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# Growth Stage Influences Mesotrione Efficacy and Fate in Two Bluegrass (*Poa*) Species

Jialin Yu and Patrick E. McCullough*

Mesotrione provides PRE and early POST control of annual bluegrass during Kentucky bluegrass establishment from seed, but applications do not effectively control multitiller plants. The physiological effects of growth stage on efficacy and the basis of mesotrione selectivity between species is not well understood. The objectives of this research were to evaluate mesotrione behavior in these species at three growth stages: pretiller (3 to 5 leaves), 1-tiller, and multitiller (5 to 7 tillers). In greenhouse experiments, a single mesotrione application at 280 g ai $ha^{-1}$ injured pretiller, 1-tiller, and multitiller annual bluegrass 54, 33, and 11% at 4 wk after initial treatment (WAIT), respectively. A sequential application of mesotrione increased injury to pretiller and 1-tiller annual bluegrass by 20 and 17% from a single treatment, respectively. Sequential mesotrione applications caused at least 14% injury to multitiller annual bluegrass and Kentucky bluegrass at all growth stages and did not reduce tillering compared to the nontreated. Annual bluegrass absorbed 34% more root-applied $^{14}C$-mesotrione than Kentucky bluegrass in hydroponic culture, but relative differences (Bq $g^{-1}$) among growth stages were not detected for both species. Averaged across growth stages, annual and Kentucky bluegrass absorbed 31 and 35% of the applied radioactivity after foliar treatments, respectively. However, averaged across species, multitiller plants metabolized approximately two times more $^{14}C$-mesotrione than pretiller and 1-tiller plants. Overall, the selectivity of mesotrione for annual bluegrass control during Kentucky bluegrass establishment results from differential levels of root absorption. Mesotrione has limited efficacy for controlling multitiller annual bluegrass due to enhanced degradation compared to pretiller and 1-tiller plants.

**Nomenclature:** Mesotrione; annual bluegrass, *Poa annua* L.; Kentucky bluegrass, *Poa pratensis* L. 'Midnight'.

**Key words:** Absorption, placement, selectivity, translocation, turfgrass.

Mesotrione brinda control de *Poa annua* en PRE y POST temprano durante el establecimiento del césped *Poa pratensis* a partir de semilla, pero las aplicaciones no controlan efectivamente plantas en el estadio de múltiples hijuelos. Los efectos fisiológicos del estadio de desarrollo sobre la eficacia y las bases de la selectividad de mesotrione entre especies no se conocen bien. Los objetivos de esta investigación fueron evaluar el comportamiento de mesotrione en estas especies en tres estadios de desarrollo: pre-hijuelo (3 a 5 hojas), 1-hijuelo, y múltiples hijuelos (5 a 7 hijuelos). En experimentos de invernadero, una aplicación sencilla de mesotrione a 280 g ai $ha^{-1}$ dañó *P. annua* en el estadio de pre-hijuelo, 1-hijuelo, y múltiples hijuelos 54%, 33, y 11% a 4 semanas después del tratamiento inicial (WAIT), respectivamente. Una aplicación secuencial de mesotrione aumentó el daño a *P. annua* en los estadios pre-hijuelo y 1-hijuelo en 20 y 17%, en comparación con el tratamiento sencillo, respectivamente. Las aplicaciones secuenciales de mesotrione causaron al menos 14% de daño a *P. annua* en el estadio de múltiples hijuelos y al césped *P. pratensis* en todos los estadios de desarrollo, y no redujo la producción de hijuelos al compararse con el testigo sin tratamiento. *P. annua* absorbió 34% más $^{14}C$-mesotrione aplicado a la raíz que *P. pratensis* en un cultivo hidropónico, pero diferencias relativas (Bq $g^{-1}$) entre los estadios de desarrollo no fueron detectadas en ninguna de las especies. Al promediar los estadios de desarrollo, *P. annua* y *P. pratensis* absorbieron 31 y 35% de la radioactividad aplicada después de los tratamientos foliares, respectivamente. Sin embargo, al promediar las especies, las plantas en el estadio de múltiples hijuelos metabolizaron aproximadamente el doble de $^{14}C$-mesotrione que las plantas en pre-hijuelos y 1-hijuelo. En general, la selectividad de mesotrione para el control de *P. annua* durante el establecimiento del césped *P. pratensis* resulta de niveles diferenciales de absorción radical. Mesotrione tiene eficacia limitada para el control de *P. annua* en el estadio de múltiples hijuelos producto de una mayor degradación del herbicida al compararse con plantas en los estadios de pre-hijuelo y 1-hijuelos.

