Physiological and Environmental Basis of Turfgrass and Weed

Response to Mesotrione Formulations

by

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ABSTRACT

Mesotrione is the first triketoine herbicide registered for use in turfgrass. Triketones prevent carotenoid biosynthesis by inhibiting the enzyme p-hydroxyphenylpyruvate dioxygenase (HPPD). Although mesotrione controls many species of grass and broadleaf weeds, it is best known for selective control of perennial grasses like creeping bentgrass (Agrostis stolonifera L.). Field trials conducted at Virginia Tech and Blacksburg Country Club determined that several programs that integrate herbicide treatment and turf seeding effectively transitioned creeping bentgrass contaminated golf roughs back to a tall fescue [Schedonorus phoenix (Scop.) Holub] monoculture. However, mature weeds require multiple mesotrione applications for effective control. This requirement is a major limitation to mesotrione's competitiveness in turfgrass markets. Several greenhouse and laboratory studies were conducted to evaluate scenarios where mesotrione rates were titrated and applied daily to mimic ascending, descending, and intervallic time-release patterns. These patterns were applied following an initial treatment to foliage or soil to mimic a potential sprayable or granular time-release formulation. These scenarios effectively controlled five targeted weed species equivalent to the standard of two broadcast sprays, regardless of initial application placement or release pattern. However, both time-release treatments and the standard
injured tall fescue based on leaf counts, plant weights, and visual phytotoxicity ratings. Additional growth chamber studies found that changes in relative humidity from 50 to 90% caused a 4- to 18-fold increase in plant phytotoxicity with a concomitant decrease in photochemical efficiency when mesotrione was applied to foliage of smooth crabgrass (*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.). Furthermore, white tissue was found predominately in the two youngest leaves when mesotrione was applied to soil, but in older leaves when applied only to foliage. Laboratory studies were conducted to evaluate interspecific differences in $^{14}$C mesotrione absorption and translocation between two plant species when applied to foliage or roots. Annual bluegrass (*Poa annua* L.) absorbed 2- to 4-fold more radioactivity than Kentucky bluegrass (*Poa pratensis* L.). Both species absorbed less radioactivity through roots than through foliage and root absorbed radioactivity was more often exuded into Hoagland's solution while foliar absorbed radioactivity was often found in other foliage.
This dissertation is dedicated to my loving family and friends, especially my parents, Ken and Linda, and my sister, Kara, for their unconditional love and support for me throughout my life.
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Chapter 1. Introduction

Many herbicides produced and marketed recently for selective and broad spectrum weed control in agronomic crops have found their niche in the turfgrass industry. Comprised of such areas as golf courses, sports fields, sod production, highway and roadside rights-of-ways, and home lawns, the turf industry makes up a multi-billion dollar annual industry (Barnes et al. 2006). This rapidly developing industry has chemical companies searching for products that improve turfgrass management. One new herbicide to reach turfgrass markets is mesotrione. Mesotrione is a herbicide that has been developed and marketed for use in maize since 2001. Research evaluating mesotrione for use in turfgrass has led to its registration as Tenacity™ herbicide in 2008 (Giese et al. 2005; Keese et al. 2005). The mode of action of mesotrione will be the first of its kind for use in turfgrass.

Mesotrione origin: Mesotrione [2-(4-mesyl-2-nitrobenzoyl)cyclohexane-1,3-dione] is chemically derived from a natural chemical secreted by the red bottlebrush (Callistemon citrinus Stapf.) plant (Cornes 2005). This chemical was discovered in 1977 when a Zeneca (now Syngenta Corp.) scientist noticed a reduction in weeds growing under the bottlebrush plant at his home in California. Analysis of the soil beneath the plant determined that an allelochemical called leptospermone, a natural polyketide previously isolated from the oils of Australian myrtaceous plants, was being exuded from the plant (Hellyer 1968; Mitchell et al. 2001). Further evaluation of pure leptospermone determined it was a moderately active herbicide that controlled several broadleaf and grassy weeds. Leptospermone and some of its synthetic analogues were patented in 1980 (Gray et al. 1980). Further research of leptospermone led to the development of the
benzoylcyclohexane-1,3-dione (triketones) herbicide chemical family (Duke et al. 2002; Mitchell et al. 2001). In June of 2001, mesotrione was first marketed in the US as the active ingredient of Callisto\textsuperscript{®} \textsuperscript{1} for use in maize (\textit{Zea mays} L.).

Mesotrione carries a nonrestrictive toxicological and environmental profile. Mesotrione has a low mammalian toxicity rating (rat oral and dermal toxicity > 5000 mg/kg body weight) (Anonymous 2009b), and in soil, microorganisms quickly degrade mesotrione reducing its leaching and groundwater contamination potential (Cornes 2005).

**Mesotrione mode of action:** Triketone herbicides, like mesotrione, act by inhibiting carotenoid biosynthesis, resulting in the decline of chlorophyll with sensitive species exhibiting tissue bleaching followed by necrosis within 3 to 5 days of application (Mayonado et al. 1989; Mitchell et al. 2001; Pallet et al. 1998, 2001; Senseman 2007; Sutton et al. 2002). Mesotrione’s active site is in the cytoplasm of the chloroplasts where it inhibits the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD) (Mitchell et al. 2001; Wakabayashi and Boger 2002), a necessary component of the biochemical pathway that converts tyrosine to plastoquinone and α-tocopherol (Shultz et al. 1985) (Figure 1). Tocopherol is an important quencher of reactive oxygen species (Kruk et al. 2005; Trebst et al. 2002). Furthermore, plastoquinone is a critical cofactor for phytoene desaturase (Mayer et al. 1990, 1992; Norris et al. 1995), an early enzyme in the carotenoid biosynthetic pathway. Depletion of plastoquinine levels consequently reduces the amount of carotenoids and causes an increase (up to 460\%) in plant tyrosine levels (Lee et al. 1997). Carotenoids of green, photosynthetically active tissue (primary carotenoids) are the light harvesting parts of the plant which act to protect the plant from photodegradation (Lichtenthaler 1987; Havaux et al. 1998). Carotenoids also function in

\textsuperscript{1} Callisto\textsuperscript{®} 4SC. Syngenta Crop Protection, P.O. Box 18300, Greensboro, NC 27419
plants to give color to fruits and flowers (secondary carotenoids) (Lichtenthaler 1987; Bartley and Scolnik 1995). This decline of carotenoid levels results in the destruction of chlorophyll which causes the characteristic bleaching symptoms often associated with the application of mesotrione and other HPPD inhibiting herbicides. A reduction in carotenoids as a result of mesotrione application results in the loss of vital photoprotective agents that prevent the formation of highly destructive singlet oxygen species (free radicals) which lead to loss of cell integrity from damage to cell membranes (Havaux et al. 1998, Schrott 1985). Bleaching responses of plant tissues following application of carotenoid biosynthesis inhibiting herbicides like mesotrione are often related to undifferentiated tissues in their developmental state (Kim et al. 2004). Kim et al. (2004) observed plant tissues developed before fluridone treatment (green tissue) turned chlorotic and necrotic within a few days of treatment, where tissues formed after treatment (differentiating tissues) emerged as white tissues and grew briefly before turning necrotic.

Intact carotenoids protect the plant from photodegradation through a de-epoxidation of violaxanthin to zeaxanthin in high light conditions within the xanthophyll cycle, the primary photoprotection cycle of higher plants (Demmig-Adams et al. 1996; Lichtenthaler 1987)(Figure 2). In low light conditions, this cycle converts zeaxanthin back to violaxanthin which functions as a light harvesting pigment. The example in Figure 2 demonstrates the vital role carotenoids play in the photochemical quenching in plants. By disrupting this pathway and preventing the desaturation of phytoene, mesotrione effectively inhibits the formation of light harvesting carotenoids which are essential to proper function of the xanthophyll cycle. Changes or mutations in the
function of carotenoids affect the photosynthetic cycle and could result in severe injury or death to the plant (Krinsky 1979). McElroy et al. (2006) studied the occurrence of zeaxanthin, antheraxanthin, violaxanthin, lutein, and epoxyluteins under high and low irradiance levels in creeping bentgrass. Their studies determined that plant xanthophyll cycle pigment (zeaxanthin and antheraxanthin) levels increased under high irradiance, emphasizing the importance of carotenoids for photoprotection.

**Mesotrione uptake and metabolism:** Mesotrione has a dissociation constant (pKa) of 3.12 at 20°C, making it a weak acid. Therefore, mesotrione solubility is dependent upon pH and ranges from 2.2 g L\(^{-1}\) at pH 4.8 to 22 g L\(^{-1}\) at pH 9 (20°C). High water solubility at a neutral pH results in ideal conditions for plant uptake and translocation (Mitchell et al. 2001). Mitchell et al. (2001) determined mesotrione is rapidly absorbed by plants, with most weeds absorbing 55 to 90% of applied product within 24 hours following foliar application. Armel et al. (2005) reported absorption in Canada thistle [Cirsium arvense (L.) Scop.] where only 26 to 44% of applied \(^{14}\)C-mesotrione was absorbed 2 HAT, and 51 to 77% absorbed 72 HAT, considerably slower than the findings of Mitchell et al. (2001) in annual weed species. Further studies on the uptake and metabolism of radioactive \(^{14}\)C mesotrione determined it to be distributed by both acropetal and basipetal movement within the plant (Mitchell et al. 2001). Herbicide mobility within the plant as well as selectivity following mesotrione application is dependent on metabolism. In tolerant species such as maize, mesotrione is rapidly metabolized to form inactive metabolites following foliar uptake before relocation to other parts of the plant (Senseman 2007). These metabolites are formed following the hydroxylation of the parent mesotrione molecule. As a result of this hydroxylation, the cyclohexane and
phenyl groups are hydrolytically split forming the immobile and nonherbicidal metabolites 4-(methylsulfonyl)-2-nitrobenzoic acid (MNBA), and 2-amino-4-(methylsulfonyl) benzoic acid (AMBA) (Armel et al. 2005) (Figure 3). In more sensitive species, mesotrione is translocated throughout the plant with symptoms typically appearing first on newly emerged and actively growing meristematic tissues.

**Environmental effects on mesotrione activity:** Past studies evaluating postemergence herbicide effectiveness at varying temperatures have shown that applications during high temperatures improve efficacy of glufosinate, glyphosate, fluazifop, and acifluorfen (Anderson et al. 1993; Kells et al. 1984; McWhorter and Azlin; 1978; Wills and McWhorter 1981). Johnson and Young (2002) observed similar responses to postemergence applications of mesotrione in velvetleaf (*Abutilon theophrasti* Medik.), ivyleaf morningglory (*Ipomoea hederacea* Jacq.), and common cocklebur (*Xanthium strumarium* L.) at 32°C. Conversely, Johnson and Young (2002) reported large crabgrass [*Digitaria sanguinalis* (L.) Scop.] and common waterhemp (*Amarathus rudis* Sauer) were more susceptible to injury from mesotrione at lower temperatures (18°C), hypothesizing that the contrasting results could be physiological differences within the plants. Velvetleaf, ivyleaf morningglory, and common cocklebur are C₃ plants whereas large crabgrass and common waterhemp are C₄ plants which may contribute to the differences observed among the plants. In a similar study evaluating large crabgrass response to mesotrione at varying temperatures and irradiance levels, McCurdy et al. (2009) reported no differences in control at 18 or 32°C. Ricker (2007) observed greatest injury to perennial ryegrass (*Lolium perenne* L.) as a result of mesotrione application at optimum growing temperatures between 18.3 and 23.8°C.
Relative humidity (RH) can also affect herbicide activity following herbicide application. Johnson and Young (2002) reported 85% RH resulted in greater plant injury to velvetleaf, large crabgrass, and common waterhemp than 30% RH following mesotrione application. Similarly, high RH increased efficacy of acifluorfen on showy rattlebox (Crotalaria spectabilis Roth) (Wills and Mcwhorter 1981) and glyphosate and sethoxydim on bermudagrass [Cynodon dactylon (L.) Pers.] (Jordan 1977; Wills 1984). However, ivyleaf morningglory was injured more at low humidity and common cocklebur was not influenced by humidity when treated with mesotrione.

Solar irradiance is another environmental factor that can influence herbicide activity within the plant. Herbicides whose active site requires photochemical quenching mechanisms or photosynthetic activity require light for activity. As mentioned earlier, McElroy et al. (2006) reported an increase in plant carotenoids within the xanthophyll cycle during periods of high irradiance; a defense mechanism of the plant to harvest reactive oxygen species before damage to chlorophylls occurs. With this in mind, McCurdy et al. (2009) evaluated the response of large crabgrass to mesotrione application at varying light levels. The results of this study did not confirm that irradiance levels changed the plants response to mesotrione.

**Mesotrione uses in corn:** Since its introduction, mesotrione’s natural selectivity and control of many important row crop weeds has had a huge impact in maize production. Mesotrione is labeled for use in field corn, production seed field corn, field corn grown for silage, yellow popcorn, sweet corn, and other listed crops (Anonymous 2009a). Generally, maize is extremely tolerant to mesotrione, but significant injury has been reported in hybrid sweet corn varieties (Bollman et al. 2008; O’Sullivan et al. 2002).
This sensitivity has been linked to the plants genotype at the Nsf1 locus (Pataky et al. 2008). A functional P-450 metabolism gene is encoded by the Nsf1 allele where the nsf1 allele is inactive (Pataky et al. 2008). Knowing a varieties genotype can be useful in determining a sweet corn hybrid’s potential for sensitivity to mesotrione application (Bollman et al. 2008).

Mesotrione controls several annual broadleaves, but has limited grass activity (Armel et al. 2003a). However, mesotrione has proven to be an important tank mix partner for other herbicides, improving its control spectrum (Armel et al. 2003a; Whaley et al. 2006). Armel et al. (2003b) observed mesotrione applied PRE controlled common ragweed (Ambrosia artemisiifolia L.), common lambsquarters (Chenopodium album L.), and smooth pigweed (Amaranthus hybridus L.) 70, 85, and 90%, respectively, 9 WAT and greater than 80% when applied POST for the same weed species. Mesotrione applied POST to maize controlled common cocklebur 79 to 98% and ivyleaf morningglory 60 to 90% 14 DAT when applied alone (Johnson et al. 2002). In combination with atrazine, increased control of both weeds was observed over mesotrione alone. Mesotrione effectively controlled barnyardgrass [Echinchloa crus-galli (L.) P. Beauv.], velvetleaf (Abutilon theophrasti Medik.), and redroot pigweed (Amaranthus retroflexus L.) when applied alone, and green foxtail (Setaria viridis L.) when combined with atrazine (Creech et al. 2004).

One of the key aspects of mesotrione is that it provides for mode of action diversity in the field and therefore reduces the potential for development of herbicide resistance in weeds (Stephenson et al. 2004). Weed resistance has been reported for commonly used herbicides including the triazines (Bandeen and McLaren 1976; Ryan
1970; Stephenson et al. 1990), acetolactate synthase (ALS) inhibitors (Hinz and Owen
1997; Sprague et al. 1997), and the glycines (Lee 2000; Powles et al. 1998; VanGessel
2001) and others. Currently, no resistance to mesotrione or cross resistance between
mesotrione and other chemical classes or modes of action is known to exist (Anonymous
2009a). Using mesotrione in crop rotations has provided an effective control alternative
for susceptible weeds showing resistance to previously established herbicide control
options (Sutton 2002).

