Chapter II

A New Site Mutation in the ALS Gene Confers Resistance to Four Classes of ALS-Inhibiting Herbicides

Abstract: Experiments were conducted to evaluate a biotype of smooth pigweed that had survived applications of sulfonylurea (SU) and imidazolinone (IMI) herbicides in a single season. The field had a history of repeated acetolactate synthase (ALS, EC 2.2.1.6 [formerly EC 4.1.3.18])-inhibiting herbicide use over several years. Whole-plant response experiments evaluated the resistant (R11) biotype and an ALS-inhibitor susceptible (S) smooth pigweed biotype to herbicides from the SU, IMI, pyrimidinylthiobenzoate (PTB), and triazolopyrimidine sulfonanilide (TP) chemical families. The R11 biotype exhibited 60- to 3,200-fold resistance to all four ALS-inhibiting herbicide chemistries compared to the S biotype. Nucleotide sequence comparison of ALS genes from R11 and S biotypes revealed a single nucleotide difference which resulted in R11 having an amino acid substitution of aspartate to glutamate at position 376, as numbered relative to the protein sequence of *Arabidopsis thaliana*. This is the first report of an amino acid substitution at this position of an ALS gene isolated from a field-selected weed biotype. To verify the role of this mutation in herbicide resistance, the ALS gene was cloned and expressed in *Arabidopsis*. Transgenic *Arabidopsis* expressing this ALS gene survived on medium containing 5 μM of imazethapyr, whereas wild-type *Arabidopsis* and transgenic *Arabidopsis* expressing an ALS gene from an ALS-inhibitor susceptible smooth pigweed biotype did not survive.
Nomenclature: Chlorimuron; cloransulam; imazethapyr; pyrithiobac; thifensulfuron; *Amaranthus hybridus* L. AMACH, smooth pigweed; *Arabidopsis thaliana*.
Key words: Acetolactate synthase, ALS, cross-resistance, herbicide resistance, imidazolinone resistance, pyrimidinylthiobenzoate resistance, sulfonylurea resistance, triazolopyrimidine resistance.

Introduction

Acetolactate synthase is the first common enzyme in the biosynthetic pathway of the branched-chain amino acids, valine, leucine, and isoleucine (Durner et al. 1990). ALS is

the target site for more than 50 commercial herbicides spanning five structurally distinct classes of chemicals (Heap 2004). These ALS-inhibiting herbicide classes include sulfonylureas (SU) (Chaleff and Mauvais 1984), imidazolinones (IMI) (Shaner et al. 1984), pyrimidinylthiobenzoates (PTB) (Stidham et al. 1991), triazolopyrimidine sulfonanilides (TP) (Gerwick et al. 1990), and sulfonylaminocarbonyltriazolinones (Santel et al. 1999).

In 1987, prickly lettuce (*Lactuca serriola* L.) was the first ALS-resistant weed species reported to be selected by ALS-inhibiting herbicides (Mallory-Smith et al. 1990). Since then, 86 weed species including monocots and dicots have been documented with resistance to one or multiple ALS-inhibiting herbicide classes (Heap 2004). Due to the widespread occurrence of ALS-resistant weed species, the ALS enzyme has received considerable attention in the weed science community.

In most cases, ALS-inhibitor resistance has resulted from an altered ALS enzyme with reduced sensitivity to the herbicides (Saari et al. 1994), but enhanced rates of herbicide metabolism have also been reported (Christopher et al. 1991, 1992; Menendez et al. 1997; Veldhuis et al. 2000). Target-site based ALS-inhibitor resistance is conferred by single amino acid substitutions (Shaner 1999), which can occur at multiple sites along the ALS gene. In total, seventeen different amino acid substitutions that confer herbicide resistance have been identified from intentionally selected plants, yeast, bacteria, and green algae or natural field-selected biotypes (Duggleby and Pang 2000). However, only five of these sites, Ala_{122}, Pro_{197}, Ala_{205}, Trp_{574}, and Ser_{653}, have been confirmed in target-site ALS-inhibitor resistance in the field-selected weed biotypes investigated.

Variable patterns of cross-resistance between ALS-inhibitor classes occur depending on the amino acid position affected and the specific substitution (Shaner 1999). Generally, cross-resistance patterns associated with an altered ALS can be classified as SU and TP resistant, IMI and PTB resistant, and SU, IMI, PTB, and TP resistant (broad cross-resistance) (Tranel and Wright 2002). Amino acid substitutions of Ala_{122} or Ser_{653} conferred resistance to IMI herbicides with low-level resistance to SUs (Bernasconi et al. 1995; Devine and Eberlein 1997; Patzoldt and Tranel 2001), whereas substitution of Pro_{197} conferred resistance to SUs (Guttieri et al. 1992), but low or no-cross resistance to IMIs. Substitution of Trp_{574} or Ala_{205} conferred broad cross-resistance (Bernasconi et al.

1995; Woodworth et al 1996); however, substitutions of Ala_{205} conferred much lower levels of resistance than Trp_{574}.