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Kentucky bluegrass is the most widely planted species for lawns, sports fields, and other recreational areas. A significant challenge for establishing Kentucky bluegrass from seed is the germination of



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annual bluegrass. This weed exhibits competitive growth with Kentucky bluegrass that often compromises turf establishment (Beard 1970). Mature annual bluegrass is unsightly, and has poor tolerances to heat, disease, and traffic stress (Beard 1970; Lush 1989). Thus, controlling annual bluegrass during Kentucky bluegrass establishment is critical for long-term successful culture.

Ethofumesate is a lipid biosynthesis inhibitor that can be applied for PRE and POST control of annual bluegrass in Kentucky bluegrass (Anonymous 2014). However, applications are injurious to Kentucky bluegrass seedlings, and provide erratic levels of annual bluegrass control (Anonymous 2014; Dickens 1979; Johnson et al. 1989). Amicarbazone is a Photosystem II inhibitor that provides POST annual bluegrass control in Kentucky bluegrass (Anonymous 2012). Fall applications can excessively injure Kentucky bluegrass and the herbicide is not recommended during establishment (McCullough et al. 2010). Primisulfuron-methyl is an acetolactate synthase inhibitor that effectively controls annual bluegrass in Kentucky bluegrass (Hart and McCullough 2007a; McCullough et al. 2015) and the herbicide has a 24(c) label in several states with limited annual use rates of no more than 40 g ai $ha^{-1}$ (Anonymous 2006).

Mesotrione is a carotenoid biosynthesis inhibitor used before, during, and after Kentucky bluegrass establishment (Dernoeden et al. 2008). Mesotrione inhibits the 4-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme that converts tyrosine to plastoquinone and α-tocopherol in carotenoid biosynthesis (Beaudegnies et al. 2009). Susceptible species exhibit foliar bleaching followed by tissue necrosis from free radical damage to cell membranes (Lee et al. 1999; McCurdy et al. 2009). Kentucky bluegrass seedlings are tolerant to mesotrione, and applications effectively control winter annual weeds during fall establishment. In New Jersey, sequential mesotrione applications at 280 g ai $ha^{-1}$ controlled annual bluegrass greater than 80% after seeding Kentucky bluegrass (Hart and McCullough 2007b). In Iowa, annual bluegrass control from sequential mesotrione applications at 190 g $ha^{-1}$ provided at least 90% ground cover of Kentucky bluegrass by the following year (Hoiberg and Minner 2010).

Although mesotrione provides PRE and early POST control of annual bluegrass, efficacy is significantly reduced when applied to mature annual bluegrass. Multiple applications of mesotrione in the fall provided inconsistent levels of annual bluegrass control across locations and years (Reicher et al. 2011). Skelton et al. (2012) also reported erratic control following mesotrione at 110 or 186 g $ha^{-1}$ applied POST five and three times in Kentucky bluegrass, respectively. The researchers noted that ten applications of mesotrione at 56 g $ha^{-1}$ controlled annual bluegrass at least 85%, but Kentucky bluegrass injury was unacceptable ($> 20\%$).

Mesotrione effectively controls immature annual bluegrass, but applications are less effective for controlling multitiller plants. Conversely, mesotrione causes negligible injury at labeled use rates on Kentucky bluegrass immediately after seeding and at all stages of growth. The differential behavior of mesotrione in these two bluegrass species is not well understood and the effects of growth stage on annual bluegrass tolerance have received limited investigation. The objectives of this research were to evaluate the efficacy, absorption, translocation, and metabolism of mesotrione in annual bluegrass and Kentucky bluegrass at three growth stages.

## Materials and Methods

**Plant Material.** Experiments were conducted at the University of Georgia in Griffin, GA. 'Midnight' Kentucky bluegrass (Preferred Seed, Buffalo, NY 14227) and annual bluegrass were seeded in 3.8-cm-diam by 20-cm-deep pots in a greenhouse set for 23/17 C (day/night). Annual bluegrass seed was collected from seedheads of indigenous populations in Griffin, GA. Soil was a mixture of sand and peat moss (80 : 20 v/v). Grasses received fertigation biweekly (MacroN 28-7-14 Sprayable Fertilizer, LESCO Inc., Cleveland, OH 44114) and were watered as needed to promote growth. Plants were selected based on growth stage including: 3- to 5-leaf stage (pretiller), 1-tiller, and 5- to 7-tiller (multitiller).

