Assessment of Herbicides for Control of Non-Native Species: Italian Ryegrass (Lolium Perenne Spp. Multiflorum), Tropical Spiderwort (Commelina Benghalensis), and Tropical Soda Apple (Solanum Viarum)

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By

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A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in Weed Science
in the Department of Plant and Soil Science

Mississippi State, Mississippi

December 2012

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Title of Study: Assessment of herbicides for control of non-native species: Italian ryegrass (*Lolium perenne* spp. *multiflorum*), tropical spiderwort (*Commelina benghalensis*), and tropical soda apple (*Solanum viarum*)

Pages in Study: 97

Candidate for Degree of Doctor of Philosophy

Herbicides were evaluated for control of non-native and invasive plants namely tropical soda apple (TSA) (*Solanum viarum*), tropical spiderwort or Benghal dayflower (*Commelina benghalensis*), and Italian ryegrass (*Lolium perenne* spp. *multiflorum*) from 2007 to 2012 in greenhouse at Plant Science Research Center, Mississippi State University, Starkville-Mississippi. In TSA study, aminopyralid at 0.88 or 0.12 kg ae/ha, and triclopyr + picloram + 2,4-D at (0.56 + 0.15 + 0.56) kg ae/ha controlled TSA seedlings emergence ranged from 83 to 96% at premergence (PRE), and 1 month after treatments (MAT). All herbicides failed to provide more than 63% control of TSA at 3 and 6 MAT. In Benghal dayflower study, bentazon, bromoxynil, chlorimuron ethyl plus tribenuron methyl, dimethenamid-P, nicosulfuron plus rimsulfuron, primisulfuron-methyl, S-metolachlor plus glyphosate plus mesotrione, and sulfosulfuron provided less than 50% control of Benghal dayflower during 6 weeks of evaluation whereas other herbicides: aminocyclopyrachlor (34-96%) ametryn (2-55%), atrazine (2-68%), diclosulam (12-67%), flumioxazin (59-83%), saflufenacil (24-78%), and sulfentrazone

(67-96%) provided variable control of Benghal dayflower. In Italian ryegrass study, 50 seeds of F1 generation of resistant biotype '49E' and susceptible biotypes 'Gulf' and 'Marshall' of Italian ryegrass which were previously grown in mixture were used. Each biotype's F1 generation responce to imazapyr at 0, 1, and 2% under PRE, early post emergence (EPOST) and late postemergence (LPOST) was evaluated. At PRE, imazapyr at 1 and 2% reduced '49E' seedlings emerged 3 and 18%, respectively, but both rates failed to have any significant impact on fresh biomass weight compared to untreated. At EPOST, survival of '49E' shoots were reduced 3 to 10% by both rate of imazapyr whereas only imazapyr at 1% caused 0.74 to 3.8 % fresh biomass reduction. At LPOST, '49E' shoot survival was reduced 9 to 12% by both rate of imazapyr whereas both rate of imazapyr reduced '49E' fresh biomass 3 to 31%. In all the PRE, EPOST, and LPOST experiments, 'Gulf' and 'Marshall' were still susceptible to the imazapyr.

DEDICATION

This dissertation is dedicated to my mother Rosina Ama Yawobani (81 years old), my late father Nelson Anku Kraka, and my grandmother Apollonian Diali Pidah (107 years old) whose sacrifices and unflinching support throughout my formative years inspired me to be a better person in all aspects of life; and also to my siblings, Rev. Fr. Raphael Tawiah, John Attah Agbeko, and Emmanuel Koku Kraka whose selfless nature, encouragement, and financial support enabled me achieve this academic milestone. I am proud of you all and I sincerely appreciate your love and kindness and may God bless you all.

ACKNOWLEDGEMENTS

To God be the glory for leading me so far to this ladder of my academic journey. First and foremost, I would like to express my heart felt appreciation to Dr. John D. Byrd Jr. my major advisor for his patience, motivation, guidance, support as well as knowledge imparted me ever since I became his graduate student thoroughout the successful completion of this Ph.D. degree. I would also want to express my profound gratitude to Dr. Howard W. Chambers, my minor advisor. Besides, I am grateful to my committee members Drs. Joseph H. Massey, John D. Madsen, and Charles T. Bryson for their time, encouragements, and comments that helped shape this study and my entire academic life at this noble academic institution.

I appreciate all the assistance and contribution given to me by Mr. James Taylor and Stanley Self (former) of Department of Plant and Soil Sciences towards my research in the greenhouse. My similar appreciation goes to Dr. Dennis Rowe, Statistician, for his assistance and guidance in my data analysis. I would also like to thank Mississippi Department of Agriculture Bureau of Plant Industry for providing necessary permit for conducting research on these invasive plants: tropical soda apple, and Benghal dayflower.

Several people who have had a colossal influence on my life deserve thanks as well, so I would like to express my gratitude to my family: Salome Tsevi and Veronica Tsevi (aunts), Mr. Martin K. Tsevi (uncle); Clara Klu, Salome Klu, Eliplim Kraka, Cephas Attah Quashi (siblings); Florence Kudzonu, Evenlyn Adjagah, Pa Christian

Cobbinah, Papa Eshun F. Cobbinah, Richard Tsevi, Jerry Yevu, and Mr. Ben K. Hotor (cousins) for their inspiration, prayers, encouragements and financial supports throughout my academic life.

Finally my sincere thanks also go to Dr. Peter Y. Ampim (for introducing me to Mississippi State University), Ms. Dzifa Kugbega, Mrs. Esenam Ampim, Mrs Suzie Yanney, Michael Yanney, Joseph F. Morrison, Mawunyo D. Owu, Francis Akwetey, Sebastian T. Agbozo, Edem Mawuko, George A. Agana, Ishahu Abubakari, Herve Sanghapi, members of African Students Association of Mississippi State University, 2007 to 2012, and Starkville Multicultural Lions Club, 2008 to 2012 for their help and encouragement.

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CHAPTER I

INTRODUCTION

Non-native species are considered, "alien or exotic species that have been introduced by people from other continents, ecosystems, or habitat to places where they do not occur and would not likely have been dispersed to by wind, water, wildlife, or other natural means" (Swearingen et al. 2010). Non-native species do not only contribute to the integrity of ecosystem but also pose negative impacts ecologically (wild fires, soil erosion, siltation of dams and estuaries, flooding water quality), socially, and economically leading to global concerns (Madren 2011). Humans contribute greatly to their success to become invasive because they continue to alter global environment, change global biogeochemical cycles, and enhance mobility of biota (Chapin et al. 2000). Despite the fact that prehistoric invasions did occur, they happened at slower frequencies and at regional levels compared with human-aided invasions which occur very frequently and also at global levels (Ricciardi 2007). Climate change which is occurring due to mostly human actions can have impacts on success of non-native species invasions. Hellmann et al. (2008) suggested five potential consequences of climate change for invasive species which include altering mechanism of their transport and introduction, altering climatic constraints on them, altering distribution of existing ones, altering impact of existing ones, and altering effectiveness of management strategies. In the continental U.S.A., the biological invasion (plants) ranked second apart from habitat destruction as threat to biodiversity loss but vice versa for Hawaii State in particular (Wilcove et al. 1998).

Non-native species in general are introduced to new environments for intentional beneficial uses whiles some of them were introduced unintentionally. This clearly shows that not all non-native species are invasive species. However, the intentionally introduced non-native species that escape their range of introduction coupled with unintentionally introduced species that are changing ecosystem structure leading to ecological, social and economic negative impacts are the major concern. Westbrooks (1998) wrote, "invasive plants have been called non-natives, exotics, aliens, non-indigenous harmful species, weeds, and a host of other names." According to Thompson (1991), "an invasive species can be considered successful if it colonizes a wide geographical area, exists over a range of localized environment conditions, and/or forms a dominant component of the habitat into which it spreads." Office of Technology Assessment (OTA) (1993), estimated more than 2000 non-indigenous plants species currently exist in the U.S.A. which is far greater than the number known 100 years ago. However, about 350 of these non-native plants are considered serious and dangerous invaders (Babbitt 1998). In the U.S.A. for instance, some introduced non-indigenous crops such as soybeans [*Glycine max* (L.) Merr.], and wheat (*Triticum aestivum* L.) are beneficial crops in mainstay agriculture (OTA 1993). Other introduced plants and their purported uses include, Johnsongrass [*Sorghum halepense* (L.) Pers.] for forage; Brazilian peppertree (*Schinus terebinthifolius* Raddi) as an ornamental; bermudagrass [*Cynodon dactylon* (L.) Pers.] for forage; kudzu [*Pueraria montana* var. *lobata* (Willd.) Maesen & S.M. Almeida] as an ornamental, erosion control, and forage; and water fern, (*Salvinia minima* Baker) as an ornamental; yet they have gone bad in cropland (Johnsongrass and bermudagrass), parks, forest and yards (Brazilian peppertree), right of ways, timber and field borders (kudzu); canals, lakes, and water ways (water fern) (Williams 1980). Estimate of acres infested by weeds in the U.S.A. alone are about 100 million with an estimated 14% spreading rate per annum (Babbitt 1998). More than three decades ago, it was also estimated that the annual loss of agricultural production in the U.S.A. due to weeds was about 10 billion US dollars excluding the cost of control (Shaw 1979). According to OTA (1993), estimated weed related annual crop loss for 46 major crops in the United States, excluding Alaska, amounted to $4.1 billion per year even though herbicides control programs were executed. However, without herbicide application, the loss would have amounted to $19.6 billion per annum. The Pimentel et al. (2000) publication titled ”Environmental and economic costs of nonindigenous species in the United States” revealed an estimated cost about 137 billion dollars (including losses, damages, and control) per year to control nonindigenous species (plants, mammals, birds, fishes, reptiles and amphibians, arthropods, mollusks and microbes). Out of this about 35 billion allocated for the control of pests in crops and pasture weeds alone. Shaw (1979) stated that farmers spent about $6.2 billion each year for chemical (58%) and cultural, ecological and biological (42%) control of weeds. When summed up, both losses due to weeds and cost of control of weeds amounted to $16.2 billion per year. The amount reported in 1979 and 1993 are less than the 35 billion dollars reported in 2000 which show increase in losses, damages, and control costs of weeds. Juliá and others (2007) reported over 12 million dollars economic loss in agriculture and non-agriculture sectors, which surpassed all other sectors combined due to yellow starthistle (*Centaurea solstitialis* L.) in Idaho’s rangelands in

2005. Lacey et al. (1989) study demonstrated how spotted knapweed (*Centaurea stoebe* L.) invaded bunchgrass led to higher surface runoff and sedimentation than non-invaded bunchgrass in Montana. In China the estimated economic loss due to invasive species in general amounted to 29.3 billon dollars per year while waterhyacinth [*Eichhornia crassipes* (Mart.) Solms] alone costs about 24% of this amount (Madren 2011). Recently, alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.] invasion of Wular Lake in Kashmir, India has been reported (Masoodi and Khan 2012). This shows that non-native species invasion is a global issue. There are many benefits and disadvantages of non-native species that have impacted the U.S.A. economy (Williams 1980; Reichard and White 2001; OTA 1993).

Many characteristics define non-native plants species that become invasive plants species. These include lack of natural controls (e.g., herbivores and diseases) which would have kept populations in check; strategies for adaptation to disturbance and diverse environmental conditions (extreme light, pH, and moisture); production of large number of seeds which ensure high seed germination success and capability (early maturity) or undergo dormancy and allow them to germinate periodically; possession of additional vegetative propagules (e.g. runners, rhizomes) that ensure their persistence and dispersal; ability to mimic crop seeds (have almost same size and shape as crop seeds) and most times harvested with crop seeds and aid their spread; production of biological toxins that suppress the growth of others; presence of morphological defenses such as prickles, spines, or thorns on their shoots or leaves that can cause physical injuries to herbivores which could keep them under control (Swearingen et al. 2010; Westbrooks 1998). Other factors that may enhance success of non-native invasive plants could be a switch from negative-plant-soil microbial in their native range to positive plant-soil microbial in their new range (Callaway et al. 2004). Comparative experiment on alien grasses [e.g. molassesgrass (*Melinis minutiflora* Beauv.), broomsedge (*Andropogen virginicus* L.) and bushy bluestem [*Andropogon glomeratus* (Walt.) B.S.P.] and native forbs e.g. Hillebrand's flatsedge (*Cyperus hillebrandii* Boeckeler var. *hillebrandii*), Oahu sedge (*Carex wahuensis* C.A. Mey.), Trans-Pecos cliffbrake [*Pellaea ternifolia* (Cav.) Link ssp. *ternifolia*] in the seasonal submontane zone of Hawaii National Park demonstrated how alien grasses recovered and covered the site more than native fobs after their stands were all subjected to young burn, old burn, and twice burn (Hughes et al. 1991). D'Antonio et al. (2000) study, further demonstrated fire impact of molassesgrass in altering ecosystem and its ability to regenerate and provide cover compared to some non-native and native grasses in Hawaii. In Australia, invasive gamba grass (*Andropogon gayanus* Kunth.) fueling fire impact on native species was reported (Setterfield et al. 2010). These multiple means which non-native invasive weeds can become successful in their introduced environment require knowledge and understanding of their biology and ecology to help determine their medium of entry, spread, establishment, and persistence in order to prevent them from becoming problematic as they are the most aggressive and troublesome weeds in the U.S.A. (Bryson and Carter 2004; Bryson 1996).

Tropical soda apple (TSA) (*Solanum viarum* Dunal) and Benghal dayflower (*Commelina benghalensis* L.) are among the Federal listed non-native, noxious and invasive weeds in the U.S.A. (Core 2002; Webster and Grey 2008). TSA's native ranges include Brazil, Paraguay, Uruguay, and Argentina (Nee 1991; Eplee and Westbrooks 1995) but were first reported in Florida in the late 1980s without any description or report of its weediness/invasiveness (Mullahey et al. 1993b, Bryson and Byrd 1995). By the early 1990s, TSA became a serious weed in pastures, native land, citrus, vegetables and sugarcane (*Saccharum officinarum* L.) fields in Florida (Mullahey and Cornell 1994). TSA has now spread in many southern states (Reed et al. 2004; Mullahey 1996; Dowler 1995). Many factors have been contributing to the persistence of TSA. Akanda et al. (1996b) reported that TSA seed germinated over wide range of environmental factors (pH, osmotic potential and temperature). TSA is not a weed alone but serves as a host of many pathogens (McGovern et al. 1994; Adkins et al. 2007a; Cárdenas et al. 2011). In the 1990s, production loss from TSA infestation based on decreased of carrying capacity and heat stress to cattle (*Bos* spp. L.) cost Florida about 11 million dollars per year (Mullahey et al. 1994). On other hand, Benghal dayflower which is native to Africa was first observed in 1928 in the U.S.A. (Faden 1993). It produces both above and underground flowers which all bear seeds (Maheshwari and Singh 1934; Holm et al. 1991). In 1967, Benghal dayflower was collected in Georgia (Duncan 1967) and was noticed in Louisiana and North Carolina in the 1990s (Thomas and Allen 1993; Krings et al. 2002). A survey of weeds in various crops in Georgia in 1998, *Commelina* spp. in general were averaged ranked as the 39th most troublesome weeds (Webster and MacDonald 2001) but in 2001, they became 9th most troublesome weeds in cotton (*Gossypium hirsutum* L.) (Webster 2001). By 2005, Benghal dayflower became the number one, third and sixth most troublesome weed in cotton, peanut *(Arachis hypogaea* L.) and soybean, respectively (Webster 2005). In Florida on other hand, *Commelina* spp. in general were not ranked among the ten most troublesome weeds in cotton and soybean in 2001 but were only ranked the 6th most troublesome weeds tobacco, (*Nicotiana* *tabacum* L.) (Webster 2001). However, in 2005, Benghal dayflower became the number one, second, and third most troublesome weed in cotton, peanut, and tobacco, respectively (Webster 2005). The estimate of annual control of Benghal dayflower in Georgia alone exceeded $1.2 million (Durham 2006). One of the factors that contributes to Benghal dayflower persistence is its ability to grow from stem cutting or nodes which also limits its mechanical control (Budd et al. 1979). In addition, in Georgia, for instance, the introduction of glyphosate-tolerant cotton led to fewer applications of residual herbicides to control cotton weeds postemergence without impact on cotton (Durham 2006).

Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] is an important cool-season annual or short-lived perennial forage grass that was introduced from Europe (Terrell 1968; Betts et al. 1992; Hannaway et al. 1999). Although it is not among the federal noxious weeds in the U.S.A., it is a serious weed in winter wheat and small grains (Liebl and Worsham 1984; 1987; Webster and MacDonald 2001). In general, Italian ryegrass is ranked among the 10 most common and troublesome weeds in wheat and small grains in southern states of the U.S.A. (Elmore 1988). In Oklahoma for instance, Italian ryegrass was not a major weed problem in winter wheat production; however, with the introduction of its cultivar ‘Marshall’, it became a winter weed in wheat production (Barnes et al. 2001). Marshall is known to possess the following features: late-maturing, erect-growing, wide-leaf and winter hardy (Arnold et al. 1981) which might contribute to its weediness with winter wheat.

Since weeds in general cause diverse problems to mankind and ecosystems, understanding weeds requires a holistic approach. Owing to the impact of weeds, the development of weed control technologies in the U.S.A. have long been nationally supported through research and programs that enhance basic understanding and knowledge of weeds through their biology, ecology, physiology, and biochemistry (Shaw 1979). In line with this, Shaw (1979) stated that developing principles and mechanisms of weed control should integrate biological, chemical, cultural, ecological, mechanical, and physical methods that would not only be safe, but avoid or minimize hazards to the environment. In general, weed control through mechanical and chemical methods have improved the quality of human existence in diverse ways due to the replacement of labor with the plow and hoe and the number of hours input by humans to produce crops as well as increase crop productions (Hill 1982; Klingman et al. 1982; Hilton 1979). However, there is public perception about pesticides usage due to groundwater and surface water contamination, damage to agricultural and natural ecosystem, farm worker safety and continuous evolution of pest resistance (Teague and Brorsen 1995; Pimentel et al. 1991). Pesticides (herbicides) usage technology and science are also developing and are becoming more efficient as application rates have been reduced from tons to kilograms per hectare, and from kilograms to grams per hectare (Hill 1982). In addition, it was estimated that there is a 3 to 4 dollar return per dollar invested in pesticide control of pests in the U.S.A. (Headley 1968; Pimentel et al. 1980). Furthermore, pesticides (herbicides) control of weeds provide the advantage in terms of minimum tillage requirement thus contributing to energy savings and reduced water pollution, enhanced usage of harvesting machines, and seed cleaning operations (Eue 1985; Baldwin and Santelmann 1980). Hyvöven et al. (2011) study on “impact of climate and landuse type on the distribution of Finish casual arable weeds in Europe” showed that apart from climate warming, lower intensity use of chemicals would enhance the invasion of new weed species. It is therefore an undeniable fact that weed control in these days' mainstay agriculture is through application of chemicals (Eue 1985).

The main objectives of these studies are to further assess herbicides for the control of non-native plants that have become problematic in natural areas and agrosystems in the U.S.A.

Tropical soda apple

Tropical soda apple (TSA) belongs to family Solanaceae, genus *Solanum* L., prickly subgroup *Leptostemonum* Dunal Bitter and section *Acanthopora* Dunal (Welman 2003). Levin et al. (2006) described *Leptostemonum* (Dunal) Bitter as a large group (ca. 350-450 species) within *Solanum* constituting almost one third of the genus, which are distributed worldwide, but are predominant in Central and South America, Africa and Australia. Members of *Leptostemonum* are also known as "spiny solanums" due to the fact that majority of the species are armed with epidermal prickles (Levin et al. 2005). TSA is native to Brazil, Paraguay, Uruguay, and Northern Argentina but has been introduced to Central America, the West Indies, and southern U.S.A. (Nee 1991; Eplee and Westbrooks 1995; Welman 2003).

TSA was first reported in Florida in 1988 (Mullahey et al. 1993b, Core 2002; Bryson and Byrd 1995) without any description or report of its weediness/invasiveness. The manner TSA was introduced into Florida was probably linked to its seed adherence to peoples' shoes or escape from cultivation (Mullahey et al. 1993b; Cuda et al. 2004). However, in the early 1990s it was reported as a serious weed in pastures, native land, citrus, vegetables, and sugarcane fields in Florida (Mullahey and Cornell 1994). More than a decade ago, the estimate of cattle production loss which include decreased carrying capacity and heat stress, due TSA infestation in Florida cost about 11 million dollars per year (Mullahey et al. 1994).

Apart from Florida where it was first found, it has apparently spread to Texas, Florida, Alabama, Georgia, North and South Carolina, Louisiana, Mississippi, Tennessee, Pennsylvania, Puerto Rico (Reed et al. 2004; Mullahey et al. 1998; Mullahey 1996; Dowler 1995; Eplee and Westbrooks 1995) and invading large areas of these states (Church and Rosskopf 2005; Patterson et al. 1997) which include improved pastures, row crops, truck crops, sod farms, citrus groves, sugarcane fields, roads, forestry, nurseries, fence lines, ditches and urban areas (Bryson and Byrd 1995; Core 2002). The main routes that aid TSA's spread include seed movement in cattle and commercial sod (Mullahey et al. 1998).

Research by Bhaskar et al. (2002) in India showed that TSA treated with phosphorus and potassium fertilizers responded positively with increased plant height, branches/plant, leaf-area index, fruiting points/plant, fruits/plant, fresh fruit, dry berry yield compared to its untreated control. Their results indicated the potential cost farmers might face with increasing infestation of TSA on both crop and forage production settings as it can compete with nutrients applied to crops and forage. The importance of arbuscula mycorrhizal fungi to general plant health and soil fertility can be found in Jeffries et al. (2003), yet greenhouse studies by Shenpagam and Selvaraj (2010) found that these arbuscula mycorrhizal fungi enhanced the growth, biomass and nutrition status of roots and leaves of TSA. TSA is not a weed in terms of nutrients, light and water competition alone. It was identified as a potential host for the root-knot nematode

[*Meloidogyne arenaria* (Neal) Chitwood] and this puts it as an economic threat to many crops in most of the southern states if not controlled (Church and Rosskopf 2005). It was also found as a reservoir of the tropical soda apple mosaic virus (TSAMV), which impacts important crops such as pepper (*Capsicum annuum* L.), and tomato (*Lycopersicon esculentum* Mill.) (Adkins et al. 2007a). TSA is also host of tomato mottle virus (TMoV) (McGovern et al. 1994). Its relative, red soda apple (*Solanum capsicoides* All.) was subsequently a host of TSAMV (Adkins et al 2007b). Recently, *Phytophthora infestans* which causes late blight on potato (*Solanum tuberosum* L.) was isolated from TSA in Colombia which indicates the possibility of it being a host of this pathogen also (Cárdenas et al. 2011).

Patterson et al. (1997) found that TSA responded positively to variable temperatures and concluded that this could aid its rapid spread in southern U.S.A. and even to adjacent states. Akanda et al. (1996b) found temperature range of 10 to 30 ^{o}C favored TSA seed germination which parallels with temperature ranges occurring in the southeastern U.S.A. per year. Bryson and Byrd (2007) also found both seeds and plants survived warmer winter in Stoneville, Mississippi and concluded that this would aid its persistence as annual weed in areas where it cannot survive winters as a perennial. TSA has also been described as an obligate weed due to its association with human activities (Mullahey et al. 1993b) which help spread it. Field studies indicated that both roots and stems of the plant store some amount of carbohydrate and these might give it the advantage of robust re-growth even after mowing and allowing it to persist in grazed pastures (Mullahey and Cornell 1994). Although TSA is a perennial shrub in tropical climates which is its native range, it is a short-lived perennial or annual in subtropical and temperate regions (Kreiser et al. 2004). Welman (2003) stated that although TSA is adapted to grow under full sunlight, it can also grow under shade. Akanda et al. (1996b) observed TSA grew vigorously and shaded pasture grasses after three months emergent. Planting depths that ranged from 0 to 6 cm or 1 to 4 cm favored its germination (Akanda et al. 1996b; Mullahey and Cornell 1994) which indicated that temperature might not be limiting factor that could cause excessive desiccation of the TSA seed.

Morphological features and agents of dispersal aid TSA persistence. TSA has prickles all over the stems and leaves (Pingle and Dnyansagar 1980) which may deter livestock from eating the leaves (Reed et al. 2004; Cuda et al. 2002). In addition, leaves are unpalatable but livestock would eat the fruit and help spread the seed to new areas through defecation (Mullahey et al. 1993b; Mullahey 1996). For instance, cattle are one of the primary agents of TSA dispersal because a single cowpat can hold 150 or more seeds (Core 2002) although seeds can also be dispersed through composted manure, contaminated pasture grass seed, sod or hay (Bryson and Byrd 1995). TSA has been on the Federal Noxious Weed List since 1995 (Core 2002).

Miller (2003) described TSA as an upright plant ranging from 90 to 180 cm in height, and remaining almost green year-round in most southern locations in the U.S.A. Mullahey et al. (1993b) described TSA leaves as pubescent, ranging from 10 to 20 cm long with 6 to 15 cm wide and are deeply divided into broad pointed lobes. There are variations between immature and mature TSA fruits. Immature fruits are smooth, round, mottled whitish- to light-green with dark green and looks like the color of a watermelon [*Citrullus lanatus* (Thunb.) Matsumura & Nakai var. *lanatus*] whereas its mature fruits are yellow, varies from 1.8 to 3.0 cm in diameter, having leathery-skin surrounding with thin-layer, looking pale green and have a scented pulp (Byrd et al. 2004). TSA seeds have also been described as being moderately flattened, covered in a mucilaginous layer that contains a glycol-alkaloid namely solasodine (Mullahey et al. 1993b). Bryson and Byrd (2007) found that its seed becomes mature to germinate before the seed color changes from green to yellow, but percentage germination was higher in yellow than green. Pingle and Dnyansager (1980) found that seedlings flowered 3 months after germination and the berries (fruits) matured within 2 months, whereas, Mullahey and Cornell (1994) observed 20 cm growth height with an average of 20 leaves per plant at 60 days after planting.

Despite the fact that TSA is considered one of the troublesome weeds in U.S.A. and other regions worldwide, in agronomic, natural and forested settings, it has beneficial uses in steroid production industries. In India, in particular the alkaloid product (solasodine) that can be extracted from TSA berries (Bhaskar et al. 2000; Pingle and Dnyansagar 1980).

Since TSA is an invasive and persistent weed, control must be approached through integrated means. One prime target is to prevent its introduction and re-introduction to areas that have not been infested. Containment must be used for areas that have infested already. Akanda et al. (1997) stated that areas that have become TSA grounds such as hammocks, roadsides, ditchbanks and areas that could be serving as possible congregation sites for wildlife and livestock to feed on its fruits should be frequently checked and controlled due to the fact that through their defecation, its seeds can be deposited. Bryson et al. (1995) addition to the integrated ways of TSA control include pulling and burning all parts of the plant, not excluding the fruits, especially when it exists as individually and has a small population. Furthermore, when it exists in large populations, repeated mowing with a combination of one or more applications of an effective herbicide would be an excellent control method. Byrd et al. (2004) additions include cutting, pilling and burning mature fruits of TSA so that its seed viability would cease. Bhaskar et al. (2000) in India found that when radish (*Raphanus sativus* L.) was grown as an intercrop with TSA, TSA's height, number of branches it produced and its dry yield of berry was reduced compared to TSA grown as a pure stand. This finding could, therefore, be integrated with other methods to control TSA. Studies conducted by Medal et al. (1999) showed that the leaf beetle, *Metriona elatior* Klug (Coleopteran: Chrysomelidae) has the potential to be used as a biological control of TSA in the U.S.A. This was based on results they obtained from field surveys on TSA from native range Argentina and Brazil as well as results from field experiments in Brazil where the leaf beetle chose TSA as their host over eggplant (*Solanum melongena* L.). Since then several other biological agent trials with defoliating leaf beetles: (*Gratiana boliviana* Spaeth), *(Gratiana graminea* Klug), *Metriona elatior* (Chrysomelidae), and flower bud weevil (*Anthonomus tenebrosus* Boheman) (Curculionidae) have been ongoing to evaluate their efficacies on TSA (Medal et al. 2002). *Gratiana boliviana* has been shown as a potential candidate for TSA control because its larvae or adults can feed on TSA's leaves, leading to the reduction of plant vigor, growth rate, and fruit production (Medal et al. 2002). To control TSA mechanically is difficult due to the fact that it can grow vigorously as well as it can regenerate from mowed plants (Call et al. 2000). Chemical control has been found to be more effective on TSA control. However, Mullahey et al. (1993a) stated that less than 90% control of TSA is not acceptable, as the plant has a high capacity to sustain numerous viable seed and produce a lot of seeds per plant. In greenhouse studies, Akanda et al. (1996a) found that triclopyr at 1.0 kg ae/ha, dicamba at 2.8 kg ae/ha, and clopyralid at 1.8 kg ae/ha controlled TSA more than 90% when applied at plant's 6-8 leaf, flower or fruit stages. Lower rates of these treatments rendered less control. Other studies also showed that treatments containing 2, 4-D ester or amine, metsulfuron, or hexazinone mixed with Tobacco Mild Green Mosaic Virus rendered 80 to 100% control of TSA (Ferrell et al. 2008). Aminopyralid, which is an auxin-like growth regulator, can be used at ranges of 0.053 kg to 0.12 kg ai /ha to control TSA effectively due to its residual effect (Ferrell et al. 2006).

Benghal dayflower

Benghal dayflower also known as tropical spiderwort is a C_3, fleshy or succulent, branched, creeping, annual or perennial herb that roots at nodes (Wilson 1981; Holm et al. 1991, Price et al. 2009). It belongs to the genus *Commelina* and in both tropical and subtropical regions, there are about 170 species of *Commelina* recognized, most of which are native to Africa (Faden 1993; Shu 2000; Maddox et al. 2007; Bryson and DeFelice 2009), whereas the family Commelinaceae itself has 34 to 50 genera and about 500 to 700 species prevalent in tropical and warm-temperature regions of the world (Burrows and Tyrl 2001). Ecotypes of Benghal dayflower differ in ploidy level. Webster et al. (2005) stated that the ecotypes found in tropical regions are hexaploid, whereas introduced ecotypes found in the southeastern U.S.A, are diploid. *Commelina* spp. in general are difficult to identify due to diversity in ecotypes and ploidy levels (Faden 1993). However, Benghal dayflower differs from climbing dayflower (*Commelina diffusa* Burm. f.) and the Asiatic dayflower (*Commelina communis* L.) for instance due to presence of hairs on the young leaves and on petioles whereas in general its purple/lavender flower color makes it differ from other dayflowers species (Ferrell et al. 2004). Benghal dayflower is a monocot and can grow up to 60 cm high (Akobundu and Agyakwa 1987; Westbrooks 1991). Its leaves are alternate with ovate to elliptic sheaths and often with red hairs at the apex, bracts subtending with funnel shaped flower (Westbrooks 1991).

