Chapter IV

Responses of Winter Wheat (*Triticum aestivum*) and Diclofop-methyl sensitive and –Resistant Italian Ryegrass (*Lolium multiflorum*) to AE F130060

Abstract: Greenhouse and laboratory experiments were conducted to investigate the response of winter wheat, two diclofop-sensitive (OR and KG), and four diclofop-resistant (EP, GT, RBG, and JB) Italian ryegrass biotypes to the experimental herbicide mixture AE F130060 03. AE F130060 at 15 or 18 g/ha without the safener AE F107892 reduced biomass of winter wheat 10 to 14% while applications made with AE F107892 did not reduce wheat biomass. AE F130060 03 at 15 or 18 g ai/ha was more effective than diclofop in reducing biomass of one diclofop-sensitive biotype and all four diclofop-resistant Italian ryegrass biotypes. However, differential responses to AE F130060 03 at 15 and 18 g/ha occurred among diclofop-resistant biotypes. With applications made at either rate, AE F130060 03 reduced biomass of OR, KG, EP, and GT from 61 to 84%. In RBG and JB populations, however, biomass was reduced only 35 to 52%. To further investigate this differential response to AE F130060 03 among Italian ryegrass biotypes, absorption, translocation, and metabolism experiments in the laboratory were conducted using diclofop-sensitive KG and diclofop-resistant JB biotypes. Absorption, translocation, and metabolism of AE F130060 00 in winter wheat treated with or without the herbicide safener AE F107892 was also included for comparison. Foliar absorption of [^{14}C] AE F130060 00 was not influenced by herbicide safener or Italian ryegrass biotype; however, Italian ryegrass biotypes absorbed at least three times more AE F130060 00 than wheat at 12, 36, and 72 h after treatment (HAT). Herbicide translocation was not a contributing factor to differential tolerance between species in this experiment, as no more than 9% of absorbed radioactivity translocated into shoots and roots of either species during the experiment. Differential metabolism was noted, however,

between winter wheat and Italian ryegrass. Greatest overall metabolism occurred in winter wheat treated with the safener AE F107892. At 72 HAT, relative amounts of parent AE F130060 00 in Italian ryegrass biotypes were nearly 1.8 times greater than in wheat that received AE F107892 and nearly 1.5 times greater than that in unsafened wheat. However, obvious differences in herbicide metabolism between diclofop-sensitive KG and diclofop-resistant JB were not evident. We hypothesize that differential sensitivity to AE F130060 00 in these biotypes is most likely due to a less sensitive acetolactate synthase, although further research is required to confirm this hypothesis.

Nomenclature: AE F130060 03 {8.3:1.7 mixture of AE F130060 00, proposed common name mesosulfuron-methyl, 2-[(4,6-dimethoxypyrimidin-2-*yl* carbamoyl)sulfamoyl]-4-methanesulfonamido)-*p*-toluic acid, plus AE F115008 00, proposed common name iodosulfuron-methyl-sodium, 4-iodo-2-[3-(4-methoxy-6-methyl-1,3,5-triazin-2-*yl*)ureidosulfonyl]benzoic acid}; AE F107892, proposed common name mefenpyr-diethyl, 1-(2,4-dichlorophenyl)-4,5-dihydro-5-methyl-1*H*-pyrazole-3,5-dicarboxylic acid; diclofop-methyl; Italian ryegrass, *Lolium multiflorum* Lam. LOLMU; winter wheat, 'Pioneer 26R24', *Triticum aestivum* L.

Key words: Diclofop resistance, differential absorption, herbicide safeners, metabolism, multiple resistance.

Introduction

Italian ryegrass (*Lolium multiflorum* Lam.) is a weed indigenous to winter wheat production in Southern and mid-Atlantic regions of the United States. Wheat grain yield reductions as high as 92% have occurred from competition with Italian ryegrass (Appleby et al. 1976; Hashem et al. 1998). Liebl and Worsham (1984) reported a 5% grain yield loss for every 10 Italian ryegrass plants/m^2. Competition from Italian ryegrass reduces wheat tillering (Appleby et al. 1976; Ketchersid and Bridges 1987) and depletes soil nitrogen and phosphorus resources

intended for wheat (Liebl and Worsham 1987; Perez-Fernandez and Coble 1998). Infestations of Italian ryegrass are common in North Carolina and Virginia wheat fields and often result in field abandonment (A. C. York, North Carolina State University; E. S. Hagood, Virginia Tech, personal communication).

