GROWTH ANALYSES AND PATTERNS OF CROSS-RESISTANCE IN FOUR
IMIDAZOLINONE-RESISTANT SMOOTH PIGWEED (*Amaranthus hybridus*)
POPULATIONS

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GROWTH ANALYSES AND PATTERNS OF CROSS-RESISTANCE IN FOUR IMIDAZOLINONE–RESISTANT SMOOTH PIGWEED (*Amaranthus hybridus*) POPULATIONS

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(ABSTRACT)

Studies were conducted in 1996 through 1999 to: (1) evaluate the responses of one imidazolinone (IMI)-susceptible (S) and four –resistant (R1, R2, R3, and R4) smooth pigweed populations to various acetolactate synthase (ALS)-inhibiting herbicides, (2) determine the mechanism of resistance, and (3) evaluate the relative growth and competitiveness of each population. Field studies were conducted in 1996 near Marion, MD, in a field with a history of repeated imazaquin use. Smooth pigweed control with IMI herbicides was < 8 percent, but control with sulfonylurea (SU) herbicides ranged from 73 to 99 percent. Follow-up greenhouse studies were used to confirm IMI resistance in the Marion, MD smooth pigweed population (R4) as well as three others (R1, R2, and R3). R populations were 730- to 1350-fold more tolerant to imazethapyr than the S population. Based on resistance ratios, all R populations displayed low-level cross-resistance to chlorimuron and negative cross-resistance to thifensulfuron, pyrithiobac, and cloransulam-methyl with R2 being the most sensitive of the R populations to pyrithiobac and cloransulam-methyl.

Absorption, translocation, and metabolism of ^{14}C-cloransulam-methyl in S and R2 populations were generally similar. Three metabolites of cloransulam-methyl with ratio of front (Rf) values approximately 0.83, 0.65, and 0.45 were isolated. The metabolite with a 0.83 Rf value increased over time as the parent molecule decreased indicating that it plays a major role in cloransulam-methyl metabolism in smooth pigweed. The other metabolites did not change significantly over time and never represented more than 5

percent of the extracted radioactivity. The identity of these metabolites has not been determined.

Using enzyme assays, it was determined that IMI resistance in R populations was due to an altered ALS that was no longer susceptible to inhibition by these herbicides. ALS from S, R1, and R2 populations responded similarly to chlorimuron and thifensulfuron, but reductions in enzyme activity by chlorimuron and thifensulfuron were significantly greater for R3 ALS than for S, R1 or R2 ALS. ALS from R2 and R3 was significantly more sensitive to inhibition by pyrithiobac compared to S ALS. Based on resistance ratios, R2 and R3 ALS were also more sensitive to inhibition by cloransulam-methyl than S ALS. Negative cross-resistance to thifensulfuron, pyrithiobac, and cloransulam-methyl in some R populations at the whole-plant level can be explained by increased sensitivity at the enzyme level.

Under noncompetitive conditions in the greenhouse, S produced 17, 23, 25, and 44 percent more biomass than R1, R2, R3, and R4 populations, respectively. S plants were also taller than R plants 17 and 21 d after planting (DAP) and displayed a faster initial rate of leaf area increase compared to all R populations. The net assimilation rate of S was significantly higher than R2 and R3 populations 24 DAP. R3 and R4 populations had significantly less chlorophyll per g of plant tissue compared to S; therefore, reduced growth in some R populations compared to S may be linked to chlorosis that generally appears early in seedling development. Biomass production in the field under competitive conditions was similar for all populations using both monoculture and mixed populations. For this reason, the differences in growth observed in the greenhouse in the S population may not confer a competitive advantage over R populations in the field.

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Introduction and Review of Literature

Resistance to ALS Inhibitors

Since the discovery of triazine-resistant common groundsel (*Senecio vulgaris* L.) in 1970 (Ryan 1970), the weed science community has witnessed a dramatic increase in the number of herbicide-resistant weed biotypes. Based on worldwide reports, more than 140 different weed species have developed resistance to 17 different chemical classes of herbicides (Heap 1999). Much of this increase has occurred in the past 10 to 15 years and can be attributed to weed biotypes that have developed resistance to acetolacate synthase (ALS)-inhibiting herbicides.

The sulfonylurea (SU) herbicides were discovered in the late 1970's (Levitt 1978) and were the first class of herbicides developed that inhibited ALS (Schloss 1990). ALS catalyzes the first common step in the biosynthesis of the branched-chain amino acids valine, leucine, and isoleucine (Durner et al. 1990). More specifically, ALS catalyzes the homologous condensation of two molecules of pyruvate to form α-acetolactate and carbon dioxide or the heterologous condensation of one molecule of pyruvate and one molecule of α-ketobutyrate to form α-aceto-α-hydroxybutyrate and carbon dioxide (Schloss 1990). These reactions are considered the first committed steps in the biosynthesis of the branched-chain amino acids.

Since the discovery of SU herbicides, more than 30 ALS-inhibiting herbicides representing five structurally distinct classes of chemistry have been developed (Simpson 1998). SU (Chaleff and Muvais 1984), imidazolinone (IMI) (Shaner et al. 1984), triazolopyrimidine sulfonanilide (TP)(Gerwick et al. 1990), pyrimidinylthiobenzoate (PB)(Stidham 1991), and sulfonylaminocarbonyltriazolinones (Santel et al. 1999) herbicides all inhibit ALS and collectively offer broad-spectrum weed control in a variety of crops including corn, cotton, soybeans, small grains, and vegetable crops.

The commercial introduction of ALS inhibitors was marked by the registration of chlorsulfuron (2-chloro-*N*[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide) in 1982 to control broadleaf weeds in small grains (Saari et al. 1994). Chlorsulfuron-resistant prickly lettuce (*Lactuca serriola* L.) (Mallory-Smith et al., 1990) was reported only five years later and reports of kochia (*Kochia scoparia* (L.) Schrad.) (Primiani et al., 1990) and Russian thistle (*Salsola iberica* Sennen & Pau) (Heap 1999) resistant to chlorsulfuron soon followed.

ALS inhibitors have been widely used in many crops during the past 10 to 15 years due, at least in part, to characteristics like low use rates, high efficacy, multi-crop selectivity, and low mammalian toxicity (Saari et al. 1994). Persistent use of these products has unfortunately resulted in the development of at least 53 different weed species resistant to ALS-inhibiting herbicides (Heap 1999). Due to cost effectiveness and efficacy, ALS inhibitors remain widely used despite dramatic increases in the number of herbicide-resistant weed biotypes. Consequently, more ALS-inhibitor resistant weed biotypes are likely to develop.

Resistance to ALS inhibitors, resulting from various laboratory techniques or from continuous selection pressure in the field, is generally due to an altered ALS that is no longer sensitive to inhibition by the herbicides (Bernasconi et al. 1995; Guttieri et al. 1996). Absorption, translocation, and metabolism are known to account for differential plant responses to herbicides (Jensen 1982). Hodges et al. (1990) demonstrated that the basis for naturally occurring plant tolerance to TP herbicides is due to differences in metabolic rates between susceptible and tolerant plants. In cases where continuous herbicide use has resulted in the development of ALS inhibitor-resistant weed populations, however, these three physiological factors are rarely listed as mechanisms of resistance. However, exceptions do exist. Resistance to ALS-inhibiting herbicides in rigid ryegrass (*Lolium rigidum* Gaud.), that developed as a result of repeated exposure to the SU herbicide chlorsulfuron, may involve both increased metabolism of the herbicide and an insensitive form of ALS (Christopher et al. 1992). In another biotype of rigid ryegrass, resistance to some SU herbicides developed following selection with diclofop-methyl (2-

chloro-*N*[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide) and the primary mechanism of resistance was determined to be increased metabolism of the herbicides (Christopher et al. 1991). In the greenhouse, Manley et al. (1998) observed a 2.5-fold greater tolerance to chlorimuron (chlorimuron, 2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl] amino] sulfonyl]benzoic acid) in an IMI-resistant biotype of smooth pigweed (*Amaranthus hybridus* L.) compared to the wild type. Rapid metabolism was later suggested to be the mechanism conferring increased tolerance to chlorimuron in the IMI-resistant biotype (Manley 1996). Therefore, resistance to ALS inhibitor may be the result of increased metabolism, an insensitive target site, or a combination of both.

Herbicide Resistance Defined

Definitions of herbicide resistance have evolved over time and vary depending on the source. Herbicide resistance is defined by the Herbicide Resistance Action Committee (HRAC) as the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type (Heap, 1999). Maxwell and Mortimer (1994) state that a plant biotype must withstand substantially higher herbicide concentrations than the wild type of the same species to be considered resistant. Neither definition specifies a herbicide rate that a biotype must withstand to be considered resistant. Earlier definitions were more specific and required that resistant weed biotypes survive herbicide applications made under normal use conditions and at normal field doses (Ashton and Monaco 1991; Gressel 1985). Such definitions fail to take into account that some weed species are controlled by herbicide applications made at lower than commercially recommended rates. For the purpose of this paper, we have elected to use the more recent definition of resistance proposed by the HRAC and accepted by the Weed Science Society of America.

Cross-resistance

Cross-resistance occurs when a plant is resistant to multiple herbicides with the same mode of action (Saari 1994). Manley (1998) noted that ALS inhibitor-resistant

weed biotypes are often cross-resistant to herbicides within the same chemical family as the selection agent but exhibit varying patterns of cross-resistance to other families of ALS inhibitors. Patterns of cross-resistance in ALS inhibitor-resistant weed biotypes are difficult to predict and are usually dependent upon one or more point mutations that exist within the DNA sequence of the ALS gene (Guttieri et al. 1996; Saari et al. 1994).

Amaranthus species resistant to ALS inhibitors

Several ALS inhibitor-resistant _Amaranthus_ species have been reported within the past 5 to 6 y. As with other ALS inhibitor-resistant weed biotypes, _Amaranthus_ species tend to display varying patterns of cross-resistance to herbicides other than the selection agent. Smooth pigweed from fields in Marion, MD with a history of repeated imazaquin (2-[4,5-dihydro-4-methyl-4-(methylethyl)-5-oxo-1_H_-imidazol-2-yl]-3-quinoline carboxylic acid) use exhibited IMI resistance and low level cross-resistance to chlorimuron and rimsulfuron (_N_-[[4,6-dimethoxy-2-pryimidinyl)amino]carbonyl]-3-(ethylsulfonyl)-2-pyridinesulfonamide) (Manley 1998). PB herbicides and other SU's controlled this population. Repeated use of imazethapyr in Clay County, Kansas resulted in a population of Palmer amaranth (_Amaranthus palmeri_ S Wats.) resistant to both SU's and IMI's (Gaeddert et al. 1997). In Douglas County, Kansas, imazthapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1_H_-imidazol-2-yl]-5ethyl-3-pyridinecarboxylic acid) or imazaquin applied to fields 3 out of 5 growing seasons selected for a biotype of common waterhemp (_Amaranthus rudis_ Sauer.) resistant to SU and IMI herbicides (Horak and Peterson 1995). It is apparent that patterns of cross-resistance cannot be predicted based on herbicide history and must be individually assessed for each weed population.

Herbicide resistance usually develops in annual weed species associated with agricultural production in temperate regions of the world (Hill, 1982). Repeated use of the same herbicide or herbicides with the same mode of action to control extremely sensitive weed species also contributes to resistance development (Anderson 1996). Based on herbicide use patterns and weed characteristics, several weed scientists

predicted in the early 1990's that resistance to ALS-inhibiting herbicides would likely develop in pigweed (*Amaranthus)* species (H.P. Wilson, personal communication).

Pigweed species are extremely sensitive to many ALS-inhibiting herbicides and possess many characteristics often associated with herbicide-resistant weed biotypes. Such characteristics include production of large numbers of seed, high fecundity, rapid development, and maturation of several generations in a single season (Hill 1982). Pigweed species are monoecious or dioecious annuals that are consistently ranked among the most common and most troublesome weed species in many crops throughout the southeastern United States (Anonymous 1997, 1998). Pigweed species are prolific seed producers capable of producing 500,000 seed or more per plant (Salisbury, 1961). Seeds germinate throughout the summer and multiple pigweed generations within the same growing season are not uncommon. Within the past 6 to 7 years, biotypes of Palmer amaranth (Horak and Peterson, 1995; Gaeddert et al. 1997; Sprague et al. 1997), redroot pigweed (*Amaranthus retroflexus* L.) (Gerwick et al. 1993; Saari et al. 1994), prostrate pigweed (*Amaranthus blitoides* S Wats.) (Saari et al. 1994), common waterhemp (Horak and Peterson 1995; Sprague et al. 1997; Hinz and Owen 1997; Lovell 1996), livid amaranth (*Amaranthus lividus* L.) (Manley et al. 1996) and smooth pigweed (Manley et al. 1996) resistant to ALS-inhibiting herbicides have been reported. In all instances, repeated use of ALS-inhibiting herbicides was documented.

Negative Cross-resistance

Negative cross-resistance occurs when a resistant weed biotype is more susceptible to other classes of herbicides than the susceptible biotype. Negative cross-resistance to various herbicides has been reported in several triazine resistant weed species (Deprado et al. 1989, 1992; Oettmeier et al. 1982) including atrazine (6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine)-resistant *Amaranthus cruentus* L. and *Amaranthus hybridus* that were controlled by lower doses of bentazon (3-(methylethyl)-(*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide) and pyridate (*O*-(6-chloro-3-phenyl-4-pryidazinyl) *S*-octyl carbonothioate) than were susceptible biotypes

(De Prado et al. 1992). Few reports of negative cross-resistance relative to ALS-inhibitor resistant weed biotypes exist. At the whole plant level, Manley et al. (1998) observed that an IMI-resistant smooth pigweed biotype could be controlled with lower doses of thifensulfuron (3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino] sulfonyl]-2-thiophenecarboxylic acid) than the susceptible biotype. ALS extracted from the IMI-resistant biotype was later determined to be more sensitive to inhibition by pyrithiobac (2-chloro-6-[(4,6-dimethoxy-2-pyrimidinyl)thio]benzoic acid) and thifensulfuron than ALS extracted from the susceptible biotype (Manley 1999).

Cloransulam-methyl

Cloransulam-methyl (3-chloro-2-[[(5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-*c*]pryimidin-2yl)sulfonyl]amino]benzoic acid), a TP herbicide, can be applied preplant incorporated (PPI), preemergence (PRE) or POST for broadleaf weed control in soybean (*Glycine max* (L.) Merr.) (Dorich and Schultz, 1997). POST applications have controlled many weeds including morningglory (*Ipomoea* spp.), common cocklebur (*Xanthium strumarium* L.), common ragweed (*Ambrosia artemisiifolia* L.) , and velvetleaf (*Abutilon theophrasti* Medicus), but control of pigweed species and common lambsquarters (*Chenopodium album* L.) has generally been unsatisfactory (Murdock et al. 1998, Nelson and Renner 1998, Oliver et al. 1997). Nelson and Renner (1998) observed 51 and 18 percent control of common lambsquarters and redroot pigweed, respectively 3 wk following POST cloransulam-methyl (18 g ai/ha) applications.

Fitness and Resistance

Fitness is one of the most important factors affecting the appearance and persistence of a herbicide-resistant weed biotype (Gressel and Segel 1990; Maxwell et al. 1990). Fitness is defined as the ability of an organism to establish, survive, and reproduce successfully (Silvertown 1982). Under natural selection, weed biotypes that are most fit produce the most offspring and dominate in the gene pool. Selection pressure imposed on a wild population to select for a given trait generally results in a fitness penalty for

selected individuals (Gressel and Segel 1982). Unnatural selection pressure imposed by repeated use of highly effective and persistent herbicides resulting in the selection of herbicide-resistant weed biotypes can be utilized as an example to illustrate this biological phenomenon. Ahrens and Stoller (1983) demonstrated that triazine-resistant smooth pigweed produced less shoot biomass and seed dry weight under competitive conditions, fixed less CO_2 under saturated light and CO_2 conditions, and exhibited a significantly lower relative growth rate (RGR) and net assimilation ratio (NAR) compared to a triazine-susceptible biotype. Conrad and Radosevich (1979) concluded that triazine-resistant redroot pigweed and common groundsel were less fit than their respective wild types under both competitive and non-competitive conditions. Conrad and Radosevich (1979) attributed reduced competitiveness in the resistant biotype to photosynthetic inefficiency and concluded that the triazine resistance trait was only of benefit to the plant where triazine herbicides are repeatedly used. Gressel and Segel (1982) suggest that one possible result of reduced fitness in triazine-resistant weed biotypes is that the selected biotypes may only continue to exist in a population where herbicide selection pressure is great enough to kill the wild type. Based on this premise, reversion to a mostly susceptible population will likely occur over time in the absence of the herbicidal selection agent.

