AN ABSTRACT OF THE DISSERTATION OF

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Abstract approved

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Carol A. Mallory-Smith

Understanding the mechanism of resistance, relative fitness and competitiveness of herbicide resistant biotypes is important to predict population dynamics and to establish resistance management strategies. This study was conducted to determine the level of resistance to acetolactate synthase (ALS) inhibitors and the mechanism of resistance of ALS-inhibitor resistant downy brome (*Bromus tectorum* L.) biotypes from Athena and Madras, Oregon (AR and MR). Based on the whole plant dose response test and ALS assay, the AR biotype was highly resistant to the sulfonylurea (SU) and sulfonylaminocarbonyl- triazolinone (SCT) herbicides but was not resistant to an imidazolinone (IMI) herbicide. DNA sequence analysis of the *als* gene in the AR biotype demonstrated a single-point mutation of the amino acid Pro_{197} to Ser. The MR biotype was moderately resistant to all ALS inhibitors tested. However, no differences in ALS sensitivity and *als* gene sequence were observed in the MR

biotype. Studies using ^{14}C-BAY MKH 6561, a SCT herbicide, showed that the half-life of BAY MKH 6561 in the MR biotype was 8.9 h which was 40% shorter than that in the Madras susceptible (MS) biotype. These data indicate that the mutation in the *als* gene is responsible for the SU and SCT herbicide resistance in the AR biotype but the relatively rapid metabolism is the mechanism of resistance for the MR biotype. Seed germination, plant growth, seed production, and competitiveness of the AR and MR biotypes were investigated and compared to their respective susceptible biotypes as components of fitness. Seeds of the AR biotype germinated 27 h earlier than seeds of the Athena susceptible (AS) biotype and reached over 60% germination when the AS biotype initially germinated at 5 C. No differences in seed germination were observed between the MR and MS biotypes at any temperature tested. Growth of the AR biotype was similar to the AS biotype under competitive and noncompetitive conditions. However, the MR biotype was less fit than the MS biotype in growth, seed production, and competitive ability. Seed production of the AR and MR biotypes was 83 and 71%, respectively, when compared to the AS and MS biotypes. Although the AR biotype produced 17% less seed than the AS biotype, the AR biotype could dominate the early season weed population, because of its early germination at low temperature and large seed size. Therefore, it is very difficult to predict population dynamics of the AR biotype in the weed population. However, the MR biotype should decrease and the population shift toward higher frequency of the MS biotype in the absence of ALS inhibitors because the MR biotype is less fit and competitive than the MS biotype.

ALS-inhibitor Resistant Downy Brome (*Bromus tectorum* L.) Biotypes in Oregon: Mechanism of Resistance, Fitness, and Competition

by

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CONTRIBUTION OF AUTHORS

Dr. Carol A. Mallory-Smith proposed, advised, and supervised all aspects of the projects. She substantially contributed to the ideas, hypotheses, interpretation of data and suggestions to the manuscripts. Dr. George W. Mueller-Warrant provided resistant downy brome seeds and edited successive drafts of the thesis chapters. Dr. Daniel A. Ball also provided resistant downy brome seeds and contributed to the ideas discussed in this work. Lynn Fandrich collaborated during the execution of the experiments in the metabolism study and reviewed the draft of the manuscript.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

Page

LIST OF FIGURES

LIST OF FIGURES (Continued)

Figure Page

LIST OF TABLES

ALS-inhibitor Resistant Downy Brome (*Bromus tectorum* L.) Biotypes in Oregon: Mechanism of Resistance, Fitness, and Competition

CHAPTER 1

General Introduction

HERBICIDE RESISTANT WEEDS

Herbicide resistance is defined as "the inherited ability of a biotype to survive and reproduce following exposure to a dose of herbicide that is normally lethal to the wild type" (Mallory-Smith, personal communication). There is no evidence that herbicides cause the genetic mutations that lead to herbicide resistance. Rather herbicide resistant weeds exist naturally in extremely small numbers within a population. These resistant plants contain a slightly different genetic makeup but remain reproductively compatible with the wild-type (Mallory-Smith et al. 1993). The continuous use of the same herbicide or herbicides acting on the same target site leads to the selection of herbicide resistant weed populations (Maxwell and Mortimer 1994). If the same herbicide is continuously used, the number of resistant weeds increases in the population. The selected resistant weeds grow and reproduce without competition with wild-type in the population. The first report in 1968 of a herbicide resistant weed was common groundsel (*Senecio vulgaris* L.) with resistance to triazine (Ryan 1970). Herbicide resistance has been reported to most of herbicide classes (18 classes) and in 163 weed species (Heap 2003).

Since commercialized in 1982, acetolactate synthase (ALS) inhibitor herbicides have been widely used in many cropping systems because of a broad spectrum of weed control activity, wide crop selectivity, relatively low usage rates, and low mammalian toxicity. However, many ALS-inhibitor resistant weed populations have been reported. The first resistance to ALS inhibitor herbicides was reported in prickly lettuce (*Lactuca serriola* L.) in 1987, only five years after the introduction of sulfonylurea (SU) herbicides (Mallory-Smith et al. 1990). ALS-inhibitor resistant kochia (*Kochia scoparia* L.) biotypes were reported at several sites soon after the identification of resistant prickly lettuce (Primiani et al. 1990). By 2003, there were 79 ALS-inhibitor resistant weed species (Heap 2003).

ALS INHIBITOR HERBICIDES

Since the introduction of SU herbicides, four other chemical classes of ALS inhibitor herbicides have been commercialized or are in development: imidazolinone (IMI), triazolopyrimidine (TP), pyrimidinyl thiobenzoate (PTB) and sulfonylamino-carbonyltriazolinone (SCT) herbicides (Tranel and Wright 2002; Heap 2003). Currently, more than 50 commercial ALS inhibitor herbicides are being used for selective weed control in many grass and broadleaf crop systems (Tranel and Wright 2002). The primary target site of these herbicides, ALS, catalyzes the condensation step of two molecules of pyruvate to form acetolactate in the biosynthetic pathway of the branched-chain amino acids, leucine, isoleucine, and valine (De Felice et al. 1974; Ray 1984).

MECHANISM OF ALS INHIBITOR RESISTANCE

Several mechanisms are responsible for herbicide resistance in plants. Reduced herbicide absorption or translocation, rapid herbicide metabolism, or insensitivity of the target site are possible mechanisms of resistance. Reduced absorption or translocation of ALS inhibitor herbicides does not appear to significantly contribute to resistance in crop or weed species (Saari et al. 1990; Anderson et al. 1998).

Metabolism

Metabolism is the most important mechanism in crop selectivity and naturally occurring tolerance to ALS inhibitor herbicides (Saari et al. 1994). Crop selectivity or naturally occurring weed tolerance to an ALS inhibitor herbicide is due to the plant's ability to metabolize an ALS inhibitor herbicide to nonphytotoxic compounds. The most common chemical reactions involved in crop selectivity to ALS inhibitor herbicides are hydroxylation, *O*-dealkylation, and deesterification (Brown et al. 1991). It has been reported that cytochrome P450 enzymes (P450s) are responsible for the hydroxylation reactions of ALS inhibitor herbicides in wheat and maize (Fear et al. 1991; Fonne-Pfister et al. 1990).

P450s are a heme-containing superfamily of enzymes in plants responsible for catalyzing NADPH- and O_2-dependent hydroxylation reactions of a wide range of structurally diverse substrates, including herbicides (Chapple 1998). P450s are recognized as the most important enzyme system for herbicide metabolism and selectivity in crops. The involvement of P450s in herbicide metabolism can be studied by inhibiting P450s with known P450 inhibitors such tetcyclasis, 1-

aminobenzotriazole (1-ABT), piperonyl butoxide (PBO) and certain organophosphate insecticides.

In contrast to ALS inhibitor metabolism and selectivity in many crops, metabolism based resistance have been demonstrated in only three weed species; annual ryegrass (*Lolium rigidum* Gaud.) from Australia, blackgrass (*Alopecurus myosuroides*) in Europe, and wild mustard (*Sinapis arvensis* L.) in Canada (Christopher et al. 1991; Menendez et al. 1997; Veldhuis et al. 2000).

Christopher et al. (1991) found that a biotype of annual ryegrass (SLR31) was resistant to the sulfonylurea herbicide chlorsulfuron, despite having a herbicide-sensitive target site. This biotype was originally selected by diclofop-methyl but was cross-resistant to several sulfonylurea and imidazolinone herbicides. Research using ^{14}C-chlorsulfuron showed that this biotype metabolized the herbicide at a faster rate than a susceptible biotype. The major chlorsulfuron metabolite was a sugar conjugate of hydroxylchlorsulfuron, formed following hydroxylation of chlorsulfuron in the phenyl ring. In seedlings of the resistant biotype, 84% of chlorsulfuron was metabolized 9 hours after herbicide treatment. However, when the herbicide was applied in combination with malathion, only 13% was metabolized 9 hours after herbicide treatment (Christopher et al. 1994). The authors suggested that a cytochrome P450 system might be responsible for the detoxification of chlorsulfuron in the annual ryegrass. Similar results were shown in an ethametsulfuron-methyl resistant wild mustard biotype. PBO, an inhibitor of cytochrome P450, decreased

ethametsulfuron-methyl metabolism by 20% in the resistant biotype (Veldhuis et al. 2000).

Target site

In most cases, resistance to ALS inhibitor herbicides is caused by an insensitive target site. Since the first target site resistance to ALS inhibitor herbicides was identified in kochia (*Kochia scoparia*), the physiological and molecular basis for ALS inhibitor resistance has been studied in many other crops and weeds (Saari et al. 1990; Heap 2003). The ALS gene of higher plants has an open reading frame of approximately 670 amino acids including a chloroplast transit peptide (CTP) (Tranel and Wright 2002). The ALS protein is encoded in nuclear DNA and translated in the cytosol as a precursor with an N-terminal CTP of approximately 85 amino acids that targets the protein to the chloroplast. After translocation of the precursor protein through the chloroplast envelope, the CTP is cleaved to form the mature ALS enzyme (Bernasconi et al. 1995; Raikhel and Chrispeels 2000). The length of ALS gene often varies among plant species because of non-conserved additions and deletions.

In order to avoid any confusion and to directly compare ALS amino acid number among plant species, it has been recommended that the corresponding amino acid sequence of Arabidopsis precursor ALS be used. The mature ALS proteins have five highly conserved domains in higher plants (Tranel and Wright 2002). It has been reported that a single amino acid substitution in any of the domain can confer resistance to ALS inhibitors. Domain A consists of 13 amino acids (AITGQVPRRMI GT) and is located near the N-terminal end of ALS gene. Almost all possible amino

acid substitutions at this site have been found in weed species (Tranel and Wright 2002). Six different amino acid substitutions for Pro_{197} have been identified in kochia biotypes resistant to ALS inhibitors. Pro_{197} mutations in the domain A of the ALS gene confer high level of resistance to SU herbicides. However, the level of resistance to IMI herbicides depends on the amino acids substituted for Pro_{197}. For example, a substitution of Pro_{197} to His, Leu or Ile conferred low to moderate level of resistance to IMI herbicides. However, Ser or Ala substitution conferred no resistance (Devine and Preston 2000). Amino acid substitution in other domains confers different patterns of cross-resistance. Trp_{574} to Leu substitution in domain B (QWED) confers very high levels of resistance to all classes of ALS inhibitors in many weed species (Tranel and Wright 2002). In contrast, the substitution of Ala_{122} or Ser_{653} confers high level of resistance to IMI herbicides but not to SU herbicides (Bernasconi et al. 1995; Patzoldt and Tranel 2001). Ala_{205} to Val mutation in domain D has been reported only in common cocklebur (*Xanthium strumarium* L.). This biotype was resistant to both SU and IMI herbicides but the levels of resistance were low (approximately 10-fold) (Woodworth et al. 1996). In addition to these point mutations, double mutations have been reported in tobacco mutant and sugarbeet somatic cell selections (Creason and Chaleff 1988; Wright et al. 1998). Mutations at both Ala_{122} and Pro_{197} of sugarbeet ALS conferred higher resistance levels to both SU and IMI herbicides than observed with either of the single mutations (Wright et al 1998).

FITNESS

Fitness of the herbicide resistant and susceptible biotypes has been identified as an important factor influencing the evolution and dynamics of herbicide resistance (Maxwell et al. 1990). Fitness is the evolutionary success of a phenotype, based on its survival and reproductive success (Radosevich et al. 1997). The relative fitness of phenotypes is determined by the survivorship of seeds, seedlings, and mature plants, in addition to reproductive success determined by pollen and seed production (Maxwell et al. 1990). When herbicides are used, the number of resistant individuals will increase in the population due to selection pressure. However, the population dynamics will be determined by the relative fitness of each biotype in the absence of herbicides. Jasieniuk et al. (1996) demonstrated that small amount of difference in fitness in the absence of herbicide selection could affect the initial frequency of resistant biotypes by 100-fold or more.

Though there are a few exceptions, triazine resistant biotypes are generally less fit than susceptible biotypes in the absence of triazine herbicides (Holt and Thill 1994). Reduced rates of PSII electron transport, quantum yields, biomass production, and competitiveness are characteristics of triazine resistant biotypes. In contrast to triazine resistant biotypes, no consistent differences in the relative fitness and competitiveness of ALS-inhibitor resistant biotypes have been measured (Saari et al. 1994).

Seed germination

Seed germination studies have been conducted using ALS-inhibitor resistant prickly lettuce and near isogenic lines of Bibb lettuce (Alcocer-Ruthling et al. 1992b;

Mallory-Smith et al. 1992). Seeds of resistant biotypes always germinated faster than susceptible biotypes. Seeds of a SU resistant kochia population also germinated faster than seeds from a susceptible population under low temperature conditions (Dyer et al. 1993; Thompson et al. 1994a). Dyer et al. (1993) proposed that a higher concentration of branched chain amino acids in seeds of the resistant population appeared to be correlated with the faster germination.

Growth, seed production, and competition

Addition series experiments were conducted to compare the relative competitiveness and growth rate of sulfonylurea herbicide resistant and susceptible prickly lettuce (Alcocer-Ruthling et al. 1992a). The susceptible biotype produced 31% more aboveground biomass and accumulated biomass 52% faster than the resistant biotype. However, the relative competitiveness appeared to be equal for both biotypes. No difference in seed production was reported between SU resistant and susceptible prickly lettuce biotypes (Alcocer-Ruthling et al 1992b). The relative fitness and competitiveness of SU resistant kochia biotypes have been compared with the susceptible biotypes (Thompson et al. 1994b). The resistant biotype had similar growth rates, seed production, and competitiveness when compared to the susceptible biotypes. Christoffoleti et al. (1992) demonstrated similar growth rate and seed production results from other SU resistant and susceptible kochia biotypes.

Catalytic competency

K_m values (pyruvate) and specific activity for ALS have been studied in weed species. There were no differences in K_m values for pyruvate using ALS isolated from

resistant and susceptible biotypes indicating there was no alteration in the pyruvate binding site on the ALS enzyme (Eberlein et al. 1999; Saari et al. 1992). However, K_m value of chlorsulfuron resistant sacred datura (*Datura innoxia* Mill) was higher than the susceptible biotype (Rathinasabapathi and King 1991). Specific activity for ALS from the resistant *Lactuca sativa* 'Bibb' lines was 46% lower than the susceptible biotype suggesting detrimental effects on enzyme function, expression, or stability (Eberlein et al. 1999).

ALS-INHIBITOR RESISTANT DOWNY BROME BIOTYPES

Downy brome (*Bromus tectorum* L.) is an important weed in several agricultural production systems throughout the Pacific Northwest and central U.S. Great Plains (Morrow and Stahlman 1984). SU herbicides have been used successfully to control downy brome in winter wheat and Kentucky bluegrass (*Poa pratensis*) fields (Geier et al. 1998; Hendrickson and Mallory-Smith 1999). However, within a 3 to 4-yr period of primisulfuron application, resistant downy brome biotypes evolved in Kentucky bluegrass research plots at Madras and seed fields at Athena, Oregon in 1997 and 1998, respectively (Mueller-Warrant et al. 1999; Ball and Mallory-Smith, 2000). Downy brome biotypes resistant to simazine, a photosystem II inhibitor, were reported in France and Spain in 1981 and 1990, respectively, but SU herbicide resistant downy brome has not been reported previously (Heap 2003).

OBJECTIVES

The goal of this research was to understand the resistance mechanism and relative fitness of SU herbicide resistant downy brome biotypes from two locations. The research had the following specific objectives:

1. To determine whether the primisulfuron-resistant downy brome biotypes were cross-resistance to other ALS inhibitor herbicides and to quantify the level of resistance to ALS inhibitor herbicides.
2. To examine the potential for an altered target site or enhanced metabolism as the mechanism of resistance of the two resistant downy brome biotypes.
3. To compare the relative fitness and competitiveness of SU resistant and susceptible downy brome biotypes in terms of seed germination, growth rate, and seed production.

CHAPTER 2

Physiological and Molecular Basis for ALS Inhibitor Resistance in Downy Brome (*Bromus tectorum* L.) Biotypes

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ABSTRACT

Primisulfuron-resistant (AR and MR) and -susceptible (AS and MS) downy brome biotypes were collected from a Kentucky bluegrass (*Poa pratensis*) seed field at Athena (AR and AS) and in research plots at Madras (MR and MS), Oregon. Studies were conducted to investigate the level of resistance and cross-resistance to other acetolactate synthase (ALS) inhibitors, and to determine the physiological and molecular basis for herbicide resistance. Whole plant bioassay studies revealed that the AR biotype was 317- and 263-fold more resistant than the AS biotype to the sulfonylurea (SU) herbicides, primisulfuron and sulfosulfuron, respectively. The AR biotype also was 425-fold more resistant than the AS biotype to BAY MKH 6561, a sulfonylaminocarbonyltriazolinone (SCT) herbicide. However, the AR biotype was not resistant to imazamox, an imidazolinone (IMI) herbicide. This cross-resistance pattern for the AR biotype was consistent with the results from the ALS enzyme assays for the AR biotype. The level of resistance for the MR biotype was 18, 9, 40, and 14 times higher than the MS biotype to primisulfuron, sulfosulfuron, BAY MKH 6561, and imazamox, respectively. However, there was no difference in ALS sensitivity to the ALS inhibitors used in these studies between the MR and MS biotypes. The nucleotide and amino acid sequence analysis of the *als* gene demonstrated a single-point mutation from C to T, conferring the exchange of the amino acid proline to serine at position 197 in the AR biotype. However, this point mutation in the *als* gene was not found in the MR biotype. This research indicates that the resistance of the AR biotype to SU and SCT herbicides is based on an altered

target site due to a point mutation in the *als* gene, while resistance in the MR biotype is a non-target site mechanism, but likely herbicide metabolism.

