

## Chapter II

### Absorption, Translocation, and Metabolism of AEF-130060 03 in Wheat, Barley and Italian Ryegrass with or without Dicamba<sup>1</sup>

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**Abstract:** Laboratory experiments were conducted to evaluate absorption, translocation and metabolism of AEF-130060 03 in wheat, barley, and Italian ryegrass. An additional objective was to evaluate how combinations of AEF-130060 03 with dicamba affect absorption, translocation, and metabolism in wheat, barley, and Italian ryegrass. Experiments were conducted in a completely randomized design, and data were subjected to a factorial analysis. The factors included for analysis were plant type, time, and presence or absence of dicamba. Italian ryegrass absorbed 2.5, 2.0, and 1.5 times the

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<sup>3</sup> Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds*, Revised 1989. Available only on computer disk from WSSA, 810 East 10<sup>th</sup> Street, Lawrence, KS 66044-8897.

amount of applied radioactivity at 24, 48, and 96 hours after treatment (HAT), respectively, than wheat or barley. Translocation of radiolabeled AEF-130060 03 from the treated leaf was low and did not differ among wheat, barley, or Italian ryegrass. The rates of AEF-130060 03 metabolism by the two cereal crops and Italian ryegrass were different. At 96 HAT, the total absorbed radioactivity metabolized by wheat, barley, and Italian ryegrass was 67, 51, and 34%, respectively. Conversely, at 96 HAT, the levels of non-metabolized AEF-130060 03 were highest in Italian ryegrass, intermediate in barley, and lowest in wheat. The lower absorption of herbicide and a more rapid rate of metabolism by wheat and barley in comparison to Italian ryegrass most likely account for differential selectivity among the three plant species. Dicamba did not influence absorption, translocation or metabolism in wheat, barley, or Italian ryegrass.

**Nomenclature:** Dicamba; diclofop-methyl; iodosulfuron, [methyl 4-iodo-2-[3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-ureidosulfonyl]benzoate, sodium salt]; mefenpyr-diethyl, [diethyl 1-(2,4-dichlorophenyl)-5-methyl-2-pyrazoline-3,5-dicarboxylate]; mesosulfuron-methyl, [Methyl-2-[3-(4,6-dimethoxypyrimidin-2-yl)ureidosulfonyl]-4-methane-sulfonamidomethylbenzoate]; Italian ryegrass, *Lolium multiflorum* (Lam.) #<sup>3</sup> LOLMU; barley, *Hordeum vulgare* (L.) # HORVX ‘Nomini’; wheat, *Triticum aestivum* (L.) ‘Jackson’.

**Additional index words:** Absorption; crop tolerance; diclofop-methyl resistance; metabolism; sulfonamide; translocation.

**Abbreviations:** ACCase, acetyl-Coenzyme A carboxylase; ALS, acetolactate synthase; CPM, counts per minute; d, days; g, gravity; HAT, hours after treatment; LSD, least

significant difference; LSS, liquid scintillation spectroscopy; MBq/g,  
megabequerels/gram; POST, postemergence; TLC, thin layer chromatography.

## Introduction

Italian ryegrass [*Lolium multiflorum* (Lam.)] is recognized throughout the southeastern United States as one of the ten most common and troublesome weeds in small grains (Webster 2000). Excellent Italian ryegrass control and increased wheat [*Triticum aestivum* (L.)] yields have resulted from the application of diclofop-methyl (Griffen 1986; Khodayari et al. 1983; Shaw and Wesley 1991). Diclofop-methyl is an aryloxyphenoxypropionate herbicide that inhibits the activity of acetyl-Coenzyme A carboxylase (ACCase) in susceptible plants (Devine and Shimabukuro 1994). ACCase is an essential enzyme that facilitates fatty acid biosynthesis (Devine and Shimabukuro 1994).

Italian ryegrass control has declined due to the development of resistance to diclofop-methyl, which has been the only treatment available for control of this species in wheat and barley [*Hordeum vulgare* (L.)]. The first occurrence of diclofop-methyl resistance in Italian ryegrass was documented in 1987 (Stanger and Appleby 1986), and resistance was confirmed in Virginia in 1993 in Brunswick County (Heap 2002). Resistance in biotypes of normally sensitive grass weeds, including Italian ryegrass, has been associated with an insensitive site of action (ACCase), which is not inhibited by aryloxyphenoxypropionate herbicides (Ahrens 1994). In 1999, collections of Italian ryegrass biotypes from Virginia were screened for diclofop-methyl resistance. Resistance was determined to be widespread and results indicated that certain biotypes exhibited up to 16-fold resistance to the normal use rate of diclofop-methyl (Morozov et al. 1999). Lack of control of Italian ryegrass has reduced small grain yield as much as 75 percent through competition

for resources coupled with harvest impairment<sup>3</sup>. Inability to control Italian ryegrass and low small grain prices has led to a reduction in small grain hectareage planted in Virginia. During the period of 1996-2000, harvested wheat hectares decreased from 111,291 to 82,962 (VDACS 2000).