Interestingly, weed biotypes that have developed resistance to ALS inhibitors may not suffer fitness penalties as severe as those observed in triazine resistant weed biotype. Thompson et al. (1994) noted similar growth rates, seed production, and competitiveness in both SU-susceptible and –resistant kochia. SU-resistant prickly lettuce produced less biomass compared to the wild type under noncompetitive conditions, but the biotypes grew similarly in competition studies (Alcocer-Ruthling et al. 1992).

Research Objectives

The objectives of this research were to: (1) evaluate the responses of one IMI-susceptible (S) and four –resistant (R1, R2, R3, and R4) smooth pigweed populations to various ALS-inhibiting herbicides, (2) determine the mechanism of resistance, (3)

determine if absorption, translocation, and metabolism of cloransulam-methyl differ in S and R2 populations, and (4) evaluate the relative growth and competitiveness of S and R populations.

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Chapter II

Imidazolinone Resistance in Several Smooth Pigweed (*Amaranthus hybridus*) Populations

Field and greenhouse studies were conducted in 1996, 1997, and 1998 to evaluate the responses of one imidazolinone-susceptible (S) and four –resistant smooth pigweed populations to various ALS-inhibiting herbicides. In field studies conducted in 1996, imidazolinone resistance was confirmed in one smooth pigweed population (R4) and no cross-resistance to the sulfonylurea herbicides CGA-277476, chlorimuron, or thifensulfuron was observed. Smooth pigweed control with cloransulam-methyl, flumetsulam, or cloransulam-methyl + flumetsulam was ≤ 35%, but control with cloransulam-methyl + flumetsulam was significantly higher than with imidazolinone herbicides. Greenhouse studies were conducted in 1997 and 1998 to investigate the response of an imidazolinone-susceptible (S) and of four imidazolinone-resistant smooth pigweed populations (R1, R2, R3, R4) to postemergence (POST) imazethapyr, chlorimuron, thifensulfuron, pyrithiobac, and cloransulam-methyl applications. Resistance to imazethapyr was confirmed in all R populations and no practical level of cross-resistance to chlorimuron, thifensulfuron, pyrithiobac, or cloransulam-methyl was detected. Based on resistance ratios, all R populations were slightly more tolerant to chlorimuron and slightly more sensitive to pyrithiobac, thifensulfuron, and cloransulam. Chlorimuron, thifensulfuron, CGA-277476, and pyrithiobac can be utilized to effectively control these imidazolinone-resistant smooth pigweed populations.

Nomenclature: CGA-277476, oxasulfuron (ISO proposed), 2-[[[[(4,6-dimethyl-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoic acid, 3-oxetanyl-ester; chlorimuron, 2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino] sulfonyl]benzoic acid; cloransulam-methyl, 3-chloro-2-[[(5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-*c*]pryimidin-2yl)sulfonyl]amino]benzoic acid; flumetsulam, *N*-(2,6-difluorophenyl)-5-methyl[1,2,4]triazolo[1,5α]pyrimidine-2-sulfonamide; imazethapyr, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5ethyl-3-pyridinecarboxylic acid; pyrithiobac, 2-chloro-6-[(4,6-dimethoxy-2-pyrimidinyl)thio]benzoic acid; thifensulfuron,

3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylic acid; smooth pigweed, *Amaranthus hybridus* L. AMACH.

Key words: Acetolactate synthase; ALS; imidazolinones, imidazolinone resistance; herbicide resistance; cross-resistance; imazaquin, 2-[4,5-dihydro-4-methyl-4-(methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolinecarboxylic acid; negative cross-resistance.

Since the discovery of triazine-resistant common groundsel (*Senecio vulgaris* L.) in 1970 (Ryan 1970), the weed science community has witnessed a dramatic increase in the number of herbicide-resistant weed biotypes. Based on worldwide reports, more than 140 different weed species have developed resistance to 17 different chemical classes of herbicides (Heap 1998). Much of this increase has occurred in the past 10 to 15 years and can be attributed to weed biotypes that have developed resistance to acetolacate synthase (ALS)-inhibiting herbicides (Heap 1999).

The introduction of ALS-inhibiting herbicides was marked by the commercialization of chlorsulfuron in 1982 to control broadleaf weeds in small grains (Saari et al. 1994). Chlorsulfuron-resistant prickly lettuce (*Lactuca serriola* L.) (Mallory-Smith et al., 1990) was reported only five years later and reports of kochia (*Kochia scoparia* (L.) Schrad.) (Primiani et al., 1990) and Russian thistle (*Salsola iberica* Sennen & Pau) (Heap 1999) resistant to chlorsulfuron soon followed. At least 53 different weed species are currently known to be resistant to ALS-inhibiting herbicides (Heap 1999).

ALS catalyzes the first common step in the biosynthesis of the branched-chain amino acids valine, leucine, and isoleucine (Durner et al 1990). Sulfonylureas (Chaleff and Muvais 1984), imidazolinones (Shaner et al., 1984), triazolopyrimidines (Gerwick et al. 1990), pyrimidinylthiobenzoates (Stidham 1991), and sulfonylaminocarbonyltriazolinones (Santel et al. 1999) are five chemically distinct classes of herbicides that inhibit ALS. Collectively these five classes represent more than 30 individual herbicides registered or soon to be registered for use on a wide variety of

crops (Santel 1999; Simpson 1998). ALS inhibitors remain widely used despite dramatic increases in the number of herbicide-resistant weed biotypes. Therefore, persistent use of ALS inhibitors and development of more ALS-inhibitor resistant weed biotypes are likely.

Herbicide resistance usually develops in annual weed species associated with agricultural production in temperate regions of the world (Hill, 1982). Repeated use of the same herbicide or herbicides with the same mode of action to control extremely sensitive weed species also contributes to resistance development (Anderson 1996). Based on herbicide use patterns and weed characteristics, several weed scientists predicted in the early 1990's that resistance to ALS-inhibiting herbicides would likely develop in pigweed (*Amaranthus)* species (H.P. Wilson, personal communication).

Pigweed species are extremely sensitive to many ALS-inhibiting herbicides and possess many characteristics often associated with herbicide-resistant weed biotypes. Such characteristics include production of large numbers of seed, high fecundity, rapid development, and maturation of several generations in a single season (Hill 1982). Pigweed species are monoecious or dioecious annuals that are consistently ranked among the most common and most troublesome weed species in many crops throughout the southeastern United States (Anonymous 1997, 1998). Pigweed species are prolific seed producers capable of producing 500,000 seed or more per plant (Salisbury, 1961). Seeds germinate throughout the summer and multiple pigweed generations within the same growing season are not uncommon. Within the past 6 to 7 years, biotypes of Palmer amaranth (*Amaranthus palmeri* S Wats.) (Horak and Peterson, 1995; Gaeddert et al. 1997; Sprague et al. 1997), redroot pigweed (*Amaranthus retroflexus* L.) (Gerwick et al. 1993; Saari et al. 1994), prostrate pigweed (*Amaranthus blitoides* S Wats.) (Saari et al. 1994), common waterhemp (*Amaranthus rudis* Sauer) (Horak and Peterson 1995; Sprague et al. 1997; Hinz and Owen 1997; Lovell 1996), livid amaranth (*Amarnthus lividus* L.) (Manley et al. 1996) and smooth pigweed (Manley et al. 1996) resistant to ALS-inhibiting herbicides have been reported. In all instances, repeated use of ALS-inhibiting herbicides was documented.

Definitions of herbicide resistance have evolved over time and vary depending on the source. Herbicide resistance is defined by the Herbicide Resistance Action Committee (HRAC) as the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type (Heap, 1999). Maxwell and Mortimer (1994) state that a plant biotype must withstand substantially higher herbicide concentrations than the wild type of the same species to be considered resistant. Neither definition specifies a herbicide rate that a biotype must withstand to be considered resistant. Earlier definitions were more specific and required that resistant weed biotypes survive herbicide applications made under normal use conditions and at normal field doses (Ashton and Monaco 1991; Gressel 1985). Such definitions fail to take into account that some weed species are controlled by herbicide applications made at lower than commercially recommended rates. For the purpose of this paper, we have elected to use the more recent definition of resistance proposed by the HRAC and accepted by the Weed Science Society of America.

Cross-resistance occurs when a plant is resistant to multiple herbicides with the same mode of action (Saari 1994). Manley (1998) noted that ALS inhibitor-resistant weed biotypes are often cross-resistant to herbicides within the same chemical family as the selection agent but exhibit varying patterns of cross-resistance to other families of ALS inhibitors. Similar observations can be made for ALS inhibitor-resistant pigweed species. Smooth pigweed from fields in Marion, MD with a history of repeated imazaquin use exhibited imidazolinone resistance and low level cross-resistance to chlorimuron and rimsulfuron (*N*-[[4,6-dimethoxy-2-pryimidinyl)amino]carbonyl]-3-(ethylsulfonyl)-2-pyridinesulfonamide) (Manley 1998). Pyrimidinlythiobenzoate herbicides and other sulfonylureas controlled this population. Repeated use of imazethapyr in Clay County, Kansas resulted in a population of Palmer amaranth resistant to both sulfonylureas and imidazolinones (Gaeddert et al. 1997). In Douglas County, Kansas use of imazthapyr or imazaquin 3 out of 5 growing seasons selected for a biotype of common waterhemp resistant to sulfonylurea and imidazolinone herbicides (Horak and Peterson 1995). It is apparent that patterns of cross-resistance cannot be

predicted based on herbicide history and must be individually assessed for each weed population.

Negative cross-resistance occurs when a resistant weed biotype is more susceptible to other classes of herbicides than the susceptible biotype. Negative cross-resistance to various herbicides has been reported in several triazine resistant weed species (Deprado et al. 1989, 1992; Oettmeier et al. 1982) including atrazine (6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine)-resistant *Amaranthus cruentus* L. and *Amaranthus hybridus* that were controlled by lower doses of bentazon (3-(methylethyl)-(*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide) and pyridate (*O*-(6-chloro-3-phenyl-4-pryidazinyl) *S*-octyl carbonothioate) than were susceptible biotypes (De Prado et al. 1992). Few reports of negative cross-resistance relative to ALS-inhibitor resistant weed biotypes exist. At the whole plant level, Manley et al. (1998) observed that an imidazolinone-resistant smooth pigweed biotype could be controlled with lower doses of thifensulfuron than the susceptible biotype. ALS extracted from the imidazolinone-resistant biotype was later determined to be more sensitive to inhibition by pyrithiobac and thifensulfuron than ALS extracted from the susceptible biotype (Manley 1999).

Objectives of this research were to (1) document imidazolinone-resistance in several smooth pigweed populations from soybean fields with a history of repeated imazaquin use, (2) determine the cross-resistance patterns within each population, and (3) examine each population to determine if negative cross-resistance to ALS inhibitors other than imazthapyr exists.

Materials and Methods

ALS Inhibitors Applied in the Field

An experiment was conducted in 1996 in Marion, MD to determine the response of imidazolinone-resistant smooth pigweed to other classes of ALS inhibitors. Imazaquin had been applied preemergence (PRE) and in combination with either trifluralin (2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl)benzenamine) or pendimethalin (*N*-(1-

ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine) for several years at this location and smooth pigweed from this location had previously been identified as imidazolinone-resistant (Manley 1996, 1998). Imazaquin (140 g ai ha^{-1}) + pendimethalin (800 g ai ha^{-1}) was applied preemergence by the producer to the entire test area prior to establishing field studies. This treatment failed to control smooth pigweed and likely eliminated any imidazolinone-susceptible plants that might have been present in the field. Studies were established subsequent to this preemergence application.

The soil was a Fallsington and Dragston fine sandy loam (Typic Ochraquults), pH 6.0 and 1.4 % organic matter. Soybeans were drilled into 18 cm rows using conventional tillage practices. Plots were 3.0 m wide by 6.1 m long with a 2.1 m by 6.1 m herbicide-treated area. Herbicide treatments were applied with a propane-powered backpack sprayer calibrated to deliver 189 L ha^{-1} with a pressure of 207 kPa using flat fan nozzles[1]. Imazaquin (140 g ha^{-1}), imazethapyr (70 g ai ha^{-1}), imazamox (2-[4.5-dihydro-4-methyl-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid) (35 g ai ha^{-1}), CGA-277476 (66 g ai ha^{-1}), chlorimuron (9 g ai ha^{-1}), thifensulfuron (4.5 g ai ha^{-1}), cloransulam-methyl (18 g ai ha^{-1}), flumetsulam (7 g ai ha^{-1}), flumetsulam (14 g ha^{-1}), and flumetsulam (7 g ha^{-1}) + cloransulam-methyl (18 g ha^{-1}) were applied POST to 5 to 10 cm tall smooth pigweed (Table 1). A nonionic surfactant[2] (0.25% v/v) was included with all treatments. Weed control was visually estimated 2.5 and 7 wk after POST applications. Control ratings were based on a 0 (no control) to 100% (complete control) scale using an untreated control as a comparison.

Plots were arranged in a randomized complete block design with 3 replications and the experiment was repeated in an adjacent location within the same field in 1996. Data were subjected to ANOVA and means were separated using Fisher's LSD test conducted at the alpha = 0.05 significance level. Data from both locations were combined because no treatment by location interaction was detected.

Seed Sources

Smooth pigweed seed were collected in the fall of 1993 from four soybean fields in Worcester County, MD that had histories of repeated imazaquin use. Seed were collected from several plants in each field that survived imazaquin applications. Seed were also collected from imidazolinone-susceptible smooth pigweed plants from the Eastern Shore Agricultural Research and Extension Center near Painter, VA. Seed were threshed and stored under refrigeration until needed.

Plant Culture in the Greenhouse

Seed from imidazolinone-susceptible (S) and -resistant (R1, R2, R3, R4) smooth pigweed populations were planted into 43 cm by 53 cm greenhouse flats[3] containing a commercial potting soil mix[4]. Flats were kept moist and placed on propagation mats[5] calibrated to maintain soil temperature at approximately 24 C. Seeds were germinated and seedlings allowed to develop for several days before being transplanted into 11.4 cm by 11.4 cm pots filled with potting soil. Four evenly sized seedlings with one visible true leaf were transplanted into each pot. Plants were maintained in the greenhouse under natural sunlight and sprinkler irrigation. Plants were fertilized[6] weekly to maintain active growth.

ALS Inhibitors Applied in the Greenhouse

Greenhouse experiments were conducted in May 1997 and 1998 to evaluate the response of S, R1, R2, R3, and R4 smooth pigweed populations to various ALS-inhibiting herbicides. Imidazolinone, sulfonylurea, and pyrimidinlythiobenzoate herbicides were applied POST with a nonionic surfactant[7] (0.25% v/v) at 0.01, 0.1, 1, 10 and 100 times commercial use rates. Cloransulam-methyl, a triazolopyrimidine herbicide, was applied at 0.1, 1, 10 and 100 times the recommended POST use rate. Herbicides and

rates were: imazethapyr at 0.7, 7, 70, 700, and 7,000 g ha^{-1}; chlorimuron at 0.09, 0.9, 9, 90 and 900 g ha^{-1}; thifensulfuron at 0.045, 0.45, 4.5, 45 and 450 g ha^{-1}; pyrithiobac at 0.7, 7, 70, 700 and 7000 g ai ha^{-1}; and cloransulam-methyl at 1.8, 18, 180 and 1800 g ha^{-1}. Herbicide treatments were made to smooth pigweed plants 7 to 9 cm tall with 5 to 8 true leaves using a compressed air, moving nozzle, greenhouse sprayer equipped with one 8002EVS[8] nozzle and calibrated to deliver 171 L ha^{-1} at 289 kPa. Shoots were harvested 21 d after treatment and dried for 5 d at 65 C before determining dry weights. Dry weights were expressed as a percent of untreated control plants. Pots were arranged in a randomized complete block design with four replications and the test was repeated.