Amaranthus species are among the annual broadleaf weeds most prone to develop herbicide resistant biotypes because of their high genetic variability, high production of rapidly germinating seed, and efficient pollen and seed distribution (Lovell et al. 1996). Since 1993, *Amaranthus* species including Palmer amaranth [*Amaranthus palmeri* (S.) Wats.] (Gaeddert et al. 1997; Horak and Peterson 1995; Sprague et al. 1997), common waterhemp (*Amaranthus rudis* Sauer) (Hinz and Owen 1997; Horak and Peterson 1995; Lovell et al. 1996), livid amaranth (*Amaranthus lividus* L.) (Manley et al. 1996), redroot pigweed (*Amaranthus retroflexus* L.), and smooth pigweed (*Amaranthus hybridus* L.) (Manley et al. 1996; Poston et al. 2000) have been confirmed to be resistant to ALS-inhibiting herbicides. For smooth pigweed, target-site ALS-inhibitor resistance has been conferred through amino acid substitutions of Ala_{122}, Trp_{574}, or Ser_{653} (Tranel et al. 2004; Whaley et al. 2003).

Smooth pigweed seed was collected in 1997 from a field in southeastern Pennsylvania that had survived treatment with both SU and IMI herbicides. The field had been treated with ALS-inhibiting herbicides repeatedly over several years. The objectives of this research were: (1) to compare the response of this resistant biotype (R11) to an ALS-inhibitor susceptible biotype (S) using herbicide representatives of SU, IMI, PTB, and TP ALS-inhibiting herbicide classes and (2) to determine the mechanism and molecular basis of R11 resistance to ALS-inhibiting herbicides.

Materials and Methods

Seed Sources

Seed from a smooth pigweed biotype with suspected resistance to ALS-inhibiting herbicides was collected in 1997 from a field in Lancaster County, Pennsylvania with a history of repeated ALS-inhibiting herbicide use. In that year, the plants were not controlled by applications of chlorimuron, thifensulfuron, imazethapyr, and imazamox. Seed was collected from approximately 40 plants that had survived all ALS herbicide treatments and was designated biotype R11. Seed from the S biotype was collected from

a field at the Eastern Shore Agricultural Research and Extension Center near Painter, VA that had no history of ALS-inhibiting herbicide use. Seed was stored at 4 C.

Whole-Plant Response Experiments

Greenhouse experiments were conducted in 2000, 2002, and 2003 to evaluate the response of R11 and S biotypes to four classes of ALS-inhibiting herbicides. Seed was sown into individual 43- by 53-cm greenhouse flats containing a commercial potting soil medium.[1] Three evenly sized seedlings with one true leaf were transplanted into 11.4- by 11.4-cm pots containing potting medium. Plants were maintained in the greenhouse under natural sunlight and sprinkler irrigation and were fertilized weekly with a complete fertilizer.[2]

Application rates for ALS-inhibiting herbicides were based on a logarithmic scale of 0.01, 0.1, 1, 10, and 100 times the registered postemergence commercial use rate. Herbicides and rates were: chlorimuron at 0.09, 0.9, 9, 90, and 900 g ai ha^{-1}; thifensulfuron at 0.045, 0.45, 4.5, 45, and 450 g ai ha^{-1}; imazethapyr at 0.7, 7, 70, 700, and 7000 g ai ha^{-1}; pyrithiobac at 0.7, 7, 70, 700, and 7,000 g ai ha^{-1}; and cloransulam at 0.18, 1.8, 18, 180, and 1,800 g ai ha^{-1}. All herbicide treatments included 0.25% v/v non-ionic surfactant.[3] Herbicides were applied to 4- to 8-cm tall smooth pigweed plants with four to six true leaves using a compressed air, moving nozzle, cabinet sprayer equipped with one 8002EVS[4] nozzle and calibrated to deliver 171 L ha^{-1} at 289 kPa. Above-ground biomass from the three plants in each pot was harvested 21 d after treatment (DAT) and dried at 65 C for 72 h. Dry biomass data were expressed as a percentage of the nontreated control within each biotype.

Experiments were arranged in a completely randomized design with four replications and were repeated three times. Dry biomass data was expressed as a percentage of nontreated controls and were subjected to ANOVA to test for treatment by run interactions. No significant interactions were present so data were pooled over repeated experiments. Non-linear regression was used to generate herbicide dose-response curves of the resistant and susceptible biotype for each herbicide. The herbicide-dose required for 50% growth reduction (GR$_{50}$) was calculated from regression equations. Resistance

ratios, GR_{50} resistant / GR_{50} susceptible, were calculated to indicate the level of resistance.

DNA Analysis

Plant Materials

The ALS gene was sequenced to determine the molecular basis for resistance. R11 and S plants were grown as described above. All plants were treated at the four- to five-leaf stage with imazethapyr at 70 g ha^{-1} plus a non-ionic surfactant at 0.25% v/v to verify herbicide resistance and homogeneity. Young leaf tissue (120 mg) was harvested for genomic DNA isolation from treated R11 plants 14 DAT and from nontreated S plants.

Isolation and Sequencing

Genomic DNA was isolated using a Qiagen DNeasy Plant Mini Kit.[5] Two polymerase chain reaction (PCR) primers were designed from *Amaranthus spp*. nucleotide sequence (GeneBank Accession number; U55852) to amplify an approximately 2 kb section of DNA from each smooth pigweed biotype. Primers used for amplification were Forward 1 and Reverse 1 listed in Table 2.1. Each PCR reaction contained 25 ng genomic DNA, 12 μM of each forward and reverse primer, 20 mM dNTPs, 0.5 μl *PfuTurbo*® DNA polymerase[6] with 1x concentration of supplied buffer in a final volume of 25 μl. PCR reactions were subjected to a 5-min denaturation at 94 C; 30 cycles of 1 min at 94 C, 1.5 min at 67 C, and 2 min at 72 C; then a final 5 min at 72 C.