Benghal dayflower produces both above and underground flowers (Maheshwari and Singh 1934; Holm et al. 1991). The flowers are of three types: male chasmosgamous (typical, open pollinated), hermaphrodite chasmogamous (typical, open pollinated) and hermaphrodite cleistogamous (typical, closed pollinated) (Maheshwari and Singh 1934; Maheshwari and Maheshwari 1955; Walker and Evenson 1985a; Webster and Grey 2008; Culpepper et al. 2004). It also has three types of branches/shoots which include negatively geotropic, positively geotropic, and diageotropic (Kaul et al. 2002). All of the branches/shoots bear flowers and leaves, except positively geotropic subterranean shoots which do not bear leaves. It reproduces through seeds and stolons and also roots at nodes on the stems (Miller 2001). An experiment carried out by Pancho (1964) to evaluate seed sizes and production capacities of common weed species in rice fields of the Philippines recorded that a plant of Benghal dayflower produced 1610 seeds. Benghal dayflower produces four types of seeds: underground (small and large) and aboveground (small and large) with varying degree of innate dormancy (Walker and Evenson 1985b). It was found that germination of these four types of seeds varied from 0-3% for small aerial seeds, 20-35% for large aerial seeds, 33% for small underground seeds and 90% for large underground seeds (Walker and Evenson 1985b). However, in separate experiments, Walker and Evenson (1985a) observed that Benghal dayflower grown from aerial seeds developed faster in aerial reproductive growth stages than underground seeds. Kaul et al. (2002) found in a reproductive effort and sex allocation strategy experiment on Benghal dayflower that the plant allocated as much as 15% of its total resources for reproduction and the majority of this, thus about 69%, for aerial branches/shoot production. Environmental factors such as temperature and light influence the germination of weedy plants. Studies have shown that when aerial small and large seeds of Benghal dayflower were exposed to both light and darkness, higher percentage of germinations were observed under light exposure than darkness (Matsuo et al. 2004). However, it was further found out that light was not necessary to enhance germination of large seeds. A recent study on Benghal dayflower's response to temperature in North Carolina showed that higher temperature (30 to 35 C) favored both above and below large seeds germination ranged from 50 to 80% compared to lower temperature 25 C for 10 to 30% and none at 20 C (Sermons et al 2008). Wilson (1981) review on Benghal dayflower showed that the plant could exhibit perennial growth and reproductive behavior in tropical and subtropical lowlands, whereas it is annual in more temperate zones. In general, *Commelina* species are more adaptable to moist, sometimes swampy and water logged conditions and these usually aid them to take advantage by growing rapidly and competing conveniently with crops plants (Wilson 1981; Holm et al. 1991).

History of Benghal dayflower in U.S.A. can be traced back to 1928 when it was first observed and by the mid-1930s, it appeared to have been established in Florida (Faden 1993; Durham 2006), was collected in Georgia in 1967 (Duncan 1967), and subsequently noticed in Louisiana and North Carolina in the early and late 1990s respectively (Thomas and Allen 1993; Krings et al. 2002). Apart from its native range, Benghal dayflower became naturalized in California, Florida, Georgia and Hawaii and other countries such as Brazil and French Guiana etc. (Faden 1992). It has spread within the naturalized states and to other states: Alabama and Missouri (Burton and York 2005; Miller 2001; Webster et al. 2005). In August 2006, it was noticed in Jackson County, Mississippi (Maddox et al. 2007).

Benghal dayflower grows vigorously in diverse habitats ranging from water saturated to dry soil with varying pH (Wilson 1981). It mostly invades cultivated lands, field borders, wet pasture-lands, gardens, grassland, roadsides, waste places, and disturbed areas (Holm et al. 1991; Evans et al. 2005; Miller 2001). In south-eastern Queensland, Australia for instance, Benghal flower has been found growing best in high soil moisture and fertility under cultivation in association with peanuts, navy or dry beans (*Phaseolus vulgaris* L.) sorghum [*Sorghum bicolor* (L.) Moench ssp. *bicolor*] Moench) and corn (*Zea mays* L.) (Walker and Evenson 1985a) whereas in India, Maheshwari and Maheshwari (1955) wrote, "*C. benghalensis* is found in abundance everywhere under moist shady places during the rains. It is known to be a pest in corn, soybean, nursery stock, and orchards (Webster et al. 2006b). Holm et al. (1991) reported it as a serious weed in 25 crops in 28 countries. It's allelopathic effects on seedling vigor of soybean and corn was reported (Singh et al. 1989). It is not a weed in terms of water and nutrients competition alone; it also serves as a host of nematode, pathogens, and viruses (Davis et al. 2006; Miller 2001; Desaeger et al. 2000). In 1998, *Commelina* species in general were averaged ranked as 39th most troublesome weeds in Georgia (Webster and MacDonald 2001). By 2001, they became 9th most troublesome weeds in cotton (Webster 2001). In four years later, Benghal dayflower was singly ranked the first, third and sixth most troublesome weed in cotton, peanut, and soybean, respectively (Webster 2005). In Georgia, 10% reduction of peanut yield was reported due to Benghal dayflower interference for 4 weeks of the growing season whereas full-season long interference resulted in 51% yield reduction (Webster et al. 2007). Its aggressive competition with cotton under drought stress was also reported (Webster et al. 2008). As of 2006, it's infestation in Georgia exceeded 80,000 ha with continued expansion potential (Webster et al. 2005, 2006a). Its rapid spread as a serious weed in southeastern U.S.A. has been linked to the adoption of glyphosate-tolerant cotton (Steptoe et al. 2006). This is due to the fact that glyphosate provides only marginally acceptable control (Price et al. 2009). Other factors contributing to its persistence include lack of residual herbicides for weed management in Roundup Ready crop programs in Georgia, resistance of producers to rotate to crops in which more effective, residual herbicides could be used, and reduced tillage agricultural practices (Prostko et al. 2005) which can suppress its survival. The estimated cost for control in Georgia alone exceeded $1.2 million per annum (Durham 2006). Benghal dayflower is designated a federal noxious weed (Webster and Grey 2008) and currently it is a noxious weed in 35 states (Vencill and Steptoe 2005).

Despite the fact that Benghal dayflower is serious weed in many agronomic settings, its beneficial uses cannot be underrated. It beneficial uses can be found in a review by Wilson (1981). In China, it is used as a medicinal herb with febrifugal, anti–inflammatory and diuretic effects (Shu 2000). In India: the juice of the flower is used as a pigment for painting on transparencies (Gokhale et al. 2004); the whole plant (leave and root) is used to treat leprosy (Joshi and Tyagi 2011), used as a mustard recipe (Yesodharan and Sujana 2007) and the plant is also used to treat bedsores, breast sores, pimples and haemorrhages (Meena et al. 2010). In Kenya, it is used for treating measles (Klauss and Adala 1994); in Uganda, the whole plant is used to treat menstrual pains and venereal disease (Oryema et al. 2010), but in Bangladesh only the root is used to treat menstrual disorder (Uddin et al. 2006). Ethnobotanical investigations by Focho et al. (2009) in Cameroun also found that an extract from a whole Benghal dayflower plant is taken orally for treatment of women facing difficulty in child delivery.

The ability of Benghal dayflower to germinate and thrive under crop canopy limits its cultural weed control strategies (Vencill and Steptoe 2005) whereas its ability to grow from stem cutting or nodes limits its mechanical control (Budd et al. 1979). There is no known biological control agent that can be used to control Benghal dayflower (Vencill and Steptoe 2005). Ferrell et al. (2004) observed that although Benghal dayflower germinates in late summer, its growth was poor under low light environment and they suggested that early planting of crops to form dense canopy before the emergence of Benghal dayflower would help suppress its growth and establishment. A temperature range of 30-35 °C improved seedling emergence of Benghal dayflower from 50 to 80% compared to 25 °C and 20 °C that supported only 10 to 30% growth and no growth, respectively (Sermons et al. 2008). Gonzalez and Haddad (1995) observed related results in Brazil where there was no germination of Benghal dayflower's seeds at temperature of 10 °C. Budd et al. (1979) study showed that stem cuttings of Benghal dayflower placed on soil surface regenerated 97% of the time while its seeds buried at 2 cm depth emerged 82% of the time, but no regeneration was observed as the depth increased from 4 to 8 cm.

Postemergence herbicides 2, 4-D, paraquat and glyphosate can control Benghal dayflower when the plant is young and actively growing (Wilson 1981; Maddox et al.

2007). Dual Magnum (S-metolachlor), Lasso (alachlor), and Outlook (dimethanamid) control Benghal dayflower effectively due to their long residual activities (Maddox et al. 2007). Underground seeds of Benghal dayflower have been found to be more susceptible to metolachlor than aerial seeds, which have been attributed to large translocation of metolachlor to the underground parts (Steptoe et al. 2006). Walker (1981) evaluated the efficacies of 2,4-D, dinoseb and bentazon applied at 0.125 kg ai/ha, 1.1 kg ai/ha and 0.96 kg ai/ha, respectively, at different growth stages of Benghal dayflower in Australia. He found that 2,4-D and dinoseb provided 92% and 100% control of Benghal dayflower at 2.5 and 5-leaf stages, respectively. However these levels of control decreased to 63% and 67%, respectively, as plants matured. He found that bentazon was the only herbicide that provided 95% control at the 2.5-leaf stage and 100% at the 5-leaf stage whereas a tank mixes of the herbicides (2,4-D + dinoseb, 2,4-D + bentazon, dinoseb + bentazon and 2,4-D + dinoseb + bentazon) controlled Benghal dayflower more than 95% at both growth stages. Clomazone applied at 1.05 kg ai/ha as a preemergence herbicide controlled Benghal dayflower by 87% and 94% three and six weeks after treatment, respectively (Webster et. al 2006a). However, when clomazone was applied at 0.84 kg ai/ha as a postemergence herbicide it only rendered 70% and 70% control at 3 and 6 weeks after treatment respectively, whereas s-metolachlor applied at 1.60 kg ai/ha rendered higher controls 90% and 96% at 3 and 6 weeks after treatment, respectively (Webster et al. 2006a).

Italian ryegrass

Italian ryegrass or annual ryegrass is a non-native species and was introduced into North America from Europe as forage grass (Betts et al. 1992). It has a winter annual life cycle and germinates either in the fall or early spring and completes its life cycle before summer (Webster and MacDonald 2001). It is found in fields, meadows, roadsides and waste places (Terrell 1968). It is distributed in the southern parts of the U.S.A.: Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Virginia (Elmore 1988). It grows in diverse soil types, heavy and temporarily waterlogged (if it has already been established) with the preferred soil pH ranges from 5.5-7.0 (Valenzuela and Smith 2002). Italian ryegrass germinates (6 to 10 days) when daytime temperature ranges from 10 to 30 ºC (Hannaway et al. 1999). It forms an extensive, dense root system, even in low fertility and acidic soils which are some of the qualities that make it a good grass for erosion control on sloping fields and grassed waterways (Valenzuela and Smith 2002). In total, there are nearly 3 million acres of Italian ryegrass with about 90% used for winter pasture in the southeast of the U.S.A. (Hannaway et al. 1999).

Italian ryegrass has been described as a diploid, outbreeding and self-incompatible plant (Wit 1958, Creemers-Molenaar and Beerepoot 1992). Although it has cultivars, some of which are still diploid (2n=2x=14) and others tetraploid (Miura et al. 2007). Italian ryegrass cultivars have been developed purposely to improve its forage yield and seed production, resistance to diseases, and tolerance to cold (Blount et al. 2009). Species in genera *Festuca* and *Lolium* are close allies and most often cross breed and produce interfertile species (Humphreys, 2009). Two closely related, important and interfertile species in the genus *Lolium* are Italian ryegrass and perennial ryegrass (*Lolium perenne* L.) for forage production (Ye et al. 1997; Fearon et al. 1983).

Italian ryegrass serves as the main forage for livestock during the winter growing season in the south-eastern U.S.A. (Venuto et al. 2004). It is often used as a “living sod” cover crop in vegetable and fruit crops (Valenzuela and Smith 2002). Because it attracts few insect pests, it has an advantage reducing insect pest levels in legume stands and many vegetable crops (Valenzuela and Smith 2002). It can also grow under conditions where other crops fail (Sattell et al. 1998). Italian ryegrass is used for planting in irrigated pastures and turf, overseeding on dormant turf during winter months, seeding on rangelands and dry pastures, and scattering from aircraft as an emergency cover for mountain areas burned by wild fire in California (McKell et al. 1969). It is seen as a good candidate due to its high degree of seedling vigor and a fast rate of growth, good growth at relatively low temperatures, abundant production of palatable forage and extensive root system, the good green color it provides to planted areas and the relatively cheap cost of the seeds. The other advantage is that Italian ryegrass carries resistance to a number of pathogens that may impact wheat in particular (Cheng and Xia, 2004). In the northwestern U.S.A. for instance, Italian ryegrass is used as hay, pasture forage, turf and for commercial seed production (Grey and Bridges 2003). In temperate regions of the world, it is widely used as forage and silage grass (Studer et al 2006; Miura et al. 2007) and in Japan; it is a preferred forage grass due to its high quality and productivity (Ikeda 2005). It also can be used to smoother weed growth due to its ability to establish rapidly, seedling vigor, and strong competitive ability against weeds (Valenzuela and Smith 2002; Sattell et al. 1998). Valenzuela and Smith (2002), wrote “Italian ryegrass’ has a vigorous root system that tenaciously holds the soil against erosion while improving soil organic matter levels, increasing water infiltration, and reducing nitrate leaching.” Sattell et al.

(1998) stated that "Italian ryegrass is a heavy nitrogen feeder and it can be used to scavenge nitrogen from the soil during the fall and winter."

Despite the fact that the Italian ryegrass has many beneficial uses as detailed earlier on, it is however, a weed in wheat and small grain crop in Georgia and North Carolina (Webster and MacDonald 2001; Liebl and Worsham 1987). In general, Italian ryegrass is ranked among the 10 most common and troublesome weeds in wheat and small grains southern states of the U.S.A. (Elmore 1988). It is a weed in Arkansas (Khodayari et al. 1983), a serious wheat weed in western Oregon (Stanger and Appleby 1989) and a weed in cotton and soybean in Mississippi (Nandula et al. 2007). Stone et al. (1998) reported that full or below ground interaction of wheat with Italian ryegrass caused reduction of wheat height, leaf number, tillering, leaf area, percent total nonstructural carbohydrate in shoot and dry weights of leaves, stems and roots at 45 and 75 days of planting compared to controls. As few as 20 Italian ryegrass per m^2 reportedly reduced winter wheat yields severely (Appleby and Brewster 1992). In Oklahoma, it was reported that a common Italian ryegrass was not a major weed problem in winter wheat production; however, with the introduction of its cultivar 'Marshall', which is winter hardy it became a winter weed in wheat production (Barnes et al. 2001). Appleby et al. (1976) also reported as much as 60% winter wheat reduction due to Italian ryegrass competition. In Australia, the presence of annual ryegrass (*Lolium rigidum* Gaudin), a relative species of Italian ryegrass caused reduction of dry matter production and grain yield of wheat by reducing the number of fertile tillers and spikelets (Reeves 1976). In the northern states of the U.S.A., Italian ryegrass most times are not sown with perennial grass or legumes if grown for forage because it may be too aggressive and may result in reduction in a stand of the slower, more desirable components of the mixture (Miller 1984). Hashem et al. (1998) reported that a mixture of Italian ryegrass and winter wheat caused reduction of winter wheat compared to monocultures. It is also a weed in wheat in Idaho, Oregon, South Carolina, Virginia, and Tennessee (Heap 2008). It is the most common *Lolium* species in Arkansas and most often has been misidentified as poison ryegrass (*Lolium temulentum* L.) or perennial ryegrass (Bond et al. 2005). Hoskins et al. (2005) found that Italian ryegrass competition from non-herbicide treated plots of wheat reduced wheat yield by 33% when compared with herbicide treated plots. Liebl and Worsham (1987) found in a greenhouse experiment that growth response of Italian ryegrass to NO_3^- and P^+ concentration was greater than wheat.