Statistical Analyses of Greenhouse Data

Data from 1997 and 1998 were pooled because no treatment by year interaction was detected. Non-linear regression analysis techniques similar to those employed by Chism et al. (1992) were used to generate dose response curves and to compare effects of individual herbicides on different smooth pigweed populations. Shoot dry weight data means expressed as a percent of the untreated control were plotted against the log of the herbicide concentration and regressed to fit one of two nonlinear regression models. Chlorimuron, thifensulfuron, and pyrithiobac data sets were regressed to fit a two parameter exponential function given here as:

$$y = B_1 * e^{(-B2*x)}$$ [1]

where y is shoot dry weight expressed as a percent of the untreated control, B_1 the reduction in shoot weight from the upper to the lower asymptote, B_2 the rate in which y reaches the lower asymptote, and x is the log of the herbicide concentration in g ha^{-1}. Equation 1 was also used to describe the response of populations S and R2 to increasing rates of imazethapyr and cloransulam-methyl, respectively. All other shoot dry weight data were regressed to fit the sigmoidal curve:

$$y = B / (1 + e^{-(x-C)/D})$$ [2]

where B, C, and D are constants, y is the shoot dry weight expressed as a percent of the untreated control, and x is the log of the herbicide concentration in g ha^{-1}. Whenever possible, a general nonlinear model was used to generate all dose response curves

associated with a specific herbicide thereby facilitating the use of pairwise comparisons to establish significant differences between regression coefficients using techniques described by Chism et al. (1992). Pseudo R^2 values were calculated to assess goodness of fit for individual regression equations. Imazethapyr, chlorimuron, thifensulfuron, pyrithiobac, and cloransulam-methyl concentrations required to reduce smooth pigweed growth by 50, 70, 70, 85, and 70 percent (GR_p's), respectively, were calculated using regression equations.

Results and Discussion

Field Studies

Imidazolinone herbicides gave little to no control of smooth pigweed in the field. Smooth pigweed control with imazaquin (140 g ha^{-1}), imazethapyr (70 g ha^{-1}), and imazamox (35 g ha^{-1}) was 8, 3, and 5 percent, respectively 2.5 wk after treatment and did not improve during the course of the experiment. No control of individual plants within the population was observed indicating a homogeneous population of imidazolinone-resistant plants. Imazaquin applied PRE and prior to study initiation served as a selection agent and likely eliminated any susceptible plants that may have existed in the test area. A complete or nearly complete shift from imidazolinone-susceptible to -resistant smooth pigweed has likely occurred at this location.

Smooth pigweed was controlled by all sulfonylurea herbicides included in the experiment. Control ratings of 99, 76, and 99 percent were recorded for CGA-277476 (66 g ha^{-1}), chlorimuron (9 g ha^{-1}), and thifensulfuron (4.5 g ha^{-1}) 7 wk after treatment, respectively. In earlier studies at this location, smooth pigweed control with six different sulfonylurea herbicides ranged from 72 percent with chlorimuron to 99 percent with pyrithiobac, thifensulfuron, and nicosulfuron (2-[[[[(4,6-dimethoxy-2-pyrimidinyl) amino]carbonyl] amino]sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide) (Manley 1998). Collectively, these data confirm that no practical level of cross-resistance to sulfonylurea herbicides exists within this imidazolinone-resistant smooth pigweed population.

Control with triazolopyrimidine herbicides was poor but cross-resistance was not suspected because control was generally higher than the untreated control. Weed control with cloransulam-methyl applied at 0.18 g ha^{-1} and flumetsulam applied at 7 g ha^{-1} was 24 and 10 percent, respectively 2.5 wk after treatment and did not improve over time. Increasing the rate of flumetsulam to 14 g ha^{-1} did not improve weed control. Weed control ratings were significantly higher when flumetsulam (7 g ha^{-1}) and cloransulam-methyl (18 g ha^{-1}) were applied in combination, but at no time during the course of the experiment was smooth pigweed control acceptable.

Greenhouse Studies

Imazethapyr Data.

Shoot dry weight data for the S population were fit to equation 1 ($R^2 = 0.94$) because an exponential decrease in shoot dry weight occurred as imazethapyr concentrations increased from 0.7 to 7000 g ha^{-1} (Figure 1). Dry weight data for all R populations were regressed to fit the sigmoidal equation described previously (equation 2). R^2 values of 0.94, 0.98, 0.95, and 0.93 were calculated for R1, R2, R3, and R4 populations, respectively. All R populations displayed high levels of resistance to imazethapyr while the S population was easily controlled. Imazethapyr applied at the commercial POST rate of 70 g ha^{-1} reduced shoot growth 93 percent in the S population. In contrast, shoot dry weight reductions of 32, 9, 23, and 26 percent were recorded in R1, R2, R3, and R4 populations, respectively. Extremely large doses of imazethapyr (7000 g ha^{-1}) were generally required to produce visible stunting in R populations. Approximately 1.4 g ha^{-1} imazethapyr were required to reduce shoot dry weight 50 percent in the S population compared to more than 1000 g ha^{-1} in all R populations (Table 2). Based on resistance ratios, all R populations were approximately 730- to 1350-fold more tolerant to imazethapyr than the S population (Table 3). Manley et al. (1998) described an imidazolinone-resistant smooth pigweed population that was >140-fold more tolerant to imazethapyr than the wild type. Our findings support those of Manley et al. (1998) and because of the high herbicide concentrations used in our studies more

accurately estimate the true level of resistance to imidazolinones present in several smooth pigweed populations that have developed during the past several years on the Eastern Shore of Virginia and Maryland. Using Fisher's LSD test (alpha=0.05), significant differences in shoot dry weight reductions between S and some R populations treated with 100 times the registered POST imazethapyr rate were detected (data not presented). This further illustrates the high level of resistance to the imidazolinone herbicides that exists within all R populations. Resistance to imazaquin was also confirmed in all R populations (data not presented).

Chlorimuron Data.

Shoot dry weight data for populations treated with chlorimuron were regressed to fit equation 1 (Figure 2). Excellent data fits were observed for all populations (Pseudo R^2 ≥ 0.87). Chlorimuron applied at 9 g ha^{-1}, a commonly accepted POST rate, reduced shoot dry weight 91, 87, 90, 91, and 87 percent in S, R1, R2, R3, and R4 populations, respectively indicating that chlorimuron would likely provide acceptable control of all populations in the field. GR_{70} values were 0.3, 1.4, 1.4, 2.1, and 1.4 g ha^{-1} for S, R1, R2, R3, and R4 populations, respectively (Table 2). Based on resistance ratios, R populations were approximately 4.7- to 7-fold more tolerant than S to chlorimuron in the greenhouse (Table 3). R3 was the most tolerant of all populations to chlorimuron based on resistance ratios and the magnitude of response to increasing rates of chlorimuron was significantly less with R3 than for S based on pairwise comparisons of regression coefficients (Tables 3 and 4). Increased tolerance to chlorimuron in R populations was most apparent at extremely low rates (Figure 2). Chlorimuron applied at 0.09 g ha^{-1} reduced shoot dry weights 64 percent in the S population compared to 40, 24, 39, and 19 percent in R1, R2, R3, and R4 populations, respectively. Differences in shoot dry weight reductions between S and R populations treated with 0.09 g ha^{-1} chlorimuron were significant based on Fisher's LSD test conducted at the alpha = 0.05 level (data not presented).

To obtain complete control of smooth pigweed under normal growing conditions with POST chlorimuron applications, a rate of 9 g ha^{-1} is generally required. Based on the definition of resistance established earlier, R populations should not be considered cross-

resistant to chlorimuron because no differences in the level of control between S and R populations was observed at a rate that would normally control smooth pigweed. Manley et al. (1998) made similar observations and described an imidazolinone-resistant smooth pigweed biotype that was also more difficult to control with low dosages of chlorimuron than the wild type (GR_{50} = 2.5). Manley et al. (1998) referred to this response as low-level cross-resistance. This term may be the most appropriate term to describe the response of our R populations to chlorimuron.

Thifensulfuron and Pyrithiobac Data.

Pseudo R^2 values ranged from 0.71 to 0.97 for thifensulfuron and pyrithiobac dry weight data fit to equation 1 (Figures 3 and 4). Thifensulfuron and pyrithiobac provided excellent control of all smooth pigweed populations and no practical level of cross-resistance to either compound was observed. Shoot dry weight reductions ≥ 86 percent were observed in all populations when thifensulfuron (4.5 g ha^{-1}) and pyrithiobac (70 g ha^{-1}) were applied at commercially recommended rates. Despite similarities in the level of control with thifensulfuron and pyrithiobac in all populations at commercial and higher use rates, significant differences in sensitivity to these herbicides between S and some R populations were detected at extremely low herbicide concentrations (Poston et al., 1998).

In the greenhouse, R and S populations generally responded similarly to thifensulfuron applications made at rates of 4 g ha^{-1} or higher based on analysis within a given rate using Fisher's LSD test (alpha = 0.05) (data not presented). GR_{70} values were 0.3, 0.2, 0.1, 0.1, and 0.1 g ha^{-1} for S, R1, R2, R3, and R4 populations, respectively (Table 2). Resistance ratios of 0.7, 0.3, 0.3, and 0.3 were recorded for R1, R2, R3, and R4 populations, respectively (Table 3). Therefore, R populations were approximately 1.4- to 3.3-fold more susceptible to thifensulfuron than the S population. Regression coefficient B_1 for R2 was significantly lower than for S (Table 4) indicating a magnitude difference between S and R2 regression lines. This means that control of R2 was greater than S at all thifensulfuron concentrations. Increased sensitivity to thifensulfuron (resistance ratio = 0.5) has also been observed in the greenhouse with another imidazolinone-resistant

smooth pigweed population (Manley et al. 1998). Increased sensitivity to thifensulfuron in at least some R populations might be interpreted as negative cross-resistance.

Pyrithiobac concentrations required to reduce shoot dry weights 85 percent compared to the untreated control ranged from 0.8 g ha^{-1} for R2 to 54 g ha^{-1} for S (Table 2). Resistance ratios calculated using GR$_{85}$ values were 0.3, 0.01, 0.2, and 0.2 for R1, R2, R3, and R4 populations, respectively (Table 3). As with thifensulfuron, this would suggest that all R populations are more susceptible to pyrithiobac than S. Differences in control between S and R populations treated with pyrithiobac were once again more apparent at extremely low rates (Figure 4). Significant differences in the level of control between S and some R populations treated with 0.7 or 7 g ha^{-1} pyrithiobac were detected using Fisher's LSD test conducted at the alpha = 0.05 level (data not shown). Pairwise comparisons of non-linear regression parameters between S and R populations revealed significantly smaller B$_1$ coefficients for all R populations compared to S (Table 4). It should also be noted that B$_1$ coefficients for both R1 and R4 were significantly larger than for R2 and that regression analysis revealed no significant change in shoot dry weight in the R2 population with increasing rates of pyrithiobac.

Cloransulam-methyl Data.

Sigmoidal dose response curves were observed for S, R1, R3, and R4 smooth pigweed populations treated with cloransulam-methyl in the greenhouse (Figure 5). Consequently, shoot dry weight data for these populations were regressed to fit equation 2. In contrast, an exponential decrease in dry weight with increasing rates of cloransulam-methyl was observed with R2 and data were more appropriately fit to equation 1. Excellent fits were observed for dry weight data from all populations fit to their respective non-linear models. Pseudo R^2 values for S, R1, R2, R3, and R4 data sets were 0.97, 0.92, 0.98, 0.94, and 0.99, respectively.

Cloransulam-methyl was the least effective herbicide for controlling smooth pigweed in the greenhouse with the exception of imazethapyr applied to R populations (Figure 5). Smooth pigweed control with 18 g ha^{-1} cloransulam-methyl POST was ≤ 71

percent in both S and R populations (Figure 5). Others have reported similar levels of smooth pigweed control with cloransulam-methyl in the greenhouse (Nelson and Renner, 1998). GR_{70} values were 130, 47, 12, 30, and 67 g ha^{-1} for S, R1, R2, R3, and R4 populations, respectively (Table2). R populations were 2- to 10-fold more susceptible to cloransulam-methyl compared to the S population based on resistance ratios (Table 3).

Concluding Remarks.

All R populations were resistant to imazethapyr but no practical level of cross-resistance to CGA-277476, chlorimuron, thifensulfuron, pyrithiobac, and cloransulam-methyl was found. The triazolopyrimidine herbicide cloransulam-methyl generally gave poor control of all five smooth pigweed populations. Sulfonylurea and pyrimidinylthiobenzoate herbicides gave excellent control of all populations when applied at commercially registered or higher use rates and can likely be used effectively to control some imidazolinone-resistant smooth pigweed populations. Although initially effective, the use of sulfonylurea or pyrimidinylthiobenzoate herbicides in situations where imidazolinones have failed may select for smooth pigweed biotypes resistant to all families of ALS-inhibitors (Hinz and Owen 1997, Horak and Peterson 1995, Sprague et al. 1997). Therefore, herbicides with modes of action other than the inhibition of ALS should be incorporated into weed management programs to control populations of smooth pigweed that have developed resistance to the imidazolinone herbicides.

Sources of Materials

[1]Teejet 8003 flat fan spray tips. Spraying Systems Co., North Avenue, Wheaton, IL 60188.

[2]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.

[3]Sutton universal greenhouse flat. Inside dimensions 51 cm x 40 cm x 5.7 cm. Wetzel, Inc., 1345 Diamond Springs Road, Virginia Beach, VA 23455.

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

Acknowledgments

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TABLE 1. Visual control ratings of imidazolinone-resistant smooth pigweed in the field near Marion, MD 2.5 and 7 wk after postemergence herbicide applications in 1996.[a,b,c,d]

		Control	
Treatment	Rate	2.5 WAT	7 WAT
	g ha^{-1}	——————%——————	
Imazaquin	140	8	0
Imazethapyr	70	3	3
Imazamox	35	5	0
CGA-277476	66	99	99
Chlorimuron	9	73	76
Thifensulfuron	4.5	99	99
Cloransulam-methyl	18	24	14
Flumetsulam	7	10	5
Flumetsulam	14	8	0
Flumetsulam + Cloransulam-metyhl	7 + 18	35	24
None		0	0
LSD$_{0.05}$		8	8

[a] Fields at this location had a history of annual imazaquin applications and smooth pigweed resistance to imidazolinone herbicides had previously been confirmed.

[b] Imazaquin (140 g ai ha^{-1}) in combination with pendimethalin (800 g ai ha^{-1}) was applied PRE to entire test area. Treatments represent follow-up POST applications.

[c] All treatments were applied with non-ionic surfactant (0.25% v/v).

[d] Visual ratings were based on a 0-99% scale with 0% indicating no control and 99% indicating complete control.

TABLE 2. Concentrations of five ALS-inhibiting herbicides required to reduce growth of one imidazolinone-susceptible (S) and four –resistant (R1, R2, R3, R4) smooth pigweed populations by a specified percentage (GR_p) as determined by shoot dry weights in the greenhouse. GR_p values were calculated using nonlinear regression equations presented in Figure legends 1-5.[a]

	GR_p				
Herbicide	S	R1	R2	R3	R4
	—————————————— g ha^{-1} ——————————————				
Imazethapyr[a]	1.4	1022	1505	1112	1890
Chlorimuron[b]	0.3	1.4	1.4	2.1	1.4
Thifensulfuron[c]	0.3	0.2	0.1	0.1	0.1
Pyrithiobac[d]	54	14.5	0.8	9.6	9.5
Cloransulam-methyl[e]	130	47	12	30	67

[a] Values presented for imazethapyr are GR_{50}'s.

[b] Values presented for chlorimuron are GR_{70}'s.

[c] Values presented for thifensulfuron are GR_{70}'s.

[d] Values presented for pyrithiobac are GR_{85}'s.

[e] Values presented for cloransulam-methyl are GR_{70}'s.

TABLE 3. Resistance ratios (R/S) of four imidazolinone-resistant smooth pigweed populations for five ALS-inhibiting herbicides based on GR_p values calculated using nonlinear regression equations presented in Figure legends 1-5.[a]

Herbicide	Resistance Ratios (R/S)[b]			
	R1/S	R2/S	R3/S	R4/S
Imazethapyr[c]	730	1080	790	1350
Chlorimuron[d]	4.7	4.7	7.0	4.7
Thifensulfuron[e]	0.7	0.3	0.3	0.3
Pyrithiobac[f]	0.3	0.01	0.2	0.2
Cloransulam-methyl[g]	0.4	0.1	0.2	0.5

[a] GR_p refers to the herbicide concentrations required to reduce plant growth by a specified percentage compared to the untreated control.