Nomenclature: Primisulfuron; sulfosulfuron; BAY MKH 6561; imazamox; downy brome, *Bromus tectorum* L. BROTE

Key words: Acetolactate synthase, herbicide resistance, cross-resistance, BROTE

INTRODUCTION

The continuous use of the same herbicide or herbicides acting on the same target site leads to the selection of herbicide resistant weed populations (Maxwell and Mortimer 1994). Since the first triazine resistant weed, common groundsel (*Senecio vulgaris* L.), was discovered, herbicide resistance has been reported to most of herbicide classes (18 classes) and in 163 weed species (Ryan 1970; Heap 2003).

Acetolactate synthase (ALS) inhibitors are one of the most important herbicide classes used in many cropping systems because of a broad spectrum of weed control activity, wide crop selectivity, and relatively low usage rates. There are five structurally diverse chemical classes of ALS inhibitors: sulfonylurea (SU), imidazolinone (IMI), triazolopyrimidines (TP), pyrimidinyl thiobenzoates (PTB) and sulfonylaminocarbonyltriazolinone (SCT) herbicides (Tranel and Wright 2002; Heap 2003). The primary target site of these herbicides, ALS, catalyzes the condensation step of two molecules of pyruvate to form acetolactate in the biosynthetic pathway of branched-chain amino acids, leucine, isoleucine, and valine (DeFelice et al. 1974; Ray 1984).

The first resistance to ALS inhibitors was reported in prickly lettuce (*Lactuca serriola* L.) in 1987, only five years after introduction of the SU herbicides (Mallory-Smith et al. 1990). Since then, 79 weed species have developed resistance to ALS inhibitors (Heap 2003). An insensitive target site is the most common resistance mechanism reported in ALS-inhibitor resistant weed species. Since the first target site resistance to ALS inhibitors was identified in kochia (*Kochia scoparia*), the

physiological and molecular basis for ALS inhibitor resistance has been studied in many weeds (Saari et al. 1990; Heap 2003).

The length of the ALS gene often varies among plant species because of non-conserved additions and deletions. However, mature ALS proteins have five separate highly conserved domains (Domain A to E) in higher plants (Boutsalis et al. 1999). In all cases, a single amino acid substitution in each domain (as underlined in the following peptide sequences) is sufficient to confer resistance to ALS inhibitors in target site based resistant weeds. Domain A consists of 13 amino acids (AITGQV<u>P</u>RRMIGT) and is located near the N-terminal end of ALS gene. All possible amino acid substitutions at Pro_{197} in domain A have been reported in weed species (Tranel and Wright 2002). Six different amino acid substitutions in Pro_{197} have been identified in several different kochia biotypes resistant to ALS inhibitors (Guttieri et al. 1995). Pro_{197} mutations in the domain A of ALS gene confer high level of resistance to SU herbicides. However, the level of resistance to IMI herbicides depends on which amino acids are substituted for Pro_{197}. For example, a substitution of Pro_{197} to His, Leu or Ile conferred low to moderate levels of resistance to IMI herbicides. However, Ser or Ala substitutions conferred no resistance to IMI herbicides (Devine and Preston 2000). Amino acid substitution in other domains confers different patterns of cross-resistance. Trp_{574} to Leu substitution in domain B (Q<u>W</u>ED) confers very high levels of resistance to all classes of ALS inhibitor in many weed species (Tranel and Wright 2002). In contrast, the substitution of Ala_{122} in domain C (VFAYPGG<u>A</u>SMEIHQALTRS) or Ser_{653} in domain E (IP<u>S</u>GG) confers

high levels of resistance to IMI herbicides but not to SU herbicides (Bernasconi et al. 1995; Patzoldt and Tranel 2001). Ala_{205} to Val mutation in domain D (AFQETP) has been reported only in common cocklebur (*Xanthium strumarium* L.). This biotype was resistant to all classes of ALS inhibitor but the levels of resistance were low (approximately 10-fold) (Woodworth et al. 1996).

In addition to these point mutations, double mutations have been reported in tobacco mutant and sugarbeet (*Beta vulgaris*) somatic cell selections (Creason and Chaleff 1988; Wright et al 1998). Two mutations in Ala_{122} in domain C and Pro_{197} in domain A of sugarbeet *als* gene conferred higher resistance levels to both SU and IMI herbicides than observed with either of the single mutations (Wright et al 1998).

Downy brome (*Bromus tectorum* L.) is an important weed in several agricultural production systems throughout the Pacific Northwest and central U.S. Great Plains (Morrow and Stahlman 1984). SU herbicides have been used successfully to control downy brome in winter wheat and Kentucky bluegrass fields (Geier et al. 1998; Hendrickson and Mallory-Smith 1999). However, within a 3 to 4-yr period of primisulfuron application, resistant downy brome biotypes evolved in a Kentucky bluegrass (*Poa pratensis*) research plots at Madras and seed fields at Athena, Oregon in 1997 and 1998, respectively (Mueller-Warrant et al. 1999; Ball and Mallory-Smith, 2000).

The objectives of this research were: to 1) characterize the level of resistance to different classes of ALS inhibitors (SU, SCT, and IMI) and 2) determine the

mechanism and molecular basis for resistance in ALS-inhibitor resistant downy brome biotypes.

MATERIALS AND METHODS

Seed source

Seeds of the Madras and Athena downy brome biotypes were collected in July 1997 and 1998, respectively. The seed samples were screened initially in the greenhouse to confirm resistance or susceptibility to primisulfuron at 40 g ai/ha (data not shown). The seeds of resistant and susceptible biotypes were vernalized at 5 C for 6 weeks and then 10 plants from each biotype were grown in the greenhouse under 25/20 C with a 16-h photoperiod. Seeds were collected after plants senesced and were combined by biotype. The seeds were cleaned and stored at room temperature until experimentation.

Whole plant bioassay

Greenhouse studies were conducted to evaluate the resistance of downy brome to four ALS inhibitors. Seeds of two primisulfuron-resistant downy brome biotypes (AR and MR) and two susceptible biotypes (AS and MS) were planted in 98 cell trays (26 ml/cell) containing commercial potting mix[1]. Plants were grown in a greenhouse with 16-h supplemental lighting and 25/20 C day/night temperature. Fourteen d later, individual plants were transplanted into 267 ml plastic pots (6 by 6 cm) containing commercial potting mix[2]. Herbicides were applied when the plants were in the 3- to 4-lf stage using an 8002 even flat fan nozzle and overhead compressed air sprayer calibrated to deliver 187 L/ha. A nonionic surfactant[3] at 0.25% (v/v) was added to all treatments. Shoots were harvested 2 wk after treatment, dried at 60 C for 48 hrs, and

weighed. The experiment was in a completely randomized design with four replications and was repeated.

ALS assay

Seedlings of the resistant and susceptible downy brome biotypes were grown in the greenhouse, as described previously. Two wk after planting, the crude enzyme was extracted from the leaves. The method for ALS extraction and assay were as described by Wright and Penner (1998). The protein concentrations of the crude enzyme extracts were determined by the Lowry procedure using a protein assay kit[4]. Acetolactate concentrations were determined by the method of Westerfield (1945). Primisulfuron, sulfosulfuron, and BAY MKH 6561 were added at 0, 0.003, 0.001, 0.03, 0.01, 0.03, and 0.1 μM for the MR, MS, and AS biotypes, and at 0, 0.01, 0.1, 0.3, 1, 3, and 10 μM in the AR biotype. Imazamox was added at 0, 0.01, 0.1, 1, 3, 10, 30 μM for all biotypes. The experiment was a completely randomized design with two replications and was repeated.

ALS gene sequencing

Seedlings of resistant and susceptible downy brome biotypes were grown in a growth chamber with 16-h light at 25 C. At the 3-lf stage, genomic DNA was extracted from the second leaf of each plant using a DNA isolation kit[5]. Seven pairs of oligonucleotide primers[6] were designed from homologous regions among the published grass family ALS sequences of *Oryza sativa*, *Lolium multiflorum*, *Hordeum*

vulgare, and *Zea mays* (NCBI GenBank: Nucleotide Sequence Database). Polymerase chain reaction (PCR) was conducted to amplify the genomic DNA using seven pairs of oligonucleotide primers. One 177-bp fragment was amplified by a pair of primers (sense: 5'-GGGCAGCACCAGATGTGGGCG-3', antisense: 5'-GTTCATGAGGAAG CTACCATC-3'). The amplified DNA fragment was cloned into the TA Cloning vector[7] and sequenced by using an automatic DNA sequencer[8] with fluorescence dye-labeled dideoxynucleotides.

The 5'- and 3'-RACE (rapid amplification of cDNA ends) PCR were performed from poly A^+ RNA to sequence cDNA of resistant and susceptible downy brome biotypes using a RACE PCR kit[9]. Total RNA was isolated from the second leaf of each biotype using RNA isolation kit[10]. Poly A^+ RNA was selected from the total RNA using poly A^+ RNA purification kit[11]. Two nested antisense primers and sense primers specific to the sequence of 177-bp fragment were used to amplify the DNA with adaptor primers. About 1.4 kb and 0.8 kb fragments were amplified from 5'- and 3'- RACE PCR, respectively. The amplified DNA fragments were cloned into the TA Cloning vector and sequenced.

Statistical analysis

Dose response curves for the whole plant bioassay were obtained by a nonlinear regression using log-logistic equations (Streibig 1988; Seefeldt et al. 1995):

$$y = C + \frac{D - C}{1 + \exp[b(\log(x) - \log(GR_{50}))]} \qquad [1]$$

where y represents shoot dry weight (percent of control) at herbicide rate x and C, D, b, and GR_{50} are empirically derived constants. C is the lower limit, D is the upper limit, b is the slope at the GR_{50}, and GR_{50} is herbicide rate required for 50% growth reduction. ALS assay data also were analyzed using the log-logistic equations. Herbicide rates required for 50% ALS enzyme activity inhibition (I_{50}) were calculated using the equations. The regression parameters from the resistant and susceptible biotypes were compared to test significant difference using a sum of square reduction test. The level of resistance from the whole plant bioassay or ALS assay study was determined by calculating the ratio of GR_{50} or I_{50} of the resistant biotypes to GR_{50} or I_{50}, respectively, of the susceptible biotypes. Analysis of variance for the whole plant bioassay and ALS assay studies showed no significant interaction between treatment and experiment, so data from the two experiments were pooled and analyzed. Statistical computations were carried out using SAS/STAT NLIN (SAS 1987).

RESULTS AND DISCUSSION

Whole plant bioassay

The dose-response experiments showed different responses to the ALS inhibitors tested for the AR and MR biotypes (Figure 2.1). The AR biotype was highly resistant to the SU herbicides (primisulfuron and sulfosulfuron) and the SCT herbicide (BAY MKH 6561) but was not resistant to the IMI herbicide (imazamox) (Figure 2.1). The level of resistance calculated by the estimated GR_{50} values showed that the AR biotype was 317-, 263-, and 425-fold more resistant than the AS biotype to the primisulfuron, sulfosulfuron, and BAY MKH 6561, respectively (Table 2.1). However, the estimated GR_{50} values for imazamox were similar between the AR and AS biotypes (Table 2.1). The MR biotype was moderately resistant to the four ALS inhibitors tested (Figure 2.1). The estimated GR_{50} value for the MR biotype was similar to the field rate of each herbicide. The level of resistance for the MR biotype was 18, 9, 40, and 14 to primisulfuron, sulfosulfuron, BAY MKH 6561, and imazamox, respectively (Table 2.1).

ALS assay

The ALS enzyme assays for the AR biotype were consistent with the results of whole plant dose-response experiments (Figure 2.2). The level of resistance from the ALS assays showed that ALS extracted from the AR biotype was over 100-fold resistant to primisulfuron, sulfosulfuron, and BAY MKH 6561 but was not resistant to imazamox (Table 2.2). Results from the ALS and whole plant assays suggest that the

mechanism of resistance for the AR biotype was an altered target site. In contrast to results from the whole plant assays, no different ALS sensitivities to four ALS inhibitors were observed between the MR and MS biotypes suggesting the resistance was not due to an insensitive enzyme (Figure 2.2).

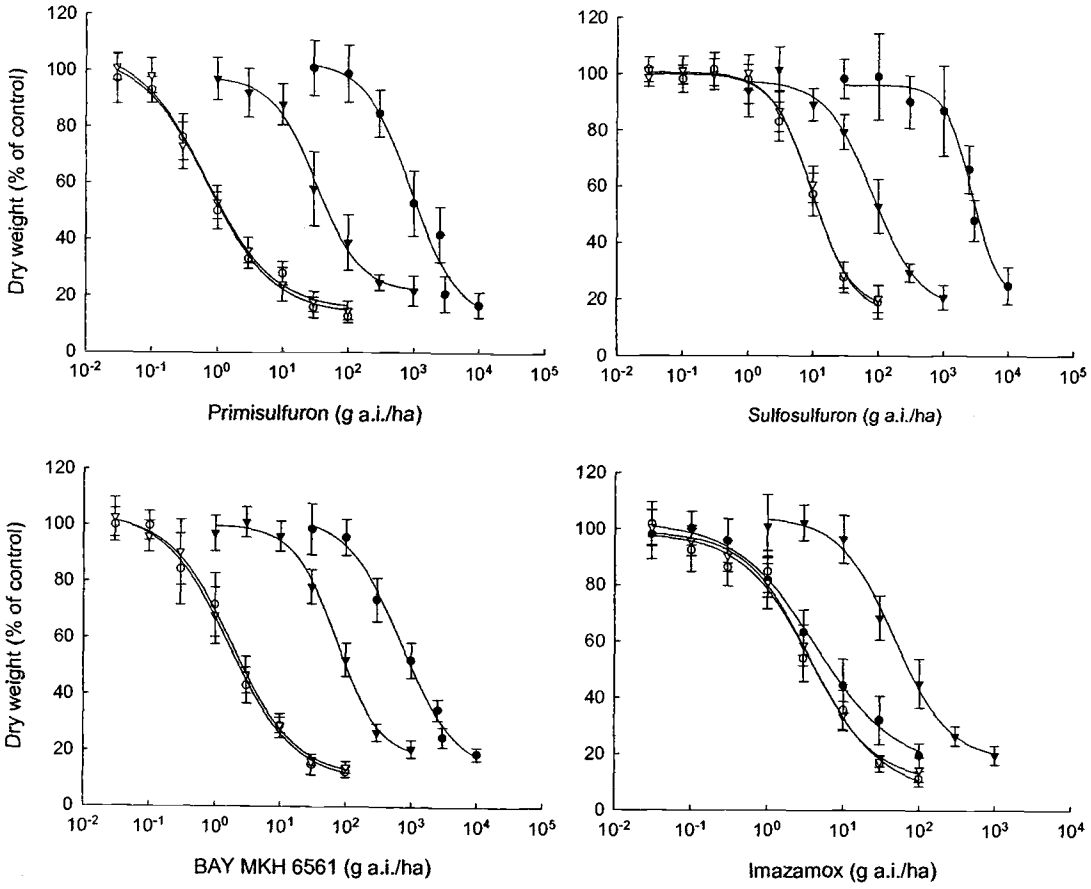

Figure 2.1. The effect of ALS inhibitors on the shoot dry weight in the AR (●), AS (○), MR (▼), and MS (▽) downy brome biotypes. Data were analyzed using the log-logistic equation. Vertical bars represent 95% confidence interval of the mean.

Table 2.1. Herbicide rates required for 50% suppression of shoot dry weight in ALS-inhibitor resistant (AR and MR) and susceptible (AS and MS) downy brome biotypes.

	GR_{50}			GR_{50}		
Herbicide	AR	AS	AR/AS[1]	MR	MS	MR/MS[1]
	- g ai/ha -			- g ai/ha -		
Primisulfuron	228 (93)[2]	0.72 (0.12)	317	32.5 (5.2)	1.85 (0.28)	18
Sulfosulfuron	2589 (69)	9.84 (1.11)	263	81.9 (13.0)	9.61 (0.95)	9
BAY MKH 6561	709 (111)	1.67 (0.28)	425	73.0 (7.5)	1.85 (0.28)	40
Imazamox	4.27 (1.0)	3.65 (0.59)	1.2	46.5 (6.9)	3.38 (0.44)	14

[1]The levels of resistance were calculated from the ratio of GR_{50} of the AR or MR biotype to the GR_{50} of the AS or MS biotype, respectively.
[2]Asymptotic standard errors for estimated GR_{50} are in parenthesis.

ALS gene sequencing

Partial *als* gene (2010-bp cDNA) were cloned and sequenced from the resistant and susceptible downy brome biotypes using 5'- and 3'-RACE PCR (Figure 2.3). This sequence included 1749-bp coding region which encoded 583 amino acid residues. The nucleotide sequences of the downy brome *als* gene showed over 90% homology with other grass family *als* nucleotide sequences. Although full length *als* gene sequences were not obtained, the sequenced region included the five highly conserved regions (Domains A to E) where mutations conferring ALS inhibitor resistance have been reported in other plant species. DNA sequence analysis of the *als* gene demonstrated a single-point mutation from C to T at nucleotide 328, conferring the exchange of the amino acid Pro_{197} to Ser in the AR biotype (Figure 2.3). However,

the nucleotide sequences of the MR biotype were the same as those of the susceptible (AS and MS) biotypes.