According to Blount et al. (2009), fall armyworms (*Spodoptera frugiperda* J.E. Smith), mole crickets *(Scapteriscus spp.*) crown rust (*Puccinia cononata* Corda), and stem rust (*Puccinia graminis* Pers: Pers: subsp. *graminicola*, Z. Urban) are some of the pests that affect Italian ryegrass productivity. Hashem et al. (2000) found that an increase in wheat density up to 800 plants/m^2 reduced Italian ryegrass seed yield by 87%. Diclofop, clodinafop, and metribuzin were found to reduce fresh weights of Italian ryegrass by 69, 71, and 62%, respectively, while imazamox or triasulfuron did not reduce Italian ryegrass weight more than 60% (Tucker et al. 2006). Khodayari et al (1983) observed in their herbicides treatment that diclofop at a rate of 1.0 kg/ha and diclofop at 1.0 kg/ha plus betazon at 1.2 kg/ha rendered excellent control of Italian ryegrass and resulted in significant wheat yield compared to untreated plots. Italian ryegrass control at four-leaf growth stage with mesosulfouron + NIS + liquid nitrogen (28%) at rate 15 g ai/ha in a wheat farm at Waco Texas, U.S.A. rendered 8% and 0% control 35 and 64 days after treatment suggesting the biotype was resistant (Ellis et al. 2008). However, species in genus *Lolium* in general have been documented to develop resistance to various families of herbicides. Italian ryegrass's resistance to glyphosate was documented in the U.S.A. (Nandula et al 2007; Perez-Jones et al. 2005) and Chile (Pérez and Kogan 2003); its relative annual ryegrass resistance to glyphosate in Australia (Powles et al. 1998; Pratley et al. 1999) and poison ryegrass and perennial ryegrass to diclofop in the U.S.A. (Kuk et al. 2000). Diclofop-methyl is an important herbicide extensively used to control ryegrass in cereal production systems worldwide (Martínez-Ghersa et al. 2004; Khodayari et al. 1983) yet many *Lolium* species have developed resistance to it. Italian ryegrass tolerance to diclofop was first reported in 1987 in Oregon, U.S.A. where it had been used to control weeds in a winter wheat field for 7 years (Stanger and Appleby 1989). Betts et al. (1992) reported that diclofop resistance biotype of Italian ryegrass collected from Oregon showed a high increase in diclofop rate (more than 15 kg/ha) required to reduce it by 50% compared to 0.16 kg/ha for the susceptible biotype. De Prado et al. (2000) observed greater translocation of diclofop-methyl in diclofop resistance Italian ryegrass biotypes compared to diclofop susceptible biotypes. They attributed the greater translocation in the resistance biotype to lack of the herbicide at any target site whiles inhibition of ACCase activity in the meristematic regions of the susceptible biotypes resulted in reduced sink demand and caused a reduction in translocation. Italian ryegrass resistance to ACCase herbicides was also reported in United Kingdom in 1990, in South Africa in 1993 (Heap 1997) and in Italy (Bravin et al. 2001) and France (De Prado et al. 2000). Perennial ryegrass became resistant to the ALS-inhibitor (sulfometuron) in the U.S.A. (1989) and rigid ryegrass resistant to the ALS-

Inhibitors sulfonylurea herbicides and glyphosate in Australia in 1984 and 1996 respectively (Heap 1997). In Texas, decreased efficacies of sulfonylurea herbicides: chlorsulfuron, triasufuron and chlorsulfuron plus metsulfuron methyl on Italian ryegrass control in wheat were reported (Tucker et al. 2006). Studies on glyphosate resistant and susceptible biotypes of Italian ryegrass in Chile showed that the former retained less glyphosate, lower foliar uptake from the abaxial leaf surface and altered translocation pattern compared to later (Michitte et al. 2007). Stanger and Appleby (1989) stated that poor application methods, unfavorable environmental conditions and other causes might be contributing factors to poor control of Italian ryegrass. Poor control of Italian ryegrass with glyphosate was observed by growers in Chile in 1999 after 8-10 years of using glyphosate to control Italian ryegrass in those fields (Perez and Kogan 2003; Michitte et al. 2007) and this was the first glyphosate resistant Italian ryegrass case in South America. Pérez et al. (2004) reported that Italian ryegrass resistance to glyphosate was not due to differential absorption, translocation or allocation of the herbicide in the plant although the susceptible Italian ryegrass translocated non-significant glyphosate from the treated leaf to the rest of the plant than the resistant population. Perez-Jones et al. (2005) studies on susceptible and resistant Italian ryegrass treated with glyphosate at 0.42 kg/ha and 0.84 kg/ha and shikimic acid and extracted 12, 24, 48, and 96 hours after treatment showed that the susceptible biotype accumulated between three to five times more shikimic acid than the resistant biotype. However, they concluded that the mechanism responsible for glyphosate resistance in Italian ryegrass is still not clear and could not be attributed to altered target site since accumulation of shikimate and sequence of the *epsp* gene. Nandula et al. (2007) research in Mississippi, U.S.A., on two suspected glyphosate tolerant and one glyphosate susceptible populations of Italian ryegrass treated with isopropylamine salt of glyphosate found that the two suspected glyphosate tolerant populations were threefold more tolerant than glyphosate susceptible populations. Comparison of contact angles of glyphosate solution on both resistant (40º to 45º) and susceptible (70º) Italian ryegrass were made in Chile (Michitte et al. 2007). The results showed that the retention of glyphosate was 35%, and uptake by the abaxial leaf surface was 40% lower in resistant Italian ryegrass than the susceptible Italian ryegrass. In addition, it was observed that more glyphosate migrated to the tip of the treated leaves of resistant Italian ryegrass than susceptible Italian ryegrass. Italian ryegrass in particular has developed resistance to several herbicides in different agronomic systems including croplands in Argentina, orchards and soybean fields in Brazil, fruit and orchards in Chile, cotton and soybean fields and orchards in Mississippi and Oregon respectively in the U.S.A. (Heap 2008). Martínez-Ghersa et al. (2004) studied the impact of Ultraviolet-B radiation on Italian ryegrass and concluded that Ultraviolet-B radiation is a weak stress factor on the efficacy of control and evolution of Italian ryegrass resistance.

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CHAPTER II

RESIDUAL EFFECTS OF HERBICIDES ON GERMINATION OF TROPICAL SODA APPLE (*Solanum viarum*)

Abstract

Tropical soda apple (TSA), (*Solanum viarum* Dunal) is an invasive, noxious, exotic, perennial weed initially found in Florida's pasture and rangeland and it has now spread to many southern states in the U.S.A. Between 2009 and 2011, greenhouse experiments were conducted at the Plant Science Research Center, Mississippi State University, Starkville, MS to evaluate residual effects of preemergence herbicides aminopyralid and tank mixtures of triclopyr plus picloram + 2,4-D on seedling emergence of TSA. Aminopyralid at 0.088 and 0.12 kg ae/ha; triclopyr at 0.56 kg ae/ha plus picloram at 0.15 kg ae/ha plus 2,4-D at 0.56 kg ae/ha were applied to potting soil and TSA seeds were planted into the treated soil at PRE, 1, 3 and 6 MAT. TSA seedlings emerged were counted from each planting time 3 to 4 WAP. At PRE, all herbicides controlled TSA seedling emergence more than 96% compared to untreated. At 1 MAT, only aminopyralid at 0.88 kg ae/ha provided at least 90% control, whereas aminopyralid at 0.12 kg ae/ha and triclopyr at 0.56 kg ae/ha plus picloram at 0.15 kg ae/ha plus 2,4-D at 0.56 kg ae/ha provided 83 and 90% control, respectively. At both 3 and 6 MAT, none of the herbicides provided more than 70% preemergence control of TSA seedlings. Taking the residual efficacies of all herbicide treatments to some extend into

consideration, repeated application will be required for effective control of TSA seedling emergence

Nomenclature: Aminopyralid, (2-pyridine carboxylic acid, 4-amino-3, 6-dichloro-); triclopyr,{[3,5,6-trichloro-2-pyridinyl)oxy]acetic acid}; picloram , 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid; 2,4-D, (2,4-dichlorophenoxy) acetic acid, Tropical soda apple, *Solanum viarum* Dunal. #[1] SOLVI.

Abbreviations: PRE preemergence; MAT: month after treatment; WAT: weeks after planting, TSA: tropical soda apple

Key words: Aminopyralid, triclopyr plus picloram + 2,4-D, TSA, MAT.

Introduction

Tropical soda apple (TSA), *Solanum viarum* Dunal is an invasive, noxious, exotic, perennial weed initially found in Florida's pasture and rangeland that is rapidly invading large areas of the southeastern U.S.A. (Akanda et al. 1996b; Church and Rosskopf 2005; Patterson et al. 1997). In these southeastern states, TSA infests rangelands, improved pastures, row crops, truck crops, sod farms, citrus groves, sugar cane fields, road sides, forestry nurseries, ditch banks, and hammocks and reducing livestock carrying capacity (Bredow et al. 2007; Gandolfo et al. 2000; Bryson and Byrd 1995; Mullahey and Cornell 1994). Apart from U.S.A., this Brazilian and Argentinean native plant has become weed in Africa, India, Nepal, West Indies, Honduras and Mexico (Bryson and Byrd 1995). TSA is known by various appellation names such as "the plant

[1] Letters followed by this symbol are a WSSA-approved computer code from Composite List of Weeds Revised 2010. Available only on computer disk from WSSA, 810 East 10th Street Lawrence, KS 660448897

from Hell” in U.S.A. or “Sodom apple” in British-speaking areas due to the fact that it was initially found near Devil Garden in Florida for the former whereas Sodom is the biblical city noted for its wickedness, respectively, for the later (Coile 1993). When TSA was first discovered in Florida in 1980’s there was no evidence of its invasiveness; however, research conducted between 1990 and 1996 on TSA proved its invasiveness and therefore necessitated restricted movement into the United States (Mullahey et al. 1998).

A study by Akanda et al. (1996b) on TSA concluded that it has potential to establish in diverse ecosystem due to its variable response to pH, planting depth, osmotic potential, light, temperature and priming treatment. In addition, Akanda et al. (1996c) found in pasture field experiment where TSA grown from seed and root, required an average number of days 55 versus 57 for former and 83 versus 89 for later to produce flowers and fruits respectively. They concluded that since TSA could produce seed in less than 90 days in both situations, any control measures implemented prior to this time would enhance its effective management strategy. In separate studies, where TSA grown from seed and root stock under natural environment in an enclosed screened cage in Tifton, Georgia, found that TSA plants initiated flowering and fruiting 60 and 120 days after germination, respectively (Dowler 1996). In Call and Coble (1998) greenhouse studies, they found that TSA reproduction increased positively with increasing phosphorous levels in soil and concluded that might be the factor responsible for TSA’s rapid infestation in U.S.A. lands. Kreiser et al. (2004) stated data was lacking on whether TSA introduction into the southern U.S.A. could be attributed to a single source. Apart from TSA appearance in Glade County, Florida in 1980s, it has now been spread to

Alabama, Georgia, Louisiana, Texas, Mississippi, North Carolina, South Carolina, Tennessee, Pennsylvania and Puerto Rico (Medal et al. 2002b; Mullahey et al. 1998). In Mississippi, TSA was only observed in two counties in 1993, but by 1995 it had spread to more than ten counties (Byrd and Bryson 1996). Infestations of TSA have rapidly spread as can be argued from the number of states infested by 2002 compared to its initial appearance in Florida just over 20 years prior. For instant, TSA infestation in Florida alone was 10,000 ha in 1990; 61,000 in 1992; 162,000 in 1993 and 500,000 in 1995 (Mullahey et al. 1993a; Mullahey et al. 1998).

The dispersal route of TSA is diverse; it includes contaminated equipment, hay, crops, seeds, composted manure and sod (Byrd et al. 2004). In addition, cattle and wildlife (birds, deer, feral hogs, and raccoons) contribute greatly to TSA spread through their fecal excretions (Medal et al. 2002a; Eplee and Westbrooks 1995). Bryson and Byrd (1995) stated that TSA population sizes and number of acres infested per site in Mississippi were directly proportional to the number of cattle imported from infested areas in Florida whereas in Texas, hay shipment from Louisiana was suspected as its introduction (Reed et. al. 2004). Bryson and Carter (2004) wrote, "Prickles, spines, thorns, and glandular hairs on stems and leaves, may protect TSA fruit from herbivores and ultimately enable the plants to produce more viable seed per plant than species that lack these specialized structure." TSA is difficult to control because it propagates by seeds and also through vegetative roots (Cuda et al. 2004) while cultural practices such as disking will favor its root fragments for dispersal (Mullahey and Cornell 1994). After emergence of TSA, flowering and mature fruit (viable seeds) were produced within 80 and 120 days respectively (Mullahey et al. 1998). TSA produces a lot of seeds per fruit and a lot of fruit per plant. Byrd et al. (2004) stated that a fruit of TSA has the potential to produce 200 to 400 seeds while a plant is capable of producing more than 200 fruit or more per year. Mullahey et al. (1993b) stated that fruit production of TSA occurs throughout the year thus from September to May and a plant is capable of producing 40,000 to 50,000 seeds per year with about 75 percent of viable seeds for germination and dispersal. Cuda et al. (2004) put the production of a plant of TSA a year at 150 fruits with mature fruits containing 400 seeds, while average seed production of a plant is 45,000 with 70% germination rate. Bryson and Byrd (2007) stated that TSA blooms and produces all year in southern Florida and other tropical climates north of the equator. It was estimated the annual impact of TSA on the livestock industry in Florida exceeds $11 million a year (Mullin et al. 2000). TSA grows vigorously in pasture and this growth characteristic can make it shades grasses within three months (Akanda et al. 1996b). For instant, TSA plants can shade bahiagrass (*Paspalum notatum* Flueggé) and cause reduced production as increased TSA populations led to decline bahiagrass production (Mullahey et. al 1999). TSA is also a potential reservoir for root-knot nematode [*Meloidogyne arenaria* (Neal) Chitwood] in Florida and other southern states of the U.S.A. (Church and Rosskopf 2005). However, TSA and its relative sticky nightshade (*Solanum sisymbriifolium* Lam.) showed severe sensitivity to blister-like growth which was documented caused as edema or oedema by (Abbas and Bryson 1996). They concluded that further studies on TSA growth, biology and life cycle based on response to this edema might lead to it effective biological control. Goats (*Capra aegagrus hircus*) are herbivores that consume TSA stems, leaves and fruits yet it has been hypothesized that ingestion of TSA caused neurologic disorder in goats (Porter et al. 2003).

Morphologically, prickles on TSA plant pose physical threat to both human and animals (Bryson and Byrd 1995). Due to tropical soda apple's rapid infestation of many livestock rangelands and also due to the fact that cattle have been recognized as one the major dispersal agent of tropical soda apple's seed, cattlemen in Florida have been involved in formulation of strategies that are enhancing effective control of its dispersal (Mullahey et al. 1998). TSA was put in Florida's Noxious Weed List in 1994 and subsequently it was placed in Federal Noxious Weed List in 1995 (Cuda et al. 2004; Bryson et al. 1995).

One of the herbicides used in this study is aminopyralid which belongs to the chemical class Pyridine Carboxylic Acid and it is formulated as liquid containing, 240 g ae/liter of aminopyralid as a salt (Carrithers et al. 2005). The recommended rate application ranges from 52.5 g ae/ha to 120 g ae/ha (Hare et al. 2005; Carrithers et al. 2005). Aminopyralid is used widely to control established broadleaf and woody weeds on pasture, rangeland, industrial vegetation management areas, non-cropland and natural areas in both Canada and U.S.A. (Hare et al 2005; Carrithers et al. 2005). The advantage of using aminopyralid is that it has systemic postemergence activity on weeds plus residual activities and it is effective at multiple stages of the plants growth[2]. Research showed that many herbicide treatments can control TSA when apply as preemergence or postemergence. Herbicide treatments that contained picloram or triclopyr controlled 8-leaf, 16-leaf or 1-year-old TSA greater than 90% at 8 weeks after treatment (WAT) (Call

[2] Milestone® Fact Sheet. Indianapolis, U.S.A. Dow AgroSciences.

et al. 2000). Akanda et al. (1996a) found that triclopyr applied at 1.0 kg ae/ha, dicamba (2.8 kg ae/ha), and clopyralid (1.8 kg ae/ha) controlled TSA more than 90% at plant's 6-8 leaf, flower or fruit stages in greenhouse experiment compared to lower rate of these treatments. Ferrell et al. (2008) found that treatments containing 2, 4-D ester or amine, metsulfuron, or hexazinone mixed with tobacco mild green mosaic virus provided 80 to 100% control of tropical soda apple. Littlefield et al. (1998) conducted studies on herbicidal activity on TSA seed soaked in rumen fluid treated with imazapic concentrations of 0, 10,100 and 1000, 10000 ppm. They observed that imazapic concentration greater 1000 ppm provided 100% control of TSA seed germination 21 days after planting compared lower concentrations. In line of this observation, they concluded that there is potential to control TSA while seed are in the rumen through administration of herbicide treated feed. Furthermore, Byrd et al. (2004) stated that emerged TSA was best controlled with treatments which contained triclopyr at rate of 1 quart per acre (2.34 L/ha) or 1 to 1.5% solution with 0.25% (v/v) nonionic surfactant while 1.5 to 2 quarts per acre (3.51 to 4.684 L/ha) or 1% solution of 2, 4-D plus picloram with 0.25% (v/v) nonionic surfactant could be used to control TSA preemergence. Picloram plus 2,4-D is used in rangeland and permanent grass pastures to selectively control many annual, biennial, and perennial broadleaf weeds[3]. However, Ferrell et al. (2006) studies on preemergent herbicides: aminopyralid applied at 0.08, 0.1 and .12 kg eg/ha , triclopyr applied at 1.1 kg ae/ha and tank mixed of 2,4-D at 1.6 kg ae/ha plus dicamba at 0.56 kg ae/ha activities on TSA showed that either rate of aminopyralid controlled TSA more

[3] Grazon P + D® specimen Label. Indianapolis, U.S.A. Dow AgroSciences.

than 97% whereas triclopyr and tank mixed 2,4-D plus dicamba failed completed rendered control at 75 DAT. Taking high seed production strategy of TSA into consideration, there is need to control its seeds in the seed bank to prevent it's appearance from year to year in crops/pasture production settings. Therefore, the objective of this study is to evaluate the residual efficacies of herbicide treatments that contain aminopyralid, and triclopyr plus picloram plus 2,4-D applied preemergence on TSA to determine the length of residual control provided by these treatments.

