Characterizing Oxadiazon Resistance and Improving Postemergence Control Programs for Goosegrass (*Eleusine indica*) in Bermudagrass (*Cynodon* spp.)

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Dissertation submitted to the faculty of
the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Plant Pathology, Physiology and Weed Science

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March 21, 2014
Blacksburg, Virginia

Keywords: goosegrass, herbicide, oxadiazon, postemergence, preemergence, topramezone

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ABSTRACT

Goosegrass is a problematic weed of golf courses, sports fields, and residential lawns that decreases playability and aesthetic quality of turf. With the recent banning of MSMA in sports fields and intensive restrictions in golf and sod production, turfgrass managers are seeking alternatives for postemergence goosegrass control and how to utilize currently labeled goosegrass control products more effectively. Studies were conducted to investigate a suspected-resistant (SR) goosegrass accession in Richmond, VA and characterize the resistance mechanism if present. The SR accession showed a hypersensitive response to oxadiazon treatment and reached maximum electrolyte leakage quicker than the susceptible (S) accession, but had significantly lower electrolyte leakage indicating less tissue damage and suggesting there is a physiological resistance mechanism within the SR accession. In absorption and translocation studies, percent oxadiazon absorption and translocation was not significantly affected by goosegrass biotype. Roots of both the S and resistant (R) biotypes contained over 95% of total detected oxadiazon, while the plant tissue above the treated foliage only contained small quantities. These results suggest that absorption or translocation is not the mechanism conferring oxadiazon resistance in the goosegrass biotype from Richmond, VA. Greenhouse and field trials were conducted to determine the lowest rate at which topramezone, with or without the addition of triclopyr, controls goosegrass while maintaining commercially-acceptable bermudagrass quality. In field trials, topramezone rate did not significantly affect goosegrass cover at 56 and

70 days after initial treatment (DAIT). All treatments reduced goosegrass cover below 3 and 7% with and without the addition of triclopyr, respectively at 70 DAIT. A significant herbicide effect on bermudagrass cultivar showed higher injury from topramezone within three weeks of application, but injury persisted longer from treatments containing triclopyr. Bermudagrass cultivars completely recovered by 4 weeks after treatment (WAT) from all treatments. Greenhouse trials were conducted to determine if goosegrass growth stage affects efficacy of nine postemergent herbicides or programs documented to have goosegrass activity. As goosegrass growth stage increased from four- to five-leaf to greater than eight-tiller stage, goosegrass control and biomass reduction decreased among all of the herbicides except topramezone and MSMA plus metribuzin at 4 and 8 WAT. These data suggest that one application of sulfentrazone is only effective for seedling stage (pre-tiller) goosegrass control; foramsulfuron, topramezone, and metribuzin suppress all growth stages of goosegrass; and diclofop, sulfentrazone plus metribuzin, fenoxaprop, and metamifop control up to three-tiller stage goosegrass.

I dedicate this work to my family, for never-ending love, support, and sacrifice.

And Belle, for being the most patient of all.

ACKNOWLEDGEMENTS

I could not have completed this degree without the encouragement and advice of so many people from home in Mississippi to here at Virginia Tech. To my major professor, Dr. Shawn Askew, thank you for the many hours of instruction, accessibility, and willingness to argue over any topic with me on a regular basis. Thank you to each and every one of my committee members: Drs. Scott Hagood, Mike Goatley, and Jacob Barney. I could not have asked for a more suitable committee to provide me with such a broad spectrum of knowledge and direction.

I owe a huge thanks to everyone at the Glade Road Research Facility for providing assistance in many ways during my time there. To Ms. Julie Keating and Mrs. Judy Fielder, thanks for your many acts of service to me and our lab at Glade Road. Thank you to each and every one of my fellow graduate students: Dr. Angela Post, Dr. Adam Smith, Kate Venner, Sandeep Rana, Dr. Larissa Smith, Dan Tekeila, and Matt Ho. To Mr. Phil and Mrs. Judy Keating and Mr. Lloyd and Mrs. Pat Hipkins, thank you very much for allowing me to hunt on your properties during my years in Blacksburg. Those were some of the few hours I was able to find stress relief.

I would especially like to thank my parents, Wayne and Diane Cox, for their continued love, support, and sacrifices that have allowed me to succeed throughout my many years of school. Through words of encouragement and countless acts of generousity, they gave me the determination, stamina, and resources to move away from my comfort zone and complete this degree. To all of my family and friends in Mississippi who have always encouraged and supported me throughout my college years, thank you.

ATTRIBUTIONS

Chapter 2 – Investigation of Oxadiazon-Resistant Goosegrass (*Eleusine indica*) in Virginia. Shawn D. Askew, Ph.D., is an associate professor of Turfgrass Weed Science in the Department of Plant Pathology, Physiology, and Weed Science at Virginia Tech. He assisted with formulating the experimental and treatment design of the greenhouse and laboratory studies, as well as statistical data analyses. David R. Spak, Ph.D., is the former northeastern U.S. technical development representative and part of the Development Administration at Bayer CropScience. He conducted this study in North Carolina as half of the greenhouse bioassay data included in this chapter.

Chapter 3. Characterizing an Oxadiazon-Resistant Goosegrass Biotype.

Eva Collakova, Ph.D., is an assistant professor and instructor in the transcriptomics, metabolomics, and fluxomics laboratory in the Department of Plant Pathology, Physiology, and Weed Science at Virginia Tech. She formulated the protocol and carried out procedure for analyzation of the goosegrass samples on the GC-MS and helped with data interpretation. Shawn D. Askew, Ph.D., is an associate professor of Turfgrass Weed Science in the Department of Plant Pathology, Physiology, and Weed Science at Virginia Tech. He assisted with formulating the experimental and treatment design of this laboratory study, as well as statistical data analyses.

Chapter 4. Goosegrass and Bermudagrass Response to Topramezone Rates and Tank Mixtures.

Shawn D. Askew, Ph.D., is an associate professor of Turfgrass Weed Science in the Department of Plant Pathology, Physiology, and Weed Science at Virginia Tech. He assisted with formulating the experimental and treatment design of the greenhouse and field studies, as well as statistical data analyses.

Chapter 5. Herbicide Efficacy as Influenced by Goosegrass Growth Stage.

Shawn D. Askew, Ph.D., is an associate professor of Turfgrass Weed Science in the Department of Plant Pathology, Physiology, and Weed Science at Virginia Tech. He assisted with formulating the experimental and treatment design of this greenhouse experiment, as well as statistical data analyses.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter I. Literature Review

Golf courses and sports fields are intensively managed turf systems utilized for a broad array of recreational sporting events within different seasons. This degree of use can allow the development of an assortment of weed problems. Goosegrass is classified as one of the five most troublesome weeds in the world (Holm et al. 1991), and currently one of the most difficult weeds to control in warm-season turfgrasses, in particular, bermudagrass sports fields and golf courses (Arrieta et al. 2009; Busey 2001; Johnson 1980b; McCarty 1991). Goosegrass is commonly found thriving in areas of high traffic with compacted soils, or where turfgrass is thin and less competitive due to unfavorable growing conditions (Carrow and Petrovic 1992; Goatley et al. 2008; Turgeon 1980; Waddington 1992; Waddington and Baker 1965). Frequent vehicular and foot traffic expose the turf and soil of golf courses and sports fields to wear and compaction. In sports fields, areas in front of players' benches, between the hash marks, along sidelines, and in front of goals are prone to more compaction and wear due to more traffic exposure (McCarty et al. 2005). On and around tee boxes and putting greens and along cart path edges are areas where most traffic occurs on golf courses (Beard 2002). Recent restrictions placed on monosodium methanearsonate (MSMA) in turfgrass, along with herbicide resistance and uniquely adaptive physiological characteristics of goosegrass, have promoted control problems for this weed in warm-season turfgrasses.

Biology. Goosegrass is a summer annual, C4 grass with a whitish to silvery appearance at the center of each plant, which is often why it is referred to as "silver crabgrass" (Bryson and DeFelice 2009; McCarty et al. 2001; Murphy 2004; Uva et al. 1997). A member of the Poaceae (grass) family and native to the Old World (Asia, Africa, and Europe), goosegrass is commonly

found thriving in temperate to tropical climates of the world (Bryson and DeFelice 2009; Holm et al. 1977; McCarty et al. 2001; Murphy 2004). Goosegrass has been classified as a significant weed in over 60 countries and 46 crops worldwide but has also been grown for hay and silage (Holm et al. 1977). In the United States, goosegrass is distributed over almost the entire country, extending from the southernmost states north to Massachusetts, along the West Coast, and including Hawaii (McCarty et al. 2001; Murphy 2004; Uva et al. 1997). Goosegrass has a clumpy growth habit resembling a rosette with shoots extending out from a central location (Uva et al. 1997). Goosegrass can also be commonly characterized by folded leaf vernation along the midvein, no auricles, flattened leaf sheaths, and a short, membranous ligule often unevenly toothed. Sparse hairs may be present around the leaf collar and on the basal portion of the leaves, especially when mature (Bryson and DeFelice 2009; McCarty et al. 2001; Uva et al. 1997). Whitish to almost translucent leaf sheaths give goosegrass its distinctive silvery appearance (Bryson and DeFelice 2009; Uva et al. 1997). Goosegrass reproduces by seed formed in two flattened rows on spikelets on two to thirteen spikes and an occasional single spike below the terminal cluster (Bryson and DeFelice 2009; McCarty et al. 2001; Uva et al. 1997). Producing three to six brownish-black seeds per spikelet (Uva et al. 1997), goosegrass can produce between 40,000 and 50,000 seeds per plant (Breeden and Brosnan 2009; Holm et al. 1977), which are often dispersed via wind, foot traffic, digestive system of animals, and commerce transportation (Holm et al. 1977). Goosegrass seeds are thought to be viable up to five years in the soil (Kranz et al. 1977). Although believed by some to be a palatable food source for grazing animals, goosegrass is not preferable as stand-alone forage and has been documented in Australia to contain enough hydrogen cyanide in immature growth stages to kill calves and sheep (Holm et al. 1977).

Goosegrass is commonly found growing in compacted and/or droughty soil conditions where moisture and oxygen levels are low (Waddington 1992; Waddington and Baker 1965). Cultivated and disturbed sites and areas where turf is thin from poor growing conditions or disease also promote goosegrass establishment (Bryson and DeFelice 2009; Carrow and Petrovic 1992; Goatley et al. 2008; McCarty et al. 2001; Turgeon 1980; Uva et al. 1997). Fluctuating temperature and light cycles favor goosegrass germination, and more fluctuating cycles in a given period of time further increase germination (Nishimoto and McCarty 1997). Goosegrass germination was less than 10% when temperatures remained constant at 20, 25, and 35 C, but increased to 99% with daily fluctuating regimes of 20 C for 16 h and 35 C for 8 h with the addition of light (Nishimoto and McCarty 1997). Chauhan and Johnson (2008) also reported greater goosegrass germination percentages with higher, alternating temperature regimes of 30/20 and 35/25 C than with 25/15 C. These fluctuating temperature and light cycles are consistent with scalped, thin, and trafficked areas common to sports turf and bare ground (Arrieta et al. 2009; Nishimoto and McCarty 1997). Goosegrass germination was not sensitive to normal levels of salt stress (less than 77.5 mM NaCl) but high water stress (osmotic potential less than -0.5 MPa) and increasing seed burial depth (greater than 1 cm) exponentially reduced germination and seedling emergence below 50% (Chauhan and Johnson 2008). Still, goosegrass germination rapidly decreased as osmotic potential decreased below -0.2 MPa, suggesting that goosegrass prefers a moist, but not wet, growing environment (Chauhan and Johnson 2008). The lack of goosegrass germination with increasing seed depth, also a common occurrence among other small-seeded weed species (Boyd and Van Acker 2004), is likely the result of hypoxic conditions and decreased gas exchange (Benvenuti 2003). Other research has shown that larger seeds often

contain higher amounts of stored carbohydrates and have greater success at germinating from deeper in the soil profile (Baskin and Baskin 1998).

History of Goosegrass Control. Monosodium methanearsonate (MSMA) plus metribuzin as a two to three application treatment 7-10 days apart has been effective for postemergence goosegrass control in bermudagrass turf for decades (Brennan et al. 1992; Busey 1999; Johnson 1980b; McCarty 1991; McElroy et al. 2007; Murdoch and Ikeda 1974; Nishimoto and Murdoch 1999; Wiecko 2000). Phytotoxic injury to bermudagrass from MSMA plus metribuzin can be intense immediately after application and can last as long as 5-8 weeks with two applications (McCarty et al. 1991; Wiecko 2000) or only 2-3 weeks when applied to immature goosegrass on bermudagrass putting greens (Johnson 1980b).

MSMA is an organic arsenical herbicide that binds tightly to soil particles (Ross and Lembi 2009; Senseman 2007) in the organic form only, which is significantly less toxic than the inorganic form (EPA 2006; Ross and Lembi 2009; MAATF 2006). Organic arsenicals were first registered in the United States for herbicidal uses in the 1950s and 1960s, and currently there are 90 end-use products containing the active ingredient MSMA (EPA 2006). MSMA is registered for weed control in cotton, turfgrass, and forestry (EPA 2013; Ross and Lembi 2009; Senseman 2007); however, restrictions are currently in place for applications of MSMA in turfgrass (EPA 2013). Approximately 3,000,000 pounds of MSMA or disodium methanearsonate (DSMA) are applied in the U.S. each year, mostly to cotton and turf, based on EPA's Screening Level Use Analysis data (EPA 2006). Although MSMA binds tightly to upper soil layers and is not readily leached by natural water flow from irrigation or rainfall (MAATF 2006; Ross and Lembi 2009; Senseman 2007), concentrations of total arsenic (non-differentiated measure of arsenic; i.e. not measured as inorganic or organic) exceeding acceptable thresholds in groundwater in Florida led

to the phasing out of MSMA as an herbicidal option in most turfgrass sites (EPA 2006). The source(s) leading to the high concentrations of arsenic in the groundwater was not determined, as quantifying how much arsenic is naturally-occurring in the environment or introduced by anthropogenic practices (MSMA, DSMA herbicide applications) is extremely difficult (EPA 2006). Still, sandy soils and high water tables near areas of high organic arsenical use likely contributed to the higher concentrations (EPA 2006). A recent lack in postemergence goosegrass control has been attributed mostly to the loss or restrictive uses of MSMA in turf.

Other factors contributing to the increase in goosegrass problems on golf courses and sports fields are the lack of turf competition due to thinning of turfgrass stands or decreases in recuperative potential resulting from lack of essential nutrients. The economic downturn and an increase in fertilizer costs over the last decade (USDA Economic Research Service 2012; USDA National Agricultural Statistics Service 2012) have hindered turf managers from supplying appropriate amounts of nutrients to the turf. From 2000 to 2012, the cost of urea and ammonium nitrate, popular sources of nitrogen used by turf managers, more than doubled (USDA Economic Research Service 2012; USDA National Agricultural Statistics Service 2012). U.S. nitrogen prices alone increased by a third in 2008 (Huang 2009). The housing market is also tied to golf course development, and the collapse in new housing developments in previous years drastically affected new golf course establishment and current course maintenance (Sweet 2008; USDA Economic Research Service 2012). A typical bermudagrass fairway or athletic field should receive 1-1.5 kg N m^{-2} yr^{-1} (Goatley et al. 2008). The revenue needed to purchase this amount of nitrogen in 2000 would buy only 66% as much in 2012.

Preemergent herbicides are key components in preventing extensive goosegrass infestations. Several families of herbicides and modes-of-action have been used for

preemergence goosegrass control. Some of the earliest herbicides used for preemergence goosegrass control were dinitroanilines such as pendimethalin, oryzalin, prodiamine, and benefin (Busey 1999). These mitosis-inhibiting herbicides (Vaughn and Lehnen, Jr. 1991) are inexpensive and have proven to be somewhat effective against germinating goosegrass seedlings (Johnson 1996a; Johnson 1997; Johnson and Murphy 1993), but they also may inhibit turfgrass rooting (Bingham 1967; Bingham and Hall 1985; Bhowmik and Bingham 1990; Busey 1999; Dickens et al. 1989; Fagerness et al. 2002; Fishel and Coats 1994; Reicher and Christians 1989; Sharpe et al. 1989). Other preemergent herbicides shown to suppress or control goosegrass include dithiopyr, indaziflam, and oxadiazon (Johnson 1980a; Ferrell et al. 2003). Oxadiazon, a shoot inhibitor (Ross and Lembi 2009; Senseman 2007), has become one of the most widely used preemergent herbicides for goosegrass control in bermudagrass due to its efficacy and safety during bermudagrass sod or sprig establishment (Bingham and Hall 1985; Bingham and Shaver 1981; Busey 1999; Dernoeden et al. 1984; Johnson 1976). Oxadiazon should only be applied to actively growing bermudagrass as a granular or to dormant bermudagrass as a granular or foliar spray to avoid turfgrass injury (Anonymous 2005; Anonymous 2007). Indaziflam is a cellulose biosynthesis inhibitor (CBI) and relatively new chemistry registered in 2010 for weed control in warm-season turf (Myers et al. 2009) under the trade name Specticle® (Anonymous 2010). Recent research documented complete, season-long goosegrass control in North Carolina with single applications of indaziflam at 48 or 64 g ai ha^{-1} and up to 84% control with 16 g ai ha^{-1} (Gannon et al. 2012). Other studies also showed complete goosegrass control for 9 weeks in the greenhouse with single applications of indaziflam at 50 g ai ha^{-1} (Askew et al. 2013) and for 27 weeks with single or split applications of 35 or 62 g ai ha^{-1} in the field (Cox et al. 2013). Although indaziflam controlled goosegrass better at 13 WAIT, goosegrass control with

indaziflam at 35 to 62 g ai ha^{-1} or split applications at a 6 wk interval of a newer formulation of dithiopyr (Dimension® 2EW) (Anonymous 2012) at 426 to 560 g ai ha^{-1} was not significantly different, 27 WAIT (Cox et al. 2013). Recent research conducted in glasshouses by Jones et al. (2013) documented significantly more phytotoxicity and stand reduction of 'Tifway' bermudagrass with applications of indaziflam at 35 to 70 g ai ha^{-1} in a sand-based soil compared to a silt-loam soil. These results suggest that golf course fairways or other bermudagrass turf stands consisting of a heavy sand base may experience an increase in injury and decrease in rooting establishment when treated with indaziflam. Dithiopyr at 0.28 kg ai ha^{-1} in combination with fenoxaprop, MSMA, or quinclorac did not control goosegrass in bermudagrass (Johnson 1996b) or Kentucky bluegrass (Johnson 1994). Wiecko (2000) reported 18 weeks of goosegrass control with two applications of dithiopyr at 0.6 kg ai ha^{-1} followed by 0.3 8 wk later; however, preemergent treatments were not as effective as multiple postemergent applications of MSMA plus metribuzin in tropical climates such as Guam and Hawaii (Wiecko 1997; Wiecko and Couillard 1999).

Several postemergence herbicides have been reported to effectively control goosegrass, but only a few of these herbicides will control mature (greater than two- to three-tiller) goosegrass. Other than MSMA plus metribuzin (Johnson 1975; Murdoch and Ikeda 1974; Nishimoto and Murdoch 1999), the following herbicides alone or in specific combinations have been used for postemergence goosegrass control in bermudagrass: foramsulfuron (Busey 2004; McCullough et al. 2012), metribuzin (Busey 2004; Johnson 1980; Murdoch and Nishimoto 1982; Nishimoto and Murdoch 1999), sulfentrazone (McCullough et al. 2012; Anonymous 2008), and diclofop (Busey 1999; McCarty 1991; Murdoch and Nishimoto 1982; Nishimoto and Murdoch 1999). Busey (2004) reported greater than 85% control of mature goosegrass with two

applications of foramsulfuron at 0.029 or 0.044 kg ai ha^{-1} plus metribuzin at 0.105 to 0.210 kg ai ha^{-1}; however, bermudagrass phytotoxicity was equivalent to that caused by MSMA plus metribuzin, which may prevent use of this program if injury persists longer than the minimum time for unacceptable turf quality set by the turf manager. Nicosulfuron, a sulfonylurea not currently labeled for turf, has also been tested for goosegrass control efficacy as a stand-alone treatment and in combination with foramsulfuron and sulfentrazone, two herbicides proven to control pre-tiller stage goosegrass. Single and combination treatments of sulfentrazone and nicosulfuron controlled mature ($\geq$5 tillers) goosegrass 84%, 9 WAIT, and significantly better than foramsulfuron at 0.04 kg ai ha^{-1} and nicosulfuron at 0.11 kg ai ha^{-1} alone and sequential applications of nicosulfuron plus foramsulfuron (McCullough et al. 2012). Two applications of nicosulfuron (0.11 kg ai ha^{-1}) plus sulfentrazone (0.42 kg ai ha^{-1}) with the addition of urea ammonium nitrate (1.5 kg ai ha^{-1}) also controlled goosegrass 80%, 12 WAIT, in a non-replicated greenhouse evaluation (author's personal observations). Sulfentrazone reacted synergistically when combined with other postemergent herbicides for goosegrass control in crops (Coles 2003), corroborating the results mentioned in the previous two studies. Single applications of diclofop controlled mature goosegrass on putting greens but not areas of higher mowing heights such as fairways (McCarty 1991; Murdoch and Nishimoto 1982). The Illoxan® 3EC (diclofop) herbicide label indicates control of goosegrass at growth stages less than 2 tillers, and diclofop should only be applied to established bermudagrass (stolons 10 cm long) (Anonymous 2004). Illoxan® is also a restricted use herbicide with a maximum use rate of 1.0 kg ai ha^{-1} and an annual limit of 1.5 kg ai ha^{-1} (Anonymous 2004), making diclofop as a stand-alone treatment mostly ineffective for controlling mature goosegrass in a fairway or athletic field (McCarty 1991). Application of diclofop at 1.7 kg ai ha^{-1} plus metribuzin at 0.56 kg ai ha^{-1} controlled mature goosegrass 90%, 7

WAT, and equivalent to control from MSMA (2.2 kg ai ha^{-1}) plus metribuzin (0.56 kg ai ha^{-1}) followed by MSMA (2.2 kg ai ha^{-1}) 1 wk later (Nishimoto and Murdoch 1999). Injury to common bermudagrass following this treatment was also equivalent to injury from MSMA plus metribuzin; however, injury was transient and not evident 3 WAT (Nishimoto and Murdoch 1999). McCarty et al. (1991) also reported unacceptable turf injury up to 2 WAT but quick recovery with an application of diclofop at 2.2 kg ai ha^{-1} plus metribuzin at 0.2 kg ai ha^{-1}.

Restrictive budgets, high fertilizer costs, and a lack of labeled products for effective postemergence goosegrass control have driven turf managers to manual removal. Hand-pulling and mechanical removal of goosegrass plants from turf, however, can be very time-consuming, labor-intensive, and often only a temporary solution since goosegrass germinates frequently throughout the summer months. This method is only cost-effective when goosegrass populations are extremely low.

Currently, postemergence goosegrass control programs for sports turf and golf courses are unclear and limited to site and/or goosegrass growth stage restrictions. Postemergent herbicides such as foramsulfuron (Revolver®) (Anonymous 2003), sulfentrazone (Dismiss® 4SC), and diclofop (Illoxan® 3EC) are popular among golf course superintendents and sports field managers, however, goosegrass growth stage, population density per surface area, budget, and environmental conditions are important factors that affect efficacy of programs utilizing these herbicides. The peer-reviewed literature does not contain specific programs for postemergence goosegrass control at different growth stages and often shows varying results across different climates. Although most of the indicated herbicide labels indicate a relative size or maturity level at which to effectively control or suppress goosegrass, more research is needed to delineate specific goosegrass growth stage(s), timings, and application frequencies that are

most efficacious for each of these herbicides. Screening of other available herbicides with activity on annual grasses should also be evaluated for possible goosegrass control.

Herbicide Resistance in Goosegrass. Goosegrass is currently reported to have resistant biotypes in eight countries and nine U. S. states, across eight herbicide families and six unique modes-of-action, and in over 11 different cropping systems (Heap 2013). Surveys conducted in 1995 and 2009 to determine the most troublesome weeds in agronomic crops within the contiguous 14 southern states of the U.S. since the introduction of genetically-modified (GM) crops revealed goosegrass ranking as the following: 15th in corn and cotton, and 34th in soybean in 2009, increasing from 25th, 24th, and 40th in 1995 within the respective crops (Webster and Nichols 2012). Goosegrass has conferred resistance to 14 herbicides including glyphosate, paraquat, metribuzin, trifluralin, pendimethalin, glufosinate, fluazifop, propaquizafop, imazapyr, sethoxydim, cyhalofop, fenoxaprop, clethodim, and haloxyfop (Heap 2013). At least two goosegrass biotypes are documented as resistant to two sites of action, acetyl CoA carboxylase (ACCase) and enolpyruvyl shikimate-3-phosphate (EPSP) synthase (Heap 2013), as well as photosystem I and glutamine synthesis inhibition (Seng et al. 2010). There have been numerous documentations, however, of cross-resistance within goosegrass biotypes (Marshall et al. 1994; Mudge et al. 1984; Vaughn et al. 1990). Goosegrass also ranks 4th out of all resistant weed species for total number of sites of action with seven, and rigid ryegrass (*Lolium rigidum* Gaudin) ranks 1st (11 total sites of action) (Heap 2013). To date, more dinitroaniline (DNA)-resistant cases of goosegrass have been found around the world than any other site of action (Heap 2013).