PCR products were purified by gel electrophoresis followed by a Qiagen Gel Purification Kit[5] and sequenced directly. Sequencing reactions included 50 ng of DNA from the gel-purified band, 2 μM of primer, and 4 μl ABI Prism® Big Dye™ Terminator[7] (v. 3.0) reagent in a final volume of 15 μl. Eight separate reactions were conducted, including four forward and four reverse primers to ensure complete coverage of both DNA strands (Table 2.1). Sequencing gels were run by the Core Laboratory Facility at the Virginia Bioinformatics Institute[8] using an ABI 377 automated sequencer. Sequences were aligned and compared using Sequencher™ 3.1 software.[9]

Arabidopsis Transformation

Isolation and Cloning

The entire ALS gene from R11 and S was isolated by PCR amplification from genomic DNA. Amplification of ALS was performed to include the start and termination codons and to introduce flanking restriction sites for the enzymes *Kpn*I (Forward underlined) and *Sac*I (Reverse underlined) using the following primers:

Forward 5'-GCA<u>GGTACC</u>ATATGGCGTCCACTTCTTCAAAC

Reverse 5'-CTA<u>GAGCTC</u>TCACTAATAAGCCCTTCTTCCATCA

PCR amplification was performed with *Taq* Master Mix[5] according to the manufacturer's recommendations. PCR reactions were subjected to 5-min denaturation at 94 C; 30 cycles of 1 min at 94 C, 1.5 min at 58 C, and 2 min at 72 C; then a final 5 min at 72 C. The amplified product was digested with *Kpn*I and *Sac*I and purified as described above. ALS genes from R11 (ALS_{R11}) and S (ALS_s) were each ligated to a *CaMV 35S* promoter with a double enhancer (Kay et al. 1987) in a pBC KS phagemid cloning vector.[10] Plasmids pBC-*35S::ALS_{R11}* and -*35S::ALS_s* were sequenced using T3 and T7 primers[10] specific for promoters flanking the pBC multiple cloning sites and four forward and four reverse primers in Table 2.1 specific for ALS to confirm that the genes were oriented correctly and that no errors existed in either amino acid sequence. The *35S::ALS_{R11}* and *35S::ALS_s* were digested from the plasmid using *Sal*I and *Sac*I and cloned into pBIB-HYG. The correct orientation of the constructs in pBIB-HYG was confirmed by restriction analysis. The pBIB-HYG-*35S::ALS_{R11}* and -*35S::ALS_s* plasmids were introduced into *Agrobacterium tumefaciens* strain GV3101 by the heat shock method. Transformed *A. tumefaciens* were selected on plates containing YEP medium supplemented with rifampicin (34 mg L^{-1}), gentamycin (25 mg L^{-1}) and kanamycin (50 mg L^{-1}).

Agrobacterium-Mediated Transformation

A. tumefaciens carrying the pBIB-HYG-*35S::ALS_{R11}* or -*35S::ALS_s* plasmids were used to transform *Arabidopsis thaliana* var. Columbia by the floral dip method (Clough and Bent 1998). T_1 seed collected from the *Arabidopsis* were surface-sterilized and plated on hygromycin (25 mg L^{-1}) selection plates at a density of approximately 1,000

seed per plate, cold-treated for 48 h and grown for 10 to 14 d at 22 C under 12 h of light. Surviving T_1 transformants were transferred to 5- by 5-cm pots containing commercial potting medium[1] and grown to seed. Seedlings were grown for approximately 14 d at 22 C under 8 h of light and plants were then subjected to 16 h of light at 22 C to induce bolting. Approximately 30 T_2 seed were plated on growth media that contained no selection agent, hygromycin at 25 mg L^{-1}, or hygromycin at 25 mg L^{-1} plus imazethapyr at 5 μM and were grown as described above. T_2 transformants that survived on hygromycin or hygromycin plus imazethapyr selection media were transferred to potting medium and grown as described above.

Arabidopsis Herbicide Response

Wild-type *Arabidopsis* var. Columbia (WT) and T_3 seed of mutant R11 and S *Arabidopsis* were plated on growth media at an approximate density of 40 seed per plate to evaluate response to imazethapyr. Treatments consisted of growth media that contained no selection agent, hygromycin at 25 mg L^{-1}, or imazethapyr at 0.5 μM or 5 μM and were grown as described above. Plant growth was visually assessed 14 d after plating.

Results and Discussion

Whole-Plant Response

Dose-response experiments were conducted in the greenhouse with representative herbicides from four ALS-inhibitor classes to confirm resistance and to determine cross-resistance patterns for the R11 biotype. Based on GR_{50} values from dry biomass expressed as a percentage of the nontreated control, the R11 biotype was 60- to 3,200-fold resistant to ALS-inhibiting herbicides relative to the S biotype (Table 2.2). At the normal use rate of all herbicides, except cloransulam, dry biomass of the S biotype was less than 3% of the nontreated, whereas dry biomass of the R11 biotype was greater than 40% of the nontreated (Figure 2.1A-E).