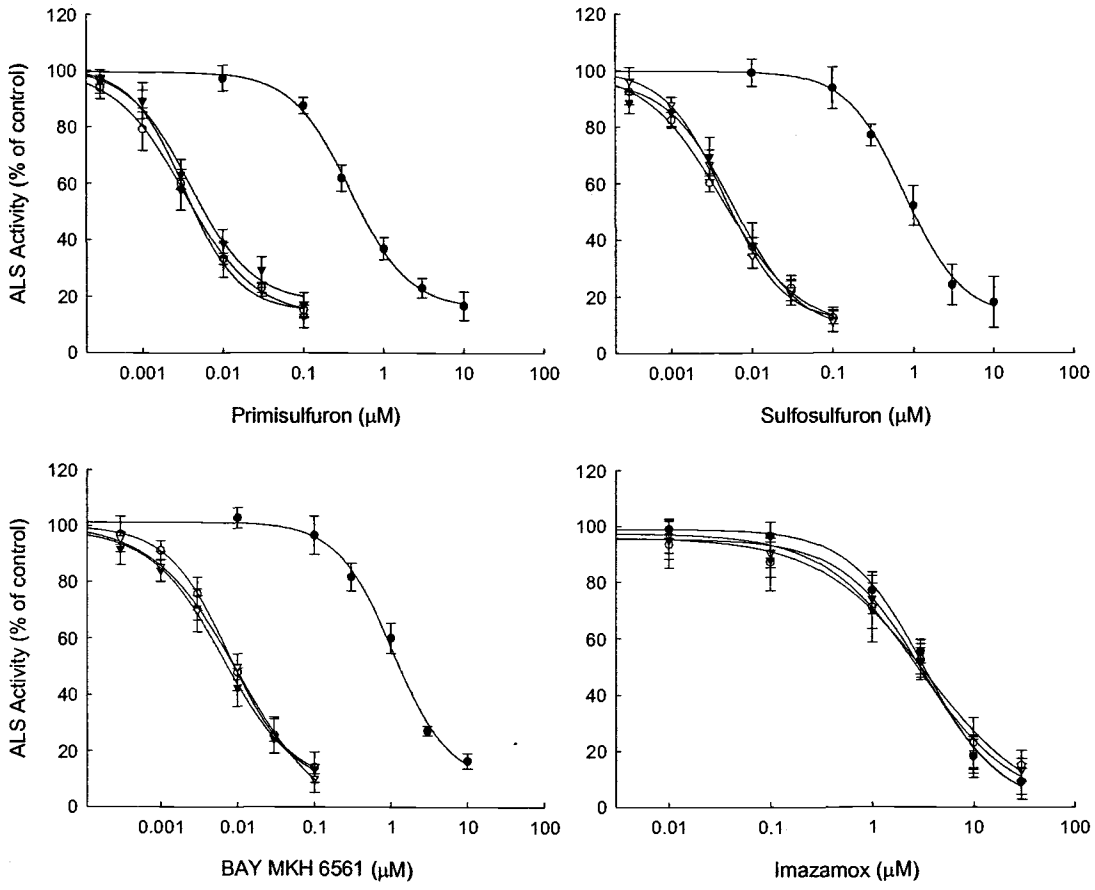

Figure 2.2. The effect of ALS inhibitors on the ALS activity in the AR (●), AS (○), MR (▼), and MS (▽) downy brome biotypes. Data were analyzed using the log-logistic equation. Vertical bars represent 95% confidence interval of the mean.

These data indicate that the proline to serine change in domain A of *als* gene is responsible for the ALS inhibitor resistance in the AR biotype, while resistance in the MR biotype is not due to a mutation in the *als* gene. The proline to serine change in

domain A of *als* gene has been reported in kochia, several *Lindernia* genera, and sugarbeet (Guttieri et al. 1995; Wright et al. 1998; Heap 2003). The sugarbeet somatic cell line (Sur), which had a proline to serine mutation in domain A, was highly resistant to SU herbicide but was not resistant to IMI herbicides (Wright et al. 1998). The level of resistance to ALS inhibitors or cross-resistance to other class of ALS inhibitors corresponds to the substituted amino acid, especially Pro_{197}, as well as the mutation site in the *als* gene (Tranel and Wright 2002).

Table 2.2. Herbicide rates required for 50% inhibition of ALS activity in ALS-inhibitor resistant (AR and MR) and susceptible (AS and MS) downy brome biotypes.

	I_{50}			I_{50}		
Herbicide	AR	AS	AR/AS[1]	MR	MS	MR/MS[1]
	- nM -			- nM -		
Primisulfuron	399 (29)[2]	3.24 (0.38)	123	3.77 (0.48)	3.23 (0.31)	1.2
Sulfosulfuron	771 (85)	4.19 (0.38)	184	6.34 (0.53)	4.55 (0.34)	1.4
BAY MKH 6561	1048 (105)	7.69 (0.73)	136	6.93 (0.56)	10.40 (2.03)	0.7
Imazamox	3181 (314)	3149 (587)	1.0	3221 (363)	2862 (432)	1.1

[1]The levels of resistance were calculated from the ratio of I_{50} of the AR or MR biotype to the I_{50} of the AS or MS biotype, respectively.
[2]Asymptotic standard errors for estimated GR_{50} are in parenthesis.

The MR biotype showed different resistance levels and cross-resistance patterns to ALS inhibitors compared to the AR biotype. Previous studies demonstrated that the injury of the MR biotype to sulfosulfuron was increased when treated with chlorpyrifos, an organophosphate insecticide, suggesting the resistance of the MR

biotype is a non-target site mechanism, but probably herbicide metabolism (Mallory-Smith et al. 1999). Metabolism studies will need to be conducted to determine if it is the mechanism of resistance for the MR biotype.

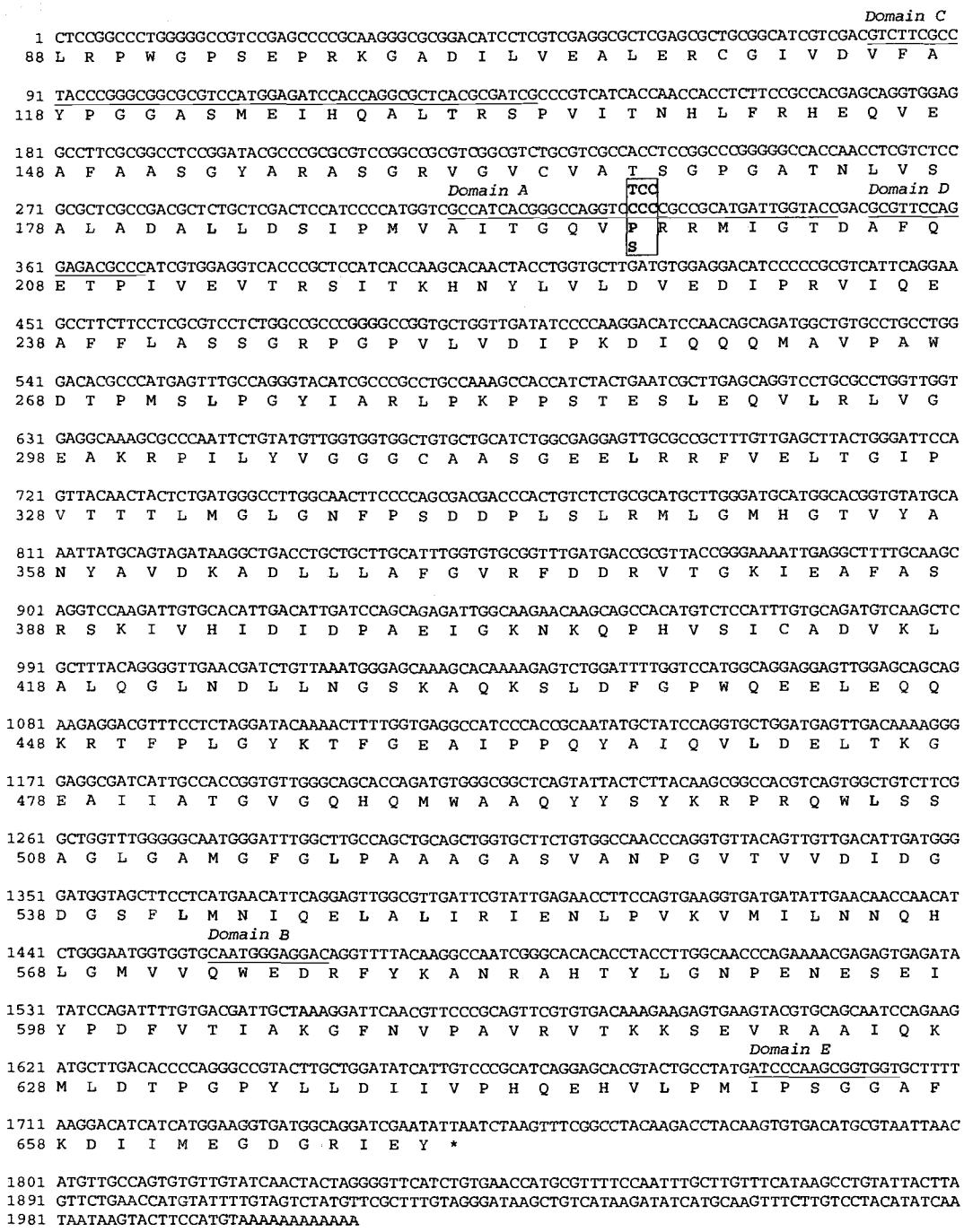

Figure 2.3. Nucleotide and deduced amino acid sequences of the *als* gene from downy brome biotypes. The underlined sequences indicate highly conserved regions (domain A, B, C, D, and E) in plant *als* genes. A box indicates a Pro to Ser mutation at amino acid 197 in Domain A in the AR biotype. Numbering of amino acids is based on the precursor *als* gene from *Arabidopsis thaliana*. The GenBank accession numbers are AF488771 and AF487459 for wild type and mutant *als* genes in downy brome, respectively (Park et al. 2002).

SOURCES OF MATERIALS

[1]Sunshine Mix #3 potting mix, Sun Gro Horticulture, Inc., 110 110th Ave. NE, Suite 490, Bellevue, WA 98004.

[2]Sunshine Mix #1 potting mix, Sun Gro Horticulture, Inc., 110 110th Ave. NE, Suite 490, Bellevue, WA 98004.

[3]Nonionic surfactant R-11 (mixture of octylphenoxypolyethoxyethanol, isopropanol, and compounded silicone), Wilbur Ellis Co., S. 2607 Southeast Blvd., Spokane, WA 99223.

[4]Protein assay kit, Sigma Diagnostics, P.O. Box 14508 St. Louis, MO 63178.

[5]Dneasy® Plant Mini Kit, QIAGEN Inc., 28159 Avenue Stanford, Valencia, CA 91355.

[6]PCR primers ordered from Invitrogen Life Technologies, 7300 Governors Way, Frederick, MD 21704.

[7]TOPO TA Cloning® Kit, Invitrogen Life Technologies, 7300 Governors Way, Frederick, MD 21704.

[8]ABI PRISM® 377 automated DNA sequencer, Perkin-Elmer Applied Biosystems, 850 Lincoln Center Drive, Foster City, CA 94404.

[9]FirstChoice™ RLM-RACE Kit, Ambion, Inc., 2130 Woodward Steet, Austin, TX 78744.

[10]RNeasy® Plant Mini Kit, QIAGEN Inc., 28159 Avenue Stanford, Valencia, CA 91355.

[11]Oligotex®, QIAGEN Inc., 28159 Avenue Stanford, Valencia, CA 91355.

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LITERATURE CITED

Ball, D. A. and C. A. Mallory-Smith. 2000. Sulfonylurea herbicide resistance in downy brome. Proc. West. Soc. Weed Sci. 53:41-42.

Bernasconi, P., A. R. Woodworth, B. A. Rosen, M. V. Subramanian, and D. L. Siehl. 1995. A naturally occurring point mutation confers broad range tolerance to herbicides that target acetolactate synthase. J. Biol. Chem. 270:17381-17385.

Boutsalis, P., J. Karotam, and S. B. Powles. 1999. Molecular basis of resistance to acetolactate synthase-inhibiting herbicides in *Sisymbrium orientale* and *Brassica tournefortii*. Pestic. Sci. 55:507-516.

Creason, G. L. and R. S. Chaleff. 1988. A second mutation enhances resistance of a tobacco mutant to sulfonylurea herbicides. Theor. Appl. Genet. 76:177-182.

DeFelice, M., J. Guardiola, B. Esposito and M. Laccarino. 1974. Structural genes for a new recognized acetolactate synthase in *Escherichia coli* K-12. Bacteriology 120:1068-1077.

Devine, M. D. and C. Preston. 2000. The molecular basis of herbicide resistance. Pages 72-104 *in* A. H. Cobb and R. C. Kirkwood, eds. Herbicides and Their Mechanisms of Action. Boca Raton, FL: Sheffield Academic Press.

Geier, P. W., P. W. Stahlman, S. D. Miller, and N. R. Hageman. 1998. MON 37500 rate and timing affects downy brome (*Bromus tectorum*) control in winter wheat (*Triticum aestivum*). Weed Sci. 46:366-373.

Guttieri, M. J., C. V. Eberlein, and D. C. Thill. 1995. Diverse mutations in the acetolactate synthase gene confer chlorsulfuron resistance in kochia (*Kochia scoparia*) biotypes. Weed Sci. 43:175-178.

Heap, I. M. 2003. International Survey of Herbicide Resistant Weeds. Herbicide Resistance Action Committee and Weed Science Society of America. Internet www.weedscience.com.

Hendrickson, P. E. and C. A. Mallory-Smith. 1999. Response of downy brome (*Bromus tectorum*) and Kentucky bluegrass (*Poa pratensis*) to applications of primisulfuron. Weed Technol. 13:461-465.

Mallory-Smith, C. A., D. C. Thill, and M. J. Dial. 1990. Identification of herbicide resistant prickly lettuce (*Lactuca serriola*). Weed Technol. 4:163-168.

Mallory-Smith, C. A., P. Hendrickson, and G. W. Mueller-Warrant. 1999. Cross-resistance of primisulfuron-resistant *Bromus tectorum* L. (downy brome) to sulfosulfuron. Weed Sci. 47:256-257.

Maxwell, B. D. and A. M. Motimier. 1994. Selection for herbicide resistance. Pages 1-26 *in* S. B. Powles and J. A. M. Holtum, eds. Herbicide Resistance in Plants: Biology and Biochemistry. Ann Arbor, MI: Lewis.

Morrow, L. A. and P. W. Stahlman. 1984. The history and distribution of downy brome (*Bromus tectorum*) in North America. Weed Sci. 32, Suppl. 1:2-6.

Mueller-Warrant, G. W., C. A. Mallory-Smith, and P. E. Hendrickson. 1999. Non-target site resistance to ALS inhibitors in downy brome. Proc. West. Soc. Weed Sci. 52:16.

Patzoldt, W. L. and P. J. Tranel. 2001. ALS mutations conferring herbicide resistance in waterhemp. Proc. N. Cent. Weed Sci. Soc. 56:67.

Ray, T. B. 1984. Site of action of chlorsulfuron. Plant Physiol. 75:827-831.

Ryan, G. F. 1970. Resistance of common groundsel to simazine and atrazine. Weed Sci. 18:614-616.

Saari, L. L., J. C. Cotterman, and M. M. Primiani. 1990. Mechanism of sulfonylurea herbicide resistance in the broadleaf weed, *Kochia scoparia*. Plant Physiol. 93:55-61.

SAS Institute. 1987. SAS/STAT™ Guide for personal computers, Version 6.0. Cary, NC: SAS Institute Inc., 1028 pp.

Seefeldt, S. S., J. E. Jensen, and E. P. Fuerst. 1995. Log-logistic analysis of herbicide dose-response relationships. Weed Technol. 9:218-225.

Streibig, J. C. 1988. Herbicide bioassay. Weed Res. 28: 479-484.

Tranel, P. J. and T. R. Wright. 2002. Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Sci. 50:700-712.

Westerfield, W. W. 1945. A colorimetric determination of blood acetoin. J. Biol. Chem. 161: 495-502.

Woodworth, A., P. Bernasconi, M. Subramanian, and B. Rosen. 1996. A second naturally occurring point mutation confers broad-based tolerance to acetolactate synthase inhibitors. Plant Physiol. 111:S105.

Wright, T. R. and D. Penner. 1998. Corn (*Zea mays*) acetolactate synthase sensitivity to four classes of ALS-inhibiting herbicides. Weed Sci. 46:8-12.

CHAPTER 3

Absorption, Translocation, and Metabolism of BAY MKH 6561 in ALS-Inhibitor Resistant Downy Brome (*Bromus tectorum* L.) Biotypes

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ABSTRACT

Acetolactate synthase (ALS)-inhibitor resistant (AR and MR) and susceptible (AS and MS) downy brome biotypes were collected from a Kentucky bluegrass (*Poa pratensis*) seed field at Athena (AR and AS) and in research plots at Madras (MR and MS), Oregon. Experiments were conducted to investigate the absorption, translocation, and metabolism of BAY MKH 6561 in the ALS-inhibitor resistant and susceptible downy brome biotypes. Absorption and translocation of applied ^{14}C-BAY MKH 6561 were similar in all biotypes. The maximum absorption was about 28% of applied herbicide at 48 hours after treatment (HAT) in all biotypes. Seventy-two HAT, about 20% of the absorbed radioactivity translocated into the shoot and less than 2% translocated into the roots in all biotypes. One major and 3 minor metabolites were identified using reverse-phase HPLC. In all biotypes, over 80% of the BAY MKH 6561 was metabolized by 72 HAT. However, BAY MKH 6561 was metabolized more rapidly in the MR biotype than in the other biotypes. The half-life of BAY MKH 6561 in the MR biotype was 8.9 h which was 40% shorter than in the MS biotype. The half-life of BAY MKH 6561 in the AR and AS biotypes was similar to the MS biotype. When ^{14}C-BAY MKH 6561 was applied with 1-aminobenzotriazole (1-ABT), a cytochrome P450 inhibitor, the rate of metabolism of BAY MKH 6561 in the MR biotype decreased from 63% to 43% at 12 HAT. These data indicate that the different sensitivity of the MR biotype to BAY MKH 6561 is due to the relatively rapid rate of BAY MKH 6561 metabolism compared to the other biotypes. It also is

proposed that cytochrome P450s are involved in the metabolic degradation of BAY MKH 6561 in the MR biotype.

Nomenclature: Downy brome, *Bromus tectorum* L. BROTE; BAY MKH 6561, methyl 2-[[[(4-methyl-5-oxo-3-propoxy-4,5-dihydro-1H-1,2,4-triazol-l-yl)carbonyl] amino]sulfonyl]benzoate sodium salt.

Key words: ALS inhibitor, herbicide resistance, absorption, translocation, metabolism, cytochrome P450, BROTE

INTRODUCTION

Since resistance to sulfonylurea (SU) herbicides was first reported in prickly lettuce (*Lactuca serriola* L.) in 1987, 79 weed species have been identified with resistance to ALS inhibitors (Mallory-Smith et al. 1990; Heap 2003). An insensitive acetolactate synthase (ALS) is the most common resistance mechanism reported in weed species. However, enhanced metabolism of ALS inhibitors has been documented in at least three weed species: annual ryegrass (*Lolium rigidum* G.), blackgrass (*Alopecurus myosuroides* H.) and wild mustard (*Brassica kaber* DC.) (Christopher et al. 1994; Menendez et al. 1997; Veldhuis et al. 2000).