Diclofop-methyl is an aryloxyphenoxypropanoate herbicide that inhibits acetyl coenzyme A carboxylase (ACCase, EC 6.4.1.2), a chloroplastic enzyme essential to fatty acid biosynthesis in susceptible monocot species (Kocher 1984; Bravin et al. 2001). Diclofop-methyl was registered for selective control of Italian ryegrass in wheat in North Carolina and Virginia in the early 1980's. Although diclofop has been very effective in controlling Italian ryegrass since its introduction, the repeated use of diclofop-methyl has selected for Italian ryegrass biotypes resistant to this herbicide. Currently, diclofop-resistant Italian ryegrass biotypes infest more than 50% of the wheat hectarage in Virginia and result in net losses in excess of $3.2 million (Hagood 2000). In addition to resistance to diclofop-methyl, many diclofop-resistant Italian ryegrass biotypes also exhibit cross-resistance to other ACCase-inhibiting herbicides such as fenoxaprop-P-ethyl and the cyclohexanedione herbicides tralkoxydim and sethoxydim (Bourgeois et al. 1997; Cocker et al. 2001; Devine and Shimabukuro 1994). For these reasons, introduction of herbicides with alternative modes-of-action is essential for profitable wheat production in areas where diclofop-resistant Italian ryegrass populations persist.

Sulfonylurea herbicides differ from ACCase-inhibiting herbicides such as diclofop by inhibiting acetolactate synthase (ALS, EC 4.1.3.18), the enzyme that catalyzes the first parallel reaction in the biosynthesis of the branched chain amino acids valine, leucine, and isoleucine (Ray 1984). Chlorsulfuron is a sulfonylurea herbicide that was registered for use in wheat in some areas of the U. S., Europe, and Australia in the early 1980's following the introduction of diclofop (Levitt et al. 1981; Liebl and Worsham 1987). Chlorsulfuron applied at 35 g ai/ha preemergence (PRE) has controlled Italian ryegrass (Griffin 1985).

Although rare, at least two Italian ryegrass biotypes in the U.S. are resistant to sulfonylurea herbicides. Taylor and Coats (1996) identified two biotypes of Italian ryegrass from rights-of-ways in Mississippi resistant to sulfometuron where this herbicide had effectively controlled Italian ryegrass previously. One of these biotypes was later determined to be resistant to other sulfonylurea herbicides. In Italy, two biotypes of Italian ryegrass exhibit six- and eight-fold resistance to diclofop and three- and 13-fold resistance to chlorsulfuron (Heap 2002).

Biotypes of diclofop-resistant annual ryegrass (*Lolium rigidum* Gaud.) from Australia also exhibit additional resistance to chlorsulfuron (Matthews et al. 1990). Some of these annual ryegrass biotypes exhibit resistance to every selective postemergence (POST) graminicide registered for use in Australia and are resistant to several herbicides that have not been released (Burnet et al. 1994; Heap and Knight 1990; Powles et al. 1990). Further, some biotypes of annual ryegrass exhibit resistance to glyphosate, a non-selective herbicide (Pratley et al. 1999). The mechanisms of multiple resistance in some of these annual ryegrass biotypes is due to altered herbicide target sites that are less sensitive to inhibition by the herbicides and/or the increased capacity of these biotypes to detoxify the herbicides (Christopher et al. 1991, 1992; Feng et al. 1999).

AE F130060 03 is an 8.3:1.7 mixture of the experimental sulfonylurea herbicides AE F130060 00 and AE F115008 00. AE F130060 00 (proposed common name mesosulfuron-methyl) is active primarily against monocotyledonous weeds while AE F115008 00 (proposed common name iodosulfuron-methyl-sodium) acts primarily against dicotyledonous weeds. This sulfonylurea mixture has effectively controlled Italian ryegrass and several winter annual dicotyledonous weed species and can be applied to winter wheat when used with the safener AE F107892 (common name mefenpyr diethyl) (Bailey et al. 2002; Crooks et al. 2002; Hand et al. 2002). AE F107892 was developed in 1993 as a crop safener for fenoxaprop-P-

ethyl, but is also an effective safener for certain other chemical classes (Hopkins 1997).

To date, we are not aware of any reports of Italian ryegrass biotypes in the U.S. that exhibit multiple resistance to diclofop and sulfonylurea herbicides. Although a similar cross-resistance phenomenon of the magnitude seen in annual ryegrass has not been documented in Italian ryegrass biotypes, Italian ryegrass is physiologically and genetically similar to annual ryegrass (Betts et al. 1992; Martinez-Ghersa et al. 1997), and the possibility of this occurrence should not be overlooked.

Results of field experiments indicate that AE F130060 03 effectively controls Italian ryegrass when applied POST at 15 g ai/ha (Bailey et al. 2002; Crooks et al. 2002). However, research is needed to verify that this herbicide mixture controls both diclofop-sensitive and -resistant Italian ryegrass biotypes in order to determine if AE F 130060 03 is a viable alternative to diclofop for control of diclofop-resistant Italian ryegrass.

