014	0.014	0.019	0.009	0.026	0.030	0.038	0.034
Nontreated	0.8787 a	0.8860 a	0.8623 a	0.8395 c	0.8198 a	0.8067 a	0.7930 a	0.7919 a
Pinoxaden alone	0.8285 c	0.8102 c	0.7907 b	0.8234 d	0.7641 b	0.6956 c	0.7215 b	0.7845 a
+ Chlorothalonil	0.8287 c	0.8407 b	0.8540 a	0.8696 a	0.7990 a	0.7703 b	0.7821 a	0.8055 a
+ Propiconazole	0.8473 b	0.8433 b	0.8442 a	0.8540 b	0.8105 a	0.7737 b	0.7962 a	0.8003 a

NDVI of 'Floratam' St. Augustinegrass								
Treatment	DAIT‡§							
	7	14	21	28	35	42	49	56
LSD	0.015	0.015	0.017	0.015	0.031	0.037	0.041	0.026
Nontreated	0.8144 a	0.8153 a	0.7980 a	0.8032 a	0.7882 a	0.7889 a	0.7634 a	0.7708 a
Pinoxaden alone	0.7954 b	0.7795 b	0.7698 b	0.7883 a	0.7351 b	0.6923 c	0.6943 b	0.7383 b
+ Chlorothalonil	0.7909 b	0.7822 b	0.7849 ab	0.7990 a	0.7489 b	0.7392 b	0.7434 a	0.7731 a
+ Propiconazole	0.7861 b	0.7803 b	0.7803 b	0.7968 a	0.7590 ab	0.7347 b	0.7441 a	0.7720 a

NDVI of 'Palmetto' St. Augustinegrass								
Treatment	DAIT‡§							
	7	14	21	28	35	42	49	56
LSD	0.040	0.045	0.043	0.038	0.050	0.052	0.040	0.035
Nontreated	0.8308 a	0.8390 a	0.8354 a	0.8348 a	0.8317 a	0.8263 a	0.8112 a	0.8018 a
Pinoxaden alone	0.7854 b	0.7631 b	0.7711 b	0.7856 b	0.7496 b	0.7111 c	0.7171 b	0.7527 b
+ Chlorothalonil	0.7974 ab	0.8047 ab	0.8209 a	0.8343 a	0.7786 b	0.7624 bc	0.7780 a	0.7995 a
+ Propiconazole	0.8136 ab	0.8032 ab	0.8100 ab	0.8186 ab	0.7871 ab	0.7702 b	0.7711 a	0.7949 a

NDVI of 'Raleigh' St. Augustinegrass								
Treatment	DAIT‡§							
	7	14	21	28	35	42	49	56
LSD	0.014	0.024	0.033	0.025	0.057	0.072	0.040	0.027
Nontreated	0.8089 a	0.8107 a	0.7779 a	0.7888 a	0.7702 a	0.7723 a	0.7411 a	0.7557 ab
Pinoxaden alone	0.7786 b	0.7261 c	0.7366 b	0.7711 a	0.7196 b	0.6760 b	0.6888 b	0.7328 b
+ Chlorothalonil	0.7850 b	0.7633 b	0.7572 ab	0.7804 a	0.7208 b	0.7123 ab	0.7356 a	0.7618 a
+ Propiconazole	0.7872 b	0.7645 b	0.7508 ab	0.7773 a	0.7278 a	0.7080 ab	0.7266 ab	0.7547 a

‡ Days after initial treatment (DAIT)
§ Column means not sharing any letter are considered significantly different according to Fisher's protected LSD (α=0.05).

Figure 3. Percent visual injury of four St. Augustinegrass cultivars across all rating dates. Data were pooled between 2018 and 2019. Means are expressed using differing symbols and are connected by different line styles. Vertical bars represent standard error (P=0.05).