[b] R/S = resistance ratio = GR_p [resistant] / GR_p [susceptible]

[c] R/S for imazethapyr = GR_{50} [resistant] / GR_{50} [susceptible]

[d] R/S for chlorimuron = GR_{70} [resistant] / GR_{70} [susceptible]

[e] R/S for thifensulfuron = GR_{70} [resistant] / GR_{70} [susceptible]

[f] R/S for pyrithiobac = GR_{85} [resistant] / GR_{85} [susceptible]

[g] R/S for cloransulam = GR_{70} [resistant] / GR_{70} [susceptible]

TABLE 4. Pairwise comparisons of nonlinear regression coefficients between one imidazolinone-susceptible (S) and four –resistant (R1, R2, R3, R4) smooth pigweed populations for chlorimuron, thifensulfuron, and pyrithiobac dose response curves.[a,b]

Population	Chlorimuron		Thifensulfuron		Pyrithiobac	
	B_1	B_2	B_1	B_2	B_1	B_2
S vs R1	ns	ns	ns	ns	*	ns
R2	ns	ns	*	ns	*	ns
R3	*	ns	ns	ns	*	ns
R4	ns	ns	ns	ns	*	ns
R1 vs R2	ns	ns	ns	ns	*	ns
R3	ns	ns	ns	ns	ns	ns
R4	ns	ns	ns	ns	ns	ns
R2 vs R3	ns	ns	ns	ns	ns	ns
R4	ns	ns	ns	ns	*	ns
R3 vs R4	ns	ns	ns	ns	ns	ns

[a] General equation: $y = B_1 * e^{(-B2*x)}$ where y is shoot dry weight expressed as a percent of the untreated control and x is the log of the herbicide concentration in g ha^{-1}.

[b] Comparisons between populations followed by an * indicate significant differences ($\alpha = 0.05$).
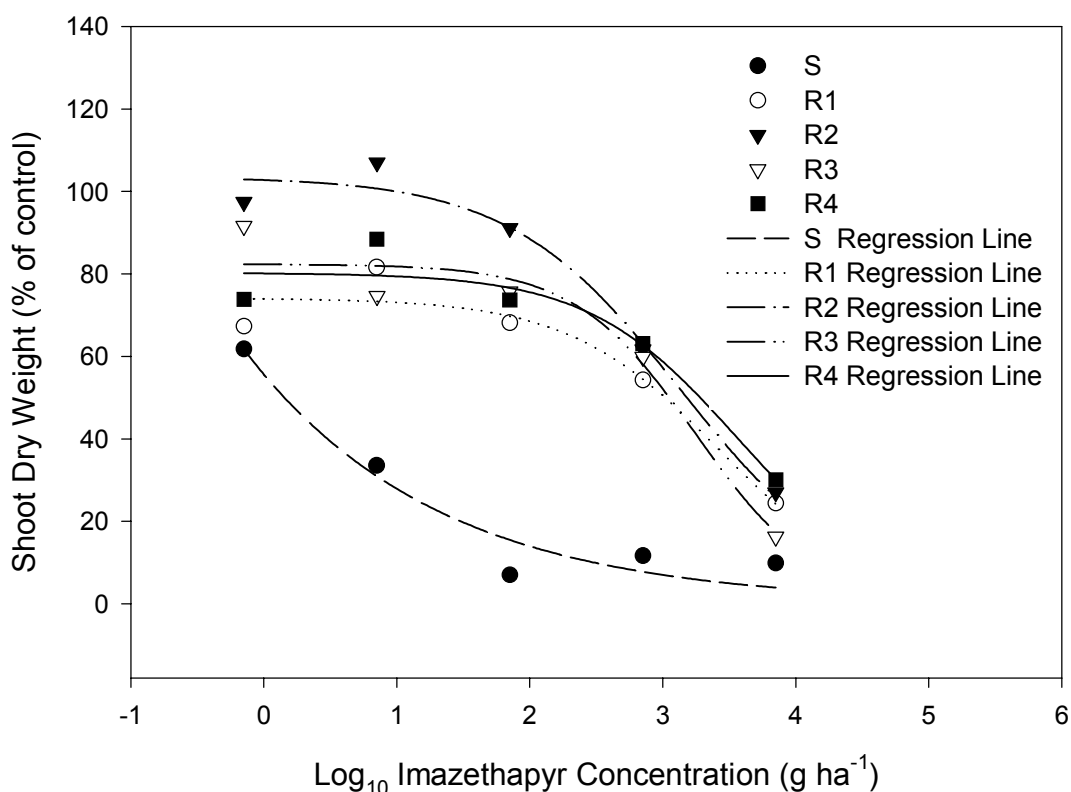
FIGURE 1. Dose response curves for shoot dry weight, as a percent of the untreated control, of one imidazolinone-susceptible (S) and four –resistant (R1, R2, R3, R4) smooth pigweed populations treated with imazethapyr in the greenhouse. GR_{50} values were calculated from nonlinear regression equations $y = 55.5 * e^{(-0.69 x)}$, $r^2 = 0.94$, $y = 74.10 / (1 + e^{-(x - 3.43) / -0.57})$, $r^2 = 0.94$, $y = 103.53 / (1 + e^{-(x - 3.13) / -0.64})$, $r^2 = 0.98$, $y = 82.34 / (1 + e^{-(x - 3.24) / -0.45})$, $r^2 = 0.95$, $y = 80.24 / (1 + e^{-(x - 3.56) / -0.56})$, $r^2 = 0.93$ for S, R1, R2, R3, and R4 populations, respectively where y is shoot dry weight expressed as a percent of the untreated control and x is the log of the herbicide concentration in g ha^{-1}. All treatments were applied POST and include a non-ionic surfactant (0.25% v/v). Plants were harvested 21 DAT.
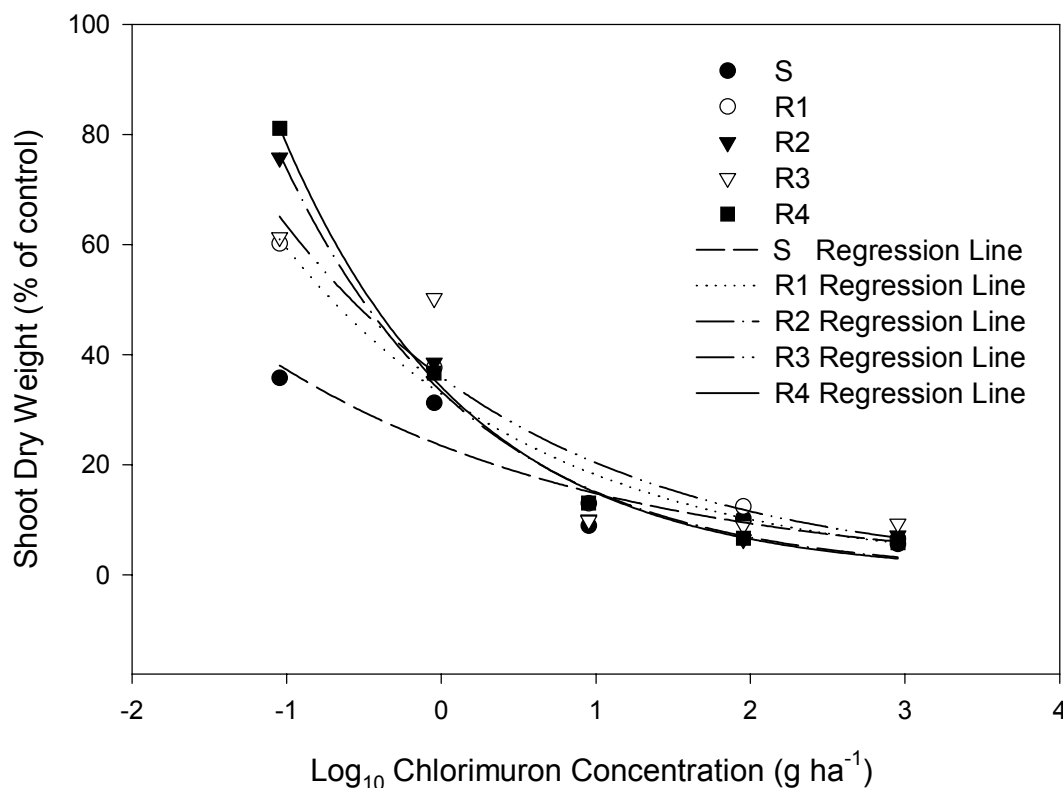
FIGURE 2. Dose response curves for shoot dry weight, as a percent of the untreated control, of one imidazolinone-susceptible (S) and four –resistant (R1, R2, R3, R4) smooth pigweed populations treated with chlorimuron in the greenhouse. GR_{70} values were calculated from nonlinear regression equations $y = 23.44*e^{(-0.46x)}$, $r^2 = 0.88$, $y = 32.83*e^{(-0.59x)}$, $r^2 = 0.97$, $y = 33.3*e^{(-0.8x)}$, $r^2 = 0.98$, $y = 35.91*e^{-(0.57x)}$, $r^2 = 0.88$, and $y = 34.15*e^{(-0.83x)}$, $r^2 = 0.99$ for S, R1, R2, R3, and R4 populations, respectively where y is shoot dry weight expressed as a percent of the untreated control and x is the log of the herbicide concentration in g ha^{-1}. All treatments were applied POST and include a non-ionic surfactant (0.25% v/v). Plants were harvested 21 DAT.
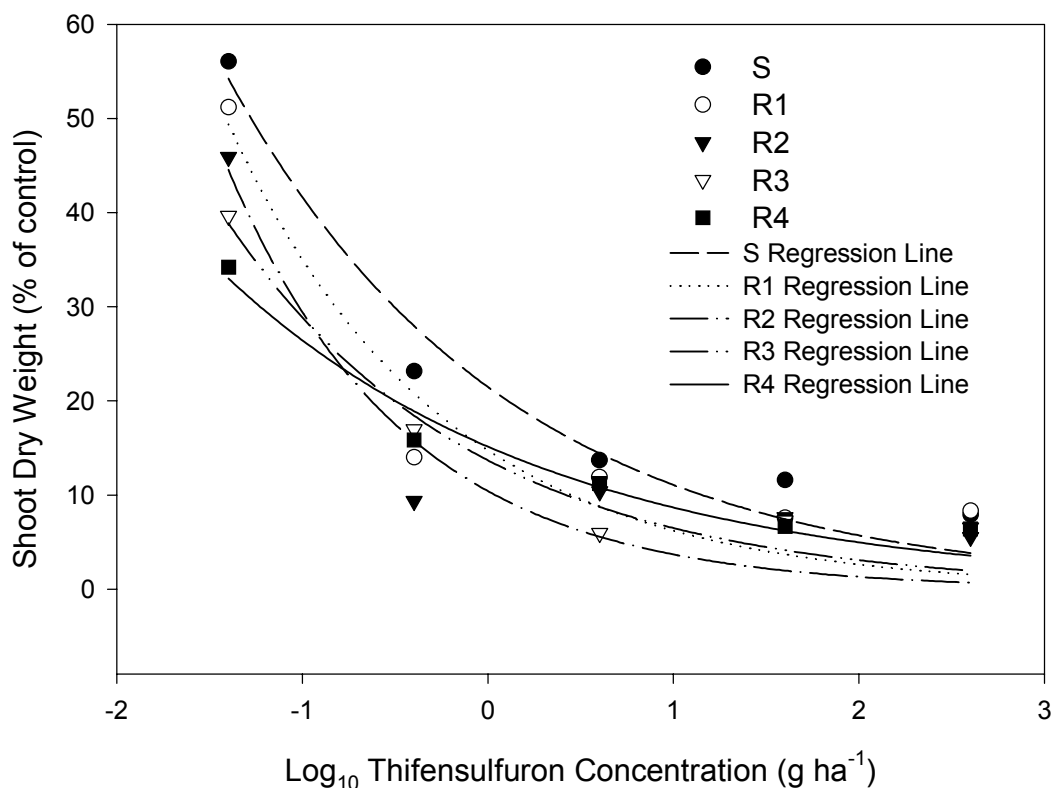
FIGURE 3. Dose response curves for shoot dry weight, as a percent of the untreated control, of imidazolinone-susceptible (S) and four –resistant (R1, R2, R3, R4) smooth pigweed populations treated with thifensulfuron in the greenhouse. GR_{70} values were calculated from nonlinear regression equations $y = 21.49*e^{(-0.66 x)}$, $r^2 = 0.96$, $y = 14.75*e^{(-0.86 x)}$, $r^2 = 0.91$, $y = 10.42*e^{(-1.04 x)}$, $r^2 = 0.89$, $y = 13.69*e^{(-0.75 x)}$, $r^2 = 0.95$, and $y = 15.15*e^{(-0.56 x)}$, $r^2 = 0.96$ for S, R1, R2, R3, and R4 populations, respectively where y is shoot dry weight expressed as a percent of the untreated control and x is the log of the herbicide concentration in g ha^{-1}. All treatments were applied POST and include a non-ionic surfactant (0.25% v/v). Plants were harvested 21 DAT.
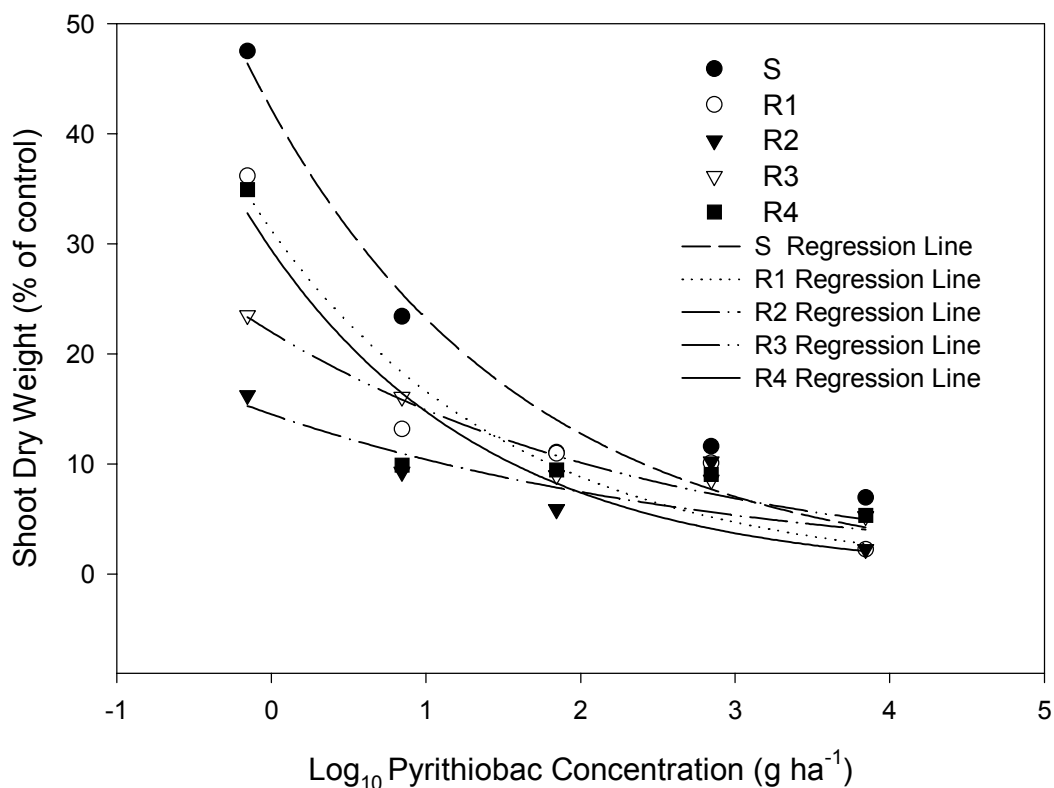
FIGURE 4. Dose response curves for shoot dry weight, as a percent of the untreated control, of imidazolinone-susceptible (S) and four –resistant (R1, R2, R3, R4) smooth pigweed populations treated with pyrithiobac in the greenhouse. GR_{85} values were calculated from nonlinear regression equations $y = 42.26*e^{(-0.6\,x)}$, $r^2 = 0.97$, $y = 31.26*e^{(-0.63\,x)}$, $r^2 = 0.92$, $y = 14.5*e^{(-0.33\,x)}$, $r^2 = 0.71$, $y = 21.97*e^{(-0.38\,x)}$, $r^2 = 0.98$, and $y = 29.45*e^{(-0.69\,x)}$, $r^2 = 0.85$ for S, R1, R2, R3, and R4 populations, respectively where y is shoot dry weight expressed as a percent of the untreated control and x is the log of the herbicide concentration in g ha^{-1}. All treatments were applied POST and include a non-ionic surfactant (0.25% v/v). Plants were harvested 21 DAT.
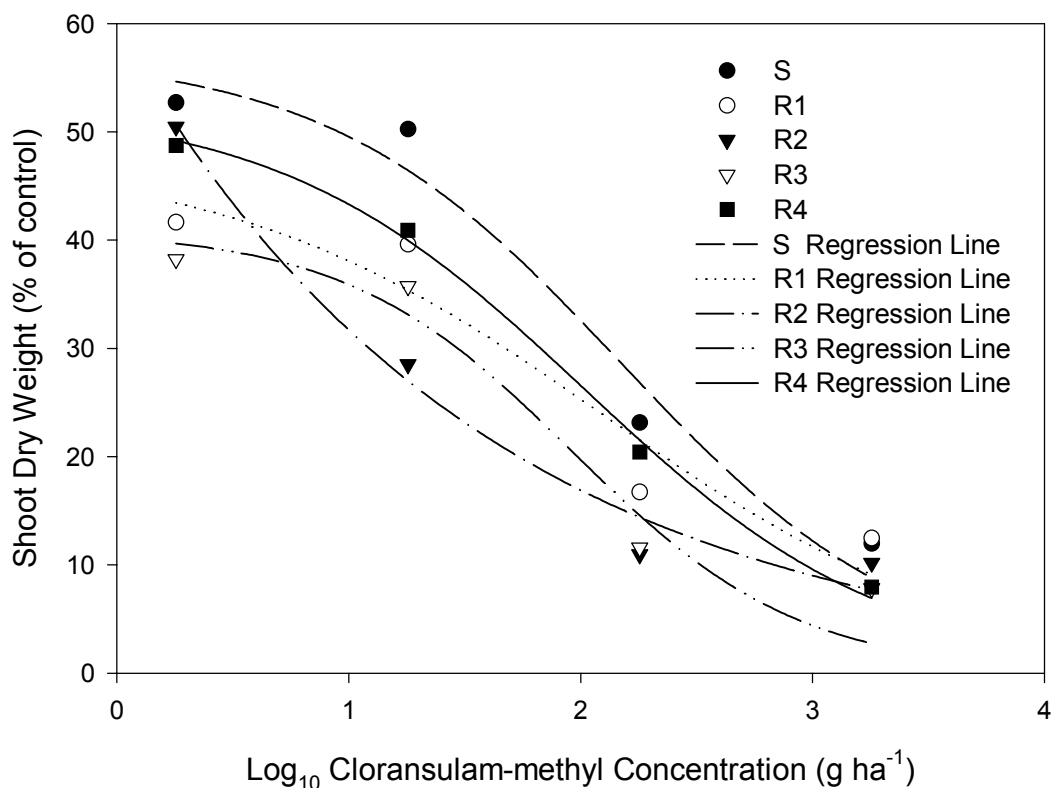
FIGURE 5. Dose response curves for shoot dry weight, as a percent of the untreated control, of imidazolinone-susceptible (S) and four –resistant (R1, R2, R3, R4) smooth pigweed populations treated with cloransulam in the greenhouse. GR_{70} values were calculated from nonlinear regression equations $y = 57.27 / (1 + e^{-(x - 2.18)/-0.63})$, $r^2 = 0.97$, $y = 47.82 / (1 + e^{-(x - 2.09)/-0.80})$, $r^2 = 0.92$, $y = 59.52*e^{(-0.63 x)}$, $r^2 = 0.98$, $y = 40.85 / (1 + e^{-(x - 1.97)/-0.49})$, $r^2 = 0.94$, $y = 52.58 / (1 + e^{-(x - 2.01)/-0.66})$, $r^2 = 0.99$ for S, R1, R2, R3, and R4 populations, respectively where y is shoot dry weight expressed as a percent of the untreated control and x is the log of the herbicide concentration in g ha^{-1}. All treatments were applied POST and include a non-ionic surfactant (0.25% v/v). Plants were harvested 21 DAT.