**Greenhouse Experiments.** Experiments were conducted to evaluate injury and tillering of annual and Kentucky bluegrass at three growth stages following single or sequential mesotrione applications. Annual bluegrass and Kentucky bluegrass at the three growth stages were treated with single or sequential applications of mesotrione (Tenacity 4SC, Syngenta Crop Protection, Greensboro, NC 27419) at 280 g



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$ha^{-1}$. A nonionic surfactant (Activator 90, Loveland Products, Inc., Greeley, CO 80632) at 0.25% v/v was included in the herbicide treatments. A nontreated check for all growth stages was included. Treatments were applied with a $CO_2$-pressured sprayer calibrated to deliver 374 L $ha^{-1}$ with a single 9504E flat-fan nozzle (TeeJet Spraying Systems, Co., Roswell, Ga 30075). The sequential treatment was applied at 2 wk after initial treatment (WAIT). Grasses were not irrigated for 24 h after treatments (HAT), but received irrigation thereafter to prevent moisture stress. Injury was visually evaluated at 2 and 4 WAIT on a percent scale where 0 equaled no injury and 100 equaled complete desiccation. Tillers were counted at 2 and 4 WAIT.

**Root Absorption and Translocation.** Experiments were conducted to investigate root absorption of $^{14}$C-mesotrione and radioactivity translocation in annual and Kentucky bluegrass at three growth stages. Plants were removed from greenhouse pots, roots were rinsed to remove soil, and plants were grown hydroponically in a 10-L plastic tank filled with a quarter-strength Hoagland solution (Hoagland and Arnon 1950). Grasses were placed through holes in the plastic lid that facilitated root submergence in the solution. The tank was covered with aluminum foil to shield roots from light and placed in a growth chamber (Percival Scientific, Inc. 505 Research Drive, Perry, IA 50220) set for 24/14 C (day/night) with a 12 h photoperiod of 350 μmol $m^{-2}$ $s^{-1}$. An aquarium pump was used to provide oxygen to the solution and plants were acclimated to hydroponic culture in the growth chamber for 72 h. This acclimation period and nutrient load were chosen for plants to resume active growth in hydroponic solution without stimulating development beyond the target growth stage.

The solution of the tank was then spiked with 170 kBq of $^{14}$C-mesotrione (109 μCi $mg^{-1}$, phenyl-ring labeled, 99% chemical purity) plus 1 μM of nonlabeled mesotrione. Plants were harvested 72 HAT. Roots were rinsed for 20 s under a stream of tap water and blotted dry with paper towels. Roots were then separated from shoots with shears and samples were oven-dried for 7 d at 40 C. Samples were then oxidized for 2 min in a biological oxidizer (OX-500, R. J. Harvey Instrument Corp., 11 Jane St., Tappan, NY 10983) and radioactivity was quantified with liquid scintillation spectroscopy (LSC) (Beckman LS 6500®, Beckman Coulter Inc., Fall River, MA 02720). Root absorption was determined by dividing the radioactivity recovered by sample dry weight. Translocation was determined by dividing the $^{14}$C recovered in shoots by the total radioactivity in the plant (roots and shoots).

**Foliar Absorption, Translocation, and Metabolism.** Experiments were conducted to evaluate foliar uptake, translocation, and metabolism of $^{14}$C-mesotrione in annual and Kentucky bluegrass at three growth stages. Plants were established in greenhouse pots as previously described and acclimated in the aforementioned growth chamber for 72 h. Grasses were prepared for radiolabeled treatments by covering the second, fully expanded leaf with flexible film (Parafilm, Bemis Company Inc., Neenah, WI 54956). A broadcast treatment of mesotrione at 280 g $ha^{-1}$ was applied as previously described. Immediately after the broadcast application, two 1 μL droplets of $^{14}$C-mesotrione containing 1.3 kBq each were applied to the second fully expanded leaf with a 10 μL microsyringe (Hamilton Co., Reno, NV 89502). Radioactive droplets were applied in the middle of the leaf and to the sides of the midrib. The spotting solution contained 0.75μg $\mu L^{-1}$ of mesotrione to simulate droplets of spray solution. A nonionic surfactant at 0.25% v/v was added to the broadcast and radioactive solutions to facilitate droplet deposition on the leaf surface.