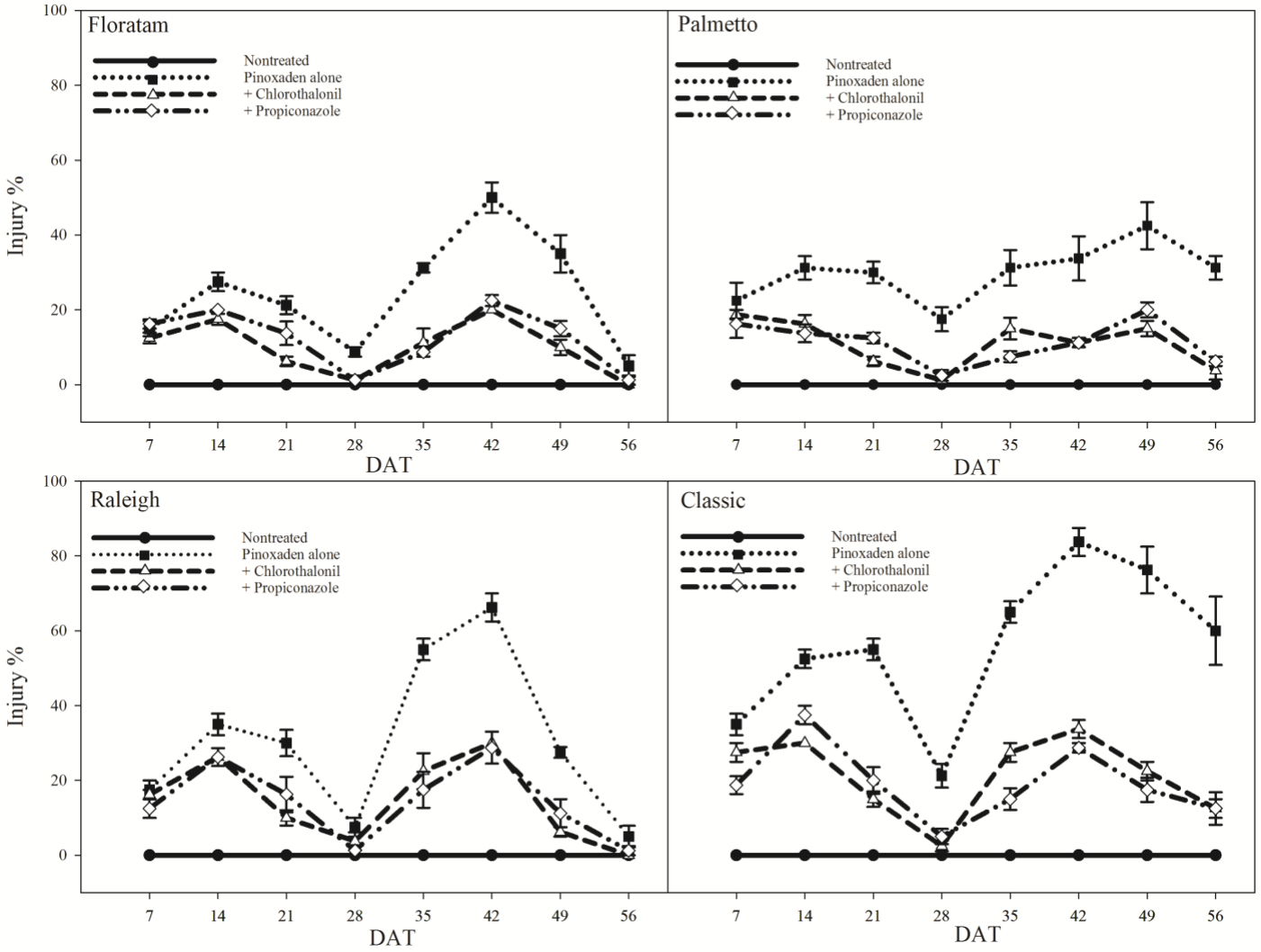


Figure 4. NDVI readings of four different St. Augustinegrass cultivars across all ratings dates in 2019. Means are expressed using differing symbols and are connected by different line styles. Vertical bars represent standard error (P=0.05).