Materials and Methods

Plant material.

Mature fruits of TSA were collected from infested pasture field in Mississippi in November 2008. The fruits were crushed, seeds removed and stored at room temperature for 6 months before commencement of this experiment in 2009. A germination viability test was conducted on 25 seed in a plastic petri-dish that contained two filter papers and was watered with 0.5 ml of tap water as needed to maintain moisture. Each petri-dish was tightly covered with its lid in order to prevent desiccation. The test was replicated four times and placed under room temperature without supplemental light. Seedlings emerged from each test were counted and recorded at 2 and 4 weeks after germination viability test. Seedlings were counted emerged upon appearance of any radicle from the seed. In all, 96% of the seeds were viable.

Greenhouse study.

Two greenhouse studies were conducted at the Plant Science Research Center, Mississippi State University in 2009 and repeated in 2010/2011 on Agro-Miracle potting mixture soil filled in styrofoam cups. For the study conducted in 2009, TSA seeds were planted March 10, 2009 and the study was concluded September 10, 2009. The study was repeated from November 11, 2010 through May 11, 2011. The temperature of the greenhouse for both studies was maintained at 25 to 30 ºC with no supplement light. Ten seed of TSA were planted about 2.5 cm below the soil surface. The planting depth used in the studies was within the documented 0 to 6 cm planting depth range (Akanda et al. 1996b) or 1 to 4 cm ranged that favored 49 to 63% germination of TSA seeds in previous studies (Mullahey and Cornell 1994). Four herbicide treatments, aminopyralid at 0.088 kg ae/ha and 0.12 kg ae/ ha, a tank mixture of triclopyr at 0.56 kg ae/ha plus picloram at 0.15 kg ae/ha plus 2, 4-D at 0.56 kg ae/ha were independently applied on March 10, 2009 for experiment I and on November 11, 2010 for experiment II to soil in the styrofoam cups. Ten TSA seeds were planted in the treated soil immediately after the herbicide treatments were applied (PRE), 1, 3 and 6 months after treatments (MAT). An untreated check was included in each planting time for comparison. All treatments were watered every other day with tap water throughout the entire studies. The experiments were conducted as a randomized block design with factorial arrangement of treatments. Each treatment by planting time for both years was replicated four times. Treatments were applied using a CO_2-pressurized back pack sprayer in 233 L/ ha spray volume and at pressure of 137 kpa with 8003VS flat fan nozzles spaced 45.7 cm apart, and at speed 3.2 km/h. Emerged seedlings from each treatment over planting time were independently counted and recorded at 3 to 6 weeks after planting (WAP). Seedlings were counted as emerged upon appearance of any part of its on soil surface. All data were expressed as percent proportion of number of 10 seed planted. Arcsine square root transformations were performed on the percent proportion germination and were subjected to analysis of variance (ANOVA) using PROC GLIMMIX (SAS 2008). Mean differences among treatments were separated by Fishers Least Significant Difference at (P=0.05) significant level.

Results and Discussion

Data from both 2009 and 2010/2011 greenhouse studies of herbicide treatments on control TSA seedling emergence were combined and analyzed as one due to the fact that 2010/2011 study was considered as a random effect (Table 2.1). Regardless, all herbicide treatments rates provided more than 96% controlled of TSA seedlings emerged at PRE compared with untreated. Triclopyr at 0.56 kg ae/ha plus picloram at 0.15 kg ae/ha plus 2, 4-D at 0.56 kg ae/ha provided almost 100% control compared to 97.5 and 99.5% of aminopyralid at 0.088 and 0.12 kg ae/ ha, respectively. At 1 MAT, only aminopyralid at 0.088 kg ae/ha provided at least 90% control compared to 83% of triclopyr plus picloram plus 2, 4-D and 67% of aminopyralid at 0.12 kg ae/ ha. The results showed that efficacy of triclopyr plus picloram + 2, 4-D, aminopyralid at 0.088 and 0.12 kg ae/ha were reduced by 17, 6.5 and 32.5% respectively at 1 MAT compared to their results at PRE. By 3 or 6 MAT, all herbicide treatments failed to render effective (>70%) control of TSA seedling and their seedlings emergence were statistical similar to seedling emergences from untreated. However, at least some numerical control of 15, 17 and 63% were observed for triclopyr at 0.56 kg ae/ha plus picloram at 0.15 kg ae/ha plus 2,4-D at 0.56 kg ae/ha, aminopyralid at 0.088 kg ae/ha and aminopyralid at 0.12 kg ae/ ha, respectively, at 3 MAT. At 6 MAT, all the herbicide treatments still provided some numerical control: 32% for triclopyr at 0.56 kg ae/ha plus picloram at 0.15 kg ae/ha plus

2,4-D at 0.56 kg ae/ha, 3% for aminopyralid at 0.088 kg ae/ha and 14% for aminopyralid at 0.12 kg ae/ha.

Apart from more than 90% control achieved for each herbicide treatment at PRE, only aminopyralid at 0.088 kg ae/ha rendered more than 90% control of TSA seedlings emergences at 1 MAT. All other treatments at 1, 3 and 6 MAT failed to provide more than 90% control. In this study, TSA seedling emergences were poorly controlled based on Mullahey et al. (1993a) proposed more 90% control as benchmark. For instance, Byrd et al. (2004) stated that a plant of TSA can produce about 200 fruits and a fruit contains range of 200 to 400 seeds. In addition, about 75% of the seeds are mostly viable for germination (Mullahey et al. 1993b). Considering this seed reproduction strategy of TSA coupled with its ability to become a weed in agronomic production settings and invasive plant in natural areas, it is therefore, imperative that it should be effectively controlled by residual herbicides so that it can be depleted from seed bank for short and long time purposes. All the herbicide treatments showed residual effect on TSA seedling emergence, although they were more effective at PRE and 1 MAT compared to 3 and 6 MAT. In the previous study, aminopyralid at both rates provided more 97% more control of TSA seedlings emergences (Ferrell et al. 2006) compared this study. Less effectiveness of aminopyralid in this study might be due to differences in soil used compared to soil used in Ferrell et al. (2006) study. Taking the residual efficacies of all herbicide treatments to some extend into consideration, their repeated application will be effective on controlling TSA seedlings emergences. In addition, since TSA can produce and flower all year around in the southern States climatic conditions (Akanda et al. 1997), this study showed that herbicide treatments can serve as a very imperative method of controlling its seedlings emergence before it matures and starts to flower or produce seeds for dispersal. Further research is also needed to understand biology and physiology of seeds of TSA as the results from untreated (control) for PRE, 1 MAT, 3 MAT and 6 MAT showed similar germination viable even though all the seeds were stored 6 months prior to the start of the study in 2009 and more than one year in 2010/2011.

Table 2.1 Percent germination rate of tropical soda apple seeds that were treated with three herbicide treatments, versus untreated reference, in greenhouse experiment at the Plant Science Research Center, Mississippi State University for 2009 and 2010/2011. [a]

Herbicide	Rate	PRE	1MAT	3MAT	6MAT
	Kg ae/ha	——% proportion germination——			
Untreated	0	40.1a	46.0a	60.0a	45.0a
Triclopyr + picloram + 2,4-D	0.56 + 0.15 + 0.56	0.0b	7.5b	50.7a	30.3a
Aminopyralid	0.088	1.0b	4.3b	49.5a	43.6a
Aminopyralid	0.12	0.2b	15.4ab	22.2a	38.4a

[a]Average over two studies. TSA, tropical soda apple; PRE, preemergence; MAT, months after treatment. Means with the same letter within the same column are not significant different, according to Fisher's Protected LSD at P = 0.05.

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CHAPTER III

EVALUATION OF HERBICIDES FOR BENGHAL DAYFLOWER (*Commelina benghalensis*) CONTROL IN THE GREENHOUSE

Abstract

Benghal dayflower (*Commelina benghalensis* L.) is a non-native, invasive and herbaceous creeping perennial plant that is rapidly invading agronomic crop, pasture and production systems of native areas in southern states of the U.S.A. Reproduction elasticity of Benghal dayflower coupled with agronomic practices such as minimum tillage (minimum cultural practices) and over-reliance on glyphosate tolerant crop productions are enhancing persistence and success of this plant in southern states. In 2008/2009 and 2012, greenhouse experiments were conducted at the Plant Science Research Center of Mississippi State University for Benghal dayflower control with fifteen postemergence herbicides. Each was independently visually rated 1 to 6 weeks after treatment (WAT) for its efficacy on Benghal dayflower. Aminocyclopyrachlor at 320 g ai/ha controlled Benghal dayflower less than 35% at 1 WAT and 56% at 2 WAT but at 4 WAT its efficacy on Benghal dayflower increased to 80% and further increased to more than 93% at 4 to 6 WAT. Benghal dayflower with sulfentrazone applied at 420 g ai/ha gave 56% control at 1 WAT, then increased to 96% control at 4 WAT, but decreased to only 67% by 6 WAT. Flumioxazin at 107 g ai/ha only controlled Benghal dayflower more than 70% at 1 to 4 WAT and decreased to 59 % at 6 WAT. Saflufenacil

at 35 g ai/ha controlled Benghal dayflower at least 78% at 1 WAT, but control decreased over all other evaluation intervals to only 24% by 6 WAT. Atrazine at 2800 g ai/ha controlled Benghal dayflower more than 60% only at 2 and 3 WAT, but less than 50% at all other WAT. All other herbicides provided less than 50% Benghal dayflower control at all WAT. Variable controlled observed for most of the herbicides was attributed to re-growth (regeneration). Based on the variable control of Benghal dayflower with some of these herbicides, it will be prudent that repeated application or use of herbicides with residual properties might be required for long term effective control of Benghal dayflower.

Nomenclature: Ametryn: 2-ethylamino-4-isopropylamino-6-methylthio-s-triazine; aminocyclopyrachlor: 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid; Atrazine: 2-chloro-4-ethylamino-6-isopropylamino-s-triazine; bentazon: (3-{1-melhylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one 2.2-dioxide);

bromoxynil :(3,5-dibromo-4-hydroxybenzonitrile); diclosulam: N-(2,6-dichlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo-[1,5-c]pyrimidine-2-sulfonamide; dimethenamid-P: (S)-2-choro-N-[(1-methyl-2-methoxy)ethyl]-N-(2,4-dimethyl-thien-3-yl)-acetamide;

flumioxazin:2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2*H*-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1*H*-isoindole-1,3(2*H*)-dione; chlorimuron ethyl plus tribenuron methyl : Ethyl 2-[[[[(4-chloro-6-methoxypyrimidin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate plus Methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)methylamino]carbonyl]amino]sulfonyl]benzoate; nicosulfuron plus rimsulfuron**:** 2-[[(4,6-dimethoxypyrimidin-2-

yl)aminocarbonyl]aminosulfonyl]-N,N-dimethyl-3-pyridinecarboxamide plus N((4,6- dimethoxypyrimidin-2-nyl) aminocarbonyl)-3-(ethylsulfonyl)-2-pyridinesulfonamide; primisulfuron-methyl: 3-[4,6-bis(difluoromethoxy)-pyrimidin-2-yl]-1-(2-methoxycarbonyl-phenylsulfonyl) urea; saflufenacil**:** N-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydr0-1(2H)pyrimidinyl)benzoyl]-N-isopropyl-N-methylsulfamide; sulfentrazone: N-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]phenyl]methanesulfonamide; sulfosulfuron: N-[[4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-2-(ethylsulfonyl)imidazo[1,2-a]pyridine-3-sulfonamide;{Sulfosulfuron}; S-metolachlor plus glyphosate plus mesotrione: acetamide,2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl]-,(S) plus glyphosate, N-(phosphonomethyl glycine plus 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione; Benghal dayflower: *Commelina benghalensis* (L). #[4] COMBE.

Abbreviation: WAT: weeks after treatment

Additional index words: Ametryn, aminocyclopyrachlor, atrazine, bentazon, bromoxynil, diclosulam, dimethenamid-P, flumioxazin, chlorimuron ethyl plus tribenuron methyl, nicosulfuron plus rimsulfuron, primisulfuron-methyl, saflufenacil, sulfentrazone, sulfosulfuron, S-metolachlor plus glyphosate plus mesotrione, Benghal dayflower.

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[4] Letters followed by this symbol are a WSSA-approved computer code from Composite List of Weeds Revised 2010. Available only on computer disk from WSSA, 810 East 10th Street Lawrence, KS 660448897

Introduction

Benghal dayflower or tropical spiderwort (*Commelina benghalensis* L.) is a fleshy, C_3, herbaceous, creeping annual or perennial plant that reproduces through seeds from aboveground and underground flowers, stolons, or roots at stem nodes and is native to some countries in Africa and Asia (Maheshwari and Singh 1934; Chivinge and Kawisi 1989; Holm et al. 1991; Steptoe et al. 2006; Price et al. 2009). In the Philippines, one Benghal dayflower produced 1610 seeds (Pancho 1964). It grows vigorously in diverse habitats ranging from water saturated to dry soil with varying pH, fields, yards, cultivated lands, disturbed areas, field borders, wet pasture-lands, gardens, grassland, roadsides and waste places (Wilson 1981; Holm et al. 1991; Faden 1993). In India, Maheshwari and Maheshwari (1955) wrote, "*C. benghalensis* are found in abundance everywhere under moist shady places during the rains." It is used as a medicinal plant and dying material (Gokhale et al. 2004; Joshi and Tyagi 2011; Oryema et al. 2010).

Uniquely, Benghal dayflower grows to form dense and pure stands, which aid it to smother out other low growing plants such as vegetables, pulses and cereals, thus making it one of the most serious weeds in the world (Wilson 1981; Holm et al. 1991). It is also a fast growing plant. Walker and Evenson (1985) found that Benghal dayflower seedlings developed rhizomes 6 weeks after emergence and the number and length of rhizomes increased as the plant aged. Benghal dayflower is not a weed in terms of water and nutrients competition alone, rather it serves as a host of nematode and pathogens such as reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira), southern root-knot nematode [*Meloidogyne incognita* (Kofoid and White) Chitwood], peanut root-knot nematodes [*Meloidogyne arenaria* (Neal) Chitwood] and fungal pathogen for southern stem rot (*Sclerotium rolfsii* Sacc.) (Desaeger et al. 2000; Davis et al. 2006). Singh et al. (1989) studies in Nigeria also found that extracts from Benghal dayflower have an allelopathic effect on seedling vigor of soybean (*Glycine max* (L.) Merr.), and corn (*Zea mays* L.).