Mesotrione uses in turfgrass: Mesotrione has been extensively tested at universities
and other research programs since it was first evaluated at Virginia Tech in 2001 for use
in turfgrass. The results of numerous research projects since then have prompted the
registration of mesotrione in turfgrass. Mesotrione has many uses as a preemergence and
postemergence herbicide in turfgrass. Mesotrione can be used to control many annual
broadleaved and grassy weeds as well as several hard to control perennial weeds.
Research evaluating mesotrione for use in turfgrass has shown that it can be safely used
in established cool and warm season turfgrass species including Kentucky bluegrass (Poa
pratensis L.), tall fescue [Schedonorus phoenix (Scop.) Holub] fine fescue (creeping red,
chewings, and hard) (Festuca spp.), perennial ryegrass (Lolium perenne L.),
centipedegrass [Eremochloa ophiuroides (Munro) Hack], and St. Augustinegrass
[Stenotaphrum secundatum (Walter) Kuntze] (Anonymous 2008; Beam et al. 2006; Jones
and Christians 2007; Willis et al. 2006a; 2007a). Mesotrione can also be used at seeding
for the previously mentioned turfgrass species except for fine fescue varieties
(Anonymous 2008; Williams et al. 2009; Willis 2006a). Preemergence applications of
mesotrione have shown to be effective for control of annual grassy weeds. However,
limited soil residual requires repeat applications for extended control. The addition of tank-mix partners has shown to improve preemergence control of annual grassy weeds. Additionally, tank-mixing mesotrione with commonly used preemergence herbicides can delay the application of preemergence herbicides and provide postemergence control as well as extended preemergence weed control (McCurdy et al. 2006). Past studies evaluating postemergence applications of mesotrione have shown it is effective in controlling annual grasses such as crabgrass (*Digitaria* spp.) (Willis et al. 2006a; McElroy et al. 2007), and goosegrass [*Eleusine indica* (L.) Gaertn.] (McElroy et al. 2007). Tank-mixing mesotrione with postemergence herbicides can also improve the spectrum of weed control provided by mesotrione alone. Ricker et al. (2006) evaluated tank-mixes of mesotrione at 0.14 kg ai/ha with bentazon at 0.28 kg ai/ha, bromoxynil at 0.28 kg ai/ha, carfentrazone-ethyl at 0.035 kg ai/ha, MSMA at 1.68 kg ai/ha, and quinclorac at 0.84 kg ai/ha for control of smooth crabgrass [*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.]. In most comparisons, mesotrione combinations with bentazon, bromoxynil, and carfentrazone synergized smooth crabgrass control when compared to mesotrione alone. Willis et al. (2007b) evaluated tank-mixes of mesotrione for improved white clover (*Trifolium repens* L.) control. Mesotrione applied at 280 g/ha was tank-mixed with bromoxynil at 560 g/ha, carfentrazone at 37 g/ha, and simazine at 2,240 g/ha. All combinations significantly improved white clover control 6 weeks after treatment over herbicides applied alone.

Mesotrione is the first herbicide to selectively control or suppress hard to control perennial grasses such as creeping bentgrass (*Agrostis stolonifera* L.), nimblewill (*Muhlenbergia schreberi* J.F. Gmel.), and bermudagrass in cool-season turfgrass. Past
control options for perennial grasses have been limited to nonselective herbicides followed by reestablishment of desirable turfgrasses (Askew and Hipkins 2006; Willis et al. 2007a). Bhowmik and Drohen (2001) first reported isoxaflutole, another HPPD-inhibiting herbicide, selectively controlled creeping bentgrass in cool-season turf. Sequential applications of mesotrione can selectively control creeping bentgrass (Beam et al. 2006; Branham et al. 2005; Jones and Christians 2007) and nimblewill (Willis et al. 2007a) in cool-season turfgrasses. Willis et al. (2006b, 2007) reported that sequential mesotrione applications along with tank mixes of triclopyr and fenoxaprop are also effective for the suppression of bermudagrass in cool season turf. These herbicide combinations evaluated for bermudagrass control also resulted in improved control of weeds not effectively controlled with mesotrione applied alone and reduced bleaching response of targeted weeds, resulting in improved turf quality (Willis et al. 2006b).

**Soil and foliar activity of mesotrione:** Although mesotrione can be absorbed through foliage or roots, the overall contribution of soil or foliar absorption of mesotrione to herbicide efficacy is unknown. McCurdy et al. (2007) compared soil and foliar applications of mesotrione and determined that treatments containing soil applied mesotrione controlled yellow nutsedge (*Cyperus esculentus* L.) more effectively. However, the methods used in this study did not take into account the amount of mesotrione actually available for plant uptake. Further studies are needed to provide a better understanding of the contribution of soil and foliar absorbed mesotrione towards herbicide efficacy.

**Granular formulations:** Granular products are one of the most popular herbicide formulations used in home lawns. Liquid spray applications are difficult to use for
people not properly trained for pesticide application. Granular formulated products are easily applied using methods that take little or no prior herbicide application experience. Current experimental granular mesotrione formulations require a second application for adequate weed control.

Ideally, herbicide applications would provide desired results following a single treatment without the need of a sequential application. In turfgrass environments, mesotrione commonly requires a sequential application for desired results. Slow release granules allow specific amounts of herbicide to be released over a given amount of time. Controlled release of herbicides also reduces the potential for leaching and offsite movement of products. Schreiber et al. (1988) reported that atrazine leaching was reduced using a controlled release starch granule (CRSG). Similarly, Boydston (1992) reported that conventional applications compared to CRSG applications containing 5.3% ai. (w/w) ^14^C-norflurazon or 6% a.i. (w/w) ^14^C-simazine retarded the leaching depths of both herbicides from 12.5 to less than 2.5cm and 15 to less than 2.5cm within a soil column for ^14^C-norflurazon and ^14^C-simazine, respectively. Other slow release formulations have been tested using products such as organic and inorganic clays (Celis et al. 2002; Hermosin et al. 2001), and ethylcellulose (Sopena et al. 2007). Adapting this technology for the production of a slow release mesotrione granule could be used to reduce the number of applications needed to control undesirable weeds, potentially eliminating the need for a sequential application.
Research Objectives

1. Determine the effectiveness of integrating mesotrione programs for the control of creeping bentgrass during seeded establishment of tall fescue.

2. Optimize mesotrione formulations for turfgrass tolerance and weed control efficacy.

3. Determine environmental influences on the soil and foliar absorption of mesotrione and their impacts on herbicide efficacy.

4. Determine the differential absorption, translocation, and metabolism of mesotrione in Kentucky bluegrass and annual bluegrass (*P. annua* L.).
Literature Cited


Figure 1: Active site of mesotrione on the 4-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme in plants. The inhibition of this enzyme prevents the formation of α-tocopherol, an important quencher of reactive oxygen species, and plastoquinone, a cofactor for phytoene desaturase and an enzyme in the carotenoid biosynthetic pathway. Figure from Armel et al. (2005).
Figure 2: Schematic of conversion pathway of primary plant carotenoids responsible for photoprotection and light harvesting. Image from McElroy et al. (2006).
Figure 3: Chemical structures of mesotrione and two of its nonherbicidal metabolites; metabolites 4-(methylsulfonyl)-2-nitrobenzoic acid (MNBA), and 2-amino-4-(methylsulfonyl) benzoic acid (AMBA). Image from Armel et al. (2005).
Chapter 2.

Integrated Approach to Creeping Bentgrass Control with Mesotrione and Seeding.

The following chapter was formatted to facilitate publication in *Weed Technology*.
Integrated Approach to Creeping Bentgrass Control with Mesotrione and Seeding

Matthew J. R. Goddard, Renee J. Keese, and Shawn D. Askew

Abstract

Mesotrione is a herbicide that offers selective control options for creeping bentgrass in cool season turf and safety at seeding of tolerant species. Research is needed to determine the effectiveness of integrating creeping bentgrass control programs utilizing mesotrione with reseeding of desirable turfgrasses. Two experiments conducted at Virginia Tech’s Turfgrass Research Center and Blacksburg Country Club in Blacksburg, VA evaluated the utility of mesotrione programs for creeping bentgrass control and reestablishment of desirable turf. Treatments included mesotrione applied at 140 g ai ha⁻¹ applied 2 weeks before seeding (WBS), at seeding, and 3 weeks after seeding (WAS), 175 g ai ha⁻¹ applied 2 WBS, at seeding, and 3 WAS, 140 g ai ha⁻¹ applied 2 WBS, at seeding, and 3 and 5 WAS, 140 g ai ha⁻¹ applied 4 and 2 WBS, at seeding, and 3 WAS, and a nontreated check. All treatments resulted in acceptable control of creeping bentgrass. Treatment programs containing four mesotrione applications or three mesotrione applications at 175 g ai ha⁻¹ were most effective at providing creeping bentgrass.

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bentgrass control. Each of the treatments allowed for seedling safety while providing weed control during establishment of seeded tall fescue.

**Nomenclature:** Creeping bentgrass, *Agrostis stolonifera* L. AGSST; mesotrione; tall fescue, *Schedonorus phoenix* (Scop.) Holub FESAR.

**Key words:** Bleaching herbicides, renovation, triketone herbicides, turfgrass injury.

**Abbreviations:** WAS, weeks after seeding; WBS, weeks before seeding
Introduction

Creeping bentgrass (*Agrostis stolonifera* L.) is a commonly managed turfgrass species on golf course putting greens, tees, and fairways. Though it is a very popular species because of its desirable surface characteristics, it commonly infests areas of managed turfgrass where it is not wanted. Cultural practices such as mowing, core aeration, verti-cutting, as well as movement of seed by flooding and animals are all methods of introducing creeping bentgrass into areas it is not wanted. Once established, creeping bentgrass can dominate turfgrass stands in home lawns, athletic fields, and golf course roughs (Branham et al., 2005), and has few control options in cool season turf.

Mesotrione is a new triketone herbicide labeled for use in turfgrass environments (Anonymous 2008). Mesotrione and other triketone herbicides cause characteristic whitening or bleaching response in sensitive plant species by inhibiting p-hydroxyphenylpyruvate dioxygenase (HPPD) (Mitchell et al. 2001; Wakabayashi and Boger 2002), a key enzyme in the carotenoid biosynthetic pathway (Shultz et al. 1985). Mesotrione controls many annual and perennial grass and broadleaf weeds, including nimblewill (*Muhlenbergia schreberi* J.F. Gmel.) (Willis et al. 2007) and creeping bentgrass (Beam et al. 2006; Branham et al. 2005; Dernoden et al. 2008) species which lack other selective control options. Additionally, mesotrione can be used during seeded establishment of tolerant species. Willis et al. (2006) evaluated several herbicides for use at seeding of tall fescue including mesotrione, siduron, and quinclorac. Mesotrione effectively controlled many grass and broadleaf weeds without injuring the tall fescue seedlings. McElroy and Breeden (2007) reported tall fescue seedlings tolerated mesotrione applied at 0.28 kg ha$^{-1}$ when applied 14 or more days following seeded
Mesotrione reduced tall fescue cover less than quinclorac (17 and 6%, respectively) 63 days after establishment. In greenhouse container trials, Williams et al. (2009) evaluated pre-plant and at planting applications of mesotrione at rates of 161, 282, 343, and 565 g ha\(^{-1}\) to ‘Treazure’ chewings fescue (Festuca rubra L. ssp. commutata Gaudin) and ‘Gallery’ perennial ryegrass (Lolium perenne L.) applied 4, 2, and 1 week pre-plant and at planting. All rates and timings were safe for seeded establishment of perennial ryegrass, but all were deemed too injurious for use when seeding chewings fescue.

Prior to the introduction of mesotrione into the turfgrass industry, control options for creeping bentgrass in cool season turfgrass included nonselective herbicides and hand removal, leaving voids in the turfgrass canopy (Branham et al. 2005). The ability to selectively control creeping bentgrass with mesotrione is a major benefit to turfgrass managers plagued with unwanted infestations. However, reestablishment programs utilizing mesotrione at seeding of desirable turfgrass species are needed. Weed competition is commonly associated with the lack of success during seeded establishment of turfgrasses. Current herbicides labeled for use in establishment of cool season turfgrasses are limited to siduron, which can be used during seeded establishment of most cool season turfgrasses for the control of select annual warm season grasses (Anonymous 2004; Christians 2007; Moshier et al. 1976; Youngner et al. 1974), and quinclorac, which can be used during establishment of several turfgrass species. However, siduron is not effective for controlling broadleaf weeds (Anonymous 2004). Based on prior research evaluating mesotrione for the selective control of creeping bentgrass in cool season turf and its observed safety during reseeding of tolerant turfgrass species, it is believed that
mesotrione used in combination with seeding will provide a viable option for the reestablishment of desirable species in creeping bentgrass contaminated areas. Determining a method of reestablishment while providing weed control can give turfgrass managers new options for controlling creeping bentgrass in areas it is not wanted. Therefore the objectives of this study are 1) to evaluate mesotrione for bentgrass control during seeded establishment of tall fescue, and 2) to determine optimum timing for controlling creeping bentgrass during seeded establishment of tall fescue.

**Materials and Methods**

Two field trials were conducted at Virginia Tech’s Turfgrass Research Center (TRC) and at Blacksburg Country Club (BCC) in Blacksburg, VA in 2007. The TRC site was a pure stand of mature ‘L-93’ creeping bentgrass maintained at a 2 cm fairway height prior to study initiation and 7.6 cm thereafter. The BCC site was a tall fescue [Schedonorus phoenix (Scop.) Holub] rough maintained at 7.6 cm and contaminated with 85% creeping bentgrass. Soil type at the TRC location was a Groseclose-Urban land complex (fine, mixed, semiactive, mesic Typic Hapludults). Soil type at the BCC location was a Purdy silt loam (Fine, mixed, active, mesic Typic Endoaquults). Each site was irrigated as needed to maintain adequate growing conditions.

A randomized complete block experimental design was used and treatments were replicated 4 times. Treatments included mesotrione applied at 140 g ai ha applied 2 weeks before seeding (WBS), at seeding, and 3 weeks after seeding (WAS) (3 LOW), 175 g ai ha applied 2 WBS, at seeding, and 3 WAS (3 HIGH), 140 g ai ha applied 2 WBS, at seeding, and 3 and 5 WAS (4 LATE), 140 g ai ha applied 4 and 2 WBS, at
seeding, and 3 WAS (4 EARLY), and a nontreated check. Trials were initiated on 
August 7, 2007 at each location. Mesotrione was applied in combination with a nonionic 
surfactant at 0.25% v/v. Treatments were applied using a CO₂ pressurized backpack 
sprayer delivering 280 L/ha at 276 kPa via 11004XR nozzles.

**Turfgrass injury and control.** Tall fescue injury, tall fescue cover, and creeping 
bentgrass control were visually rated 2 and 1 WBS, 2, 4, 5, 7, and 52 WAS. Ratings were 
recorded as a visually estimated percentage with 0% being no injury or control, and 
100% being death of all visible foliage or complete control (Frans et al. 1986).