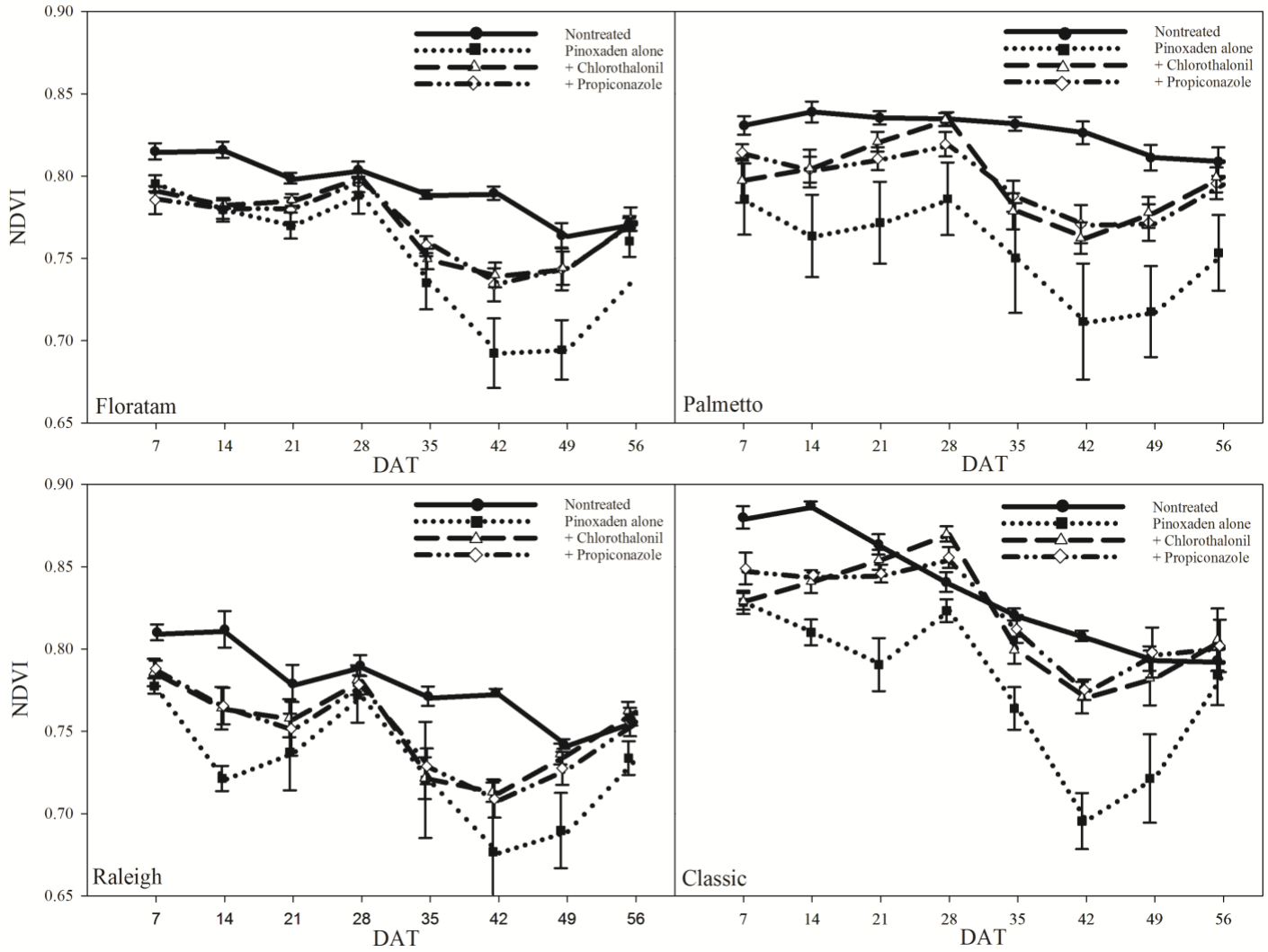


Table 8. St. Augustinegrass percent visual injury rating separated by treatment and rating date.

Percent Injury of St. Augustinegrass†					
Treatment	DAIT‡§				
	14	21	28	35	42
LSD	6.83	12.29	16.49	21.99	18.76
Nontreated	0 b	0 c	0 c	0 c	0 d
Pinoxaden alone	37.5 a	41.3 a	48.8 a	67.5 a	77.5 a
+ Halosulfuron	33.8 a	36.3 a	42.5 a	52.5 a	52.5 b
+ Metsulfuron	30 a	20 b	21.3 b	28.8 b	25 c

† A visual injury rating of 20% was considered minimally acceptable
‡ Days after initial treatment (DAIT)
§ Column means not sharing any letter are considered significantly different according to Fisher's protected LSD (α=0.05).

Table 9. St. Augustinegrass color ratings separated by treatment and rating date.

Treatment	St. Augustinegrass Color†				
	DAIT‡§				
	14	21	28	35	42
LSD	0.01	0.77	0.96	0.85	0.85
Nontreated	7 a	7 a	7 a	7 a	7 a
Pinoxaden alone	5 b	4.3 c	3.8 c	3.3 c	3 c
+ Halosulfuron	5 b	4.8 c	4.5 bc	4 c	3.8 c
+ Metsulfuron	5 b	5.8 b	5.3 b	5 b	5 b

† A color rating of 6 was considered minimally acceptable and 9 was considered optimal turfgrass
‡ Days after initial treatment (DAIT)
§ Column means not sharing any letter are considered significantly different according to Fisher's protected LSD (α=0.05).