Chapter III

Cloransulam-methyl Absorption, Translocation, and Metabolism in Imidazolinone-susceptible and –resistant Smooth Pigweed (*Amaranthus hybridus*)

Several populations of smooth pigweed with resistance to the imidazolinone herbicides have been identified in recent years. Greater control of one imidazolinone-resistant smooth pigweed population (R2) compared to the susceptible (S) wild type occurred in greenhouse studies when cloransulam-methyl was applied postemergence (POST) at 18 g/ha. Laboratory studies were conducted in 1998 to determine if differences in absorption, translocation and metabolism of cloransulam-methyl exist between the S and R2 populations. Absorption of cloransulam-methyl into the treated leaf was rapid and no significant differences between populations occurred. Translocation of ^{14}C-cloransulam-methyl out of the treated leaf was generally similar in both populations. ^{14}C-cloransulam-methyl translocated primarily to shoots above and below the treated leaf in both populations with little movement of ^{14}C-cloransulam-methyl to the roots. Metabolism of ^{14}C-cloransulam-methyl was similar in S and R2 populations. Three metabolites with ratio of front (Rf) values of approximately 0.83, 0.65, and 0.45 were detected in both populations. The metabolite with a Rf value of 0.83 increased over time and accounted for 52 and 53 percent of the extracted radioactivity 168 h after treatment in S and R2 populations, respectively. It is unlikely that absorption, translocation, and metabolism play a significant role in the differential tolerances of S and R2 to cloransulam-methyl.

Nomenclature: cloransulam-methyl, 3-chloro-2-[[(5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-*c*]pryimidin-2yl)sulfonyl]amino]benzoic acid; smooth pigweed, *Amaranthus hybridus* L. AMACH.

Key words: Herbicide resistance; negative cross-resistance; absorption; translocation; metabolism; imidazolinone resistance; ALS inhibitor; acetolactate synthase.

Cloransulam-methyl, a triazolopyrimidine sulfonanilide herbicide, can be applied preplant incorporated (PPI), preemergence (PRE) or POST for broadleaf weed control in

soybean (*Glycine max* (L.) Merr.) (Dorich and Schultz, 1997). POST applications have controlled many weeds including morningglory (*Ipomoea* spp.), common cocklebur (*Xanthium strumarium* L.), common ragweed (*Ambrosia artemisiifolia* L.) , and velvetleaf (*Abutilon theophrasti* Medicus), but control of pigweed species and common lambsquarters (*Chenopodium album* L.) has generally been unsatisfactory (Murdock et al. 1998, Nelson and Renner 1998, Oliver et al. 1997). Nelson and Renner (1998) observed 51 and 18 percent control of common lambsquarters and redroot pigweed (*Amaranthus retroflexus* L.), respectively 3 wk following POST cloransulam-methyl (18 g ai/ha) applications.

Triazolopyrimidine sulfonanilides represent one of five chemically distinct herbicide classes that inhibit acetolactate synthase (ALS) (E. C. 4.1.3.18) (Gerwick et al. 1990, Santel et al. 1999, Shaner et al. 1984, Stidham 1991). ALS is a key enzyme in the synthesis of the branched chain amino acids valine, leucine, and isoleucine and is the target site of more than 30 commercially available herbicides (Subramanian et al. 1990; Simpson 1998). Repeated use of ALS inhibitors during the past 10 to 15 years has resulted in the development of more than 50 different weed species resistant to various ALS-inhibiting herbicides (Heap 1999). Several pigweed species resistant to ALS-inhibiting herbicides have been identified in recent years (Foes et al. 1998; Hinz and Owen 1997; Horak and Peterson 1995; Lovell et al. 1996; Manley et al. 1996, 1998).

Absorption, translocation, and metabolism are known to account for differential plant responses to herbicides (Jensen 1982). Hodges et al. (1990) demonstrated that the basis for naturally occurring plant tolerance to triazolopyrimidine sulfonanilides is due to differences in metabolic rates between susceptible and tolerant plants. In cases where continuous herbicide use has resulted in the development of ALS inhibitor-resistant weed populations, however, these three physiological factors are rarely listed as mechanisms of resistance. Resistance is generally due to an altered ALS that is no longer sensitive to inhibition by ALS-inhibiting herbicides (Manley 1996; Lovell et al. 1996; Foes et al. 1998; Sprague et al. 1997).

Resistance to ALS-inhibiting herbicides in rigid ryegrass (*Lolium rigidum* Gaud.) that developed as a result of repeated exposure to the sulfonylurea herbicide chlorsulfuron (2-chloro-*N*[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide) may involve both increased metabolism of the herbicide and an insensitive form of ALS (Christopher et al. 1992). In another biotype of rigid ryegrass, resistance to some sulfonylurea herbicides developed following selection with diclofop-methyl (2-chloro-*N*[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide) and the primary mechanism of resistance was determined to be increased metabolism of the herbicides (Christopher et al. 1991). In the greenhouse, Manley et al. (1998) observed a 2.5-fold greater tolerance to chlorimuron in an imidazolinone-resistant biotype of smooth pigweed compared to the wild type. Rapid metabolism was later suggested to be the mechanism conferring increased tolerance to chlorimuron in the imidazolinone-resistant biotype (Manley 1996).

In recent greenhouse experiments, control of imidazolinone-susceptible (S) and –resistant (R2) smooth pigweed with cloransulam-methyl applied POST at 18 g ai ha^{-1} was 50 and 71percent, respectively (Poston et al. 1998). The objective of this study was to determine if differences in absorption, translocation, and metabolism of cloransulam-methyl between S and R2 could account for whole plant differences observed in the greenhouse.

Materials and Methods

Seed Sources

Smooth pigweed seed were collected in the fall of 1993 from four soybean fields in Worcester County, MD that had histories of repeated imazaquin use. Seed were collected from several plants in each field that survived imazaquin applications. Seed were also collected from imidazolinone-susceptible smooth pigweed plants from the Eastern Shore Agricultural Research and Extension Center near Painter, VA. Seed were threshed and stored under refrigeration until needed.

Plant Culture

Seed from imidazolinone-resistant (R2) and –susceptible (S) populations were planted into 43 cm by 53 cm greenhouse flats[1] containing a commercial potting soil mix[2]. Flats were kept moist and placed on propagation mats[3] calibrated to maintain soil temperature at approximately 24 C. Seed were germinated and seedlings allowed to develop for several days. Four evenly sized seedlings with one visible true leaf were transplanted into 11.4 cm by 11.4 cm pots filled with a soil mix containing Profile[4] and topsoil in a 3:1 ratio. Plants were maintained in the greenhouse under natural sunlight supplemented with metal halide lamps to deliver a photoperiod of 14 hours d^{-1}. Plants were watered as needed and fertilized[5] weekly to maintain active growth. The soil mix was washed from the roots of plants 24 hours prior to herbicide treatment and plants were transferred into glass bottles containing quarter-strength Hoagland's solution.

Herbicide Application

Leaves to be treated with ^{14}C-cloransulam-methyl were selected and covered with foil prior to herbicide application. Commercially formulated cloransulam-methyl (18 g ha^{-1}) was applied POST with a nonionic surfactant[6] (0.25% v/v) to 5- to 9-cm tall (5 to 8 true leaves) smooth pigweed plants from both S and R2 populations prior to treatment with radiolabeled herbicide. Cloransulam-methyl was applied to smooth pigweed using a compressed air, moving nozzle, greenhouse sprayer equipped with one 8002EVS[7] nozzle and calibrated to deliver 171 L ha^{-1} at 289 kPa.

Radiolabeled ^{14}C-cloransulam-methyl (radiochemical purity = 96.9%) with a specific activity of 33.4 mCi $mmol^{-1}$ was obtained from Dow Agrosciences. Immediately following application of commercially formulated cloransulam-methyl, a 20 μl spot containing approximately 0.93 kBq of ^{14}C-cloransulam-methyl and non-ionic surfactant[6] (0.25% v/v) was applied with a micropipette as several droplets to the adaxial surface of the youngest fully expanded leaf (generally the fourth true leaf) of each plant. The

amount of ^{14}C-cloransulam-methyl applied to each leaf was representative of the amount that would be expected in field applications.

Foliar Absorption and Translocation

Smooth pigweed plants were harvested 8, 24, 72, and 168 h after application (HAA) of ^{14}C-cloransulam-methyl. Roots were blotted dry and plants were sectioned into treated leaf, shoot above treated leaf, shoot below treated leaf, and roots. Treated leaves were rinsed with 10 ml of 50% methanol to remove unabsorbed herbicide. A 1 ml aliquot of leaf wash from each treated leaf was added to 10 ml of scintillation cocktail[8] and radioactivity was determined using liquid scintillation spectroscopy[9] (LSS). Individual plant parts were weighed and weights recorded. Percent absorption was calculated using the fraction of total applied radioactivity that remained in the leaf wash. Plant sections used for determining ^{14}C-cloransulam-methyl translocation were combusted in a biological oxidizer[10] after being dried at 70 C for at least 24 h. Radioactivity present as trapped $^{14}CO_2$ was quantified using LSS. Translocation was expressed as a percent of total absorbed radioactivity.

Cloransulam-methyl Metabolism

Plants were cultured, treated, and harvested as previously described and stored at –20 C until needed. Radioactivity was extracted by grinding entire plant shoots in liquid nitrogen using a mortar and pestle and adding 10 ml of 80% acetonitrile. Homogenates were centrifuged at 2000 g for 5 min. Supernatants were saved and passed through #1 Whatman filter paper and vacuum-filtered using a 0.22 micron filter[11]. Samples were air dried and resuspended in 200 μl of 80% acetonitrile. Aliquots of 100 μl from each concentrated sample were spotted onto 20- by 20-cm silica gel thin layer chromatography (TLC) plates[12] that had been activated at 110 C for 1 h prior to spotting. Ten microliters of ^{14}C-cloransulam-methyl spotting solution was also spotted onto TLC plates as a standard. Plates were developed in an ethyl acetate:acetone:glacial acetic acid:ddH2O (5:3:1:1) solution, dried, and placed on X-ray film[13] for 21 d. Film was developed and

used to determine the location of metabolite bands on TLC plates. Metabolite bands were scraped from TLC plates and radioactivity present quantified using LSS. Ratio of front (Rf) values were calculated and used to identify individual metabolites.

Statistical Analyses

Absorption and translocation studies were conducted using a completely randomized experimental design with three replications and experiments were repeated in time. Metabolism studies were not replicated but were repeated twice. Due to treatment by experiment interactions, absorption data from each experiment were analyzed separately. Translocation and metabolism data were combined because no treatment by experiment interactions were detected. Data were subjected to ANOVA and means separated using Fisher's protected LSD test conducted at the alpha = 0.05 level.

Results and Discussion

Foliar Absorption and Translocation

Absorption of ^{14}C-cloransulam-methyl was rapid in both S and R2 smooth pigweed populations (Table 1). Both S and R2 had absorbed essentially all (97 percent) of the applied radioactivity eight h after application (HAA) in experiment one. In experiment two, absorption of ^{14}C-cloransulam-methyl was initially slower with S and R2 absorbing 84 and 89 percent of the applied radioactivity eight HAA, respectively. A significant increase in foliar absorption occurred over time in both S and R2 populations with 95 and 96 percent absorption of radiolabeled cloransulam-methyl 168 HAA in S and R2 populations, respectively. At no time during the course of either experiment were significant differences in absorption between S and R2 detected. Manley (1996) documented differences in foliar absorption of nicosulfuron (2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide) 3 HAA in imidazolinone-susceptible and –resistant smooth pigweed. No significant differences between smooth pigweed populations were detected 6 HAA or later.

Therefore, small differences in foliar absorption of ^{14}C-cloransulam-methyl between S and R2 may have been detected using shorter exposure periods. However, such small differences in herbicide absorption are unlikely to result in differential response to herbicides at the whole plant level.

Translocation of ^{14}C-cloransulam-methyl out of the treated leaf was rapid in both S and R2 and data were generally variable (Table 1). At eight HAA, only 20 and 17 percent of the absorbed radioactivity remained in treated leaves of S and R2, respectively. Apoplastic and symplastic movement of ^{14}C-cloransulam-methyl occurred in both S and R2 with approximately 52 to 79 percent of absorbed radioactivity being detected collectively in shoots above and below treated leaves. Very little (**<** 2 percent of absorbed radioactivity) movement of ^{14}C-cloransulam-methyl to smooth pigweed roots occurred (data not presented). Others have also documented limited movement of foliar-applied ALS-inhibiting herbicides to plant roots (Ackley 1999; Manley 1996). Translocation of ^{14}C-cloransulam-methyl out of the treated leaf was statistically similar in S and R2 during the course of the experiment; therefore, differential whole plant responses to cloransulam-methyl between S and R2 are not associated with translocation.