Downy brome (*Bromus tectorum* L.) is a widespread and competitive weed in the Pacific Northwest. With the repeated use of primisulfuron, a SU herbicide, resistant downy brome biotypes were identified in Kentucky bluegrass (*Poa pratensis*) research plots at Madras and in a seed field at Athena, Oregon, in 1997 and 1998, respectively (Mueller-Warrant et al. 1999; Ball and Mallory-Smith 2000). Previous studies showed that the Madras resistant (MR) biotype was moderately resistant to other ALS inhibitors: sulfosulfuron, BAY MKH 6561, and imazamox. The Athena resistant (AR) biotype was highly resistant to sulfosulfuron and BAY MKH 6561 but not resistant to imazamox (Park et al. 2001). It also was shown that the mechanism of resistance for the AR biotype was an altered target site. ALS in the AR biotype was 123-fold less sensitive than that in the Athena susceptible (AS) biotype to primisulfuron. A single-point mutation conferring a proline to serine change in domain A of *als* gene was responsible for the ALS inhibitor resistance in the AR

biotype (Park et al. 2002). However, resistance in the MR biotype was not due to a mutation in the *als* gene.

Cytochrome P450 (P450) is recognized as the most important enzyme system for herbicide metabolism and selectivity in crops. P450s are inhibited by such tetcyclasis, 1-aminobenzotriazole (1-ABT), piperonyl butoxide (PBO) and certain organophosphate insecticides. Mallory-Smith et al. (1999) showed that the injury of the MR biotype to sulfosulfuron was increased when treated with chlorpyrifos, an organophosphate insecticide. The research suggested that the resistance mechanism of the MR biotype might be an enhanced metabolism.

BAY MKH 6561 is a sulfonylaminocarbonyltriazolinone (SCT) herbicide with a same mode of action to the SU herbicides which inhibit ALS. It was developed to control *Bromus* species, jointed goatgrass and several other grass and broadleaf weeds in winter wheat (Feucht et al. 1999; Scoggan et al. 1999).

The objective of this experiment was to compare the absorption, translocation, and metabolism of BAY MKH 6561 in ALS-inhibitor resistant and susceptible downy brome biotypes.

MATERIALS AND METHODS

Plant material

Seeds of two ALS-inhibitor resistant (AR and MR) downy brome biotypes and two susceptible (AS and MS) biotypes were germinated in a growth chamber with continuous light for 4 d at 25 C. Seedlings were transplanted into conical pots[1] (3.8 cm diameter by 21 cm long) filled with 30 mesh silica sand. Plants were grown in a greenhouse with 16 h photoperiod and 25/20 C day/night temperatures. Plants were surface watered daily with 25 ml half-strength Hoagland solution (Hoagland and Arnon 1938).

Absorption

Herbicide applications were made 21 days after planting (DAP) to 4 to 5 leaf stage plants. The third fully expanded leaf of each plant was marked and covered with aluminum foil. Formulated BAY MKH 6561[2] was applied at 42 g ai ha^{-1} with 0.25% (v/v) nonionic surfactant (NIS)[3]. Herbicide applications were made using an 8003 even flat fan nozzle and overhead track sprayer calibrated to deliver 187 L ha^{-1}. The plants were allowed to dry, the aluminum foil was removed and the radiolabeled treatments were applied to the protected leaf.

Radiolabeled BAY MKH 6561 (specific activity 10.2 MBq mg^{-1}, 100% purity) was dissolved with formulated BAY MKH 6561 to create the same rate as the overspray treatment. Ten 0.5 µl droplets (420 Bq per plant) were applied to the base of the protected leaf. Plants were harvested 0, 6, 12, 24, 48, and 72 h after treatment

(HAT). Treated leaves were excised and vortexed for 30 s in 5 ml 10% aqueous methanol containing 0.25% (v/v) NIS to remove any remaining ^{14}C-BAY MKH 6561. Radioactivity was quantified using liquid scintillation spectroscopy (LSS)[4]. Absorption was determined as the difference between radioactivity applied and amount recovered in the wash. The remaining shoot and roots were harvested. The treated leaf, shoot, and root tissue were then frozen in liquid nitrogen and stored at –20 for the translocation and metabolism studies.

Translocation and metabolism

Radiolabeled BAY MKH 6561was extracted from the treated leaf, shoot, and root tissue by homogenizing in 15 ml 95% aqueous methanol using a tissue homogenizer. Samples were shaken for 1 h using a wrist action shaker and centrifuged at 6,000 rpm for 20 min. The supernatant was concentrated to 1 ml using a speed vacuum and centrifuged at 14,000 rpm for 10 min. A 100 µl aliquot was taken from each supernatant and radioactivity was counted by LSS. Radioactivity from the sand and nutrient solution was combined with the radioactivity translocated into roots. Translocation of BAY MKH 6561 was considered to be the radioactivity recovered from shoot and root tissues. The data are presented as a percent of recovered radioactivity from total radioactivity absorbed by each plant.

Tissue extracts were analyzed by reverse-phase high performance liquid chromatography (HPLC)[5] using a Zorbax SB-C18 column[6] (3.5µm, 3 × 150 mm) and gradient elution. The initial mobile phase was acetonitrile (ACN):water:phosphoric

acid at 40:60:0.1 (v:v:v), followed by a linear increase to 100% ACN from 0 to 8 min. The mobile phase was held constant at 100% ACN from 8 to 20 min after the sample injection. The sample injection volume was 200 µl. Radiolabeled BAY MKH 6561 and metabolite peaks were monitored using an inline radioactive detector[7]. The parent fraction was determined by the ratio of the parent peak to the total radioactivity in the extract on a whole-plant basis.

Radiolabeled BAY MKH 6561 metabolism in combination with 1-aminobenzotriazole (1-ABT), a P450 inhibitor, was investigated in the MR biotype. Because 1-ABT is not readily absorbed through the leaf surface, 70µM 1-ABT was applied to roots with Hoagland solution 24 h before the herbicide treatment. Plants were harvested 12 HAT. Herbicide treatment and extraction procedures were the same as previously described. Whole plant extracts were analyzed by HPLC as previously described.

Data analyses

Experiments were conducted in a completely randomized design with three replications and were repeated. The experiments had similar magnitudes and constant variances, so data from the experiments were combined and analyzed. Means and 95% confidence intervals were determined from the data for absorption and translocation. In the metabolism study, the data were fit to log-logistic equations (Streibig 1988; Seefeldt et al. 1995):

$$y = C + \frac{D - C}{1 + \exp[b(\log(t) - \log(T_{50}))]} \quad [1]$$

where y represents remaining BAY MKH 6561 at time t and C, D, b, and T_{50} are empirically derived constants. C is the lower limit, D is the upper limit, b is the slope at the T_{50}, and T_{50} is half-life of BAY MKH 6561. The half-life of BAY MKH 6561 in each biotype was calculated from a regression equation and then compared between the resistant and susceptible biotypes using a sum of square reduction test. Statistical computations were carried out using SAS/STAT NLIN(SAS 1987).

RESULTS AND DISCUSSION

Absorption and translocation

Total recoveries of applied ^{14}C were greater than 96% in all downy brome biotypes (data not shown). Most of the ^{14}C-BAY MKH 6561 was absorbed within 24 h and did not significantly increase through 72 HAT (Figure 3.1). The maximum absorption was about 27% of applied 48 HAT in all biotypes. A previous study demonstrated that the absorption of ^{14}C-BAY MKH 6561 was 43 and 80% in downy brome 24 and 48 HAT, respectively (Fandrich et al. 2001). The study used a different formulation of NIS, leaf stage, biotypes, and growing conditions which may explain the difference in absorption. The majority of absorbed radioactivity remained in the treated leaf. The translocation of absorbed radioactivity into shoots and roots was similar in all biotypes (Figure 3.2). About 20% of absorbed radioactivity translocated into shoots and less than 2% translocated into roots 72 HAT. No differences in absorption and translocation were observed between the resistant and susceptible biotypes. Therefore, absorption and translocation can not account for the differential sensitivity of resistant and susceptible biotypes to ^{14}C-BAY MKH 6561.

Metabolism

Based on HPLC separation, BAY MKH 6561 had a retention time of 12.7 min, which was preceded by a major metabolite peak (M2) at 7.1 min and three minor peaks (M1, M3, and M4). Similar metabolite profiles were produced in all biotypes (Figure 3.3). M2 accounted for about 70% of all metabolites 24 HAT. Although the

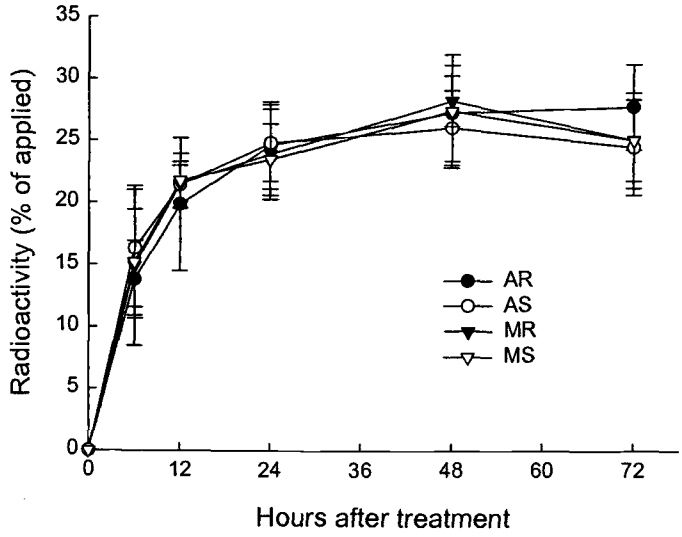

Figure 3.1. Absorption of ^{14}C-BAY MKH6561 by four downy brome biotypes through 72 h after treatment. Each point represents the mean with a 95% confidence interval of six replications.

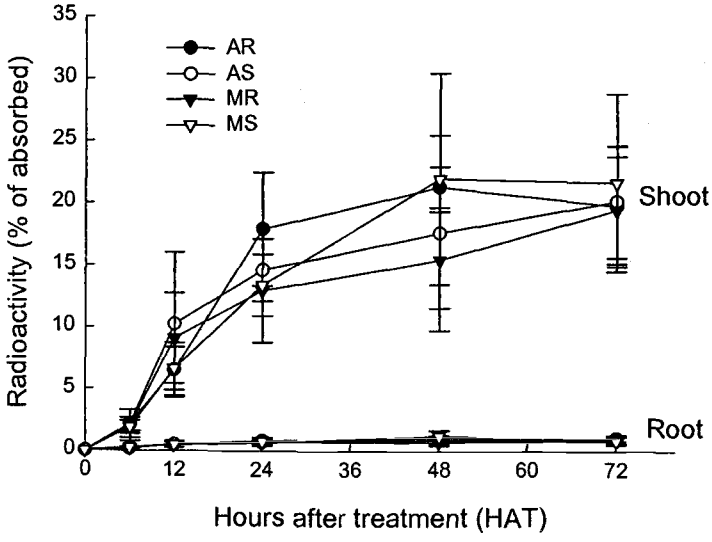

Figure 3.2. Translocation of radioactivity into shoot and roots of four downy brome biotypes through 72 h after treatment. Each point represents the mean and a 95% confidence interval of six replications.

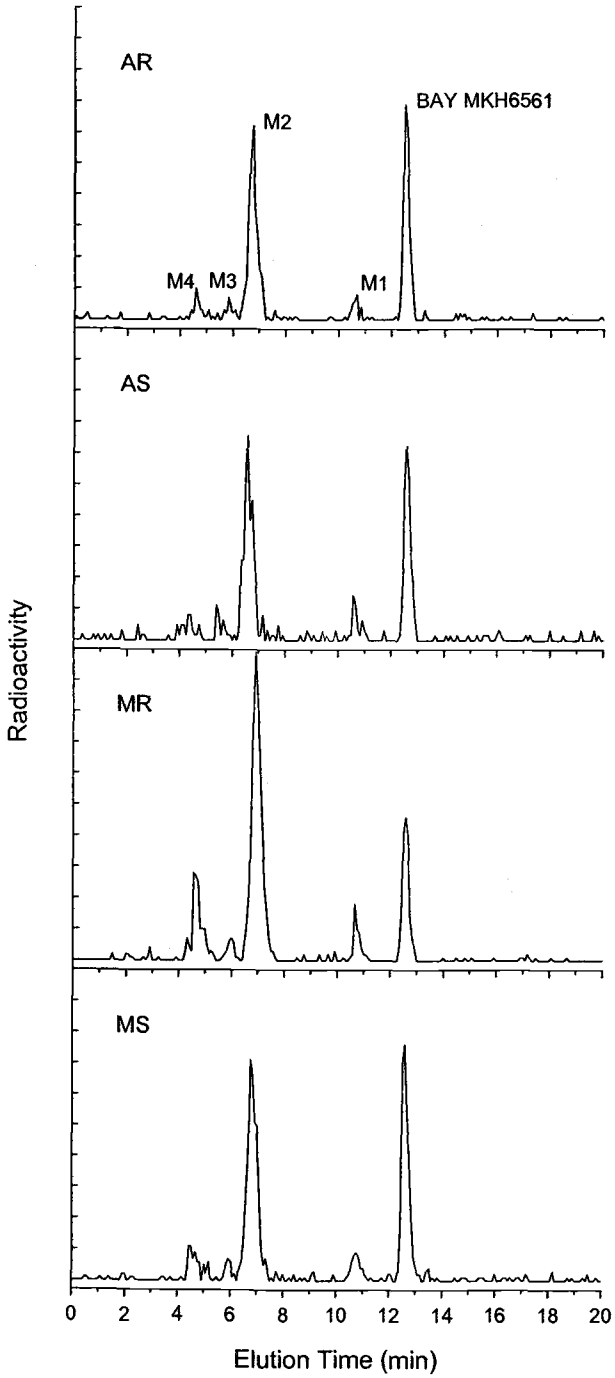

Figure 3.3. HPLC chromatograms of ^{14}C-BAY MKH6561 and metabolites (M1, M2, M3, and M4) in four downy brome biotypes 24 h after treatment.

metabolites were not identified, we suspect that the M2 was 5-hydroxyphenyl BAY MKH 6561. Fonné-Pfister et al. (1990) reported that 5-hydroxyphenyl primisulfuron is one of major metabolites of primisulfuron in maize. The major oxidation product of prosulfuron in wheat was 5-hydroxyphenyl prosulfuron (Frear and Swanson 1996). All biotypes metabolized BAY MKH 6561 rapidly and over 80% of ^{14}C was present as the metabolites 72 HAT (Figure 3.4).

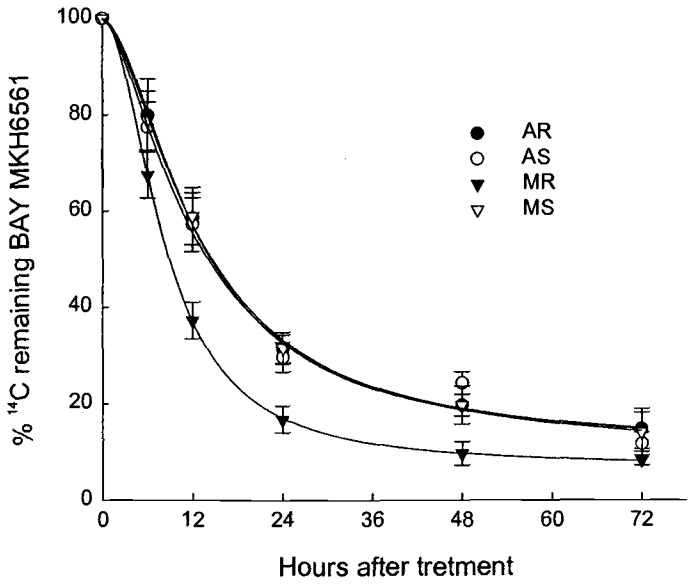

Figure 3.4. Metabolism of ^{14}C-BAY MKH6561 in four downy brome biotypes through 72 h after treatment. Each point represents the mean and a 95% confidence interval of six replications. Data were fit using the log-logistic equation.

A log-logistic equation was used to fit metabolism data using nonlinear estimation. The asymptotic R^2 was greater than 0.98 for all models and all parameter estimates were significantly different from zero ($P<0.001$). Parameter estimates

including the half-life of BAY MKH 6561 in each biotype were calculated from each equation (Table 3.1). There was no difference in the half-life between the AR, AS, and MS biotypes. However, the half-life of BAY MKH 6561 in the MR biotype was 8.9 h which was 40% shorter than in the MS biotype. The half-life of BAY MKH 6561 in the AR, AS, and MS biotypes was 14.0 h, 12.9 h, and 14.6 h, respectively. Fandrich et al. (2001) also studied the metabolism of BAY MKH 6561 in downy brome and reported a half-life of 13.1 h which was similar to those in the AR, AS, and MS biotypes.

Table 3.1. Parameter estimates for ^{14}C-BAY MKH6561 metabolism in ALS-inhibitor resistant and susceptible downy brome biotypes.

	Parameter estimates[1]			
Biotype	D	C	b	T_{50}
AR	90.5 (6.9)[2]	13.6 (2.7)	2.2 (0.5)	14.0 (1.4)
AS	96.4 (15.4)	10.6 (5.1)	1.7 (0.5)	12.9 (2.6)
MR	93.4 (14.9)	7.3 (1.5)	2.1 (0.4)	8.9 (1.6)
MS	89.6 (7.2)	12.2 (3.1)	2.1 (0.4)	14.6 (1.5)

[1]C = lower limit, D = upper limit, b = slope, and T_{50} = half life
[2]Asymptotic standard errors for estimated parameters are in parenthesis.

The rate of metabolism of BAY MKH 6561 was decreased when treated with 1-ABT in the MR biotype (Figure 3.5). When the MR biotype was treated with ^{14}C-BAY MKH 6561 with and without 1-ABT, 43 and 63% of absorbed radioactivity was metabolized 12 HAT, respectively, indicating involvement of P450s in BAY MKH

6561 metabolism. The decreased metabolism of chlorsulfuron with P450 inhibitor treatments has been reported in chlorsulfuron resistant annual ryegrass whose mechanism of resistance is enhanced metabolism (Christopher et al. 1994). Veldhuis et al. (2000) found that piperonyl butoxide (PBO), a P450 inhibitor, decreased the rate of metabolism of ethametsulfuron-methyl in the resistant wild mustard biotype and that P450 mediated metabolism might be responsible for the herbicide resistance.