The experimental herbicide combination of AEF-130060 03 plus AEF-107892 contains two experimental herbicides and a safener, respectively, of Bayer Cropscience. AEF-130060 03 contains AEF-130060 00 and AEF-115008 00 at a 5:1 ratio for postemergence (POST) use in wheat. AEF-130060 00 and AEF-115008 00 are sulfonylurea herbicides with the approved common name of mesosulfuron-methyl [Methyl-2-[3-(4,6-dimethoxypyrimidin-2-yl)ureidosulfonyl]-4-methanesulfonamidomethylbenzoate] and proposed common name of iodosulfuron-methyl-sodium [methyl 4-iodo-2-[3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)ureidosulfonyl]benzoate, sodium salt], respectively. Similar to other sulfonylurea herbicides, mesosulfuron-methyl and iodosulfuron-methyl-sodium inhibit the enzyme acetolactate synthase (ALS) in susceptible plants, which is integral in the formation of branched-chain amino acid synthesis (Anonymous a 2002; Anonymous b 2002). The AEF-107892 component of this experimental herbicide is the safener with the approved common name of mefenpyr-diethyl [diethyl 1-(2,4-dichlorophenyl)-5-methyl-2-pyrazoline-3, 5-dicarboxylate], which when absorbed by treated foliage acts by specific enhancement of herbicide degradation in the crop (Anonymous a 2002; Anonymous b 2002). Anderson et al. (2002) observed control of diclofop-methyl-resistant Italian

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<sup>3</sup> Hagood, E. S. and H. P. Wilson. 2000. Application for specific exemption in accordance with Section 18 of FIFRA to use Axiom herbicide to control Italian ryegrass in wheat. pp. 12.

ryegrass as well as annual bluegrass [*Poa annua* (L.)], wild oat [*Avena fatua* (L.)], canarygrass [*Phalaris canariensis* (L.)], downy brome [*Bromus tectorum* (L.)], and Japanese brome [*Bromus japonicus* (Thunb.) ex Murr.] with mesosulfuron-methyl applied alone at 15 g ai/ha. Wheat phytotoxicity was low and control of some mustard species was also observed when mesosulfuron-methyl was applied POST (Anderson 2002). The addition of iodosulfuron-sodium-methyl to mesosulfuron-methyl increases control of winter annual broadleaf species such as common chickweed [*Stellaria media* (L.) Vill.] and henbit [*Lamium amplexicaule* (L.)], and the wheat tolerance with the addition of this compound is not different than the tolerance to mesosulfuron-methyl applied alone (M. D. Paulsgrove, personal communication)<sup>4</sup>.

Initial investigations of AEF-130060 03 plus AEF-107892 indicated the potential of these compounds to be utilized for Italian ryegrass control in barley. Excellent Italian ryegrass control and transient phytotoxicity occurred when POST treatments of AEF-130060 03 plus AEF-107892 were applied to ‘Nomini’ barley in December (King 2002). Barley injury appeared as a general stunting. Essentially complete recovery occurred, however, by crop maturity. Barley yields in fields containing diclofop-methyl-sensitive Italian ryegrass were not significantly different between treatments of AEF-130060 03 plus AEF-107892 and diclofop-methyl (King 2002). Preliminary evaluations of mesosulfuron-methyl alone compared to mesosulfuron plus iodosulfuron did not result in differential phytotoxicity to barley.

The addition of dicamba to treatments of AEF-130060 03 and AEF-107892 resulted in a significant increase in barley phytotoxicity compared to AEF-130060 03 plus AEF-107892 applied alone (King 2002). Increased small grain phytotoxicity has occurred

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<sup>4</sup> Mary D. Paulsgrove, Product development manager, Herbicides, Bayer Cropsciences

when growth regulator herbicides such as 2,4-D were applied with carfentrazone-ethyl compared to carfentrazone-ethyl applied alone (Durgan et al. 1997). Dicamba has also reduced shattercane [*Sorghum halepense* (L.) Moench] control by decreasing the foliar absorption of imazethapyr (Hart and Wax 1996). The increased barley phytotoxicity observed when AEF-130060 03 plus AEF-107892 was combined with dicamba could result from reduced AEF-107892 absorption by barley compared to when AEF-130060 03 plus AEF-107892 was applied alone.

Differential tolerance of wheat, barley, and Italian ryegrass to AEF-130060 03 plus AEF-107892 has not been examined. For this reason, experiments were conducted to investigate absorption, translocation, and metabolism of AEF-130060 03 and AEF-107892 in wheat, barley and Italian ryegrass. An additional objective was to evaluate how AEF-130060 03 plus AEF-107892 in combination with dicamba affect absorption, translocation, and metabolism in wheat, barley, and Italian ryegrass.