Table 10. St. Augustinegrass NDVI readings separated by treatment and date.

NDVI of St. Augustinegrass					
Treatment	DAIT‡§				
	14	21	28	35	42
LSD	0.024	0.038	0.054	0.063	0.061
Nontreated	0.7401 a	0.7531 a	0.7508 a	0.7464 a	.7512 a
Pinoxaden alone	0.6656 c	0.6590 b	0.5925 c	0.5631 c	.5361 d
+ Halosulfuron	0.6821 bc	0.6825 b	0.6482 b	0.6059 c	0.6103 c
+ Metsulfuron	0.7002 b	0.7221 a	0.6985 b	0.6717 b	0.6752 b

‡ Days after initial treatment (DAIT)
§ Column means not sharing any letter are considered significantly different according to Fisher's protected LSD (α=0.05).

Figure 5. Percent visual injury of St. Augustinegrass across all rating dates. Means are expressed using differing shapes and are connected by differing line styles. Vertical bars represent standard error (P=0.05).

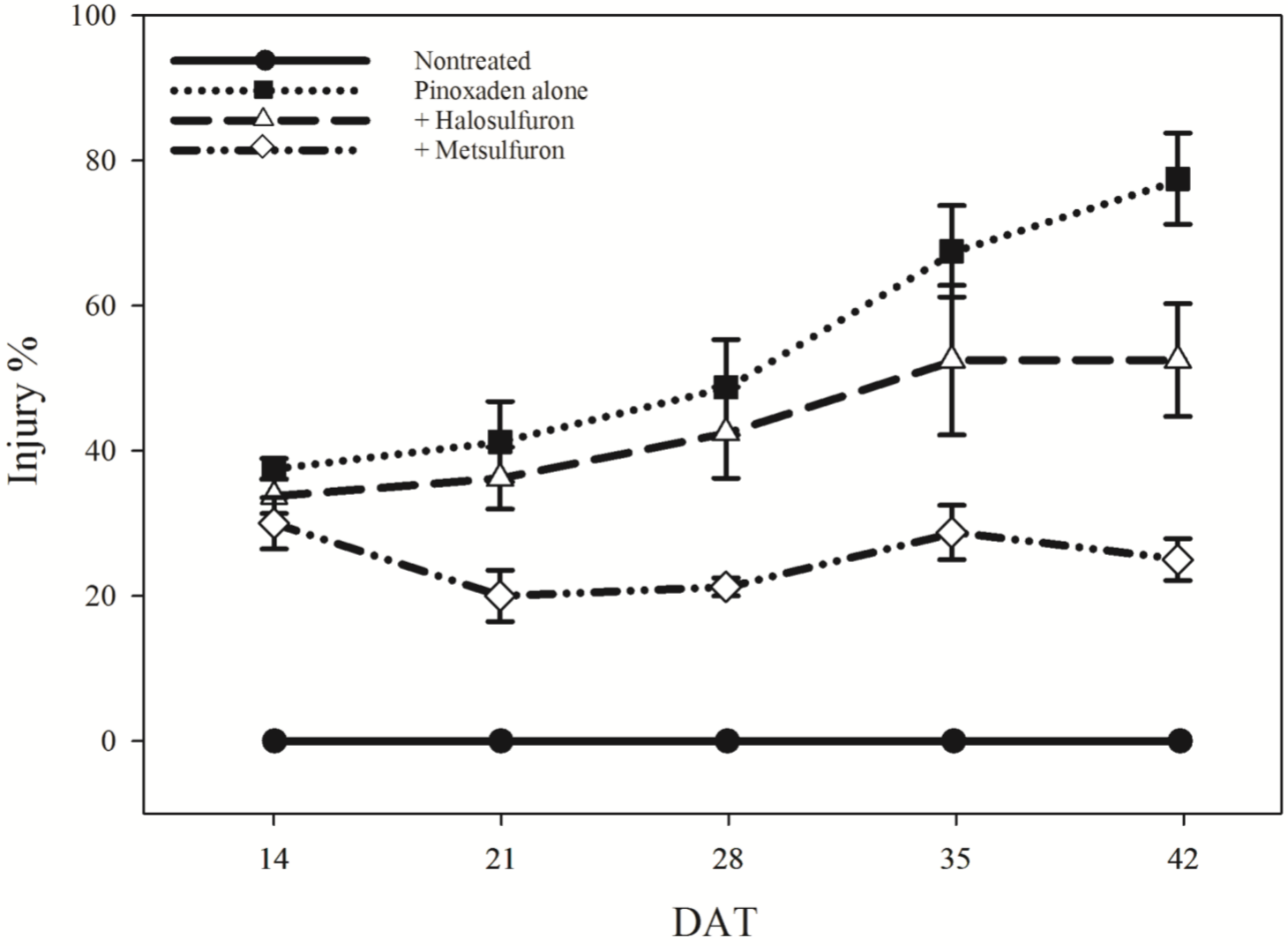


Figure 6. Color ratings of St. Augustinegrass across all rating dates. Means are expressed using differing shapes and are connected by differing line styles. Vertical bars represent standard error (P=0.05).

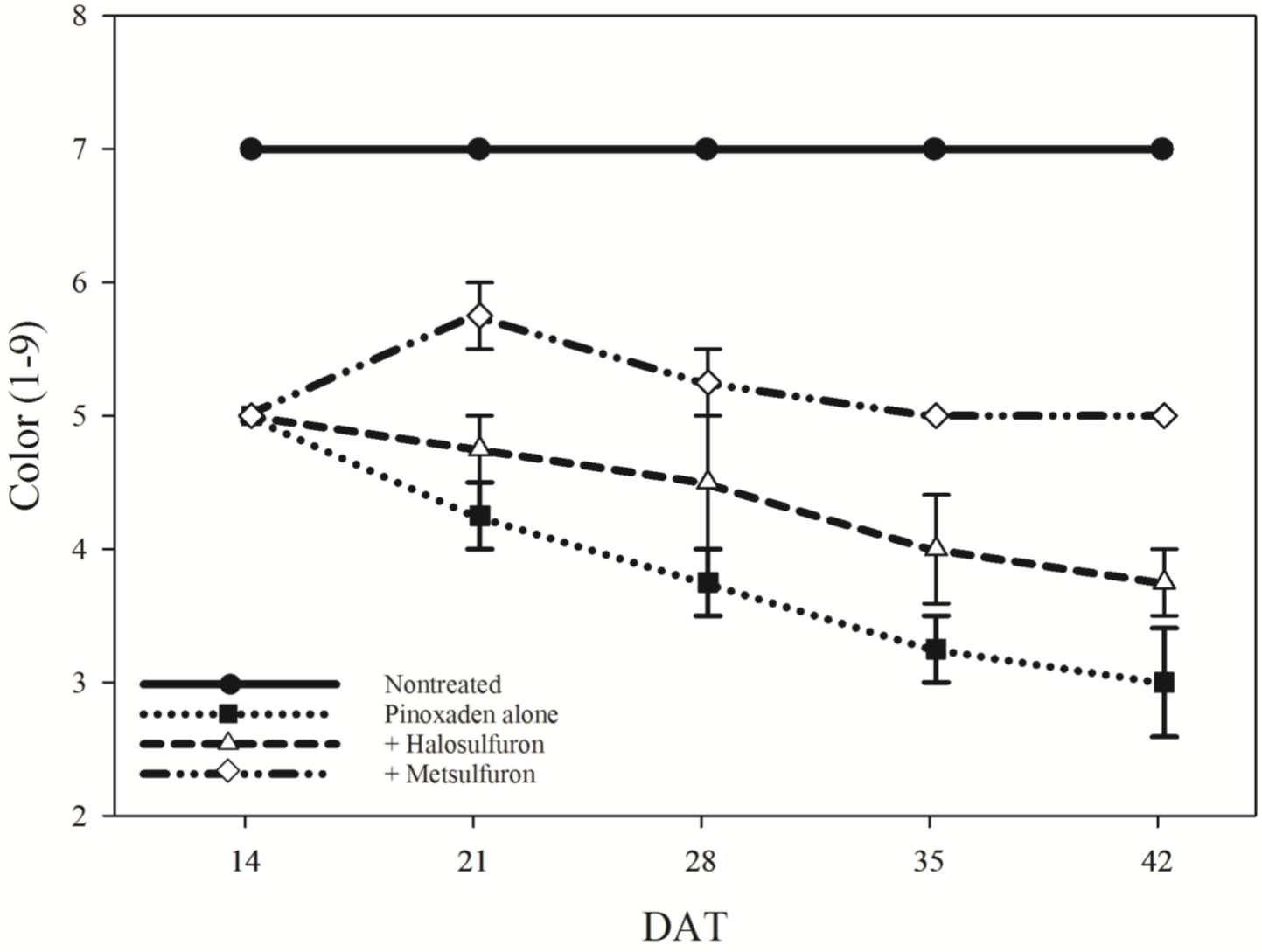


Figure 7. NDVI readings of St. Augustinegrass across all rating dates. Means are expressed using differing shapes and are connected by differing line styles. Vertical bars represent standard error (P=0.05).