In this research, the potential for differential activity of AE F130060 03 on diclofop-sensitive and –resistant Italian ryegrass biotypes was investigated in initial plant bioassay experiments in the greenhouse and subsequent absorption, translocation, and metabolism experiments in the laboratory. An additional objective was to investigate response of winter wheat to AE F130060 00 with or without the safener AE F107892 in greenhouse and laboratory experiments.

Materials and Methods

Italian Ryegrass Plant Material

In late May and early June of 1996 and 1997, Italian ryegrass seed were harvested from 29 wheat fields throughout Accomack and Northampton Co., VA, and Pasquatank Co., NC, where diclofop resistance was suspected. In initial greenhouse screens where diclofop was applied at 1680 g ai/ha POST to 3- to 4-leaf Italian ryegrass, 13 of these selections exhibited various levels of

tolerance to diclofop (data not presented). Of these 13 selections, four were chosen for use in experiments with AE F130060 03. These four selections (EP, GT, RBG, and JB) exhibited the greatest levels of diclofop resistance. A commercially-available cultivar of Italian ryegrass[1] (OR) that exhibited moderate sensitivity to diclofop was also chosen as a diclofop-sensitive comparison, while a collected population (KG) from Northampton Co., VA that exhibited the highest degree of sensitivity to diclofop was chosen as an additional diclofop-sensitive selection. The field where the KG population was collected had no record of diclofop-methyl use.

Greenhouse Experiments

Greenhouse experiments were conducted in late fall 2000 and early spring 2001. In these experiments, 18 seed of each Italian ryegrass biotype were sown 0.6 cm deep in 10- by 10-cm square pots filled with growth medium[2]. Seedlings were watered daily and fertilized weekly with water-soluble fertilizer[3] and maintained under natural lighting for the duration of the experiment. A four by six factorial experiment was used to investigate possible differential response to AE F130060 03 in the Italian ryegrass biotypes. The experiment consisted of treatment at four levels: AE F130060 03 at 15 or 18 g/ha plus AE F107892 at 30 or 36 g ai/ha, respectively, diclofop at 1120 g/ha, or nontreated; and biotype at six levels: diclofop-sensitive OR and KG, and diclofop-resistant EP, GT, RBG, and JB. All treatments were applied POST to three- to four-leaf (8-9 cm) Italian ryegrass of each biotype using a moving-nozzle research spray cabinet[4] containing a single flat-fan nozzle tip[5] that delivered 237 L/ha of spray solution at 269 kPa. Average plant height/pot and visual observations of percent mortality were recorded at 1 wk after treatment (WAT), 2.5 WAT, and 5 WAT. At 5 WAT, plant material from each pot was harvested at soil level, dried for 72 h, and weighed.

In additional greenhouse experiments, response of winter wheat to AE F130060 03 with and without AE F107892 was investigated. In wheat experiments, ten seeds of 'Pioneer 26R24' wheat were sown 0.6 cm deep in 10- by 10-cm square pots filled with growth medium[2]. Data collection and seedling maintenance were identical to Italian ryegrass experiments. Treatments for wheat included AE F130060 03 at 15 or 18 g/ha with AE F107892 at 15 or 18 g/ha, respectively, or with non-ionic surfactant (NIS) at 0.25% (v/v). All greenhouse experiments were arranged in a completely randomized design with three replications of treatments for wheat experiments and six replications for Italian ryegrass experiments. Experiments for wheat and Italian ryegrass were repeated.

Laboratory Experiments

Seed of 'Pioneer 26R24' winter wheat and diclofop-sensitive and –resistant Italian ryegrass biotypes (KG and JB, respectively, from greenhouse experiments) were sown in 10- by 10-cm square pots filled with a 1:1 mixture of vermiculite[6]:sand[7]. Seedlings were watered daily and fertilized weekly with water-soluble fertilizer[3]. When all seedlings reached the one-tiller growth stage, seedlings were removed from soil, roots were washed, and seedlings were transplanted into aluminum-foil-covered glass jars containing 100 mL of quarter-strength Hoagland's solution (pH 6.5). Seedlings were allowed to acclimate to this nutrient solution for 48 h prior to treatment. Treatments for laboratory experiments were AE F130060 00 with or without AE F107892 on winter wheat, and AE F130060 00 with AE F107892 on diclofop-sensitive or diclofop-resistant Italian ryegrass. Radiolabeled AE F130060 00 and technical-grade AE F130060 00, AE F115008, and AE F107892 used in all experiments were provided by Aventis CropScience[8]. Radiolabeled AE F130060 00 was uniformly labeled with ^{14}C in the phenyl ring (radiochemical purity of 95.1% and specific activity of 6425 MBq/g). Prior to foliar application of radiolabeled herbicide, all seedlings received a sublethal dose of non-radiolabeled, technical grade AE F130060 03 [5 g/ha (4.2 g