**Turfgrass establishment.** Each trial location was established to meet optimal tall fescue 
seeding timings in early fall. At the TRC location, a vertical mower was used in two 
directions to allow for increased seed-to-soil contact within each plot. Turf type tall 
fescue was seeded at 488 kg pure live seed (PLS) ha⁻¹ using a walk behind drop seeder. 
At the BCC location, a verti-seeder was used aggressively in three directions. This 
application physically removed approximately 60% of bentgrass present within each plot. 
Excess debris was removed from the trial area to reduce effects on seeded establishment. 
Turf type tall fescue was seeded at approximately 391 kg PLS ha⁻¹ 4 weeks after initial 
herbicide treatment.

Data variance was tested for homogeneity by plotting residuals in SAS 9.2. Data 
were subjected to a combined ANOVA with sums of squares partitioned to reflect 
location and treatment effects. Locations were considered random and the mean square 
of treatment effects was tested using the mean square associated with the random variable 
(McIntosh 1983). Means were separated using Fisher’s protected LSD tested at P = 0.05 
were appropriate.
Results and Discussion

Significant differences in trial locations were observed for creeping bentgrass control and cover. There were no significant differences in trial or replication for tall fescue cover and injury, therefore, data were pooled.

Creeping bentgrass control: Mesotrione treatment combinations controlled creeping bentgrass at both locations. Multiple mesotrione treatments prior to reseeding of the contaminated area (4 EARLY) controlled more creeping bentgrass and provided a greater advantage to tall fescue seedlings than treatments initiated later at each location (Table 1). At the TRC, 4 EARLY and 3 HIGH controlled statistically greater amounts of creeping bentgrass than the other treatments by 2 weeks after seeding (WAS) (Table 1). 4 LATE and 3 LOW treatments were consistently lower in creeping bentgrass control throughout the study until 7 WAS when 4 LATE reached its peak bentgrass control.

At BCC, mesotrione treatments controlled creeping bentgrass more than the TRC location (Table 1). By 2 WAS, all treatments controlled creeping bentgrass greater than 86%. 4 LATE controlled creeping bentgrass (96.3%) more than 3 LOW (86.3%), but these treatments were similar by 4 WAS. By 5 WAS, all treatments controlled greater than 90% of creeping bentgrass.

Creeping bentgrass cover: At TRC, creeping bentgrass cover was reduced by all treatments 1 WBS (Figure 1; Table 2). 4 EARLY reduced creeping bentgrass cover when compared to 3 LOW with exception of 4 WAS. All treatments reduced creeping bentgrass cover to less than 15% by 5 WAS. However, no treatment completely
controlled creeping bentgrass at the TRC location. Nontreated plots maintained a consistent cover around 95% creeping bentgrass.

At BCC, treatments reduced creeping bentgrass cover more than the TRC location (Figure 2; Table 2). By 2 WBS, 4 EARLY reduced creeping bentgrass cover 40%. At seeding, creeping bentgrass cover was reduced to less than 50% in each plot. Aggressive verti-cutting reduced much of the creeping bentgrass at this location. Verti-cutting alone removed approximately 65% of creeping bentgrass from the nontreated check. This aggressive verti-cutting allowed for a reduction in creeping bentgrass thatch and provided for good seed-to-soil contact of tall fescue. Removal of creeping bentgrass by the verti-cutting applications allowed greater control of remaining creeping bentgrass, providing less competition to the newly established seedlings than at the TRC location. By 2 WAS, creeping bentgrass cover was reduced to less than 10% in all treatments. Creeping bentgrass cover continued to decline in all herbicide treated plots as the trial progressed. Competition with newly seeded tall fescue kept creeping bentgrass from completely recovering. Creeping bentgrass cover remained at 40% throughout the remainder of the study.

**Tall fescue cover:** Tall fescue cover did not differ among mesotrione treatments until 5 WAS (Figure 3; Table 2). However, all treatments contained significantly greater tall fescue cover than the nontreated by 2 WAS as a result of creeping bentgrass competition with young seedlings. All treatments steadily increased in tall fescue cover as the trials progressed. At 7 WAS, tall fescue cover in 3 LOW treated plots increased to 55% where 4 EARLY increased to 70%, but 4 EARLY treatments remained superior to 3 LOW in tall fescue cover.
By one year after seeding, differences in tall fescue cover were observed (Table 1). At the TRC location, tall fescue cover was greatest for 4 EARLY (86%) but was not different from 4 LATE (78%) or 3 HIGH (76%). Tall fescue cover was significantly less in 3 LOW one year after seeding (73%) than 4 EARLY. In nontreated plots, tall fescue seedlings emerged and reached 26% cover 7 WAT but did not persist one year after seeding due to creeping bentgrass cover (Figure 3; Table 1). At the BCC location, all treatments were equal and contained greater than 97% tall fescue cover one year after seeding (Table 1). Because of the verti-cutting severity at seeding, creeping bentgrass cover loss reduced existing turfgrass competition with new seedlings and allowed for some tall fescue seedlings to survive in contrast to the TRC location. 69% tall fescue cover was achieved one year after seeding in these nontreated plots.

**Tall fescue injury:** Mesotrione injured tall fescue briefly after each application but injury never exceeded 23% and was considered acceptable in all cases (Table 3). All treated plots had fully recovered by 11 weeks after initial treatment with exception of the 4 LATE treatment combination.

Aggressive verti-cutting increased tall fescue establishment rates at BCC. Sequential mesotrione treatments initiated 2 WBS increased tall fescue cover slightly over mesotrione treatments initiated 1 WBS. Mestrione controlled creeping bentgrass more when applied three times at high rates or four times at low rates. Differences between mesotrione programs, however, were small and all programs effectively transitioned infested areas back to a predominately tall fescue turf. Prior to the registration of mesotrione for use in turfgrasses, few options were available to turfgrass mangers for weed control during seeded establishment. Attributes such as pre and
postemergence weed control efficacy and seedling safety make mesotrione a valuable product for use in establishment of tall fescue and other tolerant species.
Sources of Materials

1 Tenacity® Herbicide, Syngenta Crop Protection, Inc., Greensboro, NC 27419-8300 USA.

2 Chem-Stik Nonionic Spreader-Sticker, Precision Laboratories, Inc. Waukegan, IL 60085 USA.

3 11004XR Extended Range Flat Fan Spray Tip, TeeJet Technologies, P.O. Box 7900, Wheaton, IL 60187-7900, USA.

4 Southern Lawn Turf Type Tall Fescue Blend, Landscape Supply, Inc. 101 Madison Ave, Roanoke, VA 24027, USA.

5 Scotts AccuGreen 3000 Model Drop spreader, The Scotts Company LLC, 14111 Scottslawn Rd., Marysville, OH 43041, USA.

6 Jacobsen Verti-Seeder Model 2201, Jacobsen, A Textron Company. 11108 Quality Drive, Charlotte, NC 28273, USA.

7 SAS 9.2 software, SAS Institute Inc., Cary, NC 27513-2414, USA.
Literature Cited


Table 1: Effect of mesotrione treatment programs on creeping bentgrass control -2, -1, 2, and 7 WAS, and tall fescue cover 52 WAS at the Blacksburg Country Club (BCC) and Turfgrass Research Center (TRC) trial locations.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Creeping bentgrass control</th>
<th>Tall fescue cover</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BCC</td>
<td>TRC</td>
</tr>
<tr>
<td></td>
<td>-2</td>
<td>-1</td>
</tr>
<tr>
<td>3 LOW&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 HIGH</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4 LATE</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4 EARLY</td>
<td>37.5</td>
<td>31</td>
</tr>
<tr>
<td>Nontreated</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>LSD</td>
<td>2</td>
<td>5.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Abbreviations: BCC, Blacksburg Country Club; TRC, Turfgrass Research Center; NS, not significant at P = 0.05; WAS, weeks after seeding;
\textsuperscript{b} mesotrione applied at 140 g ai ha\textsuperscript{-1} applied 2 weeks before seeding (WBS), at seeding, and 3 weeks after seeding (WAS) (3 LOW), 175 g ai ha\textsuperscript{-1} applied 2 WBS, at seeding, and 3 WAS (3 HIGH), 140 g ai ha\textsuperscript{-1} applied 2 WBS, at seeding, and 3 and 5 WAS (4 LATE), 140 g ai ha\textsuperscript{-1} applied 4 and 2 WBS, at seeding, and 3 WAS (4 EARLY)
Table 2: Line equations and $R^2$ values for regression analysis found in Figures 1, 2, and 3.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Figure 1 Equation</th>
<th>Figure 1 $R^2$</th>
<th>Figure 2 Equation</th>
<th>Figure 2 $R^2$</th>
<th>Figure 3 Equation</th>
<th>Figure 3 $R^2$</th>
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</thead>
<tbody>
<tr>
<td>3 LOW$^a$</td>
<td>$y = 15.73e^{0.12x}$</td>
<td>0.87</td>
<td>$y = 49.14e^{-0.14x}$</td>
<td>0.48</td>
<td>$y = 114.74e^{-0.42x}$</td>
<td>0.96</td>
</tr>
<tr>
<td>3 HIGH</td>
<td>$y = 11.65e^{0.16x}$</td>
<td>0.94</td>
<td>$y = 45.13e^{-0.2x}$</td>
<td>0.58</td>
<td>$y = 1774.2e^{-1.5x}$</td>
<td>0.65</td>
</tr>
<tr>
<td>4 LATE</td>
<td>$y = 7.45e^{0.19x}$</td>
<td>0.96</td>
<td>$y = 67.93e^{-0.22x}$</td>
<td>0.91</td>
<td>$y = 106.59e^{-0.4x}$</td>
<td>0.83</td>
</tr>
<tr>
<td>4 EARLY</td>
<td>$y = 12.56e^{0.16x}$</td>
<td>0.92</td>
<td>$y = 35.71e^{-0.18x}$</td>
<td>0.44</td>
<td>$y = 77.93e^{-0.11x}$</td>
<td>0.93</td>
</tr>
<tr>
<td>Nontreated</td>
<td>$y = 4.28e^{0.17x}$</td>
<td>0.97</td>
<td>$y = 91.28e^{0.01x}$</td>
<td>0.53</td>
<td>$y = 97.03e^{-0.1x}$</td>
<td>0.54</td>
</tr>
</tbody>
</table>

$^a$ mesotrione applied at 140 g ai ha$^{-1}$ applied 2 weeks before seeding (WBS), at seeding, and 3 weeks after seeding (WAS) (3 LOW), 175 g ai ha$^{-1}$ applied 2 WBS, at seeding, and 3 WAS (3 HIGH), 140 g ai ha$^{-1}$ applied 2 WBS, at seeding, and 3 and 5 WAS (4 LATE), 140 g ai ha$^{-1}$ applied 4 and 2 WBS, at seeding, and 3 WAS (4 EARLY).
Table 3: Effect of mesotrione treatment program on tall fescue injury pooled over location.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>-2</th>
<th>-1</th>
<th>2</th>
<th>4</th>
<th>5</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 LOW&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>1.3</td>
<td>21.5</td>
<td>6.3</td>
<td>1.3</td>
<td>0</td>
</tr>
<tr>
<td>3 HIGH</td>
<td>0</td>
<td>2.5</td>
<td>20.9</td>
<td>8.8</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>4 LATE</td>
<td>0</td>
<td>2.5</td>
<td>20.5</td>
<td>5.6</td>
<td>2.5</td>
<td>6.3</td>
</tr>
<tr>
<td>4 EARLY</td>
<td>3.8</td>
<td>8.8</td>
<td>23.1</td>
<td>6.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LSD</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>1.9</td>
<td>NS</td>
<td>1.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Abbreviations: NS, not significant at P = 0.05.

<sup>b</sup> Mesotrione applied at 140 g ai ha<sup>-1</sup> applied 2 weeks before seeding (WBS), at seeding, and 3 weeks after seeding (WAS) (3 LOW), 175 g ai ha<sup>-1</sup> applied 2 WBS, at seeding, and 3 WAS (3 HIGH), 140 g ai ha<sup>-1</sup> applied 2 WBS, at seeding, and 3 and 5 WAS (4 LATE), 140 g ai ha<sup>-1</sup> applied 4 and 2 WBS, at seeding, and 3 WAS (4 EARLY)
Figure 1: Effects of mesotrione treatment combinations including 3 LOW, 3 HIGH, 4 LATE, 4 EARLY, and the nontreated comparison on creeping bentgrass cover at the Turfgrass Research Center location. Regression equations and $R^2$ values for each variable can be found in Table (3).

* indicates significant differences among herbicide treatments.
Figure 2: Effects of mesotrione treatment combinations including 3 LOW, 3 HIGH, 4 LATE, 4 EARLY, and the nontreated comparison on creeping bentgrass cover at the Blacksburg Country Club location. Regression equations and $R^2$ values for each variable can be found in Table (3).
Figure 3: Effects of mesotrione treatment combinations including 3 LOW, 3 HIGH, 4 LATE, 4 EARLY, and the nontreated comparison on tall fescue cover. Trial locations were similar so data were pooled for the first year data. Regression equations and $R^2$ values for each variable can be found in Table (3).

\[\text{LSD} = \text{NS} \quad \begin{array}{cccc}
7.2 & 8.1 & 8.6^* & 9.6^*
\end{array}\]

\[\text{LSD} = \text{NS} \quad \begin{array}{cccc}
7.2 & 8.1 & 8.6^* & 9.6^*
\end{array}\]

* Indicates significant differences among herbicide treatments
Chapter 3.

Optimizing Mesotrione Formulations for Turfgrass Tolerance and Weed Control

The following chapter was formatted to facilitate publication in *Weed Science*. 
Optimizing Mesotrione Formulations for Turfgrass Tolerance and Weed Control

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Abstract

Mesotrione typically requires multiple applications to control susceptible weeds in turfgrass. Since mesotrione is absorbed by both foliage and roots, a timed-release formulation could eliminate the need for multiple applications. Two experiments conducted at Virginia Tech’s Glade Road Research Facility in Blacksburg, VA evaluated simulated release patterns for a potential timed-release mesotrione formulation. A soluble concentrate formulation of mesotrione was titrated to produce a stepwise change in mesotrione rates that were applied daily to mimic predetermined release patterns over a three week period. These time-release scenarios were compared to a standard foliar broadcast treatment of mesotrione at 280 g ai ha$^{-1}$ applied twice at three week intervals, and a nontreated check. Mesotrione applied in the simulated release patterns controlled creeping bentgrass (Agrostis stolonifera L.), goosegrass (Eleusine indica L), nimblewill (Muhlenbergia scaber J.F. Gmel.), smooth crabgrass (Digitaria ischaemum Schreb. ex Muhl), and white clover (Trifolium repens L.) statistically similar to the standard

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treatment in every comparison. However, each simulated release pattern injured tall fescue 2- to 7-fold greater than the standard comparison treatment. Soil and foliar initiated treatments were statistically similar in most comparisons. Data suggest a timed-release mesotrione formulation can control weeds equivalent to two broadcast applications, but more research is needed to elucidate proper timings and release patterns to minimize turf injury.