Dinitroaniline Resistance. The first case of herbicide-resistant goosegrass was reported in 1973 by Dr. Harold Coble in North Carolina (Heap 2013). A population was found to be unaffected

by trifluralin in cotton fields near the southeastern portion of North Carolina and northeastern South Carolina. It is also believed that goosegrass biotypes resistant to one dinitroaniline herbicide such as trifluralin are likely cross-resistant to other members of the DNA family (Heap 2013; Powles and Yu). High levels of dinitroaniline resistance have been observed in goosegrass biotypes with an α-tubulin gene mutation and Thr-239-Ile substitution (Mudge et al. 1984). This particular substitution also enables cross-resistance to phosphoroamidate and pyridine herbicides but negative cross-resistance to some carbamates (Powles and Yu 2010). An intermediate level (I) of dinitroaniline-resistant goosegrass has also been documented in South Carolina (Vaughn et al. 1990). The intermediate biotype is resistant to trifluralin by ~50 times the labeled rate; however, the resistant (R) biotype can tolerate a 1,000- to 10,000-fold labeled rate of trifluralin (Vaughn et al. 1990). A Met-268-Thr mutation was found to endow lower-level dinitroaniline resistance in goosegrass biotypes (Yamamoto et al. 1998). Regardless of resistance level, target-site dinitroaniline resistance is inherited as a recessive gene (Jasieniuk et al. 1994; Tian et al. 2006; Wang et al. 1996; Zeng and Baird 1997; 1999) and only homozygous plants survive herbicide treatment at labeled rates. When evaluated under noncompetitive conditions and compared to dinitroaniline-susceptible biotypes, a fitness cost did not seem to be associated with dinitroaniline-resistant goosegrass (Harris et al. 1995; Murphy et al. 1986).

Glyphosate Resistance. Glyphosate-resistant goosegrass has been reported in five countries and within six cropping systems (Heap 2013; Kaundun et al. 2008; Lee and Ngim 2000; Mueller et al. 2011). Glyphosate at rates of 210 g ae ha^{-1} or higher controlled a glyphosate-susceptible (GS) biotype in Tennessee greater than 90%, 21 days after treatment (DAT) (Mueller et al. 2011); however, a glyphosate-resistant (GR) biotype was controlled only 12% at the same rates (Mueller et al. 2011). I_{50} values associated with the GR biotype were three to ten times greater

than those of the GS biotype in regards to fresh weight of goosegrass harvested 21 DAT (Mueller et al. 2011). A goosegrass biotype in Malaysia was found to be 8 to 12-fold resistant to glyphosate when treated with 5.76 kg ai ha^{-1} and compared to susceptible plants in a four-year-old orchard (Lee and Ngim 2000). A $prolyl^{106}$ point mutation to serine (P106S) was found to confer glyphosate resistance in a goosegrass biotype in Davao, Mindanao Island, Philippines (Kaundun et al. 2008). Extensive testing of the two biotypes at different goosegrass growth stages and glyphosate rates produced a strong correlation ($P < 0.001$) between presence of the mutated P106S in EPSP synthase and the resistant plants (Kaundun et al. 2008). No ^{14}C-glyphosate absorption or translocation patterns were observed between all genotypes of resistant and susceptible goosegrass populations at that location, indicating the resistance was due to an altered binding site (Kaundun et al. 2008). Other studies have indicated a significant mechanism of glyphosate resistance in goosegrass to be an altered EPSPS with a cytosine to thymine replacement in the resistant allele (Baerson et al. 2002). Furthermore, a study by Ng et al. (2004) revealed that glyphosate resistance in goosegrass is inherited by a resistant EPSPS allele, or a single, nuclear, incompletely dominant gene.

Graminicide Resistance. A suspected graminicide-resistant and susceptible biotypes were evaluated for resistance to several graminicides in controlled environmental conditions (Marshall et al. 1994). The resistant biotype survived treatments of 50 g ai ha^{-1} with clethodim, tralkoxydim, sethoxydim, fluazifop-butyl, fenoxaprop-ethyl, and diclofop methyl. I_{50} values associated with the resistant biotype suggested that rates exceeding 4 kg ai ha^{-1} of fluazifop-butyl did not control 50% of the population, and the resistant biotype was more resistant to arloxyphenoxypropionate than cyclohexanedione herbicides (Marshall et al. 1994). A previous study showed a 50% reduction in translocation of sethoxydim in goosegrass when tank-mixed

with bentazon, a photosystem II inhibitor (Rhodes and Coble 1984). Clethodim was antagonized by the addition of glufosinate and decreased goosegrass control by 52% when applied as a tank-mix; however, applying clethodim at least seven days prior to glufosinate or fourteen days after achieved effective control of one- to two-tiller goosegrass (Burke et al. 2005).

Paraquat and Glufosinate Resistance. A 30-fold, paraquat-resistant biotype of goosegrass was confirmed in Manatee County, Florida after years of repeated applications in tomato fields (Buker et al. 2002). In addition, a goosegrass population growing in a bitter gourd field in Air Kuning, Perak, Malaysia was reported to be 3.4- to 3.6-fold more resistant to treatments of glufosinate and paraquat than the susceptible biotype after a minimum of six sequential applications of these herbicides over the previous four years (Seng et al. 2010). These results were the first to demonstrate multiple resistances of goosegrass to two, non-selective herbicides in field conditions (Seng et al. 2010).

Herbicide-Resistant Goosegrass in Turf. The only documented occurrences of herbicide-resistant goosegrass in a turfgrass environment reported to date originated on the island of Kauai, Hawaii in 2003 with triazines (Brosnan et al. 2008) and in eastern Georgia on a golf course with dinitroanilines (Heap 2013). Two biotypes demonstrated a resistance level of 100 to 200 times that of susceptible biotypes when treated with metribuzin at 0.28 kg ai ha^{-1} plus MSMA at 2.2 kg ai ha^{-1} once, followed by an additional application of MSMA seven days later (Brosnan et al. 2008). Applications of MSMA alone did not control either biotype (Brosnan et al. 2008). The biotypes uncontrolled by the asymmetrical metribuzin also responded equivalently to treatments with the symmetrical simazine (Brosnan et al. 2008), suggesting cross-resistance to triazine herbicides. Resistance to these two different structures of triazines has been characterized with a glycine-serine substitution in the photosystem II (Devine and Shukla 2000; Senseman 2007).

Glyphosate applied at 1.1 kg ae ha^{-1} or foramsulfuron at 0.045 kg ai ha^{-1}, however, controlled the metribuzin-resistant biotype (Brosnan et al. 2008). This case of resistance can likely be attributed to the perennial nature of goosegrass that often occurs in tropical climates such as Hawaii. In this case, plants are not eradicated in the winter months, goosegrass frequently germinates throughout the year, and multiple applications are required to achieve consistent control (Brosnan et al. 2008).

Pathogens of Goosegrass. Several pathogens have been identified on goosegrass and characterized. Figliola et al. (1988) isolated two pathogens inducing a leaf spot on goosegrass. *Bipolaris setariae* (Saw.) was the fungal pathogen characterized for severely diseased goosegrass in field plots (Figliola et al. 1988). A second pathogen, *Piricularia frisea* (Cke.) Sacc., infected goosegrass plants the following spring in a greenhouse (Figliola et al. 1988). Further tests indicated no threat of these pathogens to dicotyledenous plants and low levels of infection on corn (Figliola et al. 1988). Although these pathogens have potential as biocontrol agents for goosegrass based on host specificity (family), sporulation, preferable environmental conditions, and pathogenicity (Figliola et al. 1988), no additional data was found regarding their progression as potential biocontrol agents. A leaf spotting and flecking disease of goosegrass was also reported by Luttrell (1957) as *Drechslera nodulosum*. Sugawara and Strobel (1987) further evaluated this pathogen and one of its metabolites, tryptophol. No implications of biological control with this pathogen were mentioned thereafter. Likewise, *Drechslera cynodontis* was reported to have pathogenic activity on goosegrass and also responded favorably when combined with a 0.25X rate of glyphosate as a potential combination treatment (Chia 2011). No further studies were found regarding this pathogen. Samples were collected and analyzed of goosegrass plants exhibiting leaf spots and necrosis while growing in a mature stand of zoysiagrass (*Zoysia*

japonica Steud.) in Blacksburg, VA. Fungal structures appeared to indicate infection of *Drechslera catenaria* (author's observations and personal communication with David McCall, Virginia Tech Turfgrass Pathologist), a close relative of previously mentioned pathogens also producing leaf spot and "melting out" symptoms in grasses (Smiley et al. 2005). No additional research was conducted with this pathogen to determine if it possessed the potential to be a biological control agent for goosegrass; however, reducing pesticide applications and the potential for point-source pollution of nearby waterways could increase favorability with golf courses if biological control organisms are found to be environmentally and economically efficient for goosegrass control. Decreasing pesticide input into the environment, especially in sand-based systems, near waterways, and adjacent to residential areas, alone warrants future research to elucidate if *D. catenaria* is effective as a biological control agent of goosegrass.

Current Goosegrass Control in Bermudagrass and Future Research. Since the banning of MSMA for turfgrass uses, a large demand has occurred for effective and economical goosegrass control in bermudagrass turf. Few preemergent herbicides control goosegrass well enough to preclude the use of postemergence techniques. Pursuant to this, these preemergent herbicides are often not a feasible control option due to the need for frequent repair of turf in sports turf with seeding, sprigging, and sodding. Oxadiazon is widely used in bermudagrass due to its safety during sprigging. Recent observations near Richmond, VA showing a lack of goosegrass control with oxadiazon has caused testing to be initiated to determine if oxadiazon resistance can be confirmed in this area. If oxadiazon resistance does in fact exist, a postemergence control program would become even more essential for turf managers and offer the most flexible approach to controlling goosegrass in bermudagrass sports fields, as long as visual quality of the turf is not compromised when aesthetic value of the field is crucial.

Although there are several postemergent herbicides which control goosegrass to some degree, control is often inconsistent and limited to small, pre-tiller stage plants. These smaller plants may not be noticeable in the turf canopy, and once these plants become a visible problem after reaching a larger growth stage they can no longer be controlled with currently labeled herbicides. After the release of topramezone (Pylex™ herbicide, BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709) (Anonymous 2013) for turfgrass use in June 2013, goosegrass control at any growth stage is now no longer a problem in cool season turfgrasses (Smith et al. 2013). Pylex™ is also labeled for bermudagrass control in cool-season turfgrasses; however, the rate and number of sequential applications needed to achieve bermudagrass control (one to three applications at 30 g ae ha^{-1}) is significantly more than the labeled recommendation for goosegrass control (one application at 24 g ae ha^{-1}) (Anonymous 2013). With such a substantial difference in total topramezone applied, more research is needed to determine if low rates of topramezone can be utilized to control goosegrass in bermudagrass without injuring the turf beyond a commercially-acceptable threshold.

Research Objectives

The objectives of this research were to 1) investigate a suspected oxadiazon-resistant goosegrass accession, 2) characterize the oxadiazon-resistant goosegrass accession, if present, 3) begin to understand absorption, translocation, and metabolism of oxadiazon within wild-types and the suspected oxadiazon-resistant goosegrass accession, 4) evaluate goosegrass and bermudagrass response to topramezone rates and tank mixtures, and 5) evaluate several herbicides used for postemergence goosegrass control as influenced by goosegrass growth stage.

Literature Cited

Anonymous (2003) Revolver herbicide label. Bayer Environmental Sciences, 2 T. W. Alexander Drive, Research Triangle Park, NC 27709.

Anonymous (2004) Illoxan herbicide label. Bayer Environmental Sciences, 2 T. W. Alexander Drive, Research Triangle Park, NC 27709.

Anonymous (2005) Ronstar G product label. Bayer Environmental Sciences, Research Triangle Park, NC 27709.

Anonymous (2007) Ronstar Flo product label. Bayer Environmental Sciences, Research Triangle Park, NC 27709.

Anonymous (2008) Dismiss 4SC herbicide label. Philadelphia, PA: FMC Corp.

Anonymous (2010) Specticle 20WSP product label. Bayer Environmental Sciences, Research Triangle Park, NC 27709.

Anonymous (2012) Dimension 2EW product label. Dow AgroSciences, LLC, Indianapolis, IN 46268.

Anonymous (2013) Pylex herbicide product label. BASF Corporation, Research Triangle Park, NC 27709.

Arrieta C, Busey P, and Daroub S (2009) Goosegrass and bermudagrass competition under compaction. Agron J 101(1):11-16

Askew SD, Cox MC, and Spak DR (2013) A suspected oxadiazon-resistant goosegrass population in Virginia. Page 247 *in* Proceedings of the 66th Southern Weed Science Society Annual Meeting. Houston, TX: Southern Weed Science Society of America.

Baerson SR, Rodriguez DJ, Tran M, Feng Y, Biest A, and Dill GM (2002) Glyphosate-resistant goosegrass. Identification of a mutation in the target enzyme 5-enolpyruvylshikimate-3-phosphate synthase. Plant Physiol 129:1265-1275

Baskin CC and Baskin JM (1998) Seeds: Ecology, biogeography, and evaluation of dormancy and germination. San Diego: Academic. 666 p.

Beard JB (2002) Turf management for golf courses. 2nd edn. Ann Arbor Press, Chelsea, MI. Pp. 181, 299, 565, 571, and 574.

Benvenuti S (2003) Soil texture involvement in germination and emergence of buried weed seeds. Agron J 95:191-198

Bhowmik PC and Bingham SW (1990) Preemergence activity of dinitroaniline herbicides used for weed control in cool-season turfgrasses. Weed Technol 4:387-393

Bingham SW (1967) Influence of herbicides on root development of bermudagrass. Weeds 15:363-365

Bingham SW and Hall, III JR (1985) Effects of herbicides on bermudagrass (*Cynodon* spp.) sprig establishment. Weed Sci 33:253-257

Bingham SW and Shaver RL (1981) Goosegrass (*Eleusine indica*) control during bermudagrass (*Cynodon dactylon*) establishment. Weed Sci 29(1):11-16

Boyd N and Van Acker R (2004) Seed and microsite limitations to emergence of four annual weed species. Weed Sci 52:571-577

Breeden G and Brosnan JT (2009) W170-Goosegrass (*Eleusine indica*). Knoxville, TN: The University of Tennessee Agricultural Extension Service. 5p.

Brennan BM, Higashi AK, and Murdoch CL (1992) Estimated pesticide use on golf courses in Hawaii. Honolulu, HI: Hawaii Institute of Tropical Agriculture and Human Resources Research Extension Series 137. 16p.

Brosnan JT, Nishimoto RK, and DeFrank J (2008) Metribuzin-resistant goosegrass (*Eleusine indica*) in bermudagrass turf. Weed Technol 22:675-678

Bryson CT and DeFelice MS, ed (2009) Weeds of the South. Athens, GA: University of Georgia Press. P. 374.

Buker, III RS, Steed ST, and Stall WM (2002) Confirmation and control of a paraquat-tolerant goosegrass (*Eleusine indica*) biotype. Weed Technol 16:309-313

Burke IC, Askew SD, Corbett JL, and Wilcut JW (2005). Glufosinate antagonizes clethodim control of goosegrass (*Eleusine indica*). Weed Technol 19:664-668

Busey P (1999) Goosegrass and golf don't mix. GCM September 63-66.

Busey P (2001) Goosegrass most difficult weed in South Florida. Fla. Turf Digest 18:24-27.

Carrow RN and Petrovic AM (1992) Effects of traffic on turfgrasses. Pp. 285-330 in D. V. Waddington et al. (ed.) Turfgrass. Agron. Monogr. 32. ASA, CSSA, and SSSA, Madison, WI.

Chauhan BS and Johnson DE (2008) Germination ecology of goosegrass (*Eleusine indica*): an important grass weed of rainfed rice. Weed Sci 56:699-706

Chia SZ (2009) *Drechslera cynodontis* as a potential bioherbicide for controlling goosegrass (*Eleusine indica*). Master's thesis. Putra, Malaysia: University of Putra.

Coles J (2003) Efficacy of herbicide combination on burley and dark-air cured tobacco. M.S. thesis. Bowling Green, KY: Western Kentucky University.

Cox MC, Venner K, and Askew SD (2013) Crabgrass and goosegrass control with dithiopyr and indaziflam. Page 242 *in* Proceedings of the 66th Southern Weed Science Society Annual Meeting. Houston, TX: Southern Weed Science Society of America.

Dernoeden PH, Watschke TL, and Mathias JK (1984) Goosegrass (*Eleusine indica*) control in turf in the transition zone. Weed Sci 32:4-7

Devine MD and Shukla A (2000) Altered target sites as a mechanism of herbicide resistance. Crop Prot 199:881-889

Dickens R, Sharpe SS, and Turner DL (1989) Herbicide effects on tensile strength and rooting of zoysiagrass sod. J Prod Agric 2:369-373

[EPA] Environmental Protection Agency-Office of Prevention, Pesticides, and Toxic Substances (2006) Reregistration Eligibility Decision for MSMA, DSMA, CAMA, and Cacodylic Acid. Washington, DC: U. S. Environmetal Protection Agency. Accessed: August 2013.

[EPA] Environmental Protection Agency (2013) Organic Arsenicals. Washington, DC: U. S. Environmetal Protection Agency. http://www.epa.gov/oppsrrd1/reregistration/organic_arsenicals_fs.html Accessed: August 2013.

Fagerness MJ, Yelverton FH, and Cooper RJ (2002) Bermudagrass *Cynodon dactylon* (L.) Pers. and zoysiagrass (*Zoysia japonica*) establishment after preemergence herbicide applications. Weed Technol 16:597-602

Ferrell JA, Murphy TR, and Vencill WK (2003) Tolerance of winter-installed tall fescue (*Festuca arundinacea*) and hybrid bermudagrass (*Cynodon transvaalensis* x *C. dactylon*) sod to herbicides. Weed Technol 17:521-525

Figliola SS, Camper ND, and Ridings WH (1988) Potential biological control agents for goosegrass (*Eleusine indica*). Weed Sci 36:830-835

Fishel FM and Coats GE (1994) Bermudagrass (*Cynodon dactylon*) sod rooting as influenced by preemergence herbicides. Weed Technol 8:46-49

Gannon TW, Yelverton FH, and Warren LS (2012) Indaziflam for weed control in warm-season turf. http://www.turffiles.ncsu.edu/PDFFiles/005302/INDAZIFLAM_FOR_WEED_CONTROL_IN_WARM-SEASON_TURF.pdf. Accessed August 19, 2013.

Goatley M, Askew S, Ervin E, McCall D, Studholme B, Schultz P, and Horvath B (2008) Sports Turf Management in the Transition Zone. Virginia: Pocahontas Press, Inc. Pp. 64, 76.

Harris JR, Gossett BJ, and Toler JE (1995) Growth characteristics of selected dinitroaniline-resistant and –susceptible goosegrass (*Eleusine indica*) population. Weed Technol 9(3):561-567

Heap I (2013) The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Accessed September 20, 2013.

Holm LG, Plucknett DL, Pancho JV, and Herberger JP (1977) The World's Worst Weeds: Distribution and Biology. Honolulu: University of Hawaii Press. Pp. 609.

Holm LG, Plucknett DL, Pancho JV, and Herberger JP (1991) The World's Worst Weeds. Malabar, Florida: Kriegen.

Huang W (2009) Factors contributing to the recent increase in U. S. fertilizer prices, 2002-08. AR-33, Economic Research Service, Washington, DC: U.S. Department of Agriculture.

Jasieniuk M, Brule-Babel AL, Morrison IN (1994) Inheritance of trifluralin resistance in green foxtail (*Setaria viridis*). Weed Sci 42:123-127

Johnson BJ (1976) Bermudagrass tolerance to consecutive butralin and oxadiazon treatments. Weed Sci 24(3):302-305

Johnson BJ (1980a) Root growth of southern turf cultivars as affected by herbicides. Weed Sci 28:526-528

Johnson BJ (1980b) Goosegrass (*Eleusine indica*) control in bermudagrass turf. Weed Sci 28(4):378-381

Johnson BJ (1994) Herbicide programs for large crabgrass and goosegrass control in Kentucky bluegrass turf. HortScience 29(8):876-879

Johnson BJ (1996a) Reduced rates of preemergence and postemergence herbicides for large crabgrass (*Digitaria sanguinalis*) and goosegrass (*Eleusine indica*) control in bermudagrass (*Cynodon dactylon*). Weed Sci 44:585-590

Johnson BJ (1996b) Tank-mixed postemergence herbicides for large crabgrass (*Digitaria sanguinalis*) and goosegrass (*Eleusine indica*) control in bermudagrass (*Cynodon dactylon*) turf. Weed Technol 10(4):716-721

Johnson BJ (1997) Reduced herbicide rates for large crabgrass (*Digitaria sanguinalis*) and goosegrass (*Eleusine indica*) control in bermudagrass (*Cynodon dactylon*). Weed Sci 45(2):283-287

Johnson BJ and Murphy TR (1993) Summer weed control with herbicides in turfgrasses. Georgia Agric. Res. Bul. 411.

Jones PA, Brosnan JT, Kopsell DA, and Breeden GK (2013) Soil type and rooting depth affect hybrid bermudagrass injury with preemergence herbicides. Crop Sci 53:660-665

Kaundun SS, Zelaya IA, Dale RP, Lycett AJ, Carter P, Sharples KR, and McIndoe E (2008) Importance of the P106S target-site mutation in conferring resistance to glyphosate in a goosegrass (*Eleusine indica*) population from the Philippines. Weed Sci 56:637-646

Kranz JH, Schmutterer H, and Koch W (1977) Diseases, Pests, and Weeds in Tropical Crops. New York: J. Wiley. 585 p.

Lee LJ and Ngim J (2000) A first report of glyphosate-resistant goosegrass (Eleusine indica (L) Gaertn) in Malaysia. Pest Manage Sci 56:336-339

Luttrell ES (1957) *Helminthosporium nodulosum* and related species. Phytopathology 47(9):540-548

[MAATF] Methane Arsonic Acid Research Task Force (2006) The Environmental Fate of Monosodium Methanearsonate (MSMA), 2-EF: Washington, D. C. http://www.maatf.com/docs/pdf/2_E-Fate_031113_EF.pdf

Marshall G, Kirkwood RC, and Leach GE (1994) Comparative studies on graminicide-resistant and susceptible biotypes of *Eleusine indica*. Weed Research 34:177-185

McCarty LB (1991) Goosegrass (*Eleusine indica*) control in bermudagrass (*Cynodon* spp.) turf with diclofop. Weed Sci 39:255-261

McCarty LB, Everest JW, Hall DW, Murphy TR, and Yelverton F (2001) Color Atlas of Turfgrass Weeds. Chelsea MI: Ann Arbor Press. P.45.

McCarty LB, Miller LC, and Colvin DL (1991) Bermudagrass (Cynodon spp.) cultivar response to diclofop, MSMA, and metribuzin. Weed Technol 5(1):27-32

McCarty B, Miller G, Waltz C, and Hale T (2005) Designing, constructing, and maintaining bermudagrass sports fields. EC 698/SP 361/Bull. 1292. Clemson Univ., Clemson, SC.

McCullough PE, de Barreda DG, and Raymer P (2012) Nicosulfuron use with foramsulfuron and sulfentrazone for late summer goosegrass (*Eleusine indica*) control in bermudagrass and seashore paspalum. Weed Technol 26(2):376-381

McElroy JS, Robinson DK, Samples T, Sorochan JC, and Breeden G (2007) Weed Management Recommendations for Professional Turfgrass Managers: Athletic Fields, Golf Courses, Commercial Lawns and Turfgrass Sod. Knoxville, TN: University of Tennessee Agricultural Extension Service #PB1539. 54 p.

Mudge LC, Gossett BJ, and Murphy TR (1984) Resistance of goosegrass (*Eleusine indica*) to dinitroaniline herbicides. Weed Sci 32:591-594

Mueller TC, Barnett KA, Brosnan JT, and Steckel LE (2011) Glyphosate-resistant goosegrass (*Eleusine indica*) confirmed in Tennessee. Weed Sci 59:562-566

Murdoch CL and Ikeda D (1974) Goosegrass control in bermudagrass turf with combinations of MSMA and *s*-triazines. Agron J 66:712-714

Murdoch CL and Nishimoto RK (1982) Diclofop for goosegrass control in bermudagrass putting greens. HortScience 17:914-915

Murphy TR (2004) Weeds of Southern Turfgrasses. Gainesville, FL: University of Florida IFAS Extension. P.41.