Plants (roots and shoots) were harvested 6 d after treatment (DAT). This harvest timing was chosen from previous research with $^{14}$C-mesotrione (Abit and Al-Khatib 2009; Armel et al. 2005) and from pilot experiments that indicated turfgrasses require at least 3 d to show significant levels of metabolism (PE McCullough, personal observation). The treated leaf was excised and then rinsed with 10 ml of methanol inside a 20-ml scintillation vial. The base of the leaf was held with forceps and rinsate was applied towards the leaf tip with a 5-ml pipette on the leaf surface. This methodology was chosen from pilot experiments that completely removed adsorbed $^{14}$C immediately after treatment and previous research with $^{14}$C-mesotrione on grain sorghum [*Sorghum bicolor* (L.) Moench.] (Abit and Al-Khatib 2009). Roots were then separated from nontreated shoots with shears and samples were stored at −20 C until analysis.



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Table 1. Injury of annual bluegrass and 'Midnight' Kentucky bluegrass with mesotrione at 280 g ai $ha^{-1}$ in two greenhouse experiments, Griffin, GA. Results were pooled over experimental runs.

| | | Injury (2 WAIT)[c] | | Injury (4 WAIT) | |
|---|---|---|---|---|---|
| Growth stage[a] | Application[b] | Annual bluegrass | Kentucky bluegrass | Annual bluegrass | Kentucky bluegrass |
| | | % | | | |
| Pretiller | Single | 32 | 5 | 54 | 6 |
| | Sequential | | | 74 | 12 |
| 1-tiller | Single | 20 | 4 | 33 | 2 |
| | Sequential | | | 50 | 5 |
| Multitiller | Single | 15 | 2 | 11 | 0 |
| | Sequential | | | 14 | 3 |
| | $LSD_{0.05}$ | 3 | 3 | 5 | 5 |

[a] Multitiller plants had 5 to 7 tillers at application.

[b] The sequential treatment was applied 2 wk after the initial application.

[c] Abbreviation: WAIT, weeks after initial treatment.

The treated leaf and nontreated parts (nontreated shoots and roots) were minced and homogenized separately in 20 ml of methanol (FSH 125, Fisher Scientific LLC, 300 Industry Drive, Pittsburg, PA 15275) for 30 s. Samples were then sonicated in water for 8 h at room temperature, centrifuged for 10 min, and the supernatant was transferred to separate tubes. This procedure was repeated with an additional 20 ml of methanol and the supernatants were combined. Plant residue was then placed in water sonication for approximately 6 h in 20 ml of methanol plus 1% formic acid, centrifuged, and the supernatants were combined. Radioactivity of the supernatant for the treated leaf and the rest of the plant were quantified from 4-ml aliquots using LSC. Residue was dried for 72 h in the hood and then combusted in the oxidizer to quantify extraction efficiency. The supernatant from the treated leaf and nontreated parts were then combined to evaluate whole plant metabolism.

The supernatant was then evaporated in a forced-air hood. Samples were resuspended in 40 μL of methanol and spotted on 20- by 20-cm thin layer chromatography (TLC) plates. The TLC plates were developed to 16 cm in a glass chamber using chloroform:methanol (1 : 9). The plates were air-dried and metabolites were detected with a radio-chromatogram scanner (BioScan System 200 Imaging Scanner, Bioscan, 4590 MacArthur Boulevard NW, Washington, DC 20007) connected to a computer equipped with Laura Chromatography Data Collection and Analysis Software® (LabLogic System, Inc. 1040 E Brandon Blvd Brandon, FL 33511).

Residue from the treated leaf and nontreated parts were oxidized separately for 2 min in a biological oxidizer and radioactivity was quantified with LSC. Foliar absorption was quantified by dividing the total radioactivity recovered in the supernatant and residue by the total $^{14}C$ applied. Translocation was determined by dividing the total radioactivity recovered in nontreated shoots and roots from the total radioactivity recovered in the plant.

**Experimental Design and Data Analysis.** The design for the greenhouse experiment was a randomized complete block with five replications. The design for all laboratory experiments was completely randomized with five replications. Two runs were conducted for all experiments. Data were subjected to analysis of variance using the General Linear Model Procedure in SAS (SAS v. 9.3, SAS Institute Inc., Cary, NC 27513) and means were separated with Fisher's LSD test at $\alpha = 0.05$. When experiment-by-treatment interactions were not detected, results were pooled over runs.