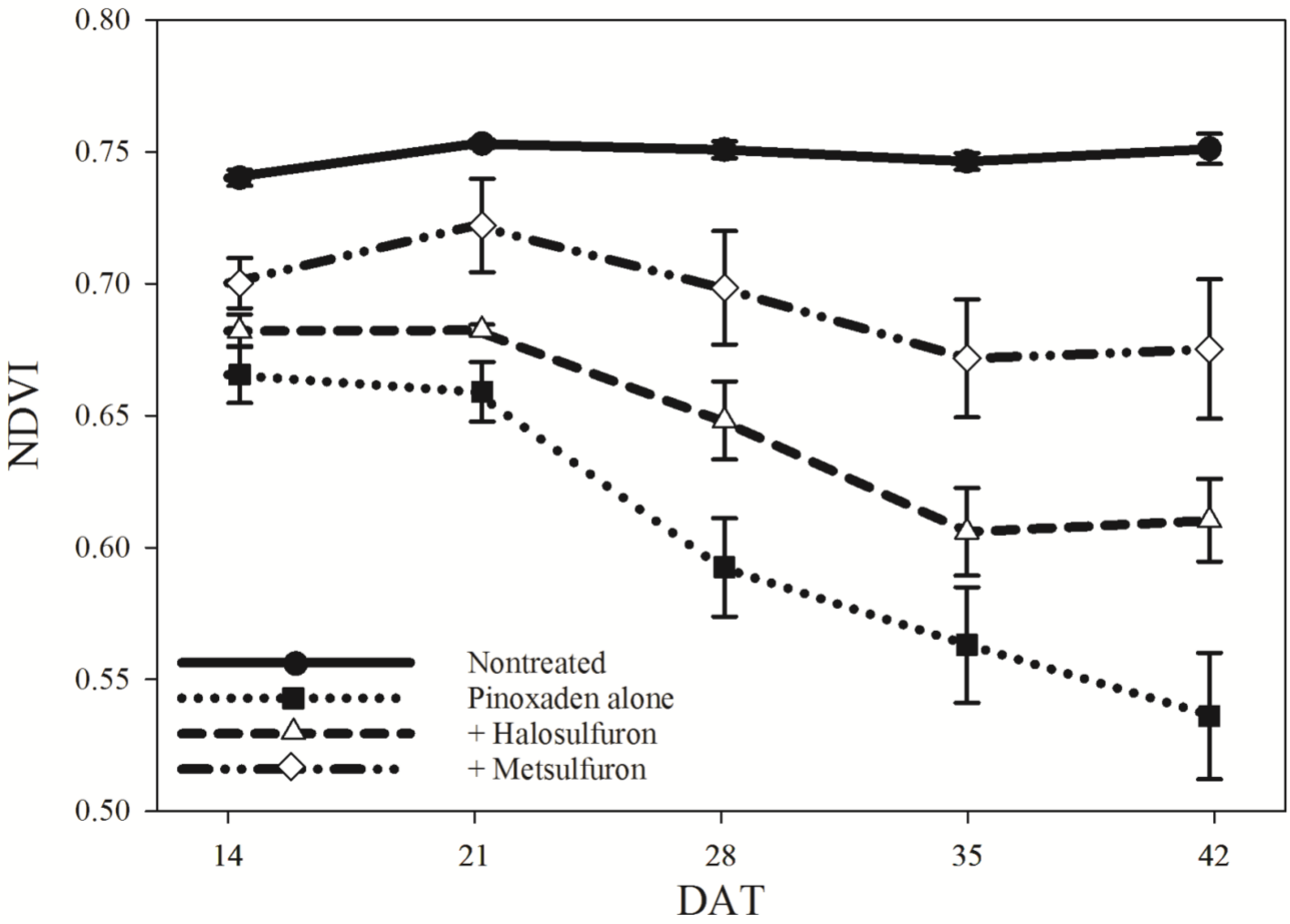


Table 11. Treatments applied to smooth crabgrass (*Digitaria sanguinalis*) to test for antagonism. All treatments included surfactant at 0.5% v/v. All treatments were reapplied 14 days after the initial treatment.