Murphy TR, Gossett BJ, and Toler JE (1986) Growth and development of dinitroaniline-susceptible and –resistant goosegrass (*Eleusine indica*) biotypes under noncompetitive conditions. Weed Sci 34:704-710

Myers DF, Hanrahan R, Michel J, Monke B, Mudge L, Olsen C, Parker A, Smith J, and Spak D (2009) Indaziflam/BCS AA170717-A new herbicide for preemergent control of grasses and broadleaves in turf and ornamentals. Proc Southern Weed Sci Soc 62:393

Ng CH, Ratnam W, Surif S, and Ismail BS (2004) Inheritance of glyphosate resistance in goosegrass (*Eleusine indica*). Weed Sci 52:564-570

Nishimoto RK and McCarty LB (1997) Fluctuating temperature and light influence seed germination of goosegrass (*Eleusine indica*). Weed Sci 45:426-429

Nishimoto RK and Murdoch CL (1999) Mature goosegrass (*Eleusine indica*) control in bermudagrass (*Cynodon dactylon*) turf with a new metribuzin-diclofop combination. Weed Technol 13:169-171

Powles SB and Yu Q (2010) Evolution in action: plants resistant to herbicides. Annu Rev Plant Biol 61:317-347

Reicher ZJ and Christians NE (1989) Establishment of Kentucky bluegrass sod following application of herbicides. HortScience 24:799-801

Rhodes, Jr. GN and Coble HD (1984) Influence of bentazon on absorption and translocation of sethoxydim in goosegrass (*Eleusine indica*). Weed Sci 32:595-597

Ross MA and Lembi CA (2009) Applied Weed Science: Including the Ecology and Management of Invasive Species. 3rd edn. Upper Saddle River, NJ: Pearson Education, Inc. Pp. 258-259.

Seng CT, Lun LV, San CT, and Sahid IB (2010) Initial report of glufosinate and paraquat multiple resistance that evolved in a biotype of goosegrass (*Eleusine indica*) in Malaysia. Weed Bio And Manage 10:229-233

Senseman SA (2007) Herbicide Handbook. 9th ed. Champaign, IL: Weed Sci Soc Am Pp. 377-379.

Sharpe SS, Dickens R, and Turner DL (1989) Herbicide effects on tensile strengths and rooting of bermudagrass (*Cynodon dactylon*) sod. Weed Technol 3:353-357

Smiley RW, Dernoeden PH, and Clarke BB (2005). Compendium of Turfgrass Diseases, 3rd edn. St. Paul, MN: APS Press. Pp.60-67.

Smith AN, Cox MC, Askew SD, and Miller K (2013) Topramezone for crabgrass and goosegrass control in cool season turf. Page 3 *in* Proceedings of the 66th Southern Weed Science Society Annual Meeting. Houston, TX: Southern Weed Science Society of America.

Sugawara F and Strobel GA (1987) Tryptophol, a phytotoxin produced by Drechslera nodulosum. Phytochemistry 26(5):1349-1351

Sweet D (2008) Golf industry in trouble as economy worsens: Housing market's collapse hampers development of new courses. http://www.nbcnews.com/id/28021989/ns/business-sports_biz/t/golf-industry-trouble-economy-worsens/#.Ug57l9KG2So

Tian X, Delye C, Darmency H (2006) Molecular evidence of biased inheritance of trifluralin herbicide resistance in foxtail millet. Plant Breed 125:254-258

Turgeon AJ (1980) Turfgrass Management. Reston Publishing Co., Inc., Reston, VA. Pp. 330-335.

[USDA] United States Department of Agriculture Economic Research Service (2012) Fertilizer Use and Price. Washington, DC: U.S. Department of Agriculture. http://www.ers.usda.gov/data-products/fertilizer-use-and-price.aspx#26727

[USDA-NASS] United States Department of Agriculture National Agricultural Statistics Service (2012) Average U.S. farm prices of selected fertilizers, 2003-2012. Washington, DC: U.S. Department of Agriculture. www.nass.usda.gov/Statistics_by_State/Minnesota/.../Annual.../91.pdf

Uva RH, Neal JC, and DiTomaso JM (1997) Weeds of the Northeast. Ithaca, NY: Cornell University.

Vaughn KC and Lehnen, Jr., LP (1991) Mitotic disrupter herbicides. Weed Sci 39(3):450-457

Vaughn KC, Vaughn MA, and Gossett BJ (1990) A biotype of goosegrass (*Eleusine indica*) with an intermediate level of dinitroaniline herbicide resistance. Weed Technol 4(1):157-162

Waddington DV (1992) Soils, soil mixtures, and soil amendments. Pp. 331-383 in D. V. Waddington et al. (ed.) Turfgrass. Agron. Monogr. 32. ASA, CSSA, and SSSA, Madison, WI.

Waddington DV and Baker JH (1965) Influence of soil aeration on the growth and chemical composition of three grass species. Agron J 57:253-258

Wang T, Fleury A, Ma J, Darmency H (1996) Genetic control of dinitroaniline resistance in foxtail millet (*Setaria italica*). J Hered 87:423-426

Webster TM and Nichols RL (2012) Changes in the prevalence of weed species in the major agronomic crops of the southern United States: 1994/1995 to 2008/2009. Weed Sci 60:145-157

Wiecko G (2000) Sequential herbicide treatments for goosegrass control in bermudagrass (*Cynodon dactylon*) turf. Weed Technol 14:686-691

Wiecko G and Couillard A (1999) Sequential postemergence/preemergence treatments for crabgrass control in bermudagrass turf. J Environ Hortic 17:234-239

Yamamoto E, Zeng LH, Baird WV (1998) Alpha-tubulin missense mutations correlate with antimicrotubule drug resistance in *Eleusine indica*. Plant Cell 10:297-308

Zeng L and Baird WV (1997) Genetic basis of dinitroaniline herbicide resistance in a highly resistant biotype of goosegrass (*Eleusine indica*). J Hered 88:427-432

Zeng L and Baird WV (1999) Inheritance of resistance to antimicrotubule dinitroaniline herbicides in an "intermediate" resistant biotype of *Eleusine indica* (*Poaceae*). Am J Bot 86:940-947

Chapter II. Investigation of Oxadiazon-Resistant Goosegrass (*Eleusine indica*) in Virginia

Michael C. Cox, Shawn D. Askew, and David. R. Spak[1]

Oxadiazon-resistance has not been documented in any species to date. Although successful for the previous two decades, a lack of goosegrass control with oxadiazon at rates of 3.4 to 4.5 kg ai ha^{-1} during 2009 and 2010 at a golf course near Richmond, VA prompted studies to determine if escaped goosegrass plants had become resistant to oxadiazon. Greenhouse trials conducted in Blacksburg, VA and Clayton, NC evaluated oxadiazon at rates of 0.03 to 4.5 kg ai ha^{-1} to determine control of goosegrass plants grown from seed of suspected-resistant plants at the aforementioned golf course. Laboratory bioassays measured electrolyte leakage associated with the concentration of oxadiazon applied to suspected oxadiazon-resistant (SR) and oxadiazon-susceptible (S) goosegrass accessions. Data from the Virginia Tech study indicated that oxadiazon rates required to achieve 95% control were 28 times greater for the SR than the S accessions. Likewise, S accessions were 24, 45, 87, and 105 times more susceptible to oxadiazon than SR plants in the studies conducted by Bayer in North Carolina. The SR accession had significantly higher electrolyte leakage from total plant tissue than the S accessions, but appreciable tissue damage to SR foliage still occurred. The data suggest that goosegrass collected from Richmond, VA is a resistant biotype, and physiological resistance to oxadiazon only partially explains the lack of control.

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NOMENCLATURE: Oxadiazon; goosegrass, *Eleusine indica* (L.) ELEIN.

KEYWORDS: bermudagrass, oxadiazole, PPO inhibitor, preemergence.

INTRODUCTION

Several cases of protoporphyrinogen-oxidase (PPO) resistance have been documented in the last two decades; however, no resistance to oxadiazon has been reported (Heap 2013). Additionally, all PPO-resistance cases have been from broadleaf weeds only, no grass species, and only from postemergence applications. Common waterhemp (*Amaranthus rudis* Sauer) was the first documented PPO-resistant weed, with occurrences being reported in Kansas, Illinois, Missouri, and Iowa from 2001 to 2009 (Legleiter and Bradley 2008; Owen 2009; Patzoldt et al. 2005; Shoup et al. 2002). However, high tolerances to PPO inhibitors such as oxyfluorfen have been observed in common chickweed [*Stellaria media* (L.) Vill.] (Matsumoto et al. 1999) and ivyleaf morningglory (*Ipomoea hederacea* Jacq.) with acifluorfen and lactofen (Higgins et al. 1988). In China, a population of Asian copperleaf (*Acalypha australis* L.) infesting soybean was found to be resistant to fomesafen in 2011 (Heap 2013). Flixweed [*Descurainia sophia* (L.) Webb. ex Prantl] resistant to carfentrazone was also reported in China in 2011 while growing in winter wheat (Heap 2013). In South America, wild poinsettia (*Euphorbia heterophylla* L.) (Trezzi et al. 2005) and pigweed (*Amaranthus quitensis* Kunth.) (Heap 2013) were documented as resistant to several PPO inhibitors in 2004 and 2005, respectively. There are normally thought to be three primary mechanisms by which plants develop herbicide resistance (Dyer et al. 1993), but the complex mechanism of action of PPO inhibitors (Duke et al. 1991; Nandihalli and Duke 1993) provides up to six mechanisms by which resistance could potentially evolve: 1) reduced uptake or sequestration of herbicide, 2) rapid metabolism of herbicide, 3) altered binding site, 4) rapid metabolism of protoporphyrinogen IX or protoporphyrin IX, 5) inactivation of the resistant enzyme that converts protoporphyrinogen IX to protoporphyrin IX, and 6) resistance to toxic

oxygen species due to high levels of antioxidants and enzymes that fight these toxins (Duke et al. 1997). Previous research indicated differential metabolism as the mechanism by which several crops and weeds displayed differential responses to sulfentrazone (Dayan et al. 1996; Duke et al. 1991).

Oxadiazon is a shoot inhibitor and member of the oxadiazole family of herbicides (Senseman 2007) that is primarily used as a preemergent herbicide. Oxadiazon controls annual grasses and broadleaves including goosegrass (*Eleusine indica* L.), green foxtail (*Setaria viridis* (L.) Beauv.), crabgrass (*Digitaria* spp.), carpetweed (*Mollugo verticillata* L.), and Florida pusley (*Richardia scabra* L.) in warm-season turf and several crops (Anonymous 1973; Bailey and Simmons 1979; Dernoeden and Krouse 1991; Johnson 1993; Johnson and Murphy 1987, 1989, 1993; Ross and Lembi 2009; Senseman 2007). It is an important herbicide in Virginia because the state boasts a total of 336 golf courses (National Golf Federation 2011), half of which have bermudagrass fairways (USDA/NASS 2006). Oxadiazon is commonly used in warm-season turf for its excellent preemergence goosegrass control and turf safety when applied approximately one week before sprigging (WBS) or two weeks after sprigging (WAS) and thereafter (Bingham and Hall, III 1985; Bingham and Schmidt 1983; Bingham and Shaver 1980; Brecke et al. 2010; Dernoeden et al. 1984; Johnson 1976a, 1976b; Johnson 1985). Specifically in Virginia and the northernmost bermudagrass-growing regions of the U.S., turf managers are heavily reliant upon oxadiazon due to short growing seasons that restrict bermudagrass establishment and recovery time. Over the last three years, oxadiazon has been applied to approximately 850 hectares per year in Virginia as the herbicide Ronstar® (Jeff Michel, Green and Industrial Vegetation Marketing Manager for Bayer Cropscience, personal communication), totaling between 2,860 to 3,810 kg oxadiazon applied annually across the state. Unlike root-inhibiting herbicides which

pose a long-term bermudagrass injury threat, injury from oxadiazon is transient and does not hinder stolon or root establishment and growth, which is especially crucial in the transitional climate zones where spring dead spot disease, incited by *Ophiosphaerella* spp., and winterkill are often problematic. Past research indicated $\geq$ 80% control of goosegrass with oxadiazon applied at 3.4 kg ai ha^{-1} (McCarty and Weinbrecht 1997). Still, previous observations have shown a delay in spring greenup and transient phytotoxicity to bermudagrass with applications of oxadiazon (Callahan and High, Jr. 1990; Johnson 1994; Johnson 1976a) at 3.36 kg ai ha^{-1} or higher in early spring. Brecke and others (2010) reported most warm-season turfgrass injury "at sprigging" (AS), although injury ratings never exceeded 23% for oxadiazon.

During 2009 and 2010, a golf course superintendent in Richmond, VA began to experience a lack of goosegrass control with oxadiazon applied at 3.4 to 4.5 kg ai ha^{-1}. This golf course had exclusively used oxadiazon for preemergence goosegrass control for 21 consecutive years. However, in the last four years, numerous goosegrass plants emerged on treated fairways within one to two months of oxadiazon application. It is possible that resistance to oxadiazon has developed at this location due to many years of exclusive and consecutive use of this herbicide, through selection of naturally-occurring resistant goosegrass plants in the population. The objectives of this study were to 1) determine if the suspected-resistant (SR) goosegrass accession at this specific location is, in fact, resistant to oxadiazon, and 2) determine if the SR goosegrass accession demonstrates a different physiological response to oxadiazon than a susceptible (S) accession. A difference in physiological response between the SR and S accessions would indicate a true resistance mechanism present in the SR accession and that lack of control with oxadiazon is not due to other biotic or abiotic factors at that specific location.

MATERIALS AND METHODS

Greenhouse Bioassay. Three greenhouse and laboratory experiments were conducted from 2012 to 2013 to determine oxadiazon efficacy in goosegrass sampled from the suspected population. Two studies were implemented at the Glade Road Research Facility (GR) of Virginia Tech in Blacksburg, VA and one at the Bayer CropScience research facility in Clayton, North Carolina. In 2011, Virginia Tech and Bayer CropScience independently sampled escaped goosegrass plants and seed and tested them to determine response to oxadiazon rates as well as other herbicides. Six unique populations were tested. Seed of native goosegrass populations were collected near Clayton, NC; Blacksburg, VA; and on the golf course in Richmond, VA in areas known to have no history of oxadiazon use to serve as susceptible (S) comparisons. Two mature goosegrass plants collected from oxadiazon-treated fairways and one non-fairway site with a history of inconsistent oxadiazon applications on the Richmond, VA golf course served as seed donors for three, suspect populations that were evaluated in the study by Bayer. A composite of seedheads from plants growing at random locations on previously oxadiazon-treated fairways at the same golf course were collected for use in the Virginia Tech trial. Collected seedheads were allowed to dry at room temperature. After drying, seeds were separated from the seedheads and removed from the chaff by rubbing with fine grit sandpaper and shaking away the debris.

Goosegrass seeds were planted and treated as described in Beckie et al. (2000) but slightly modified to better fit a topdressed, golf course fairway scenario. Approximately 100

seeds were evenly spread over a soil-mix (3 part sand: 1 part potting soil) packed lightly, then irrigated with enough water to allow soil to settle and drain for 24 hours. Seed was then topdressed lightly with approximately 0.32 cm dry sand and irrigated lightly to encourage seed/soil settling. Pots were treated with Ronstar® FLO (Ronstar® FLO, Bayer Environmental Sciences, Research Triangle Park, NC 27709) (380 g oxadiazon L^{-1}) in a spray chamber equipped with flat fan nozzles at a spray volume of 373 L ha^{-1}. Treatments included oxadiazon applied at rates of 0.03, 0.07, 0.17, 0.56, 1.1, 2.2, 3.4, and 4.5 kg ai ha^{-1}. Indaziflam (Specticle® FLO, Bayer Environmental Sciences, Research Triangle Park, NC 27709) at 0.05 kg ai ha^{-1} and prodiamine (Barricade®, Syngenta Crop Protection, LLC, Greensboro, NC 27419-8300) at 0.84 kg ai ha^{-1} were included as reference standards. All treatments were replicated four times. Pots were then placed in a greenhouse under 427-530 µmol m^{-2} s^{-1} lighting with a 14-hour photoperiod per day, a day/night temperature range of 27/18 ˚C, and automatic irrigation to encourage seed germination. The study was conducted at Bayer CropScience in Cary, NC on January 30 and March 14, 2012 and repeated at Virginia Tech in Blacksburg, VA on March 20, 2012. Emerged seedlings were counted weekly. Seedling counts were transformed to percent reduction relative to counts in untreated pots and oxadiazon rate responses were fit to the hyperbolic function with the following equation using PROC Nlin in SAS®:

$$Y = (iX)/(1 + \left(\frac{iX}{100}\right)) \quad [1]$$

where Y represents goosegrass seedling population reduction (%) and X represents oxadiazon rate (kg ai ha^{-1}).

Estimated parameters, *i* (rate at which goosegrass seedling population reaches 100% reduction) and *a* (asymptote, or 100% seedling population reduction), were subjected to ANOVA or were used to calculate LC_{50} and LC_{95} values that were also subjected to ANOVA. Final data

were determined to be normal using the NORMAL option in PROC UNIVARIATE and Shapiro-Wilk statistic. Data were subject to ANOVA in SAS® 9.2 (SAS Institute Inc, Cary, NC) with sums of squares partitioned to reflect the goosegrass accession by herbicide rate factorial design and trials, which were considered random. Main effects and interactions were tested using mean square error associated with the random variable interaction (McIntosh 1983). Means were separated with Fisher's Protected LSD test at P = 0.05.

Electrolyte Leakage Study. Two S goosegrass accessions and the SR_1 (selfed progeny of SR_0) generation of the originally collected goosegrass accession plants were tested for differences in electrolyte leakage associated with treatment of oxadiazon. The SR_1 seed was collected from surviving, mature SR_0 plants after treatment with oxadiazon at 1.68 and 3.36 kg ai ha^{-1} in a greenhouse screening, similar to procedures used by Anderson et al. (1998). Electrolyte leakage studies have been used for decades in agronomic crops and turfgrasses to assess plant cell injury associated with environmental stresses and herbicide treatment (Anderson et al. 1988, 2002; Cardona et al. 1997; Dayan et al. 1997a and 1997b; Fry et al. 1991; Koo et al. 1994; Miller and Dickens 1996; Shashikumar and Nus 1993; Vanstone and Stobbe 1977; Zhang and Ervin 2009; Zhang et al. 2006). Differences in plant cultivar or biotype response to light-activated herbicides such as protoporphyrinogen oxidase (PPO)- and photosystem-inhibitors can be easily observed due to cell membrane permeability and damage, resulting in leakage of internal electrolytes (Dayan et al. 1997b; Duke and Kenyon 1993). If confirmed, a significant difference in electrolyte leakage between the SR and two S goosegrass accessions could be indicative of a physiological mechanism for resistance to oxadiazon (Beckie et al. 2000). Electrolyte leakage procedures in this experiment were similar to those described by Dayan et al. (1997b), Sherman et al. (1991), Koo et al. (1994), Zhang and Ervin (2009), and others, although slightly modified

to better fit the limitations of this study. Plants at the two- to three-leaf stage of both S goosegrass accessions and the SR accession were treated with three replications of oxadiazon (Ronstar® FLO herbicide) at 0, 5.790, 57.90, 579.0, and 5,790 µM oxadiazon and placed on P5 grade, 7 cm Fisherbrand® filter paper (Fisher Scientific, Pittsburgh, PA 15275) saturated with 1 mL of the respective solution. The 57.90 µM rate represents the concentration of herbicide a germinating seedling would encounter if oxadiazon at the maximum labeled rate of 3.4 kg ai ha^{-1} was uniformly distributed to a 1-cm depth in soil. Oxadiazon is tightly bound to soil and organic matter (Carringer et al. 1975), has low water solubility (0.7 mg L^{-1}), and does not readily leach below approximately 1 cm in a sand/clay system typical of non-modified golf courses and sports field soils (Ambrosi and Helling 1976). Samples were then incubated in complete darkness for 12 hours. Plants were removed from dark incubation and placed in growth chambers with light at 265 µmol^{-2} m^{-2} s^{-1} of photosynthetically active radiation (PAR) and a temperature of 25 to 30 °C for 8 hours to activate the herbicide. After light incubation, samples were removed from Petri plates, lightly rinsed with distilled water to remove any herbicide or salt residues present on the leaf surfaces, and placed in 15-mL centrifuge tubes containing 8 mL of distilled water. Samples were placed on an automatic shaker for 4 hours at 25 °C. Sample solutions were then removed, and an initial electrolyte leakage reading was taken (EC_1) using an electrical conductivity meter (VWR SR601C Symphony Meter, VWR International, Radnor, PA). Samples were then autoclaved at 121 °C for 30 min. After cooling to room temperature, a second electrical conductivity reading (EC_2) was taken. The electrolyte leakage (EL) is expressed as a percent by the following equation:

$$\left(\frac{EC1}{EC2}\right) x\ 100 \qquad [2]$$

where EC_1 represents the initial electrical conductivity measurement after herbicide treatment but before samples were autoclaved and EC_2 represents the second electrical conductivity measurement taken after samples were autoclaved. The experiment was repeated three weeks later. Electrolyte leakage percentages and oxadiazon rate responses were fit to the hyperbolic function using PROC Nlin in SAS® 9.2 (SAS Institute Inc, Cary, NC). Estimated *i* (rate at which goosegrass seedling reaches maximum electrolyte leakage value) values and *a* (asymptote, or maximum electrolyte leakage value) values were subjected to ANOVA to test for differences in concentration required to induce a significant increase in electrolyte leakage between goosegrass accessions. Prior to ANOVA, data were determined to be normal using the NORMAL option in PROC UNIVARIATE and Shapiro-Wilk statistic. Sums of squares were partitioned to reflect the goosegrass accession by oxadiazon concentration factorial design, and trials which were considered random. Main effects and interactions were tested using mean square error associated with the random variable interaction (McIntosh 1983). Means were separated using Fisher's Protected LSD test at P = 0.05.

RESULTS AND DISCUSSION

Greenhouse Bioassay. Seedling population reductions for all seed sources fit the hyperbolic function in response to oxadiazon rate with S plants having rapid ascent to 100% reduction compared to slower ascents from SR plants (Figure 1, Table 1). Estimated *i* values from S seed were several orders of magnitude higher than *i* values from SR plants (Table 1). The SR population in Virginia Tech trials required an estimated 0.485 and 16.6 kg ai ha^{-1} oxadiazon to control 50 and 95%, respectively, of the population at 9 WAT while the Virginia Tech S seed

required only $1.15e^{-09}$ and 0.587 kg ai ha^{-1}, respectively (Table 1). These data indicate that oxadiazon rates required to achieve commercially-acceptable control (95%) (Laurence Mudge, Bayer, personal communication) were 28 times greater for the SR than the S accessions. Likewise, S accessions were 24, 45, 87, and 105 times more susceptible to oxadiazon than SR plants in the studies conducted by Bayer in North Carolina (Table 1). Goosegrass plants were never observed in pots treated with prodiamine or indaziflam at any time in any study, suggesting no cross resistance to these classes of chemistry (Tables 2 and 3). Similar levels of resistance to acifluorfen and lactofen have been reported in common waterhemp when applied postemergence (Shoup et al. 2002; Patzoldt et al. 2005). A goosegrass biotype resistant to the preemergent trifluralin was considered to be resistant at an intermediate level (50-fold resistant) compared to other goosegrass biotypes boasting a 1,000 to 10,000-fold level of resistance to trifluralin (Vaughn et al. 1990). Based on our results, the goosegrass collected from the Richmond golf course is resistant to oxadiazon; however, the mechanism of resistance is not clear. Since it may be possible that goosegrass can avoid oxadiazon in soil via several methods (e.g., delayed germination, rapid soil degradation, etc.), further evaluations were conducted to elucidate if the mechanism for resistance is physiological.

Electrolyte Leakage Study. Although oxadiazon is a preemergent herbicide, goosegrass seedlings should display phytotoxicity after exposure to oxadiazon in the upper few millimeters of the soil profile but before necrosis and death. If a physiological mechanism of oxadiazon resistance exists, oxadiazon applied at labeled rates should damage S leaf tissue more than SR leaf tissue.

Trial interactions were nonsignificant ($P > 0.05$) so data were pooled over trial. The SR goosegrass accession demonstrated a quicker ascent (*i*) to maximum electrolyte leakage (*a*) than

the S accessions (Table 2, Figure 2). Still, the SR goosegrass accession had significantly less electrolyte leakage than the S accession based on estimated asymptote values from the ANOVA (Table 2, Figure 2). Although the *i* values of SR and S accessions were significantly different, standard error values were often several magnitudes higher than the estimated *i* value itself ($\leq$ 13,995), whereas standard error associated with *a* values were minimal ($\leq$ 5.3). Significantly higher *i* values suggest that the SR goosegrass accession exhibited a hypersensitive response when exposed to oxadiazon; however, total electrolyte leakage and cell membrane damage was surpassed by the S accession (Table 2, Figure 2), suggesting that the SR accession is resistant to oxadiazon by a physiological mechanism.