## Results and Discussion

**Greenhouse Experiments.** Growth stage-by-treatment interactions were detected for annual bluegrass and Kentucky bluegrass injury; thus, results are presented across all combinations. At 2 WAIT, mesotrione injured annual bluegrass 32, 20, and 15% at the pretiller, 1-tiller, and multitiller growth stage, respectively (Table 1). Kentucky bluegrass was injured no more than 5% at all growth stages.



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Table 2. Tiller count for annual bluegrass and 'Midnight' Kentucky bluegrass treated with mesotrione at 280 g ai $ha^{-1}$ in two greenhouse experiments, Griffin, GA. Results were pooled over experimental runs.

| | | Tiller count (2 WAIT)[c] | | Tiller count (4 WAIT) | |
|---|---|---|---|---|---|
| Growth stage[a] | Application[b] | Annual bluegrass | Kentucky bluegrass | Annual bluegrass | Kentucky bluegrass |
| | | No. $plant^{-1}$ | | | |
| Pretiller | Nontreated | 1.2 | 1.0 | 2.4 | 1.2 |
| | Single | 1.0 | 1.1 | 1.0 | 1.8 |
| | Sequential | | | 1.0 | 2.0 |
| | $LSD_{0.05}$ | NS | NS | 0.4 | NS |
| 1-tiller | Nontreated | 4.4 | 3.8 | 6.4 | 4.9 |
| | Single | 3.0 | 3.6 | 3.8 | 4.7 |
| | Sequential | | | 4.2 | 4.6 |
| | $LSD_{0.05}$ | 1.3 | NS | 2.0 | NS |
| Multitiller | Nontreated | 9.4 | 7.2 | 13.7 | 8.5 |
| | Single | 8.7 | 7.6 | 13.0 | 8.9 |
| | Sequential | | | 13.1 | 9.7 |
| | $LSD_{0.05}$ | NS | NS | NS | NS |

[a] Multitiller plants had 5 to 7 tillers at application.

[b] The sequential treatment was applied 2 wk after the initial application.

[c] Abbreviations: NS, not significant; WAIT, weeks after initial treatment.

At 4 WAIT, single mesotrione applications injured annual bluegrass 54, 33, and 11% at the pretiller, 1-tiller, and multitiller growth stage, respectively. Sequential applications increased injury 20 and 17% from single treatments on pretiller and 1-tiller annual bluegrass, respectively. The sequential treatment only injured multitiller annual bluegrass 14% at 4 WAIT and was similar to injury from the single application. The sequential treatment injured pretiller Kentucky bluegrass more than tillered plants, but injury did not exceed 12%.

Growth stage-by-treatment interactions were detected for tiller counts at both dates; thus, results are presented across all combinations. At 2 WAIT, mesotrione reduced annual bluegrass tiller count 32% compared to the nontreated when applied at the 1-tiller growth stage (Table 2), but other growth stages were similar to the nontreated. At 4 WAIT, differences between single and sequential applications were not detected for tiller counts. Mesotrione inhibited tillering of annual bluegrass 58 and 38% compared to the nontreated when applied at the pretiller and 1-tiller growth stage, respectively (Table 2). Mesotrione did not inhibit tillering of multitiller annual bluegrass plants compared to the nontreated. Kentucky bluegrass tillering was not inhibited following single and sequential mesotrione applications at all growth stages 2 and 4 WAIT.

Results suggest mesotrione has the greatest efficacy on annual bluegrass prior to reaching a multitiller growth stage. Kentucky bluegrass has superior tolerance to mesotrione compared to annual bluegrass, and treatments did not inhibit tillering from the nontreated at any growth stage. These results support previous observations on the differential tolerance levels of annual bluegrass to mesotrione at various stages of maturity in field experiments (Reicher et al. 2011; Skelton et al. 2012). POST applications of mesotrione at 140 and 210 g $ha^{-1}$ applied 4 wk after emergence did not reduce Kentucky bluegrass cover from the nontreated (Venner 2011). Similarly, Askew and Beam (2002) reported no injury to Kentucky bluegrass following sequential mesotrione applications. Annual bluegrass injury and growth inhibition from mesotrione would likely give Kentucky bluegrass a competitive growth advantage during seedling establishment.

Mesotrione efficacy for controlling immature annual bluegrass is also consistent with the susceptibility of other weeds at various growth stages. Canada thistle [*Cirsium arvense* (L.) Scop.] was more susceptible to mesotrione at the rosette stage compared to the bolting stage (Armel et al. 2005). Researchers have also noted that mesotrione efficacy on smooth [*Digitaria ischaemum* (Schreb.) ex Muhl.] and large crabgrass [*D. sanguinalis* (L.)