## **Materials and Methods**

### **Plant Material**

In the fall of 2001 and winter of 2002, ‘Jackson’ wheat, ‘Nomini’ barley, and Italian ryegrass<sup>5</sup> seeds were planted in a 1:1 mixture of commercial potting soil<sup>6</sup> and vermiculite<sup>7</sup> in 10-cm by 10-cm square plastic pots, with one seed per pot. Italian

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<sup>5</sup> Landscape supply, Inc. P. O. Box 12706, Roanoke, VA. 24027

<sup>6</sup> Premier Promix, Premier Horticulture Inc. Red Hill, Pa. 18076

<sup>7</sup> Vermiculite, The Schunder Co. P. O. Box 513, Metuchen, NJ. 08840-0513

ryegrass seed used in the experiments was screened for resistance to diclofop-methyl and AEF-130060 03 plus AEF-107892 prior to the initiation of the experiments and plants were determined to be susceptible to each material applied at typical field use rates. Seedlings were grown in a greenhouse, watered as needed, and fertilized with a commercial fertilizer<sup>8</sup> once. Seedlings of wheat and barley were grown to the four-leaf stage, while Italian ryegrass plants were grown to the 3-tiller stage to allow successful spotting of radioactive materials. Tillers were removed from Italian ryegrass plants and remaining Italian ryegrass plants had 3 to 4 leaves and were approximately the same size as wheat and barley plants. All plant species were then transferred to glass jars wrapped in aluminum foil containing 100 ml of quarter-strength Hoagland's nutrient solution (pH 6.8). Seedlings were then allowed to acclimate in this medium for 7 d prior to the initiation of the experiments. An additional 50 ml of quarter-strength Hoagland's nutrient solution was added to the jars one day before herbicide treatment to compensate for transpiration and evaporation losses.

### **<sup>14</sup>C labeled herbicide**

Absorption, translocation and metabolism experiments were conducted with radiolabeled AEF-130060 03, which was supplied by Bayer Cropsciences. This herbicidal mixture contained 50% [phenyl -U-<sup>14</sup>C] radiolabeled mesosulfuron-methyl, 10% non-labeled iodosulfuron and 40% inerts. Therefore, results of the experiments only describe absorption, translocation and metabolism of mesosulfuron-methyl, without reference to the activity of iodosulfuron-methyl-sodium and the safener, mefenpyr-

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<sup>8</sup> Peters 20-20-20, Scotts-Sierra Horticultural Products, 14111 Scottslawn Rd. Marysville, OH. 43041



diethyl. All results regarding iodosulfuron-methyl-sodium and mefenpyr-diethyl have to be inferred. The specific activity of [<sup>14</sup>C]mesosulfuron-methyl was 6425 MBq/g.

### **Herbicide Absorption and Translocation**

Half of the hydroponically grown seedlings of each species were treated with 0.0038, and 0.0074 kg ai/ha of AEF-130060 03 plus AEF-107892, respectively, while the other half received AEF-130060 03 plus AEF-107892 in combination with 0.035 kg ai/ha of the dimethylamine salt of dicamba. AEF-130060 03 contains mesosulfuron-methyl and iodosulfuron-methyl-sodium at a ratio of 5:1. These herbicide rates were approximately 25% of the field use rate. Both of these herbicide applications were also applied in combination 0.4 and 0.6 L/ha of methylated sunflower oil<sup>9</sup> and 28% urea ammonium nitrate solution, respectively. These sublethal herbicide treatments were applied to stimulate plant metabolism prior to [<sup>14</sup>C]mesosulfuron-methyl application. These herbicide treatments were applied with a stationary track sprayer<sup>10</sup> containing a single flat-fan nozzle tip<sup>11</sup> that delivered 234 L/ha of spray solution at 269 kPa.

[<sup>14</sup>C]mesosulfuron-methyl was initially dissolved in a 50:50 solution of acetone and double-distilled water. Approximately 4 hours after initial herbicide treatment, all plants received a 10  $\mu$ l droplet of [<sup>14</sup>C]AEF-130060 03, which was applied to the adaxial surface of the newest fully expanded leaf. Each 10  $\mu$ l droplet consisted of 90.8 % water, 8.7 % [<sup>14</sup>C]AEF-130060 dissolved in a 50:50 solution of acetone and double-distilled water, and

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<sup>9</sup> Sun Wet, Brewer International, P.O. Box 6006, Vero Beach, FL. 32961

<sup>10</sup> Allen Machine Works, 607 E. Miller Road, Midland, MI. 48640

<sup>11</sup> Teejet, 8001E, Spraying Systems Co.<sup>®</sup>, P.O. Box 7900, Wheaton, IL. 60189