**Additional index words:** Bleaching herbicides, triketone herbicides, slow release, turfgrass injury.

**Abbreviations:** WAIT, weeks after initial treatment;

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⁶ Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds*, Revised May 2006. Available at http://wssa.net/Weeds/ID/Names.htm.
Introduction

For effective control of most mature weeds targeted by mesotrione, initial herbicide application must be followed by a sequential application approximately 3 weeks later (Anonymous 2008b). This aspect of mesotrione is one of its limitations. Ideally herbicide applications would provide desired results following a single treatment without the need for a sequential application. Slow release formulations allow specific amounts of herbicide to be released over a given amount of time. Controlled release of herbicides also reduces the amount of leaching and offsite movement of products. Schreiber et al. (1988) reported that atrazine leaching was reduced using a controlled release starch granule (CRSG). Similarly, Boydston (1992) reported that conventional applications compared to CRSG applications containing $^{14}$C-norflurazon or $^{14}$C-simazine retarded the leaching depths of both herbicides. Other slow release formulations have been tested using products such as organic and inorganic clays (Celis et al. 2002; Hermosin et al. 2001), and ethylcellulose (Sopena et al. 2007).

Generally, granular herbicides are less effective for weed control than foliar sprays. Granular herbicides have limited opportunity to enter the plant through foliar absorption, especially when the foliage is dry. Thus, granular herbicides typically rely on soil absorption and must be root absorbed to obtain desired results. Davis and Ahrens (1981) reported greater control of white clover (Trifolium repens L.) and smooth hawkweed (Hieracium laevigatum Willd.) when granules containing 2,4-D plus mecoprop or dicamba were mixed with fertilizer and applied when moisture was present on plant foliage. Mesotrione is absorbed by plant roots and foliage and is active when used as a preemergence (PRE) and postemergence (POST) herbicide (Anonymous 2008a;
Because of its soil activity, mesotrione is less dependent on leaf moisture for absorption. Goddard et al. (2007) reported dew presence at the time of application did not influence the control of dandelion (*Taraxacum officinale* G.H. Weber ex Wiggers) or white clover by combination granules of mesotrione plus fertilizer, but dew was necessary for effective control with granules containing fertilizer plus 2,4-D and MCPP.

Adapting slow release technology for production of a mesotrione formulation could be used to reduce the number of applications needed to control undesirable weeds in turfgrass stands, potentially eliminating the need for a sequential application. Because mesotrione can be absorbed by plants through foliage or roots, either sprayable or granular formulations could be produced. Therefore the objectives of this study are 1) to determine the optimum mesotrione release pattern to maintain acceptable turfgrass tolerance and herbicide efficacy, and 2) to determine if application placement at initial application affects herbicide efficacy.

**Materials and Methods**

Two greenhouse experiments were conducted at the Virginia Tech Glade Road Research Facility at Blacksburg, VA in 2007 and 2008. Mature plugs of turf type tall fescue* [Schedonorus phoenix* (Scop.) Holub], nimblewill (*Muhlenbergia schreberi* J.F. Gmel.) and creeping bentgrass (*Agrostis stolonifera* L.) were taken from the field and thinned to 2 to 3 tillers. Smooth crabgrass [*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.], goosegrass [*Eleusine indica* (L.) Gaertn.] and white clover (*Trifolium repens* L.) were seeded into flats for establishment. Seedlings were size selected for 2-3 leaf stage.
crabgrass and goosegrass, and 1 fully expanded leaf for white clover. Mature plants were washed to remove any excess soil before being transplanted into 10 by 10 cm pots containing steam sterilized sand and soil (50:50 by vol). Soil type was a Grosclose-Urban Land complex (Fine, mixed, semiactive, mesic, Typic Haplustolls) with 1.8% organic matter and pH 6.6. Once transplanted, plants were irrigated as needed to maintain adequate growing conditions. Each pot was fertilized biweekly using a 20-20-20 water soluble fertilizer. Plants were allowed to acclimate in the greenhouse for 1 week before treatments were initiated.

For these experiments, a randomized complete block experimental design was used, and treatments were replicated four times. Treatments included a nontreated check, a standard, and a three by two factorial arrangement that mimicked three hypothetical release scenarios applied following an initial application to foliage or soil. The initial treatment was mesotrione at 280 g ai ha$^{-1}$ applied broadcast to foliage and soil or syringed directly to soil. The release scenarios that followed consisted of an additional 280 g ai ha$^{-1}$ mesotrione titrated into different rates applied daily to match predetermined ascending, descending, and intervallic patterns over a three week period. The standard treatment consisted of two broadcast applications of mesotrione at 280 g ai ha$^{-1}$ applied at three week intervals. These rates are the maximum application rates for tall fescue turf.

**Herbicide Application.** Treatments were applied daily for 21 days to mimic the release pattern of each slow release granule scenario (Figure 1). Plants were watered as needed throughout the study. Both foliar treatments for the standard comparison and initial foliar treatments, where applicable, for the scenarios were applied using a hand held, CO$_2$ pressurized boom equipped with 11004XR$^3$ spray tips and delivering 280 L ha$^{-1}$ at 206.8
kPa. Each foliar treatment was applied with a non-ionic surfactant at 0.25% v/v. For all slow release scenarios, predetermined mesotrione rates were mixed in 5 ml water and syringed to soil daily following initial treatment.

**Data Collection.** Turfgrass injury, leaf counts by color (green, pale, white, or necrotic), tiller counts, and control of creeping bentgrass, goosegrass, nimblewill, smooth crabgrass, and white clover were evaluated weekly following herbicide application. Green leaves are defined as those leaves with no visible injury symptoms, pale leaves are defined as leaves with mild to moderate injury symptoms present (foliar chlorosis or bleaching), white leaves are defined as leaves having severe tissue injury (completely white), and necrotic leaves are defined as leaves that are completely dead. These data are reported as a percentage of the number of leaves per plant. Tiller counts were taken for smooth crabgrass and goosegrass only. Injury and control ratings were recorded as a visually estimated percentage with 0% indicating no injury or control, and 100% indicating complete death of all visible foliage or complete control (Frans et al. 1986). After 6 weeks, all above ground vegetative growth was removed, oven dried at 65°C for 48 hours, and weighed. Variance was tested for homogeneity by plotting residuals in SAS 9.2. Data were subjected to a combined ANOVA with sums of squares partitioned to reflect trial effects and the factorial treatment arrangement. Location was considered random, and mean square of treatment effects were tested using mean square associated with the random variable (McIntosh 1983). Appropriate means were separated using Fisher’s Protected LSD at P = 0.05. Comparison treatments were measured against appropriate main effect levels or interacting factors with single-degree-of-freedom contrasts after appropriate effects were identified in the factorial analysis.
Results and Discussion

Weed control: At 3 and 6 weeks after initial treatment (WAIT), trial, scenario, and application placement were insignificant for creeping bentgrass, goosegrass, nimblewill, and white clover control, therefore, data were pooled for comparison to the standard and check (Table 1). Regardless of scenario or application placement, treatments designed to mimic time-release effectively controlled creeping bentgrass, goosegrass, nimblewill, and white clover greater than 87% 6 WAIT and equivalent to the standard treatment. For smooth crabgrass, differences were observed for trial and application placement (Table 1). In trial 2 regardless of release pattern, initial treatment to soil only controlled smooth crabgrass 91% and significantly greater than initial treatment to foliar plus soil (58%).

Tall fescue injury: Tall fescue injury was significantly influenced by initial application placement but not by trial or scenario (Table 1). Initial soil applications injured tall fescue more than foliage plus soil applications at 3 and 6 WAIT. All treatments designed to mimic time-release patterns injured tall fescue 2- to 7-fold greater than the standard comparison treatment. Comparison treatments injured tall fescue 7 and 18% at 3 and 6 WAIT, respectively, where each scenario averaged over 34% injury, regardless of application placement. This increased injury could have occurred since more mesotrione was available to plants between 0 and 3 WAIT. Had release patterns been initiated one to two weeks after initial treatments, tall fescue injury may have been decreased. More research is needed to assess other release patterns to reduce turf injury.

Leaf color: To facilitate analysis and discussion, pale, white, green, and necrotic leaf count, data were converted to percent discolored leaves using the equation 

\[ \frac{P + W + G + N}{P + W + G + N} \times 100 \]
\[ \frac{N}{T} \times 100 \], where \( P \) is the number of pale, white, and necrotic leaves, respectively, and \( T \) is the total number of leaves per plant. Trial repetition, application placement, and mesotrione release pattern had no effect on percent discolored leaves of nimblewill, smooth crabgrass, and white clover 2 WAIT, therefore data were pooled for comparison to the standard and check (Table 2). Regardless of initial application placement or release pattern, time-release scenarios discolored 46, 76 and 72\% of nimblewill, smooth crabgrass, and white clover leaves, respectively and equivalent to the standard mesotrione treatment 2 WAIT. Both the average of time-release treatments and the standard had more discolored leaves than the nontreated check. Tall fescue injury was influenced by time-release scenarios and trial but not by initial application placement (Table 2). The standard mesotrione comparison treatment injured tall fescue 37 and 18\% in trials 1 and 2, respectively. In each case, injury from the standard comparison was less than the injury sustained from the simulated release patterns. In trial 1, the simulated release patterns did not differ from each other, but in trial 2, the rate descending release pattern resulted in the most discolored leaves 2 WAIT. Although time-release scenarios were insignificant for tall fescue injury data in Table 1, the percent leaf discoloration indicates increased mesotrione rate in the first three weeks could be responsible for tall fescue discoloration observed in these trials. The rate descending pattern applies the equivalent of over 525 g ai/ha mesotrione in the first four days due to the logarithmic pattern chosen. It may be possible to reduce turfgrass injury by delaying time-release treatments for at least one to two weeks after the initial application but more research is needed to test this hypothesis. A three-way interaction in trial, application placement, and mesotrione release pattern was observed for creeping bentgrass 2 WAIT (Table 3).
In trial 1, complete control of creeping bentgrass was observed for each application placement, release pattern, and standard mesotrione comparison treatment. In trial 2, differences in application placement and release pattern were observed. For the rate ascending release pattern, foliar initiating treatments controlled smooth crabgrass 80% and more than soil initiating treatments (69%). This foliar initiated rate ascending treatment was also more effective than the foliar applied rate descending, the soil applied rate intervallic, and the standard comparison treatments. Since smooth crabgrass is one of the most important weeds in turfgrass markets (Webster 2004), these data suggest the rate ascending formulation may have advantages over other time-release patterns.

Goosegrass leaf discoloration did not differ between treatments (Table 3).

**Fresh weights:** Trial repetition, application placement, and mesotrione release pattern had no effect on plant fresh weights for creeping bentgrass, nimblewill, smooth crabgrass and white clover 7 WAT, therefore data were pooled (Table 4). In each case, the nontreated plant weights were significantly greater than the standard comparison or the average of application placement and mesotrione release pattern, which were statistically similar. In each case, mesotrione treatments were effective at controlling these species. The average of application placement and mesotrione release pattern reduced plant weights 93, 90, 99, and 98% where the standard treatment reduced plant weights 94, 86, 100, and 95% for creeping bentgrass, nimblewill, smooth crabgrass, and white clover, respectively. For goosegrass, effects of application placement were similar, therefore data were pooled. Standard mesotrione application and the mesotrione release patterns were similar and reduced plant weights 86% or greater when compared to the nontreated check (Table 4). Mesotrione release scenarios reduced tall fescue fresh weights at least
51%. Each of these release scenarios were more injurious than the standard mesotrione treatment. Standard mesotrione treatments reduced tall fescue weights by 20% but were not different from the nontreated.

In these studies, injury was noticed and reported in Table 2 for the nontreated plants within nimblewill, smooth crabgrass, and white clover species. This injury was not expected and is difficult to explain. Effort was made during these trials to reduce the potential for herbicide movement from one plant to the other. Each daily treatment was carefully syringed onto the soil surface of each pot to reduce contact with the plant foliage and prevent herbicide splash during application. Once daily treatments were absorbed into the soil profile, each pot was lightly hand watered to reduce herbicide movement during watering events but some movement could have occurred from soil splash. Potential volatility of mesotrione has been observed in field and greenhouse trials at Virginia Tech. Highly sensitive species to mesotrione such as *Oxalis sp.* and small *Brassica sp.* located in areas around field plots have been injured although herbicide was applied with a shield to reduce drift potential. Though volatility is suspected, no known reports of mesotrione volatility exist. However, researchers evaluating other bleaching herbicides have reported volatility in their trials (Halstead and Harvey 1986; Locke et al. 1996; Thelen et al. 1988).

Tall fescue was injured more by treatments designed to mimic time-release scenarios than by the standard comparison. This occurrence was unexpected but could be due to increased herbicide rate in the time release treatments. The three release scenarios were chosen based on discussion with Syngenta formulation chemist (L. Galiano, personal communication). The ascending, descending, and intervallic patterns are
indicative of various formulation technologies available to formulation chemists. Each comes with associated costs. By knowing the extent of bioefficacy from each pattern, economically viable choices could be made for any future work on time-release mesotrione formulations. The patterns were designed to apply 280 g ai ha\(^{-1}\) initially and another 280 g ai ha\(^{-1}\) over the next 21 days. Since the standard mesotrione broadcast treatment consists of two 280 g ai ha\(^{-1}\) treatments at a 21 day interval, it may have been better to initiate the release patterns at 2 WAIT and have them extend over a period of time between 2 and 5 WAIT.

These slow release products could have an application in turfgrass markets. Development of a slow release granule would reduce time and costs to the applicator as it could negate the need for a sequential mesotrione application. Results of these studies indicate that mesotrione can control a variety of weeds when released into the soil environment in different ways. Further studies on the specific release patterns and mesotrione rates are needed for adequate control of targeted weeds while ensuring turfgrass safety.

**Acknowledgements**

The authors would like to thank Syngenta Crop Protection for supporting this research.
Sources of Materials

1 Southern Lawn Turf Type Tall Fescue Blend, Landscape Supply, Inc. 101 Madison Ave, Roanoke, VA 24027, USA.

2 Peters Professional 20-20-20 General Purpose Water Soluble Fertilizer, The Scotts Company, 14111 Scottslawn Road, Marysville, OH 43041

3 11004XR Extended Range Flat Fan Spray Tip, TeeJet Technologies, P.O. Box 7900, Wheaton, IL 60187-7900, USA

4 Chem-Stik Nonionic Spreader-Sticker, Precision Laboratories, Inc. Waukegan, IL 60085 USA.

5 SAS 9.2 software, SAS Institute Inc., Cary, NC 27513-2414, USA.
Literature Cited

Greensboro, NC 27419-8300 33 p.

Greensboro, NC 27419-8300 14 p.


clays as carriers for controlled release of the herbicide hexazinone. Agricul and
Food Chem. 50(8):2324-2330.