AE F130060 00 plus 0.8 g AE F115008)] using the moving-nozzle research spray cabinet[4] previously described. This sublethal dose is approximately 33% of the field use rate and was applied to stimulate the metabolism of seedlings without interfering with absorption, translocation, or metabolism of subsequent [^{14}C] AE F130060 00 applications. Eighteen wheat seedlings also received 30 g/ha of technical-grade AE F107892 while the remaining wheat seedlings received NIS[9] at 0.25% vol./vol. All Italian ryegrass seedlings received AE F107892 at 30 g/ha.

Prior to radiolabeled applications, [^{14}C] AE F130060 00 was diluted to an aqueous solution in 80% acetone containing 0.5% (vol./vol.) Tween 20[10]. One h following non-radiolabeled applications, all seedlings were spotted on the newest fully-expanded leaf with 10 µL of radiolabeled solution containing 3.7 ± 0.1 kBq [^{14}C] AE F130060 00. Seedlings of each species and treatment were harvested 12, 36, and 72 h after treatment with [^{14}C] AE F130060 00. At each harvest, 24 seedlings were randomly collected (six wheat seedlings that received the safener AE F107892, six wheat that received only non-ionic surfactant, six diclofop-sensitive Italian ryegrass, and six diclofop-resistant Italian ryegrass). Of the six seedlings collected from each treatment, two plants were used for absorption and translocation, two plants were used for X-ray autoradiography, and two plants were used for metabolism experiments. To determine if exudation of AE F130060 00 out of root tissue occurred, a 1-mL aliquot of Hoagland's solution was collected from each jar at each harvest period, mixed with 10 mL scintillation cocktail[11], and radioactivity was determined using liquid scintillation spectrometry[12] (LSS). Unabsorbed [^{14}C] AE F130060 00 was removed from treated leaves of each seedling at each harvest time by washing the treated leaf in a 5-mL wash solution containing methanol:water (1:1 by vol.) and 0.1% Tween 20. A 1-mL aliquot from each leaf wash was mixed with 10 mL scintillation cocktail, and the total unabsorbed radioactivity was determined by LSS.

Absorption, Translocation, and X-ray Autoradiography

Seedlings collected for absorption and translocation experiments were separated into treated leaf, remaining shoot, and root tissues and dried for 72 h at 60 C. Radioactivity in each portion was determined by LSS with scintillation cocktail after combustion in a biological sample oxidizer[13]. Seedlings used for X-ray autoradiography were pressed for 1 wk and then placed on X-ray film[14] for 21 d.

Metabolism

Seedlings collected for metabolism experiments were separated into treated leaf, remaining shoot, and root tissues and stored at –20 C prior to extraction. For extraction, each plant portion was homogenized in 5 mL of acetonitrile:water (4:1 by vol.) and the insoluble plant material was separated by centrifugation[15] at 14,000 x *g* for 10 min. Extraction and centrifugation were repeated three times, supernatants were pooled, evaporated to dryness with a N-evaporator[16], and redissolved in 400 µL acetonitrile. Two hundred µL of these extracts were then spotted on silica gel thin-layer chromatography (TLC) plates[17], which had been prepared by baking for 1 h at 110 C. Ten µL of [^{14}C] AE F130060 00 and nonlabeled AE F130060 00 standards were cochromatographed with the plant extracts, and the TLC plates were developed in a solvent system of chloroform:absolute ethanol:glacial acetic acid (43:55:5 % by vol.). Following development, TLC plates were dried and observed under ultraviolet light. Radioactivity distribution in lanes of each TLC plate was then scanned using a TLC plate scanner[18] and analyzed using the 13-point cubic peak finder function with <1% peak rejection in accompanying analytical software[19]. Absorption, translocation, and metabolism experiments were arranged in a completely randomized design with two replications for each treatment within each harvest time and all experiments were repeated.

Experimental Design and Analysis

Data from all experiments were subjected to factorial analysis of variance in SAS[20] with factor sums of squares partitioned accordingly (Steel et al. 1997). Factors subject to analysis in greenhouse experiments included herbicide treatment and Italian ryegrass biotype. All laboratory data were also subjected to factorial analysis of variance with partitioning of sums of squares. Factors subject to analysis in laboratory experiments were species (wheat or Italian ryegrass), treatment (AE F107892 or NIS for wheat and diclofop sensitivity or resistance for Italian ryegrass), and time (12, 36, or 72 h). Appropriate means for all data were separated using Fisher's protected LSD (α=0.05). Analysis of variance indicated sufficient homogeneity between greenhouse experiments and between laboratory experiments; therefore, data are presented as averages of the two greenhouse experiments for wheat, the two greenhouse experiments for Italian ryegrass, and the two laboratory experiments.