Previous researchers have also utilized electrolyte leakage experiments to analyze oxadiazon activity (Duke et al. 1989), test for herbicide resistance (Nimbal et al. 1995), and also separate response differences to cellular membrane disrupting herbicides within cultivars of the same species (Dayan et al. 1997a and 1997b; Ekmekci and Terzioglu 2005). Ekmekci and Terzioglu (2005) reported approximately 80-95, 20-60, and 12-30% electrolyte leakage of wild and cultivated wheats after exposure to 60, 30, and 15 µM paraquat, respectively. Koo et al. (1994) observed 476% electrolyte leakage ($\mu S\ g^{-1}$ fresh weight as compared to the untreated) from susceptible smooth crabgrass (*Digitaria ischaemum* L.) and only 83% from a resistant smooth crabgrass biotype at 72 hours after treatment of 0.5 kg ha^{-1} quinclorac. Although a useful tool in detecting resistance to cellular membrane-disrupting herbicides, electrolyte leakage studies do not always reveal a significant difference between resistant and susceptible biotypes (Nimbal et al. 1995), suggesting that resistance mechanisms may be active at other locations in the plant (Duke et al. 1997).

Oxadiazon is a PPO inhibitor with no documented cases of annual grass resistance globally. This is the first reported case of herbicide resistance development of an annual grass to oxadiazon; still, other reports have recently been made suggesting a lack of goosegrass control with oxadiazon at a few locations in North Carolina (Drs. Fred Yelverton and Travis Gannon, North Carolina State University, personal communication). The potential to develop resistance is still relatively low given the approximate 20 years of use, but resistance management strategies should be implemented before options become limited. Future research should further elucidate the physiological resistance mechanism and also determine if correlations can be made between gene mutations and occurrence of oxadiazon resistance.

ACKNOWLEDGEMENTS

The authors would like to thank Scott Moulden at The Foundry Club for supplying us with goosegrass seed and plants for this study. Many thanks also go to Drs. Xunzhong Zhang and Yiming Liu for advice and lab space in conducting the electrolyte leakage experiment.

LITERATURE CITED

Ambrosi D and Helling CS (1977) Leaching of oxadiazon and phosalone in soils. J Agric Food Chem 25:215-217

Anderson JA, Kenna MP, and Taliaferro CM (1988) Cold hardiness of 'Midiron' and 'Tifgreen' bermudagrass. HortScience 23:748-750

Anderson DD, Roeth FW, and Martin AR (1998) Discovery of a primisulfuron-resistant shattercane (*Sorghum bicolor*) biotype. Weed Technol 12(1):74-77

Anderson JA, Taliaferro CM, and Martin DL (2002) Freezing tolerance of bermudagrass: vegetatively propagated cultivars intended for fairway and putting green use, and seed-propagated cultivars. Crop Sci 42:975-977

Anonymous (1973) Oxadiazon technical data bulletin. Rhodia Inc., Monmouth Junction, New Jersey.

Bailey RE and Simmons JA (1979) Oxadiazon for weed control in woody ornamentals. Weed Sci 27:396-400

Beckie HJ, Heap IM, Smeda RJ, and Hall LM (2000) Screening for herbicide resistance in weeds. Weed Technol 14:428-445

Bingham SW and Hall, III JR (1985) Effects of herbicides on bermudagrass (*Cynodon* spp.) sprig establishment. Weed Sci 33:253-257

Bingham SW and Schmidt RE (1983) Turfgrass establishment after application of preemergence herbicides. Agron J 75:923-926

Bingham SW and Shaver RL (1980) Goosegrass control in bermudagrass. Proc Int Turfgrass Conf 3:237-245

Brecke BJ, Stephenson, IV DO and Unruh JB (2010) Timing of oxadiazon and quinclorac application on newly sprigged turfgrass species. Weed Technol 24:28-32

Callahan LM and High, Jr. JW (1990) Herbicide effects on bermudagrass lawn recovery and crabgrass control during spring root decline in the North-South transition zone. J Amer Soc Hort Sci 115:597-601

Cardona CA, Duncan RR, and Lindstrom O (1997) Low temperature tolerance assessment in paspalum. Crop Sci 37:1283-1291

Carringer RD, Weber JB, and Monaco TJ (1975) Absorption-desorption of selected pesticides by organic matter and montmorillonite. J Agric Food Chem 23:568-572

Dayan FE, Duke SO, Reddy KN, Hamper BC, and Leschinsky KL (1997a) Effects of isoxazole herbicides on protoporphyrinogen oxidase and porphyrin physiology. J Agric Food Chem 45:967-975

Dayan FE, Weete JD, Duke SO, and Hancock HG (1997b) Soybean (*Glycine max*) cultivar differences in response to sulfentrazone. Weed Sci 45(5):634-641

Dayan FE, Weete JD, and Hancock HG (1996) Differential sensitivity to sulfentrazone by sicklepod (*Senna obtusifolia*) and coffee senna (*Cassia occidentalis*). Weed Sci 44:12-17

Dernoeden PH and Krouse JM (1991) Selected crabgrass control evaluations for Maryland in 1990. Proc Northeastern Weed Sci Soc 45:117-118

Dernoeden PH, Watschke TL, and Mathias JK (1984) Goosegrass (*Eleusine indica*) control in turf in the transition zone. Weed Sci 32:4-7

Duke SO and Kenyon WH (1993) Peroxidizing activity determined by cellular leakage. Pages 61-69 *in* Boger and Sandmann, eds. Target Assays for Modern Herbicides and Related Phytotoxic Compounds. Boca Raton, FL: CRC Press, Inc.

Duke SO, Lee HJ, Duke MV, and others (1997) Mechanisms of resistance to protoporphyrinogen oxidase-inhibiting herbicides. Pages 155-160 *in* R. De Prado, J. Jorrin, and L. Garcia-Torres, eds. Weed and Crop Resistance to Herbicides. Norwell, MA: Kluwer Academic.

Duke SO, Lydon J, Becerril JM, Sherman TD, Lehnen, Jr., LP and Matsumoto H (1991) Protoporphyrinogen Oxidase-Inhibiting Herbicides. Weed Sci 39:465-473

Duke SO, Lydon J, and Paul RN (1989) Oxadiazon activity is similar to that of p-nitro diphenyl ether herbicides. Weed Sci 37:152-160

Dyer WE, Hess FD, Holt JS, and Duke SO (1993) Potential benefits and risks of herbicide-resistant crops produced by biotechnology. Horticultural Reviews 15:367-408

Ekmekci Y and Terzioglu S (2005) Effects of oxidative stress induced by paraquat on wild and cultivated wheats. Pestic Biochem and Physiol 83:69-81

Fry JD, Lang NS, and Clifton RGP (1991) Freezing resistance and carbohydrate composition of 'Floratam' St. Augustinegrass. HortScience 26:1537-1539

Heap, I. The international survey of herbicide resistant weeds. Online. Internet. Saturday, February 09, 2013. Available www.weedscience.org.

Higgins JM, Whitwell T, Corbin FT, Carter, Jr. GE and Hill, Jr. HS (1988) Absorption, translocation, and metabolism of acifluorfen and lactofen in pitted morningglory (*Ipomoea lacunosa*) and ivyleaf morningglory (*Ipomoea hederacea*). Weed Sci 36:141-145

Johnson BJ (1976a) Bermudagrass tolerance to consecutive butralin and oxadiazon treatments. Weed Sci 24(3):302-305

Johnson BJ (1976b) Herbicides for seasonal weed control in turfgrasses. Agron J 68:

717-720

Johnson BJ (1985) Response of four bermudagrass (Cynodon dactylon) cultivars to dates of oxadiazon treatments. Weed Sci 33:371-375

Johnson BJ (1992) Frequency of herbicide treatments for summer and winter weed control in turfgrasses. Weed Sci 30:116-124

Johnson BJ (1993) Differential large crabgrass control with herbicides in tall fescue and common bermudagrass. HortScience 28(10):1015-1016

Johnson BJ (1994) Response of Tifway bermudagrass and tall fescue turfgrasses to preemergence herbicides. J Environ Hort 12(1):19-23

Johnson BJ and Murphy TR (1987) Control of large crabgrass and goosegrass in warm-season turfgrasses. Georgia Agr Res Bul 364

Johnson BJ and Murphy TR (1989) Summer annual weed control in turfgrass. Georgia Agr Res Bul 388

Johnson BJ and Murphy TR (1993) Summer weed control with herbicides in turfgrasses. Georgia Agr Res Bul 411

Koo SJ, Neal JC, and DiTomaso JM (1994) Quinclorac-induced electrolyte leakage in seedling grasses. Weed Sci 42(1):1-7

Legleiter TR and Bradley KW (2008) Glyphosate and multiple herbicide resistance in common waterhemp (*Amaranthus rudis*) populations from Missouri. Weed Sci 56:582-587

Matsumoto H, Kashimoto Y, and Warabi E (1999) Basis for common chickweed (*Stellaria media*) tolerance to oxyfluorfen. Pestic Biochem Physiol 64:47-53

McCarty LB and Weinbrecht JS (1997) *Cynodon dactylon* x *C. transvaalensis* cv.

Tifway sprigging establishment and weed control following pre-emergence herbicide use. Int Turfgrass Soc Res J 8:507-515

McIntosh, M. S. 1983. Analysis of combined experiments. Agron J 75:153–155

Miller GL and Dickens R (1996) Potassium fertilization related to cold resistance in bermudagrass. Crop Sci 36:1290-1295

Nandihalli UB and Duke SO (1993) The porphyrin pathway as a herbicide target site. Pages 62-78 *in* Duke et al. eds, Pest Control with Enhanced Environmental Safety. ACS Symposium Series; Washington, D.C: American Chemical Society.

National Golf Federation (2011) Tee Time: Fore! Find out which states are rich in golf courses. http://www.aarp.org/entertainment/arts-leisure/info-08-2011/golf- courses.html. Accessed December 20, 2013.

Nimbal CI, Shaw DR, Duke SO, and Byrd, Jr. JD (1995) Response of MSMA-resistant and –susceptible common cocklebur (*Xanthium strumarium*) biotypes to cotton (*Gossypium hirsutum*) herbicides and cross-resistance to arsenicals and membrane disruptors. Weed Technol 9:440-445

Owen MK (2009) The evolution of herbicide resistant weeds in Iowa: description, implications and solutions. Pages 65-70 in Proceedings of the integrated crop management conference. Ames, IA: Iowa State University.

Patzoldt WL, Tranel PJ, and Hager AG (2005) A waterhemp (*Amaranthus tuberculatus*) biotype with multiple resistance across three herbicide sites of action. Weed Sci 53:30-36

Ross MA and Lembi CA (2009) Applied Weed Science: Including the Ecology and

Management of Invasive Species. 3rd edn. Upper Saddle River, NJ: Pearson Education, Inc. P. 275.

Senseman SA, ed (2007) Herbicide Handbook. 9th ed. Champaign, IL: Weed Sci Soc Am Pp. 212-213

Shashikumar K and Nus JL (1993) Cultivar and winter cover effects on bermudagrass cold acclimation and crown moisture content. Crop Sci 33:813-817

Sherman TD, Becerril JM, Matsumoto H, Duke MV, Jacobs JM, Jacobs NJ, and Duke SO (1991) Physiological basis for differential sensitivities of plant species to protoporphyrinogen oxidase-inhibiting herbicides. Plant Physiol 97:280-287

Shoup, DE, Al-Khatib K, and Peterson DE (2003) Common waterhemp (*Amaranthus rudis*) resistance to protoporphyrinogen oxidase-inhibiting herbicides. Weed Sci 51:145-150

Trezzi MM, Felippi CL, Mattei D, Silva HL, Nunes AL, Debastiani C, Vidal RA, and Marques A (2005) Multiple resistance of acetolactate synthase and protoporphyrinogen oxidase inhibitors in Euphorbia heterophylla biotypes. J Environ Sci Health, B, Pesticides, food contaminants, and agricultural wastes 40:101-109

[USDA-NASS] U.S. Department of Agriculture-National Agricultural Statistics Service 2006. Virginia's turfgrass industry. Virginia Field Office. Richmond, VA: U.S. Department of Agriculture. P.13.

Vanstone DE and Stobbe EH (1977) Electrolyte conductivity: a rapid measure of herbicide injury. Weed Sci 25(4): 352-354

Vaughn KC, Vaughn MA, and Gossett BJ (1990) A biotype of goosegrass (Eleusine

indica) with an intermediate level of dinitroaniline herbicide resistance. Weed Technol 4:157-162

Zhang X and Ervin E (2009) Physiological assessment of cool-season turfgrasses under ultraviolet-B stress. HortScience 44(6):1785-1789

Zhang X, Ervin E, and LaBranche AJ (2006) Metabolic defense responses of seeded bermudagrass during acclimation to freezing stress. Crop Sci 46:2598-2605

Table 1. Estimated parameter, (*i*), from hyperbolic regression of percent reduction in goosegrass seedling count as affected by oxadiazon rate and concentration of oxadiazon required to reduce goosegrass population by 50% (LC_{50}) and 95% (LC_{95}) associated with SR and S goosegrass accessions[a].

Goosegrass seed source	*i* value[bc]	Oxadiazon LC_{50}[c]	Oxadiazon LC_{95}[c]
		—— kg ai ha^{-1} ——	
NC-S1	7.571E+10 a[c]	1.321E-09 c	0.0900 d
NC-S2	8.497E+10 a	1.177E-09 c	0.1000 d
VT-S	8.707E+10 a	1.149E-09 c	0.5870 cd
NC-SR-ICP	2150 b	0.04651 b	2.577 bcd
NC-SR2	470.0 b	0.2128 a	4.552 bcd
NC-SR-15	222.0 b	0.4505 a	8.782 abc
NC-SR-16	214.0 b	0.4673 a	10.54 ab
VT-SR	206.0 b	0.4854 a	16.58 a

[a]Abbreviations: SR, suspected-resistant; S, susceptible; NC-S1, collected by Bayer and susceptible accession 1; NC-S2, collected by Bayer and susceptible accession 2; VT-S, collected by Virginia Tech and susceptible accession 1; NC-SR-ICP, collected by Bayer and suspected-resistant accession from non-fairway site, "in caddie pass"; NC-SR2, collected by Bayer and

suspected-resistant accession from fairway #2; NC-SR-15, collected by Bayer and suspected-resistant accession from fairway #15; NC-SR-16, collected by Bayer and suspected-resistant accession from fairway #16; VT-SR, collected by Virginia Tech and a mixture of suspected-resistant accession seeds from fairways #1, 2, 9, 15, and 16.

[b]*i* values predicted from hyperbolic function using PROC Nlin in SAS® performed on replicates by regressing oxadiazon rates.

[c]Means followed by the same letter within a column do not significantly differ according to Fisher's Protected LSD test at P = 0.05.

Table 2. Estimated parameter, (i)[b], from hyperbolic regression of electrolyte leakage in goosegrass seedlings and maximum electrolyte leakage (a) as affected by oxadiazon concentration associated with SR and S goosegrass accessions[a].

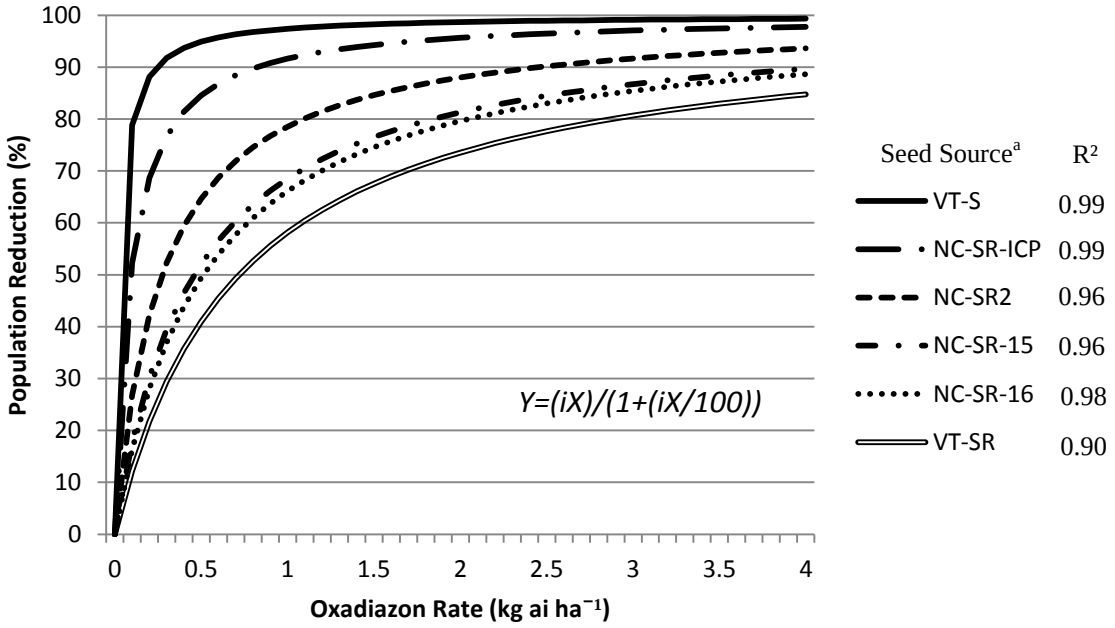
Accession	i^{bc}	a^{c}
SR	153.8a	13.5c
S	28.48b	19.1a

[a]Abbreviations: SR, suspected-resistant; S, susceptible; EL, electrolyte leakage; LSD, least significant difference.

[b] i and a values were subjected to ANOVA and used to approximate the parameters electrolyte leakage/ppm and maximum electrolyte leakage, respectively.

[c]Means followed by the same letter within a column do not significantly differ according to Fisher's Protected LSD test at P = 0.05.

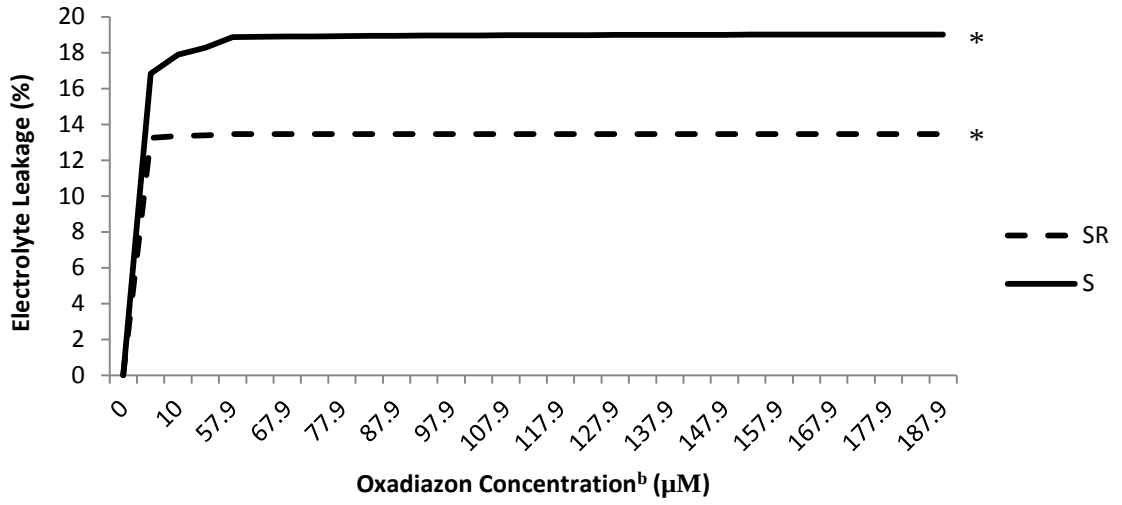
Figure 1. Goosegrass population reduction as affected by oxadiazon rate, 9 WAT.



[a]Abbreviations: SR, suspected-resistant; S, susceptible; VT-S, collected by Virginia Tech and susceptible accession 1; NC-SR-ICP, collected by Bayer and suspected-resistant accession from non-fairway site, "in caddie pass"; NC-SR2, collected by Bayer and suspected-resistant accession from fairway #2; NC-SR-15, collected by Bayer and suspected-resistant accession from fairway #15; NC-SR-16, collected by Bayer and suspected-resistant accession from fairway #16; VT-SR, collected by Virginia Tech and a mixture of suspected-resistant accession seeds from fairways #1, 2, 9, 15, and 16.

Population reduction values based on predicted *i* values from hyperbolic function using PROC Nlin in SAS® performed on replicates by regressing oxadiazon rates. Subsequent predicted values were subjected to ANOVA and LSD means separation at P = 0.05.

Figure 2. Electrolyte leakage hyperbolic regression of S[a] and SR goosegrass accessions as affected by oxadiazon concentration.



*Significant difference between goosegrass accessions ($P < 0.05$).

[a]Abbreviations: S, susceptible; SR, suspected resistant.

[b] Oxadiazon concentration was truncated on the x-axis for graph clarity and due to insignificant increases in hyperbola regressions of goosegrass accessions at concentrations exceeding 15 ppm. Electrolyte leakage values based on predicted *i* values from hyperbolic function using PROC Nlin in SAS® performed on replicates by regressing oxadiazon rates. Subsequent predicted values were subjected to ANOVA and LSD means separation at $P = 0.05$.

Chapter III. Characterizing an Oxadiazon-Resistant Goosegrass (*Eleusine indica*) Biotype

Michael C. Cox, Eva Collakova, and Shawn D. Askew[1]

Laboratory studies were conducted to determine if limited absorption or translocation could be the mechanism by which a goosegrass biotype from Richmond, VA developed oxadiazon resistance. Percent oxadiazon absorption was not significantly affected (P = 0.95) by goosegrass biotype. The S and R goosegrass plant tissue both absorbed 3.0% of total detected oxadiazon. Methanol washes accounted for the rest of the unabsorbed, detected oxadiazon as the WT and R goosegrass washes had 97% oxadiazon. Goosegrass biotype by plant part sample interaction was significant (P = 0.02), indicating that different amounts of oxadiazon were translocated to tissue above the treated foliage and the roots. The amount of translocated oxadiazon to tissue above the treated foliage or to the roots was not significantly different between goosegrass biotypes (P = 0.09), however. The roots of the S and R biotypes contained 93 and 99%, respectively, of the total amount of detected oxadiazon. The foliage above treatment location for the S and R biotypes contained 7.0 and 0.70%, respectively, of the total amount of detected oxadiazon. These data suggest that absorption or translocation is not likely to be the mechanism of resistance to oxadiazon in the R goosegrass biotype from Richmond, VA. Field-level and physiological resistance of oxadiazon has been confirmed with this goosegrass biotype; however, a specific mechanism of resistance has not been identified. It is also not clear at this time as to

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whether this is an isolated or widespread resistant population. Turf managers should implement alternative modes-of-action to preserve herbicide stewardship and prevent further spread of herbicide-resistant goosegrass.

NOMENCLATURE: oxadiazon; goosegrass; *Eleusine indica* L. Gaertn.; bermudagrass; *Cynodon dactylon* L.

KEYWORDS: absorption; biotype; gas chromatography (GC); mass spectrometry (MS); metabolism; PPO inhibitor; translocation.

INTRODUCTION

Goosegrass [*Eleusine indica* (L.) Gaertn.] is classified as one of the five most troublesome weeds in the world (Holm et al. 1991) and currently one of the hardest to control weeds in warm-season turfgrasses of temperate and tropical climates, particularly, in bermudagrass athletic fields and golf courses (Arrieta et al. 2009; Busey 2001; Johnson 1980; McCarty 1991). Few preemergent herbicides provide adequate control of goosegrass in bermudagrass turf. Oxadiazon is a member of the oxadiazole family and a shoot-inhibiting herbicide (Senseman 2007). Oxadiazon controls weeds by inhibiting protoporphyrinogen IX oxidase (Protox), an enzyme of chlorophyll and heme biosynthesis that converts Protox to protoporphyrin IX (Proto) (Duke et al. 1991; Jacobs and Jacobs 1987; Matringe et al. 1989; Witkowski and Halling 1989). Primarily used as a preemergent herbicide, oxadiazon has been utilized commercially for control of annual grasses and broadleaves including goosegrass (*Eleusine indica* L.), green foxtail [*Setaria viridis* (L.) Beauv.], crabgrass (*Digitaria* spp.), carpetweed (*Mollugo verticillata* L.), Florida pusley (*Richardia scabra* L.), in warm-season turf, and other crops since it was registered in the late 1970's (Anonymous 1973; Bailey and Simmons 1979; Johnson 1993; Johnson and Murphy 1987, 1989, 1993; Ross and Lembi 2009; Senseman 2007). Oxadiazon is commonly used in warm-season turf for its excellent preemergence goosegrass control and turf safety when applied approximately 1 week before sprigging (WBS) or 2 weeks after sprigging (WAS) and thereafter (Bingham and Hall, III 1985; Bingham and Schmidt 1983; Bingham and Shaver 1980; Brecke et al. 2010; Dernoeden et al. 1984; Johnson 1976; Johnson 1985). Past research indicated 80% or greater control of goosegrass with oxadiazon applied at 3.4 kg ai ha^{-1} (McCarty and Weinbrecht 1997).