0.5% Tween 20<sup>12</sup> and contained an approximate radioactivity of 3,800 Bq per microliter. Thirty-two plants of each species were spotted, half of which were previously treated with AEF-130060 03 plus AEF-107892 alone and the other half treated with AEF-130060 03 plus AEF-107892 in combination with dicamba. Four plants of each species treated with AEF-130060 03 plus AEF-107892 alone and four plants of each species treated with AEF-130060 03 plus AEF-107892 in combination with dicamba were randomly selected for harvest 6, 24, 48, and 96 h after treatment (HAT). After each harvest all plants were divided into treated leaf, foliage above treated leaf, foliage below treated leaf, and roots. The treated leaf of each plant was washed in 10 ml of 50:50 acetonitrile and double-distilled water solution containing 0.1% (v/v) Tween 20 for 30 seconds to remove unabsorbed [<sup>14</sup>C]AEF-130060 03. A 1-ml sample of each leaf wash solution was mixed with 10 ml of scintillation cocktail<sup>13</sup> and subjected to liquid scintillation spectrometry<sup>14</sup>(LSS) to quantify the total unabsorbed radioactivity. Two plants of each species treated with AEF-130060 03 plus AEF-107892 alone and two plants of each species treated with AEF-130060 03 plus AEF-107892 in combination with dicamba were then frozen in a -20 C freezer for use in the metabolism studies. Remaining plant parts were dried for 72 h at 45 C and combusted with a biological sample oxidizer<sup>15</sup>. Samples were subjected to LSS in order to determine the amount of

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<sup>12</sup> Polysorbate 20 (polyoxyethylene [20] sorbitan monolaurate), ICI America, Inc., Wilmington, DE. 19899.

<sup>13</sup> ScintiVerse<sup>®</sup> BD, Fisher Scientific, Fair Lawn, NJ 07410

<sup>14</sup> Liquid scintillation counter, Beckman LS 5000TA model, Beckman Instruments, 4300 N.Harbor Boulevard, Fullerton, CA. 92634

<sup>15</sup> Biological oxidizer model 307, Packard Instrument Co., 2200 Warrenville Road, Downers Grove, IL.

absorbed radioactivity. Absorption of [ $^{14}\text{C}$ ]AEF-130060 03 was expressed as a percentage of the applied radioactivity and was calculated by dividing the amount of  $^{14}\text{C}$  from the respective plant section by the sum of the  $^{14}\text{C}$  from the leaf wash of each seedling and the  $^{14}\text{C}$  recovered from all oxidized plant sections. Translocation of [ $^{14}\text{C}$ ]AEF-130060 03 was expressed as a percentage of the absorbed radioactivity and was calculated by dividing the amount of  $^{14}\text{C}$  from the respective plant section by the total amount of  $^{14}\text{C}$  recovered from all oxidized plant parts. Experiments were repeated twice and each treatment contained two replications.

### **Herbicide Metabolism**

Plants used in the metabolism experiments were treated and harvested with the same procedure as described in the absorption and translocation experiment. Results from the absorption and translocation study indicated that only treated leaves contained sufficient radioactivity to allow analysis of metabolites. Treated leaves were removed from the freezer and ground in liquid nitrogen. Homogenized tissue was extracted with 5 ml of 4:1 acetonitrile and water and centrifuged at 13,000 g for 10 min. Supernatant was removed and pellet was resuspended in another 5 ml of 4:1 acetonitrile and water and centrifuged again. This procedure was repeated one more time to assure acceptable recovery of absorbed [ $^{14}\text{C}$ ]AEF-130060 03 within the treated leaf. All three supernatants were combined and then evaporated to near dryness on a sample evaporator<sup>16</sup>. Remaining materials were then redissolved in 400  $\mu\text{l}$  of acetonitrile. Two hundred  $\mu\text{l}$  of the final extract of each treated leaf was then spotted on silica gel thin-layer

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<sup>16</sup> N-Evap 111, Organomation Associates, Inc., 266 River Road West, Berlin, MA. 01503

chromatography (TLC) plates<sup>17</sup> in addition to 10  $\mu$ l of the [<sup>14</sup>C]AEF-130060 03 standard and 10  $\mu$ l of the non-labeled AEF-130060 03 standard. Each plate contained 6 lanes, which consisted of 4 treated leaf samples and the two standards. TLC plates were developed in a solvent system of 45:55:10 chloroform, ethanol, and glacial acetic acid by volume. TLC plates were analyzed with a radiochromatogram scanner<sup>18</sup> to determine radioactive positions of metabolite and parent compounds. Winscan software<sup>19</sup> was used to calculate peak areas with equation parameters set to 13-point cubic smoothing and rejection of peaks that were below 1.5% of total radioactivity. Parent material was identified as the peak observed in the [<sup>14</sup>C]AEF-130060 03 standard. Metabolite peaks were summed to allow calculation of the percentage of metabolism and were expressed as a percentage of the absorbed radioactivity recovered in the TLC analysis of the treated leaf of each representative sample. Metabolism experiments were repeated twice with two replications per experiment.