Biotype R11 was 3,261- and 1,300-fold resistant to chlorimuron and thifensulfuron, respectively, relative to the S biotype (Table 2.1). R11 dry biomass was reduced 50% with rates of chlorimuron and thifensulfuron at 587 and 26 g ha^{-1}, respectively, while S

dry biomass was reduced 50% at 0.18 and 0.02 g ha^{-1}, respectively. Based on resistance ratios, the R11 biotype was 60- and 213-fold resistant to imazethapyr and pyrithiobac, respectively, relative to the S biotype. Approximately 0.9 g ha^{-1} of imazethapyr and 0.6 g ha^{-1} of pyrithiobac were required to reduce dry biomass 50% in the S biotype compared to 55 and 128 g ha^{-1}, respectively, in the R11 biotype. Cloransulam applied at the normal use rate of 18 g ha^{-1} was not effective in controlling either biotype (Figure 2.1E). Complete control of the S biotype was obtained with cloransulam at 1,800 g ha^{-1}, but dry biomass of the R11 biotype was only reduced to 46% of the nontreated at this rate. Resistance ratios indicated that biotype R11 was 194-fold resistant to cloransulam relative to biotype S. It is concluded from these results that R11 is resistant to herbicide representatives from four classes of ALS-inhibiting herbicides.

Molecular Basis of Resistance

Results from the dose-response experiments suggested that the resistance mechanism was an altered ALS enzyme due to the high levels of resistance observed. To identify the molecular basis for resistance, 2 kb of the ALS gene from biotypes R11 and S were sequenced and compared. Primers for sequencing were located at approximately 500 bp intervals in forward and reverse directions, so in most cases provided 2- to 4-fold coverage of the gene. Only one nucleotide difference existed between R11 and S ALS genes, which resulted in an amino acid substitution of aspartate (GAT codon) to glutamate (GAA codon) at position 376, as numbered relative to the *Arabidopsis thaliana* ALS amino acid sequence. The aspartate at this position is highly conserved among all known wild type enzymes (Bedbrook et al. 1995). Falco et al. (1989) reported that the substitution of aspartate with glutamate at postion 376 conferred SU resistance in yeast subjected to high selection pressure. Substitution of Asp$_{376}$ with asparagine, valine, cysteine, glycine, proline, serine, or tryptophan also resulted in an SU-resistant ALS enzyme, but responses to other classes of ALS-inhibiting herbicides were not evaluated (Bedbrook et al. 1995).

Plant fitness costs associated with an altered ALS enzyme could decrease the likelihood or rate of weed populations developing resistance (Tranel and Wright 2002). Rajcan et al. (2003) indicated that several Powell amaranth [*Amaranthus powellii* (S.)

Wats.] biotypes with an amino acid substitution of Trp_{574} to Leu exhibited slow development, reduced biomass and leaf area, and distorted leaves relative to ALS inhibitor-susceptible biotypes. Visually, the R11 biotype exhibited normal growth relative to the S biotype. Preliminary results conducted under noncompetitive conditions in the greenhouse indicated that in the first 42 d of plant development, no differences were detected between R11 and S biomass accumulation and leaf area, however, R11 inflorescences emerged at least one wk earlier compared to S. Others have noted similar growth characteristics between ALS inhibitor-susceptible and -resistant species under noncompetitive and competitive conditions (Blackshaw et al. 1994; Christoffoleti et al. 1997; Thompson et al. 1994)

Transgenic *Arabidopsis* Response

It is interesting that the Asp_{376} mutation has not been reported from any other herbicide resistant weed species. To verify that resistance in the R11 biotype is indeed due to this mutation, genes encoding the ALS enzymes from R11 and S biotypes were expressed in *Arabidopsis*. The entire ALS gene including transit and signal peptides from R11 and S biotypes of smooth pigweed were fused to the *CaMV 35S* promoter and transformed into *Arabidopsis*. Four lines of transgenic *Arabidopsis* expressing an ALS gene from R11 (AR11) and S (AS) smooth pigweed were assayed on hygromycin and imazethapyr selection media.

Growth between WT, AS, and AR11 lines differed (Figure 2.2). On each respective selective medium, WT plants failed to grow, but AS and AR11 lines exhibited evidence of being transformed and having increased imazethapyr resistance. Lines of AS and AR11 displayed a segregation ratio of 3:1 and each respective line responded similarly to hygromycin and imazethapyr. Both transgenic lines showed resistance to imazethapyr at 0.5 μM, but only AR11 survived imazethapyr at 5 μM. The survival of AS on 0.5 μM of imazethapyr could be attributed to overexpression of ALS. Tourneur et al. (1993) reported that gene fusion to the *CaMV 35S* promoter with a double enhancer increased enzymatic activity by up to 12-fold. However, the survival of only AR11 at 5 μM of imazethapyr strongly supports this gene as the reason for resistance.

We conclude that an aspartate to glutamate substitution at position 376 of the R11 ALS gene is responsible for the broad cross-resistance of 60- to 3,200-fold in smooth pigweed (Table 2.2). Other amino acid substitutions at Trp_{574} and Ala_{205} have also been reported to confer broad cross-resistance to ALS-inhibiting herbicides. Substitution of Trp_{574} has conferred resistance levels of 10- to 10,000-fold, while resistance levels of an Ala_{205} substitution were approximately 10-fold. This is the first reported case of ALS-inhibiting herbicide resistance in a field-selected biotype due to an amino acid substitution of aspartate to glutamate at position 376. Further experiments are being conducted on the enzyme level, through expression in *E. coli*, to examine enzyme activity in the presence of various concentrations of ALS-inhibiting herbicides, cofactors, and branched-chain amino acids.

Sources of Materials

[1] Pro-Mix BX. Premier Horticulture, Inc., Red Hill, PA 18076.

[2] Peters 20-20-20 professional soluble plant food. Wetzel, Inc., 1345 Diamond Springs Road, Virginia Beach, VA 23455.