Previous research has described absorption and translocation of oxadiazon in rice (*Oryza sativa* L.) and barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] (Achhireddy et al. 1984a, b; Ishizuka et al. 1975) and peanuts (*Arachis hypogaea* L.) (Bingham et al. 1980). Ishizuka et al. (1975) observed only 5% of applied radiolabeled oxadiazon was taken up by rice roots in a solution culture or paddy system at 10 days after treatment (DAT), and readily tranlsocated to old and young leaves. Studies by Achhireddy et al. (1984a, b), however, showed little to no translocation of radiolabeled oxadiazon in rice and barnyardgrass leaves.

Although uptake of oxadiazon may be limiting, a study by Chauhan and Johnson (2011) showed application of oxadiazon at 1.0 to 1.5 kg ha^{-1} reduced rice shoot biomass by approximately 10 mg $plant^{-1}$ at both rates and in saturated soils or aerobic soils. Total shoot biomass from plants treated with 1.0 and 1.5 kg ha^{-1} was 70 and 60 mg $plant^{-1}$, respectively, in aerobic conditions and only 60 and 50 mg $plant^{-1}$, respectively, in saturated soils likely suggesting that higher soil moisture levels increase absorption and translocation of oxadiazon in grasses. Bingham et al. (1980) documented higher concentrations of ^{14}C oxadiazon (0.65 ppmw) in both roots and shoots of peanut than in the hypocotyl or cotyledon at 131 days after planting (DAP); however, hulls contained 0.98 ppmw oxadiazon at 131 DAP, mostly due to direct contact with the treated soil solution. ^{14}C oxadiazon concentration also increased by 25% in peanut shoots from 30 to 61 DAP, suggesting an increase in uptake and translocation of oxadiazon as roots grew laterally throughout the treated soil (Bingham et al. 1980). Although the nuts matured in the treated layer of the soil profile, they contained the least concentration of ^{14}C oxadiazon of all plant parts at 131 DAP (Bingham et al. 1980).

Although there are normally thought to be three primary mechanisms by which plants develop herbicide resistance (Dyer et al. 1993), the complex mechanism of action of PPO

inhibitors (Duke et al. 1991; Nandihalli and Duke 1993) provides up to six mechanisms by which resistance could potentially evolve: 1) reduced uptake or sequestration of the herbicide, 2) rapid metabolism of the herbicide, 3) target site mutation, 4) rapid metabolism of protoporphyrinogen IX or protoporphyrin IX to non-photodynamic compounds, 5) inactivation of the resistant enzyme that converts protoporphyrinogen IX to protoporphyrin IX, and 6) resistance to toxic oxygen species due to high levels of antioxidants and enzymes that fight these toxins (Duke et al. 1997). Previous research indicated differential metabolism as the mechanism by which several crops and weeds displayed different responses to sulfentrazone (Dayan et al. 1996; Duke et al. 1991), also a PPO-inhibiting herbicide. Common chickweed (*Stellaria media* L.) exhibited increased tolerance to the diphenyl ether oxyflurofen, likely due to a decreased sensitivity to singlet oxygen and by sequestration of the herbicide to inactive sites away from the chloroplasts (Matsumoto et al. 1999). Achhireddy et al. (1984) observed minimal translocation of ^{14}C-oxadiazon in barnyardgrass and rice from the treated leaf at 7 DAT, but the LC_{50} of barnyardgrass was 250 ppmv while rice was only slightly injured at oxadiazon rates exceeding 500 ppmv.

Herbicide resistance mechanisms in goosegrass, such as absorption, translocation, and metabolism, have also been characterized in previous work. In a greenhouse evaluation, Everman et al. (2009) reported 50 and 76% absorption of ^{14}C-glufosinate into 5-10 cm goosegrass plants. ^{14}C-sethoxydim was equally absorbed and translocated to apical and basal leaves by centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.] and goosegrass 6 hours after treatment (HAT); however, 81 to 98% of remaining ^{14}C in goosegrass extracts were ^{14}C-sethoxydim and only 1% in centipedegrass leaves and roots, suggesting decreased metabolism as the mechanism for sethoxydim susceptibility in goosegrass (McCarty et al. 1990). Plants with

pubescent leaves have been documented to use decreased herbicide absorption as a resistance mechanism (Higgins et al. 1988); however, the glabrous surface of goosegrass leaves should not be capable of providing such a mechanism. A paraquat-resistant goosegrass biotype in Florida (Buker III et al. 2002) possibly survived herbicide treatments due to reduced translocation as was the case in reported paraquat-resistant horseweed (Lehoczki et al. 1992; Smisek et al. 1998).

In a previous study, a suspected-resistant goosegrass biotype from Richmond, VA demonstrated resistance to oxadiazon up to 105 times that of the tested susceptible biotype in greenhouse screenings and a significant reduction in electrolyte leakage from total plant tissue compared to the susceptible biotype (Cox et al. 2014). Further testing is warranted with this goosegrass biotype to elucidate possible resistance mechanisms to oxadiazon. The objective of this study was to test absorption and translocation of oxadiazon within a resistant goosegrass biotype and a susceptible biotype to determine if a difference in absorption and/or translocation exists to further characterize this case of oxadiazon resistance.

MATERIALS AND METHODS

Oxadiazon Absorption. Goosegrass seedlings at the four- to five-leaf growth stage grown from seed collected from an oxadiazon-resistant (R) F2 generation and an oxadiazon-susceptible (S) biotype were spot-treated with 2 µL of Ronstar® Flo herbicide (Bayer Cropscience, Research Triangle Park, NC) in the center of the top two, fully-expanded leaves, totaling 4 µL of Ronstar® Flo herbicide per seedling. This amount of Ronstar® Flo herbicide delivered 1.52 mg oxadiazon to each plant, or 0.760 mg oxadiazon per treated leaf. Treated seedlings were floated in water baths via foam plugs for 24 hours at 25 °C to allow for herbicide absorption. Seedlings were

then washed five times to remove any herbicide residue from the leaf surface by placing them in Falcon tubes filled with 3 mL methanol and gently upending the tube for several seconds before removing each seedling. All methanol washes were pooled into one 50-mL Falcon tube and stored at 3 °C until further analysis. Washed seedlings were each transferred to a 2-mL Eppendorf tube and stored at -62 °C until lyophilization.

Oxadiazon Translocation. Goosegrass seedlings at the four- to five-leaf growth stage grown from seed collected from the resistant F2 generation and a S accession were spot-treated with 6 µL of Ronstar® Flo herbicide (Bayer Cropscience, Research Triangle Park, NC) in the center of the bottom (older) three leaves (2 µL per leaf). This amount of Ronstar® Flo herbicide delivered 2.28 mg oxadiazon to each plant, or 0.760 mg oxadiazon per treated leaf. Treated seedlings were floated in water baths via foam plugs for 24 hours at 25 °C to allow for herbicide absorption and translocation. Goosegrass tissue was excised and separated in subsamples as plant tissue above treated leaves and roots. Treated plant tissue was not analyzed since the seedlings could not be washed in methanol to prevent contamination with unabsorbed oxadiazon.

Extraction Procedure. For both experiments, plant samples were lyophilized on a Labconco® FreezeDry System (Labconco®, Kansas City, MO) for 72 hours to remove any water in the plant tissues. Dry weights for each plant sample were recorded, and subsamples were subsequently placed into 1.5-mL Eppendorf tubes containing three, 4-mm glass beads. Samples were placed on a paint shaker for ten minutes first without and then with the addition of 200 µl of chloroform. Samples were centrifuged for ten seconds at maximum speed to bring the liquid down from the lids, and 200 µl of water was then added. Samples were vortexed and centrifuged again at maximum speed for five minutes to enable partitioning of oxadiazon into the organic chloroform phase and interfering sugars into the aqueous phase. One hundred µl of the chloroform phase

was transferred to a 250-µl glass insert placed in a 2-mL glass vial used for gas chromatography-mass spectrometry (GC-MS). One µl was injected in a pulsed splitless mode on an Agilent® 7890A series GC equipped with a 30-m DB-5MS-DG column (0.25 mm x 0.25 µm with a 10-m pre-column (Agilent® Technologies, Santa Clara, CA). The MS was an Agilent 5975C series single quadruple MS (Agilent® Technologies, Santa Clara, CA). The inlet temperature was set to 280 °C. The GC program consisted of holding the temperature at 180 °C for 2 min and then increasing it to 320 °C at 20 °C/min and holding at 320 °C for 2 min. For MS, the mass-to-charge (m/z) ratio range was from 160 to 360.

Final data were determined to be normal using the NORMAL option in PROC UNIVARIATE and Shapiro-Wilk statistic. Data were subjected to ANOVA in SAS® 9.2 (SAS Institute Inc, Cary, NC) with sums of squares partitioned to reflect the herbicide by goosegrass biotype factorial design for the absorption study and herbicide by goosegrass biotype by plant part subsample factorial design for the translocation study and trials, which were considered random. Main effects and interactions were tested using mean square error associated with the random variable interaction (McIntosh 1983). Means were separated with Fisher's Protected LSD test at P = 0.05.

RESULTS AND DISCUSSION

Oxadiazon Absorption. Oxadiazon eluted from the column in all samples from 7.35-7.37 min (Figure 1). Percent oxadiazon absorption was not significantly affected (P = 0.95) by goosegrass biotype. Based on technical standards, approximately 99% of oxadiazon was recovered in plants or plant washes. Data are presented as percentage of recovered in Table 1. The S and R

goosegrass plant tissue both absorbed 3.0% of total detected oxadiazon and were not significantly different (Table 1). The methanol washes accounted for the rest of the unabsorbed, detected oxadiazon as the S and R goosegrass washes both had 97% oxadiazon (Table 1). These data suggest that absorption is not likely to be the mechanism of resistance to oxadiazon in the R goosegrass biotype from Richmond, VA. McCarty et al. (1990) reported only 2.0% absorption of ^{14}C-sethoxydim into a susceptible goosegrass biotype. Everman et al. (2009) observed up to 76% absorption of ^{14}C-glufosinate into a glufosinate-resistant goosegrass biotype, further suggesting that absorption is not a probable mechanism of herbicide resistance in goosegrass biotypes.

Oxadiazon Translocation. Goosegrass biotype by plant part interaction was significant (P = 0.02), indicating that different concentrations of oxadiazon were translocated to tissue above the treated foliage or to the roots. Translocation of oxadiazon to tissue above the treated foliage or to the roots was not significantly different between goosegrass biotypes (P = 0.09), however (Table 2). The roots of the S and R biotypes contained 93 and 99%, respectively, of the total amount of absorbed oxadiazon (Table 2). The tissue above treated foliage for the S and R biotypes contained 7.0 and 1.0%, respectively, of the total amount of absorbed oxadiazon (Table 2). Based on these data, oxadiazon appears to be primarily basipetally translocated from the leaves, where absorbed, to the roots and following the "source to sink" dynamic (Ross and Lembi 2009). Senseman (2007) describes oxadiazon movement within plants to be limited toward the growing points but accumulating in mature plant structures. Previous work by Achhireddy et al. (1984a, b) also corroborates these data as little to no acropetal translocation of oxadiazon was observed in leaves of rice and barnyardgrass. In addition, Bingham et al. (1980) documented high concentrations of oxadiazon in roots of peanut at 131 days after harvest.

Although no absorption or translocation of oxadiazon differences could be ascertained in this study between the S and R goosegrass biotypes, oxadiazon appeared to behave similarly in these biotypes as in other grass species in previous research. These data suggest that translocation of oxadiazon, like the S, is efficient in the R goosegrass biotype and does not appear to be the mechanism responsible for resistance to oxadiazon. Future research should determine what mechanism is responsible for this case of resistance. Field-level and physiological resistance of oxadiazon has been confirmed with this goosegrass biotype; however, a specific mechanism of resistance has not been identified. It is also not clear at this time as to whether this is an isolated or widespread resistance phenomenon or occurrence. Turf managers should implement alternative modes-of-action to preserve herbicide stewardship and prevent further spread of herbicide-resistant goosegrass.

ACKNOWLEDGEMENTS

The authors would like to thank Andrew Schneider for assistance with preparation of the goosegrass samples for the GC-MS and generously allowing us space and time in the lab.

LITERATURE CITED

Achhireddy NR, Kirkwood RC, and Fletcher WW (1984a) The uptake and distribution of MCPA and oxadiazon in excised leaves. J Pestic Sci 9:611-615

Achhireddy NR, Kirkwood RC, and Fletcher WW (1984b) Oxadiazon absorption, translocation, and metabolism in rice (*Oryza sativa*) and barnyardgrass (*Echinochloa crus-galli*). Weed Sci 32(6):727-731

Anonymous (1973) Oxadiazon technical data bulletin. Rhodia Inc., Monmouth Junction, New Jersey.

Arrieta C, Busey P, and Daroub S (2009) Goosegrass and bermudagrass competition under compaction. Agron J 101(1):11-16

Bailey RE and Simmons JA (1979) Oxadiazon for weed control in woody ornamentals. Weed Sci 27:396-400

Bingham SW and Hall, III JR (1985) Effects of herbicides on bermudagrass (*Cynodon* spp.) sprig establishment. Weed Sci 33:253-257

Bingham SW and Schmidt RE (1983) Turfgrass establishment after application of preemergence herbicides. Agron J 75:923-926

Bingham SW and Shaver RL (1980) Goosegrass control in bermudagrass. Proc Int Turfgrass Conf 3:237-245

Bingham SW, Shaver RL, and Guyton CL (1980) Peanut uptake and metabolism of [^{14}C] oxadiazon from soil. J Agric Food Chem 28:735-740

Brecke BJ, Stephenson, IV DO and Unruh JB (2010) Timing of oxadiazon and quinclorac application on newly sprigged turfgrass species. Weed Technol 24:28-32

Buker III RS, Steed ST, and Stall WM (2002) Confirmation and control of a paraquat tolerant goosegrass (*Eleusine indica*) biotype. Weed Technol 16(2):309-313

Busey P (2001) Goosegrass most difficult weed in South Florida. Fla. Turf Digest 18:24-27

Chauhan BS and Johnson DE (2011) Growth response of direct-seeded rice to oxadiazon and bispyribac-sodium in aerobic and saturated soils. Weed Sci 59:119-122

Cox MC, Collakova E, and Askew SD (2014) Physiological response of five goosegrass populations to oxadiazon. Page 23 *in* Proceedings of the 68th Annual Meeting of the Northeastern Weed Science Society. Philadelphia, PA: Weed Science Society of America.

Dayan FE, Weete JD, and Hancock HG (1996) Differential sensitivity to sulfentrazone by sicklepod (*Senna obtusifolia*) and coffee senna (*Cassia occidentalis*). Weed Sci 44:12-17

Dernoeden PH, Watschke TL, and Mathias JK (1984) Goosegrass (*Eleusine indica*) control in turf in the transition zone. Weed Sci 32:4-7

Duke SO, Lee HJ, Duke MV, and others (1997) Mechanisms of resistance to protoporphyrinogen oxidase-inhibiting herbicides. Pages 155-160 *in* R. De Prado, J. Jorrin, and L. Garcia-Torres, eds. Weed and Crop Resistance to Herbicides. Norwell, MA: Kluwer Academic.

Duke SO, Lydon J, Becerril JM, Sherman TD, Lehnen, Jr., LP and Matsumoto H (1991) Protoporphyrinogen Oxidase-Inhibiting Herbicides. Weed Sci 39:465-473

Dyer WE, Hess FD, Holt JS, and Duke SO (1993) Potential benefits and risks of herbicide resistant crops produced by biotechnology. Hort Reviews 15:367-408

Everman WJ, Mayhew CR, Burton JD, York AC, and Wilcut JW (2009) Absorption,

translocation, and metabolism of ^{14}C-glufosinate in glufosinate-resistant corn, goosegrass (*Eleusine indica*), large crabgrass (*Digitaria sanguinalis*), and sicklepod (*Senna obtusifolia*). Weed Sci 57:1-5

Higgins JM, Whitwell T, Corbin FT, Carter, Jr. GE, and Hill, Jr. HS (1988) Absorption, translocation, and metabolism of acifluorfen and lactofen in pitted morningglory (*Ipomoea lacunosa*) and ivyleaf morningglory (*Ipomoea hederacea*). Weed Sci 48:347-355

Holm LG, Plucknett DL, Pancho JV, and Herberger JP (1991) The World's Worst Weeds. Malabar, Florida: Kriegen.

Ishizuka K, Hirata H, and Fukunaga K (1975) Absorption, translocation, and metabolism of oxadiazon in rice plants. Agric Biol Chem 39:1431-1446

Jacobs JM and Jacobs NJ (1987) Oxidation of protoporphyrinogen to protoporphyrin, a step in chlorophyll and haem biosynthesis. Purification and partial characterization of the enzyme from barley organelles. Biochem J 244:219-224

Johnson BJ (1976) Herbicides for seasonal weed control in turfgrasses. Agron J 68:717-720

Johnson BJ (1980) Goosegrass (*Eleusine indica*) control in bermudagrass turf. Weed Sci 28(4):378-381

Johnson BJ (1985) Response of four bermudagrass (*Cynodon dactylon*) cultivars to dates of oxadiazon treatments. Weed Sci 33:371-375

Johnson BJ (1993) Differential large crabgrass control with herbicides in tall fescue and common bermudagrass. Hort Sci 28(10):1015-1016

Johnson BJ and Murphy TR (1987) Control of large crabgrass and goosegrass in warm-season turfgrasses. Georgia Agr Res Bul 364

Johnson BJ and Murphy TR (1989) Summer annual weed control in turfgrass. Georgia Agr Res Bul 388

Johnson BJ and Murphy TR (1993) Summer weed control with herbicides in turfgrasses. Georgia Agr Res Bul 411

Lehockzi E, Laskay G, Gaal I, and Szigeti Z (1992) Mode of action of paraquat in leaves of paraquat-resistant *Conyza canadensis* L Cronq Plant Cell Environ 15:531-539

Matringe M, Camadro JM, Labette P, and Scalla R (1989) Protoporphyrinogen oxidase as a molecular target for diphenyl ether herbicides. Biochem. J. 260:231-235.

Matsumoto H, Kashimoto Y, and Warabi E (1999) Basis for common chickweed (*Stellaria media*) tolerance to oxyfluorfen. Pest Biochem and Physiol 64:47-53

McCarty LB (1991) Goosegrass (*Eleusine indica*) control in bermudagrass (*Cynodon* spp.) turf with diclofop. Weed Sci 39:255-261

McCarty LB, Higgins JM, Corbin FT, and Whitwell T (1990) Absorption, translocation, and metabolism of sethoxydim in centipedegrass and goosegrass. J Amer Soc Hort Sci 115(4):605-607

McCarty LB and Weinbrecht JS (1997) *Cynodon dactylon* x *C. transvaalensis* cv. Tifway sprigging establishment and weed control following pre-emergence herbicide use. Int Turfgrass Soc Res J 8:507-515

McIntosh, M. S. 1983. Analysis of combined experiments. Agron. J. 75:153–155.

Nandihalli UB and Duke SO (1993) The porphyrin pathway as a herbicide target site. Am Chem Soc Symposium Series 524:62-78

Ross MA and Lembi CA (2009) Applied Weed Science: Including the Ecology and

Management of Invasive Species. 3rd edn. Upper Saddle River, NJ: Pearson Education, Inc. Pp. 82, 258-259.

Senseman SA (2007) Herbicide Handbook. 9th ed. Champaign, IL: Weed Sci Soc Am Pp. 212-213.

Smisek A, Doucet C, Jones M, and Weaver S (1998) Paraquat resistance in horseweed (*Conyza canadensis*) and Virginia pepperweed (*Lepidium virginicum*) from Essex county, Ontario. Weed Sci 46:200-204

Witkowski DA and Halling BP (1989) Inhibition of plant protoporphyrinogen oxidase by herbicide acifluorfen-methyl. Plant Physiol 90:1239-1242

Table 1. Percentage of total detected oxadiazon absorbed by the plant or collected in methanol washes (unabsorbed) in two goosegrass biotypes.

Biotype	Sample	Oxadiazon abundance
		——— % of detected ———
S[a]	Plant	3.0[b] b
R	Plant	3.0 b
S	Wash	97 a
R	Wash	97 a

[a]Abbreviations: S, susceptible biotype; R, resistant biotype.

[b]Means followed by the same letter within a column do not significantly differ according to Fisher's Protected LSD test at P = 0.05.

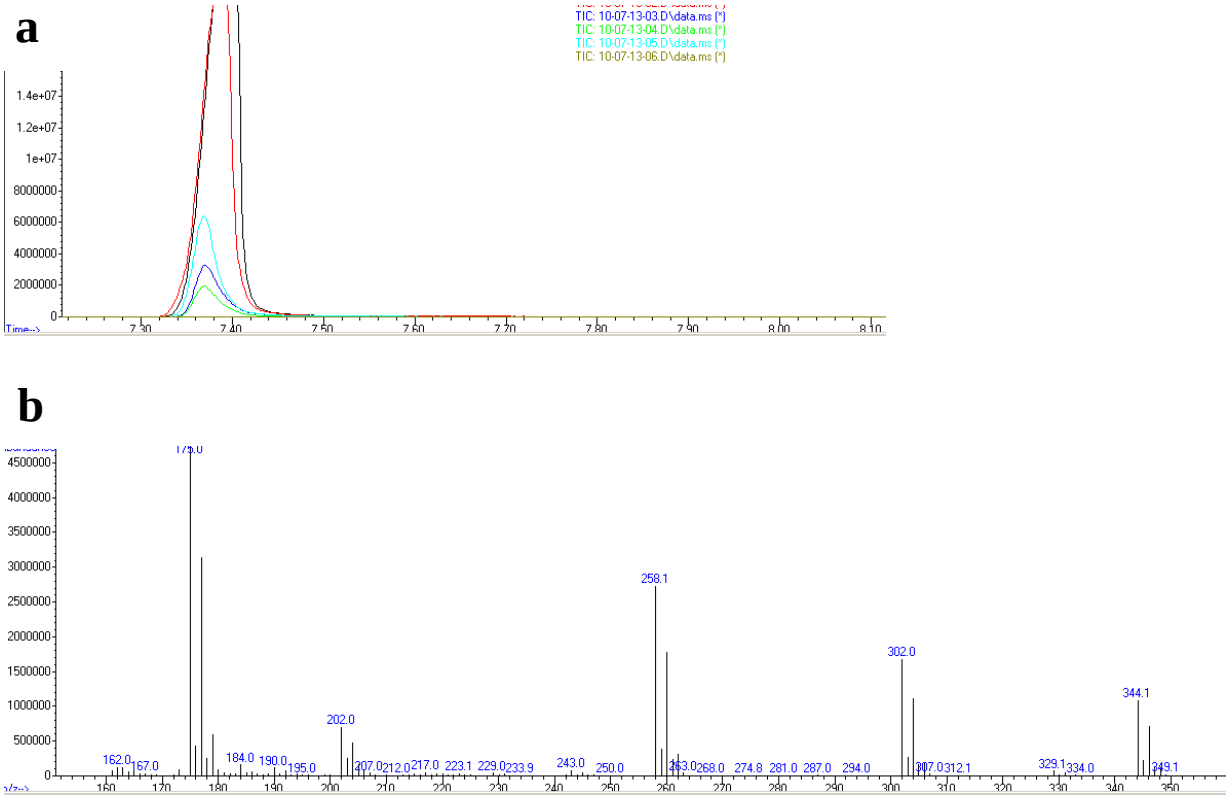
Table 2. Percentage of total detected oxadiazon translocated to sampled plant parts of two goosegrass biotypes.

Biotype	Plant part	Oxadiazon abundance
		——— % of detected ———
S[a]	above treated foliage	7.0[b] b
R	above treated foliage	0.70 b
S	Roots	93 a
R	Roots	99 a

[a]Abbreviations: S, susceptible biotype; R, resistant biotype.

[b]Means followed by the same letter within a column do not significantly differ according to Fisher's Protected LSD test at P = 0.05.

Figure 1. Chromatogram of goosegrass samples and subsequent methanol washes illustrating (a) distinct oxadiazon abundance peaks and a characteristic oxadiazon spectrum (b).



Chapter IV. Goosegrass and Bermudagrass Response to Topramezone Rates and Tank Mixtures

Michael C. Cox and Shawn D. Askew[1]

Greenhouse and field trials were conducted in the spring and summer of 2013 to determine the lowest rate at which topramezone, with or without the addition of triclopyr, controls goosegrass while maintaining commercially-acceptable bermudagrass quality. Results from the dose-response greenhouse study were analyzed and herbicide rates were refined for field studies. In field trials, topramezone rate did not significantly affect goosegrass cover ($P > 0.05$) 56 and 70 days after initial treatment (DAIT), so data were pooled over topramezone rates. There was a significant trial by triclopyr interaction ($P < 0.05$) at 56 and 70 DAIT, likely attributed to regrowth of goosegrass at the zoysiagrass site when triclopyr was not added. All treatments reduced goosegrass cover below 3 and 7% with and without the addition of triclopyr, respectively at 70 DAIT. A significant herbicide effect ($P < 0.05$) on bermudagrass cultivar showed higher injury from topramezone within three weeks of application, but injury persisted longer from treatments containing triclopyr. All bermudagrass cultivars recovered completely by 4 weeks after treatment (WAT). These data suggest that topramezone effectively controls mature goosegrass, and the addition of triclopyr at 140 g ae ha^{-1} seems to suppress regrowth as well as eliminate whitening symptoms. Although all bermudagrass cultivars recovered by 4

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WAT, these topramezone and triclopyr rates still appear to be higher than required to achieve goosegrass control and maintain acceptable bermudagrass quality.