### **Experimental Design and Analysis**

Absorption, translocation and metabolism experiments were arranged in a completely randomized design, and data were subjected to a three-way factorial analysis of variance. The factors subjected to analysis were plant type, time, and presence or absence of

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<sup>17</sup> Thin layer chromatography plates, Silica Gel 60 F<sub>254</sub> TLC plates, EM Science, 480 Democrat Road, Gibbstown, NJ. 08027

<sup>18</sup> BioScan System 200 Imaging Scanner, Bioscan Inc., 4590 MacArthur Blvd. NW, Washington, D. C. 20007

<sup>19</sup> LabLogic<sup>®</sup> Win-Scan Radio TLC Version 2.2(5) 32-bit, Distributed by BioScan, 4590 MacArthur Blvd. NW, Washington, D. C. 20007

dicamba. Homogeneity of variance evaluation indicated a non-significant interaction effect between experiments. For this reason, data are presented as an average of the two experiments. Means were separated using the least significant difference (LSD) test at the 5% level of probability.

## **Results and Discussion**

### **Herbicide Absorption and Translocation**

Initial absorption of [<sup>14</sup>C]mesosulfuron-methyl in all three plant species was relatively slow, where less than 20 percent of the radioactivity was absorbed at 6 HAT, and no significant differences between plant species were observed (Figure 1). At the later harvest times however, Italian ryegrass had absorbed greater than 2.5, 2.0, and 1.5 times the amount of applied radioactivity at 24, 48, and 96 HAT, respectively, than wheat or barley. These results are in contrast to those reported by Askew and Wilcut (2002) and Carey et al. (1997), where increased absorption of the sulfonylurea herbicides nicosulfuron, primisulfuron and CGA-362622 was observed in tolerant species as compared to susceptible plant species. The addition of dicamba to applications of AEF-130060 03 plus AEF-107892 did not affect absorption of [<sup>14</sup>C]mesosulfuron-methyl in wheat, barley, or Italian ryegrass compared to treatments of AEF-130060 03 plus AEF-107892 applied alone (Figure 2). Throughout all phases of the experiments, no differences were observed when dicamba was added to AEF-130060 03 plus AEF-107892 within individual plant species. Therefore, results of translocation and metabolism experiments were averaged over both experiments, and combined over

dicamba treatments and repetitions, and means are comprised of a total sample size of eight.

Slight differences between wheat, barley, and Italian ryegrass were observed with respect to translocation of [ $^{14}\text{C}$ ]mesosulfuron-methyl out of the treated leaf at some harvest timings (Table 1). The movement of absorbed radioactivity to the roots of wheat and barley was significantly higher at 6 and 24 HAT than in Italian ryegrass (Table 1). At 24, 48 and 96 HAT, only about 2% of the absorbed radioactivity was found in the shoots below the treated leaf in wheat and barley. In Italian ryegrass however, radioactivity translocating to the shoots below increased with time and reached 6% at 96 HAT, which was significantly higher than in wheat or barley. Generally, however, over 90% of the total absorbed [ $^{14}\text{C}$ ]mesosulfuron-methyl was contained in the treated leaf of each plant species throughout the duration of the experiment (Table 1). Absorbed radioactivity contained within the treated leaf increased as time elapsed in barley and wheat (Table 1). In contrast, absorbed radioactivity contained in the treated leaf of Italian ryegrass decreased with time (Table 1). Treated leaves of Italian ryegrass often contained as much as 3 times more [ $^{14}\text{C}$ ]mesosulfuron-methyl at the 4 harvest timings compared to wheat or barley (Data not shown). These results indicated that, while some differences among plant species occurred, selectivity does not appear to be associated with differential translocation of mesosulfuron-methyl out of the treated leaf.

### **Herbicide Metabolism**

Due to confidentiality constraints the identification of metabolites will not be discussed, and Rf values and metabolite structures and conjugations will not be defined.