Absorption, translocation, and metabolism did not explain the differential sensitivity of BAY MKH 6561 between the AR and AS biotype. Different sensitivities of the MR and MS biotypes to BAY MKH 6561 were not due to altered absorption, translocation, nor as previously reported ALS sensitivity. However, resistance of the MR biotype was due to relatively rapid rate of BAY MKH 6561 metabolism and was related to P450s.

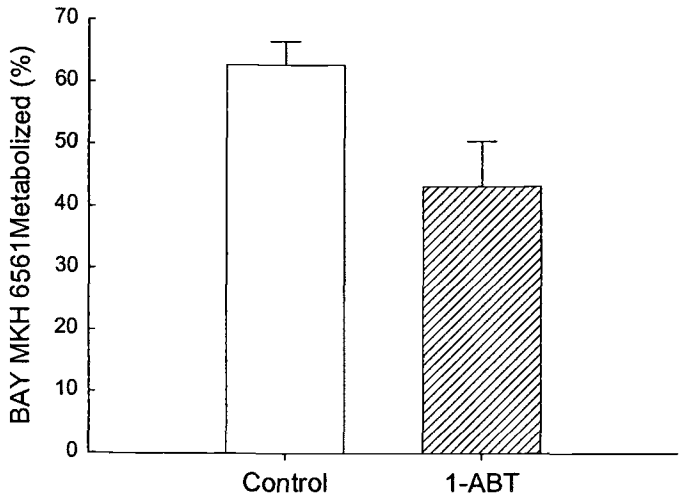

Figure 3.5. The effect of 1-ABT on metabolism of BAY MKH6561 in the MR biotype. Vertical bars represent 95 % confidence interval for the mean of 6 replicates.

SOURCES OF MATERIALS

[1]Stuewe and Sons, Inc., 2290 S.E. Kiger Island Drive, Corvallis, OR 97333-9461.

[2]Bayer Corporation, 8400 Hawthorn Road, P.O. Box 4913, Kansas City, MO 64120.

[3]Nonionic surfactant R-11 (mixture of octylphenoxypolyethoxyethanol, isopropanol, and compounded silicone), Wilbur Ellis Co., S. 2607 Southeast Blvd., Spokane, WA 99223.

[4]Packard Instrument Co., 800 Research Parkway, Meriden, CT 06450.

[5]High performance liquid chromatography, Hitachi Ltd., Tokyo, Japan.

[6]Mac-Mod Analytical, Inc., 127 Commons Ct., Chadds Ford, PA 19317.

[7]INUS Systems, Inc., 5809 North 50th St., Tampa, FL 33610.

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LITERATURE CITED

Ball, D. A. and C. A. Mallory-Smith. 2000. Sulfonylurea herbicide resistance in downy brome. Proc. West. Soc. Weed Sci. 53:41-42.

Christopher, J. T., C. Preston, and S. B. Powles. 1994. Malathion antagonizes metabolism-based chlorsulfuron resistance in *Lolium rigidum*. Pestic. Biochem. and Physiol. 49:172-182.

Fandrich, L., S. K. MacDonald, S. J. Nissen, P. Westra, and H. J. Santel. 2001. Absorption and fate of BAY MKH 6561 in jointed goatgrass and downy brome. Weed Sci. 49:717-722.

Feucht, D., K. H. Mueller, A. Wellmann, and H. J. Santel. 1999. BAY MKH 6561-a new selective herbicide for grass control in wheat, rye and triticale. Proc. Br. Crop Prot. Conf. Weeds 1:53-58.

Heap, I. M. 2003. International Survey of Herbicide Resistant Weeds. Herbicide Resistance Action Committee and Weed Science Society of America. Internet www.weedscience.com.

Hoagland, D. R. and D. I. Arnon. 1938. The water culture method of growing plants without soil. Californian Agricultural Experimental Station Circular Number 347.

Mallory-Smith, C. A., D. C. Thill, and M. J. Dial. 1990. Identification of herbicide resistant prickly lettuce (*Lactuca serriola*). Weed Technol. 4:163-168.

Mallory-Smith, C. A., P. Hendrickson, and G. W. Mueller-Warrant. 1999. Cross-resistance of primisulfuron-resistant *Bromus tectorum* L. (downy brome) to sulfosulfuron. Weed Sci. 47:256-257.

Menendez, J. M., R. De Prado, M. D. Devine. 1997. Chlorsulfuron cross-resistance in a chlorotoluron-resistant biotype of *Alopercurus myosuroides*. Proc. Br. Crop Prot. Conf. Weeds 1: 319-324.

Mueller-Warrant, G. W., C. A. Mallory-Smith, and P. E. Hendrickson. 1999. Non-target site resistance to ALS inhibitors in downy brome. Proc. West. Soc. Weed Sci. 52:16.

Park, K. W., C. A. Mallory-Smith, G. W. Mueller-Warrant and D. A. Ball. 2001. Growth characteristics of sulfonylurea-resistant and –susceptible downy brome biotypes. Proc. West. Soc. Weed Sci. 54:12.

Park, K. W., C. A. Mallory-Smith, G. W. Mueller-Warrant and D. A. Ball. 2002. Point mutation confers sulfonylurea resistance to one downy brome (*Bromus tectorum*) biotype but not in another. Proc. West. Soc. Weed Sci. 55:11.

SAS Institute. 1987. SAS/STAT™ Guide for personal computers, Version 6.0. Cary, NC: SAS Institute Inc., 1028 pp.

Scoggan, A. C., H. J. Santel, J. W. Wollam, and R. D. Rudolph. 1999. BAY MKH 6561-a new herbicide for grass and broadleaf control in cereals. Proc. Br. Crop Prot. Conf. Weeds 1:93-98.

Seefeldt, S. S., J. E. Jensen, and E. P. Fuerst. 1995. Log-logistic analysis of herbicide dose-response relationships. Weed Technol. 9:218-227.

Streibig, J. C. 1988. Herbicide bioassay. Weed Res. 28: 479-484.

Veldhuis, L. J., L. M. Hall, J. T. O'Donovan, W. Dyer, and J. C. Hall. 2000. Metabolism-based resistance of a wild mustard (*Sinapis arvensis* L.) biotype to ethametsulfuron-methyl. J. Agric. Food Chem 48:2986-2990.

CHAPTER 4

Differential Fitness and Competition of ALS-Inhibitor Resistant and Susceptible Downy Brome (*Bromus tectorum* L.) Biotypes

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ABSTRACT

The relative fitness and competitive ability of herbicide resistant biotypes are important to predict population dynamics when the selection pressure is absent. Studies were conducted to determine the relative fitness and competitiveness of two ALS-inhibitor resistant downy brome biotypes (AR and MR) compared to their susceptible biotypes (AS and MS). Seed germination of AR and MR biotypes was compared with AS and MS biotypes at 5, 15, and 25 C. The AR biotype germinated 27 h earlier than the AS biotype and had reached over 60% germination when the AS biotype initially germinated at 5 C. No differences in germination were observed between MR and MS biotypes at any temperature. Under noncompetitive greenhouse conditions, growth of the AR biotype was similar to the AS biotype in shoot dry weight, leaf area, and plant height. However, the MR biotype produced 33 and 15% less shoot dry weight and leaf area, respectively and was 23% shorter than the MS biotype. Seed production of the AR and MR biotypes was 83 and 71%, respectively when compared to the AS and MS biotypes. However, seeds of the AR biotype were larger than those of the AS biotype. Competition between biotypes was evaluated using replacement series experiments at one density (100 plants m^{-2}) and five planting ratios (100:0, 75:25, 50:50, 25:75, and 0:100). No competition was observed in shoot dry weight, leaf area, and plant height between the AR and AS biotypes. Similar results were observed between the AS and MS biotypes. However, the MR biotype was less competitive than the MS and AR biotypes. The shoot dry weight and leaf area of individual MR biotype plants were about 50% of the MS and AR biotypes in

any planting mixture. These results suggest that populations of the MR biotype will decrease and shift toward higher frequency of the MS biotype in the absence of ALS inhibitors. However, it will be difficult to predict population dynamics of the AR biotype in the field.

Nomenclature: Downy brome, *Bromus tectorum* L. BROTE;

Key words: Herbicide resistance, ALS inhibitor, fitness, replacement series, competition, BROTE

INTRODUCTION

The continuous use of the same herbicide or herbicides acting on the same target site leads to the selection of herbicide resistant weed populations (Maxwell and Mortimer 1994). The selected resistant weeds grow and reproduce without any competition with wild-type in the population. If the same herbicide is continuously used, the number of resistant weeds increases in the population. Since the first herbicide resistant weed was reported in common groundsel (*Senecio vulgaris*), 163 resistant weed species have been reported (Ryan 1970; Heap 2003). Resistance occurs in most herbicide classes.

Fitness is the evolutionary success of a phenotype, based on its survival and reproductive success (Radosevich et al. 1997). Fitness of herbicide resistant and susceptible biotypes has been identified as an important factor influencing the evolution and dynamics of herbicide resistance (Maxwell et al. 1990). The population dynamics will be determined by the relative fitness of each biotype in the absence of herbicides. Jasieniuk et al. (1996) demonstrated that small difference in fitness in the absence of herbicide selection could affect the initial frequency of resistant biotypes by 100-fold or more. The relative fitness of phenotypes is determined by the survivorship of seeds, seedlings, and mature plants and reproductive success determined by pollen and seed production, in addition to plant competition (Maxwell et al. 1990).

Though there are a few exceptions, triazine resistant biotypes are generally less fit than susceptible biotypes in the absence of triazine herbicides (Holt and Thill 1994).

Reduced rates of PSII electron transport, quantum yields, biomass production, and competitiveness are characteristics of triazine resistant biotypes. In contrast to triazine resistant biotypes, no consistent differences in the relative fitness of ALS-inhibitor resistant biotypes have been measured (Saari et al. 1994).

Increased germination rates have been observed in ALS-inhibitor resistant biotypes. The resistant near-isogenic line of Bibb lettuce germinated faster than the susceptible line (Mallory-Smith et al. 1992). Dyer et al. (1993) showed that seeds of a sulfonylurea resistant kochia population germinated faster than seeds from a susceptible population at 4.6 C but not at 10.5 C. They proposed that elevated levels of branched chain amino acids in the resistant seed could be responsible for the faster germination. Thompson et al. (1994a) found that the difference in the rate of germination between resistant and susceptible biotypes of kochia (*Kochia scoparia*) was temperature-dependent with the resistant biotypes germinating faster at 8 C and 18 C but not at 28 C.

A sulfonylurea (SU) herbicide susceptible prickly lettuce (*Lactuca serriola*) biotype produced 31% more shoot dry weight and accumulated biomass 52% faster than the resistant biotype (Alcocer-Ruthling et al. 1992a). No difference in growth rates was observed in SU herbicide resistant and susceptible kochia biotypes (Thompson et al. 1994b; Christoffoleti et al 1997). SU herbicide susceptible *Arabidopsis thaliana* lines produced about 25% more seeds than the resistant lines, especially in nutrient-poor condition (Purrington and Bergelson 1997). No difference in seed production was observed between resistant and susceptible biotypes in prickly

lettuce and kochia (Alcocer-Ruthling et al. 1992b; Thompson et al 1994b). The relative competitiveness of SU herbicide resistant biotypes were compared with the susceptible biotypes in prickly lettuce and kochia (Alcocer-Ruthling et al. 1992b; Thompson et al 1994b). The resistant biotype had similar competitiveness when compared to the susceptible biotypes in both plant species.

With repeated use of primisulfuron, ALS-inhibitor resistant downy brome (*Bromus tectorum*) biotypes were identified at Madras and Athena, Oregon (Mueller-Warrant et al. 1999; Ball and Mallory-Smith 2000). The mechanism of resistance for the Athena resistant (AR) biotype was an altered target site, while resistance in the Madras resistant (MR) biotype was due to relatively rapid metabolism (Park et al. 2002; Park et al. 2003). The objective of this research was to determine the relative fitness of two different resistant (AR and MR) biotypes to ALS inhibitors, when compared to susceptible (AS and MS) biotypes with regard to seed germination, growth rate, seed production, and competitiveness. This information will add insight into potential fitness differences between the resistant and susceptible biotypes. Different fitness, if it exists, could be helpful in developing resistance management strategies.

MATERIALS AND METHODS

Plant material

Seeds of AR and AS biotypes were collected from two different Kentucky bluegrass seed fields near Athena, Oregon in 1997. Seeds of MR and MS biotypes were harvested in a Kentucky bluegrass (*Poa pratensis*) field research study at Madras, Oregon in 1998. Seed samples were screened to confirm resistance or susceptibility to primisulfuron in the greenhouse with rates equivalent to 40 g ai/ha primisulfuron (data not shown). The seed was vernalized at 5 C for 6 weeks and then 10 plants from each biotype were grown in the greenhouse under 25/20 C with a 16-h photoperiod. After the plants senesced, seeds from 10 plants were collected by biotype and combined. The seeds were cleaned and stored at room temperature until experimentation.

Germination test

Seed germination of Athena (AR vs. AS) and Madras (MR vs. MS) biotypes was compared at constant 5, 15, and 25 C in germination chambers[1] with 24 h light. Fifty seeds of each biotype were placed on two Whatman No. 2 filter papers in 10 cm diameter plastic petri dishes. Three ml of distilled water was placed in each petri dish and additional water was added as needed. At 6 hr intervals, germinated seeds were counted and removed from the petri dishes. A seed was counted as germinated when the length of radicle was greater than the seed diameter. After 3 wks, the viability of ungerminated seeds was tested with tetrazolium chloride (Peters 2000).

Experiments were conducted in a completely randomized design with four replications and were repeated. The same seed sources and germination chambers[1] were used for both experiments. The cumulative germination was calculated with the Weibull equation (Brown and Mayer 1988; Shafii et al. 1991):

$$y = M\,[1\text{-}\exp\,(-\,(K\,(t\text{-}L)\,)^{C})\,] \qquad [1]$$

where y is the cumulative germination percentage at time t and M, K, L and C are empirically derived constants. M is the maximum cumulative germination, K is the rate of increase, L is the lag in germination, and C is the shape parameter. Single and joint hypothesis of parameter estimates were compared between resistant and susceptible biotype models in all temperature regimes.

Germination parameters and standard errors for all biotypes at each temperature were similar and so data were combined over experiments. All parameter estimates were significantly different from zero ($P<0.001$) based on asymptotic t tests, suggesting the model was reasonable and all parameters were required in the model (data not shown). The lack of fit tests showed that there was no significant lack of fit in any models with the lowest p value of 0.369 indicating that the regression functions fit the data (data not shown).

Comparative growth and seed production

Seeds of each biotype were vernalized at 5 C for 6 wks. The seedlings were transplanted into individual plastic pots (15 cm diameter × 20 cm deep) containing commercial potting mix[2] fertilized with Osmocote[3]. Plants were grown in the

greenhouse under 25/20 C with a 16-h photoperiod. A plant of each biotype from each replication were harvested at 5 day intervals. The first harvest was at 15 days after transplanting (DAT) and finished 80 DAT, resulting in 14 harvest dates. Plants were cut at the soil surface and leaf area and plant height were measured. Leaf area was determined with a Li-COR stationary leaf area meter[4]. The plants were dried at 75 C for 72 hr and weighed for shoot dry weight. Ten plants of each biotype were retained for seed production. After plants senesced, seeds of each biotype were harvested and cleaned. The number and weight of seeds per plant were measured.

The experiment was a randomized complete block design with four replications and was repeated. Experiments were begun August, 2000 and 2001, respectively. The plant height, leaf area, and shoot dry weight data collected from both experiments were combined.

Shoot dry weight (W) at time t was fit using the Richards function:

$$W = A(1 \pm \exp(b-kt))^{-1/n} \quad [2]$$

where parameter A is the asymptotic maximum shoot dry weight, b is the time when the curve rises above zero, k is a rate constant, and n is the shape of the curve (Richards 1959; Hunt 1982). Leaf area and plant height data were also fit to Richards function. The variability of the data increased as the time increased. To overcome unequal variance, the data for the shoot dry weight were analyzed by weighed nonlinear squares with 1 time^{-3} and the data for the leaf area and plant height were analyzed by weighted nonlinear squares with 1 time^{-2}. The seed number and seed

weight in Athena and Madras resistant and susceptible biotypes were compared using Fisher's Protected LSD test at a significance level of 0.05.

Competitive growth

The competition studies were conducted with the following combinations: AR:AS, MR:MS, AR:MR, and AS:MS biotypes. Seeds of each biotype were vernalized at 5 C for 6 weeks and then planted in 98 cell trays (26 ml/cell). Fourteen days after planting (DAP) at the 3- to 4-lf stage, plants were transplanted into pots (40×40×30cm; 0.16m^2) containing a commercial potting mix[2] fertilized with 50 g of Osmocote[3] per pot. Competition between biotypes was evaluated using a replacement series experiment at 5 planting ratios (100:0, 75:25, 50:50, 25:75, and 0:100) and at a planting density of 100 plants m^{-2} (16 plants per pot). Plants were grown in a greenhouse with 12 h supplemental lighting and 20/15 C day/night temperature. The experiment was conducted in a randomized complete block design with four replications and was repeated. Experiments were begun November, 2001 and February 2002, respectively. Pots were rearranged within each block every 5 d to reduce light variation. Plants were cut at the soil surface 60 DAT. Individual plant height and leaf area was measured. Four plants per pot were measured for leaf area, and then multiplied by 4 to obtain a total leaf area per pot.

Plants were dried at 75 C for 72 hr and weighed for shoot dry weight. No edge effect was found based on a Student's t-test. Individual plant height, leaf area, and shoot dry weight data were subjected to ANOVA. The experiment by treatment

interaction was not significant and data from the repeated experiments were combined. Total shoot dry weight and leaf area were compared to the theoretical yields for equal competitive ability using 95% confidence intervals. Statistical computations were carried out using SAS/STAT, IML (SAS 1987).

RESULTS AND DISCUSSION

Germination test

Seeds of all downy brome biotypes germinated more than 95% at 5, 15, and 25 C (Figures 4.1 and 4.2). Ungerminated seeds from the 5 and 15 C treatments were viable (94 and 88% viable, respectively), based on the tetrazolium test. No viable seeds remained from the 25 C treatment because seeds deteriorated. There was no difference in the number of viable seeds between resistant and susceptible biotypes (data not shown).

The maximum cumulative germination between AR and AS was not different at 15 and 25 C (Table 4.1). Although a contrast procedure showed that the maximum cumulative germination was different between AR and AS biotypes at 5 C, the difference was only 2.5% (Table 4.1). The maximum cumulative germination between MR and MS biotypes was not different at any temperature regime (Table 4.2).