NOMENCLATURE: Topramezone; goosegrass, *Eleusine indica* (L.) Gaertn. ELEIN. bermudagrass, *Cynodon dactylon* (L.) Pers. CYNDA.

KEYWORDS: bleaching, HPPD inhibitor, triclopyr, warm-season.

INTRODUCTION

Goosegrass is an annual, grassy weed that is extremely problematic in turf and especially warm-season turfgrasses due to its bunch-type growth habit, conspicuous seedheads, and ability to grow in dry, compacted areas (Goatley et al. 2008; McCarty 1991; Turgeon 2005). Several postemergent herbicides have been utilized for control of goosegrass in warm-season turfgrass (Busey 2004; Johnson 1975; Johnson 1980; McCarty 1991; Nishimoto and Murdoch 1999); however, most of the indicated herbicide labels, except monosodium methanearsonate (MSMA) and metribuzin (Sencor®), do not claim effective goosegrass control at growth stages exceeding early-tillering (Anonymous 2007; Anonymous 2012). Even so, MSMA has recently been banned or heavily restricted in turf (EPA 2006, 2013), thus it is no longer a widely available option for weed control. Although mostly transient, turf phytotoxicity is also a concern with some of the aforementioned herbicides used for postemergence goosegrass control (Busey 1999; Johnson 1996; McCalla et al. 2004; McCarty 1991; Nishimoto and Murdoch 1999; Wiecko 2000).

Several triketone herbicides have been shown to control annual, grass weeds (Bollman et al. 2008; Johnson and Young 2002; Goddard et al. 2010), as well as suppress bermudagrass (Brosnan and Breeden 2013; Cox et al. 2013). Likewise, using these HPPD inhibitors for annual grassy weed control in bermudagrass turf may result in some level of turf phytotoxicity depending on specific herbicide, rate, and application frequency (Brosnan et al. 2011; Elmore et al. 2011a; Elmore et al. 2011b; Kopsell et al. 2010). Inhibitors of HPPD cause “bleaching” symptoms in susceptible plants, specifically in newer tissue (Grossmann and Ehrhardt 2007; Goddard et al. 2010), which present an aesthetic dilemma where the turf may be highly-visible to

the public eye. Previous research has shown that tank-mixing HPPD inhibitors with triclopyr significantly reduces bleaching symptoms (Brosnan and Breeden 2013; Cox et al. 2013). Preliminary research suggests topramezone controls goosegrass at rates much lower than those required for other susceptible weed species (Askew 2012). We hypothesized that low rates of topramezone may control goosegrass better than commercially-available products and the addition of triclopyr at reduced rates may prevent associated discoloration. The objective of this study was to elucidate the minimum rate at which topramezone and/or topramezone plus triclopyr controls goosegrass without injuring bermudagrass beyond a commercially-acceptable threshold.

MATERIALS AND METHODS

Optimizing Topramezone and Triclopyr Rates. An experiment was conducted concurrently in two different greenhouses at the Glade Road Research Facility (GR) in Blacksburg, VA in May 2013. A randomized complete block (RCB) experimental design was utilized and replicated three times in two greenhouses for a total of six replications. Goosegrass seed collected from a local population with no history of herbicide treatment was spread evenly in flats filled with a 2:1 ratio by volume of sand/silt loam [Duffield silt loam (fine-loamy, mixed, active, mesic, Ultic Hapludalfs)-Ernest silt loam (fine-loamy, mixed, superactive, mesic Aquic Fragiudults) complex with 6.6 pH] that was previously sterilized in an autoclave at 115 ˚C for 30 min to minimize weed competition and disease pressure. Flats were placed in the greenhouse under automatic irrigation and 427-530 µmol m^{-2} m^{-2} s^{-1} PAR lighting with a 14-hour photoperiod per day and a day/night temperature range of 27/18 ˚C to encourage germination.

As goosegrass seedlings reached the two to three-leaf growth stage, they were carefully removed from the germination flats and transplanted into 4.0-cm by 20-cm deep conetainers (SC-10 Super Cell Ray Leach "Cone-tainers"™, Hummert International, 1415 N. W. Moundview Drive, Topeka, Kansas 66618) filled with a similar 2:1 sand:soil mixture. Approximately 144 goosegrass seedlings, 72 seedlings per trial run, were then allowed adequate time to acclimate and reach the two- to three-tiller growth stage before herbicide treatment. The two- to three-tiller growth stage was chosen as the treatment timing in this study because this stage of goosegrass growth appears to be the most noticeable in a stand of turf and stage at which a turf manager would likely begin a postemergence goosegrass control program.

'Tifway' bermudagrass cores were collected in February 2013 while still dormant. Two to three dormant bermudagrass shoots with attached stolons were planted in each of 144 conetainers filled with a 2:1 sand:soil mixture as described earlier. Bermudagrass conetainers were amended with Osmocote® 14-14-14 to provide sufficient nutrients over the duration of the study and encourage growth. Bermudagrass was mowed as needed to promote density and maintain appropriate height of cut (1.3 cm). One-third of the recommended labeled rate of Miracle-Gro® 20-20-20 fertilizer was applied to both goosegrass and bermudagrass every three to four weeks during the study to prevent nutrient deficiency and leaf discoloration.

Herbicide treatments included topramezone (Pylex™ herbicide, BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709) at 0, 2.46, 6.14, 12.3, 18.4, and 24.6 g ae ha^{-1} alone and combined with triclopyr (Turflon® Ester Ultra herbicide, Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN 46268) at 0, 140, 280, and 560 g ae ha^{-1}. Foramsulfuron (Revolver® herbicide, Bayer Environmental Sciences, 26 Davis Drive, Research Triangle Park, NC 27709) at 43.7 g ai ha^{-1}, diclofop (Illoxan® herbicide, Bayer Environmental Sciences, 26

Davis Drive, Research Triangle Park, NC 27709) at 1,140 g ai ha^{-1}, and MSMA (MSMA 6 Plus herbicide, Drexel Chemical Company, P.O. Box 13327, Memphis, TN 38113-0327) plus metribuzin (Sencor® herbicide, Bayer Environmental Sciences, 26 Davis Drive, Research Triangle Park, NC 27709) at 2,240 plus 560 g ai ha^{-1}, respectively, were included as industry standard comparisons, along with an untreated check. Each treatment was applied twice with the initial application made on April 11, 2013 and the second application three weeks later. Green color, control/injury, chlorophyll content (mg m^{-2}), and chlorophyll fluorescence ratio F735/F700 (CFR) ratings were taken at 3, 7, 14, 21, 28, 35, 42, 49, and 56 days after initial treatment (DAIT) in the greenhouse study. Goosegrass and bermudagrass green color was evaluated based on a scale from 0-9, where 0 = no green tissue and 9 = complete green tissue. Goosegrass control and bermudagrass injury ratings were recorded as visually-estimated percentages, with 0% being no injury and 100% being death of all visible foliage (Frans et al. 1986). Goosegrass control over time was converted to area under the control progress curve (AUCPC) using the following equation as Campbell and Madden (1990) used for disease epidemiology and Askew et al. (2013) described for weediness over time in a turfgrass comparison study:

$$\partial = \sum_{i=1}^{ni-1} \left(\frac{(y_i + y_{(i-1)})}{2} (t_{(i+1)} - t_{(i)}) \right). \quad [1]$$

where ∂ represents AUCPC, *i* is ordered sampling date, *ni* is the number of sampling dates, *y* is goosegrass control rating at a given date, and *t* is time in days. Final data were determined to be normal using the NORMAL option in PROC UNIVARIATE and Shapiro-Wilk statistic. Data were subjected to ANOVA in SAS® 9.2 (SAS Institute Inc, Cary, NC) with sums of squares partitioned to reflect the herbicide by rate factorial design and trials, which were considered random. Main effects and interactions were tested using mean square error

associated with the random variable interaction (McIntosh 1983). Appropriate means were separated with Fisher's Protected LSD test at P = 0.05.

Chlorophyll content and CFR data were collected with a chlorophyll fluorometer (CCM-300 Chlorophyll Content Meter, Opti-Sciences, 8 Winn Avenue, Hudson, NH 03051) that measures chlorophyll content based on the emission ratio of red chlorophyll fluorescence from 700 nm to 710 nm and the far red emission fluorescence value from 730 nm to 740 nm with a peak at 735 nm. One reading was taken per treatment, which produced a chlorophyll content and CFR value. Readings on goosegrass plants were made by placing the sensor, outfitted with a clip for easy sensor-leaf attachment, on the first fully-expanded leaf on the mother shoot. Readings were taken on bermudagrass by attaching the sensor to the minimum number of bermudagrass leaves required to obtain a reading by the chlorophyll fluorometer. Bermudagrass leaves from the center of each conetainer that best represented its appearance were chosen for chlorophyll content readings. All plant material was harvested at the soil line at 8 weeks after initial treatment (WAIT) and dried at 50 °C for approximately 72 hours. Plant dry weight was then recorded. Data were tested for homogeneity of variance and subjected to an ANOVA test where P = 0.05 (SAS 2004).

Goosegrass Control in the Field. Three sites comprised a field study conducted at the Turfgrass Research Center (TRC) in Blacksburg, VA beginning on June 27, 2013. A RCB design replicated three times was the experimental design for the studies and repeated over three different locations. The soil at all three trial locations was a Groseclose urban land complex (clayey, mixed, mesic, Typic Hapludalfs) with 6.3 pH. One study site was a mixed stand of Zoysiagrass (*Zoysia japonica* Steud.) infested with multi-tiller goosegrass and smooth crabgrass [*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.]. The second site was a creeping bentgrass

fairway previously strip-killed with glyphosate and void areas seeded with goosegrass seed two weeks after herbicide application. The third site was a fallow area with no turf competition and high goosegrass pressure (35-75% goosegrass cover per plot). A condensed treatment arrangement via results of the greenhouse study was chosen based on goosegrass control and bermudagrass tolerance. Herbicide treatments for this study included: topramezone at 6.14 and 12.3 g ae ha^{-1} with and without triclopyr at 140 g ae ha^{-1}. All treatments included a methylated seed oil surfactant at 0.5% v/v, and an untreated check was included for comparison. Initial applications for site one were made on June 27, 2013 and repeated three weeks later. Initial applications for sites two and three were made on July 24, 2013 and repeated three weeks later. Goosegrass and smooth crabgrass green cover and control were visually-estimated, along with zoysiagrass green cover and injury, at 0, 3, 7, 14, 21, 28, 42, 56, and 70 days after initial treatment (DAIT). Normalized difference vegetation index (NDVI) was also recorded at each rating date using a Crop Circle™ Model ACS-210 (Holland Scientific Inc., 6001 South 58th Street, Lincoln, NE 68516). Data were tested for homogeneity of variance and subjected to an ANOVA test in SAS® (SAS Institute Inc., Cary, NC) where P = 0.05. Means were separated according to Fisher's Protected LSD test.

Bermudagrass Cultivar Tolerance. Soil at the bermudagrass study location was a Groseclose urban land complex (clayey, mixed, mesic, Typic Hapludalfs) with a 6.3 pH. Fertilizer (146.5 kg N ha^{-1}) was applied to the TRC bermudagrass field trial location in three applications over a 3-month time span and while the study was underway to mimic normal fertilization and maintenance practices of a bermudagrass sports field or golf course fairway. Herbicide treatments, as indicated previously in the goosegrass field study, were applied to 31 bermudagrass varieties (Table 1) as a randomized complete block split-plot experimental design,

where main plot was bermudagrass cultivar and each of five subplots were herbicide treatment. Plots were 1.8 by 1.8 m, and treatments were replicated three times. Bermudagrass stunting, whitening, and overall injury were visually-estimated at 0, 3, 7, 14, 21, 28, 42, 56, and 70 DAIT. Normalized difference vegetation index (NDVI) was also recorded at each rating date using a Crop Circle™. Data were tested for homogeneity of variance and subjected to an ANOVA test in SAS® where P = 0.05. Means were separated according to Fisher's Protected LSD test.

RESULTS AND DISCUSSION

Optimizing Topramezone and Triclopyr Rates. The ANOVA indicated a significant trial by topramezone interaction ($P < 0.05$) at 8 WAIT, showing greater goosegrass control in the first trial location than the second (data not shown). Mean temperature for the first trial location was 29 ˚C and 23 ˚C for the second trial location, suggesting that higher temperature increases topramezone activity. Johnson and Young (2002) reported greater control of velvetleaf [*Abutilon theophrasti* (L.) Medic.] and common cocklebur (*Xanthium strumarium* L.) with mesotrione, another HPPD inhibitor, at 32 ˚C versus 18 ˚C. Previous research with glyphosate, fluazifop, and acifluorfen has also shown that higher temperatures generally increase foliar herbicide efficacy (Anderson et al. 1993; Kells et al. 1984; McWhorter and Azlin 1978; Wills and McWhorter 1981), likely due to increased absorption and translocation within the plant (Legg 1983; Price 1983). Light intensity was also different for each trial location, likely affecting topramezone activity. Trial site 1 light intensity was 530 µmol m^{-2} m^{-2} s^{-1}, where a greater topramezone effect was observed, and 427 µmol m^{-2} m^{-2} s^{-1} at trial site 2. Others have observed greater activity of atrazine (Mayasich et al. 1986) and similar herbicides that inhibit

photosynthetic processes or carotenoid biosynthesis directly or indirectly (Dodge 1982) with increasing light intensity. Triketone herbicides, such as topramezone, promote photo-oxidative stress and indirectly inhibit carotenoid biosynthesis in plants (Senseman 2007) such that an increase in light intensity could likely increase their activity.

A significant topramezone by triclopyr interaction ($P < 0.05$) was noted when goosegrass control was analyzed over time using the area under the progress curve (Figure 1). Goosegrass control significantly increased when topramezone rate increased from 6.14 to 12.3 g ae ha^{-1} with triclopyr at 140 g ae ha^{-1} (Table 2, Figure 1a), but did not significantly increase when topramezone rate increased to 18.4 g ae ha^{-1} with 140 g ae ha^{-1} of triclopyr (Table 2, Figure 1a). Goosegrass control did not significantly differ when topramezone rate was 6.14 or 12.3 g ae ha^{-1} and triclopyr rate was 140 or 280 g ae ha^{-1} (Table 3, Figure 1b); hence, we chose the lower triclopyr rate for field experiments to minimize bermudagrass injury. Goosegrass dry biomass was significantly affected by topramezone rate ($P < 0.05$) at 61 DAIT (Figure 2). For every gram increase in topramezone rate, goosegrass biomass reduction increased by 3.3% when compared to the untreated check (Figure 2). Topramezone at 24.6 g ae ha^{-1} reduced goosegrass biomass better than all industry standards at 61 DAIT (Figure 2). Topramezone at 12.3 g ae ha^{-1} reduced goosegrass biomass equivalently to diclofop and MSMA plus metribuzin at 61 DAIT (Figure 2). All rates of topramezone except 2.46 and 6.14 g ae ha^{-1} reduced goosegrass biomass more than foramsulfuron at 61 DAIT (Figure 2). Although one application of foramsulfuron at the maximum labeled rate (43.7 g ai ha^{-1}) was used in this study, these data are similar to those reported by Busey (2004) with two applications of foramsulfuron at 29.0 g ai ha^{-1} not effectively controlling multi-tiller goosegrass 4 to 5 weeks after initial treatment (WAIT). MSMA plus metribuzin did, however, control multi-tiller goosegrass better than foramsulfuron alone in the

study by Busey (2004), which our study also corroborates. In a study by McCarty (1991), diclofop at 1.1 kg ai ha^{-1} controlled goosegrass (91%) 3 WAIT at a 1.3-cm mowing height, but plants recovered (65%) by 5 WAIT. Nishimoto and Murdoch (1999) documented only 19% control of goosegrass at 7 WAT with diclofop at 1.7 kg ai ha^{-1}. In our study, diclofop also demonstrated short-lived control as goosegrass biomass reduction was only around 60% at 61 DAIT (Figure 2), which we believe would have been significantly higher at previous rating dates in the study based on visual control data (data not shown). The decrease in goosegrass control was likely due to many plants recovering or generating new growth after the single application by 61 days. Effective goosegrass control often requires sequential herbicide applications (Busey 2004; Johnson 1980; Wiecko 2000); therefore, multiple applications and bermudagrass tolerance were taken into consideration when topramezone rates were selected for goosegrass and bermudagrass field trials. To keep bermudagrass injury at a minimum, topramezone rates at 12.3 g ae ha^{-1} and lower were chosen for use in field trials.

Goosegrass Control in the Field. Topramezone at 6.14 and 12.3 g ae ha^{-1} reduced goosegrass cover 66% or greater with or without the addition of triclopyr at 70 days after initial treatment (DAIT) (Table 4); however, a significant trial by triclopyr interaction ($P < 0.05$) at 56 and 70 DAIT indicated an increase in goosegrass cover at trial location one (zoysiagrass site) when triclopyr was not added (Table 4). This increase in goosegrass cover, or goosegrass regrowth, at the zoysiagrass site could possibly be due to less spray coverage reaching smaller goosegrass plants below the dense turfgrass canopy, resulting in less goosegrass suppression and/or death later in the study. Past research has also demonstrated better suppression of grasses when triclopyr was tank-mixed with topramezone (Brosnan and Breeden 2013; Cox et al. 2013). Unlike the zoysiagrass site, the other two locations had little to no turfgrass canopy and minimal

spray interception by other weeds such as smooth crabgrass. It is likely that goosegrass control at locations with dense turfgrass and/or higher mowing heights will suffer if herbicide application techniques to improve spray coverage are not practiced. Goosegrass was completely controlled at the fallow and creeping bentgrass sites 70 DAIT (Table 4), suggesting that 6.14 or 12.3 g ae ha^{-1} of topramezone with or without the addition of triclopyr controls goosegrass in two applications three weeks apart when spray coverage is not inhibited by a dense turfgrass.

Bermudagrass Cultivar Tolerance. A significant herbicide main effect ($P < 0.05$) was detected from 3 to 56 DAIT (Figure 3) across bermudagrass cultivars. Topramezone at 12.3 g ae ha^{-1} with or without triclopyr visually-injured bermudagrass significantly more than 6.14 g ae ha^{-1} at all assessment dates except 28, 56, and 70 DAIT (Figure 3). Topramezone at 12.3 g ae ha^{-1} plus triclopyr at 140 g ae ha^{-1} only injured bermudagrass above the commercially-acceptable threshold (30%) at 14 and 42 DAIT (Figure 4a), and caused maximum phytotoxicity approximately two weeks after each application date. Although bermudagrass injury with topramezone alone was significantly higher than with the addition of triclopyr, injury was transient and recovery was complete by four weeks after each application (Figures 3 and 4). These observations are similar to those documented by Elmore et al. (2011b), where topramezone treatments at rates over double those used in our study still did not injure bermudagrass above 15% past 35 days after treatment (DAT). Treatments including triclopyr, however, severely stunted bermudagrass and injury was 13-17% 56 DAIT (Figure 3). Brosnan and Breeden (2013) also reported higher and more persistent injury to bermudagrass from topramezone plus triclopyr treatments than topramezone alone; however, topramezone rates used in our study did not exceed 12.3 g ae ha^{-1}, resulting in much lower overall injury when compared to those used in the study by Brosnan and Breeden (2013). It is also evident that there is an

additive effect on bermudagrass injury when combining topramezone with triclopyr, since triclopyr applied alone may injure bermudagrass by 30% or more (Brosnan and Breeden 2013) and since it has also been used as a tank-mix component with other bermudagrass-controlling herbicides such as fenoxaprop, fluazifop, and mesotrione (Cox et al. 2013; Cudney et al. 1997; Lewis et al. 2010; McElroy and Breeden 2006).

A significant bermudagrass cultivar by triclopyr interaction ($P < 0.05$) was observed when bermudagrass injury was analyzed over time using the area under the progress curve to assess total injury over 70 days (Figure 4). Although some bermudagrass cultivars are more sensitive to topramezone or topramezone plus triclopyr than others (Figure 4), there are no visual characteristic trends across cultivars that suggest a correlation between turfgrass density and topramezone or triclopyr sensitivity (author's personal observations and personal communication with Dr. Michael Goatley, Jr., Virginia state extension turfgrass specialist).

Due to a broad range of needs concerning goosegrass pressure and coverage, environmental conditions, bermudagrass cultivars, mowing heights, etc., the most effective topramezone program for goosegrass control in bermudagrass turf cannot be determined at this time. Many factors contribute to topramezone efficacy and bermudagrass injury; hence, the most appropriate topramezone program will vary across regions and turfgrass management practices. If aesthetics and visual bermudagrass quality without a short recovery restriction are of primary concern, a topramezone program including triclopyr would likely be a more suitable program since triclopyr significantly reduces whitening symptoms of topramezone. However, if only a short window is available for goosegrass control and prolonged injury to bermudagrass is a concern, perhaps triclopyr should not be included due to extensive stunting and "browning" of many bermudagrass cultivars. For small goosegrass populations, spot treatments with

topramezone plus triclopyr may also be an option as only a minimum area of bermudagrass would be affected. Following this study, a non-replicated demonstration in a bermudagrass cv. Patriot fairway illustrated a significant reduction in bleaching from topramezone with rates of triclopyr as low as 30 and 75 g ae ha^{-1} (author's personal observations). Triclopyr injured bermudagrass in the field studies more than the greenhouse studies such that we did not find the optimal triclopyr rate for use in bermudagrass. Future research should further evaluate triclopyr rates lower than our field rate of 140 g ae ha^{-1} to significantly reduce whitening symptoms but not compromise goosegrass control or commercially-acceptable bermudagrass quality. Subsequent work should also determine if topramezone rates can be reduced even further and applied over a season with sequential treatments to maintain goosegrass control but prevent substantial bermudagrass injury.

ACKNOWLEDGEMENTS

We would like to thank Camden Shelton, Tori Kellinger, Tyler Brewer, Adam Smith, and Sandeep Rana for assistance in material procurement and establishment of these studies.

LITERATURE CITED

Anderson DM, Swanton CJ, Hall JC, and Mersey BG (1993) The influence of temperature and relative humidity on the efficacy of glufosinate-ammonium. Weed Res 33:139-147

Anonymous (2012) MSMA 6 Plus herbicide label. Drexel Chemical Company, Memphis, TN 38113-0327.

Anonymous (2007) Sencor® herbicide label. Bayer CropScience, Research Triangle Park, NC 27709.

Askew SD (2012) Weed control in cool-season turfgrass with topramezone. Page 139 *in* Proceedings of the 65th Southern Weed Science Society. Houston, TX: Weed Science Society of America.

Askew WB, Goatley, Jr. JM, Askew SD, Hensler KL, and McKissack DR (2013) A comparison of turfgrasses for cemeteries and other low-input areas. Int Turfgrass Soc Res J 12:245-250.

Bollman JD, Boerboom CM, Becker RL, and Fritz VA (2008) Efficacy and tolerance to HPPD-inhibiting herbicides in sweet corn. Weed Technol 22:666-674

Brosnan JT and Breeden GK (2013) Bermudagrass control with topramezone and triclopyr. Weed Technol 27:138-142

Brosnan JT, Kopsell DA, Elmore MT, Breeden GK, and Armel GR (2011) Changes in 'Riviera' bermudagrass [*Cynodon dactylon* (L.) Pers.] carotenoid pigments after treatment with three p-hydroxyphenylpyruvate dioxygenase-inhibiting herbicides. HortScience 46:493-498

Busey P (1999) Goosegrass and golf don't mix. Golf Course Management 67:63-66

Busey P (2004) Goosegrass (*Eleusine indica*) control with foramsulfuron in bermudagrass (*Cynodon* spp.) turf. Weed Technol 18:634-640

Campbell CL and Madden LV (1990) Introduction to Plant Disease Epidemiology. New York: J. Wiley. Pp 192-193.

Cox MC, Askew SD, Venner KA, and Miller K (2013) Selective bermudagrass control in cool season turf with topramezone. Page 67 *in* Proceedings of the 66th Annual Northeastern Weed Science Society meeting. Philadelphia, PA: Weed Science Society of America.

Cudney, D. W., C. L. Elmore, V. A. Gibeault, and J. S. Reints. 1997. Common bermudagrass (*Cynodon dactylon*) management in cool-season turfgrass. Weed Technol 11:478-483

Dodge AD (1982) The role of light and oxygen in the action of photosynthesis inhibitor herbicides. Pages 57-77 *in* Moreland DE, St. John JB, and Hess FD, ed. Biochemical responses induced by herbicides. Washington, D.C.: American Chemical Society.