Results and Discussion

Greenhouse Experiments

Italian ryegrass experiments

Analysis of variance for Italian ryegrass biomass data indicated a significant herbicide treatment by biotype interaction for biomass production; therefore, data are presented accordingly. Levels of diclofop sensitivity and resistance were generally confirmed by biomass production following treatment with diclofop at 1120 g/ha. However, OR was significantly less sensitive to diclofop than KG. Within diclofop-treated Italian ryegrass, diclofop reduced biomass production by 43% in OR and by 69% in KG (Table 4.1). Differential sensitivity between OR and KG biotypes may not be surprising, since OR was a commercially-available cultivar originating from Oregon where diclofop resistance was first reported (Stanger and Appleby 1989) and is now common in

that region. In diclofop-resistant biotypes, however, biomass production was reduced no more than 7% by diclofop.

AE F130060 03 at 15 or 18 g/ha reduced biomass of one diclofop-sensitive biotype (OR) and all diclofop-resistant biotypes more than diclofop. AE F130060 03 at 15 g/ha was as effective as 18 g/ha in reducing biomass of five of the six Italian ryegrass biotypes. Although AE F130060 03 at 15 or 18 g/ha reduced Italian ryegrass biomass at least 35% in all biotypes, differential responses occurred in two diclofop-resistant biotypes. AE F130060 03 at either 15 or 18 g/ha reduced biomass in both diclofop-sensitive populations and diclofop-resistant EP and GT from 61 to 84%. In diclofop-resistant RBG and JB, however, biomass production was reduced only 35 to 52%. These same general trends were also seen in data for percent mortality at 5 WAT (data not presented). In general, diclofop-sensitive KG was the most sensitive to AE F130060 03, while diclofop-resistant JB was the least sensitive. Therefore, these biotypes were selected for absorption, translocation, and metabolism experiments in the laboratory.

Wheat experiments

In wheat experiments, AE F130060 03 at 15 or 18 g/ha without the safener AE F107892 resulted in wheat biomass reductions of 10 to 14% compared to nontreated controls (data not presented). AE F130060 03 at 15 or 18 g/ha with AE F107892 did not reduce wheat biomass compared to nontreated controls.

Laboratory Experiments

Herbicide absorption and translocation

Total absorption of AE F130060 00 was influenced by plant species ($F = 190.72$; $P = <0.0001$) and time ($F = 34.56$; $P = <0.0001$). Since AE F130060 00 absorption was not influenced by herbicide safener in wheat or by biotype in Italian ryegrass, absorption data for wheat were averaged over safener or NIS

and absorption data for Italian ryegrass were averaged over biotype (Figure 4.1). Absorption of [^{14}C] AE F130060 00 by Italian ryegrass was 33% at 12 h after treatment, 61% after 12 h, and 81% after 72 h. In wheat, however, absorption after 72 h was only 23%. Within each harvest time, Italian ryegrass absorbed significantly more [^{14}C] AE F130060 00 than wheat. These differences were approximately five-fold after 12 and 36 h, and more than three-fold after 72 h. Differential absorption is obviously a contributing factor in differential tolerance of winter wheat and Italian ryegrass to AE F130060 00. Baird et al. (1989) also noted differential absorption as a factor in differential tolerance of bahiagrass (*Paspalum notatum* Fluegge) and centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.] to sulfometuron, another sulfonylurea herbicide.

Translocation of AE F130060 00 out of the treated leaf and into shoots and roots was not influenced by plant species, treatment within species, or time. Across all of these factors, amounts of AE F130060 00 remaining in treated leaves was at least 91% of total absorbed AE F130060 00 and total translocation out of the treated leaf never exceeded 9% of total absorbed AE F130060 00 (Table 4.2). Askew et al. (2002) also noted very little translocation of the sulfonylurea herbicide CGA362622 out of treated leaves of tolerant cotton (*Gossypium hirsutum* L.) and jimsonweed (*Datura stramonium* L.), and susceptible peanut (*Arachis hypogaea* L.) and sicklepod [*Senna obtusifolia* (L.) Irwin and Barnaby].

There was no measurable radioactivity present in 1-mL aliquots of Hoagland's solution taken from jars at any harvest period. Therefore, it was concluded that root exudation of AE F130060 00 following treatment was negligible or did not occur.