Historical records on Benghal dayflower revealed that it was first observed in 1928 in U.S.A. and by the mid-1930s it appeared to have been established in Florida (Faden 1993; Durham 2006). As far as its geographical distribution is concerned, it has been found in Alabama, California, Hawaii, Georgia, North Carolina and Missouri (Burton and York 2005; Webster et al. 2005) and was recently found in Jackson County, Mississippi in August 2006 (Maddox et al. 2007). According to Faden (1993) there are nine *Commelina* species that occur in the U.S.A. and six of them which include: Benghal dayflower; Carolina dayflower (*Commelina caroliniana* Walter), Asiatic dayflower, (*Commelina communis* L.); spreading dayflower (*Commelina diffusa* Burm f.), rat's ear (*Commelina forskaolii* Vahl), and African dayflower (*Commelina nigritana* Benth) are considered non-native whereas the other three, birdbill dayflower (*Commelina dianthifolia* Delile), whitemouth dayflower (*Commelina erecta* L.) and Virginia dayflower (*Commelina virginica* L.), are native. However, Benghal dayflower is different from other *Commelina* species because it is the only known *Commelina* species in the U.S.A. with underground flowers (Durham 2006). In addition, it can often be misidentified for spreading dayflower when viewed from aboveground. But it is distinguished from spreading dayflower due to presence of hairs on its young leaves and petioles (Ferrell et al. 2004).

Benghal dayflower is dispersed through movement of infested equipment, animals (grazing cattle and wildlife) and harvested crops (Webster et al. 2006b). Apparently Benghal dayflower invades and poses a threat to crop production in the southern U.S.A. (Sermons et al. 2008; Durham 2006) and it is now considered a noxious, exotic, and invasive plant in the U.S.A. (Prostko et al. 2005). In Georgia, for instance, the introduction of glyphosate-tolerant cotton (*Gossypium hirsutum* L.) has led to fewer applications of residual herbicides to control cotton weeds postemergently without impact on cotton (Prostko et al. 2005). However, this practice is also enhancing persistence of Benghal dayflower which reproduces through underground seeds and stolon/rhizome development (Stamps 2007). As much as 10 to 51% reduction of peanut (*Arachis hypogaea* L.) yield was reported in Georgia, U.S.A., due to Benghal dayflower's interference in the first four weeks and full interference with it during growing season, respectively (Webster et al. 2007). Additional greenhouse studies documented that aggressive competition of Benghal dayflower with cotton under drought stress (Webster and Grey 2008). The estimated cost for control of this invasive plant in Georgia alone exceeded $1.2 million per annum (Durham 2006). Benghal dayflower is a difficult to control weed due to its ability to grow in diverse habitats, its sprawling habit and ability to root readily at the nodes (Wilson 1981; Holm et al. 1991; Vencill and Steptoe 2005). The ability of Benghal dayflower to germinate and thrive under the crop canopy limits its cultural weed control strategies (Vencill and Steptoe 2005). However, herbicide treatments that contain 2, 4-D, paraquat, and glyphosate applied postemergence can control it, especially juvenile plants that are actively growing (Wilson 1981; Maddox et al. 2007). In addition, S-metolachlor, alachlor, and dimethanamid provide some control of Benghal dayflower due to their residual activities (Maddox et al. 2007). Steptoe et al. (2006) stated that underground seeds of Benghal dayflower are more susceptible to metolachlor than aerial seeds due to more likely large translocation of metolachlor to the underground flowers. Previous studies by (Walker 1981) in Australia when efficacies of herbicide treatments that contained 2,4-D (0.125 kg ai/ha), dinoseb (1.1 kg ai/ha) and bentazon (0.96 kg ai/ha) were applied at 2.5 and 5-leaf stages of Benghal dayflower development, reported 2,4-D and dinoseb provided 92% and 100%, control, respectively. However, control was reduced to 63% (2,4-D) and 67% (dinoseb) when applied to mature plants. In the United States, Culpepper et al. (2004) observed 70 and 78 % control of Benghal dayflower with glyphosate (0.84 kg ai/ha) and MSMA (0.84 kg ai/ha) when each applied alone as a late-postemergence-directed at 3WAT respectively. However, they observed that when glyphosate was mixed with flumioxazin (0.036 kg ai/ha) or MSMA was mixed with flumioxazin (0.036 kg ai/ha) Benghal dayflower control increased to 85 and 89% respectively at 3 WAT. Webster et al. (2006a) observed 87% and 94% control of Benghal dayflower treated premergence with clomazone at 1.05 kg ai/ha 3 and 6 WAT, respectively. In contrast, they observed reduced control (70%) when clomazone at 0.84 kg ai/ha was applied as postemergence at the same weeks after treatment as in preemergence. Webster and others (2006a) also found that S-metolachlor applied at 1.60 kg ai/ha provided 90% and 96% control of Benghal dayflower at 3 and 6 WAT, respectively.

The objective of this study is to evaluate residual effect of 15 postemergence herbicide treatments (Table 3.1) on Benghal dayflower control.

Materials and Methods

A greenhouse experiment was initiated in November 2008 and repeated in February 2012 at the Plant Science Research Center of Mississippi State University on Benghal dayflower grown in Agro-Miracle® potting mixture soil in styrofoam (50 cm diameter by 14 cm size) cups. Fifteen postemergence herbicide treatments were evaluated for efficacy on Benghal dayflower in the 2008/2009 experiment, but in the repeated experiment, fourteen postemergence herbicide treatments were evaluated. The temperature of the greenhouse was maintained at 25 to 30 C for the studies with no supplemental lighting. Prior to the experiment, Benghal dayflower stem cuttings with 3 to 4 nodes were collected from a corn farm in George County, Mississippi and transplanted in the greenhouse until they became established at about 6 weeks after planting. However, for the repeated experiment in 2012, Benghal dayflower was established from the seed due to failure of all planted stem cuttings to germinate. The seed planted initially also failed to germinate at one month after planting. However, seeds were later soaked in commercial bleach[5] for 7 minutes and washed thoroughly. When the seed was planted, seedling emergence was observed 7 days after planting. Each pot was watered every other day. Treatments were arranged in a randomized complete block design and replicated four times. Herbicide treatments applied were saflufenacil at 35 g ai/ha, S-metolachlor plus glyphosate plus mesotrione at 1170 + 1170 + 120 g ai/ha , bromoxynil at 560 g ai/ha, dimethenamid-P at 1100 g ai/ha, bentazon at 1120 kg ai/ha, nicosulfuron plus rimsulfuron at (26.3 + 13.1) g ai/ha , flumioxazin at 107 g ai/ha, diclosulam at 26 g

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[5] CLOROX. Regular-Bleach. Clorox Company, 1221 Broadway, Oakland, CA 94612.

ai/ha, sulfosulfuron at 3.52 g ai/ha, aminocyclopyrachlor (applied as DPX-MAT28®) at 320 g ai/ha, sulfentrazone at 420 g ai/ha, chlorimuron ethyl plus tribenuron methyl at 52 + 16 g ai/ha , ametryn at 1192 g ai/ha, atrazine at 2800 g ai/ha and primisulfuron-methyl at 39.9 kg ai/ha in 2008 experiment whereas diclosulam was not included in repeated experiment in 2012. In both experiments, saflufenacil was applied with crop oil concentrate at 0.01% V/V, other treatments, except dimethenamid-P, sulfentrazone, and atrazine, were applied with non-ionic surfactant at 0.25% V/V.

All treatments were applied with a CO_2-pressurized backpack sprayer in 233.4 L/ha spray volume and at a pressure of 137 kpa. The spray boom was equipped with 8003VS flat fan nozzles, spaced at 45.7 cm, and at ground speed of 3.2 km/h.

Percent Benghal dayflower control on a scale of 0 equal to no injury and 100% equal to complete mortality, was visually rated at 1 to 6 weeks after treatment (WAT). In the 2008 experiment, the visual rating started on May 19, 2009 and in the repeated experiment, it started on March 30, 2012. The repeated experiment was considered as a random effect; therefore, both experiments were combined and analyzed as one data. Data were expressed as percent reduction of the untreated and arcsine root square transformation was performed on them. The transformed data were then subjected to analysis of variance (ANOVA) and analyzed as repeated measurement using PROC GLIMMIX in SAS (2008). The transformed data did not influence the interpretation of the results; therefore, the results were presented untransformed. An untreated control was not included in data analysis for each evaluation period. Differences among means were separated using Fisher's Protected LSD at ($P = 0.05$) significant level.

Results and Discussion

Combined analysis of the data over 6 weeks of ratings showed significant differences ($P < 0.0001$). Tests for main effects, both treatments ($P < 0.0001$) and weeks of evaluations ($P < 0.0001$) were significant and test for the interaction between treatment and weeks of evaluations ($P < 0.0001$) was also significant; therefore, the data were presented weekly for all treatments (Table 3.1).

At 1 WAT, a significant difference ($P < 0.0001$) occurred among the herbicide treatments (Table 3.1). Benghal dayflower control ranged from 5% with bromoxynil applied at 560 g ai/ha to 79% with flumioxazin applied at 107 g ai/ha. Saflufenacil applied at 35 g ai/ha (77%) provided statistically similar control to (79%) flumioxazin. All other treatments failed to provide at least 60% control of Benghal dayflower at the initial evaluation period. While only sulfentrazone applied at 420 g ai/ha provided up to 56% control of Benghal flower at this evaluation, other treatments ametryn at 1192 g ai/ha, atrazine at 2800 g ai/ha, aminocyclopyrachlor at 320 g ai/ha, S-metolachlor plus glyphosate plus mesotrione at 1170 + 1170 + 120 g ai/ha, and chlorimuron ethyl plus tribenuron methyl at 52 + 16 g ai/ha provided 47, 41, 34, 26 and 26% decreased control, respectively. Furthermore, bromoxynil at 560 g ai/ha, dimethenamid-P at 1100 g ai/ha, bentazon at 1120 g ai/ha, nicosulfuron plus rimsulfuron at 26.3 + 13.1 g ai/ha, diclosulam at 26 g ai/ha, sulfosulfuron at 3.52 g ai/ha, and primisulfuron-methyl at 39.9 g ai/ha all failed to control Benghal dayflower by at least 13%.

At 2 WAT, significant differences ($P < 0.0001$) were also observed (Table 3.1**).** Flumioxazin, sulfentrazone, atrazine and saflufenacil controlled Benghal dayflower 83, 80, 64 and 62%, respectively, with no statistical differences in control level of these treatments. Flumioxazin, sulfentrazone, and atrazine improved Benghal dayflower control whereas saflufenacil decreased control compared to flumioxazin (79%), sulfentrazone (56%), atrazine (41%), and saflufenacil (77%) at 1 WAT. All other herbicide treatments: S-metolachlor plus glyphosate plus mesotrione, bromoxynil, dimethenamid-P, bentazon, nicosulfuron plus rimsulfuron, diclosulam, sulfosulfuron and primisulfuron-methyl provided less than 26% control of Benghal dayflower, except ametryn (55%), aminocyclopyraclhlor (55%) and chlorimuron ethyl plus tribenuron methyl (43%).

At 3 WAT, significant differences ($P < 0.0001$) were again observed (Table 3.1). Control of Benghal dayflower provided by sulfentrazone, aminocyclopyrachlor, flumioxazin, atrazine, saflufenacil was 93, 82, 81, 68, and 60%, respectively. In addition, control with dimethenamid-P, nicosulfuron plus rimsulfuron, and diclosulam increased to 16, 26, and 27% compared to 1, 10, and 9% observed at 2 WAT, respectively. However, Benghal dayflower control with ametryn at this evaluation period drastically decreased to 31% compared to its rating of 47% at 1 WAT and 55% at 2 WAT. S-metolachlor plus glyphosate plus mesotrione, bromoxynil, dimethenamid-P, bentazon, nicosulfuron plus rimsulfuron, sulfosulfuron, and primisulfuron-methyl failed to provide at least 27% control at this evaluation period; however chlorimuron ethyl plus tribenuron methyl which maintained 42% control which is almost similar to its 43% control at 2 WAT.

Significant differences ($P < 0.0001$) were also observed at 4 and 5 WAT, respectively (Table 3.1). Benghal dayflower was consistently controlled more than 95% with aminocyclopyrachlor at 4 and 5 WAT. Although sulfentrazone provided similar control of Benghal dayflower as aminocyclopyrachlor at 4 WAT, its efficacy decreased to 11% at 5 WAT as re-growth of the plant was observed. Similar observations were recorded for saflufenacil and flumioxazin. Although Benghal dayflower control provided by saflufenacil was 57% and flumioxazin was 74% at 4 WAT, by 5 WAT, these control levels reduced to 44 and 62%, respectively. Diclosulam provided 49% control at 4 WAT but 64% at 5 WAT. All other herbicide treatments S-metolachlor plus glyphosate plus mesotrione, bromoxynil, dimethenamid-P, bentazon, nicosulfuron plus rimsulfuron, sulfosulfuron, chlorimuron ethyl plus tribenuron methyl, ametryn, atrazine, and primisulfuron-methyl rendered less than 50 and 40% control at 4 and 5 WAT, respectively.

By the 6 WAT final evaluation, there remained significant differences ($P < 0.0001$) among treatments. Benghal dayflower control was excellent at more than 90% with aminocyclopyrachlor. Diclosulam control of Benghal dayflower was 67% at 6 WAT, but this level of control was not acceptable. Similarly, Benghal dayflower control with nicosulfuron plus rimsulfuron increased from 34% at 5 WAT to 37% at 6 WAT. Although Benghal dayflower control with flumioaxazin was 59% and with sulfosulfuron 67% at this evaluation, the pattern showed a slight decrease in efficacy for flumioaxazin and a drastic decrease for sulfosulfuron compared to 62 and 85% at 5 WAT, respectively. Again, Benghal dayflower control with salflufenacil, S-metolachlor plus glyphosate plus mesotrione, bromoxynil and dimethenamid-P hardly improved 25%. Chlorimuron ethyl plus tribenuron methyl only provided 15% control of Benghal dayflower, whereas other treatments ametryn, atrazine, bentazon, primisulfuron-methyl, and sulfosulfuron provided less than 10% control at this evaluation period.

In this study, flumioxazin provided consistent control of Benghal dayflower more than 70% at 1 to 4 WAT, but Benghal dayflower began to re-grow by 5 WAT. Previous work by Culpepper et al. (2004) showed that glyphosate, or MSMA applied alone as a late-postemergence-directed treatment did not control Benghal dayflower effectively, but when mixed with flumioxazin, control improved compared to glyphosate, or MSMA applied alone at 3 WAT. While Benghal dayflower control with flumioxazin was not the highest in the duration evaluations, it was more consistent compared to aminocyclopyrachlor whose efficacy only started to peak from 3 WAT and sulfentrazone from 2 WAT. Control of Benghal dayflower with saflufenacil ranged from 57 to 78% at 1 to 4 WAT, but decreased to 24% by the termination of the study. Benghal dayflower control with atrazine was only above 60% at 2 and 3 WAT while 2 to 41% were observed at other time intervals after treatments. In all evaluations, regeneration of Benghal dayflower was observed. Based on observations from this study, it can therefore be suggested that repeated application of herbicides will be required for long term effective suppression of the Benghal dayflower due to its reproduction elasticity through underground, aboveground seeds, and regeneration from any fragment parts into consideration. However, the excellent controlled of Benghal dayflower with aminocyclopyrachlor at the final evaluation, coupled with variable good to excellent control with flumioxazin, sulfentrazone, and salflufenacil at various evaluation periods in this study provided evidence that timely repeated applications are essential for Benghal dayflower control. Further research will be needed in the greenhouse or field to ascertain efficacies of some of the herbicides that provided variable control of Benghal dayflower, and particular attention should be placed on sequential applications of aminocyclopyrachlor, sulfentrazone, and flumioxazin due to their consistent and good to excellent efficacy.

Table 3.1 Percent Benghal dayflower control with 15 individual herbicides evaluated at 1 to 6 WAT averaged over two experiments (2009 and 2012) in greenhouse at the Plant Science Research Center, Mississippi State University.