Frans, R. E., R. Talbert, D. Marx, and H. Crowley. 1986. Experimental design and
techniques for measuring and analyzing plant responses to weed control practices.
Champaign, IL: Southern Weed Science Society.

formulation on mesotrione efficacy for lawn weed control. Proc. Northeast. Weed
Sci. Soc. 61:84


Table 1: Effect of initial mesotrione placement on tall fescue injury and smooth crabgrass control in two trials averaged over time-release scenarios. Placement, scenario, and trial were insignificant for creeping bentgrass, goosegrass, nimblewill, and white clover so data were pooled for comparison with the standard treatment\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Species</th>
<th>Initial placement\textsuperscript{c}</th>
<th>3 WAIT\textsuperscript{b}</th>
<th>6 WAIT</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>Trial 1</td>
<td>Trial 2</td>
<td>Average</td>
</tr>
<tr>
<td>Tall fescue</td>
<td>Soil only</td>
<td>34*</td>
<td></td>
<td></td>
<td>35*</td>
</tr>
<tr>
<td></td>
<td>Foliar + soil</td>
<td>48*</td>
<td></td>
<td></td>
<td>51*</td>
</tr>
<tr>
<td></td>
<td>LSD (0.05)</td>
<td>10</td>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Smooth crabgrass</td>
<td>Soil only</td>
<td></td>
<td>60</td>
<td>91</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Foliar + soil</td>
<td></td>
<td>57</td>
<td>58</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>LSD (0.05)</td>
<td></td>
<td>NS</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>Goosegrass</td>
<td></td>
<td>55</td>
<td></td>
<td></td>
<td>87</td>
</tr>
<tr>
<td>Creeping bentgrass</td>
<td></td>
<td>69</td>
<td></td>
<td></td>
<td>89</td>
</tr>
<tr>
<td>Nimblewill</td>
<td></td>
<td>49</td>
<td></td>
<td></td>
<td>91</td>
</tr>
<tr>
<td>White clover</td>
<td></td>
<td>81</td>
<td></td>
<td></td>
<td>97</td>
</tr>
</tbody>
</table>
a Means followed by a * are different from the standard comparison treatment (2 applications of mesotrione applied at 280 g ai ha\(^{-1}\) at 3 week intervals). Standard treatments controlled creeping bentgrass 61 and 86\%, goosegrass 41 and 80\%, nimblewill 53 and 79\%, smooth crabgrass 72 and 100\%, tall fescue 7 and 18\%, and white clover 71 and 91\% at 3 and 6 WAT, respectively.

b Abbreviations: WAIT, week after initial treatment.

c Initial placement indicates the initial mesotrione treatment of 280 g ai ha\(^{-1}\) was applied only to soil (to mimic a granular treatment) or to foliar + soil (to mimic a broadcast spray). Regardless of the initial application placement, all time-release scenarios were achieved by diluting titrated mesotrione rates in 5 ml water and syringing them to soil daily over a three week period.
Table 2: Average effect of time-release scenarios and application placement compared to standard treatment and nontreated check on percentage discolored leaves of nimblewill, smooth crabgrass, and white clover and effect of release pattern and trial on the percentage discolored leaves of tall fescue\(^a\).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nimblewill</th>
<th>Smooth crabgrass</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>White clover</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avg. time-release(^b)</td>
<td>46</td>
<td>76</td>
<td>--</td>
<td>--</td>
<td>72</td>
</tr>
<tr>
<td>Standard</td>
<td>47</td>
<td>57</td>
<td>37</td>
<td>18</td>
<td>69</td>
</tr>
<tr>
<td>Nontreated</td>
<td>13</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Rate ascending(^c)</td>
<td>60</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate descending</td>
<td>55</td>
<td>51</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate intervallic</td>
<td>53</td>
<td>38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>16</td>
<td>25</td>
<td>14</td>
<td>7</td>
<td>31</td>
</tr>
</tbody>
</table>

\(^a\) Percentage discolored leaves based on leaf counts of necrotic, pale, and white leaves converted to a percentage of total leaves per plant.
Significant differences were not observed among the factorial structure of mesotrione time-release scenarios and application placement for nimblewill, smooth crabgrass, and white clover, therefore data were pooled for comparison to the nontreated check and standard treatment (2 applications of mesotrione applied at 280 g ai ha\(^{-1}\) at 3 week intervals).

Time-release scenarios included mesotrione rate titrations that were added to 5 ml water and syringed to soil each day during a three week period. The titrated rates totaled 280 g ai ha\(^{-1}\) and were applied in a logarithmic ascent between day 2 and day 21 ("rate ascending"), a logarithmic descent between day 2 and day 21 ("rate descending"), and a curved pattern between day 17 and day 21 ("intervallic").
Table 3: Effect of release pattern and application placement of mesotrione on discolored leaves of creeping bentgrass and goosegrass. Significant differences in trial were observed for creeping bentgrass.

<table>
<thead>
<tr>
<th></th>
<th>Creeping bentgrass</th>
<th>Goosegrass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
</tr>
<tr>
<td>Foliar + soil</td>
<td>Rate ascending 100</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Rate descending 100</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>Rate intervallic 100</td>
<td>76</td>
</tr>
<tr>
<td>Soil only</td>
<td>Rate ascending 100</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Rate descending 100</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Rate intervallic 100</td>
<td>67</td>
</tr>
<tr>
<td>Foliar + soil</td>
<td>Standard 100</td>
<td>69</td>
</tr>
<tr>
<td>Nontreated</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>---</td>
<td>10</td>
</tr>
</tbody>
</table>

*Initial placement indicates the initial mesotrione treatment of 280 g ai ha\(^{-1}\) was applied only to soil (to mimic a granular treatment) or to foliar + soil (to mimic a broadcast spray). Regardless of the initial application placement, all time-release scenarios were achieved by diluting titrated mesotrione rates in 5 ml water and syringing them to soil daily over a three week period. Time-release patterns included mesotrione rate titrations that were added to 5 ml water and syringed to soil each day during a three week period. The titrated rates totaled 280 g ai ha\(^{-1}\) and were applied in a logarithmic ascent between*
day 2 and day 21 ("rate ascending"), a logarithmic descent between day 2 and day 21 ("rate descending"), and a curved pattern between day 17 and day 21 ("intervallic").
Table 4: Average effect of time-release patterns and application placement compared to standard treatment and nontreated check on above-ground fresh plant weights of creeping bentgrass, nimblewill, smooth crabgrass, and white clover and effect of release pattern on above-ground fresh plant weights of goosegrass and tall fescue.

<table>
<thead>
<tr>
<th>Release pattern</th>
<th>Creeping bentgrass</th>
<th>Goosegrass</th>
<th>Nimblewill</th>
<th>Smooth crabgrass</th>
<th>Tall Fescue</th>
<th>White clover</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avg. time-release(^a)</td>
<td>0.26</td>
<td>--</td>
<td>0.13</td>
<td>0.04</td>
<td>--</td>
<td>0.03</td>
</tr>
<tr>
<td>Standard</td>
<td>0.24</td>
<td>0.62</td>
<td>0.18</td>
<td>0</td>
<td>1.88</td>
<td>0.09</td>
</tr>
<tr>
<td>Nontreated</td>
<td>3.74</td>
<td>4.37</td>
<td>1.33</td>
<td>4.19</td>
<td>2.32</td>
<td>1.86</td>
</tr>
<tr>
<td>Rate ascending(^b)</td>
<td>0.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate descending</td>
<td>0.32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate intervallic</td>
<td>0.37</td>
<td></td>
<td></td>
<td></td>
<td>1.19</td>
<td></td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>0.48</td>
<td>0.77</td>
<td>0.34</td>
<td>0.87</td>
<td>0.51</td>
<td>1.16</td>
</tr>
</tbody>
</table>

\(^a\) Significant differences were not observed among the factorial structure of mesotrione time-release scenarios and application placement for creeping bentgrass nimblewill, smooth crabgrass, and white clover, therefore data were pooled for comparison to the nontreated check and standard treatment (2 applications of mesotrione applied at 280 g ai ha\(^{-1}\) at 3 week intervals).
Time-release patterns included mesotrione rate titrations that were added to 5 ml water and syringed to soil each day during a three week period. The titrated rates totaled 280 g ai ha$^{-1}$ and were applied in a logarithmic ascent between day 2 and day 21 ("rate ascending"), a logarithmic descent between day 2 and day 21 ("rate descending"), and a curved pattern between day 17 and day 21 ("intervallic").
Figure 1: Diagram of simulated mesotrione release patterns consisting of mesotrione rate
titrations that were added to 5 ml water and syringed to soil each day during a three week
period. The titrated rates total 280 g ai ha$^{-1}$. These treatments followed an initial
treatment of mesotrione at 280 g ai ha$^{-1}$ that was applied to soil (mimic of granular
formulation) or as a foliar spray (mimic of sprayable formulation).
Chapter 4.

Application Placement and Relative Humidity Affects Smooth Crabgrass and Tall Fescue Response to Mesotrione

The following chapter was formatted to facilitate publication in *Weed Science*. This work was accepted for publication in *Weed Science* in 2010 (manuscript num. WS-09-107R)
Application Placement and Relative Humidity Affects Smooth Crabgrass and Tall Fescue Response to Mesotrione

Matthew J. R. Goddard, John B. Willis, and Shawn D. Askew

Abstract

Much research has been conducted on mesotrione activity on crops and weeds, but information is lacking with regard to the relative contribution of soil and foliar absorption of mesotrione. Three experiments conducted at Virginia Tech’s Glade Road Research Facility in Blacksburg, VA evaluated the effects of 50 and 90% relative humidity (RH) on the activity of mesotrione applied to foliage, soil, and soil plus foliage. Tall fescue (Schedonorus phoenix (Scop.) Holub) injury ranged from 0 to 21% and was significant in 6 of 20 comparisons. Three of these injury events were caused by soil plus foliar applications, which were always more injurious than foliar only applications, which were more injurious than soil only applications. Both application placement and RH significantly influenced smooth crabgrass (Digitaria ischaemum (Schreb.) Schreb. ex Muhl.) responses to mesotrione. Smooth crabgrass phytotoxicity was lowest when mesotrione was applied only to foliage and highest when mesotrione was applied to soil

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and foliage. Increasing RH from 50 to 90% caused a 4- to 18-fold increase in plant phytotoxicity when mesotrione was applied only to foliage. By dissecting the plant canopy, it was noted at 14 days after treatment when averaged over RH that white leaves comprise 16% of leaves when only foliage was treated and 55 and 62% when applied to soil plus foliage and soil only, respectively. Furthermore, white tissue was found predominately in the two youngest leaves when mesotrione was applied to soil or both soil and foliage, but in older leaves when applied only to foliage. Data indicate mesotrione entering plants through soil travels quickly to growing points and has an equal or greater impact on plant phytotoxicity than foliar absorbed mesotrione. In addition, foliar absorption of mesotrione appears to significantly increase in plants with increasing RH, but does not move rapidly to growing points.

**Nomenclature:** Mesotrione; smooth crabgrass, *Digitaria ischaemum* Schreb. *ex* Muhl. DIGIS; tall fescue, *Schedonorus phoenix* (Scop.) Holub FESAR.

**Key words:** Bleaching herbicides; photochemical efficiency; relative humidity; triketone herbicides; turfgrass injury.

**Abbreviations:** DAT, days after treatment; RH, relative humidity
Introduction

Mesotrione is a new herbicide registered for use in turfgrass (Anonymous 2008b) that exhibits inconsistent weed control in variable environmental conditions (Askew et al. 2007; Johnson and Young 2002). Mesotrione can sometimes turn desirable, otherwise tolerant turfgrass species white (Askew et al. 2007; Goddard et al. 2007). Formulations of mesotrione have been developed for foliar spray and granular application. Herbicides differ greatly in their mode of action and mechanisms of entry into the plant. Some herbicides rely primarily on foliar absorption for their activity (Fuerst and Vaughn 1990; Sprankle et al. 1975a; 1975b), while others work mainly through soil absorption (Molin and Khan 1997) or a combination of both soil and foliar absorption (Lamoureux and Rusness 1995; Thompson and Slife 1969). Mesotrione can be absorbed through plant foliage or roots and exhibits both preemergence (PRE) and postemergence (POST) activity (Anonymous 2008a; 2008b; Armel et al. 2003; Johnson et al. 1999; Young et al. 1999). Therefore, mesotrione can be applied as a foliar broadcast or directly to the soil. However, the overall contribution of soil or foliar absorption of mesotrione to herbicide efficacy is unknown.

Herbicide applications have shown to be influenced by environmental factors such as temperature (Anderson et al. 1993; Johnson and Young 2002; Kells et al. 1984; McWhorter and Azlin; 1978; Wills and McWhorter 1981), relative humidity (RH) (Johnson and Young 2002; Jordan 1977; Wills 1984; Wills and McWhorter 1981), and soil fertility levels (Cathcart et al. 2004; Mithila et al. 2008). Anderson et al. (1993) reported green foxtail [Setaria viridis (L.) Beauv.] produced less ammonia following glufosinate application at 800 g ha⁻¹ as temperatures decreased, while barley (Hordeum
vulgare L. cv. 'Samson'), a tolerant species, was not affected. The same study reported green foxtail survival following application of glufosinate at 100 g ha$^{-1}$ when RH was 40% and death when RH was 95%. Johnson and Young (2002) observed similar responses to postemergence applications of mesotrione during high temperatures in velvetleaf (*Abutilon theophrasti* Medic.), ivyleaf morningglory (*Ipomoea hederacea* Jacq.), and common cocklebur (*Xanthium strumarium* L.) when treated at 32°C. Conversely, Johnson and Young (2002) reported large crabgrass [*Digitaria sanguinalis* (L.) Scop.] and common waterhemp (*Amaranthus rudis* Sauer) were more susceptible to injury from mesotrione at lower temperatures (18°C) and hypothesized that the contrasting results could be physiological differences between the species. Velvetleaf, ivyleaf morningglory, and common cocklebur are C$_3$ plants while large crabgrass and common waterhemp are C$_4$ plants. However, McCurdy et al. (2009) reported large crabgrass control by mesotrione was not affected by temperature or irradiance levels. Ricker et al. (2007) observed greatest injury to perennial ryegrass as a result of mesotrione application at optimum growing temperatures between 18.3 and 23.8°C.

Like temperature, RH seems to affect injury following herbicide application. High RH levels (85%) resulted in greater plant injury to velvetleaf, large crabgrass, and common waterhemp than low RH (30%) following mesotrione application (Johnson and Young 2002). Similarly, high RH levels increased efficacy of acifluorfen on showy crotalaria (*Crotalaria spectabilis*) (Wills and McWhorter 1981) and glyphosate and sethoxydim on bermudagrass (*Cynodon dactylon*) (Jordan 1977; Wills 1984). However, RH did not influence common cocklebur response to mesotrione application (Johnson and Young 2002).
Based on observations in field studies at Virginia Tech and a review of literature, it is believed that RH levels will influence the amount of turfgrass injury following mesotrione application. Determining the environmental factors that affect mesotrione activity within the plant can help us understand injury responses observed in the field. Granular applications may have limited foliar absorption when leaves are dry, and little is known about mesotrione bioefficacy in turf when applied only to soil. Therefore the objectives of this study are 1) to determine the relative contribution of soil and foliar absorption to mesotrione efficacy, and 2) to determine the effects of RH on soil and foliar activity of mesotrione in tall fescue and smooth crabgrass.