[3] Induce nonionic low-foam wetter/spreader adjuvant with 90% principal functioning agents as a blend of alkyl aryl polyoxylkane ether free fatty acids. Setre Chemical Company, Memphis, TN 38137.

[4] Teejet 8002EVS flat fan spray tip. Spraying Systems Co., North Avenue, Wheaton, IL 60188.

[5] QIAGEN Inc., 28159 Avenue Stanford, Valencia, CA 91355-1106.

[6] Roche Diagnostics GmbH, Roche Molecular Biochemicals, Sandhofer Strasse 116, D-68305 Mannheim, Germany.

[7] PE Corporation, PE Biosystems, 850 Lincoln Center Drive, Foster City, CA 94404.

[8] Virginia Bioinformatics Institute, Core Laboratory Facility, Washington Street Blacksburg, VA 24061-0477.

[9] Gene Codes Corp, 640 Avis Drive, Ann Arbor, MI 48108.

[10] Stratagene, 11011 North Torrey Pines Road, La Jolla, CA 92037.

Acknowledgments

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Table 2.1. Oligonucleotide primer sequences used in sequencing reactions. Primers were located at approximately 500 bp intervals in forward and reverse directions to sequence both ALS DNA strands of susceptible and resistant smooth pigweed biotypes.

Primer	Sequence
Forward 1	5'-TCCTCGCCGCCCTCTTCAAATC
Forward 2	5'-GTCCGGGTGCTACTAATCTTGTTT
Forward 3	5'-TTGCTAGTACTTTAATGGGGTTGG
Forward 4	5'-CGAAGGGCGATGCGGTTGTAAG
Reverse 1	5'-CAGCTAAACGAGAGAACGGCCAG
Reverse 2	5'-GCATCTGGTCGAGCAACAGCAG
Reverse 3	5'-GTCACTCGATCATCAAACCTAACC