Rather, quantification of the degradation rate of the parent material to the sum of the metabolites will be the primary focus. At 96 HAT, three identifiable metabolites were observed. The quantity and Rf values of the three metabolites were similar among the three plant species (Data not shown). At 6 HAT the treated leaves of Italian ryegrass contained almost 4 times as much [<sup>14</sup>C]mesosulfuron-methyl compared to wheat or barley plants (Table 2). A small amount of metabolites was identified in the treated leaves of Italian ryegrass at 6 HAT, but no metabolites were identified in wheat or barley at 6 HAT. There was, however, no significant difference in metabolism observed among plant species at 6 HAT. The amount of total radioactivity at 96 HAT was higher in Italian ryegrass than in wheat or barley (Table 2). The total amount of radioactivity contained within the treated leaves was not significantly different between wheat and barley, where 17695 and 19444 CPM were present in each plant species, respectively, at 96 HAT. Significant differences in parent compound content, however, were observed between each plant species at 96 HAT. Parent compound content of the three species was Italian ryegrass > barley > wheat. The amounts of radioactivity at 96 HAT associated with the metabolites of [<sup>14</sup>C]mesosulfuron-methyl, however, were not different among plant species (Table 2). All three species contained the same amount of metabolites at 96 HAT; however, metabolites were produced at different rates. Calculation of the ratio of the metabolites to the total indicated that 67, 51 and 34% of the total radioactivity absorbed as [<sup>14</sup>C]mesosulfuron-methyl by the treated leaf was metabolized by wheat, barley, and Italian ryegrass, respectively. This result suggested that the rapidity of metabolism may be another mechanism associated with differential selectivity between the three plant species.

These results indicated that the primary mechanism for the selectivity between wheat, barely and Italian ryegrass was associated with increased herbicide absorption by Italian ryegrass, which is a sensitive species. A secondary mechanism may include a more rapid rate of metabolism of the absorbed herbicide by wheat and barley. Differential absorption of sulfonylurea herbicides does not usually account for selectivity among species (Carey et al. 1997; Askew and Wilcut 2002; Ma 1997; Beyer et al. 1988; Eberlein et al. 1989). Ackley et al. (1999) however, indicated that differences in early uptake and translocation might have resulted in differential sensitivity of eastern black nightshade [*Solanum ptycanthum* (Dun.)], hairy nightshade [*Solanum sarrachoides* (Sendtner)], and black nightshade [*Solanum nigrum* (L.)] to rimsulfuron. Generally, the primary mechanism of naturally occurring resistance to ALS inhibitors is a metabolic alteration of the active ingredient that prevents lethal herbicide levels from reaching the target site (Saari et al. 1994). Differential metabolism rates resulted in differential tolerance between plant species with other sulfonylurea herbicides such as CGA-362622, prosulfuron, nicosulfuron, and primisulfuron (Askew and Wilcut 2002; Ma 1997; Carey et al. 1997). This occurs because the more tolerant plant quickly degrades the active ingredient to non-toxic metabolites.

The reduced total amount of herbicide within wheat and barley coupled with the more rapid rate of metabolism than Italian ryegrass is most likely the reason for differential selectivity between the three plant species. Therefore it would be conceivable that any factor that increases the absorption of AEF-130060 03 by Italian ryegrass could result in greater control. No difference in absorption, translocation or metabolism occurred due to the addition of dicamba to AEF-130060 03 plus AEF-107892. The stunting that occurs



in barley in field situations due to applications of AEF-130060 03 plus AEF-107892 may be reflected in increased amounts of parent compound in the treated leaf in comparison to wheat and a slightly reduced rate of metabolism (Table 2). Increases in the quantity of safener may increase tolerance of AEF-130060 03 by barley. Safeners, however, are often characterized by a high degree of botanical and chemical specificity (Hatzios, 1997). Therefore, increasing safener ratios may not be effective. Barley phytotoxicity may also be reflected in differential absorption, translocation and metabolism of iodosulfuron-methyl-sodium in comparison to wheat.

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Figure 1. Absorption of [ $^{14}\text{C}$ ] mesosulfuron-methyl in wheat, barley, and Italian ryegrass at 6, 24, 48, and 96 h after treatment. Error bars represent the standard error of the mean. Asterisk indicates significant differences between Italian ryegrass and wheat and barley at the  $P = 0.05$  significance level.