Herbicide Metabolism

Since translocation of AE F130060 00 out of the treated leaf was no more than 9% of total absorption in any species, treatment, or time, metabolism procedures were conducted only on treated leaf tissue. Metabolism of AE F130060 00 in treated leaves was influenced by species ($F = 56.79$, $P = <0.0001$) and time ($F = 26.39$, <0.0001). Within each species, metabolism in wheat was influenced by the safener AE F107892 ($F = 5.59$, $P = 0.032$) and time ($F = 23.61$, $P = <0.0001$). Metabolism in Italian ryegrass, however, was influenced only by time ($F = 4.59$, $P = 0.028$) and did not differ between diclofop-sensitive and –resistant Italian ryegrass biotypes.

Wheat that received AE F107892 prior to the radiolabeled application contained less parent AE F130060 00 at each harvest time than wheat that received only NIS with no safener prior to application (Table 4.3). Amounts of parent AE F130060 00 in wheat that received AE F107892 were 82.5, 58.5, and 49% of total recovered ^{14}C at 12, 36, and 72 HAT, respectively. In wheat that was not safened prior to radiolabeled application, treated leaves contained 94.9, 66.8, and 58.8% parent AE F130060 00 at 12, 36, and 72 HAT, respectively.

In both Italian ryegrass biotypes, relative amounts of parent AE F130060 00 in treated leaves at 12 and 36 HAT were similar to that in nonsafened wheat at 12 HAT. At 72 HAT, relative amounts of parent AE F130060 00 were 86.3 and 92.2% in diclofop-sensitive and –resistant Italian ryegrass, respectively. Amounts of parent AE F130060 00 in either Italian ryegrass biotype was nearly 1.5 times more than that in unsafened wheat and nearly 1.8 times more than that in safened wheat at 72 HAT. Although amounts of parent AE F130060 decreased from 97.4% at 12 HAT to 86.3% at 72 HAT in diclofop-sensitive Italian ryegrass, relative amounts of parent herbicide did not decrease significantly over time in diclofop-resistant Italian ryegrass. However, amounts of parent herbicide in treated leaves of either biotype were similar at each harvest time.

Based on metabolism data, it can be concluded that differences in metabolism also play a role in differential tolerance to AE F130060 00 between winter wheat and Italian ryegrass and that the safener AE F107892 increases metabolism of AE F130060 00 in winter wheat. Although there were generally no differences in AE F130060 00 metabolism between the two Italian ryegrass biotypes, relative amounts of parent herbicide were at least 1.5 times greater in Italian ryegrass than in wheat at 72 HAT. However, metabolism data are relative amounts of recovered ^{14}C that were attributed to parent AE F130060 00 and one must also consider the substantial differences in overall absorption of AE F130060 00 that occurred between winter wheat and Italian ryegrass.

Based on laboratory data, differential tolerance between winter wheat and Italian ryegrass is due to differential absorption as well as differential metabolism between these two species. Although absorption was similar between wheat treated with AE F107892 or NIS only and between diclofop-sensitive and –resistant Italian ryegrass, Italian ryegrass absorbed at least three-fold more AE F130060 00 than wheat at any harvest time. Differential tolerance was not due to differential translocation of AE F130060 00 since at least 91% of the herbicide remained in the treated leaves of both species. Metabolism experiments showed that AE F130060 00 is more rapidly degraded in wheat than in Italian ryegrass and that the most rapid degradation occurs in wheat that is safened with AE F107892. However, there were generally no differences in AE F130060 00 metabolism between the diclofop-sensitive and diclofop-resistant Italian ryegrass biotypes used in the laboratory experiment.

In the vast majority of cases of diclofop resistance in grass species, resistance is due to an altered ACCase that is no longer sensitive to diclofop (Cocker et al. 2001; Devine 1997; Evenson et al. 1994; Gronwald et al. 1992; Joseph et al. 1990; Maneechote et al. 1997; Marles et al. 1993; Masooji et al. 1992). However, other mechanisms may exist, such as overproduction of ACCase (Bradley et al. 2001). Conversely, resistance to the sulfonylurea herbicide

chlorsulfuron in annual ryegrass is not associated with an insensitive ALS but was due to enhanced metabolism of the herbicide (Christopher et al. 1991; Matthews et al. 1990; Preston et al. 1996). In other research, Burnet et al. (1994) reported that a population of annual ryegrass (VLR69) resistant to several ALS-inhibiting herbicides including imazaquin, chlorsulfuron, triasulfuron, and sulfometuron contained a subpopulation that possessed an ALS that was less sensitive to inhibition by chlorsulfuron than ALS extracted from susceptible plants. In another annual ryegrass biotype, WLR1, the basis for resistance to sulfometuron was also a less-sensitive target enzyme, although this biotype also had increased capacity to detoxify chlorsulfuron (Christopher et al. 1992). In the research reported here, however, obvious differences in the metabolism of AE F130060 00 between the two Italian ryegrass biotypes were not evident.