**Materials and Methods**

Three greenhouse experiments were conducted at the Virginia Tech Glade Road Research Facility in Blacksburg, VA in 2008. Smooth crabgrass seedlings and mature turf type tall fescue\(^1\) thinned to 2 or 3 tillers were transplanted into 10 by 10 cm pots containing steam sterilized sand and soil (50:50 by vol). The soil was a Groseclose-Urban Land complex (Fine, mixed, semiactive, mesic, Typic Hapludults) with 1.8% organic matter and a pH of 6.6. Plants were irrigated as needed to maintain favorable growing conditions. Each of the pots was fertilized weekly using a 20-20-20\(^2\) water soluble fertilizer. Plants were allowed to acclimate in the greenhouse for 1 week before being moved into growth chambers. Two environmental growth chambers\(^3\) were used and calibrated to deliver 50% (observed mean 50.6%, SD 1.5) and 90% (observed mean 88.9%, SD 1.0) RH levels. Each growth chamber was calibrated for 600 µmoles m\(^2\)s\(^{-1}\) PAR using a quantum meter light sensor\(^4\) during 14 hour days, maintained at average
day/night temperatures of 26.7/18.3°C (observed SD 0.15/0.19°C, respectively). Once in the growth chambers, plants were allowed to acclimate for three days before herbicide treatment. Pots were removed from growth chambers for herbicide treatments and promptly returned. Following treatment, pots were subirrigated to reduce the occurrence of herbicide movement caused by water splash from overhead irrigation, and were left in growth chambers for three days before returning them to the greenhouse. After returning the treated pots to the greenhouse, plants continued to receive subirrigation for 1 week.

For these experiments, a split-plot experimental design was used and treatments were replicated three times. Treatments were split between two RH levels (main plot factors), each containing three herbicide placements and a nontreated comparison (subplot factors), resulting in eight total treatments. This study was temporally repeated three times between October 27 and November 10, 2008. Herbicide placements included soil plus foliage (standard foliar broadcast), foliage only, and soil only. Mesotrione was applied at 280 g ai ha⁻¹ using a moving-nozzle, compressed-air greenhouse sprayer⁵ equipped with a single even-flow nozzle⁶ and calibrated to deliver 374 L ha⁻¹ 331 kPa. A non-ionic surfactant⁷ at 0.25% v/v was added to mesotrione in all cases.

**Herbicide Application.** Soil plus foliage applications were applied by allowing the moving nozzle to move over pots without any shields on foliage or soil. For soil only treatments, plants were covered with a 13mm test tube to prevent herbicide treatments from contacting the foliage while allowing normal soil application. For foliar only treatments, the soil surrounding the plant was completely covered using a 13mm thick cotton roll cut to fit each pot to prevent herbicide treatments from contacting the soil while allowing normal foliar application. Treated pots were allowed to dry for 15
minutes, test tubes or cotton fabric was removed, and plants were returned to their respective growth chambers for an additional three days.

**Turfgrass injury and control.** Turfgrass injury and crabgrass control were evaluated as indicators of herbicide efficacy. Ratings were recorded 3, 7, 14, and 21 days after treatment (DAT) as a visually estimated percentage with 0% being no injury or control, and 100% being death of all visible foliage or complete control (Frans et al. 1986). At the end of the experiment, all above ground vegetative growth was removed, oven dried at 65°C for 48 hours, and weighed.

**Photochemical efficiency measurement.** Photochemical efficiency (PE) was determined 3 and 7 DAT by measuring chlorophyll fluorescence with a dual wavelength fluorometer⁸. Relative PE (Fv/Fm) was measured using the ratio of variable fluorescence to maximum fluorescence at 690 nm. Chlorophyll fluorescence was measured using the single leaf method, similar to the method described by Xu et al. (2002), by taking readings on the newest fully emerged leaf only.

**Canopy composition.** Additional ratings included leaf counts by color (green, pale, or white) and tiller counts 3, 7, 14, and 21 DAT (Appendix A). Green leaves are defined as those leaves with no visible injury symptoms (healthy leaves), pale leaves are defined as leaves with mild to moderate injury symptoms (foliar chlorosis or not more than 10% bleaching), and white leaves are defined as leaves having severe tissue injury (over 70% white). These data are reported as a percentage of the total amount of leaves per plant. Tiller counts were taken on smooth crabgrass plants only.

Since white tissue is most conspicuous in a turfgrass setting, leaf position measurements were taken to specify where white or pale leaves occurred in the plant.
canopy. White and pale leaves were counted and their position on the respective tiller was recorded. The average of the sums of these counts was used to analyze where injury occurs. An average value of 1 would indicate that most leaves were characterized as the newest leaf, while an average of 2 would indicate most leaves were observed at the second position from the terminal.

**Dry biomass.** Dry biomass was recorded 21 DAT by clipping each plant at the soil surface and placing them into previously dried and weighed paper bags. Once bagged, samples were placed in a drying oven at 65°C for 48 hours, and weighed.

Variance was tested for homogeneity by plotting residuals in SAS 9.2. Data were subjected to a combined ANOVA with sums of squares partitioned to reflect trial effects and the split-plot treatment structure. Main plots were tested using the mean square of replication by main plot interaction while subplot main effects were tested using the mean square of their interaction with the random variable, trial (McIntosh 1983). Treatments were replicated within species as subsamples and over time for trials. Means were separated using Fisher’s protected LSD tested at P = 0.05.

**Results and Discussion**

There were no significant differences in trial or replication; therefore, data were pooled for photochemical efficiency, canopy composition, injury, leaf position, and dry biomass.

**Plant injury.** Data for 3, 7, 14, and 21 DAT were pooled over trials for both tall fescue and smooth crabgrass. Observed turfgrass injury for tall fescue never exceeded 21% and was deemed acceptable for all treatment options (Table 1). Soil plus foliar treatments
injured tall fescue greater than soil only treatments 3 DAT and foliar and soil only treatments 7 DAT. When RH was increased from 50 to 90%, tall fescue injury increased significantly from 2 to 8%, 3 to 21%, and 3 to 19% in soil plus foliage treatments 3, 7, and 14 DAT, respectively, and 1 to 7% in soil only treatments 7 DAT (Table 1).

Smooth crabgrass response to mesotrione application placement varied greatly between treatments and RH levels (Table 2). Soil plus foliar treatments were most injurious 3 and 7 DAT (27 and 48%, respectively) in 50% RH. Soil plus foliar applications injured smooth crabgrass significantly more 14 DAT when treated in 90% RH (76%) than in 50% RH (61%). Soil only treatments were equivalent to soil plus foliar treatments 14 DAT for 50% RH, but were significantly lower at 90% RH, suggesting foliar absorption of mesotrione is affected more by RH. This trend was most apparent when observing the responses of smooth crabgrass to foliar only treatments in different RH levels. When treated in a 50% RH environment, foliar only treatments injured smooth crabgrass most 3 DAT (8%) and were the least effective treatment throughout the study. However, RH levels influenced foliar only treatments greater than other treatment placements. Significantly more activity was observed in 90% RH than in 50% RH at each rating date following application (Table 2). These treatments were statistically similar to soil plus foliar applications at each evaluation except 21 DAT. Mesotrione activity increased from a 4-fold difference 3 DAT to 9-, 18-, and 15-fold difference at 7, 14, and 21 DAT, respectively. Preliminary work by McCurdy et al. (2007) compared soil, foliar, and soil plus foliar applications of mesotrione and determined that the treatments containing soil applied mesotrione were more effective at controlling yellow nutsedge (*Cyperus esculents* L.) and large crabgrass. However, this
study did not evaluate differing environmental conditions and their effects on the efficacy of mesotrione applications.

**Photochemical efficiency.** Photochemical efficiency (PE) data collected 3 and 7 DAT also indicate a significant influence of RH on the effects of mesotrione application placement (Table 3). Photochemical efficiency of nontreated plants was not affected by RH, regardless of species. Soil plus foliar applications in 90% RH 3 DAT was the only significant reduction in PE among treatments for tall fescue. No differences due to RH levels were observed for tall fescue.

Significant differences in smooth crabgrass PE were recorded for soil plus foliar and foliar only treatments 3 DAT. Soil plus foliar treatments reduced smooth crabgrass photochemical efficiency from 0.28 to 0, while foliar only treatments reduced PE from 0.61 to 0.03 in 50% to 90% RH, respectively. By 7 DAT, soil plus foliar treatments were reduced to 0.1 in 50% RH and remained at 0 in 90% RH. Foliar only and soil only treatments remained similar to the nontreated check (0.62 and 0.52, respectively) in 50% RH while a significant reduction was noticed in 90% RH (0 and 0.1, respectively).

**Canopy composition.** Discoloration of plant leaves following mesotrione application varied among treatments and RH levels (Table 4). Significant differences in the percentage of green leaves plant⁻¹ occurred at all rating dates when comparing between RH treatments. The application placement main effect showed a reduction in the total amount of leaves plant⁻¹ compared to the nontreated check for soil plus foliar and foliar only treatments 14 DAT and all treatments 21 DAT. A reduction in the amount of green leaves over time was noticed for all treatments and all RH levels except for foliar only treatments in 50% RH (Figure 1; Table 5). This effect is mirrored by the increase in
percentage of pale or white leaves over time. For most treatments, injury following mesotrione application occurred in the newest emerged leaf tissue. In most cases, this injury was noted on the first 2 leaves of each crabgrass tiller with pale leaves occurring at earlier emerging leaf positions (Table 6). This observation was true for soil only and soil plus foliar application placements. However, this was not true of foliar only applied mesotrione. Whitening occurred in earlier developing leaves, suggesting differential translocation following foliar absorption. Using radioactive mesotrione, Mitchell et al. (2001) reported mesotrione is both acropetally and basipetally translocated through the plant. Differential absorption and translocation of mesotrione following entry at different plant parts could be causing differential symptom development in smooth crabgrass leaf canopies.

**Dry biomass.** ANOVA indicated that neither treatment nor RH significantly affected tall fescue dry biomass (P > 0.05, data not shown). However, smooth crabgrass dry biomass was affected by treatment and RH (Table 7). When treated in 50% RH, soil plus foliar and soil only treatments significantly reduced smooth crabgrass dry biomass (0.13 and 0.23g, respectively) compared to the nontreated check (0.49g). Foliage only treatments did not affect smooth crabgrass dry biomass (0.53g) in 50% RH. When treated in 90% RH, soil plus foliar, foliar only and soil only treatments all reduced dry biomass (0.07, 0.17, and 0.19g, respectively) compared to the nontreated check (0.58g). Increased RH levels caused a significant reduction in smooth crabgrass dry biomass when only the foliage was treated. These data support the results from photochemical efficiency and leaf position measurements reported in Tables 3 and 6; further indicating foliar
absorption of mesotrione is more affected by RH levels at the time of treatment than soil applied mesotrione.

Mesotrione activity is significantly greater in tall fescue and smooth crabgrass when treated in high RH than in low RH environments. Both soil and foliar absorption are important for mesotrione efficacy. Foliar absorption is most affected by varying RH levels. High RH and rewetting of the plant foliage by dew or rainfall following applications can increase herbicide absorption into the plant (Hull 1970; Thompson and Slife 1969).

Tall fescue injury was not greater than 21% in these studies, but increased RH tended to increase tall fescue injury response to mesotrione by several orders of magnitude. These data suggest turfgrass injury is more likely during periods of high humidity. Differences in herbicide placement indicate both root and foliar absorption contribute to mesotrione efficacy for smooth crabgrass control. Root absorption tended to be more influential than foliar absorption. Adequate soil moisture appears to be an important factor for mesotrione activity (Ricker 2006). Differences in tissue symptoms within smooth crabgrass canopies indicate that root absorbed mesotrione causes white tissue in newer leaves that are formed after herbicide application. In other studies, tank mixtures of mesotrione and triclopyr have nearly eliminated white symptoms in susceptible leaves via temporary stunting leaf growth (Goddard et al. 2007b; Willis and Askew 2007; Willis et al. 2008). Our data help explain this interaction with triclopyr by characterizing the placement of white foliage predominately in newer leaves. The fact that white tissue predominantly occurs in newer leaves also supports work that suggests perennial ryegrass injury is most likely under optimum growing conditions (Ricker 2006)
and not a continuous positive correlation to temperature as others have suggested (Johnson and Young 2002). Future work will evaluate absorption, translocation, and metabolism of $^{14}$C mesotrione in turfgrass and weeds to better understand application placement effects on white tissue occurrence in plant canopies.
Sources of Materials

1 Southern Lawn Turf Type Tall Fescue Blend, Landscape Supply, Inc. 101 Madison Ave, Roanoke, VA 24027, USA.

2 Peters Professional 20-20-20 General Purpose Water Soluble Fertilizer, The Scotts Company, 14111 Scottslawn Road, Marysville, OH 43041

3 M series growth chambers model M-12, Environmental Growth Chambers, 510 East Washington Street, Chagrin Falls, OH 44022, USA.

4 Quantum Meter light sensor model LQM50-3, Spectrum Technologies, Inc. 12360 South Industrial Dr. East, Plainfield, IL 60585, USA.

5 Allen Track Sprayer, Allen Machine Works, 607 East Miller Road, Midland, MI 48640, USA.

6 8001EVS Even Flat Spray Tip, TeeJet Technologies, P.O. Box 7900, Wheaton, IL 60187-7900, USA

7 Chem-Stik Nonionic Spreader-Sticker, Precision Laboratories, Inc. Waukegan, IL 60085 USA.

8 OS-50 Dual Wavelength Chlorophyll Fluorometer, Opti-Sciences, Inc., Tyngsboro, MA 01879, USA.

9 SAS 9.2 software, SAS Institute Inc., Cary, NC 27513-2414, USA
Literature Cited


Ricker, D. B. 2006. Elucidating influence of temperature and environmental stress on turfgrass response to mesotrione and evaluation of potential synergistic


Table 1: The effect of mesotrione placement and relative humidity on tall fescue injury.

<table>
<thead>
<tr>
<th>Mesotrione placement</th>
<th>3 DAT\textsuperscript{a}</th>
<th>7 DAT</th>
<th>14 DAT</th>
<th>21 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50% RH</td>
<td>90% RH</td>
<td>50% RH</td>
<td>90% RH</td>
</tr>
<tr>
<td>Soil + foliar</td>
<td>2</td>
<td>8\textsuperscript{a} \textsuperscript{b}</td>
<td>3</td>
<td>21\textsuperscript{*}</td>
</tr>
<tr>
<td>Foliar only</td>
<td>3</td>
<td>6</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Soil only</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>LSD</td>
<td>NS</td>
<td>2.9</td>
<td>NS</td>
<td>7.6</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Abbreviations: DAT, days after treatment; RH, relative humidity; NS, not significant at P \leq 0.05.

\textsuperscript{b} * indicates a significant difference across humidity levels for that treatment at P \leq 0.05.
Table 2: The effect of mesotrione placement and relative humidity on smooth crabgrass injury.

<table>
<thead>
<tr>
<th>Mesotrione placement</th>
<th>Smooth crabgrass injury</th>
<th>3 DAT(^a)</th>
<th>7 DAT</th>
<th>14 DAT</th>
<th>21 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50% RH</td>
<td>90% RH</td>
<td>50% RH</td>
<td>90% RH</td>
</tr>
<tr>
<td>Soil + foliar</td>
<td></td>
<td>27</td>
<td>37</td>
<td>48</td>
<td>58</td>
</tr>
<tr>
<td>Foliar only</td>
<td></td>
<td>8</td>
<td>35*(^b)</td>
<td>7</td>
<td>62***</td>
</tr>
<tr>
<td>Soil only</td>
<td></td>
<td>3</td>
<td>23</td>
<td>19</td>
<td>49</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>11.5</td>
<td>13.6</td>
<td>20.9</td>
<td>5.1</td>
</tr>
</tbody>
</table>

\(^a\) Abbreviations: DAT, days after treatment; RH, relative humidity; NS, not significant at P ≤ 0.05.