Treatment	Rate
	g ai ha^{-1}
Nontreated	-
Pinoxaden	71.35
Pinoxaden + halosulfuron	71.35 + 3.36
Halosulfuron	3.36
Pinoxaden + metsulfuron	71.35 + 31.52
Metsulfuron	31.52
Pinoxaden + chlorothalonil	71.35 + 7355

Table 12. Smooth crabgrass (*Digitaria sanguinalis*) control separated by treatment and rating date.

Percent Smooth Crabgrass Control†				
Treatment	DAIT‡§			
	14	28	42	56
LSD	30.28	22.32	20.81	24.62
Nontreated	0 c	0 b	0 c	0 c
Pinoxaden alone	65 a	82.75 a	87.5 a	72.5 a
+ Halosulfuron	60 a	63.75 a	62.5 b	45 b
Halosulfuron alone	0 c	0 b	1.25 c	0 c
+ Metsulfuron	20 bc	6.25 b	8.75 c	0 c
Metsulfuron alone	5 c	0 b	7.5 c	0 c
+ Chlorothalonil	36.25 ba	75 a	65 b	25 b

† Percent control above 70 percent was considered acceptable

‡ Days after initial treatment (DAIT)

§ Column means not sharing any letter are considered significantly different according to Fisher's protected LSD (α=0.05).

Figure 8. Percent control of smooth crabgrass (*Digitaria sanguinalis*) across all rating dates. Means are expressed using differing shapes and are connected by differing line styles. Vertical bars represent standard error (P=0.05).