Cloransulam-methyl Metabolism

Approximately 99 percent of standard ^{14}C-cloransulam-methyl could be associated with a Rf value of 0.98 (data not presented). TLC analysis also revealed a second much fainter band with a Rf value of approximately 0.94 that was also associated with the parent compound. Physical separation of the two bands was difficult, therefore the two bands were combined and for the purpose of discussion will be identified as a single metabolite with a Rf value of 0.98 (Table 2). Less than one percent of the recovered radioactivity remained at the origin (Rf value = 0.0) following development of TLC plates. Three metabolites with Rf values of 0.83, 0.65, and 0.43 were detected in both S and R populations. Metabolites with Rf values of 0.43 and 0.65 were considered minor metabolites because at no time during the course of the experiment did either metabolite account for more than 5.1 percent of the recovered radioactivity. Furthermore,

no significant increase or decrease in the level of these metabolites occurred over time. In contrast, a significant increase in the metabolite with a Rf value of 0.83 occurred over time in both S and R2 populations. This increase was paralleled by a corresponding decrease in parent compound (Rf = 0.98). The identity of this metabolite has not been determined, but it is likely that this metabolite plays a key role in the metabolism of cloransulam-methyl in smooth pigweed. By 168 HAA, metabolite with a Rf value of 0.83 accounted for 52 and 53 percent of the recovered radioactivity in S and R2 populations, respectively. At no time during the course of the experiment were significant differences in the level of any metabolite between S and R2 populations detected.

The level of cloransulam-methyl metabolism by smooth pigweed observed in our studies may partially explain the intermediate level of control often observed with POST applications in the field. Others have suggested that rate of herbicide metabolism is the major factor responsible for selectivity of triazolopyrimidine herbicides (Hodges et al. 1990). Tolerant species were able to metabolize >80 percent of the parent herbicide within 8 HAA compared to <20 percent in more susceptible species. In our studies, S and R2 smooth pigweed populations metabolized cloransulam-methyl 30 and 38 percent, respectively, within the same time period.

The identity of these metabolites has not been determined and due to limited information on cloransulam-methyl metabolism in plants it is difficult to speculate as to their identity without further research. In redroot pigweed, Hodges et al. (1990) detected three metabolites of a different triazolopyrimidine herbicide (N-(2,6-dichlrophenyl)-5,7-dimethyl-1,2,4-triazolo[1,5-a]pryimidine-2-sulfonanilide) and determined that two of the three metabolites were common to nine different plant species. Hodges et al. (1990) also concluded that formation of the major metabolites likely involved methyl hydroxylation or hydroxylation of the aniline ring followed by glucose conjugation. Due to structural similarities between cloransulam-methyl and the triazolopyrimidine investigated by Hodges et al. (1990), similar metabolic pathways may exist in smooth pigweed. Further studies are needed to confirm this hypothesis.

Based on our results, it is unlikely that absorption, translocation, and metabolism play a significant role in the differential tolerances of S and R2 to cloransulam-methyl. Differences in sensitivity of S and R2 ALS to cloransulam-methyl may be responsible for the whole plant differences observed in the greenhouse and will be investigated in future studies.

Sources of Materials

[1]Sutton universal greenhouse flat. Inside dimensions 51 cm x 40 cm x 5.7 cm. Wetzel, Inc., 1345 Diamond Springs Road, Virginia Beach, VA 23455.

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

[3]Heat-A-Matic propagation mats. E. C. Geiger, Inc., 1104 W. Roslyn Rd., Colonial Heights, VA 23834.

[4]Profile™, a granular porous ceramic for use as a soil modifier. Applied Materials Corp., Buffalo Grove, IL 60089.

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

[6]Ortho X-77 nonionic surfactant with 80% principal functioning agents as: alkylarylpolyoxy ethylene glycols, free fatty acids, and isopropanol. Valent USA Corp., 1333 North California Boulevard, Walnut Creek, CA 94596-8025.

[7] Teejet 8001EVS flat fan spray tip. Spraying Systems Co., North Avenue, Wheaton, IL 60188.

[8] Scintiverse. Fisher Scientific, 50 Fadem Road, Springfield, NJ 07081-3193.

[9] Liquid Scintillation Spectrometer, Beckman Model LS-5000TA. Beckman Instrument Co., Fullerton, CA 92634.

[10] Packard Sample Oxidizer Model 307. Packard Instrument Co., Downers Grove, IL 60515.

[11] Millipore 0.22 micron vacuum filter. Fisher Scientific, 50 Fadem Road, Springfield, NJ 07081-3193.

[12] Silica Gel 60 F_{254} precoated TLC plates. EM Science, 480 Democrat Road, Gibbstown, NJ 08027.

[13] Kodak X-OMAT AR film. Eastman Kodak Co., Rochester, NY 14650.

Acknowledgements

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Table 1. Absorption and translocation of foliar-applied ^{14}C-cloransulam-methyl in imidazolinone-susceptible and -resistant smooth pigweed after 8, 24, 72, and 168 hour exposure .[a]

Population	Time after application	Absorption[b] I[d]	II	Translocation[c] Treated leaf	Shoot above treated leaf	Shoot below treated leaf
	H	— % of applied —		——— % of absorbed radioactivity ———		
S	8	97	84	20	33	28
	24	98	94	17	40	39
	72	99	92	26	33	28
	168	98	95	33	27	25
R2	8	97	89	17	39	31
	24	96	94	35	28	25
	72	98	95	32	30	28
	168	99	96	30	31	23
LSD (0.05)[e]		2	9	ns	ns	12

[a] All treatments contained 0.25% v/v nonionic surfactant.

[b] Due to treatment by study interactions, absorption means are listed individually by study and are the average of three replications.

[c] Translocation means are the average of two studies.

[d] I and II refer to first and second studies, respectively.

[e] Means were separated using Fisher's protected LSD at the 0.05 significance level.

Table 2. Metabolism of foliar-applied ^{14}C-cloransulam-methyl in imidazolinone-susceptible and -resistant smooth pigweed after 8, 24, 48, and 168 hours exposure.[a, b]

Population	Time after application	Rf values of detected metabolites[c]				
		0.0	0.43	0.65	0.83	0.98
	H	————————% of recovered radioactivity————————				
S	8	0.1	1.5	2.7	26	70
	24	0.2	2.5	4.6	47	46
	72	0.2	2.7	4.0	41	52
	168	0.2	2.9	4.5	53	39
R2	8	0.2	2.3	3.5	32	62
	24	0.1	2.4	4.5	44	49
	72	0.2	3.1	5.1	50	42
	168	0.2	2.6	3.5	52	42
LSD (0.05)[d]		ns	1.5	2.2	15	18

[a] All treatments contained 0.25% v/v nonionic surfactant.

[b] Means are the average of three studies.

[c] Approximately 99% of standard ^{14}C-cloransulam-methyl migrated to a Rf value of 0.98. TLC analysis revealed another fainter band with a Rf value of approximately 0.94 that was also associated with the parent compound. This band could not be physically separated from the band with a Rf value of 0.98 and the two bands were combined and are referred to as the metabolite with a Rf value of 0.98.

[d] Means were separated using Fisher's protected LSD at the 0.05 significance level.

Chapter IV

Responses of Acetolactate Synthase (ALS) from One Imidazolinone-susceptible and Three –resistant Smooth Pigweed (*Amaranthus hybridus*) Populations to Various ALS Inhibitors

As a follow-up to greenhouse studies, acetolactate synthase (ALS) (EC 4.1.3.18) was extracted from one imidazolinone-susceptible (S) and three imidazolinone-resistant (R1, R2, and R3) smooth pigweed populations and activity was assayed in the presence of imazethapyr, chlorimuron, thifensulfuron, pyrithiobac, and cloransulam-methyl. ALS inhibitor concentrations required to reduce enzyme activity a specified percentage compared to the untreated control (I_p's) were determined for each herbicide and resistance ratios were calculated. I_{50}'s of >35 µM imazethapyr were calculated for all R populations compared to approximately 3.4 µM for the S population thereby confirming resistance to the imidazolinones at the enzyme level. With chlorimuron, thifensulfuron, pyrithiobac, and cloransulam-methyl data sets, pairwise comparisons of regression coefficients were used to determine significant differences between regression lines. Using this technique, it was established that ALS from R3 was more sensitive to inhibition by chlorimuron and thifensulfuron than was S ALS. Also, ALS from R2 and R3 displayed increased sensitivity to pyrithiobac compared to ALS extracted from the S population. This increased sensitivity in R2 and R3 could be interpreted as negative cross-resistance. Based on resistance ratios, increased sensitivity of R2 and R3 ALS to cloransulam-methyl also occurred. We have confirmed resistance to imazethapyr at the enzyme level in all R populations and documented negative cross-resistance in some R populations to ALS inhibitors other than imazethapyr.

Nomenclature: Chlorimuron, 2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino] sulfonyl]benzoic acid; cloransulam-methyl, 3-chloro-2-[[(5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-*c*]pryimidin-2yl)sulfonyl]amino]benzoic acid; imazethapyr, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5ethyl-3-pyridinecarboxylic acid; pyrithiobac, 2-chloro-6-[(4,6-dimethoxy-2-pyrimidinyl)thio]benzoic acid; thifensulfuron, 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-

yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylic acid; smooth pigweed, *Amaranthus hybridus* L. AMACH.

Key words: Acetolactate synthase; ALS; imidazolinones, imidazolinone resistance; herbicide resistance; cross-resistance; imazaquin, 2-[4,5-dihydro-4-methyl-4-(methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolinecarboxylic acid; negative cross-resistance.

The sulfonylurea (SU) herbicides were discovered in the late 1970's (Levitt 1978) and were the first class of herbicides developed that inhibited ALS (Schloss 1990). ALS catalyzes the homologous condensation of two molecules of pyruvate to form α-acetolactate and carbon dioxide or the heterologous condensation of one molecule of pyruvate and one molecule of α-ketobutyrate to form α-aceto-α-hydroxybutyrate and carbon dioxide (Schloss 1990). These reactions are considered the first committed steps in the biosynthesis of the branched-chain amino acids valine, leucine, and isoleucine.

Since the discovery of SU herbicides, more than 30 ALS-inhibiting herbicides representing five structurally distinct classes of chemistry have been developed (Simpson 1998). SU (Chaleff and Muvais 1984), imidazolinone (IMI) (Shaner et al. 1984), triazolopyrimidine sulfonanilide (TP)(Gerwick et al. 1990), pyrimidinylthiobenzoate (PB)(Stidham 1991), and sulfonylaminocarbonyltriazolinone herbicides (Santel et al. 1999) all inhibit ALS and collectively offer broad-spectrum weed control in a variety of crops including corn, cotton, soybeans, small grains, and vegetable crops.

ALS inhibitors have been widely used in many crops during the past 10 to 15 years due at least in part to characteristics like low use rates, high efficacy, multi-crop selectivity, and low mammalian toxicity (Saari et al. 1994). Persistent use of these products has unfortunately resulted in the development of at least 53 different weed species resistant to ALS-inhibiting herbicides (Heap 1999). Resistance to ALS inhibitors resulting from various laboratory techniques or from continuous selection pressure in the field is generally due to an altered ALS that is no longer sensitive to inhibition by the

herbicides (Bernasconi et al. 1995; Guttieri et al. 1996). Patterns of cross-resistance in ALS inhibitor-resistant weed biotypes are difficult to predict and are usually dependent upon one or more point mutations that exist within the DNA sequence of the ALS gene (Guttieri et al. 1996; Saari et al. 1994). Manley et al. (1998) noted that ALS inhibitor-resistant weed biotypes are often cross-resistant to herbicides within the same chemical family as the selection agent but exhibit varying patterns of cross-resistance to other families of ALS inhibitors.

Similar observations can be made for ALS inhibitor-resistant *Amaranthus* species that have been reported within the past 5 to 6 y. Smooth pigweed from fields in Marion, MD with a history of repeated imazaquin use exhibited IMI resistance and low level cross-resistance to chlorimuron and rimsulfuron (*N*-[[4,6-dimethoxy-2-pryimidinyl) amino]carbonyl]-3-(ethylsulfonyl)-2-pyridinesulfonamide) (Manley et al. 1998). PB herbicides and other SU herbicides controlled this population. Repeated use of imazethapyr in Clay County, Kansas resulted in a population of Palmer amaranth (*Amaranthus palmeri*) resistant to both SU and IMI herbicides (Gaeddert et al. 1997). In Douglas County, KS, imazthapyr or imazaquin applied to fields 3 out of 5 growing seasons selected for a biotype of common waterhemp (*Amaranthus rudis*) resistant to SU and IMI herbicides (Horak and Peterson 1995). It is apparent that patterns of cross-resistance cannot be predicted based on herbicide history and must be individually assessed for each weed population.

In recent greenhouse studies, we have characterized several IMI-resistant smooth pigweed populations (Poston et al. 1998). Based on resistance ratios, these populations displayed low-level cross-resistance to chlorimuron and increased sensitivity (negative cross-resistance) to thifensulfuron, pyrithiobac, and cloransulam-methyl. The objectives of this study were to: (1) examine the response of ALS extracted from one IMI-susceptible and three IMI-resistant smooth pigweed populations to imazethapyr, chlorimuron, thifensulfuron, pyrithiobac, and cloransulam-methyl, (2) determine if differences in enzyme sensitivity can account for whole plant responses observed in the greenhouse, and (3) determine if ALS enzyme sensitivity is the basis for IMI resistance.

Materials and Methods

Seed Sources

Smooth pigweed seed were collected in the fall of 1993 from four soybean fields in Worcester County, MD that had histories of repeated imazaquin use. Seed were collected from several plants in each field that survived imazaquin applications. Seed were also collected from IMI-susceptible smooth pigweed plants from the Eastern Shore Agricultural Research and Extension Center near Painter, VA. Seed were threshed and stored under refrigeration until needed.

Plant Culture in the Greenhouse

Seed from S, R1, R2, and R3 smooth pigweed populations were planted into 43 by 53 cm greenhouse flats[1] containing a commercial potting soil mix[2]. Flats were kept moist and placed on propagation mats[3] calibrated to maintain soil temperature at approximately 24 C. Seed were germinated and seedlings allowed to develop for several days before being transplanted into new flats. Fifty-four seedlings with one visible true leaf were transplanted at equidistant spacings into new flats. Plants were maintained in the greenhouse under natural sunlight and sprinkler irrigation and fertilized[4] weekly to maintain active growth.

ALS Assays

Smooth pigweed plants were grown to 5 to 10 cm tall using methods described above. Plant material was harvested, ALS extracted, and activity assayed in the presence of various ALS inhibitors using modifications of methods previously employed by Chaleff and Muvais (1984). ALS was extracted from all populations and activity was assayed in the presence of imazethapyr (0.0035, 0.035, 0.1, 0.35, 3.5, 10, and 35 μM),

chlorimuron (0.0024, 0.024, 0.072, 0.24, 2.4, 7.2, and 24 μM), thifensulfuron (0.0026, 0.026, 0.08, 0.26, 2.6, 8, and 26 μM), pyrithiobac (0.0029, 0.029, 0.09, 0.29, 2.9, 9, and 29 μM), and cloransulam-methyl (0.0023, 0.23, 0.07, 0.23, 2.3, 7, and 23 μM). All herbicides were dissolved in dimethyl sulphoxide (DMSO) and diluted to final assay concentrations with potassium phosphate buffer. Approximately 10 g of fresh plant material were harvested by removing the top one third of several plants. Plant material was immediately placed in a cold beaker that was kept on ice, chopped into smaller pieces, and mixed thoroughly. A 1 g sample was homogenized in a cold glass cell grinder with two volumes of extraction buffer (potassium phosphate, 100 mM; pH 7.5; magnesium chloride, 0.5 mM; glycerol 100 ml L^{-1}; sodium pyruvate, 1 mM; flavin adenine dinucleotide [FAD], 10 μM; thiamine pyrophosphate [TPP], 0.5 mM). The homogenate was centrifuged at 14,000 g and at 4 C for 5 min. The supernatant was diluted with extraction buffer (1:6 v:v) and kept on ice until assayed.