We hypothesize that differential response to AE F130060 03 between the diclofop-sensitive KG and diclofop-resistant JB Italian ryegrass biotypes is likely due to an altered ALS in JB that is less sensitive to AE F130060. Alteration of the target enzyme ALS is the most common mechanism of resistance in several ALS-inhibitor-resistant weed species including kochia (*Kochia scoparia* L.), prickly lettuce (*Lactuca serriola* L.), Russian thistle (*Salsola iberica* L.), perennial ryegrass (*Lolium perenne* L.), and common chickweed [*Stellaria media* (L.) Cyrillo] (Mallory-Smith et al. 1990; Primiani et al. 1990; Saari et al. 1989a, 1989b). The JB biotype could contain both an altered ACCase and an altered ALS that confers decreased sensitivity to both diclofop and AE F130060. Further research is required to confirm this hypothesis.

Sources of Materials

[1]Oregon Grown Premium Quality Grass Seed. Italian ryegrass, variety not stated. Wetsel, Inc., 1345 Diamond Springs Road, Virginia Beach, VA 76618.

[2]Metro-Mix 500, Scotts-Sierra Horticultural Products Co., Marysville, OH 43040.

[3]Peters Professional General Purpose 20-20-20. Scotts-Sierra Horticultural Products Company, 14111 Scottslawn Rd., Marysville, OH 43041.

[4]Allen Machine Works. 607 E. Miller Road, Midland, MI 48640.

[5]Spraying Systems Co.® P. O. Box 7900, Wheaton, IL 60189.

[6]Horticultural vermiculite sterile growing media, medium grade. The Schundler Company, P. O. Box 513, Metuchen, NJ 08840-0513.

[7]Quikrete all-purpose sand. The Quikrete Companies, Atlanta, GA 30329.

[8]Aventis Pharma, Chem. Dev./Radiosynthesis Analytical Lab. Frankfurt, Germany.

[9]X-77 spreader, a mixture of alkylarylpolyoxyethylene glycols, free fatty acids, and isopropanol. Valent USA Corporation, 1333 North California Boulevard, Walnut Creek, CA 94596-8025.

[10]Polysorbate 20 (polyoxyethylene [20] sorbitan monolaurate), ICI America, Inc., Wilmington, DE 19899.

[11]Scintiverse BD scintillation cocktail. Fisher Scientific, Fair Lawn, NJ 07410.

[12]Liquid scintillation counter, Beckman LS 5000TA model, Beckman Instruments, 4300 N. Harbor Boulevard, Fullerton, CA 92634.

[13]Packard Model 307 biological oxidizer, Packard Instrument Co. 2200 Warrenville Road, Downer’s Grove, IL 60515.

[14]X-OMAT diagnostic film, Eastman Kodak Company, Rochester, NY 14650.

[15]Sorvall RC-58 refrigerated superspeed centrifuge with SS-34 rotor. Sorvall-Kendro Laboratory Products, L.P., 31 Pecks Lane, Newtown, CT 06470-2337.

[16]Meyer N-EVAP analytical evaporator. Organomation Associates, Inc., P. O. Box 5 Tpk. Sta., Shrewsbury, MA 01545.

[17]Silica Gel 60F_{254} precoated glass plates for thin layer chromatography. EM Science, 480 Democrat Road, Gibbstown, NJ 08027.

[18]BioScan System 200 Imaging Scanner with BioScan Autochanger 1000. BioScan, Inc. 4590 MacArthur Blvd. N.W., Washington, DC 20007.

[19]Win-Scan Software, ver. 1.6. LabLogic, St. Johns House, 131 Psalter Lane, Sheffield S11 8UX, England.

[20]Statistical Analysis Systems (SAS) software, Version 7.0, SAS Institute, Inc., Box 8000, SAS Circle, Cary, NC 27513.

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Table 4.1. Interaction of herbicide treatment and Italian ryegrass biotype on biomass production of diclofop-sensitive and –resistant Italian ryegrass.[a]

		Biomass production						
		Italian ryegrass biotype						
Herbicide	Application	Diclofop-sensitive		Diclofop-resistant				
treatment	rate	OR	KG	EP	GT	RBG	JB	LSD (0.05)[d]
	g/ha	% of nontreated control[b]						
AE F130060 03[c]	15	39	27	29	27	53	65	14
AE F130060 03	18	21	23	16	20	52	48	14
Diclofop	1120	57	31	93	97	100	94	25
	LSD (0.05)[e]	28	NS	13	15	18	20	

[a]Values represent the mean of two experiments with six replications per experiment.