Seeds of the AR biotype germinated 27, 8, and 5 h sooner than the AS biotype at 5, 15, and 25 C, respectively (Table 4.1). However, the difference was significant only at 5 C. There was no difference in the onset of germination between MR and MS biotypes at any temperature regime (Table 4.2).

The rate of germination for all biotypes increased as temperature increased (Tables 4.1 and 4.2). There were no differences in the rate of germination between resistant and susceptible biotypes at any temperature regime. The F statistics comparing the joint hypothesis of all parameters between AR and AS biotypes

decreased as temperature increased and provided no significant difference at 25 C (Table 4.1). These results confirmed that differences between AR and AS biotypes germination processes were greater as temperatures decrease. This difference might be important because downy brome germinates during the late fall or winter when temperatures are cooler. Similar results were observed in SU resistant Bibb lettuce isoline and kochia species (Mallory-Smith et al. 1992; Thompson et al. 1994a). However, the germination between MR and MS biotypes was similar at all temperatures tested (Table 4.2).

Comparative growth and seed production

Shoot dry weight, leaf area, and plant height of the AR biotype were similar to those of the AS biotype under noncompetitive conditions (Figure 4.3). The MS biotype produced more shoot dry weight and leaf area than the MR biotype (Figure 4.4). The MS biotype was taller than the MR biotype early in development. The asymptotes (A) calculated from the Richards function showed that the MR biotype produced 33% less shoot dry weight and 15% less leaf area than the MS biotype (Table 4.3). The plant height of the MR biotype was 77% of the MS biotype. Other parameter estimates (b, k, and n) from shoot dry weight, leaf area, and plant height models were similar among all resistant and susceptible biotypes indicating similar rate and shape of the growth curves in all biotypes (Table 4.3).

The AR and MR biotypes produced 17 and 29% less seed than the AS and MS biotypes, respectively (Table 4.4). However, total weight of seeds were similar

between AR and AS biotypes, because seeds of the AR biotype were heavier than those of the AS biotype. The heavier seed weight of the AR biotype may be related to the early germination at low temperature.

Competitive growth

When the AR and AS biotypes were grown in mixtures under competitive conditions, the shoot dry weight per pot of the AR biotype was similar to that of the AS biotype (Figure 4.5A). The lines for the shoot dry weight per pot of the AR and AS biotypes were similar and correspond the theoretical response of two biotypes having equal competitive fitness. A similar result was observed in the AS:MS mixture (Figure 4.5D). ANOVA for individual shoot dry weight showed no ratio effect in AR:AS and AS:MS mixtures indicating no competition between the biotypes (Table 4.5). However, the shoot dry weight of the MR biotype was significantly reduced when grown with the MS or AR biotype (Figure 4.5B and C). Ratio effects in MR:MS and AR:MR mixtures were significant indicating competition between biotypes (Table 4.5). The shoot dry weight of the MR biotype was greatest in monoculture and decreased as its proportion decreased in MR:MS and AR:MR mixtures, while the shoot dry weight of the MS and AR biotypes increased (Table 4.6). The results from the leaf area per pot were similar to those from the shoot dry weight in all mixtures (Figure 4.6). ANOVA for individual plant leaf area showed that only the MR:MS mixture had a ratio effect indicating competition between biotypes (Table 4.5). The individual leaf area of the MR biotype at 25% of the

mixture with the MS biotype decreased 30% compared to leaf area in monoculture. Whereas the leaf area of the MS biotype at 25% of the mixture increased 28% when compared to monoculture (100%) of each biotype (Table 4.7). ANOVA for individual plant height showed no difference in any mixture (Table 4.5). The plant height of each biotype in all mixtures was constant at all proportions (Table 4.8). These results indicate that the AR, AS, and MS biotype are equally competitive but the MR biotype is less competitive than the MS and AR biotypes.

The AR biotype, whose mechanism of resistance is an altered ALS binding site, reached about 60% germination before the AS biotype began germination at 5 C (Figure 4.1). Although, the AR biotype produced fewer seeds than the AS biotype, the seeds were larger. However, there were no differences in shoot dry weight, leaf area, and plant height between AR and AS biotypes. The AR biotype also was equally competitive to the AS biotype. Thus, it appears that the trait conferring resistance to ALS inhibitors in the AR biotype does not result in a growth penalty. Similar results were observed in a SU resistant kochia biotype, whose mechanism of resistance is an altered ALS binding site (Thompson et al. 1994a, 1994b).

No germination differences were observed between the MR and MS biotypes. However, the MR biotype was less fit than the MS biotype in growth, seed production, and competition.

Different mechanisms of ALS inhibitor resistance in the AR and MR biotypes appear to influence there relative fitness and competitive ability. Although the AR biotype produced 17 % less seeds than the AS biotype, the AR biotype might

dominate early in the season, because of its early germination at low temperature and large seed size. Therefore, it is difficult to predict the population dynamics of the AR biotype in the weed population. However, the MR biotype should decrease and the population shift toward a higher frequency of the MS biotype in the absence of ALS inhibitors because the MR biotype is less fit and competitive than the MS biotype.

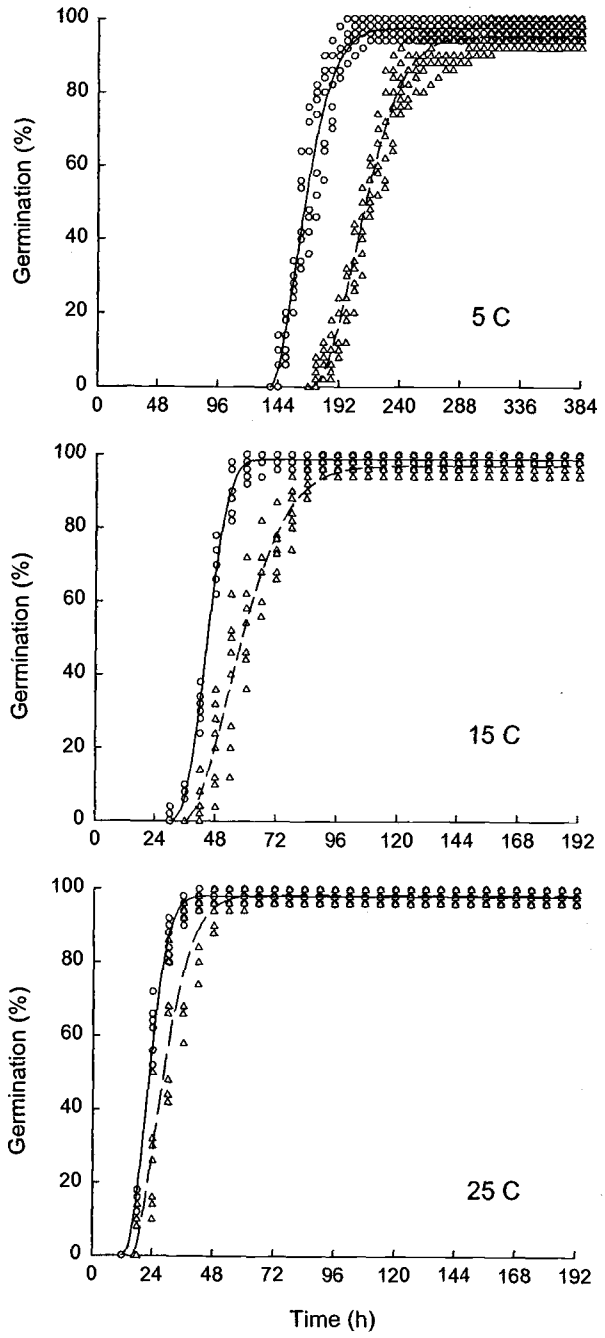

Figure 4.1. Cumulative germination percentage for AR ○ and AS △ downy brome biotypes at 5, 15, and 25 C. Solid lines are regression curves for the AR biotype and dashed lines are regression curves for the AS biotype.

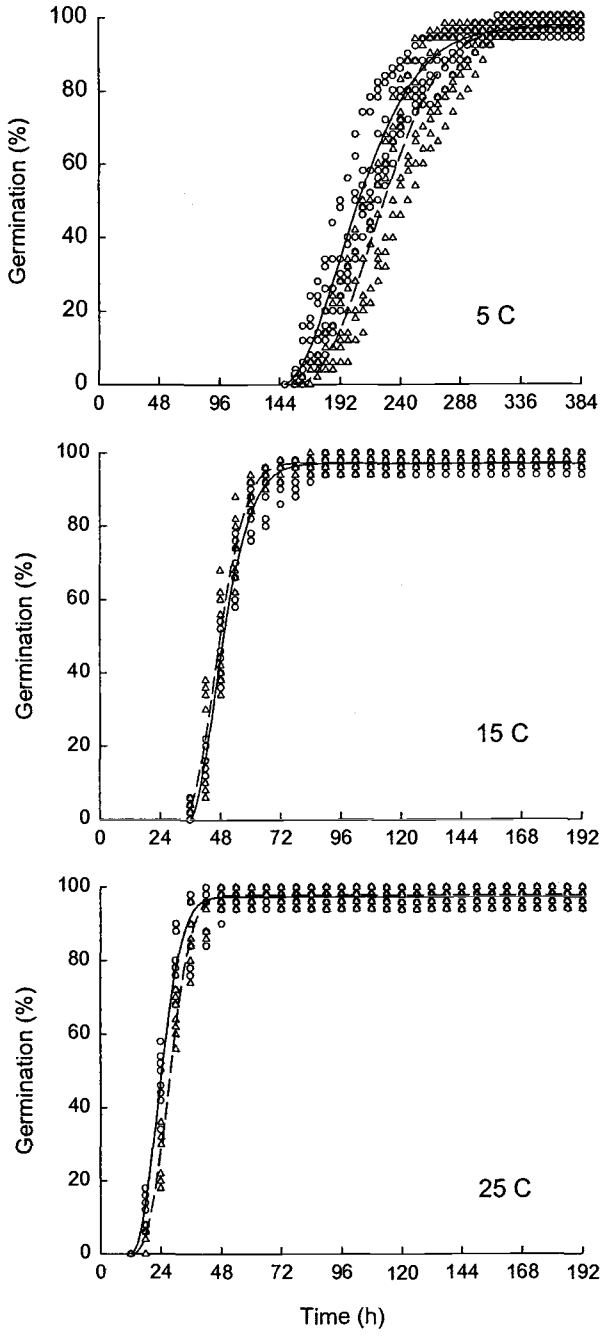

Figure 4.2. Cumulative germination percentage for MR ○ and MS △ downy brome biotypes at 5, 15, and 25 C. Solid lines are regression curves for the MR biotype and dashed lines are regression curves for the MS biotype.

Table 4.1. Parameter estimates, the asymptotic standard errors (SE), and contrasts for each parameter for germination models of AR and AS downy brome biotypes at temperatures 5, 15, and 25 C.

		Parameter estimates		
Temp.	Parameter[1]	AR (SE)	AS (SE)	F (Pr>F)
5 C	M	97.43 (0.335)	94.97 (0.412)	4.34 (0.038)
	K	0.03 (0.002)	0.02 (0.002)	0.72 (0.396)
	L	137.80 (2.287)	164.70 (4.577)	4.24 (0.040)
	C	1.76 (0.162)	2.30 (0.236)	30.73 (0.001)[2]
15 C	M	98.52 (0.289)	97.19 (1.053)	0.39 (0.536)
	K	0.06 (0.006)	0.04 (0.004)	0.52 (0.473)
	L	29.02 (1.845)	36.51 (2.818)	0.52 (0.473)
	C	2.79 (0.349)	1.71 (0.253)	17.41 (0.001)
25 C	M	98.22 (0.215)	97.36 (0.311)	0.01 (0.979)
	K	0.11 (0.007)	0.07 (0.004)	0.24 (0.622)
	L	15.41 (0.434)	20.00 (0.813)	2.14 (0.145)
	C	1.41 (0.106)	1.56 (0.120)	1.49 (0.206)

[1]M: maximum cumulative germination, K: rate of increase, L: lag in germination, and C: shape parameter.

[2]Contrast for joint hypothesis of parameter estimates (M, K, L, and C)

Table 4.2. Parameter estimates, the asymptotic standard errors (SE), and contrasts for each parameter for germination models of MR and MS downy brome biotypes at temperatures 5, 15, and 25 C.

		Parameter estimates		
Temp.	Parameter[1]	MR (SE)	MS (SE)	F (Pr>F)
5 C	M	96.87 (0.771)	97.41 (0.591)	0.01 (0.950)
	K	0.02 (0.001)	0.01 (0.001)	0.41 (0.522)
	L	149.30 (4.964)	158.20 (5.186)	0.47 (0.494)
	C	1.73 (0.172)	2.26 (0.165)	3.48 (0.008)[2]
15 C	M	97.38 (0.419)	96.81 (0.506)	0.16 (0.690)
	K	0.08 (0.005)	0.06 (0.008)	1.20 (0.274)
	L	38.76 (0.762)	32.50 (2.570)	1.00 (0.318)
	C	1.29 (0.112)	2.12 (0.371)	0.42 (0.797)
25 C	M	97.50 (0.333)	98.16 (0.283)	0.63 (0.429)
	K	0.09 (0.007)	0.07 (0.005)	0.53 (0.428)
	L	15.01 (0.797)	16.09 (0.926)	1.02 (0.313)
	C	1.58 (0.165)	1.94 (0.168)	7.72 (0.001)

[1]M: maximum cumulative germination, K: rate of increase, L: lag in germination, and C: shape parameter.

[2]Contrast for joint hypothesis of parameter estimates (M, K, L, and C)

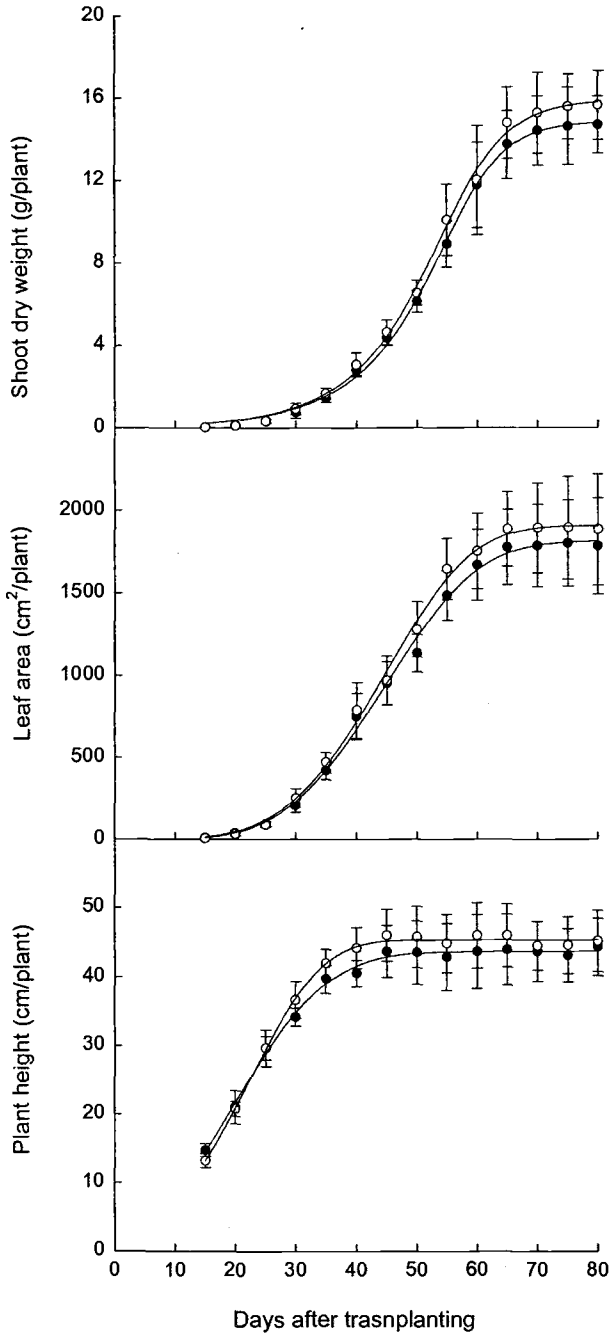

Figure 4.3. Regression curves and 95% confidence intervals for shoot dry weight, leaf area, and plant height of AR ● and AS ○ downy brome biotypes under noncompetitive conditions.

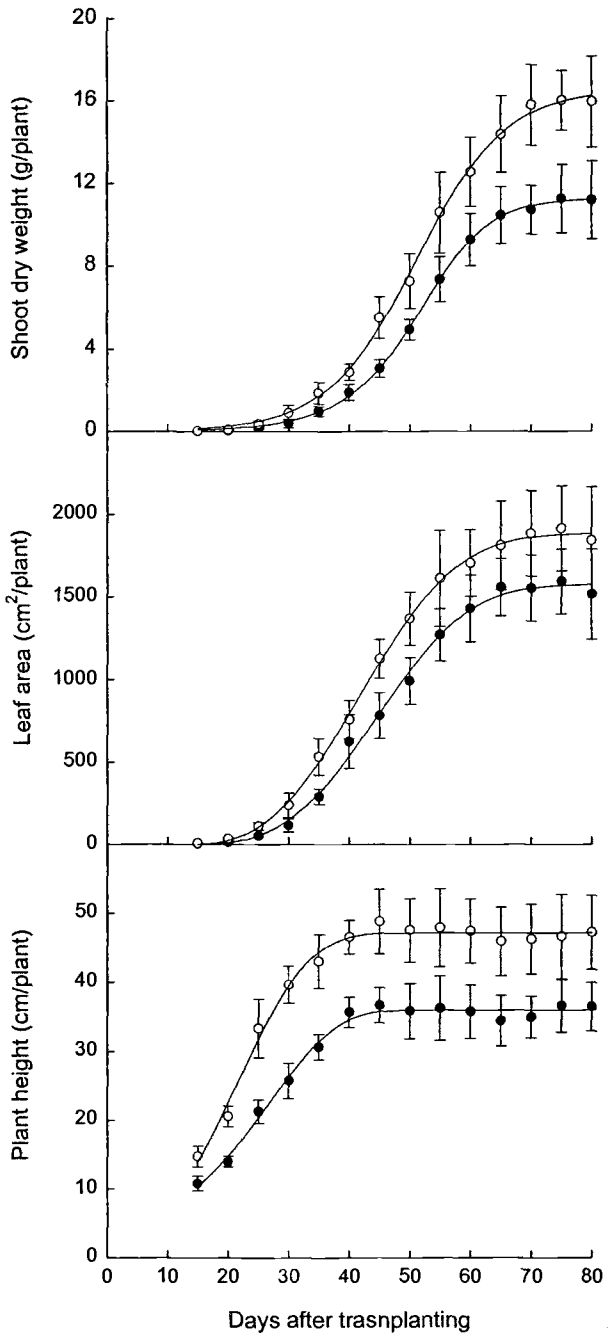

Figure 4.4. Regression curves and 95% confidence intervals for shoot dry weight, leaf area, and plant height of MR ● and MS ○ downy brome biotypes under noncompetitive conditions.