Assays were conducted in 96-well flat-bottom microtiter plates. ALS activity was assayed in a total volume of 100 μl containing 70 μl crude enzyme preparation, 10 μl herbicide solution, and 20 μl ALS reaction buffer (potassium phosphate, 105 mM; pH 7.0; TPP, 2.5 mM; FAD, 14.3 mM; sodium pyruvate, 100 mM). Following incubation at 30 C for 2 h, the enzymatic reaction was terminated by adding 10 μl of 3M H_2SO_4 to the reaction mixture and incubating again at 60 C for 15 min. This process also ensured the complete conversion of acetolactate to acetoin. Creatine (5 g L^{-1} in water, 100 μl) and α-naphthol (50 g L^{-1} in 2.5 N NaOH, 100 μl) were added and mixtures were incubated at 60 C for 30 min to accelerate color development. Reaction mixtures were allowed to cool and acetoin content was determined by reading the absorbance at 525 nm using a Bio-Rad plate reader[5]. Background colors were determined for each enzyme extract using wells that had received 10 μl 3N H_2SO_4 prior to adding reaction buffer. These values were subtracted from reaction absorbance values. Acetoin formation resulting from non-ALS catalyzed reactions was also taken into account and reaction absorbance values corrected accordingly. ALS activities are presented as a percent activity of wells receiving no herbicide.

Statistical Analyses

Three replications were used for assays and experiments were repeated. Data were combined across experiments because treatment by experiment interactions were not detected. Non-linear regression analysis techniques similar to those employed by Chism et al. (1992) were used to generate dose response curves and to compare effects of individual herbicides on ALS extracted from different smooth pigweed populations. Absorbance data expressed as a percent of untreated controls were regressed to fit one of two non-linear equations. The three-parameter sigmoidal function given here as:

$$y = a / (1 + e^{(-(x-c)/b)}) \tag{1}$$

where y is the absorbance expressed as a percent of the untreated control, a, b, and c are constants, and x is the $\log_{10}$ of the herbicide concentration in μM was used to describe the responses of S and R2 ALS to increasing imazethapyr concentrations. A linear model:

$$y = a + bx \tag{2}$$

where y is absorbance expressed as a percent of the untreated control, a is the x intercept, b is slope, and x is the $\log_{10}$ of the herbicide concentration was used to describe the responses of R1 and R3 ALS to increasing rates of imazethapyr. Chlorimuron, thifensulfuron, pyrithiobac, and cloransulam-methyl data were all regressed to fit the two-parameter exponential function given here as:

$$y = a * e^{(-bx)} \tag{3}$$

where y is absorbance expressed as a percent of the untreated control, a and b are constants, and x is the $\log_{10}$ of the herbicide concentration in μM. Pairwise comparisons between regression coefficients were made using techniques described by Chism et al. (1992) to determine if regression equations were significantly different from one another. Pseudo R^2 values were calculated to assess goodness of fit for individual regression equations. Imazethapyr, chlorimuron, thifensulfuron, pyrithiobac, and cloransulam-methyl concentrations inhibiting ALS activity 50, 70, 60, 60, and 50 percent (I_p's), respectively were calculated from regression equations. I_p values were selected individually for each herbicide to ensure that concentrations derived from regression

equations fell within the herbicide concentrations used in our experiments. Resistance ratios ($I_{p\,resistant}$/$I_{p\,susceptible}$) for ALS inhibition were calculated using I_p values.

Results and Discussion

Imazethapyr Data

The responses of S and R2 ALS to increasing imazethapyr concentrations were sigmoidal and consequently data points were regressed to fit equation 1 (Figure 1). Based on pseudo R^2 values of 0.96 for S data and 0.90 for R2 data, equation 1 adequately described the response of both populations to imazethapyr. No significant decrease and a slight linear decrease in R1 and R4 ALS activity, respectively occurred as imazethapyr concentrations increased from 0.0035 µM to 35 µM. Activity of R1, R2, and R3 ALS was reduced 23, 23, and 15 percent, respectively by the highest imazethapyr concentration utilized in our assays (35 µM) compared to 70 percent reduction in S ALS activity. Approximately 3.4 µM imazethapyr were required to reduce activity of S ALS 50 percent compared to more than 35 µM for R ALS (Table 1). Estimates of imazethapyr I_{50}'s for R ALS calculated using regression equations and extrapolated outside of the rate ranges utilized in our assays were unrealistic and therefore not utilized. We have determined based on resistance ratios that ALS from all R populations is at least 10.3-fold more tolerant to imazethapyr than S ALS (Table 2). Of course this is an unrealistically low estimate. In the greenhouse for example, R1, R2, and R3 were 730-, 1080-, and 790-fold more tolerant to imazethapyr indicating that actual resistance ratios at the enzyme level are much higher than 10.3 (Chapter 2). Manley et al. (1999b) estimated that ALS from another IMI-resistant smooth pigweed population was 109,000-fold more tolerant to imazethapyr than ALS extracted from a wild type population. This value may be unrealistically high due to the inability of regression models to accurately predict values outside of the rate ranges used in assays. Using *in vivo* assays, Lovell et al.

(1996) estimated >500-fold resistance at the enzyme level in a biotype of common waterhemp (*Amarathus rudis* Sauer.)

Chlorimuron Data

Pseudo R^2 values ranged from 0.62 to 0.88 for chlorimuron data fit to equation 3 (Figure 2). Chlorimuron was the most potent inhibitor used in our experiments with greater than 45 percent reduction in the activity of ALS from all populations by the lowest chlorimuron concentration (0.0024 μM) used in the assay (Figure 2). Pairwise comparisons of non-linear regression coefficients revealed that ALS from S, R1, and R2 populations responded similarly to chlorimuron (Table 3). In contrast, R3 ALS was significantly more sensitive to inhibition by chlorimuron compared to ALS from all other populations. R3 ALS activity declined at a faster rate and to a lower overall level as a result of increasing chlorimuron concentrations compared to the activity of ALS extracted from S, R1, and R2 (Figure 2). Chlorimuron concentrations reducing ALS activity 70 percent ranged from 0.009 μM for R3 to 0.08 μM for S (Table 1). Resistance ratios based on I_{70} values were 0.4, 0.8, and 0.1 for R1, R2, and R3 ALS, respectively (Table 2) suggesting that ALS from all R populations is slightly more sensitive to inhibition by chlorimuron than ALS from the S population with R3 ALS being the most sensitive. These findings do not agree with responses observed at the whole plant level where R1, R2, and R3 populations were approximately 5- to 7-fold more tolerant to chlorimuron in the greenhouse (Chapter 2). Manley et al. (1998) characterized an imidazolinone-resistant smooth pigweed population that was 2.5 times more tolerant to chlorimuron in the greenhouse compared to the wild type. Increased tolerance to chlorimuron at the whole plant level was not attributed to sensitivity differences at the enzyme level (Manley et al. 1999b). Similar to our findings, ALS from the wild type was more tolerant to chlorimuron than R ALS. Manley et al. (1999a) later attributed greater whole plant tolerance in the R population to increased chlorimuron metabolism within 3 h of application.

Thifensulfuron Data

Pseudo R^2 values of 0.78, 0.94, 0.88, and 0.92 were calculated for thifensulfuron data fit to equation 3 (Figure 3). The activity of ALS from all populations decreased exponentially with increasing thifensulfuron concentrations. Thifensulfuron concentrations ≥ 0.08 μM reduced ALS activity in all populations more than 65 percent. Based on pairwise comparisons of regression coefficients, ALS from all populations responded similarly to thifensulfuron with R3 as an exception (Table 3). As with chlorimuron, R3 ALS activity declined at a faster rate and to a lower overall level as a result of increasing thifensulfuron concentrations compared to ALS from the S and R2 populations. R3 ALS differed from R1 ALS only in the magnitude of response to thifensulfuron. Thifensulfuron I_{60} values were 0.03, 0.07, 0.1, and 0.03 for S, R1, R2, and R3 ALS, respectively (Table 1). Based on resistance ratios, ALS from R1 and R2 populations was 2.3- to 3.3-fold more tolerant to thifensulfuron compared to S ALS, but R3 and S ALS responded similarly to thifensulfuron based on a resistance ratio of 1.0 (Table 2). However, interpretations based solely on resistance ratios calculated from I_p values should always be interpreted carefully because they represent only one specific point on a dose response curve and therefore do not take into account differences in sensitivity that may exist at other points on a curve. For example, resistance ratios based on thifensulfuron I_{70}'s or I_{80}'s rather than I_{60}'s would likely reflect the increased sensitivity of R3 ALS to thifensulfuron.

The increased sensitivity of R3 ALS to thifensulfuron compared to S ALS that was detected using pairwise comparisons of regression coefficients may partially explain whole-plant responses to thifensulfuron. In the greenhouse, R3 was slightly more sensitive to thifensulfuron based on a resistance ratio of 0.4 calculated from GR_{70} values (Chapter 2). At the whole-plant level, R2 was also more sensitive than S to thifensulfuron based on both pairwise comparisons of regression coefficients and resistance ratios (Chapter 2). Differences in enzyme sensitivity, therefore, cannot account for the increased sensitivity of R2 to thifensulfuron at the whole-plant level.

Pyrithiobac Data

Pyrithiobac data fit well to equation 3 with pseudo R^2 values ranging from 0.69 for R1 to 0.88 for R2 (Figure 4). Visual assessment of Figure 4 reveals that reductions in ALS activity were generally greater for R ALS compared to S ALS especially at pyrithiobac concentrations <0.29-μM. Pairwise comparisons of regression coefficients revealed significant differences in the magnitude of R2 and R3 ALS response to pyrithiobac compared to S ALS (Table 3). The response of R1 ALS to pyrithiobac was similar to S ALS and the rate at which ALS activity decreased with increasing pyrithiobac concentrations was similar in all populations. Pyrithiobac concentrations required to reduce ALS activity 60 percent were 1.3, 0.2, 0.04, and 0.002 μM for S, R1, R2, and R3 ALS, respectively (Table 1). Resistance ratios calculated using these values were 0.2, 0.03, and 0.002 for R1, R2, and R3 populations, respectively (Table 2). R1, R2, and R3 ALS were 5-, 33-, and 500-fold more sensitive, based on these values, to inhibition by pyrithiobac than S ALS, respectively. In other studies, ALS extracted from an IMI-resistant smooth pigweed population was found to be 3-fold more sensitive to pyrithiobac compared to ALS from a wild type population (Manley et al. 1999b). These findings explain very well the increased sensitivity of R populations to pyrithiobac observed in the greenhouse where R populations were approximately 5- to 30 fold more sensitive to pyrithiobac compared to S (Chapter 2).

Cloransulam-methyl Data

Cloransulam-methyl data regressed to fit equation 3 resulted in pseudo R^2 values of 0.67, 0.56, 0.71, and 0.70 for S, R1, R2, and R3 ALS dose response curves, respectively (Figure 5). Pairwise comparisons of regression coefficients revealed that the response of ALS from R populations to increasing cloransulam-methyl concentrations was similar to the response of S ALS (Table 3). The magnitude of response to increasing rates of cloransulam-methyl was significantly smaller for R2 and R3 ALS compared to R1 ALS. Based on concentrations required to reduce enzyme activity 50 percent, relative ALS sensitivity to cloransulam-methyl was R2>R3>S>R1 (Table 1). Resistance ratios of

7.0, 0.04, and 0.1 were recorded for R1, R2, and R3 ALS, respectively (Table 2). Resistance ratios were 0.08 and 0.2 for R2 and R3 populations, respectively in the greenhouse (Chapter 2). These values coincide well with the resistance ratios from our current study and may explain slightly better control of R2 and R3 populations in the greenhouse by postemergence cloransulam-methyl applications. Absorption, translocation, and metabolism of cloransulam-methyl in S and R2 populations were similar thus ruling out these factors as contributing to increased sensitivity to cloransulam-methyl in the R2 population (Chapter 3). At the whole-plant level, R1 was also slightly more sensitive to cloransulam compared to S based on resistance ratios (Chapter 2). The seven-fold greater tolerance of R1 ALS to cloransulam-methyl does not coincide with these findings indicating that factors other than enzyme sensitivity may be contributing to differences in sensitivity at the whole-plant level.

Concluding Remarks

We have determined that high-level resistance to IMI herbicides in three smooth pigweed populations is due to an altered ALS that is no longer sensitive to inhibition by these herbicides. Low-level cross-resistance to chlorimuron was not explained by differences in enzyme sensitivity and must be the result of other factors. Increased sensitivity to thifensulfuron in the R3 population observed in the greenhouse may be due to increased sensitivity of R3 ALS to thifensulfuron. Likewise, the better control of R populations by pyrithiobac and cloransulam-methyl in the greenhouse may be due to differential tolerance at the enzyme level. Greater control of some R populations at the whole-plant level and greater reductions in the activity of some R ALS by classes of ALS inhibitors other than the imidazolinones may be interpreted as negative cross-resistance.

The patterns of cross-resistance established by our work and the work of Manley et al. (1998, 1999b) are dissimilar to those reported for other pigweed species. Lovell et al. (1996) characterized a biotype of common waterhemp that was 130- and 520-fold more tolerant to imazethapyr at the whole-plant and enzyme levels, respectively. Interestingly, this biotype displayed greater tolerance to thifensulfuron and chlorimuron

than to imazethapyr at both the whole-plant and enzyme levels despite the fact that resistance was selected for only by IMI herbicides. It should be noted, however, that Lovell et al. (1996) did not rule out the possibility that SU herbicides may have been used in years preceding their available field history records or that resistant seed or pollen could have been brought in from other locations. Sprague et al. (1997) documented patterns of cross-resistance similar to those of Lovell et al. (1996) in biotypes of both common waterhemp and Palmer amaranth (*Amaranthus palmeri* S Wats.) that had been treated with repeated annual applications of both IMI and SU herbicides. IMI resistance and cross-resistance to thifensulfuron and flumetsulam (*N*-(2,6-difluorophenyl)-5-methyl[1,2,4]triazolo[1,5-α]pyrimidine-2-sulfonamide) has also been reported in a biotype of common waterhemp from Bond Co., Illinois that also displays resistance to atrazine (6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine) (Foes et al. 1998). Complete field histories were not provided for this location, but it is suspected that the selection agent was a commercial premix of atrazine and imazethapyr. Selection for imidazolinone resistance in our smooth pigweed populations was exclusively by imazaquin. Differential patterns of cross-resistance in smooth pigweed compared to other pigweed species may be related to the selection agent used or to the frequency at which a particular mutation exists within a given pigweed species.

Foes et al. (1998) determined that broad-range resistance to IMI, SU, and TP herbicides was the result of a mutation in the ALS enzyme resulting in an amino acid substitution at position 569 from tryptophan in the susceptible to leucine in the resistant biotype. The identical mutation has been shown to confer resistance to both IMI and SU herbicides in two other common waterhemp biotypes (Schmenk et al. 1997; Woodworth et al. 1996). The tryptophan to leucine mutation has also been shown to produce the same patterns of cross-resistance in common cocklebur (*Xanthium strumarium* L.) from Missouri and Pioneer[®] 3180 IR corn (Bernasconi et al. 1995). The patterns of cross-resistance in our smooth pigweed populations and of the Manley et al. (1998) biotype are similar to those displayed by IMI-resistant common cocklebur from Mississippi and ICI 8532 IT corn where an alanine to threonine substitution at position 57 of the ALS amino acid sequence was documented (Greaves et al. 1993; Bernasconi et al. 1995). However,

the level of IMI resistance displayed by our smooth pigweed populations appears far greater than that displayed by Mississippi cocklebur or ICI® 8532 IT corn. Therefore, it is possible that the mutation(s) conferring IMI resistance in our smooth pigweed populations is different from those currently characterized.

Sources of Materials

Acknowledgments

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TABLE 1. Concentrations of five ALS-inhibiting herbicides causing a specified percent reduction in activity (I_p's) of ALS extracted from one imidazolinone-susceptible (S) and three –resistant (R1, R2, and R3) smooth pigweed populations as determined by absorbance values collected in the laboratory. I_p values were calculated using nonlinear regression equations presented in Figure legends 1-5.[a]

| Herbicide | I_p | | | |
	S	R1	R2	R3
	μM			
Imazethapyr[b]	3.4	>35[c]	>35	>35
Chlorimuron[d]	0.08	0.03	0.06	0.009
Thifensulfuron[e]	0.03	0.07	0.1	0.03
Pyrithiobac[e]	1.3	0.2	0.04	0.002
Cloransulam-methyl[b]	0.1	0.7	0.004	0.01

[a] I_p = herbicide concentration required to reduce enzyme activity a specified percentage compared to the untreated control.

[b] Values presented are I_{50}'s.

[c] ALS from R1, R2, and R3 did not respond or responded very little to increasing imazethapyr concentrations. Consequently, the regression models utilized could not accurately predict imazethapyr concentrations required to inhibit ALS activity 50% compared to the untreated control. It can only be established that these values lie above 35 μM, the highest imazethapyr concentration used in the assay.

[d] Values presented are I_{70}'s.