[b]Data presented as the percent of dry weight of nontreated controls for each population.

[c]AE F130060 03 applications made with AE F107892 at 30 or 36 g/ha.

[d]LSD values for rows within a level of herbicide treatment.

[e]LSD values for columns within a level of Italian ryegrass population.

Table 4.2. Translocation of [^{14}C] AE F130060 00 in winter wheat and Italian ryegrass.

Species	Treatment	HAT	Absorbed [^{14}C] AE F130060		
			Treated Leaf	Shoots	Roots
			% of absorbed[ab]		
Wheat	Safener	12	94.9 ± 1.8 a	2.9 ± 1.1 b	2.2 ± 0.8 b
Wheat	Safener	36	91.0 ± 4.5 a	5.1 ± 2.2 b	3.9 ± 2.4 b
Wheat	Safener	72	94.8 ± 0.7 a	4.0 ± 0.6 b	1.2 ± 0.3 c
Wheat	No safener	12	91.8 ± 2.0 a	4.3 ± 2.1 b	3.9 ± 0.4 b
Wheat	No safener	36	93.4 ± 2.2 a	4.6 ± 1.8 b	2.0 ± 0.7 b
Wheat	No safener	72	95.0 ± 1.4 a	4.1 ± 1.2 b	0.8 ± 0.3 b
Italian ryegrass	Diclofop-sensitive	12	94.8 ± 2.2 a	3.8 ± 1.8 b	1.4 ± 0.5 b
Italian ryegrass	Diclofop-sensitive	36	96.9 ± 0.7 a	2.4 ± 0.7 b	0.7 ± 0.2 b
Italian ryegrass	Diclofop-sensitive	72	97.7 ± 0.6 a	1.9 ± 0.5 b	0.4 ± 0.2 b
Italian ryegrass	Diclofop-resistant	12	96.9 ± 0.4 a	1.8 ± 0.3 b	1.2 ± 0.2 b
Italian ryegrass	Diclofop-resistant	36	97.6 ± 0.6 a	1.4 ± 0.2 b	0.9 ± 0.4 b
Italian ryegrass	Diclofop-resistant	72	97.1 ± 0.3 a	2.2 ± 0.2 b	0.7 ± 0.2 c

[a]Values represent the mean of two experiments ± standard error as a percent of absorbed radioactivity.

[b]Means followed by the same letter within a row do not differ according to Fisher’s protected LSD (α=0.05).

Table 4.3. Metabolism of AE F130060 00 in winter wheat and Italian ryegrass as influenced by time, herbicide safener, and Italian ryegrass biotype.

Harvest time (HAT[c])	Parent [^{14}C] AE F130060 00[ab]			
	Winter wheat		Italian ryegrass	
	Safener	No safener	Diclofop-sensitive	Diclofop-resistant
	% of ^{14}C recovered[d]			
12	82.5 ± 7.3 aB	94.9 ± 4.1 aA	97.4 ± 0.9 aA	98.3 ± 1.0 aA
36	58.5 ± 3.5 bA	66.8 ± 7.4 bA	92.7 ± 0.3 bA	93.4 ± 2.0 aA
72	49.0 ± 1.5 bB	58.8 ± 3.1 bA	86.3 ± 1.4 cA	92.2 ± 3.6 aA

[a]Means represent the pooled average of two experiments as a percentage of total radioactivity attributed to parent AE F130060 00 ± standard errors. For time comparisons, means within a column followed by the same lowercase letter do not differ according to Fisher's protected LSD (α=0.05). For treatment comparisons within species, means within a row within each harvest time and species followed by the same uppercase letter do not differ according to Fisher's protected LSD (α=0.05).

[b]R_f value of parent AE F130060 00 was 0.85 in the solvent system used (chloroform:ethanol:glacial acetic acid, 43:55:5 % by vol.).

[c]HAT = h after treatment.

[d]Metabolism data presented as a percentage of recovered radioactivity from treated leaves attributed to parent AE F130060 00.

CAPTIONS FOR FIGURES

Figure 4.1. Absorption of [^{14}C] AE F130060 00 by winter wheat and Italian ryegrass. Data for winter wheat are averaged over wheat treated with AE F107892 (safener) or with non-ionic surfactant (no safener). Data for Italian ryegrass are averaged over diclofop-sensitive and –resistant populations. Asterisks denote a difference at the 0.05 significance level.

Bailey, *Figure 4.1.*