\(^b\) *, **, and *** indicate a significant difference across humidity levels for that treatment at P ≤ 0.05, 0.01, and 0.001, respectively.
Table 3: The effect of mesotrione placement and relative humidity (RH\textsuperscript{a}) on photochemical efficiency (Fv/Fm) of tall fescue and smooth crabgrass 3 and 7 days after treatment (DAT).

<table>
<thead>
<tr>
<th>Mesotrione placement</th>
<th>Tall fescue</th>
<th>Smooth crabgrass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 DAT</td>
<td>7 DAT</td>
</tr>
<tr>
<td></td>
<td>50% RH</td>
<td>90% RH</td>
</tr>
<tr>
<td>Nontreated</td>
<td>0.61</td>
<td>0.66</td>
</tr>
<tr>
<td>Soil + foliar</td>
<td>0.63</td>
<td>0.44</td>
</tr>
<tr>
<td>Foliar only</td>
<td>0.61</td>
<td>0.55</td>
</tr>
<tr>
<td>Soil only</td>
<td>0.59</td>
<td>0.60</td>
</tr>
<tr>
<td>LSD</td>
<td>NS</td>
<td>0.13</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Abbreviations: DAT, days after treatment; RH, relative humidity; NS, not significant at \( P \leq 0.05 \).

\textsuperscript{b} * and ** indicate a significant difference across humidity levels for that treatment at \( P \leq 0.05 \), and 0.01, respectively.
Table 4: The main effects of treatment and relative humidity on canopy composition of smooth crabgrass. The partition of green, pale, and white colored leaves following mesotrione application is indicated as percent of total number of smooth crabgrass leaves at 3, 7, 14, and 21 days after treatment (DAT\(^a\)).

<table>
<thead>
<tr>
<th>Mesotrione placement</th>
<th>Green leaves</th>
<th>Pale leaves</th>
<th>White leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3(^b)</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Nontreated</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Soil + foliar</td>
<td>50</td>
<td>27.6</td>
<td>13.5</td>
</tr>
<tr>
<td>Foliar only</td>
<td>58.3</td>
<td>52.5</td>
<td>52.4</td>
</tr>
<tr>
<td>Soil only</td>
<td>74.8</td>
<td>57.7</td>
<td>22.9</td>
</tr>
<tr>
<td>LSD</td>
<td>13.7</td>
<td>11.8</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative humidity</td>
<td>50%</td>
<td>82.3</td>
<td>71.6</td>
</tr>
<tr>
<td>90%</td>
<td>59.3</td>
<td>47.3</td>
<td>35.9</td>
</tr>
<tr>
<td>LSD</td>
<td>13.7</td>
<td>8.2</td>
<td>7.3</td>
</tr>
</tbody>
</table>

\(a\) Abbreviations: DAT, days after treatment; NS, not significant at P = 0.05.

\(b\) Data were averaged at 3, 7, 14, and 21 DAT.
Table 5: Line equations and $R^2$ values for smooth crabgrass canopy composition regression analysis found in Figure 1.

<table>
<thead>
<tr>
<th>Mesotrione placement</th>
<th>Canopy composition</th>
<th>50% RH $^a$</th>
<th>Equation</th>
<th>$R^2$</th>
<th>90% RH</th>
<th>Equation</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated</td>
<td>Green $^b$</td>
<td>y = 100</td>
<td>NS</td>
<td></td>
<td>y = 100</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pale</td>
<td>y = 0</td>
<td>NS</td>
<td></td>
<td>y = 0</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>White</td>
<td>y = 0</td>
<td>NS</td>
<td></td>
<td>y = 0</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>y = 15.69(\ln(x) + 6.78)</td>
<td>0.89</td>
<td></td>
<td>y = 15.71(\ln(x) + 5.34)</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Soil + foliar</td>
<td>Green</td>
<td>y = -34.52(\ln(x) + 55.25)</td>
<td>0.96</td>
<td></td>
<td>y = -26.60(\ln(x) + 43.34)</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pale</td>
<td>y = -7.64(\ln(x) + 33.82)</td>
<td>0.97</td>
<td></td>
<td>y = 3.42(\ln(x) + 34.41)</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>White</td>
<td>y = 42.19(\ln(x) + 10.93)</td>
<td>0.98</td>
<td></td>
<td>y = 23.21(\ln(x) + 22.26)</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>y = 4.77(\ln(x) + 7.24)</td>
<td>0.95</td>
<td></td>
<td>y = -0.60(\ln(x) + 8.75)</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Foliar only</td>
<td>Green</td>
<td>y = 13.78(\ln(x) + 74.90)</td>
<td>0.99</td>
<td></td>
<td>y = -4.91(\ln(x) + 33.85)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pale</td>
<td>y = -13.78(\ln(x) + 25.10)</td>
<td>0.99</td>
<td></td>
<td>y = -18.57(\ln(x) + 42.60)</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>White</td>
<td>y = 0</td>
<td>NS</td>
<td></td>
<td>y = 14.37(\ln(x) + 25.26)</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>y = 14.27(\ln(x) + 6.61)</td>
<td>0.87</td>
<td></td>
<td>y = 4.51(\ln(x) + 7.54)</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>Soil only</td>
<td>Green</td>
<td>y = -55.35(\ln(x) + 100.55)</td>
<td>0.90</td>
<td></td>
<td>y = -32.01(\ln(x) + 55.79)</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pale</td>
<td>y = 10.78(\ln(x) + 1.76)</td>
<td>0.94</td>
<td></td>
<td>y = -11.45(\ln(x) + 37.25)</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>White</td>
<td>y = 44.57(\ln(x) - 2.31)</td>
<td>0.88</td>
<td></td>
<td>y = 43.46(\ln(x) + 7.0)</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>y = 10.36(\ln(x) + 6.40)</td>
<td>0.95</td>
<td></td>
<td>y = 8.58(\ln(x) + 8.21)</td>
<td>0.94</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Abbreviations: RH, relative humidity.

$^b$ Green, pale, and white leaf variables are reported as a percent of the total number of leaves plant$^{-1}$.

Total leaf variables are reported as the total amount of living leaves plant$^{-1}$.
Table 6: Mesotrione placement and relative humidity effects on the positions of white and pale leaves within the smooth crabgrass canopy.

A value of 1 indicates the newest expanding leaf and larger values indicate older leaves found lower in the plant canopy.

<table>
<thead>
<tr>
<th>Mesostrione placement</th>
<th>White leaves</th>
<th></th>
<th>Pale leaves</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50% RH (^a)</td>
<td>90% RH</td>
<td>50% RH</td>
<td>90% RH</td>
</tr>
<tr>
<td>Soil + foliar</td>
<td>1.5</td>
<td>1.6</td>
<td>2.6</td>
<td>2.9* (^b)</td>
</tr>
<tr>
<td>Foliar only</td>
<td>--</td>
<td>2.8</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Soil only</td>
<td>1.3</td>
<td>1.4</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>LSD</td>
<td>0.48</td>
<td>0.69</td>
<td>0.97</td>
<td>0.45</td>
</tr>
</tbody>
</table>

\(^a\) Abbreviations: RH, relative humidity.

\(^b\) * indicate a significant difference across humidity levels for that treatment at \(P \leq 0.05\).
Table 7: Effect of mesotrione placement and relative humidity on smooth crabgrass dry biomass pooled over locations 21 DAT\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Mesotrione placement</th>
<th>50% RH (g)</th>
<th>90% RH (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated</td>
<td>0.49</td>
<td>0.58</td>
</tr>
<tr>
<td>Soil + foliar</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>Foliar only</td>
<td>0.53</td>
<td>0.17\textsuperscript{b}</td>
</tr>
<tr>
<td>Soil only</td>
<td>0.23</td>
<td>0.19</td>
</tr>
<tr>
<td>LSD</td>
<td>0.17</td>
<td>0.16</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Abbreviations: DAT, days after treatment; RH, relative humidity.

\textsuperscript{b} ** indicate a significant difference across humidity levels for that treatment at $P \leq 0.01$. 
Figure 1: Effects of mesotrione application placement including nontreated, soil plus foliar, foliar only, and soil only in 50% relative humidity (A, B, C, and D, respectively) and 90% relative humidity (E, F, G, and H, respectively). ‘Green’, ‘pale’, and ‘white’ leaves are shown as a percentage of total plant canopy composition. ‘Total’ refers to the total numbers of leaves plant$^{-1}$. Regression equations and R$^2$ values for each variable can be found in Table (5).
Chapter 5.

Differential Absorption and Translocation of Mesotrione in Kentucky Bluegrass

(_Poa pratensis_ L.) and Annual Bluegrass (_Poa annua_ L.)

The following chapter was formatted to facilitate publication in _Weed Science_.

Differential Absorption and Translocation of Mesotrione in Kentucky Bluegrass

(Poa pratensis L.) and Annual Bluegrass (Poa annua L.)

Matthew J. R. Goddard, J. R. James, Darren W. Lycan, and Shawn D. Askew

Abstract

Mesotrione was recently registered for use in US turfgrass. Turfgrass tolerances vary and white discoloration may sometimes occur on more sensitive species. Annual bluegrass is severely injured by normal rates of mesotrione while Kentucky bluegrass is among the most tolerant of desirable turfgrass species. Little is known about absorption or translocation of mesotrione in these species or turfgrasses in general. Laboratory studies were conducted to evaluate interspecific differences in mesotrione activity between these two species when 14C mesotrione is applied to foliage or roots. Annual bluegrass consistently absorbed more radioactivity than Kentucky bluegrass. Both plants absorbed less radioactivity through roots than through foliage and root absorbed radioactivity was more often exuded into Hoagland's solution while foliar absorbed radioactivity was often found in other foliage. Interspecific differences in response to mesotrione could partially

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be due to the 4- and 2-fold increase in foliar absorption by annual bluegrass (46 to 50%) over Kentucky bluegrass (11 to 29%). Studies are underway to elucidate differences in metabolism rates between the two species.

**Nomenclature:** mesotrione; annual bluegrass, *Poa annua* L. (POAAN), Kentucky bluegrass, *Poa pratensis* L. (POAPR) ‘Midnight’.

**Key words:** bleaching herbicides, HPPD inhibiting herbicides, triketone.

**Abbreviations:** HAT, hours after treatment; PRE, preemergence; POST, postemergence.
Introduction

Mesotrione is a new herbicide registered for use in select cool and warm-season turfgrasses. A member of the triketone family of herbicides, mesotrione acts by inhibiting carotenoid biosynthesis, resulting in tissue bleaching of sensitive species (Mitchell et al. 2001; Pallet et al. 1998, 2001; Sutton et al. 2002). Mesotrione is rapidly absorbed by susceptible species and it is readily translocated via xylem and phloem throughout the plant (Mitchell et al. 2001). Past studies have evaluated $^{14}$C mesotrione and confirmed its selectivity among similar species and other plants is due to differential absorption rates and herbicide metabolism (Abit and Al-Khatib 2009; Armel et al. 2005; Mitchell et al. 2001). Similarly, Kentucky bluegrass (Poa pratensis L.) and annual bluegrass (P. annua L.) demonstrate a differential tolerance to mesotrione application with Kentucky bluegrass being tolerant to mesotrione applications. Studies evaluating weed control and tolerance of Kentucky bluegrass following preemergence (PRE) and postemergence (POST) mesotrione applications have reported consistent results indicating safety following treatment (Beam et al. 2006; Jones and Christians 2007; Willis et al. 2007). Conversely, annual bluegrass has shown sensitivity to PRE and POST applications of mesotrione (Anonymous 2008; Hart 2007; Minner et al. 2008). Preemergence and POST herbicidal control options are limited for annual bluegrass populations infesting cool season turf (Dernoeden and Turner 1988; Hart and McCullough 2007).

Although studies evaluating plant response to mesotrione applications in varying environmental conditions have been conducted, none of them have examined absorption, translocation, and metabolism in turfgrass or annual bluegrass. A better understanding of
the response of tolerant turfgrass species following mesotrione application in comparison to more sensitive species is needed to understand why differential responses occur. The objective of this experiment was to determine the rates of absorption and translocation of mesotrione in Kentucky bluegrass and annual bluegrass in an attempt to elucidate reasons for differential plant response to mesotrione in similar species.

**Materials and Methods**

Mature ‘Midnight’ Kentucky bluegrass and annual bluegrass were selected from field grown populations, thinned to one tiller, and transplanted in a 100% sand media. Plants were fertilized daily with a 20-20-20 water soluble fertilizer and allowed to grow for 4 days. Healthy plants were then size selected and transplanted into a 50 ml centrifuge vial containing half strength Hoagland’s Modified Basal Salt Solution, and transferred to a growth chamber. Environmental conditions were monitored using a Hobo Data Logger. Average nighttime and daytime temperatures were 27 and 25 C, respectively. Supplemental lighting was provided and average irradiance was 250 µmol m⁻² sec⁻¹ photosynthetically active radiation using a mix of incandescent and fluorescent lights. Plants acclimated for 3 days before treatment. Treatments were arranged in a split-split-plot design containing six harvest timings as main plots, a two by two factorial arrangement containing two plant species and two application placements as sub plots, and five plant partitions as sub-sub plots. The study was repeated in time with the first trial initiated July 10, 2009 and the second trial initiated on September 16, 2009.

**Herbicide application:** Radiolabeled samples were provided by Syngenta Crop Protection, Inc. Treatment solution consisted of 1,3-Cyclohexanedione, 2[4-
(methylsulfonyl)-(ring-U-\textsuperscript{14}C)-2-nitrobenzoyl] (mesotrione, Figure 1) with 98.4% purity and a specific activity of 4070 kBq mg\textsuperscript{-1} dissolved in acetonitrile and 0.25% v/v nonionic surfactant\textsuperscript{6}. Foliar absorption and translocation of \textsuperscript{14}C mesotrione was determined by applying four 1-\textmu l droplets of treatment solution to the adaxial side of the newest fully expanded leaf blade on each plant. Root absorption and translocation were determined by applying four 1-\textmu l droplets of treatment solution into a 1.5 ml centrifuge tube\textsuperscript{7} suspended in but separated from the growing media containing a single plant root and 1.5 ml half strength Hoagland’s Modified Basal Salt Solution. The 4 \textmu l of treatment solution contained 9.17 kBq radioactivity (Appendix B).

**Plant harvest:** Treated plants were harvested at 0.17, 4, 24, 48, 96, and 144 hours after treatment (HAT). Treated leaves and roots were excised and shaken in 6 ml deionized water for 30 seconds then rinsed with 3 ml methanol. The 1.5-ml vials containing treated solution for treated roots were also emptied into the rinse solution and the vials themselves rinsed with 1 ml methanol. Hoagland's solution was adjusted to 50 ml and a 10-ml aliquot was removed for radioactivity detection of root exudates. Rinse solutions and Hoagland’s solution were collected in 20-ml liquid scintillation vials\textsuperscript{8}. Each vial was then dried to less than 2 ml using a heated water bath and forced air to concentrate the radioactivity. Once volume was below 2 ml, 16 ml scintillation cocktail\textsuperscript{9} was added to each vial and analyzed with a liquid scintillation spectrometer (LSS)\textsuperscript{10}.