Reverse 4	5'-CTTGGTAATGGATCGAGTTACCTC

Table 2.2. Summary of whole-plant responses for susceptible (S) and resistant (R11) smooth pigweed biotypes to five ALS-inhibiting herbicides. Values were obtained by non-linear regression of dry biomass expressed as a percentage of the nontreated control.

Herbicide	Class[a]	GR_{50}[b]		RS_{GR}[c]
		S	R11	
		g ha^{-1}		
Chlorimuron	SU	0.18	587	3,261
Thifensulfuron	SU	0.02	26	1,300
Imazethapyr	IMI	0.91	55	60
Pyrithiobac	PTB	0.60	128	213
Cloransulam	TP	3.94	764	194

[a] Abbreviations: SU, sulfonylurea; IMI, imidazolinone; PTB, pyrimidinylthiobenzoate; TP, triazolopyrimidine.
[b] GR_{50} refers to the herbicide dose (in g ai ha^{-1}) required for 50% biomass reduction compared to the nontreated control.
[c] Resistance to susceptibility ratios (RS) were calculated by dividing the GR_{50} of the resistant biotype by the GR_{50} of the susceptible biotype.
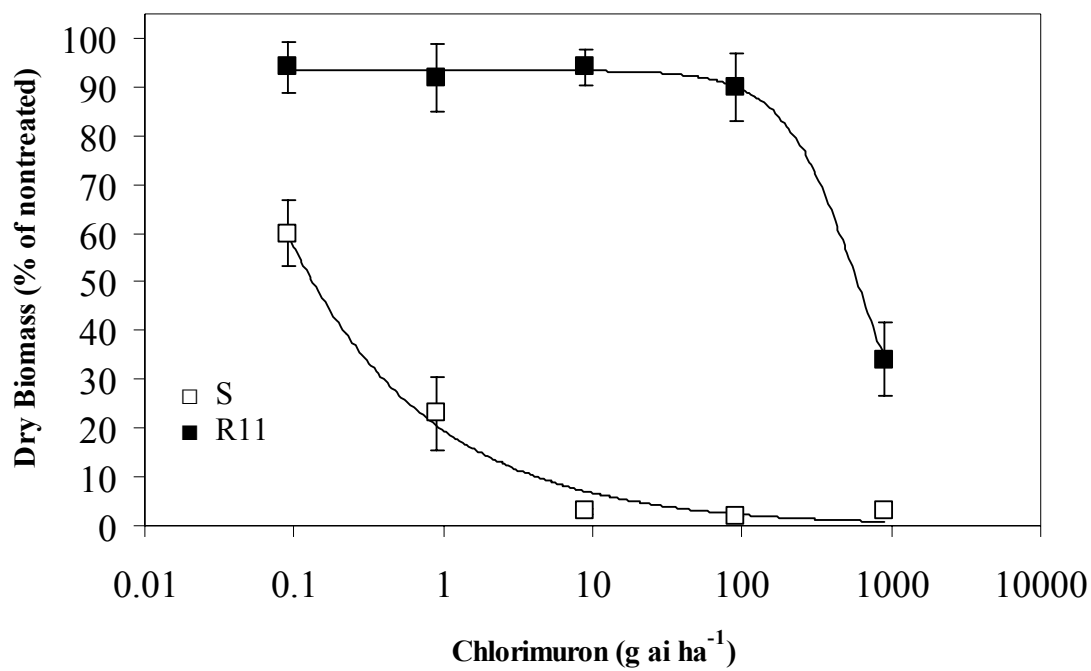
Figure 2.1A. Whole-plant response of susceptible (□, S) and resistant (■, R11) smooth pigweed biotypes to the sulfonylurea herbicide chlorimuron. Dry biomass was expressed as a percentage of the nontreated control. Symbols in graphs are means ± SE of four replications and repeated in triplicate.
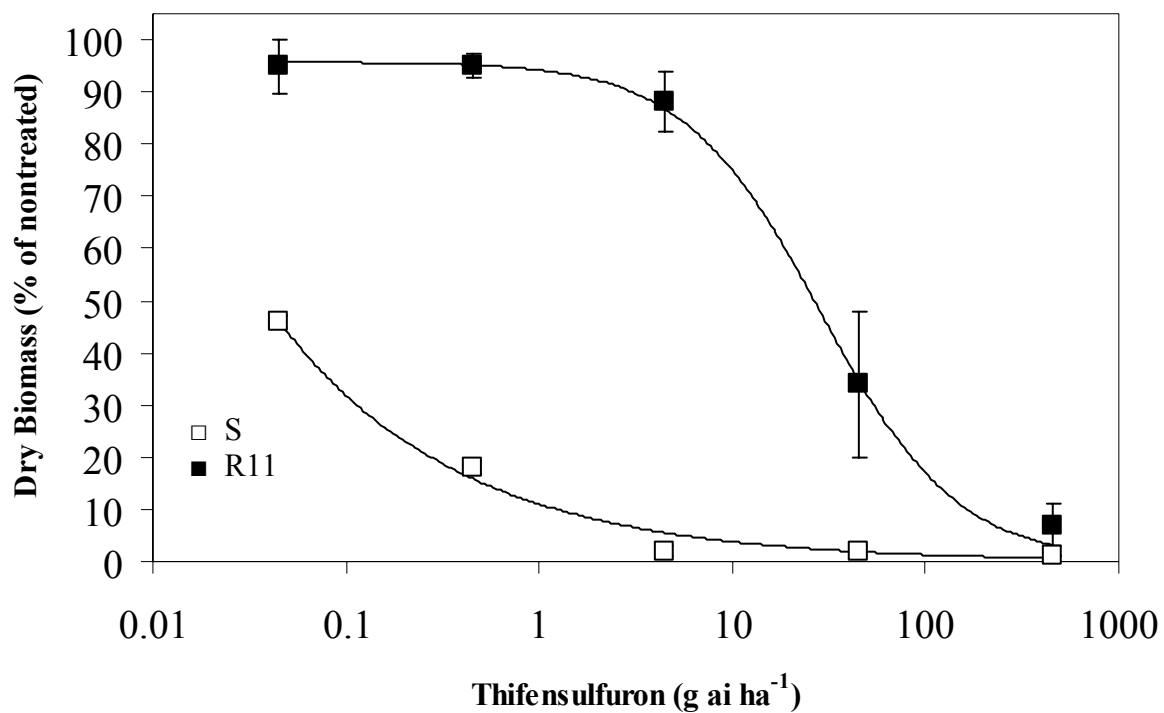
Figure 2.1B. Whole-plant response of susceptible (□, S) and resistant (■, R11) smooth