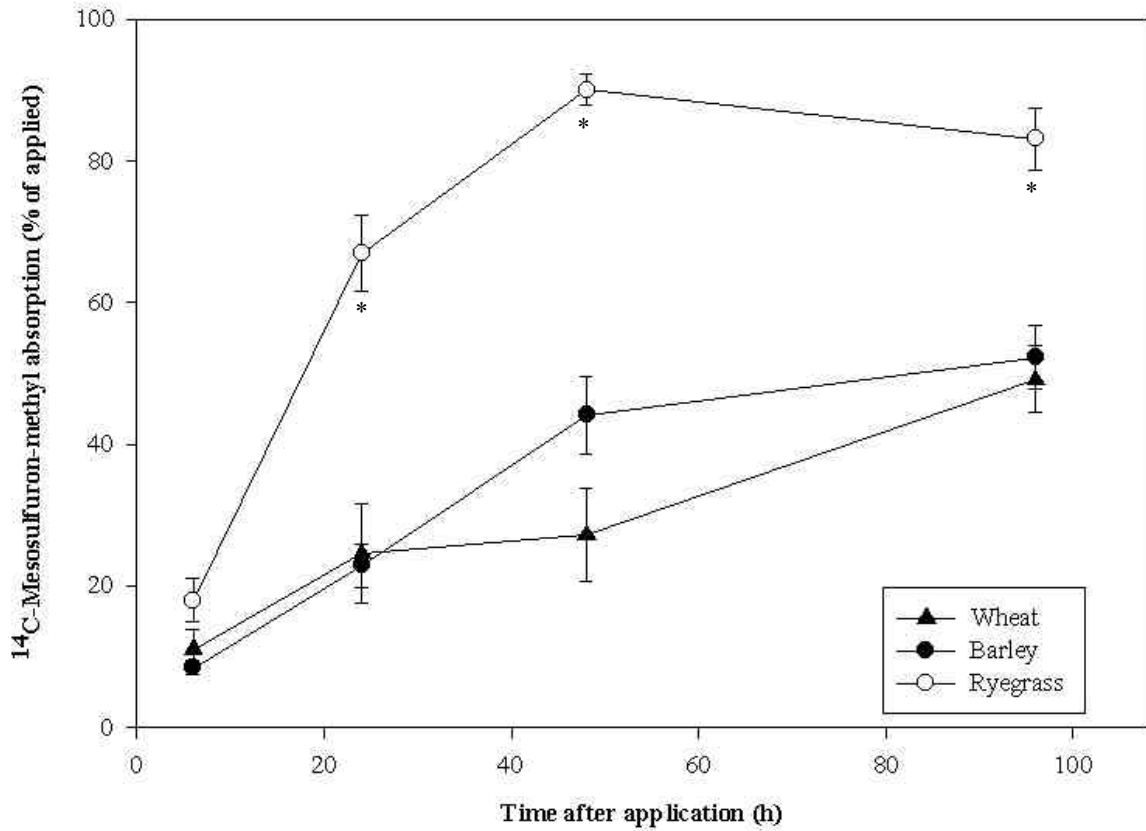


Figure 2. Absorption of [<sup>14</sup>C] mesosulfuron-methyl in wheat, barley and Italian ryegrass with or without dicamba at 6, 24, 48, and 96 h after treatment. Error bars represent the standard error of the mean.