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Table 3. Root absorption and translocation of radioactivity to shoots for annual bluegrass and 'Midnight' Kentucky bluegrass at 72 h after treatment with $^{14}$C-mesotrione in two experiments, Griffin, GA. Results were pooled over experimental runs.

| | Absorption | Translocation | |
|---|---|---|---|
| | Bq $g^{-1}$ dry plant wt | Bq $g^{-1}$ dry shoot wt | % of $^{14}$C absorbed |
| Species | | | |
| Annual bluegrass | 479 | 444 | 66 |
| Kentucky bluegrass | 357 | 332 | 71 |
| $LSD_{0.05}$ | 117 | 107 | NS[a] |
| Growth stage[b] | | | |
| Pretiller | 465 | 357 | 60 |
| 1-tiller | 393 | 394 | 72 |
| Multitiller | 404 | 412 | 73 |
| | NS | NS | 9 |

[a] Abbreviation: NS, not significant.

[b] Pretiller (3 to 5 leaves), 1-tiller, and multitiller (5 to 7 tillers).

Scop.] was reduced on multitiller plants compared to seedlings (McCurdy et al. 2008; Whaley et al. 2006). The effects of growth stage also influence the susceptibility of weeds to other herbicides. For example, Chism et al. (1992) reported that southern crabgrass [*Digitaria ciliaris* (Retz.) Koel.] at pretiller and two- to four-tiller stages were more susceptible to quinclorac than at the flowering stage. Kells et al. (1984) noted that fluazifop caused greater injury to quackgrass [*Elytrigia repens* (L.) Desv. ex B.D. Jackson, now *Elymus repens* (L.) Gould] at 2- to 3-leaf stage than at a 5- to 6-leaf stage.

The tolerance of annual and Kentucky bluegrass to mesotrione at the multitiller stage suggests that applications could be used for selectively controlling other weeds, such as crabgrass (*Digitaria* spp.), in mixed stands. However, annual bluegrass exhibits significantly more susceptibility to mesotrione prior to tillering, compared to after establishment, and applications could be injurious. The selectivity for controlling pretiller annual bluegrass suggests mesotrione has differential behavior in these species that is influenced by growth stage.

**Absorption and Translocation.** Species-by-growth stage interaction was not detected for absorption or translocation of root-applied $^{14}$C-mesotrione; thus, results are presented by main effect (Table 3). At 72 h of root exposure to nutrient solution containing $^{14}$C-mesotrione, annual bluegrass accumulated more $^{14}$C in whole plant and shoots per dry weight than Kentucky bluegrass. Annual bluegrass accumulated 479 and 444 Bq $g^{-1}$, while Kentucky bluegrass accumulated 357 and 332 Bq $g^{-1}$ in whole plant and shoots, respectively. For both species, differences were not detected for relative absorption (Bq $g^{-1}$) of $^{14}$C-mesotrione in whole plant and shoots among growth stages. For both species, pretiller plants translocated approximately 10% less of the root-absorbed radioactivity to shoots than tillered plants, but differences between species were not detected.

Uptake and subsequent transport from roots has been attributed to the selectivity of other herbicides for annual bluegrass control in turfgrass. For example, annual bluegrass had greater root absorption of $^{14}$C-amicarbazone than creeping bentgrass (*Agrostis stolonifera* L.) and tall fescue [*Festuca arundinacea* Shreb., now *Lolium arundinacea* (Schreb.) S. J. Darbyshire] (Yu et al. 2013). In other experiments, annual bluegrass had similar root absorption of $^{14}$C-primisulfuron-methyl to Kentucky bluegrass, but the transport of primisulfuron acid to shoots was greater in annual bluegrass due to slower metabolism (McCullough et al. 2015). Soil uptake might be critical for mesotrione selectivity on annual bluegrass seedlings during Kentucky bluegrass establishment. Seeded areas typically take several weeks for Kentucky bluegrass to reach greater than 50% ground coverage, and applications could have minimal spray retention on immature foliage.