Table 4.3. Parameter estimates for shoot dry weight, leaf area, and plant height of AR, AS, MR, and MS downy brome biotypes under noncompetitive conditions.

	Parameter estimates			
Parameter[1]	AR	AS	MR	MS
Shoot dry weight				
A	15.75 (0.75)[2]	16.93 (0.80)	11.51 (0.55)	17.21 (0.76)
b	6.17 (1.51)	5.50 (1.34)	7.75 (2.23)	4.79 (1.19)
k	0.12 (0.02)	0.11 (0.02)	0.15 (0.03)	0.11 (0.02)
n	0.82 (0.27)	0.71 (0.24)	0.97 (0.38)	0.58 (0.20)
Leaf area				
A	1906 (79.9)	2012 (85.2)	1647 (73.1)	1940 (74.2)
b	2.79 (1.56)	2.88 (1.63)	2.71 (2.14)	3.17 (1.63)
k	0.10 (0.02)	0.10 (0.02)	0.10 (0.02)	0.11 (0.02)
n	0.31 (0.24)	0.31 (0.26)	0.23 (0.28)	0.34 (0.27)
Plant height				
A	43.8 (0.80)	45.7 (0.65)	36.2 (0.62)	47.2 (0.83)
b	3.78 (1.64)	4.94 (1.41)	8.22 (2.66)	6.79 (2.40)
k	0.16 (0.04)	0.19 (0.04)	0.25 (0.07)	0.25 (0.07)
n	1.50 (0.84)	1.76 (0.66)	3.70 (1.39)	2.63 (1.17)

[1]A: asymptotes, b: time when the curve rises above zero, k: rate constant, and n: shape parameter.
[2]Asymptotic standard errors for estimated parameters are in parenthesis.

Table 4.4. Seed production for AR, AS, MR, and MS downy brome biotypes under noncompetitive conditions.[1]

Biotype	Total seed	Seed weight	
	no. plant^{-1}	g 300 seeds^{-1}	g plant^{-1}
AR	2390 b	0.89 a	6.54 a
AS	2883 a	0.73 b	6.61 a
MR	2211 b	0.76 b	5.16 b
MS	3126 a	0.74 b	7.19 a

[1]Means within a column followed by the same letter are not significantly different as determined by the Fisher's protected LSD test (P =0.05).

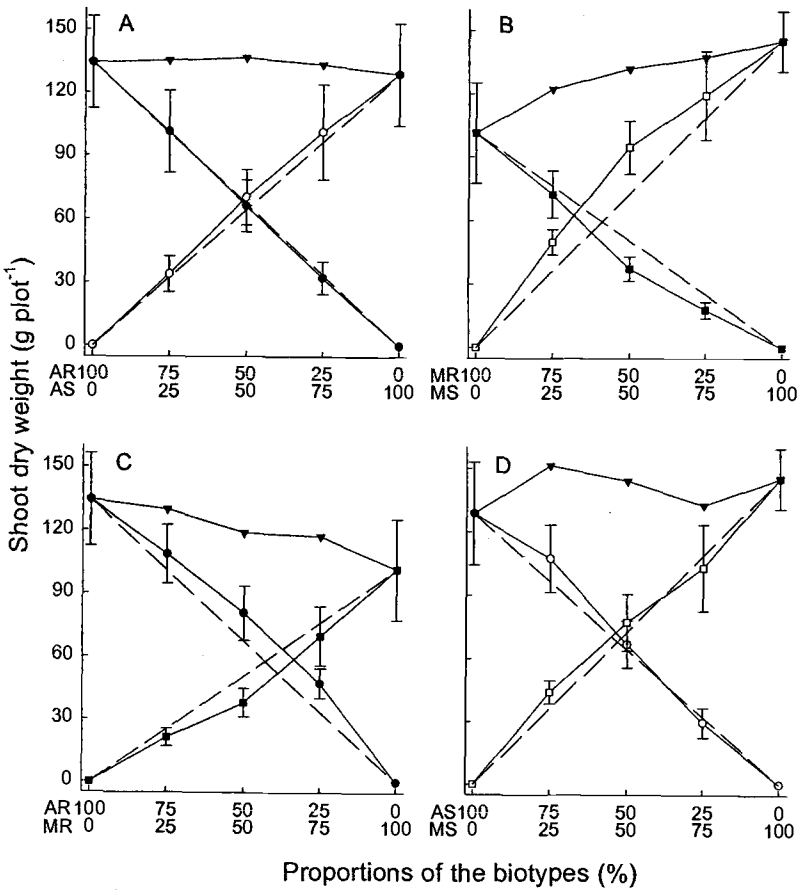

Figure 4.5. Shoot dry weight per pot produced by resistant or susceptible downy brome biotypes (AR ●, AS ○, MR ■, MS □, and total ▼) grown at five proportions and a constant density (100 plants pot^{-1}). Dashed lines indicate theoretical shoot dry weight when the biotypes are equally competitive. Vertical bars represent 95% confidence intervals of the mean.

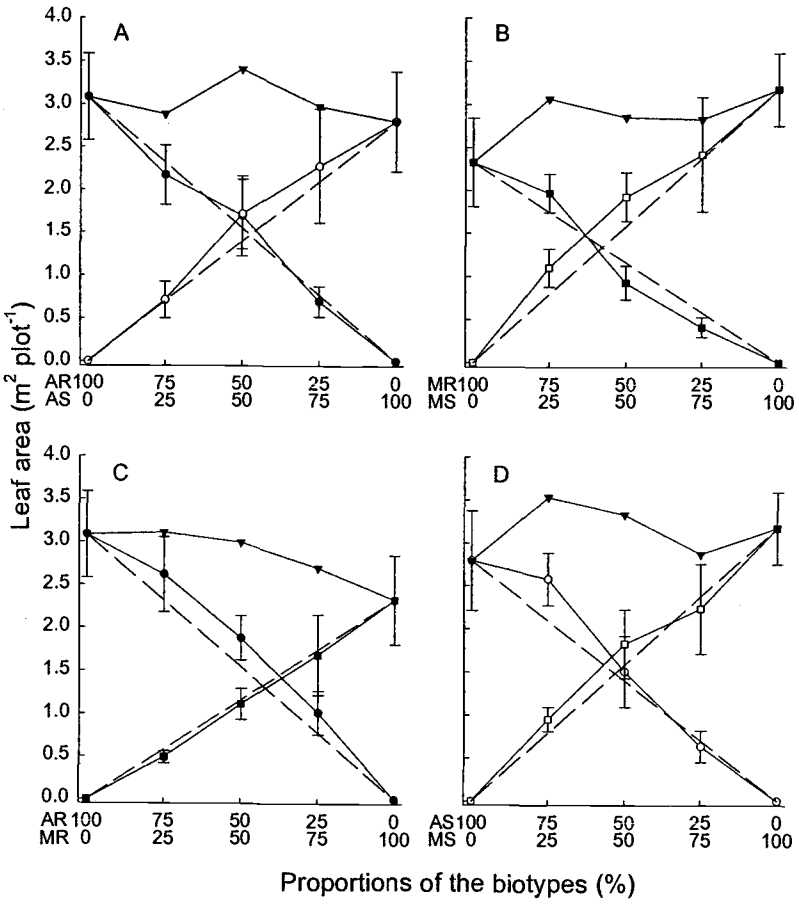

Figure 4.6. Leaf area per pot produced by resistant or susceptible downy brome biotypes (AR ●, AS ○, MR ■, MS □, and total ▼) grown at five proportions and a constant density (100 plants pot^{-1}). Dashed lines indicate theoretical shoot dry weight when the biotypes are equally competitive. Vertical bars represent 95% confidence intervals of the mean.

Table 4.5. Analysis of variance table on individual shoot dry weight, leaf area, and plant height for four combinations in a replacement series experiments.

	Shoot dry weight					Leaf area					Plant height				
Source	d.f.	AR:AS	MR:MS	AR:MR	AS:MS	d.f.	AR:AS	MR:MS	AR:MR	AS:MS	d.f.	AR:AS	MR:MS	AR:MR	AS:MS
Block	3	**[1]	**	**	**	3	**	*	NS	**	3	*	**	**	NS
Proportion	4	NS	**	**	NS	4	NS	*	NS	NS	4	NS	NS	NS	NS
Error a	12					12					12				
Biotype	1	NS	**	**	**	1	NS	**	**	NS	1	NS	**	**	**
Proportion×biotype	2	NS	**	**	**	2	NS	**	NS	**	2	NS	*	*	**
Error b	617					137					617				
Total	639					159					639				

[1]NS not significant; * $P \leq 0.05$; ** $P \leq 0.01$

Table 4.6. Individual shoot dry weight for ALS-inhibitor resistant (AR and MR) and susceptible (AS and MS) downy brome biotypes in four combinations of a replacement series experiment at five proportions.

		Proportions of mixture (%)				
		100	75	50	25	0
Mixture	Biotype	0	25	50	75	100
		(g plant^{-1})				
AR : AS	AR	8.4 (0.40)[1]	8.5 (0.56)	8.3 (0.77)	8.0 (1.20)	0
	AS	0	8.5 (0.70)	8.8 (0.89)	8.4 (1.24)	8.0 (0.41)
MR : MS	MR	6.3 (0.37)	6.0 (0.33)	4.7 (0.35)	4.5 (0.62)	0
	MS	0	12.5 (1.20)	11.8 (0.71)	10.0 (0.59)	9.1 (0.33)
AR : MR	AR	8.4 (0.40)	9.1 (0.52)	10.1 (0.72)	11.8 (1.32)	0
	MR	0	5.3 (0.65)	4.8 (0.42)	5.8 (0.41)	6.3 (0.37)
AS : MS	AS	8.0 (0.41)	9.1 (0.62)	8.4 (0.65)	7.6 (1.06)	0
	MS	0	11.0 (0.96)	9.7 (0.71)	8.7 (0.57)	9.1 (0.33)

[1]95% confidence intervals are in parenthesis.

Table 4.7. Individual plant leaf area for ALS-inhibitor resistant (AR and MR) and susceptible (AS and MS) downy brome biotypes in four combinations of a replacement series experiment at five proportions.

		Proportions of mixture (%)				
		100	75	50	25	0
Mixture	Biotype	0	25	50	75	100
		(cm^2 $plant^{-1}$)				
AR : AS	AR	1927 (163)[1]	1807 (194)	2111 (439)	1739 (444)	0
	AS	0	1774 (534)	2139 (370)	1894 (305)	1745 (185)
MR : MS	MR	1453 (158)	1640 (148)	1158 (179)	1022 (287)	0
	MS	0	2752 (554)	2408 (258)	2015 (291)	1982 (172)
AR : MR	AR	1927 (163)	2179 (215)	2347 (259)	2530 (638)	0
	MR	0	1226 (179)	1389 (178)	1403(218)	1453 (158)
AS : MS	AS	1745 (185)	2148 (255)	1881 (382)	1594 (460)	0
	MS	0	2384 (352)	2281 (351)	1861 (237)	1982 (172)

[1]95% confidence intervals are in parenthesis.

Table 4.8. Individual plant height for ALS-inhibitor resistant (AR and MR) and susceptible (AS and MS) downy brome biotypes in four combinations of a replacement series experiment at five proportions.

		Proportions of mixture (%)				
		100	75	50	25	0
Mixture	Biotype	0	25	50	75	100
		(cm plant^{-1})				
AR : AS	AR	61.6 (0.85)[1]	59.1 (1.26)	60.5 (1.78)	59.7 (2.15)	0
	AS	0	60.2 (2.40)	61.9 (1.82)	59.5 (1.43)	60.3 (0.94)
MR : MS	MR	46.2 (0.93)	47.6 (1.28)	48.4 (1.36)	47.1 (1.81)	0
	MS	0	54.8 (2.19)	60.4 (2.22)	56.8 (1.14)	54.8 (1.02)
AR : MR	AR	61.6 (0.85)	60.1 (1.43)	63.6 (1.91)	61.5 (1.75)	0
	MR	0	47.3 (2.37)	47.1 (1.28)	47.1 (1.01)	46.2 (0.93)
AS : MS	AS	60.3 (0.94)	62.3 (1.44)	62.0 (1.43)	57.8 (2.14)	0
	MS	0	58.3 (2.50)	58.2 (1.41)	54.7 (1.08)	54.8 (1.02)

[1]95% confidence intervals are in parenthesis.

SOURCES OF MATERIALS

[1]Controlled Environment Chamber, Hoffman Manufacturing, Inc., P.O. Box 547 Albany, OR 97321

[2]Sunshine Mix #1 potting mix, Sun Gro Horticulture, Inc., 110 110th Ave. NE, Suite 490, Bellevue, WA 98004.

[3]OSMOCOTE® (14-14-14), Southern Agricultural Insecticides, Inc., P.O. Box 218 Palmetto, FL 34220.

[4]LI-COR 3100 Leaf Area Meter, Li-COR Inc., 4421 Superior St., Lincoln, NE 68504.

LITERATURE CITED

Alcocer-Ruthling, M., D. C. Thill, and B. Shafii. 1992a. Differential competitiveness of sulfonylurea resistant and susceptible prickly lettuce (*Lactuca serriola*). Weed Technol. 6:303-309.

Alcocer-Ruthling, M., D. C. Thill, and B. Shafii. 1992b. Seed biology of sulfonylurea-resistant and -susceptible biotypes of prickly lettuce (*Lactuca serriola*). Weed Technol. 6:858-864.

Ball, D. A. and C. A. Mallory-Smith. 2000. Sulfonylurea herbicide resistance in downy brome. Proc. West. Soc. Weed Sci. 53:41-42.

Brown, R. F. and D. G. Mayer. 1988. Representing cumulative germination. 2. The use of the Weibull function and other empirically derived curves. Ann. Bot. 61:127-138.

Christoffoleti, P. J., P. Westra, and F. Moore III. 1997. Growth analysis of sulfonylurea-resistant and –susceptible kochia (*Kochia scoparia*). Weed Sci. 45:691-695.

Dyer, W. E., P. W. Chee, and P. K. Fay. 1993. Rapid germination of sulfonylurea-resistant *Kochia scoparia* accessions is associated with elevated seed levels of branched chain amino acids. Weed Sci. 41:18-22.

Heap, I. M. 2003. International Survey of Herbicide Resistant Weeds. Herbicide Resistance Action Committee and Weed Science Society of America. Internet www.weedscience.com.

Holt, J. S. and D. C. Thill. 1994. Growth and productivity of resistant plants. Page 299-316 *in* S. B. Powles and J. A. M. Holtum, eds. Herbicide resistance in plants: Biology and Biochemistry. Ann Arbor, MI: Lewis.

Hunt, R. 1982. Plant Growth Curves: The Functional Approach to Plant Growth Analysis. Baltimore, Maryland: Edward Arnold.

Jasieniuk, M., A. L. Brûlé-Babel, and I. N. Morrison. 1996. The evolution and genetics of herbicide resistance in weeds. Weed Sci. 44:176-193.

Mallory-Smith, C. A., D. C. Thill M, Alcocer-Ruthling, and C. Thompson. 1992. Growth comparisons of sulfonylurea resistant and susceptible biotypes. Proc. of the First International Weed Control Congress 2:301-303.

Maxwell, B. D. and A. M. Motimier. 1994. Selection for herbicide resistance. Page 1-26 in S. B. Powles and J. A. M. Holtum, eds. Herbicide resistance in plants: Biology and Biochemistry. Ann Arbor, MI: Lewis.

Maxwell, B. D., M. L. Roush, and S. R. Radosevich. 1990. Predicting the evolution and dynamics of herbicide resistance in weed populations. Weed Technol. 4:2-13.

Mueller-Warrant, G. W., C. A. Mallory-Smith, and P. E. Hendrickson. 1999. Non-target site resistance to ALS inhibitors in downy brome. Proc. West. Soc. Weed Sci. 52:16.

Park, K. W., C. A. Mallory-Smith, G. W. Mueller-Warrant and D. A. Ball. 2002. Point mutation confers sulfonylurea resistance to one downy brome (*Bromus tectorum*) biotype but not in another. Proc. West. Soc. Weed Sci. 55:11.

Park, K. W., L. Fandrich, and C. A. Mallory-Smith. 2003. Absorption, translocation, and metabolism of BAY MKH 6561 in ALS-inhibitor resistant- and susceptible-downy brome (*Bromus tectorum*) biotypes. Proc. West. Soc. Weed Sci. In press.

Peters, J., ed. 2000. Tetrazolium testing handbook: contribution no. 29 to the handbook on seed testing. Association of Official Seed Analysts.

Purrington C. B. and J. Bergelson. 1997. Fitness consequences of genetically engineered herbicide and antibiotic resistance in *Arabidopsis thaliana*. Genetics 145:807-814.

Radosevich, S. R., J. S. Holt, and C. Ghersa. 1997. Weed Ecology, Implications for management. 2nd ed. New York: J. Wiley, pp 69-102.

Richards, F. J. 1959. A flexible growth function for empirical use. J. Exp. Bot. 10:290-300.

Ray, T. B. 1970. Site of action of chlorsulfuron. Plant Physiol. 75:827-831.

Saari, L. L., J. C. Cotterman, and D. C. Thill. 1994. Resistance to acetolactate synthase inhibiting herbicides. Page 83-139 *in* S. B. Powles and J. A. M. Holtum, eds. Herbicide Resistance in Plants: Biology and Biochemistry. Ann Arbor, MI: Lewis.

SAS Institute. 1987. SAS/STAT™ Guide for personal computers, Version 6.0. Cary, NC: SAS Institute Inc., 1028 pp.

Shafii, B., W. J. Price, J. B. Swenson, and G. A. Murray. 1991. Nonlinear estimation of growth curve models for germination data analysis. Pages 19-36 *in* G. A.

Milliken and J. R. Schwenke, eds. Proc. of 1991 Kansas State Univ. Conf. on Appl. Statistics in Agric. Kansas State Univ., Manhattan, KS 66506.

Thompson, C. R., D. C. Thill, and B. Shafii. 1994a. Germination characteristics of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). Weed Sci. 42:50-56.