pigweed biotypes to the sulfonylurea herbicide thifensulfuron. Dry biomass was

expressed as a percentage of the nontreated control. Symbols in graphs are means ± SE
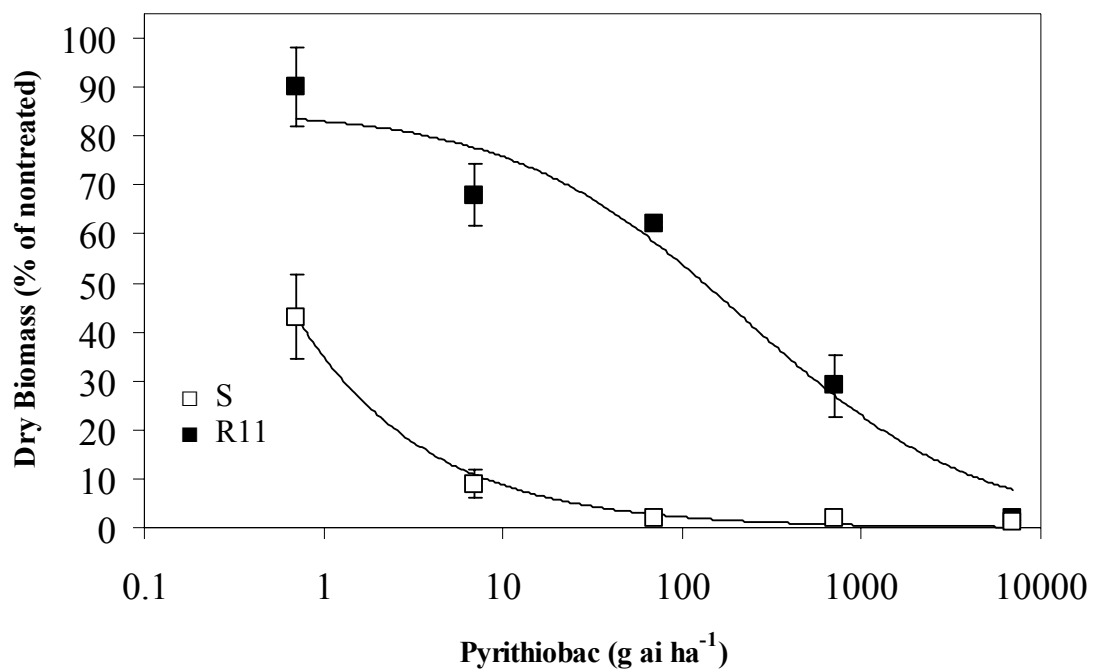
of four replications and repeated in triplicate.
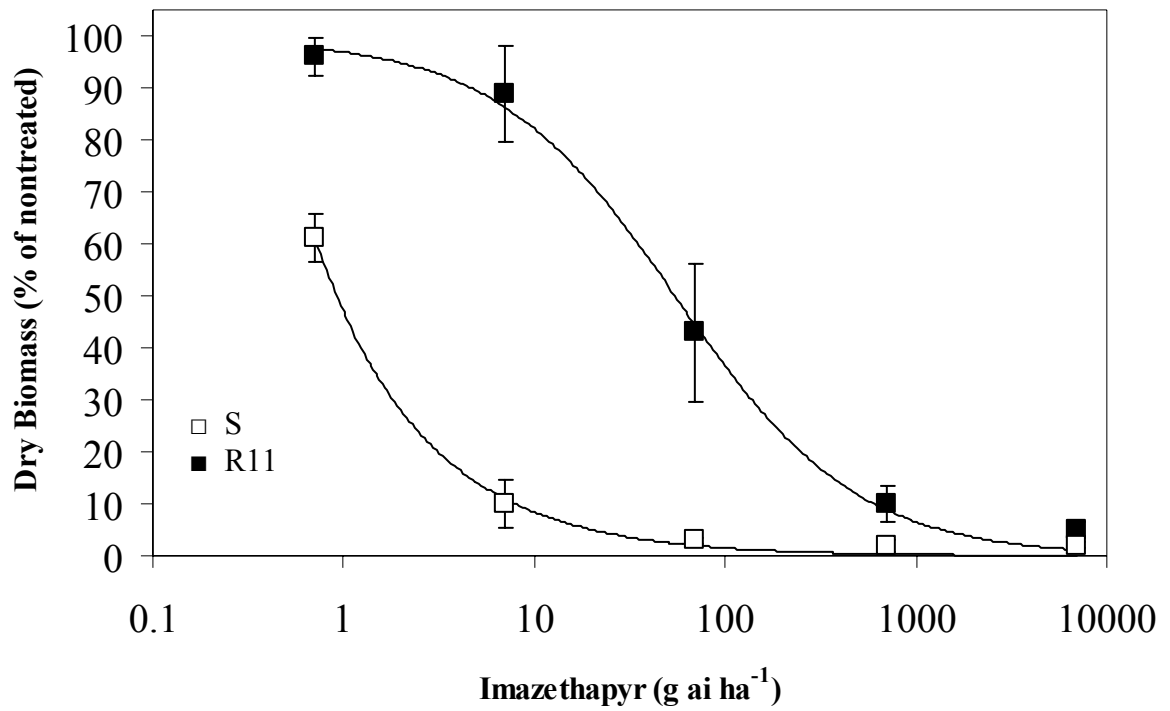
Figure 2.1C. Whole-plant response of susceptible (□, S) and resistant (■, R11) smooth

pigweed biotypes to the imidazolinone herbicide imazethapyr. Dry biomass was

expressed as a percentage of the nontreated control. Symbols in graphs are means ± SE

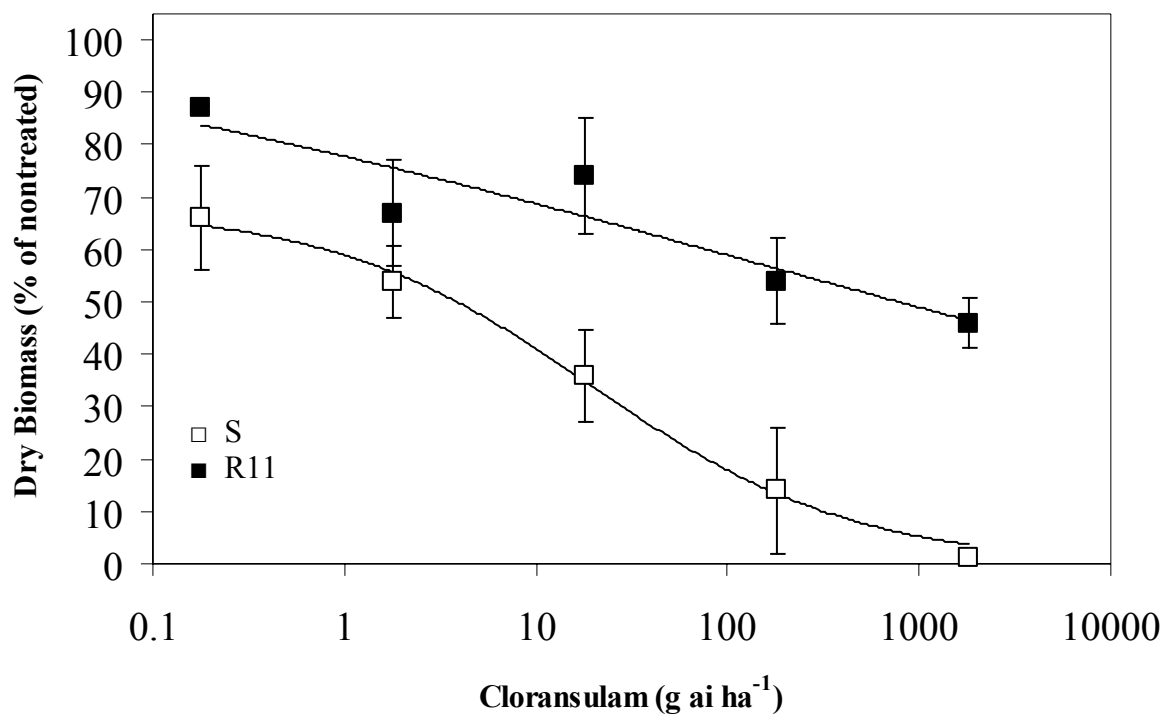of four replications and repeated in triplicate.