Herbicides	Rate	1	2	3	4	5	6
	g ai/ha	% control WAT[a]					
Saflufenacil**	0.035	78ba	62bac	60bcd	57cb	44dc	24bcd
S-metolachlor +	1170 +	26dfe	25dfe	26egf	44cd	38edc	21bcd
Glyphosate +	1170 +						
Mesotrione	120						
Bromoxynil	560	5g	3fg	5g	15fg	23gefdc	22bcd
Dimethenamid-P	1100	7g	1g	16gf	37ced	30efdc	25bcd
Bentazon	1120	12gfe	12ef	10gf	20fged	6gfh	3d
Nicosulfuron +	26.3 + 13						
Rimsulfuron		12gf	10efg	26egf	44cd	34edc	37bc
Flumioxazin	107	79a	83a	81ba	74b	62bc	59b
*Diclosulam	26	12gfe	9efg	27egfd	49cbd	64bac	67ba
Sulfosulfuron	3.52	12gfe	15ef	17egf	24fed	10gefh	7cd
Aminocyclopyrachlor	320	34dce	55bc	82ba	96a	96a	94a
Sulfentrazone	420	56bc	80ba	93a	96a	85ba	67ba
Chlorimuron Ethyl +	52 + 16	26dfe	43dc	42ecd	37ced	15gefdh	15cd
Tribenuron methyl							
Ametryn	1192	47dc	55bc	31efd	17fge	4gh	2d
Atrazine	2800	41dc	64bac	68bc	33fed	4gh	2d
Primisulfuron-methyl	39.9	8gf	9efg	7g	5g	1h	4d

** Saflufenacil was applied with Crop oil concentrate (COC) 0.01 % V/V. All the other treatments were applied with Nonionic surfactant (NIS) added at 0.25% (v/v) except dimethenamid-P, sulfentrazone and atrazine

WAT: Weeks after treatment.

[a] Means with the same letter within the same column are not significant different, according to Fisher's Protected LSD at P = 0.05.

* Herbicide was not included in 2012 experiment.

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CHAPTER IV

RESPONSE F1 GENERATIONS OF ALS RESISTANT AND SUSCEPTIBLE BIOTYPES OF ITALIAN RYEGRASS (*Lolium perenne* L. ssp. *multiflorum*) HARVESTED FROM MIXTURE TO IMAZAPYR

Abstract

Greenhouse studies were done in 2007/2008 and repeated in 2008/2009 at the Plant Science Research Center, Mississippi State University to determine gene flow among ALS confirmed resistant ‘49E’ and susceptible ‘Marshall’ and ‘Gulf’ biotypes of Italian ryegrass [*Lolium multiflorum* Lam.] F1 generations. Each biotype F1 generation’s response to imazapyr applied preemergence (PRE) at rates of 0, 1 and 2% by volume was evaluated by seedling emergence (SE) and seedling fresh biomass weights (SFBW). Responses to early (EPOST) and late (LPOST) postemergence applications of the same rates of imazapyr were also evaluated by shoot survival (SS) and shoot fresh biomass weights (SHFBW). In the PRE study, regardless of imazapyr rate of either 1 or 2% reduced ‘Marshall’ over 90% and ‘Gulf’ over 69% as evaluated by SE and SFBW. By comparison, the resistant biotype ‘49E’ SE was reduced 3 and 18% by applications of imazapyr at 1 and 2%, respectively, in experiment I, without significant reduction in SFBW in experiment II. In EPOST study, either at 1 or 2% of imazapyr reduced ‘Marshall’ SS over 82% and it’s SHFBW between 75 to 90%. ‘Gulf’SS was also reduced over 75% and it’s SHFBW between 66 to 80% by either at 1 or 2% applications of

imazapyr. The resistant ’49E’ SS was reduced 2 to 9.5% in experiment I and 0 to 4.1% in repeated experiment by either at 1 and 2% of imazapyr. However, both rates of imazapyr failed to reduce it’s SHFBW, averaged over the two experiments. In LPOST study, ‘49E’SS was reduced 9 to 11% regardless of imazapyr rates, but there was no differences in their corresponding SHFBW in experiment I. In experiment II, at 1 and 2% of imazapyr, ‘49E’ SS was reduced 10% and 0%, respectively. In contrast, both rates of imazapyr reduced ‘49E’ SHFBW 26 to 30%. The susceptible ‘Gulf’ and ‘Marshall’ SS were reduced 70 to 75% and 83 to 86%, respectively, in experiment II at 1 or 2% of imazapyr compared to over 90% for both biotypes in experiment I. Applications of both 1 and 2% of imazapyr caused less SHFBW reductions in ‘Gulf’ and ‘Marshall’ in experiment II compared to their SHFBW in all studies. Evaluation of ‘49E’ under all the studies (PRE, EPOST, EPOST) showed some reduced fitness response to imazapyr treatments. The results therefore showed that with cessation of herbicide treatments (ALS herbicides), gene movements from Italian ryegrass susceptible biotype could reduce fitness of resistant biotype to some extent.

Nomenclature : Imazapyr: (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid); Italian ryegrass, *Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot #[6] LOPEM2.

Additional index words: Biotype; 49E (resistant); Marshall (susceptible), Gulf (susceptible).

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[6] Letters followed by this symbol are a WSSA-approved computer code from Composite List of Weeds Revised 2010. Available only on computer disk from WSSA, 810 East 10th Street Lawrence, KS 660448897

Abbreviations: SE, seedlings emergence; SS, shoot survival; SFBW, seedlings fresh biomass weights; SHFBW, shoots fresh biomass weights; PRE, preemergence; EPOST, early postemergence; LPOST, late postemergence; ALS, acetolactate synthase.

Introduction

Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] is an important cool-season annual or short-lived perennial forage grass that was introduced into North America from Europe (Terrell 1968; Betts et al. 1992; Hannaway et al. 1999). It germinates either in the fall or early spring and completes its life cycle before summer (Webster and MacDonald 2001). According to Balasko et al. (1995), it grows in diverse soil types from sand to clay with the preference soil pH range from 5.5 to 8.0, however, its optimum growth usually occurs at pH 5.7 and above due to poor nutrients availability and likely aluminum toxicity which may associate with lower pHs. In addition to its adaptability, Valenzuela and Smith (2002) stated that it does well on heavy, temporarily waterlogged soils if it has already been established. Italian ryegrass belongs to genus *Lolium* and is among three outbreeding species in the genus (Terrell 1968) and it is also a diploid with chromosome number (2n=14) (Balasko et al. 1995). This plant has been reported as a weed in many production settings. In Georgia, it is considered a troublesome weed in grain (Webster and MacDonald 2001); in North Carolina, a major weed in wheat (*Triticum aestivum* L.) and small grain crops (Liebl and Worsham 1984; 1987) and in Idaho, Oregon, South Carolina, Virginia and Tennessee, a winter weed in wheat (Heap 2008). Italian ryegrass competition with winter wheat led to as much as about 60% reduction in grain yield (Appleby et al. 1976). Since Italian ryegrass is considered a weed in those production settings, many herbicides have been used successfully to control it until some populations/ biotypes developed herbicide resistant. Italian ryegrass tolerance to diclofop was first reported in Oregon, U.S.A. in 1987, in a winter wheat field treated with diclofop for 7 years (Stanger and Appleby 1989). Subsequently, its populations/ biotypes resistance and tolerance to glyphosate was reported in Chile (Perez and Kogan 2003; Michitte et al. 2007) and in the USA (Perez-Jones et al. 2005, Nandula et al. 2007; Jasieniuk et al. 2008). In studies by Perez-Jones et al. (2005), where both susceptible and resistant biotypes of Italian ryegrass treated with glyphosate at 0.42 and 0.84 kg ha^{-1}, and shikimic acid extracted from them at 12, 24, 48, and 96 hours after treatment proved that the susceptible biotype accumulated between three to five times more shikimic acid than the resistant biotype. In Chile where contact angles of glyphosate spray solution was compared on both resistant (40 to 45º) and susceptible (70º) Italian ryegrass biotypes, Michitte et al. (2007) found that the retention of glyphosate uptake by the abaxial leaf surface was 40% lower in the resistant biotype than the susceptible biotype. In addition, they found that more glyphosate migrated to the tip of the treated leaves of resistant than susceptible biotypes. In Texas, Tucker et al. (2006) reported decreased efficacies of ALS (acetolactate synthase) inhibiting herbicides (sulfonylurea) chlorsulfuron, triasulfuron, and chlorsulfuron plus metsulfuron methyl on Italian ryegrass in wheat field.

The response of Italian ryegrass biotypes to imazapyr were evaluated in this study. Imazapyr belongs to the imidazolinone herbicide family, which generally control plants by inhibiting the enzyme acetolactate synthase (ALS) also known as an acetohydroxyacid synthase (AHAS) (Tan et al. 2005; Ahrens 1994). There are four additional herbicide families: sulfonylureas, triazolopyrimidines, pyrimidinylthiobenzoates and sulfonylamino-carbonyltriazolinones which inhibit ALS (Mallory-Smith and Retzinger 2003). The ALS or AHAS enzyme is responsible for production of branched chain amino acids (valine, leucine and isoleucine) (Cox 1996; Ahrens 1994; Cobb 1992). Therefore, inhibition of any of these proteins denies the plants necessary nutrients and necessary biochemical processes that allow the plants to survive. ALS inhibition herbicides have advantages because they can be applied foliar or to soil, are potent, selective, render broad-spectrum inhibition of plant growth, they are applied in low dosages (grams instead of kilograms per hectare) and have very low mammalian toxicity (Cobb 1992). Plants resistant to ALS herbicides have long been predicted. Cobb (1992) stated that due to fact that sulfonylureas and imidazolinone are such potent inhibitors of ALS and also could have soil residual activity under certain conditions, plants would have tendencies to develop resistant to them. Furthermore, in a review of resistance of weeds to ALS inhibiting herbicides, Tranel and Wright (2002) stated that weeds resistant to ALS herbicides can either come from homozygous or heterozygous resistant alleles despite the fact that ALS functions in plastid, it is also a nuclear gene and its resistant alleles can be disseminated by both pollen and seed.

Imazapyr is mostly translocated through xylem to accumulate in leaf tips and becomes available for phloem loading and redistribution within the plant (Little and Shaner 1991). Imazapyr can persist in soils and has a half-life that ranges from 25 to 142 days depending on soil type and environmental conditions (Ahrens 1994).

Due to high selectivity of most herbicides which enhances the evolution of herbicide resistance in most weeds, it has long been predicted that stopping herbicides use for years would allow the susceptible weed population to re-establish and reduce the fitness of the resistant weed populations (Gressel and Segel 1978). Roush and others (1990) stated that “research on fitness and gene flow processes that link the disciplines of genetics and physiology with ecology will help explain the population dynamics of herbicide resistance.” Field and greenhouse experiments were conducted on emergence patterns of both diclofop-methyl resistant and susceptible biotypes of Italian ryegrass in Oregon (Ghersa et al. 1994a). The results showed that diclofop-methyl susceptible biotypes emerged earlier than resistant biotypes. Ghersa et al. (1994b) conducted a study to evaluate whether or not cross fertilization of diclofop-methyl resistant ryegrass could be controlled with susceptible ryegrass biotype. They found that the timing and abundance of both ovule production and pollen release from the susceptible biotype of Italian ryegrass plants were faster than the resistant biotypes. Their study provided evidence that gene flow could be a potential way to reduce resistance evolution of diclofop-methyl in Italian ryegrass population within wheat-ryegrass cropping systems where herbicide application stopped and the field planted with susceptible biotype.

The objective of this study was to screen efficacy of imazapyr rates on F1seed generation of a resistant Italian ryegrass selection (‘49E’) and two susceptible (‘Marshall’ and ‘Gulf’) biotypes previous grown in mixture in field and evaluate whether resistance genes were transferred among them. The specific objective included evaluate the efficacy of imazapyr rates applied as preemergence (PRE) on seedling emergence; early postemergence (EPOST) on seedlings survivorship and late postemergence (LPOST) on shoot survivorship of all F1 generations of the three biotypes in separate studies. The final objective was to determine the impact of imazapyr rates on fresh biomass weights of all specific objectives described above.

Materials and Methods

Experiments were conducted in 2007/2008 and repeated in 2008/2009 to determine gene movement among ALS confirmed 49E resistant and two outsourced susceptible 'Marshall' and 'Gulf' susceptible Italian ryegrass biotypes. Research was conducted in a greenhouse at the Plant Science Research Center, Mississippi State University. The '49E' represents the Italian ryegrass biotype that survived sulfometuron (ALS herbicide) applications along U. S. Highway 49E in Holmes County Mississippi located 8 km north of Eden, Mississippi (Taylor and Coats 1996) whereas 'Marshall' and 'Gulf' represent susceptible biotypes purchased from Oktibbeha County Co-op, Mississippi (Taylor, Personal Communication, December 2007). Based on knowledge of out-breeding or cross pollination reproduction strategies of species in *Lolium* genus in particular Italian ryegrass, all above biotypes were grown in adjacent plots in the same field and replicated four times. F1 generation seeds were harvested from each biotype, stored separately and later used for these studies. In all experiments, fifty seeds of each biotype F1 generation were planted in 50 by 14 cm styrofoam cup filled with Redi-earth Plug and Seedling Mix series consisting of Canadian sphagnum peat moss, horticulture grade vermiculite, dolomitic limestone (for pH adjustment of the materials in this soil less mix) and wetting agent purchased from Sungro Horticulture Distribution Inc. and replicated 8 times. All seeds planted were covered with 0.60 cm of soil. Imazapyr [(2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid)] rates at 1% and 2% (V/V) were independently applied to each separate study as follows preemergence (PRE) immediately after seeds were planted; early postemergence (EPOST) when seedlings reached growth height 10 cm and late postemergence (LPOST) when seedlings reached growth height 30-40 cm. For PRE, each F1 generation seeds of Italian ryegrass biotypes were planted in October 26, 2007 (experiment I) and repeated in October 30, 2008 (experiment II). Seedling emergence was counted and fresh biomass determined for each biotype at 34 and 32 days after planting and treatment in experiment I and II, respectively. In the EPOST experiment, F1 generation seeds of Italian ryegrass biotypes were also planted on October 26, 2007 and repeated on October 30, 2008. However, 19 and 21 days after planting (DAP), imazapyr treatments were applied to them in experiment I and II, respectively. Shoot survivors from each biotype were counted at 113 and 51 days after treatment (DAT) for experiment I and II, respectively. Fresh shoot biomass weights were determined immediately after the shoot survivors (live plants) were counted. In LPOST, seeds of each F1 biotype were independently planted on the same day as planted in both PRE and EPOST. Imazapyr treatments were applied 82 and 76 DAP for experiment I and II, respectively. However, shoot survivors were counted and their fresh biomass weights were determined for each biotype per each imazapyr treatment at 50 and 71 DAP in experiment I and II, respectively. In all studies (PRE, EPOST and LPOST), an untreated was included each biotype and seedlings/shoots were clipped at soil surface level. All soil was watered before planting and watered again after planting. This is to help incorporate imazapyr to the soil for PRE experiment. All treatments were applied with a CO_2-pressurised backpack sprayer equipped with 8003VS flat fan nozzles, spaced 45.7 cm along the boom, 45.7 cm above each biotype and at ground speed of 3.2 km/h. Water was the herbicide carrier and the sprayer was calibrated to deliver 94 L/A.

The experimental design of each experiment was split plot design with Italian ryegrass F1 biotypes: '49E', 'Gulf' and 'Marshall' as whole plots and imazapyr rates (0, 1, and 2%) as subplots with 8 replications. Treatments were watered throughout as needed in order to maintain moisture level.

All data except fresh biomass weights were express as percent proportion of the number of 50 seeds planted. Arcsine root square transformations were performed on percent proportion data and were subjected to analyses of variance (ANOVA) using PROC GLIMIX (SAS 2008). However the transformation did not improve efficiency of the data, so the data are presented in original proportion percent germination.