Elmore MT, Brosnan JT, Kopsell DA, and Breeden GK (2011a) Methods of assessing bermudagrass (*Cynodon dactylon* L.) responses to HPPD inhibiting herbicides. Crop Sci. 51:2840-2845.

Elmore MT, Brosnan JT, Kopsell DA, Breeden GK, and Mueller TC (2011b) Response of hybrid bermudagrass (*Cynodon dactylon* x *C. transvaalensis*) to three HPPD-inhibitors. Weed Sci 59:458-463

[EPA] Environmental Protection Agency-Office of Prevention, Pesticides, and Toxic Substances (2006) Reregistration Eligibility Decision for MSMA, DSMA, CAMA, and Cacodylic Acid. Washington, DC: U. S. Environmetal Protection Agency. Accessed: August 2013.

[EPA] Environmental Protection Agency (2013) Organic Arsenicals. Washington, DC: U. S. Environmetal Protection Agency. http://www.epa.gov/oppsrrd1/reregistration/organic_arsenicals_fs.html Accessed: August 2013.

Frans R, Talbert R, Marx D, and Crowley H (1986) Experimental design and techniques for measuring and analyzing plant responses to weed control practices. Pages 29-46 *in* Camper ND, ed. Research methods in weed science. Illinois: Southern Weed Sci Soc

Goatley M, Askew S, Ervin E, McCall D, Studholme B, Schultz P, and Horvath B (2008) Pages 76-77 *in* Sports Turf Management in the Transition Zone, Blacksburg, VA: Pocahontas Press, Inc.

Goddard MJR, Willis JB, and Askew SD (2010) Application placement and relative humidity affects smooth crabgrass and tall fescue response to mesotrione. Weed Sci 58:67-72

Grossmann K and Ehrhardt T (2007) On the mechanism of action and selectivity of the corn herbicide topramezone: a new inhibitor of 4-hydroxyphenylpyruvate dioxygenase. Pest Manag Sci 63:429-439

Johnson BJ (1975) Postemergence control of large crabgrass and goosegrass in turf. Weed Sci 23:404-409

Johnson BJ (1980) Goosegrass (*Eleusine indica*) control in bermudagrass (*Cynodon dactylon*) turf. Weed Sci 28:378-381

Johnson BJ (1996) Reduced rates of preemergence and postemergence herbicides for large crabgrass (*Digitaria sanguinalis*) and goosegrass (*Eleusine indica*) control in bermudagrass (*Cynodon dactylon*). Weed Sci 44:585-590

Johnson BC and Young BG (2002) Influence of temperature and relative humidity on the foliar activity of mesotrione. Weed Sci 50:157-161

Kells JJ, Meggitt WF, and Penner D (1984) Absorption, translocation, and activity of fluazifop-p butyl as influenced by plant growth stage and environment. Weed Sci 32: 143-149

Kopsell DA, Brosnan JT, Armel GR, and McElroy JS (2010) Mesotrione induced increases in bermudagrass tissue pigment concentrations. HortScience 45:1559-1562

Legg BJ (1983) Micrometeorology and the influence of local variations of environment on plant growth and herbicide performance. Asp Appl Biol 4:15-31

Lewis, D. F., J. S. McElroy, J. C. Sorochan, T. C. Mueller, T. J. Samples, and G. K. Breeden. 2010. Efficacy and safening of arloxyphenoxypropionate herbicides when tank-mixed with triclopyr for bermudagrass control in zoysiagrass turf. Weed Technol 24:489-494

Mayasich JM, Karlander EP, and Terlizzi, Jr. DE (1986) Growth responses of *Nannochloris oculata* droop and *Phaeodactylum tricornutum* bohlin to the herbicide atrazine as influenced by light intensity and temperature. Aquatic Toxicol 8:175-184

McCalla Jr., JH, Richardson MD, Karcher DE, and Boyd JW (2004) Tolerance of seedling bermudagrass to postemergence herbicides. Crop Sci 44:1330-1336

McCarty LB (1991) Goosegrass (*Eleusine indica*) control in bermudagrass (*Cynodon* spp.) turf with diclofop. Weed Sci 39:255-261

McElroy, J. S. and G. K. Breeden. 2006. Triclopyr safens the use of fluazifop and fenoxaprop on zoysiagrass while maintaining bermudagrass suppression. Online. Appl. Turfgrass Sci. doi:10.1094/ATS-2006-0502-01-RS.

McIntosh, M. S. 1983. Analysis of combined experiments. Agron J 75:153–155

McWhorter CG and Azlin WR (1978) Effects of environment on the toxicity of glyphosate to johnsongrass (*Sorghum halepense*) and soybeans (*Glycine max*). Weed Sci 26:605-608

Nishimoto RK and Murdoch CL (1999) Mature goosegrass (*Eleusine indica*) control in bermudagrass (*Cynodon dactylon*) turf with a metribuzin-diclofop combination. Weed Technol 13:169-171

Price CE (1983) The effect of environment on foliage uptake and translocation of herbicides. *In*: (Van Oorshot J. L. P., ed.) Aspects of Applied Biology 4. Influence of Environmental Factors on Herbicide Performance and Crop and Weed Biology, Wellesbourne, UK: Association of Applied Biologists, pp. 157-169.

Senseman SA (2007) Herbicide Handbook. 9th ed. Champaign, IL: Weed Sci Soc Am Pp. 12-13

Turgeon AJ (2005) Page 252 *in*: Turfgrass Management. Pearson Education, Inc., New Jersey.

Wiecko G (2000) Sequential herbicide treatments for goosegrass (*Eleusine indica*) control in bermudagrass (*Cynodon dactylon*) turf. Weed Technol 14:686-691

Wills GD and McWhorter CG (1981) Effect of environment on the translocation and toxicity of acifluorfen to showy crotolaria (*Crotolaria spectabilis*). Weed Sci 29:397-401

Table 1. Bermudagrass cultivars[a] tested for topramezone rate response and tolerance.

BAR 7CD5 (S)[b]	Princess 77 (S)	SWI-1070 (S)
IS-01-201 (S)	PSG 91215 (S)	SWI-1081 (S)
IS-CD10 (S)	PSG 94524 (S)	SWI-1083 (S)
J-720 (Hollywood) (S)	PSG 9Y2OK (S)	SWI-1113 (S)
OKC 1119 (Latitude 36) (V)	PSG PROK (S)	SWI-1117 (S)
Midlawn (V)	PST-R6FLT (S)	SWI-1122 (S)
OKC 1134 (Northbridge) (V)	RAD-CD1 (S)	Tifway (V)
Numex Sahara (S)	Riviera (S)	Veracruz (S)
OKS 2004-2 (S)	PSG 9BAN (Royal Bengal) (S)	Yukon (S)
Patriot (V)	Sunsport (S)	
Premier (V)	SWI-1057 (S)	

[a]Cultivars were randomized within 2 by 2 m plots across three replications.

[b]Abbreviations: (S), seeded; (V), vegetative.

Table 2. Goosegrass area under the control progress curve (AUCPC) regression equations and r^2 values for triclopyr rate as affected by increasing topramezone rate.

Triclopyr rate	Equation	r^2
g ae ha^{-1}		
0	y = 544.9ln(x) + 2003	0.74
140	y = 359.5ln(x) + 2585	0.65
280	y = 219.7ln(x) + 3119	0.52
560	y = 269.1ln(x) + 3299	0.81

Table 3. Goosegrass area under the control progress curve (AUCPC) regression equations and r^2 values for topramezone rate as affected by increasing triclopyr rate.

Topramezone rate	Equation	r^2
g ae ha^{-1}		
0	y = 164.7ln(x) + 1102	0.86
2.46	y = 128.8ln(x) + 1431	0.73
6.14	NS	NS
12.3	NS	NS
18.4	NS	NS
24.6	NS	NS

Table 4. Goosegrass cover in field trials at 56 and 70 days after initial treatment (DAIT) and three trial locations, averaged over topramezone rate without and with triclopyr at 140 g ae ha^{-1}.

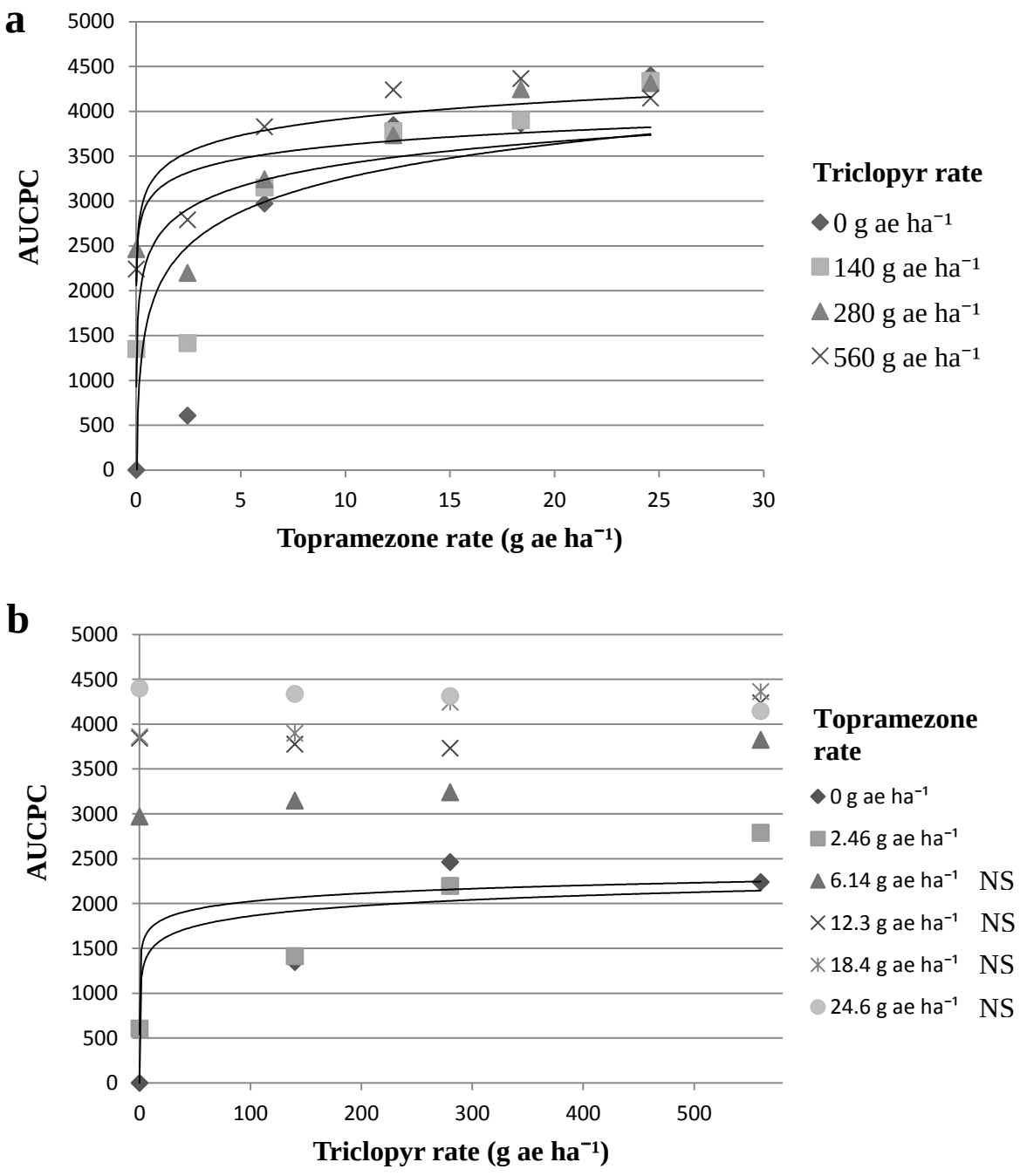
Treatment	Rate	Location 1		Location 2		Location 3	
		DAIT					
	g ae ha^{-1}	56	70	56	70	56	70
		Goosegrass cover (%)					
Topramezone	-	3.5a[bc]	6.3a	0a	0a	0a	0a
Topramezone + Triclopyr	140	1.7b	2.7b	0a	0a	0a	0a
Untreated check[a]	-	25	19	25	19	25	19

[a]Means from the untreated check were averaged over all three trial locations and included for comparison, but not included in the factorial treatment statistical analyses.

[b]Means were separated using Fisher's Protected LSD test at P = 0.05.

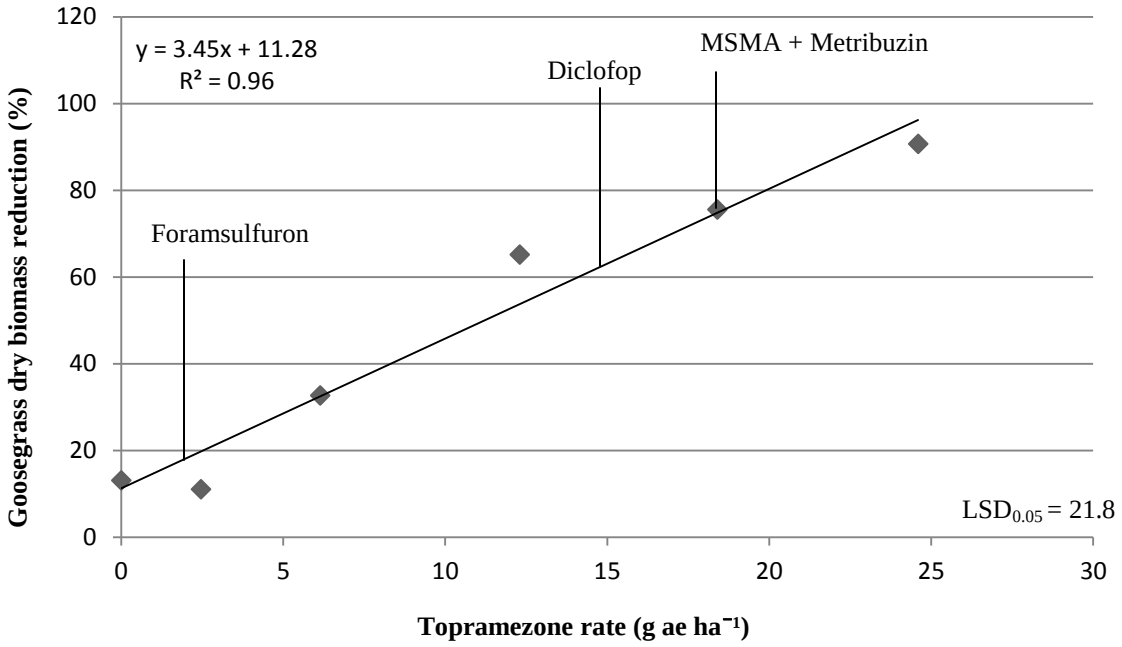
[c]Means followed by the same letter within the same location and assessment date do not significantly differ according to Fisher's Protected LSD test at P = 0.05.

Figure 1. Area under the goosegrass control progress curve (AUCPC) for each triclopyr rate as affected by increasing topramezone rate (a) and each topramezone rate as affected by increasing triclopyr rate (b).



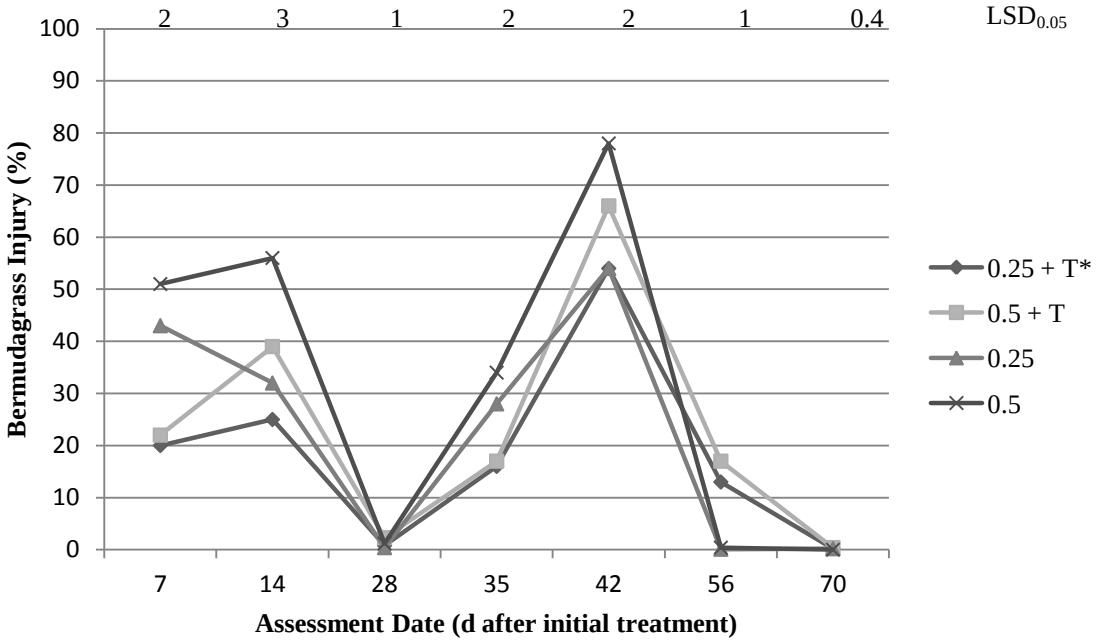
*Regression equations and r^2 values are in Tables 2 and 3.

Figure 2. Goosegrass dry biomass reduction as influenced by topramezone rate and averaged over triclopyr at 61 days after initial treatment.



Goosegrass biomass reduction means for foramsulfuron, diclofop, and MSMA plus metribuzin were included as standard comparisons with topramezone rate and averaged over triclopyr rate. Standard comparisons were not included in the statistical analyses of goosegrass biomass reduction as affected by topramezone/triclopyr programs.

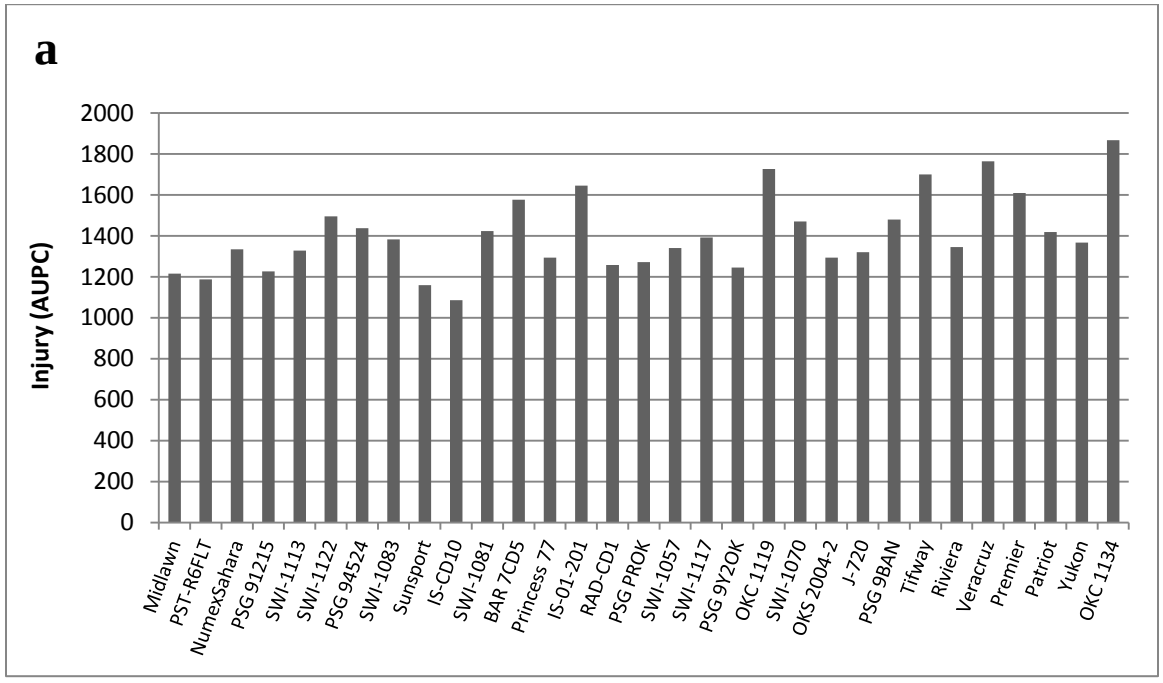
Figure 3. Visual estimates of bermudagrass injury[a] over time as influenced by topramezone with and without triclopyr.

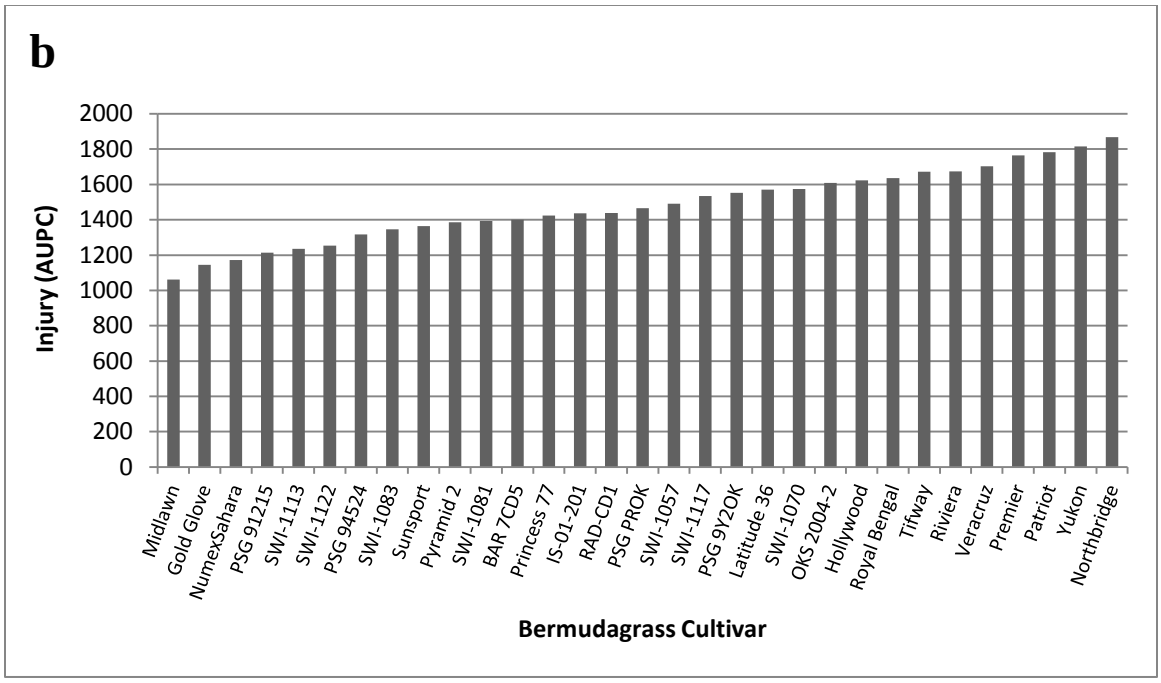


*Abbreviations: T, triclopyr; UTC, untreated check.

[a]Bermudagrass injury was pooled over 31 cultivars.

Figure 4. Area under the injury progress curve for bermudagrass cultivars as influenced by topramezone with (a) or without (b) triclopyr.





Chapter V. Herbicide Efficacy as Influenced by Goosegrass Growth Stage

Michael C. Cox and Shawn D. Askew[1]

Greenhouse trials were conducted to determine if and to what extent goosegrass growth stage affects efficacy of nine, postemergent herbicides or programs documented to have goosegrass activity. There was a significant herbicide by goosegrass growth stage interaction ($P < 0.05$) at 4 and 8 weeks after treatment (WAT) for visual goosegrass control and also at 8 WAT for goosegrass dry weight. As goosegrass growth stage increased from four- to five-leaf to greater than eight-tiller stage, goosegrass control and biomass reduction decreased among all of the herbicides tested in this experiment except topramezone and MSMA plus metribuzin. Topramezone, metamifop, diclofop, and MSMA plus metribuzin controlled goosegrass at the greater than eight-tiller stage 77, 80, 94, and 99%, respectively, and better than all other treatments, 8 WAT. Similar trends were observed with goosegrass dry weights except that weights with foramsulfuron were statistically equivalent to those with topramezone at 8 WAT. Diclofop and MSMA plus metribuzin controlled goosegrass regardless of growth stage. These data suggest that one application of sulfentrazone is only effective for seedling stage (pre-tiller) goosegrass control; foramsulfuron, topramezone, and metribuzin suppress all growth stages of goosegrass; and diclofop, sulfentrazone plus metribuzin, fenoxaprop, and metamifop control up to three-tiller stage goosegrass. Future research should further evaluate herbicides labeled for goosegrass control in bermudagrass turf at different growth stages in the field to improve these

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control distinctions in natural environmental conditions and with the addition of turfgrass competition.

NOMENCLATURE: diclofop; fenoxaprop; foramsulfuron; metribuzin; MSMA; metamifop; sulfentrazone; topramezone; goosegrass, *Eleusine indica* (L.) Gaertn. ELEIN; bermudagrass, *Cynodon dactylon* (L.) Pers. CYNDA.