Figure 2.1D. Whole-plant response of susceptible (□, S) and resistant (■, R11) smooth

pigweed biotypes to the pyrimidinylthiobenzoate herbicide pyrithiobac. Dry biomass

was expressed as a percentage of the nontreated control. Symbols in graphs are means ±

SE of four replications and repeated in triplicate.

Figure 2.1E. Whole-plant response of susceptible (□, S) and resistant (■, R11) smooth

pigweed biotypes to the triazolopyrimidine sulfonanilide herbicide cloransulam. Dry

biomass was expressed as a percentage of the nontreated control. Symbols in graphs are

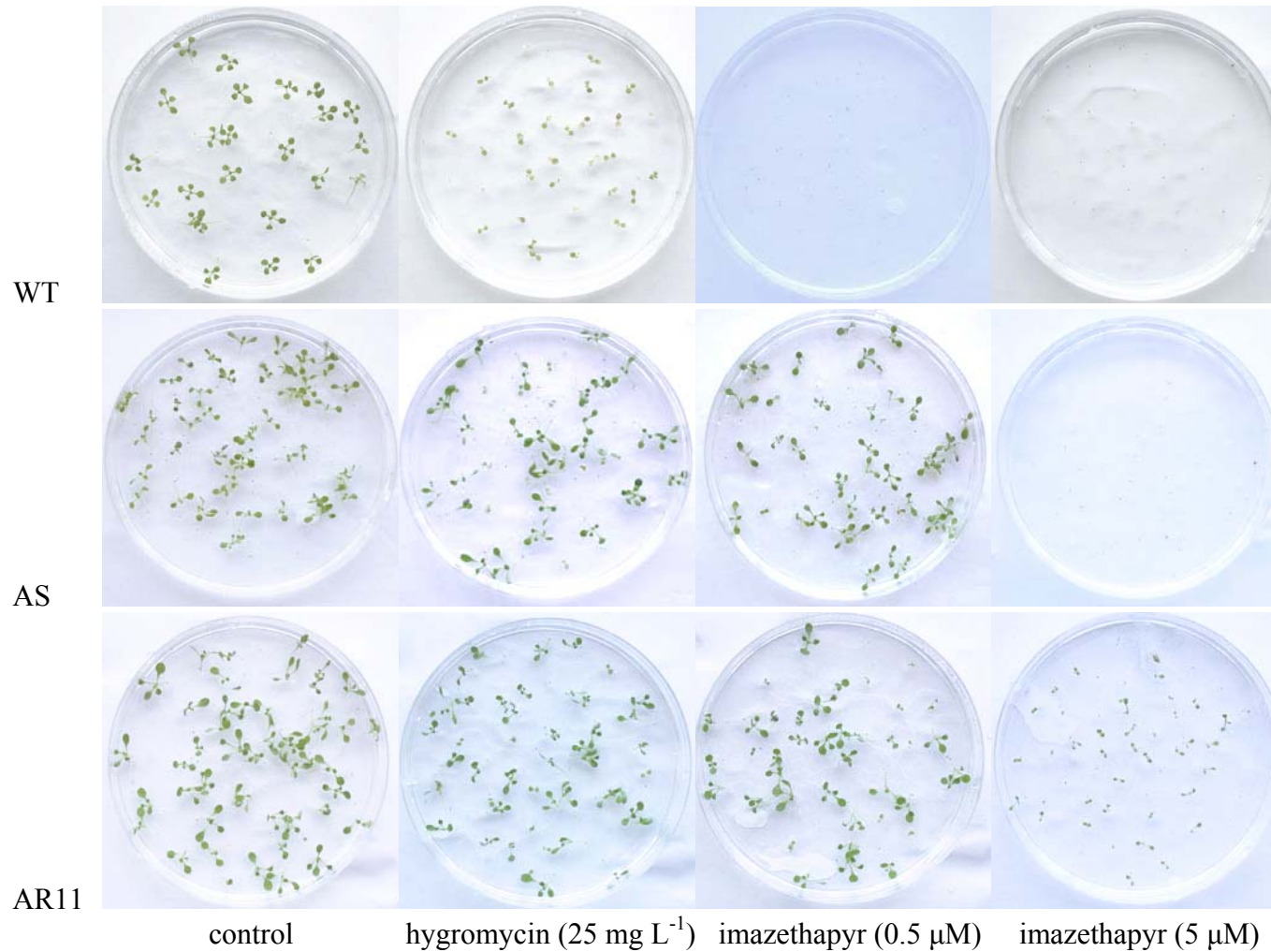means ± SE of four replications and repeated in triplicate.

| | control | hygromycin (25 mg L^{-1}) | imazethapyr (0.5 μM) | imazethapyr (5 μM) |

Figure 2.2. *Arabidopsis thaliana* seedling growth on media that contained no selection agent, hygromycin at 25 mg L^{-1}, or imazethapyr at 0.5 or 5 μM. Growth of WT (row WT) and transgenic *Arabidopsis* expressing the ALS gene from an ALS inhibitor-susceptible (row AS) and -resistant (row AR11) smooth pigweed biotype are shown.