Results and Discussion

PRE seedlings emergence study

A treatment by biotype and year interaction occurred, therefore seedlings emerged were presented as separate years (I and II) Table 4.1. In experiment I, seedlings emerged from untreated pots was higher in '49E' (62.5%) than 'Gulf' (46.7%) and 'Marshall' (33.9%) but in experiment II, 'Gulf' (76.2%) was higher than '49E' (57.6%) and 'Marshall' (53.8%). In both experiments, seedlings emerged from 'Marshall' was the lowest. In pots treated with imazapyr at 1 and 2%, seedlings emerged from '49E' was reduced 3 and 18%, respectively in experiment I, however in experiment II, there was no difference in seedlings emerged from 49E under both rates of imazapyr compared with untreated. Regardless of time, the two rates of imazapyr provided more than 70 to 95% seedling emergence reduction for 'Gulf' and almost 100% for 'Marshall' in both experiments. Differences observed in seedlings emerged in 49E in experiment I and II at both rates of imazapyr could probably be due to differences in genes segregation in biotype. As Tranel and Wright (2002) review on ALS herbicides resistance in weeds revealed, either homozygous or heterozygous ALS resistant alleles could covey resistance. Soignier (1995) studies on inheritance of resistance to imazaquin, also an ALS herbicide, in common cocklebur (*Xanthium strumarium* L.) biotype in Missssissippi also confirmed that a single incomplete –dominant gene was responsible for imazaquin resistant. This conclusion was reached after he evaluated responses of crosses between resistant and susceptible biotypes. Therefore some decrease in seedlings emerged in 49E in experiment I could be attributed to susceptible alleles accepted by heterozygous alleles of '49E' from susceptible biotype either 'Gulf' or 'Marshall'. High susceptibility of 'Marshall', high susceptibility of 'Gulf' and decreased seedlings emerged of resistant '49E' in response to imazapyr treatments, indicated that the later accept genes from the formers rather than vice versa. This result is also in line with Ghersa et al. (1994b) studies where they found diclofop-methyl susceptible Italian ryegrass biotype's ovule production and pollen release occurred earlier than the resistant biotypes; therefore, the former has more potential to cross fertilize the later than vice versa within wheat: ryegrass cropping systems.

PRE fresh biomass weights

There was no significant interaction between biotypes by treatments and by years for fresh biomass weights in the PRE experiment, therefore the data were pooled (Table 4.2). Fresh biomass weights obtained from 'Gulf' (9.0 g) was statistically similar to '49E' (8.3 g) and both better than 'Marshall' (7.0 g) in untreated checks. However, 'Gulf' fresh biomass weight was 8% higher than 49E numerically. Lower fresh biomass weight of 'Marshall' compared to '49E' and 'Gulf' could be attributed to reduced 27 and 28% of seedlings emerged, respectively. Competition for nutrients might have also prevented both '49E' and 'Gulf' from obtaining higher fresh biomass weights corresponding to their superior seedlings emerged compared to 'Marshall.' Imazapyr at either 1% or 2% failed completely to cause any reduction of '49E' fresh biomass weight. However imazapyr rates with increasing order caused fresh biomass weight reductions as much as 73 to 77% in 'Gulf' and 96 to 98% in 'Marshall'. Results further show that 'Marshall' is highly susceptible to imazapyr injury, while 'Gulf' is susceptible and 49E is highly resistant.

EPOST shoots survival study

Shoot survivors in untreated checks were '49E' (77.4%) > 'Gulf' (60.0%) > 'Marshall' (35.7%) in experiment I, whereas in experiment II, both 49E and 'Gulf' have similar shoot survivors, but still better than 'Marshall' (62.4%) (Table 4.3). There were no differences among 49E shoot survivors at 0, 1 and 2% of imazapyr in experiment I statistically. However numerically, 9.3 and 3.4% of 49E shoots were controlled by 1 and 2% imazapyr, respectively. In contrast, '49E' shoot survivors were higher at 2% than 0 and 1% of imazapyr rate in experiment II. Despite the fact that there was no statistical significant difference between '49E' shoot survivors at 0 and 1% of imazapyr rate, the later suppressed shoot survivors at least 3%. Imazapyr at 1 and 2% suppressed shoot survivors of 'Gulf' 76 to 86% and 'Marshall' 84 to 96%, respectively, in experiment II. Furthermore, imazapyr at 1 and 2% rates suppressed 'Gulf' shoots survival at least 91 to 95% and almost 100% control of 'Marshall' in experiment II. The '49E' biotype could still be resistant because is known that either dominant homozygote or dominant heterozygote alleles of ALS herbicides can confirm resistant (Tranel and Wright 2002, Soignier 1995). Assuming most '49E' biotype population having ALS resistant dominant homozygote alleles before accepting alleles from homozygote recessive susceptible biotypes, the F1 progeny will all be dominant heterozygote alleles which can confirm resistant. On other hand, if the 49E biotype used in this study contains some dominant heterozygote alleles of ALS and accept alleles from homozygote recessive susceptible biotypes, the '49E's F1 progeny will have 50% dominant heterozygote alleles which will still confirm resistance whereas other 50% will reduce fitness and make it susceptible. Other factors such as limited uptake of imazapyr due to increasing structural carbohydrate component of the biotypes as they mature could contribute to their shoots abilities to withstand the impact of imazapyr treatment rates. It is also known that herbicides are more effective on grass when they are young and actively growing as their growth points are more exposed than when they are becoming mature and their leaves cover up the growth points.

EPOST fresh shoots biomass weights

In experiment I, fresh shoots biomass weights of both '49E' (26.8 g) and 'Gulf' (29.3 g) were similar and at the same time higher than 'Marshall' (22.1 g) at 0% imazapyr (Table 4.4). Numerically, fresh biomass weight of 'Gulf' was 8.5% greater than 49E and 24.6% greater than 'Marshall', whereas '49E' was 17.5% greater than 'Marshall'. In contrast, at 0% imazapyr in experiment II, fresh biomass of all the three biotypes was statistically the same. However, '49E' biotype was inferior to both 'Gulf' and 'Marshall'. Regardless in both experiment I and experiment II, all rates of imazapyr failed to reduce fresh biomass weights of 49E. At 1% rate of imazapyr only 0.75% in experiment I and 3.5% in experiment II fresh biomass weights of 49E were reduced, whereas the same rate of imazapyr reduced fresh biomass weight of 'Gulf' 67.2 to 82.9% in experiment I and 75 to 76% in experiment II. At 1% of imazapyr, fresh biomass weight of 'Marshall' was reduced 71% in experiment I and 88% in experiment II whereas at 2% of imazapyr, reduction was 73% in experiment I and 90% in experiment II.

LPOST shoots survival study

In experiment I, imazapyr at 0%, '49E' biotype shoots survival was greater than > 23.9% for 'Gulf' and also more than 53% for 'Marshall' (Table 4.5). However in experiment II, '49E' shoots survived were only greater than 3.5% for 'Gulf' and 3.4% for 'Marshall', respectively. At 1 and 2% of imazapyr, 11 and 9.2% in experiment I, and 10.5 and 0% in experiment II reductions of '49E' seedlings survivors were observed. Regardless, both rates of imazapyr reduced 'Gulf' significantly at least 96% in experiment I. In contrast, both rates of imazapyr reduced 'Gulf' seedlings survivors 72 to 75% in experiment II. On the other hand, at 1 or 2% of imazapyr, 'Marshall' shoots survivors were highly suppressed, more than 95% in experiment I compared to less than 90% in experiment II for both rates. Reduced efficacy of all rates of imazapyr on 'Marshall' in 2009 could be attributed to higher density of seedlings survivors before treatments were applied. The higher density may lead to some seedlings not adequately receive the treatment.

LPOST shoots fresh biomass weights

Regardless at 0% imazapyr, '49E' fresh biomass weight was more than 'Gulf' and 'Marshall' in both experiment I and experiment II (Table 4.6). No significant differences were observed among 0, 1 and 2% imazapyr on 49E in experiment I. However 2% of imazapyr caused 3% reduction of 49E fresh biomass weight. In contrast, 1 and 2% imazapyr significantly reduced 49E fresh biomass weight in experiment II. These observations of fresh biomass reductions or reduce fitness of 49E at 1 and 2% of imazapyr showed that it might acquire some susceptible genes in its seeds from the field before planting. Both ‘Gulf’ (29.2 g) and ‘Marshall’ (27.6 g) have statistically similar fresh biomass weight at 0% imazapyr in experiment I. However, in experiment II, fresh biomass weights obtained from ‘Gulf’ (12.9 g) and ‘Marshall’ (12.7 g) were less than in experiment I. Regardless 1 or 2% imazapyr caused equal 82% reduction of ‘Gulf’ fresh biomass weight in experiment I compared to 35.7% for former and 52% for later in experiment II. At 1 and 2% of imazapyr ‘Marshall’ fresh biomass weight 87% and 82% in experiment I whereas in experiment II, 63% and 74% with respect to increasing order of imazapyr treatment. Inconsistent efficacies of both rates of imazapyr on ‘Gulf’ compared to ‘Marshall’ showed that the former has greater incidence of acquiring the tolerant alleles.

The studies showed that gene flow from susceptible biotypes might have caused reduction in seedlings emergence of 49E in PRE experiment I under imazapyr rates. On other hand, it could be possible that ‘49E’ gene population is still segregating and that some seeds produced in 49E populations are still susceptible to imazapyr while other offspring are resistant. However, the effects were variable in fresh biomass weights. Furthermore, imazapyr rates have less effects on 49E shoots survivorship and fresh biomass reductions in both EPOST and LPOST studies. In these studies, genotypes of all the Italian ryegrass biotypes were not determined to confirm their homozygous or heterozygous alleles for resistance or susceptibility before they were planted in a mixture. It will be, therefore be prudent that future research should determine the genotypes of both resistant and susceptible biotypes before planting in mixture so that gene flow can be clearly determined. In addition the biotypes used in this research were based on F1 generations which may limit significant gene segregation. In future, crosses should be done so that F2, F3, or F4 biotypes could be used.

Table 4.1 Influence of imazapyr applied PRE on Italian ryegrass biotypes seedlings emerged at 34 (Exp. I) and 32 (Exp. II) DAT in greenhouse at the Plant Science Research Center, Mississippi State University.

		Exp. I			Exp. II		
Herbicide	Rate	49E	Gulf	Marshall	49E	Gulf	Marshall
		% proportion germination/ cup[a]					
Imazapyr	0	62.5aA	46.7aB	33.9aC	57.9bB	76.2aA	53.8aB
	1	60.6abA	3.3bA	0.0bC	61.0bA	14.5bB	0.8bC
	2	51.3bA	2.0bB	0.18bB	74.6aA	15.0bB	0.1bC

PRE: preemergence; DAT: days after treatments, Exp: experiment, Exp. I: planted in October 26, 2007, Exp. II: planted in October 30, 2008

[a] Lowercase letters represent Fisher's Protected LSD at P = 0.05 between imazapyr rates within Italian ryegrass biotypes in the column. Uppercase letters represent Fisher's Protected LSD at P = 0.05 between Italian ryegrass biotypes within imazapyr rates in the row.

Table 4.2 Influence of imazapyr applied PRE on Italian ryegrass biotypes seedlings fresh biomass weights average over Exp. I + Exp. II in greenhouse at the Plant Science Research Center, Mississippi State University.

Herbicide	Rate	49E	Gulf	Marshall
	g	% seedlings fresh biomass weight/cup[a]		
Imazapyr	0	8.3 aA	9.0 aA	7.0a B
	1	9.9 aA	2.4 bB	0.2 bC
	2	8.5 aA	2.0 bB	0.1 bC

PRE: preemegence; nonionic surfactant (NIS) was added at 0.025 (v/v); Exp: experiment; Exp. I: planted in October 26, 2007; Exp. II: planted in October 30, 2008
[a] Lowercase letters represent Fisher's Protected LSD at P = 0.05 between imazapyr rates within Italian ryegrass biotypes in the column. Uppercase letters represent Fisher's Protected LSD at P = 0.05 between Italian ryegrass biotypes within imazapyr rates in the row.

Table 4.3 Influence of imazapyr applied EPOST on Italian ryegrass biotypes shoot survivorship at 113 (exp. I) and 51 DAT (exp. II) in greenhouse at the Plant Science Research Center, Mississippi State University

		Exp.I			Exp.II		
Herbicide	Rate	49E	Gulf	Marshall	49E	Gulf	Marshall
		% shoot proportion survival/ cup[a]					
Imazapyr	0	77.4aA	60.0aB	35.7aC	75.6ba A	72.4aA	62.4aB
	1	70.2aA	14.3bB	5.7bC	72.4bA	5.6bB	1.4bC
	2	74.8baA	8.4bB	3.2bC	82.9aA	3.9bB	1.2bB

EPOST: early postemergence; DAT: days after treatments; Exp: experiment; Exp. I: planted in October 26, 2007; Exp. II: planted in October 30, 2008; nonionic surfactant was added at 0.025 (v/v)
[a] Lowercase letters represent Fisher's Protected LSD at P = 0.05 between imazapyr rates within Italian ryegrass biotypes in the column. Uppercase letters represent Fisher's Protected LSD at P = 0.05 between Italian ryegrass biotypes within imazapyr rates in the row.

Table 4.4 Influence of imazapyr applied EPOST on Italian ryegrass shoot fresh biomass weights at 113 (Exp. I) and 51 (Exp. II) DAT in greenhouse at the Plant Science Research Center, Mississippi State University

		Exp. I			Exp. II		
Herbicide	Rate	49E	Gulf	Marshall	49E	Gulf	Marshall
		Shoot fresh biomass (g) / cup[a]					
Imazapyr	0	26.8bA	29.3aA	22.1.7aB	10.5aA	12.1aA	12.0aB
	1	26.6bA	9.6bB	6.3bB	10.1aA	3.0bB	1.4bC
	2	30.8aA	5.3 bB	6.0bB	11.4aA	2.9bB	1.2bB

EPOST: early postemergence; DAT: days after treatments; Exp: experiment; Exp. I: planted in October 26, 2007; Exp. II: planted in October 30, 2008; nonionic surfactant (NIS) was added at 0.025 (v/v)
[a] Lowercase letters represent Fisher's Protected LSD at P = 0.05 between imazapyr rates within Italian ryegrass biotypes in the column. Uppercase letters represent Fisher's Protected LSD at P = 0.05 between Italian ryegrass biotypes within imazapyr rates in the row.

Table 4.5 Influence of imazapyr applied LPOST on Italian ryegrass biotypes shoot survival at 50 (Exp. I) and 71 (Exp. II) DAT in greenhouse at the Plant Science Research Center, Mississippi State University.

		Exp. I			Exp. II		
Herbicide	Rate	49E	Gulf	Marshall	49E	Gulf	Marshall
		% germination/ cup[a]					
Imazapyr	0	65.3aA	50.3aB	30.7aC	74.0ba A	71.4aA	71.5aA
	1	57.6aA	2.5bB	0.5bC	66.2bA	19.6bB	11.0bB
	2	59.3baA	2.3bB	1.1bC	77.4aA	17.8bB	9.2bB

LPOST: late postemergence; DAT: days after treatments; Exp: experiment; Exp. I: planted in October 26, 2007; Exp. II: planted in October 30, 2008; nonionic surfactant (NIS) was added at 0.025 (v/v).
[a] Lowercase letters represent Fisher's Protected LSD at P = 0.05 between imazapyr rates within Italian ryegrass biotypes in the column. Uppercase letters represent Fisher's Protected LSD at P = 0.05 between Italian ryegrass biotypes within imazapyr rates in the row.

Table 4.6 Influence of imazapyr applied LPOST on Italian ryegrass fresh biomass weights at 50 (Exp. I) and 71 (Exp. II) DAT in greenhouse at the Plant Science Research Center, Mississippi State University.

		Exp. I			Exp. II		
Herbicide	Rate	49E	Gulf	Marshall	49E	Gulf	Marshall
		Fresh biomass (g)/ cup[a]					
Imazapyr	0	26.4aA	29.2aA	27.6aA	18.6aA	12.9aB	12.7aB
	1	30.1aA	5.1bB	3.5bB	12.8bA	8.3bB	4.7bC
	2	25.6aA	5.1 bB	4.9bB	13.5bA	6.2bB	3.3bB

LPOST: late postemergence; DAT: days after treatments; Exp: experiment; Exp. I: planted in October 26, 2007; Exp. II: planted in October 30, 2008; Nonionic surfactant (NIS) was added at 0.025 (v/v).

[a] Lowercase letters represent Fisher's Protected LSD at P = 0.05 between imazapyr rates within Italian ryegrass biotypes in the column. Uppercase letters represent Fisher's Protected LSD at P = 0.05 between Italian ryegrass biotypes within imazapyr rates in the row.

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