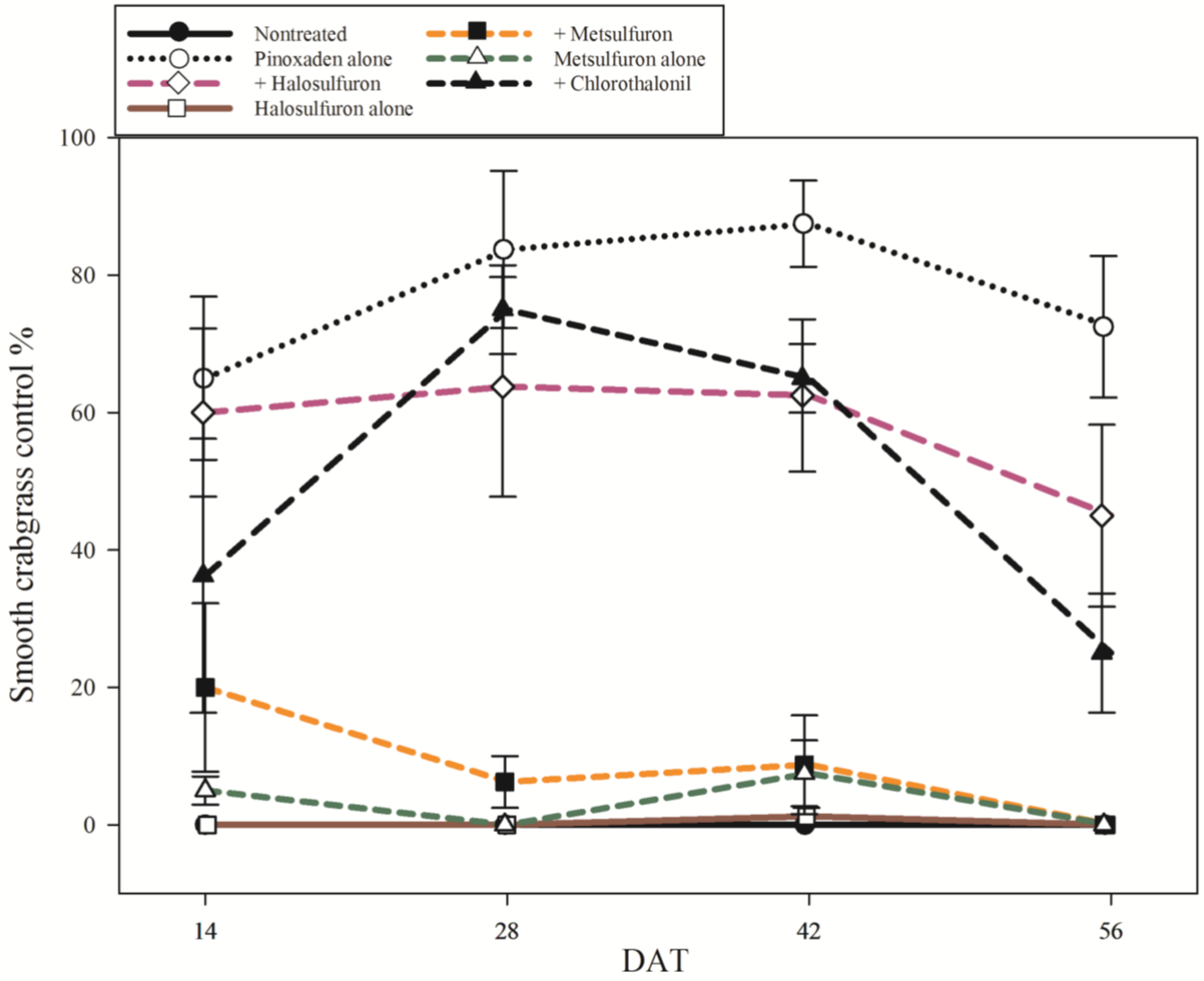


Table 13. Percent coverage of smooth crabgrass (*Digitaria sanguinalis*) per plot separated by treatment and rating date.

Percent Smooth Crabgrass Coverage†				
Treatment	DAIT‡§			
	14	28	42	56
LSD	26.51	24.34	27.59	26.17
Nontreated	65 ab	65 b	68.75 b	90 a
Pinoxaden alone	25 d	16.25 c	12.5 c	27.5 c
+ Halosulfuron	32.5 cd	23.75 c	33.75 c	55 b
Halosulfuron alone	80 a	90 a	98.75 a	100 a
+ Metsulfuron	57.5 abc	70 ab	90 ab	100 a
Metsulfuron alone	62.5 ab	77.5 ab	92.5 ab	100 a
+ Chlorothalonil	41.25 bcd	17.5 c	35 c	75 ab

† Percent coverage above 30 percent was considered unacceptable
‡ Days after initial treatment (DAIT)
§ Column means not sharing any letter are considered significantly different according to Fisher's protected LSD (α=0.05).

Figure 9. Injury observed on smooth crabgrass when pinoxaden is applied in a tank mixture with chlorothalonil.

Table 14. Treatments applied to St. Augustinegrass (*Stenotaphrum secundatum*) to test for antagonism. All treatments included surfactant at 0.5% v/v.

Treatment	Rate
	g ai ha^{-1}
Nontreated	-
Pinoxaden	77
Pinoxaden	156
Pinoxaden	310
Pinoxaden + halosulfuron	77 + 3.36
Pinoxaden + halosulfuron	156 + 3.36
Pinoxaden + halosulfuron	310 + 3.36
Pinoxaden + metsulfuron	77 + 31.52
Pinoxaden + metsulfuron	156 + 31.52
Pinoxaden + metsulfuron	310 + 31.52
Pinoxaden + chlorothalonil	77 + 7355
Pinoxaden + chlorothalonil	156 + 7355
Pinoxaden + chlorothalonil	310 + 7355

Table 15. St. Augustinegrass percent injury and weight, at the end of the trial, separated by treatment. Means were pooled between both runs of this experiment. Low, medium and high rates of pinoxaden were 77, 156, and 310 g ai ha^{-1}.

Treatment	injury§	weight§
	%	g
LSD	15.10	3.95
Nontreated	0 f	20.55 a
Pinoxaden low	30.83 cd	6.79 def
Pinoxaden mid	33.33 cd	7.60 def
Pinoxaden high	58.33 b	4.36 f
low + Halosulfuron	8.33 ef	11.81 bc
mid + Halosulfuron	25 d	10.17 cd
high + Halosulfuron	45 bc	7.30 def
low + Metsulfuron	7.5 ef	14.80 b
mid + Metsulfuron	19.17 de	10.37 cd
high + Metsulfuron	20.83 de	8.36 cde
low + Chlorothalonil	19.17 de	11.70 bc
mid + Chlorothalonil	33.33 cd	8.98 cde
high + Chlorothalonil	75 a	5.52 ef

§ Column means not sharing any letter are considered significantly different according to Fisher's protected LSD (α=0.05).

Figure 10. Injury to St. Augustinegrass when pinoxaden is applied in a tank mixture with chlorothalonil.

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