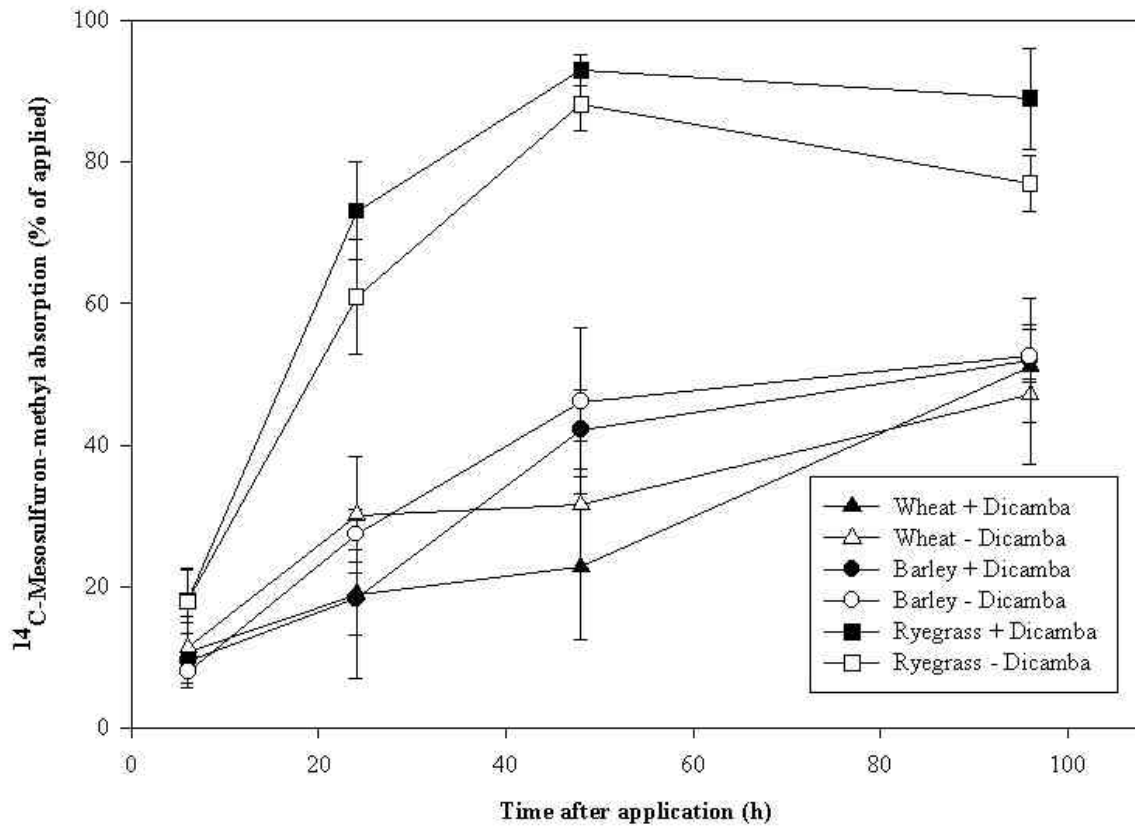


Table 1. Translocation of [<sup>14</sup>C] mesosulfuron-methyl from the treated leaf in wheat, barley, and Italian ryegrass at 6, 24, 48 and 96 (HAT).

| Time | [ <sup>14</sup> C] mesosulfuron-methyl absorption <sup>a</sup> |            |              |           |              |            |              |           |                  |            |              |           |
|------|--|------------|--------------|-----------|--------------|------------|--------------|-----------|------------------|------------|--------------|-----------|
|      | Wheat  |            |              |           | Barley       |            |              |           | Italian Ryegrass |            |              |           |
|      | Treated leaf   | Leaf above | Shoots below | Roots     | Treated leaf | Leaf above | Shoots below | Roots     | Treated leaf     | Leaf above | Shoots below | Roots     |
| HAT  | ----- % -----  |            |              |           |              |            |              |           |                  |            |              |           |
| 6    | 89.5 ± 3.0   | 1.2 ± 0.4  | 2.9 ± 1.2    | 6.4 ± 2.1 | 83.7 ± 3.7   | 1.2 ± 0.1  | 4.7 ± 1.9    | 8.4 ± 2.6 | 97.3 ± 1.2       | 0.4 ± 0.04 | 0.8 ± 0.8    | 1.5 ± 0.9 |
| 24   | 95.1 ± 0.9   | 0.5 ± 0.04 | 1.9 ± 0.5    | 2.5 ± 0.7 | 96.1 ± 0.8   | 0.4 ± 0.1  | 1.5 ± 0.5    | 2.0 ± 0.5 | 97.6 ± 0.4       | 0.3 ± 0.1  | 1.4 ± 0.4    | 0.8 ± 0.2 |
| 48   | 91.5 ± 2.0   | 0.7 ± 0.2  | 2.2 ± 0.6    | 5.7 ± 1.9 | 96.1 ± 0.7   | 0.5 ± 0.1  | 1.1 ± 0.2    | 2.3 ± 0.5 | 95.9 ± 0.9       | 0.4 ± 0.1  | 2.6 ± 0.7    | 1.2 ± 0.4 |
| 96   | 95.9 ± 0.6   | 0.7 ± 0.1  | 1.9 ± 0.5    | 1.5 ± 0.3 | 96.5 ± 0.7   | 0.4 ± 0.1  | 1.7 ± 0.5    | 1.5 ± 0.3 | 90.6 ± 1.3       | 1.2 ± 0.1  | 6.0 ± 0.9    | 2.3 ± 0.4 |

<sup>a</sup> Values represent percentage of absorbed [<sup>14</sup>C]mesosulfuron-methyl as means ± standard errors.

Table 2. Quantification of the total radioactivity and the degradation of [<sup>14</sup>C] mesosulfuron-methyl from the parent compound to metabolites in the treated leaf of wheat, barley and Italian ryegrass at 6 and 96 HAT<sup>a</sup>.

| Plant              | 6 HAT                        |                 |             | 96 HAT              |                 |             |
|--------------------|------------------------------|-----------------|-------------|---------------------|-----------------|-------------|
|                    | Total Radioactivity          | Parent Compound | Metabolites | Total Radioactivity | Parent Compound | Metabolites |
|                    | ----- CPM <sup>b</sup> ----- |                 |             |                     |                 |             |
| Wheat <sup>c</sup> | 2956 B                       | 2956 B          | 0 A         | 17695 B             | 5794 C          | 11901 A     |
| Barley             | 2016 B                       | 2016 B          | 0 A         | 19444 B             | 9253 B          | 10091 A     |
| Italian Ryegrass   | 11760 A                      | 11409 A         | 351 A       | 25776 A             | 16822 A         | 8954 A      |

<sup>a</sup> Means are the average of both experiments combined over dicamba treatments and repetitions.

<sup>b</sup> Quantification is based on the CPM of [<sup>14</sup>C]mesosulfuron-methyl by radiochromatogram scanner.

<sup>c</sup> Values followed by the same letter do not significantly differ, LSD = 0.05.