Species-by-growth stage interactions were not detected for foliar absorption and translocation after 144 h. Foliar absorption levels averaged 31% (± 1 SEM) and 35% (± 1) of the applied radioactivity for annual and Kentucky bluegrass, respectively, but differences among growth stages were not detected (data not shown). Abit and Al-Khatib (2009) reported no differences in foliar absorption of $^{14}$C-mesotrione in tolerant and susceptible sorghum hybrids. Annual bluegrass has exhibited greater foliar uptake of $^{14}$C-amicarbazone, $^{14}$C-bispyribac-sodium, and $^{14}$C-ethofumesate than creeping bentgrass (Kohler and Branham 2009; Lycan and Hart 2006; Yu et al. 2013). Foliar absorption levels of mesotrione might be inconsequential for the selectivity between annual and Kentucky bluegrass.

Research on foliar uptake of mesotrione in grasses at various growth stages is limited. Canada thistle in the rosette stage absorbed 5 to 10% more



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Table 4. Translocation of radioactivity for annual bluegrass and 'Midnight' Kentucky bluegrass at 144 h after foliar-applied of $^{14}$C-mesotrione in experiments, Griffin, GA. Results were pooled over experimental runs.

| | Translocation[a] |
|---|---|
| | % of $^{14}$C absorbed |
| Species | |
| Annual bluegrass | 27 |
| Kentucky bluegrass | 32 |
| $LSD_{0.05}$ | NS[b] |
| Growth stage[c] | |
| Pretiller | 41 |
| 1-tiller | 27 |
| Multitiller | 20 |
| $LSD_{0.05}$ | 11 |

[a] Results represent the total radioactivity recovered in roots and nontreated shoots.

[b] Abbreviation: NS, not significant.

[c] Pretiller (3 to 5 leaves), 1-tiller, and multitiller (5 to 7 tillers).

Table 5. Metabolism of $^{14}$C-mesotrione in annual bluegrass and 'Midnight' Kentucky bluegrass at 144 h after treatment in two experiments, Griffin, GA. Results were pooled over experimental runs.

| | | Metabolites | |
|---|---|---|---|
| | Parent | Polar | Nonpolar |
| | % of $^{14}$C extracted | | |
| Species | | | |
| Annual bluegrass | 35 | 56 | 9 |
| Kentucky bluegrass | 32 | 59 | 9 |
| $LSD_{0.05}$ | NS[a] | NS | NS |
| Growth stage[b] | | | |
| Pretiller | 39 | 49 | 12 |
| One tiller | 41 | 48 | 11 |
| Multitiller | 21 | 75 | 4 |
| $LSD_{0.05}$ | 7 | 9 | 4 |

[a] Abbreviation: NS, not significant.

[b] Pretiller (3 to 5 leaves), 1-tiller, and multitiller (5 to 7 tillers).

foliar-applied $^{14}$C-mesotrione than at the bolting stage (Armel et al. 2005). The speed of mesotrione uptake was not measured in annual and Kentucky bluegrass due to the number of plants and growth stages that were evaluated. Perhaps annual bluegrass absorbs mesotrione more quickly than Kentucky bluegrass and warrants further investigation.

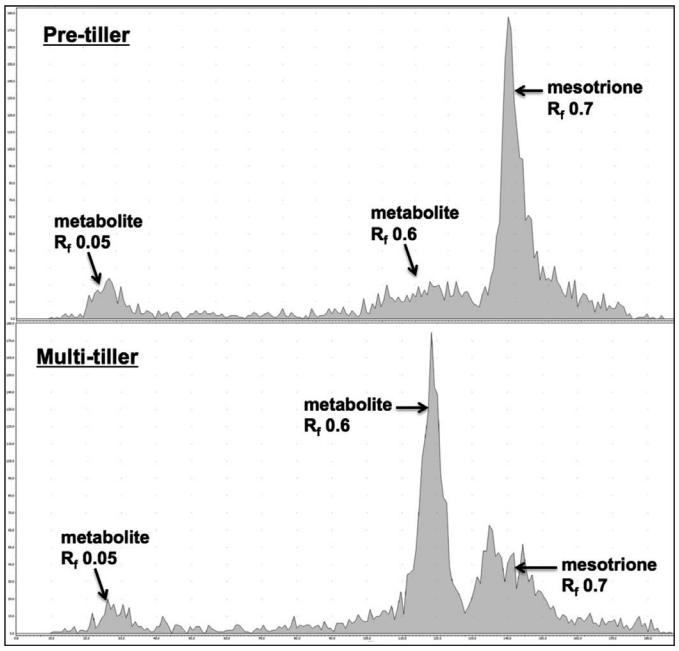



Figure 1. Radiochromatogram scans of the major metabolites detected in pretiller and multitiller Kentucky bluegrass.