Thompson, C. R., D. C. Thill, and B. Shafii. 1994b. Growth and competitiveness of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). Weed Sci. 42:172-179.

CHAPTER 5

CONCLUSIONS

These studies were conducted to determine the mechanism of resistance of the AR and MR downy brome biotypes and their relative fitness compared to susceptible biotypes to estimate population dynamics of resistant biotypes when herbicides are not used. The AR and MR biotypes evolved at Madras and Athena, Oregon in 1997 and 1998 and are the first reported downy brome biotypes resistant to ALS inhibitors. The AR biotype was highly resistant to the SU and SCT herbicides but was not resistant to the IMI herbicide. ALS assay experiments revealed that the AR biotype was over 100-fold more resistant to the SU and SCT herbicides but was not resistant to the IMI herbicide. DNA sequence analysis of the *als* gene demonstrated a single-point mutation from C to T at nucleotide 328, conferring the exchange of the amino acid Pro_{197} to Ser in the AR biotype. These data indicate that the proline to serine change in domain A of *als* gene is responsible for the ALS inhibitor resistance in the AR biotype. Different cross-resistance pattern and level of resistance have been reported in many ALS-inhibitor resistant biotypes, whose mechanism of resistance is an altered target site. The proline to serine change in domain A of *als* gene has been reported in kochia, several *Lindernia* genera, and sugarbeet (Guttieri et al. 1995; Wright et al. 1998; Heap 2003). Resistance to SU herbicides but no resistance to IMI herbicides

were observed in the sugarbeet somatic cell line where Pro_{197} to Ser point mutation occurred (Wright et al. 1998).

The MR biotype was moderately resistant to all ALS inhibitors tested. However, no differences in ALS sensitivity and *als* gene sequence were observed between the MR and MS biotypes suggesting the resistance was not due to an insensitive ALS enzyme. Moderate level of resistance and broad range of herbicide resistance are the general characteristics of metabolism based resistant plant biotypes. Studies using ^{14}C-BAY MKH 6561 confirmed that the mechanism of resistance for the MR biotype was relatively rapid metabolism of ALS inhibitors. The half-life of BAY MKH 6561 in the MR biotype was 8.9 h which was 40% shorter than that in the MS biotype. The rapid metabolism of BAY MKH 6561 in the MR biotype was related to a P450 based on the P450 inhibitor study.

The relative fitness of resistant biotypes can be determined by the survivorship of seeds, seedlings, and mature plants and reproductive success in addition to plant competition. The AR biotype germinated 27 h earlier than the AS biotype and had reached over 60% germination when the AS biotype initially germinated at 5 C. Shoot dry weight, leaf area, and plant height of the AR biotype were similar to those of the AS biotype under competitive and noncompetitive conditions. However, the AR biotype produced 17% less seed than the AS biotype, although total weight of seeds were similar between AR and AS biotypes, because seeds of the AR biotype were heavier than those of the AS biotype.

No differences in germination were observed between MR and MS biotypes at any temperature. However, the MR biotype produced 33% less shoot dry weight and 15% leaf area than the MS biotype under noncompetitive conditions. The MR biotype produced 29% less seeds than the MS biotype. Under competitive conditions, the MR biotype was less competitive than the AR biotype as well as the MS biotype.

The two different downy brome biotypes resistant to ALS inhibitors had different mechanism of resistance: insensitive target site and enhanced metabolism. These studies also showed the different relative fitness and competitiveness between the AR and MR biotypes. Although the AR biotype produced 17 % less seeds than the AS biotype, the AR biotype could dominate early season of weed population, because of its early germination at low temperature and large seed size. Therefore, it is very difficult to predict population dynamics of the AR biotype in the weed population. However, the MR biotype should decrease and the population shifts toward higher frequency of the MS biotype in the absence of ALS inhibitors because the MR biotype is less fit and competitive than the MS biotype.

Future research on gene flow and fitness should investigate the following areas: 1) the mechanism of inheritance of ALS inhibitor resistance in the AR and MR biotypes; and 2) the relative competitiveness of the AR and MR biotypes under field conditions.

BIBLIOGRAPHY

Alcocer-Ruthling, M., D. C. Thill, and B. Shafii. 1992a. Differential competitiveness of sulfonylurea resistant and susceptible prickly lettuce (*Lactuca serriola*). Weed Technol. 6:303-309.

Alcocer-Ruthling, M., D. C. Thill, and B. Shafii. 1992b. Seed biology of sulfonylurea-resistant and -susceptible biotypes of prickly lettuce (*Lactuca serriola*). Weed Technol. 6:858-864.

Anderson, D. D., S. J. Nissen, and A. R. Martin, F. W. Roeth. 1998. Mechanism of primisulfuron resistance in a shattercane (*Sorghum bicolor*) biotype. Weed Sci. 46:158-162.

Ball, D. A. and C. A. Mallory-Smith. 2000. Sulfonylurea herbicide resistance in downy brome. Proc. West. Soc. Weed Sci. 53:41-42.

Bernasconi, P., A. R. Woodworth, B. A. Rosen, M. V. Subramanian, and D. L. Siehl. 1995. A naturally occurring point mutation confers broad range tolerance to herbicides that target acetolactate synthase. J. Biol. Chem. 270:17381-17385.

Boutsalis, P., J. Karotam, and S. B. Powles. 1999. Molecular basis of resistance to acetolactate synthase-inhibiting herbicides in *Sisymbrium orientale* and *Brassica tournefortii*. Pestic. Sci. 55:507-516.

Brown, H. M. 1990. Mode of action, crop selectivity, and soil relations of the sulfonylurea herbicides. Pestic. Sci. 29:263-281.

Brown, H. M., R. F. Dietrich, W. H. Kenyon, and F. T. Lichtner. 1991. Prospects for the biorational design of crop selective herbicides. Page 847-856 *in* Brighton Crop Protection Conference-Weeds: The British Crop Protection Council.

Brown, R. F. and D. G. Mayer. 1988. Representing cumulative germination. 2. The use of the Weibull function and other empirically derived curves. Ann. Bot. 61:127-138.

Chapple, C. 1998. Molecular-genetic analysis of plant cytochrome P450-dependent monooxygenases. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:311-343.

Christoffoleti, P. J. and P. Westra. 1992. Competition and coexistence of sulfonylurea resistant and susceptible kochia (*Kochia scoparia*) biotypes in unstable environments. Weed Sci. Soc. Am. Abstr. 32:17.

Christoffoleti, P. J., P. Westra, and F. Moore III. 1997. Growth analysis of sulfonylurea-resistant and –susceptible kochia (*Kochia scoparia*). Weed Sci. 45:691-695.

Christopher, J. T., C. Preston, and S. B. Powles. 1994. Malathion antagonizes metabolism-based chlorsulfuron resistance in *Lolium rigidum*. Pestic. Biochem. and Physiol. 49:172-182.

Christopher, J. T., S. B. Powles, D. R. Liljegren, and J. A. M. Holtum. 1991. Cross resistance to herbicides in annual ryegrass (*Lolium rigidum*). II. Chlorsulfuron resistance involves a wheat-like detoxification system. Plant Physiol. 100:1036-1043.

Creason, G. L. and R. S. Chaleff. 1988. A second mutation enhances resistance of a tobacco mutant to sulfonylurea herbicides. Theor. Appl. Genet. 76:177-182.

DeFelice, M., J. Guardiola, B. Esposito and M. Laccarino. 1974. Structural genes for a new recognized acetolactate synthase in *Escherichia coli* K-12. Bacteriology 120:1068-1077.

Devine, M. D. and C. Preston. 2000. The molecular basis of herbicide resistance. Page 72-104 *in* A. H. Cobb and R. C. Kirkwood, eds. Herbicides and Their Mechanisms of Action. Boca Raton, FL: Sheffield Academic Press.

Dyer, W. E., P. W. Chee, and P. K. Fay. 1993. Rapid germination of sulfonylurea-resistant *Kochia scoparia* accessions is associated with elevated seed levels of branched chain amino acids. Weed Sci. 41:18-22.

Eberlein, C. V., M. J. Guttieri, P. H. Berger, J. K. Fellman, C. A. Mallory-Smith, D. C. Thill, R. J. Baerg, and W. R. Belknap. 1999. Physiological consequences of mutation for ALS-inhibitor resistance. Weed Sci. 47:383-392.

Fandrich, L., S. K. MacDonald, S. J. Nissen, P. Westra, and H. J. Santel. 2001. Absorption and fate of BAY MKH 6561 in jointed goatgrass and downy brome. Weed Sci. 49:717-722.

Feucht, D., K. H. Mueller, A. Wellmann, and H. J. Santel. 1999. BAY MKH 6561-a new selective herbicide for grass control in wheat, rye and triticale. Proc. Br. Crop Prot. Conf. Weeds 1:53-58.

Fonne-Pfister, R., J. Gaudin, K. Kreuz, K. Ramsteiner, and E. Ebert. 1990. Hydroxylation of primisulfuron by an inducible cytochrome P450-dependent monooxygenase system from maize. Pestic. Biochem. Physiol. 37:165-173.

Frear, D. S., H. R. Swanson, and F. W. Thalacker. 1991. Induced microsomal oxidation of diclofop, triasulfuron, chlorsulfuron, and linuron in wheat. Pestic Biochem. Physiol. 41:274-287.

Geier, P. W., P. W. Stahlman, S. D. Miller, and N. R. Hageman. 1998. MON 37500 rate and timing affects downy brome (*Bromus tectorum*) control in winter wheat (*Triticum aestivum*). Weed Sci. 46:366-373.

Guttieri, M. J., C. V. Eberlein, and D. C. Thill. 1995. Diverse mutations in the acetolactate synthase gene confer chlorsulfuron resistance in kochia (*Kochia scoparia*) biotypes. Weed Sci. 43:175-178.

Heap, I. M. 2003. International Survey of Herbicide Resistant Weeds. Herbicide Resistance Action Committee and Weed Science Society of America. Internet www.weedscience.com.

Hendrickson, P. E. and C. A. Mallory-Smith. 1999. Response of downy brome (*Bromus tectorum*) and Kentucky bluegrass (*Poa pratensis*) to applications of primisulfuron. Weed Technol. 13:461-465.

Hoagland, D. R. and D. I. Arnon. 1938. The water culture method of growing plants without soil. Californian Agricultural Experimental Station Circular Number 347.

Holt, J. S. and D. C. Thill. 1994. Growth and productivity of resistant plants. Page 299-316 *in* S. B. Powles and J. A. M. Holtum, eds. Herbicide resistance in plants: Biology and Biochemistry. Ann Arbor, MI: Lewis.

Hunt, R. 1982. Plant Growth Curves: The Functional Approach to Plant Growth Analysis. Baltimore, Maryland: Edward Arnold.

Jasieniuk, M., A. L. Brûlé-Babel, and I. N. Morrison. 1996. The evolution and genetics of herbicide resistance in weeds. Weed Sci. 44:176-193.

Mallory-Smith, C. A., D. Thill, and D. Morishita. 2000. Herbicide-resistant weeds and their management. PNW 437 Univ. of Idaho, Moscow, ID

Mallory-Smith, C. A., D. C. Thill M, Alcocer-Ruthling, and C. Thompson. 1992. Growth comparisons of sulfonylurea resistant and susceptible biotypes. Proc. of the First International Weed Control Congress 2:301-303.

Mallory-Smith, C. A., D. C. Thill, and M. J. Dial. 1990. Identification of herbicide resistant prickly lettuce (*Lactuca serriola*). Weed Technol. 4:163-168.

Mallory-Smith, C. A., P. Hendrickson, and G. W. Mueller-Warrant. 1999. Cross-resistance of primisulfuron-resistant *Bromus tectorum* L. (downy brome) to sulfosulfuron. Weed Sci. 47:256-257.

Maxwell, B. D. and A. M. Motimier. 1994. Selection for herbicide resistance. Page 1-26 *in* S. B. Powles and J. A. M. Holtum, eds. Herbicide Resistance in Plants: Biology and Biochemistry. Ann Arbor, MI: Lewis.

Maxwell, B. D., M. L. Roush, and S. R. Radosevich. 1990. Predicting the evolution and dynamics of herbicide resistance in weed populations. Weed Technol. 4:2-13.

Menendez, J. M., R. De Prado, M. D. Devine. 1997. Chlorsulfuron cross-resistance in a chlorotoluron-resistant biotype of *Alopercurus myosuroides*. Proc. Br. Crop Prot. Conf. Weeds 1: 319-324.

Morrow, L. A. and P. W. Stahlman. 1984. The history and distribution of downy brome (*Bromus tectorum*) in North America. Weed Sci. 32, Suppl. 1:2-6.

Mueller-Warrant, G. W., C. A. Mallory-Smith, and P. E. Hendrickson. 1999. Non-target site resistance to ALS inhibitors in downy brome. Proc. West. Soc. Weed Sci. 52:16.

Park, K. W., C. A. Mallory-Smith, G. W. Mueller-Warrant and D. A. Ball. 2002. Point mutation confers sulfonylurea resistance to one downy brome (*Bromus tectorum*) biotype but not in another. Proc. West. Soc. Weed Sci. 55:11.

Park, K. W., L. Fandrich, and C. A. Mallory-Smith. 2003. Absorption, translocation, and metabolism of BAY MKH 6561 in ALS-inhibitor resistant- and susceptible-downy brome (*Bromus tectorum*) biotypes. Proc. West. Soc. Weed Sci. In press.

Park, K-W., C. A. Mallory-Smith, G. W. Mueller-Warrant and D. A. Ball. 2001. Growth characteristics of sulfonylurea-resistant and –susceptible downy brome biotypes. Proc. West. Soc. Weed Sci. 54:12.

Patzoldt, W. L. and P. J. Tranel. 2001. ALS mutations conferring herbicide resistance in waterhemp. Proc. N. Cent. Weed Sci. Soc. 56:67.

Peters, J., ed. 2000. Tetrazolium testing handbook: contribution no. 29 to the handbook on seed testing. Association of Official Seed Analysts.

Primiani, M. M., J. C. Cotterman, and L. L. Saari. 1990. Resistance of kochia (*Kochia scoparia*) to sulfonylurea and imidazolinone herbicides. Weed Technol. 4:169-172.

Purrington C. B. and J. Bergelson. 1997. Fitness consequences of genetically engineered herbicide and antibiotic resistance in *Arabidopsis thaliana*. Genetics 145:807-814.

Radosevich, S. R., J. S. Holt, and C. Ghersa. 1997. Weed Ecology, Implications for management. 2nd ed. New York: J. Wiley, pp 69-102.

Raikhel, N. and M. J. Chrispeels. 2000. Protein sorting and vesicle traffic Page 160-2001 in B. B. Buchanan, W. Gruissem, and R. L. Jones eds. Biochemistry & Molecular Biology of Plants: American Society of Plant Physiologists.

Rathinasabapathi, B. and J. King. 1991. Herbicide resistance in *Datura innoxia*. Kinetic characterization of acetolactate synthase from wild type and sulfonylurea variants. Plant Physiol. 96:255-261.

Ray, T. B. 1984. Site of action of chlorsulfuron. Plant Physiol. 75:827-831.

Richards, F. J. 1959. A flexible growth function for empirical use. J. Exp. Bot. 10:290-300.

Ryan, G. F. 1970. Resistance of common groundsel to simazine and atrazine. Weed Sci. 18:614-616.

Saari, L. L., J. C. Cotterman, and D. C. Thill. 1994. Resistance to acetolactate synthase inhibiting herbicides. Page 83-139 *in* S. B. Powles and J. A. M. Holtum, eds. Herbicide Resistance in Plants: Biology and Biochemistry. Ann Arbor, MI: Lewis.

Saari, L. L., J. C. Cotterman, and M. M. Primiani. 1990. Mechanism of sulfonylurea herbicide resistance in the broadleaf weed, *Kochia scoparia*. Plant Physiol. 93:55-61.

Saari, L. L., J. C. Cotterman, W. F. Smith, and M. M. Primiani. 1992. Sulfonylurea herbicide resistance in common chickweed, perennial ryegrass, and Russian thistle. Pestic. Biochem. Physiol. 42:110-118.

SAS Institute. 1987. SAS/STAT™ Guide for personal computers, Version 6.0. Cary, NC: SAS Institute Inc., 1028 pp.

Scoggan, A. C., H. J. Santel, J. W. Wollam, and R. D. Rudolph. 1999. BAY MKH 6561-a new herbicide for grass and broadleaf control in cereals. Proc. Br. Crop Prot. Conf. Weeds 1:93-98.

Seefeldt, S. S., J. E. Jensen, and E. P. Fuerst. 1995. Log-logistic analysis of herbicide dose-response relationships. Weed Technol. 9:218-227.

Shafii, B., W. J. Price, J. B. Swenson, and G. A. Murray. 1991. Nonlinear estimation of growth curve models for germination data analysis. Pages 19-36 *in* G. A. Milliken and J. R. Schwenke, eds. Proc. of 1991 Kansas State Univ. Conf. on Appl. Statistics in Agric. Kansas State Univ., Manhattan, KS 66506.

Streibig, J. C. 1988. Herbicide bioassay. Weed Res. 28: 479-484.

Thompson, C. R., D. C. Thill, and B. Shafii. 1994a. Germination characteristics of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). Weed Sci. 42:50-56.

Thompson, C. R., D. C. Thill, and B. Shafii. 1994b. Growth and competitiveness of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). Weed Sci. 42:172-179.

Tranel, P. J. and T. R. Wright. 2002. Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Sci. 50:700-712.

Veldhuis L. J., L. M. Hall, J. T. O'Donovan, W. Dyer, and J. C. Hall. 2000. Metabolism-based resistance of a wild mustard (*Sinapis arvensis* L.) biotype to ethametsulfuron-methyl. J. Agric. Food Chem 48:2986-2990.

Westerfield, W. W. 1945. A colorimetric determination of blood acetoin. J. Biol. Chem. 161: 495-502.

Woodworth, A., P. Bernasconi, M. Subramanian, and B. Rosen. 1996. A second naturally occurring point mutation confers broad-based tolerance to acetolactate synthase inhibitors. Plant Physiol. 111:S105.

Wright, T. R. and D. Penner. 1998. Corn (*Zea mays*) acetolactate synthase sensitivity to four classes of ALS-inhibiting herbicides. Weed Sci. 46:8-12.

Wright, T. R., N. F. Bascomb, S. F. Sturner, and D. Penner. 1998. Biochemical mechanism and molecular basis for ALS-inhibiting herbicide resistance in sugarbeet (*Beta vulgaris*) somatic cell selections. Weed Sci. 46:13-23.