KEYWORDS: foliar, postemergence, tiller.

INTRODUCTION

Goosegrass is classified as one of the five most troublesome weeds in the world (Holm et al. 1991) and currently one of the hardest to control weeds in warm-season turfgrasses of temperate and tropical climates, in particular, bermudagrass athletic fields and golf courses (Arrieta et al. 2009; Busey 2001; Johnson 1980; McCarty 1991). Monosodium methanearsonate (MSMA) plus metribuzin as a two to three application treatment 7 to 10 days apart has been effective for postemergence goosegrass control in bermudagrass turf for decades (Brennan et al. 1992; Busey 1999; Johnson 1980; McCarty 1991; McElroy et al. 2007; Murdoch and Ikeda 1974; Nishimoto and Murdoch 1999; Wiecko 2000). MSMA is registered for weed control in cotton, turfgrass, and forestry situations (EPA 2013; Ross and Lembi 2009; Senseman 2007); however, restrictions are currently in place for applications of MSMA in turfgrass (EPA 2013). A recent lack in postemergence goosegrass control has been attributed mostly to the loss or restrictive uses of MSMA in turf.

Several postemergent herbicides have been reported in peer-reviewed literature to effectively control goosegrass, but only a few of these herbicides will control goosegrass at growth stages exceeding two to three tillers. Other than MSMA plus metribuzin (Johnson 1975; Murdoch and Ikeda 1974; Nishimoto and Murdoch 1999), the following herbicides alone or in specific combinations have been used for postemergence goosegrass control in bermudagrass: foramsulfuron (Busey 2004; McCullough et al. 2012), metribuzin (Busey 2004; Johnson 1980; Nishimoto and Murdoch 1999), sulfentrazone (McCullough et al. 2012; Anonymous 2008), and diclofop (Busey 1999; McCarty 1991; Nishimoto and Murdoch 1999).

Busey (2004) reported greater than 85% control of mature goosegrass with two applications of foramsulfuron at 0.029 or 0.044 kg ai ha^{-1} plus metribuzin at 0.105 to 0.210 kg ai ha^{-1}; however, bermudagrass phytotoxicity was equivalent to MSMA plus metribuzin, which may prevent turf managers from using this program if recovery time is restricted. Single and combination treatments of sulfentrazone and nicosulfuron controlled mature goosegrass 84% at 9 weeks after initial treatment (WAIT), and significantly better than foramsulfuron at 0.04 kg ai ha^{-1} and nicosulfuron at 0.11 kg ai ha^{-1} alone and sequential applications of nicosulfuron plus foramsulfuron (McCullough et al. 2012). Nicosulfuron is not currently labeled for use in turfgrass. Single applications of diclofop controlled mature goosegrass on putting greens but not areas with higher mowing heights such as fairways (McCarty 1991; Murdoch and Nishimoto 1982). Illoxan® is also a restricted use herbicide with a maximum use rate of only 1.02 kg ai ha^{-1} per application and rates of 1.53 kg ai ha^{-1} yr^{-1} are required for control of young goosegrass (Anonymous 2004), making diclofop as a stand-alone treatment mostly ineffective for controlling mature goosegrass in a bermudagrass fairway or sports field (McCarty 1991). Application of diclofop at 1.7 kg ai ha^{-1} plus metribuzin at 0.56 kg ai ha^{-1} as a tank-mix controlled mature goosegrass 90% at 7 weeks after treatment (WAT) and equivalent to control with MSMA (2.2 kg ai ha^{-1}) plus metribuzin (0.56 kg ai ha^{-1}) followed by MSMA (2.2 kg ai ha^{-1}) 1 wk later (Nishimoto and Murdoch 1999). Injury to common bermudagrass following this treatment was also equivalent to or slightly less than MSMA plus metribuzin; however, injury was transient and not evident 3 WAT (Nishimoto and Murdoch 1999).

The most effective postemergence goosegrass control program in bermudagrass sports and golf turf is currently not clearly elucidated. Generally, many other factors are involved in choosing the most appropriate and effective treatment for controlling goosegrass. In sports

fields, small “windows” between sports events often allow turf managers to use only those herbicides that will not injure the turfgrass or prevent recovery. Postemergent herbicides such as foramsulfuron, sulfentrazone, and diclofop are very popular among golf course superintendents and sports field managers, however, goosegrass growth stage, population density, budget, and environmental conditions are other important factors that affect efficacy of these herbicides. Peer-reviewed literature does not describe specific programs for postemergence goosegrass control at different growth stages and often shows varying results across different climates. Although most herbicide labels indicate a relative size or maturity level at which they effectively control or suppress goosegrass, more research is needed to delineate specific goosegrass growth stage(s), timings, and application frequencies that are most efficacious for each of these herbicides as results have been shown to vary. Screening of other herbicides with activity on annual grasses should also be evaluated for possible goosegrass control.

The objective of this research was to elucidate any goosegrass growth stage limitations of herbicides previously documented to control goosegrass and/or those currently labeled for postemergence goosegrass control.

MATERIALS AND METHODS

Goosegrass seed collected from a local population in Montgomery County, Virginia with no history of herbicide treatment was spread evenly in flats filled with a 2:1 ratio by volume of sand/silt loam soil that was previously sterilized in an autoclave at 115 ˚C for 30 minutes to minimize weed competition and disease pressure. Flats were placed in two separate greenhouses under automatic irrigation and lighting with a 14-hour photoperiod per day and a day/night

temperature range of 27/18 ˚C to encourage germination. When plants reached a two- to four-leaf growth stage, they were carefully removed from germination flats and transplanted into cone-tainers (SC-10 Super Cell Ray Leach "Cone-tainers™", Hummert International, 1415 N. W. Moundview Drive, Topeka, Kansas 66618) filled with a similar 2:1 sand:soil medium. Plants were allowed one week to acclimate before herbicide treatment. A randomized complete block experimental design with a 10 by 3 factorial treatment design was used for this study. Goosegrass plants were separated into three growth stage categories: four- to five-leaf, two- to three-tiller, and greater than eight-tiller. Treatments included a single foliar application of the following herbicides on October 16, 2013 to two replications of this study conducted in separate greenhouses: foramsulfuron (Revolver® herbicide, Bayer Environmental Science, 2 T. W. Alexander Drive, Research Triangle Park, NC 27709) at 43.7 g ai ha^{-1} plus a methylated seed oil surfactant at 0.5% v/v, sulfentrazone (Dismiss® Turf herbicide, FMC Corporation, 1735 Market Street, Philadelphia, PA 19103) at 420 g ai ha^{-1}, diclofop (Illoxan® 3EC herbicide, Bayer Environmental Science, 2 T. W. Alexander Drive, Research Triangle Park, NC 27709) at 1,140 g ai ha^{-1}, metribuzin (Sencor® herbicide, Bayer Environmental Science, 2 T. W. Alexander Drive, Research Triangle Park, NC 27709) at 560 g ai ha^{-1}, sulfentrazone (same rate as above) plus metribuzin at 280 g ai ha^{-1}, fenoxaprop (Acclaim® Extra herbicide, Bayer Environmental Science, 2 T. W. Alexander Drive, Research Triangle Park, NC 27709) at 45 g ai ha^{-1} plus a nonionic surfactant at 0.25% v/v, topramezone at 12.3 g ae ha^{-1} (Pylex™ herbicide, BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709), metamifop (SAH-001 10% EC, Summit Agro International Ltd., Tokyo, Japan) at 400 g ai ha^{-1}, and monosodium methanearsonate (MSMA 6 Plus herbicide, Drexel Chemical Company, P.O. Box 13327, Memphis, TN 38113-0327) at 2,240 g ai ha^{-1} plus metribuzin at 560 g ai ha^{-1}. An untreated

check was included for comparison. Plants were fertilized with foliar applications of Miracle-Gro® 20-20-20 as needed to maintain growth throughout the duration of the study. Plants were placed under automatic irrigation, a 27/18 ˚C day/night temperature regime, and a 530 µmol m^{-2} s^{-1} 14-hour photoperiod per day. Goosegrass color and control were assessed at 7, 14, 28, 35, 42, 49, and 56 days after initial treatment (DAIT). Goosegrass green color was evaluated based on a scale from 0-9, where 0 = no green tissue and 9 = complete green tissue. Goosegrass control ratings were recorded as visually-estimated percentages, with 0% being no injury and 100% being death of all visible foliage (Frans et al. 1986). All plant material was harvested at the soil line at 56 DAIT and dried at 50 ˚C for approximately 72 hours. Aboveground dry weight was recorded for data analysis. Final data were determined to be normal using the NORMAL option in PROC UNIVARIATE and Shapiro-Wilk statistic. Data were subjected to ANOVA in SAS® 9.2 (SAS Institute Inc, Cary, NC) with sums of squares partitioned to reflect the herbicide by goosegrass growth stage factorial design and trials, which were considered random. Main effects and interactions were tested using mean square error associated with the random variable interaction (McIntosh 1983). Appropriate means were separated with Fisher's Protected LSD test at $P = 0.05$.

RESULTS AND DISCUSSION

Herbicide efficacy was significantly affected by goosegrass growth stage, as indicated by significant herbicide by goosegrass growth stage interactions ($P < 0.05$) at 4 and 8 weeks after treatment (WAT) (Table 1) and also at 8 WAT for goosegrass dry weight (Table 2). Goosegrass at the four- to five-leaf stage was controlled 82 to 100% by foramsulfuron, sulfentrazone,

diclofop, sulfentrazone plus diclofop, fenoxaprop, metamifop, and MSMA plus metribuzin, which was significantly better than control from all other treatments, 8 WAT (Table 1). Although topramezone at 12.3 g ae ha^{-1} controlled four- to five-leaf stage goosegrass 83% at 4 WAT and provided statistically equivalent control to the previously mentioned treatments, goosegrass recovery rendered this treatment ineffective for all goosegrass growth stages at 8 WAT according to visual control ratings (Table 1) and dry weight measurements (Table 2). Contrary to these observations, one application of topramezone at 12.3 g ae ha^{-1} or two applications at 6.14 g ae ha^{-1} controlled multi-tiller goosegrass in unpublished greenhouse and field studies (author's personal observations). Although it has been documented that topramezone will control goosegrass at rates exceeding 12.3 g ae ha^{-1} (Anonymous 2013; Smith et al. 2013), it is currently only labeled for use in cool-season turf (Anonymous 2013). Our objective, however, was to determine how it performs compared to other industry standards when used at rates tolerated by bermudagrass turf. In this greenhouse study, the lack of goosegrass control with topramezone may be due to lower light intensity in a greenhouse setting compared to natural sunlight, no desirable turfgrass competition, and less lateral root growth for absorbing maximum amounts of the herbicide, as the plants were grown in conetainers only four cm in diameter. Nevertheless, topramezone controlled goosegrass at the greater than eight-tiller stage 77% at 8 WAT, which was equivalent to or greater than control with all other treatments except MSMA plus metribuzin (Table 1). These data corroborate results documented by McCarty (1991) where diclofop controlled multi-tiller goosegrass by greater than 90% in a single application. Wiecko (2000) indicated that two sequential applications of MSMA plus metribuzin at 2.2 plus 0.14 kg ai ha^{-1} were needed to control goosegrass above 97% at 8 to 10 weeks after initial treatment (WAIT); however, Nishimoto and Murdoch (1999) showed greater than 90%

goosegrass control at 7 WAT with only one application of MSMA plus metribuzin at 2.2 plus 0.56 kg ai ha^{-1}. These data also suggest that topramezone still controls multi-tiller goosegrass better than other herbicides labeled for use in bermudagrass turf, although it appears to not be more efficacious than diclofop or MSMA plus metribuzin when applied at 12.3 g ae ha^{-1}, which is half of the recommended label rate. Similar trends were observed with goosegrass dry weights except that weights with foramsulfuron were statistically equivalent to those with topramezone at 8 WAT (Table 2). Foramsulfuron at 0.044 kg ai ha^{-1} did not control goosegrass above 76% in studies conducted by Busey (2004) except when combined with metribuzin as a tank-mix. Nevertheless, bermudagrass phytotoxicity levels exceeded 30% even 4 WAIT with this treatment combination (Busey 2004), likely decreasing its vitality for bermudagrass turf managers.

As goosegrass growth stage increased from four- to five-leaf to greater than eight-tiller stage, goosegrass control and biomass reduction decreased among all of the herbicides tested in this experiment except topramezone and MSMA plus metribuzin (Tables 1 and 2). Still, these data suggest that MSMA plus metribuzin controls goosegrass better than all other herbicides except diclofop based on biomass reduction and regardless of growth stage (Table 2); nevertheless, control with metamifop was equivalent to control with diclofop and MSMA plus metribuzin based on visual ratings (Table 1). Metamifop is not registered for use in turfgrass at this time, however.

Foramsulfuron, metribuzin, and topramezone controlled goosegrass 63-86%, 55-62%, and 59-77%, respectively, and growth stage did not significantly affect control at 8 WAT (Table 1). Sulfentrazone controlled four- to five-leaf goosegrass 85% and significantly better than at all other growth stages 8 WAT. (Table 1). Diclofop, sulfentrazone plus metribuzin, fenoxaprop, and metamifop controlled four- to five-leaf and two- to three-tiller stage goosegrass 100%, 68-82%,

99-100%, and 100%, respectively, and significantly better than control of eight-tiller or greater goosegrass 8 WAT (Table 1). Although MSMA plus metribuzin only controlled two- to three-tiller goosegrass 84% and significantly less than control of four- to five-leaf goosegrass (100%), it controlled greater than eight-tiller goosegrass 99%, 8 WAT, which was equivalent to control at four- to five-leaf plants (Table 1).

These data suggest that one application of sulfentrazone is only effective for seedling stage (pre-tiller) goosegrass control; foramsulfuron, topramezone, and metribuzin suppress all growth stages of goosegrass; and diclofop, sulfentrazone plus metribuzin, fenoxaprop, and metamifop control up to three-tiller stage goosegrass. It is apparent that many factors contribute to the success or failure of goosegrass control with these herbicides, as seen when comparing varying conditions between greenhouse and field trials. Inconsistent results are often the norm with goosegrass control studies, and many herbicides perform differently across varying geographical regions as well due to different temperature regimes, precipitation amounts, humidity levels, soil types, and other factors. The results from this study, however, do provide better distinctions for choosing the most appropriate herbicide(s) based on goosegrass growth stage. Future research should further evaluate herbicides labeled for goosegrass control in bermudagrass turf at different growth stages in the field to improve these control distinctions in natural environmental conditions and with the addition of turfgrass competition.

ACKNOWLEDGEMENTS

The authors would like to thank Chris Dowdy and Tori Kellinger for assistance in experimental setup and harvesting and weighing samples. Special thanks also go to Tyler Brewer and Kevin Hensler for irrigation assembly.

LITERATURE CITED

Anonymous (2004) Illoxan herbicide label. Bayer Environmental Sciences, 2 T. W. Alexander Drive, Research Triangle Park, NC 27709.

Anonymous (2008) Dismiss 4SC herbicide label. FMC Corporation, 1735 Market Street, Philadelphia, PA 19103.

Anonymous (2013) Pylex herbicide product label. BASF Corporation, Research Triangle Park, NC 27709.

Arrieta C, Busey P, and Daroub S (2009) Goosegrass and bermudagrass competition under compaction. Agron J 101(1):11-16

Brennan BM, Higashi AK, and Murdoch CL (1992) Estimated pesticide use on golf courses in Hawaii. Honolulu, HI: Hawaii Institute of Tropical Agriculture and Human Resources Research Extension Series 137. 16p.

Busey P (1999) Goosegrass and golf don't mix. GCM September 63-66

Busey P (2001) Goosegrass most difficult weed in South Florida. Fla Turf Digest 18:24 27

Busey P (2004) Goosegrass (Eleusine indica) control with foramsulfuron in bermudagrass (Cynodon spp.) turf. Weed Technol 18(3):634-640

[EPA] Environmental Protection Agency (2013) Organic Arsenicals. Washington, DC: U. S. Environmetal Protection Agency. http://www.epa.gov/oppsrrd1/reregistration/organic_arsenicals_fs.html Accessed: August 2013.

Frans R, Talbert R, Marx D, and Crowley H (1986) Experimental design and techniques for measuring and analyzing plant responses to weed control practices. Pages 29-46 *in* Camper ND, ed. Research methods in weed science. Illinois: Southern Weed Sci Soc

Holm LG, Plucknett DL, Pancho JV, and Herberger JP (1991) The World's Worst Weeds. Malabar, Florida: Kriegen.

Johnson BJ (1975) Postemergence control of large crabgrass and goosegrass in turf. Weed Sci 23:404-409

Johnson BJ (1980) Goosegrass (*Eleusine indica*) control in bermudagrass turf. Weed Sci 28(4):378-381

McCarty LB (1991) Goosegrass (*Eleusine indica*) control in bermudagrass (*Cynodon* spp.) turf with diclofop. Weed Sci 39:255-261

McCullough PE, de Barreda DG, and Raymer P (2012) Nicosulfuron use with foramsulfuron and sulfentrazone for late summer goosegrass (*Eleusine indica*) control in bermudagrass and seashore paspalum. Weed Technol 26(2):376-381

McElroy JS, Robinson DK, Samples T, Sorochan JC, and Breeden G (2007) Weed Management Recommendations for Professional Turfgrass Managers: Athletic Fields, Golf Courses, Commercial Lawns and Turfgrass Sod. Knoxville, TN: University of Tennessee Agricultural Extension Service #PB1539. 54 p.

McIntosh, M. S. 1983. Analysis of combined experiments. Agron J 75:153–155

Murdoch CL and Ikeda D (1974) Goosegrass control in bermudagrass turf with combinations of MSMA and *s*-triazines. Agron J 66:712-714

Nishimoto RK and Murdoch CL (1999) Mature goosegrass (*Eleusine indica*) control in bermudagrass (*Cynodon dactylon*) turf with a new metribuzin-diclofop combination. Weed Technol 13:169-171

Ross MA and Lembi CA (2009) Applied Weed Science: Including the Ecology and Management of Invasive Species. 3rd edn. Upper Saddle River, NJ: Pearson Education, Inc. Pp. 258-259.

Senseman SA (2007) Herbicide Handbook. 9th ed. Champaign, IL: Weed Sci Soc Am Pp. 377-379.

Smith AN, Cox MC, Askew SD, and Miller K (2013) Topramezone for crabgrass and goosegrass control in cool season turf. Page 3 in Proceedings of the 66th Annual Southern Weed Science Society meeting. Houston, TX: Weed Science Society of America.

Wiecko G (2000) Sequential herbicide treatments for goosegrass control in bermudagrass (*Cynodon dactylon*) turf. Weed Technol 14:686-691

Table 1. Goosegrass control as influenced by herbicide and goosegrass growth stage at 4 and 8 weeks after treatment (WAT).

Treatment	Rate	Growth Stage						
		4-5 leaf		2-3 tiller		>8 tiller		
		Assessment Date						
		4 WAT	8 WAT	4 WAT	8 WAT	4 WAT	8 WAT	8 WAT
	g ai ha^{-1}	Control (%)[a]						LSD
Foramsulfuron[c]	43.7	90ab	83a	72bc	86ab	62cd	63cd	28
Sulfentrazone	420	85ab	85a	18d	10c	15e	20e	23
Diclofop[d]	1,140	100a	100a	100a	100a	82b	94ab	3
Metribuzin[d]	560	74b	55b	62c	62b	74bc	54d	39
Sulf[b] + Metrib	420 + 280	88ab	82a	68c	68b	69bc	48d	34
Fenoxaprop[d]	45.0	99a	100a	95ab	99a	52d	45d	26
Topramezone[c]	12.3	83ab	59b	69bc	61b	84b	77bc	26
Metamifop[d]	400	100a	100a	99a	100a	80b	80abc	13
MSMA + Metrib	2,240 + 560	100a	100a	94ab	84ab	100a	99a	13
UTC	-	0c	0c	0d	0c	0e	0f	0

[a]Means followed by the same letter within a specific goosegrass growth stage and assessment date do not differ significantly according to Fisher's Protected LSD test (P = 0.05).

[b]Abbreviations: sulf, sulfentrazone; metrib, metribuzin; UTC, untreated check.

[c]A methylated seed oil surfactant was included with this treatment at 0.5% v/v as according to label specifications.

[d]A nonionic surfactant was included with this treatment at 0.25% v/v as according to label specifications.

Table 2. Goosegrass dry weight as influenced by herbicide and goosegrass growth stage at 8 weeks after treatment (WAT).

		8 WAT		
		Growth Stage		
Treatment	Rate	4-5 lf[b]	2-3 tiller	>8 tiller
	g ai ha^{-1}		g pot^{-1} [a]	
Foramsulfuron[c]	43.7	0.046bc	0.095cd	0.44cd
Sulfentrazone	420	0.075 bc	0.63 a	0.72 a
Diclofop[d]	1,140	0.0021 c	0.095 cd	0.28 de
Metribuzin[d]	560	0.11 b	0.19 bc	0.45 c
Sulf + Metrib	420 + 280	0.055 bc	0.16 bcd	0.62 ab
Fenoxaprop[d]	45.0	0.0047 c	0.051 cd	0.50 bc
Topramezone[c]	12.3	0.098 b	0.27 b	0.41 cd
Metamifop[d]	400	0.00082 c	0.0043 d	0.45 c
MSMA + Metrib	2,240 + 560	0.00047 c	0.068 cd	0.14 e
UTC	-	0.41 a	0.66 a	0.74 a

[a]Means followed by the same letter within a specific goosegrass growth stage do not significantly differ according to Fisher's Protected LSD test (P = 0.05).

[b]Abbreviations: lf, leaf stage; sulf, sulfentrazone; metrib, metribuzin; UTC, untreated check.

[c]A methylated seed oil surfactant was included with this treatment at 0.5% v/v as according to label specifications.

[d]A nonionic surfactant was included with this treatment at 0.25% v/v as according to label specifications.

APPENDIX A. Supplemental Images.

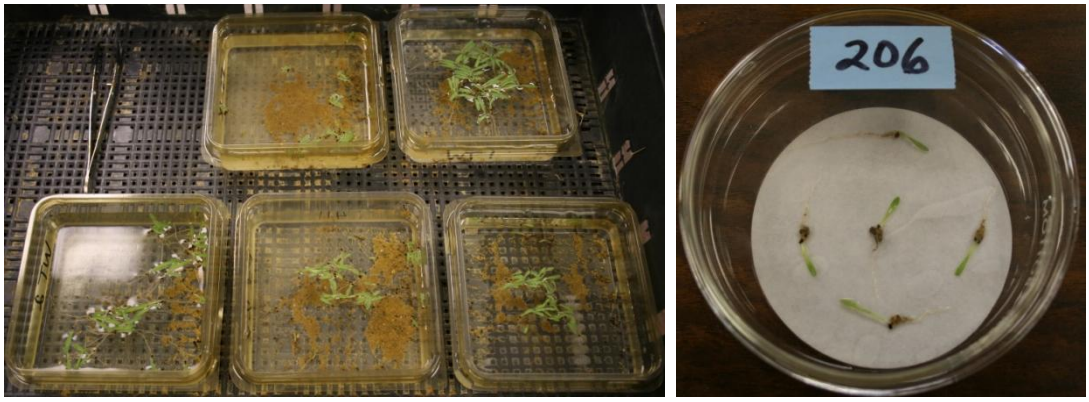

Image 1. Goosegrass seedlings separated by accession (left), treated with oxadiazon by dipping into respective herbicide solution, and placed on herbicide-saturated filter paper in Petri plate (right) during electrolyte leakage experiment.

Image 2. *Eleusine indica* seedlings after 3 weeks acclimation in the greenhouse and before herbicide application in the topramezone and triclopyr dose-response study.

Image 3. *Cynodon dactylon* cores after 3 weeks acclimation in the greenhouse and before herbicide application.

Image 4. Goosegrass and bermudagrass cores sorted by replicate before herbicide application in a spray chamber.

Image 5. Untreated bermudagrass (left) and bermudagrass treated with topramezone alone at 12.3 g ae ha^{-1} (right), 14 days after treatment (DAT).

Image 6. Untreated bermudagrass (left) and bermudagrass treated with topramezone at 12.3 g ae ha^{-1} plus triclopyr at 240 g ae ha^{-1} (right), 14 days after treatment (DAT).

Image 7. Superior goosegrass control after two applications of topramezone at 6.14 g ae ha^{-1}, 8 weeks after initial treatment (WAIT).

Image 8. Response of 31 bermudagrass varieties to topramezone (6.14 and 12.3 g ae ha^{-1}) with (brown subplots) and without triclopyr (white subplots) (240 g ae ha^{-1}) programs, 14 days after second application (peak injury).

Image 9. Complete recovery of 31 bermudagrass varieties after two sequential applications of topramezone (6.14 and 12.3 g ae ha^{-1}) with and without triclopyr (240 g ae ha^{-1}) programs, 28 days after second application.

Image 10. Goosegrass growth stages and treatments randomized within replications before herbicide application in the growth chamber.