Species-by-growth stage interaction was not detected for translocation of foliar-absorbed $^{14}$C-mesotrione. Annual and Kentucky bluegrass translocated 27 to 32% of radioactivity out of the treated leaf (Table 4). In previous research, the majority of radioactivity was also recovered in the treated leaf of grain sorghum hybrids after 7 d (Abit and Al-Khatib 2009). Annual and Kentucky bluegrass have exhibited similar translocation of $^{14}$C-primisulfuron-methyl, despite the differential tolerance levels to applications (McCullough et al. 2015). Researchers have also noted greater translocation of radioactivity from $^{14}$C-amicarbazone and $^{14}$C-bispyribac-sodium in annual bluegrass compared to creeping bentgrass (Lycan and Hart 2006; Yu et al. 2013). Results suggest that translocation patterns do not explain the selectivity of mesotrione in these bluegrass species.

Tillered grasses translocated 24% of the absorbed radioactivity to nontreated shoots and roots. Pretiller plants translocated 41% of the $^{14}$C absorbed, which was greater than both tillered growth stages tested. The efficacy of mesotrione for controlling seedling annual bluegrass could be attributed to translocation after foliar uptake. Armel et al. (2005) noted higher levels of radioactivity translocation from $^{14}$C-mesotrione in rosette Canada thistle compared to the bolting stage. Although Kentucky bluegrass had less injury to mesotrione than annual bluegrass, pretiller plants had more injury than tillered plants for both species. Greater



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translocation following the foliar absorption could contribute to the susceptibility of pretiller plants to mesotrione injury, but results do not explain the differential tolerance levels between species.

**Metabolism.** Extraction of radioactivity from plants averaged 87% (± 0.6) (data not shown). The $R_f$ of parent mesotrione was 0.7 and two major metabolites were identified in both species at $R_f$ 0.05 and 0.6 after foliar uptake (Figure 1). Growth stage-by-species interaction was not detected for metabolism of $^{14}$C-mesotrione. Annual bluegrass and Kentucky bluegrass had similar metabolism of $^{14}$C-mesotrione (65 to 68%) (Table 5). This range in mesotrione degradation is similar to previous reports in grain sorghum after 7 d (Abit and Al-Khatib 2009). However, parent herbicide levels in pretiller and 1-tiller plants were approximately two times greater than multitiller plants (40 vs. 21%). Compared to the multitiller stage, pretiller and 1-tiller plants averaged 26% fewer polar metabolites and approximately three times greater nonpolar metabolite levels.

Metabolism was noted as the primary basis for differential response of crops to mesotrione (Abit and Al-Khatib 2009; Ma et al. 2013; Mitchell et al. 2001). Abit and Al-Khatib (2009) reported a tolerant sorghum hybrid metabolized 7% more foliar-absorbed mesotrione compared to a susceptible hybrid. Enhanced metabolism of mesotrione has been reported in corn (*Zea mays* L.) from cytochrome P450 monooxygenase-mediated detoxification mechanisms (Hawkes et al. 2001). Pataky et al. (2009) reported the mutation of a cytochrome P450 gene, referred to as *nsf1* or *ben1*, resulted in reduced enzyme activity and increased sensitivity of corn to cytochrome P450-metabolized herbicides, including mesotrione and nicosulfuron. Ma et al. (2013) noted corn is naturally tolerant to mesotrione due to a rapid rate of P450-catalyzed ring hydroxylation. This supposition was supported by increased corn injury by mesotrione following the addition of malathion, a cytochrome P450 monooxygenase inhibitor. Perhaps multitiller plants of annual and Kentucky bluegrass have higher concentrations of P450 enzymes than pre- and 1-tiller plants, which contribute to a more rapid degradation of mesotrione. The role of metabolism does not explain the relative differences in tolerance to mesotrione between annual and Kentucky bluegrass.

Mesotrione can be used for selective annual bluegrass control during Kentucky bluegrass establishment from seed. However, efficacy is significantly limited for controlling mature annual bluegrass plants. The selectivity of mesotrione between these species is attributed to differential root-absorption levels. Metabolism is probably inconsequential for mesotrione selectivity between these species, but multitiller annual bluegrass might have greater degradation than pretiller or 1-tiller plants. Further research is needed to analyze differences in the susceptibility levels of HPPD enzymes to inhibition by mesotrione. Research is also warranted to quantify differential levels of uptake between Kentucky bluegrass cultivars with varying levels of susceptibility to mesotrione injury.

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