**\textsuperscript{14}C extraction:** At each harvest time, plant parts were separated into treated leaf or root, other foliage, and other roots, promptly placed in a freezer, and stored to prevent further metabolism of the herbicide within the plant. The plant material was then macerated using liquid nitrogen and a mortar and pestle. Five ml of extraction solution (4:1 ratio
acetonitrile:water) was added to the macerated plant tissue and homogenized using the mortar and pestle. Ground plant material and extraction solution were suction filtered using a Buchner funnel and 70 mm qualitative filter papers. Mortar and pestle were each rinsed using 5 ml extraction solution and filtered. The resulting extract was dried to less than 2 ml before adding 16 ml scintillation cocktail and radioactivity determined by LSS as described previously.

Total percentage recovery of $^{14}$C was calculated by combining the $^{14}$C activity recovered in treated washes, harvested plant parts, and Hoagland’s solution, and dividing by the total $^{14}$C activity applied. Percent translocation was determined by dividing the sum of radioactivity from plant parts by the total radioactivity absorbed.

Data were subjected to ANOVA with sums of squares partitioned to reflect the split-split-plot design, the factorial subplot arrangement, and trial effects. Homogeneity of variance was tested based on visual inspection of plotted residuals and appropriate transformations were performed to satisfy assumptions of ANOVA using SAS 9.2. Trial effects were considered random and mean squares were tested appropriately based on the treatment design (McIntosh 1983). Where main plot effects were significant, regressions were used to explain the relationship of measured responses over time. Effects of species and plant parts were separated via Fisher’s Protected LSD test at $P \leq 0.05$.

**Results and Discussion**

A four way interaction between trial, plant species, plant partition, and application placement was observed for the percentage of $^{14}$C recovered from treated plants. To
describe this complex interaction, regressions were used to show trends over time between various plant parts, species, and application placement (Figure 2, Table 2). In addition, the three-way interaction of trial, plant species, and plant partition, which was also significant, is shown in Table 1 averaged over time to explain differences between plant species and placement for various plant parts (Table 1). The trial interaction was apparently due to differences in Kentucky bluegrass translocation of root-absorbed radioactivity to foliage compared to exudates in Hoagland's solution. In trial 1, 26% radioactivity was exuded into Hoagland's solution and only 2% was found in foliage (Table 1). Conversely, in trial 2, 13% of radioactivity moved to foliage and only 2% was found in Hoagland's solution. A similar difference between trials was noted for radioactivity exuded into Hoagland's solution by annual bluegrass (Table 1). These trends can be noted in Figure 2E and Figure 2F by the curvilinear response of percent radioactivity in Hoagland's that curves upward over time with a concomitant decrease in percent radioactivity in the wash. Note the same trend does not occur in trial 2. These differences between trials can't be explained but it was generally noted that plant-to-plant variability in absorbed and translocated radioactivity was higher when radioactivity was absorbed through roots compared to foliage (data not shown). Trends in radioactivity absorbed and translocated after foliar application were generally consistent between trials (Table 1, Figure 2).

More radioactivity was absorbed by annual bluegrass in both trials when radioactive mesotrione was foliar applied and in a single comparison when radioactive mesotrione was applied to roots (Table 1). These results are consistent with those of Kohler and Branham (2002) where annual bluegrass absorbed 3 times more ethofumesate
than creeping bentgrass or perennial ryegrass (*Lolium perenne* L.). However, Beam and Askew (2007) reported similar absorption rates of $^{14}$C prohexadione-calcium in Kentucky bluegrass and annual bluegrass and concluded that increased absorption of prohexadione-calcium in these two species contributed to selectivity over creeping bentgrass and perennial ryegrass. Their results contrast with this study as annual bluegrass consistently absorbed more radioactivity following mesotrione application than Kentucky bluegrass.

Greater absorption of radioactivity was observed when treatments were foliar applied than root applied, regardless of species (Table 1). These results seem to conflict with field and greenhouse studies that indicate root absorption contributes as much or more to weed control than foliar absorption (Goddard et al. 2010; McCurdy et al. 2007). In the current study, only one root was exposed to mesotrione and the benefit of nonabsorbed product washing from treated leaves down to soil, as occurs in the field, did not occur in this experiment. More radioactivity was observed in the treated foliage than in the treated roots for Kentucky bluegrass and annual bluegrass for each comparison (Table 1). These differences are only partially explained by differences in fresh weights of these plant parts. Treated leaves averaged 8 µg while treated roots averaged 4 µg (data not shown). The increase in foliar absorption also contributed to increased radioactivity moving into other parts of the plant for annual bluegrass. In the first trial, foliar absorbed $^{14}$C was greater in annual bluegrass for treated leaf, foliage, and Hoagland's (Table 1, Figure 2). Wash collected from the treated leaf also indicated a 4- and 2-fold increase in foliar absorption by annual bluegrass (46 and 50%) over Kentucky bluegrass (11 and 29%) in the first and second trials, respectively. When radioactive mesotrione was applied to roots, an increase in absorption was only noticed for annual bluegrass in trial 1.
In the first trial, significantly greater radioactivity was absorbed into plant tissues for annual bluegrass than Kentucky bluegrass when applied to the leaf (Figure 2). However, a similar amount of radioactivity was absorbed by each species when applied to roots. When foliar absorbed, much of the recovered radioactivity was found in other plant partitions. For foliar treated annual bluegrass, most of the activity was recovered in the treated leaf with greater than 20% recovered from other plant foliage by 96 hours after treatment. For root-treated plants, much of the activity recovered that had moved out of the treated area had moved into the Hoagland’s solution. This phenomenon happened in both trials for Kentucky bluegrass treated roots and the first location for annual bluegrass. For annual bluegrass in the second trial, most of the activity was recovered from the treated wash, with approximately 10% found in other roots by 144 hours after treatment.

The increased sensitivity of annual bluegrass to mesotrione applications is likely due in part to herbicide absorption. Though Kentucky bluegrass readily absorbed applied mesotrione, annual bluegrass consistently absorbed more radioactivity into the treated leaf and thus, into other parts of the plant. The fact that more radioactivity was absorbed by foliage than by roots could be due to limitations in study design. It is difficult to balance the need to measure lateral mobility from one plant root to another while simultaneously measuring bulk root absorption. Previous work has indicated that mesotrione activity is highly dependent on root absorption (Goddard et al. 2010; McCurdy et al. 2007). It is believed that in some cases, root health might have contributed to the decrease in absorption noticed in these trials. Additional work is underway to assess the radiochemical purity in various extractions from this study.
(Appendix C). These data, when collected, will allow us to identify the nature of radioactivity detected in various plant parts and elucidate how metabolism contributes to interspecific differences in *Poa* spp. response to mesotrione.

**Acknowledgements**

The authors would like to thank Syngenta Crop Protection, Inc. for providing the $^{14}$C mesotrione used and their support of this project.
Sources of Materials

1. Peters Professional 20-20-20 General Purpose Water Soluble Fertilizer, The Scotts Company, 14111 Scottslawn Road, Marysville, OH 43041

2. 50 ml Flat Top Cap Polypropylene Centrifuge Tube, Corning Incorporated, Corning, NY 14831, USA

3. Hoagland’s Modified Basal Salt Mixture, MP Biomedicals, LLC, 29525 Fountain Pkwy., Solon, Ohio 44139, USA

4. HOBO RH/Temp/Light/External Data Logger model H08-004-02, Onset Computer Corporation, Pocasset, MA 02559-3450, USA

5. Sun System Tek Light 48, Sunlight Supply, Inc., Vancouver, WA, USA.

6. Chem-Stik Nonionic Spreader-Sticker, Precision Laboratories, Inc. Waukegan, IL 60085 USA.

7. Fisherbrand 1.5 ml Flat Top Polypropylene Microcentrifuge Tubes, Fisher Scientific, Pittsburg, PA 15275, USA

8. Wheaton Glass 20 ml Scintillation vials (O.D. x H (with cap): 28 x 61mm), Fisher Scientific, Pittsburgh, PA 15275, USA


10. LS 6500 Multi-Purpose Scintillation Counter, Beckman Coulter, Inc., Fullerton, California 92634-3100, USA

11. 70mm qualitative filter paper circles, Whatman International, Ltd., Maidstone, Kent, ME14 2LE, UK

12. SAS 9.2 software, SAS Institute Inc., Cary, NC 27513-2414, USA
Literature Cited


Beam, J. B. and S. D. Askew. 2007. Fate of prohexadione-calcium in annual bluegrass (Poa annua) and three turfgrasses. Weed Sci. 55:541-545.


Table 1: Effects of herbicide placement, plant species, plant part and trial on percentage of $^{14}$C recovered from treated plants after application of radiolabeled mesotrione.

<table>
<thead>
<tr>
<th>Placement</th>
<th>Plant part</th>
<th>Kentucky bluegrass</th>
<th>Annual bluegrass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
</tr>
<tr>
<td>Foliar</td>
<td>Treated</td>
<td>5.2*†</td>
<td>16*†</td>
</tr>
<tr>
<td></td>
<td>Other foliage</td>
<td>3.9†</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>Other roots</td>
<td>0.2†</td>
<td>1.4*</td>
</tr>
<tr>
<td></td>
<td>Wash</td>
<td>88.8†</td>
<td>71.3†</td>
</tr>
<tr>
<td></td>
<td>Hoagland’s</td>
<td>1.8*</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>LSD (0.05)</td>
<td>3</td>
<td>9.3</td>
</tr>
<tr>
<td>Root</td>
<td>Treated</td>
<td>0.4*†</td>
<td>0.7*</td>
</tr>
<tr>
<td></td>
<td>Other foliage</td>
<td>2</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>Other roots</td>
<td>0.5</td>
<td>0.4*</td>
</tr>
<tr>
<td></td>
<td>Wash</td>
<td>70.7</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Hoagland’s</td>
<td>26.2*</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>LSD (0.05)††</td>
<td>14.6</td>
<td>13.8</td>
</tr>
</tbody>
</table>

* Indicates significant difference between plant application placement in a given trial, plant part, and species.

† Indicates significant difference between plant species in a given trial, plant part, and placement.
Table 2: Line equations and $R^2$ values for plant canopy composition regression analysis presented in Figure 1.

<table>
<thead>
<tr>
<th>Location</th>
<th>Species</th>
<th>Plant partition</th>
<th>Foliar applied</th>
<th>Root applied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Equation</td>
<td>$R^2$</td>
</tr>
<tr>
<td>Trial 1</td>
<td>KBG</td>
<td>Treated</td>
<td>$y = 3.47\ln(x) + 1.86$</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Foliage</td>
<td>$y = 4.88\ln(x) - 0.73$</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Roots</td>
<td>$y = 0.3087\ln(x) - 0.0956$</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wash</td>
<td>$y = -10.02\ln(x) + 98.41$</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hoagland’s</td>
<td>$y = 2.31\ln(x) - 0.41$</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ABG</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treated</td>
<td>$y = 9.417\ln(x) + 17.183$</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Foliage</td>
<td>$y = 14.1\ln(x) + 1.56$</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Roots</td>
<td>$y = 0.3\ln(x) + 0.63$</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wash</td>
<td>$y = -28.01\ln(x) + 80.88$</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hoagland’s</td>
<td>$y = 4.19\ln(x) - 0.43$</td>
<td>0.70</td>
</tr>
<tr>
<td>Trial 2</td>
<td>KBG</td>
<td>Treated</td>
<td>$y = 14.23\ln(x) + 2.38$</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Foliage</td>
<td>$y = 10.18\ln(x) - 2.27$</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Roots</td>
<td>$y = 1.25\ln(x) + 0.2$</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wash</td>
<td>$y = -31.19\ln(x) + 101.19$</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hoagland’s</td>
<td>$y = 5.36\ln(x) - 1.2$</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ABG</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treated</td>
<td>$y = 21.82\ln(x) + 6.89$</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Foliage</td>
<td>$y = 16\ln(x) - 1.78$</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Roots</td>
<td>$y = 0.96\ln(x) + 0.22$</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wash</td>
<td>$y = -47.15\ln(x) + 95.54$</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hoagland’s</td>
<td>$y = 8.28\ln(x) - 0.87$</td>
<td>0.78</td>
</tr>
</tbody>
</table>

a Abbreviations: ABG, annual bluegrass; KBG, Kentucky bluegrass; NS, not significant

b Plant partitions are described as treated part, other foliage, other roots, treated wash, and Hoagland’s growing solution.
Figure 1: Chemical structure of 1,3-cyclohexanedione, 2-[4-(methylsulfonyl)-(ring-U-14C)-2-nitrobenzoyl] (mesotrione). $^{14}$C-labeled carbon occurs randomly around six positions of the phenyl ring indicated by an asterisk.
Figure 2: Interaction of mesotrione placement, trial, harvest timing, and plant species on percent recovered radioactivity. Regressions of percent recovered radioactivity over time for various plant parts are shown for mesotrione applied to Kentucky bluegrass and annual bluegrass foliage (A and B, respectively in trial 1 and C and D, respectively in trial 2) and for mesotrione applied to Kentucky bluegrass and annual bluegrass roots (E and F, respectively in trial 1 and G and H, respectively in trial 2). Regression equations and $R^2$ values for each variable are presented in Table (2).
APPENDIX A

Canopy Composition
Figure 1: Total amount of plant leaves by color (green, pale, white) following mesotrione application were determined for each plant species as indicated in the following image.
Figure 2: Differential whitening was observed following mesotrione application to different parts of the plant. Whitening occurred first on the newest leaves when soil applied, and on older leaves when foliar applied.
Appendix B

$^{14}$C Mesotrione Treatment
Figure 1: Diagram of plant partitions relating to $^{14}$C mesotrione application placement (A), and treatment of Kentucky bluegrass leaf with four 1µl drops of $^{14}$C mesotrione (B).
Figure 2: Treatment of plant roots separated by a 1.5 ml centrifuge vial (A), and diagram of plant partitions relating to $^{14}$C mesotrione application placement (B).
APPENDIX C

$^{14}$C Metabolism Methods
Differential Metabolism of $^{14}$C Mesotrione in Kentucky Bluegrass (*Poa pratensis* L.) and Annual Bluegrass (*Poa annua* L.)

**Metabolism:** Annual bluegrass and Kentucky bluegrass plants were treated with $^{14}$C mesotrione and homogenized as described in Chapter 5. Metabolism of $^{14}$C mesotrione will be determined by using half of the extraction fluid not used in the absorption and translocation studies. These samples will be dried and resuspended in 500 µl acetonitrile. Once suspended, 100 µl of this volume will be applied to a normal phase thin layer chromatography (TLC) plate and developed in a solvent system of chloroform : acetonitrile : water (10:5:1 v/v). The samples from each plant part will be developed in separate lanes beside a comparison of dilution stock of radiolabeled mesotrione and its metabolites as references. The radiolabeled bands will be analyzed using a Bioscan imaging scanner and the relative contributions of each band will be expressed as a percentage. This method allows for the total amount of parent mesotrione and its metabolites to be determined over time in different plant parts.

**Sources of Materials**

1. Silica Gel 60 F$_{254}$ 20 x 20cm Thin Layer Chromatography Plates, EM Industries, Inc., Gibbstown, NJ, 08027, USA.

2. Bioscan System 200 Imaging Scanner, Bioscan, Inc., NW Washington, D.C., 20007, USA.