[e] Values presented are I_{60}'s.

TABLE 2. Resistance ratios (R/S) of three imidazolinone-resistant smooth pigweed populations for five ALS-inhibiting herbicides based on I_p values calculated using nonlinear regression equations presented in Figure legends 1-5.[a]

Herbicide	Resistance Ratios (R/S)[b]		
	R1/S	R2/S	R3/S
Imazethapyr[c]	>10.3	>10.3	>10.3
Chlorimuron[d]	0.4	0.8	0.1
Thifensulfuron[e]	2.3	3.3	1.0
Pyrithiobac[e]	0.2	0.03	0.002
Cloransulam-methyl[c]	7.0	0.04	0.1

[a] I_p refers to the herbicide concentration required to reduce enzyme activity a specified percentage compared to the untreated control.

[b] R/S = resistance ratio = $I_{p\ resistant} / I_{p\ susceptible}$

[c] R/S = resistance ratio = $I_{50\ resistant} / I_{50\ susceptible}$

[d] R/S = resistance ratio = $I_{70\ resistant} / I_{70\ susceptible}$

[e] R/S = resistance ratio = $I_{60\ resistant} / I_{60\ susceptible}$

TABLE 3. Pairwise comparisons of nonlinear regression coefficients between ALS from one imidazolinone-susceptible (S) and three –resistant (R1,R2, and R3) smooth pigweed populations for imazethapyr, chlorimuron, thifensulfuron, pyrithiobac, and cloransulam-methyl dose response curves.[a, b]

	Herbicide							
	Chlorimuron		Thifensulfuron		Pyrithiobac		Cloransulam-methyl	
Population	a	b	a	b	a	b	a	b
S vs.								
R1	ns	ns	ns	ns	ns	ns	ns	ns
R2	ns	ns	ns	ns	*	ns	ns	ns
R3	*	*	*	*	*	ns	ns	ns
R1 vs.								
R2	ns	ns	ns	ns	ns	ns	*	ns
R3	*	*	*	ns	*	ns	*	ns
R2 vs.								
R3	*	*	*	*	ns	ns	ns	ns

[a] Comparisons between populations followed by an * indicate significant differences ($\alpha = 0.05$).

[b] General equation: $y = a * e^{(-bx)}$

FIGURE 1. Dose response curves for ALS activity, as a percent of the untreated control, of ALS extracted from one imidazolinone-susceptible (S) and three –resistant (R1, R2, and R3) smooth pigweed populations treated with imazethapyr in the laboratory. I_{50} values were calculated from regression equations $y = 110.4 / (1 + e^{(- (x - 0.4) / -0.7)})$, $r^2 = 0.96$; $y = 86.5 + -4.1*x$, $r^2 = 0.41$; $y = 106.8 / (1 + e^{(- (x - 1.7) / -0.18)})$, $r^2 = 0.90$; and $y = 91.4 + -5*x$, $r^2 = 0.65$ for S, R1, R2, and R3 ALS, respectively.
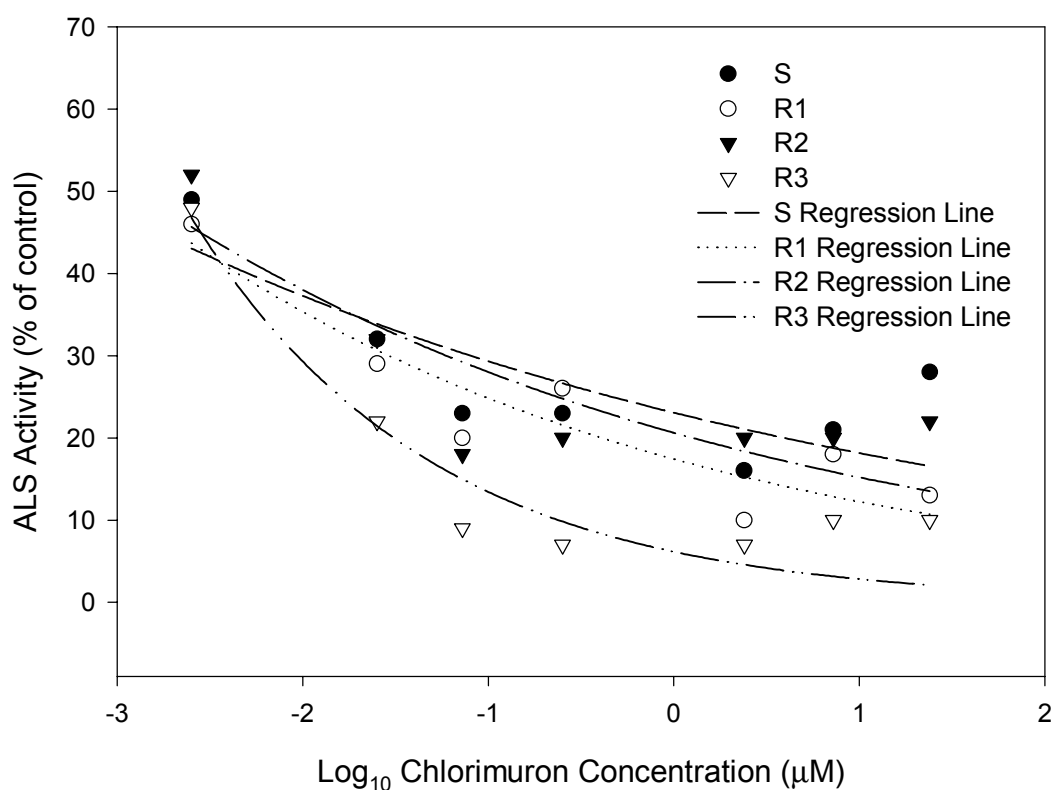
FIGURE 2. Dose response curves for ALS activity, as a percent of the untreated control, of ALS extracted from one imidazolinone-susceptible (S) and three –resistant (R1, R2, R3) smooth pigweed populations treated with chlorimuron in the laboratory. I_{70} values were calculated from nonlinear regression equations $y = 22.98 * e^{(-0.24x)}$, $r^2 = 0.62$; $y = 17.28 * e^{(-0.35x)}$, $r^2 = 0.86$; $y = 20.42 * e^{(-0.31x)}$, $r^2 = 0.69$; and $y = 6.15 e^{(-0.81x)}$, $r^2 = 0.88$ for S, R1, R2, and R3 ALS, respectively.
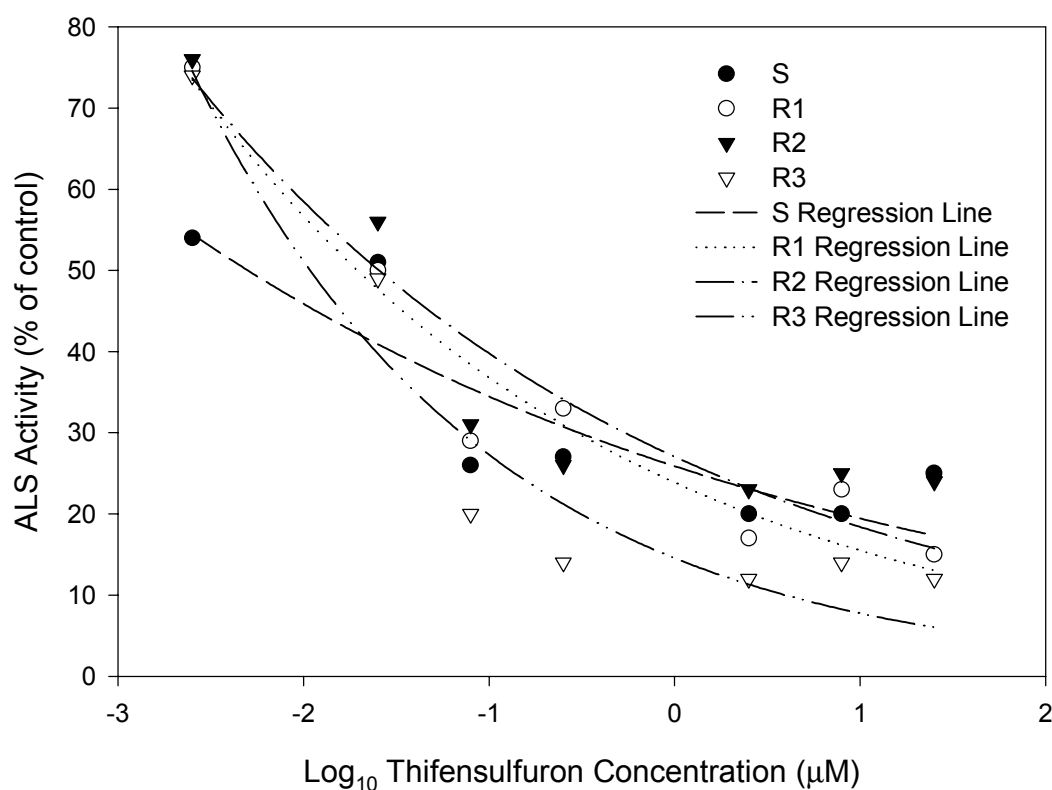
FIGURE 3. Dose response curves for ALS activity, as a percent of the untreated control, of ALS extracted from one imidazolinone-susceptible (S) and three –resistant (R1, R2, R3) smooth pigweed populations treated with thifensulfuron in the laboratory. I_{60} values were calculated from nonlinear regression equations $y = 26.03 * e^{(-0.28x)}$, $r^2 = 0.78$; $y = 23.84 * e^{(-0.44x)}$, $r^2 = 0.94$; $y = 27.50 * e^{(-0.38x)}$, $r^2 = 0.88$; and $y = 14.68\ e^{(-0.63x)}$, $r^2 = 0.92$ for S, R1, R2, and R3 ALS, respectively.
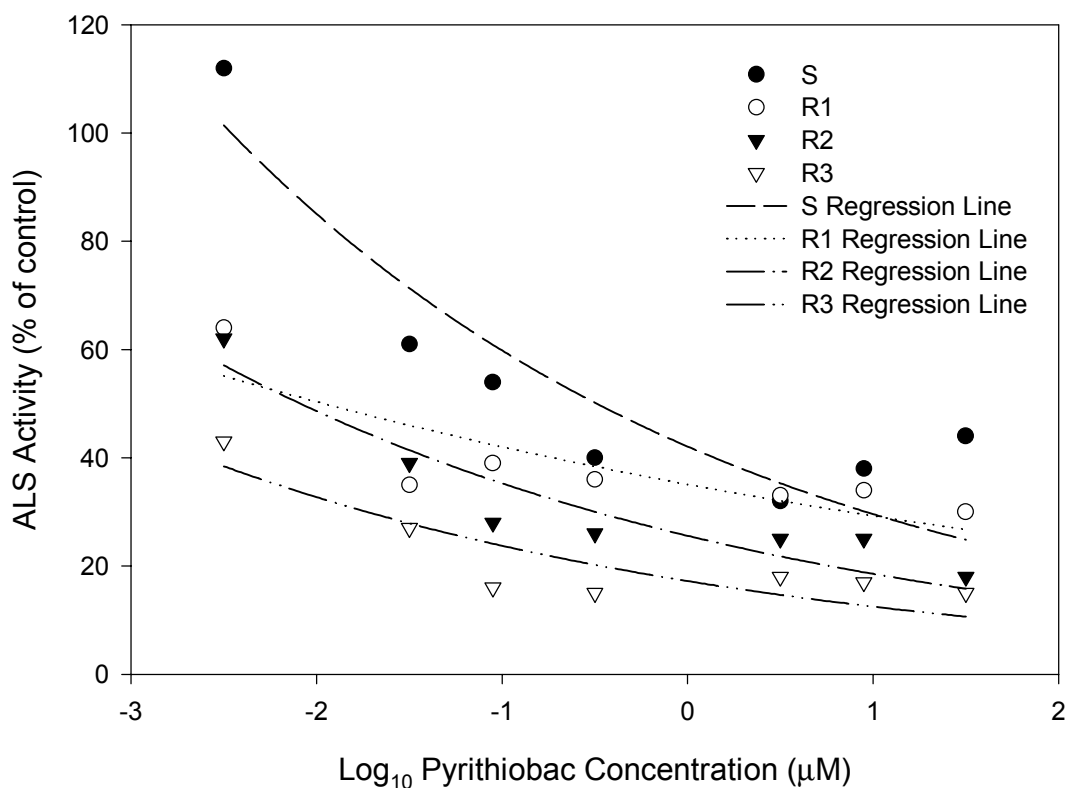
FIGURE 4. Dose response curves for ALS activity, as a percent of the untreated control, of ALS extracted from one imidazolinone-susceptible (S) and three –resistant (R1, R2, R3) smooth pigweed populations treated with pyrithiobac in the laboratory. I_{60} values were calculated from nonlinear regression equations $y = 41.69 * e^{(-0.35x)}$, $r^2 = 0.82$; $y = 34.81 * e^{(-0.18x)}$, $r^2 = 0.69$; $y = 25.54 * e^{(-0.32x)}$, $r^2 = 0.88$; and $y = 17.21 e^{(-0.32x)}$, $r^2 = 0.74$ for S, R1, R2, and R3 ALS, respectively.
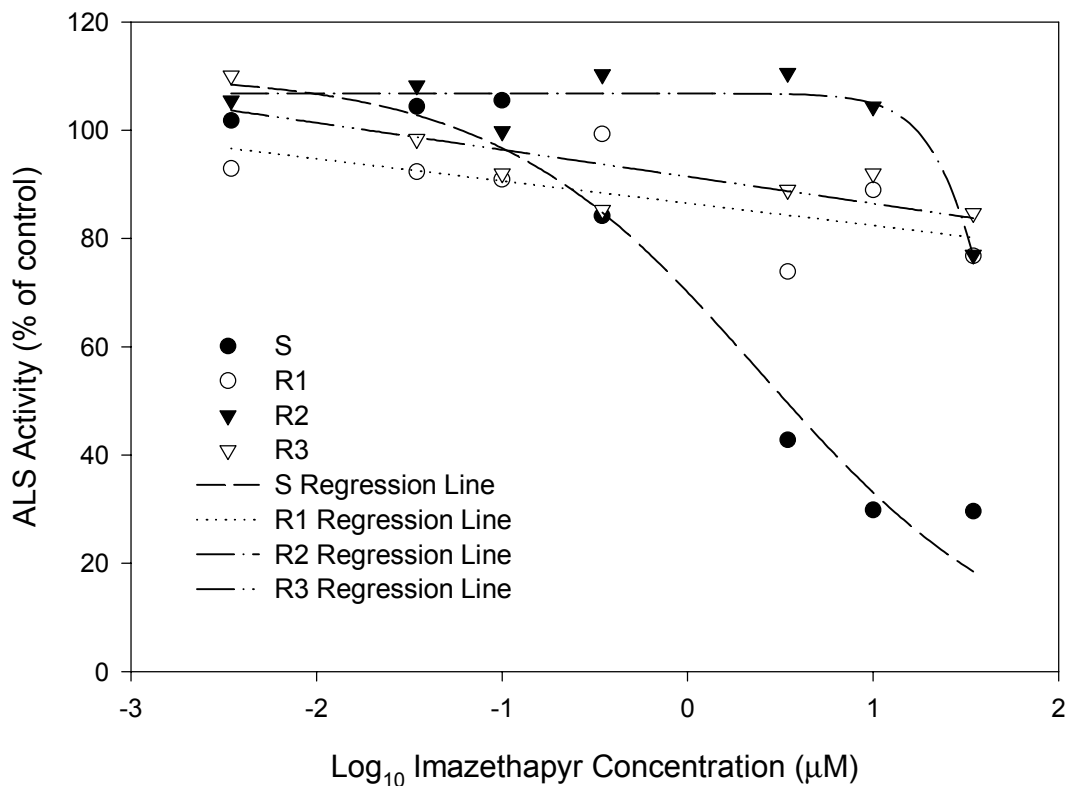
FIGURE 5. Dose response curves for ALS activity, as a percent of the untreated control, of ALS extracted from one imidazolinone-susceptible (S) and three –resistant (R1, R2, R3) smooth pigweed populations treated with cloransulam-methyl in the laboratory. I_{50} values were calculated from nonlinear regression equations $y = 38.03 * e^{(-0.30x)}$, $r^2 = 0.67$; $y = 48.98 * e^{(-0.14x)}$, $r^2 = 0.56$; $y = 25.02 * e^{(-0.28x)}$, $r^2 = 0.71$; and $y = 34.24\ e^{(-0.19x)}$, $r^2 = 0.70$ for S, R1, R2, and R3 ALS, respectively.
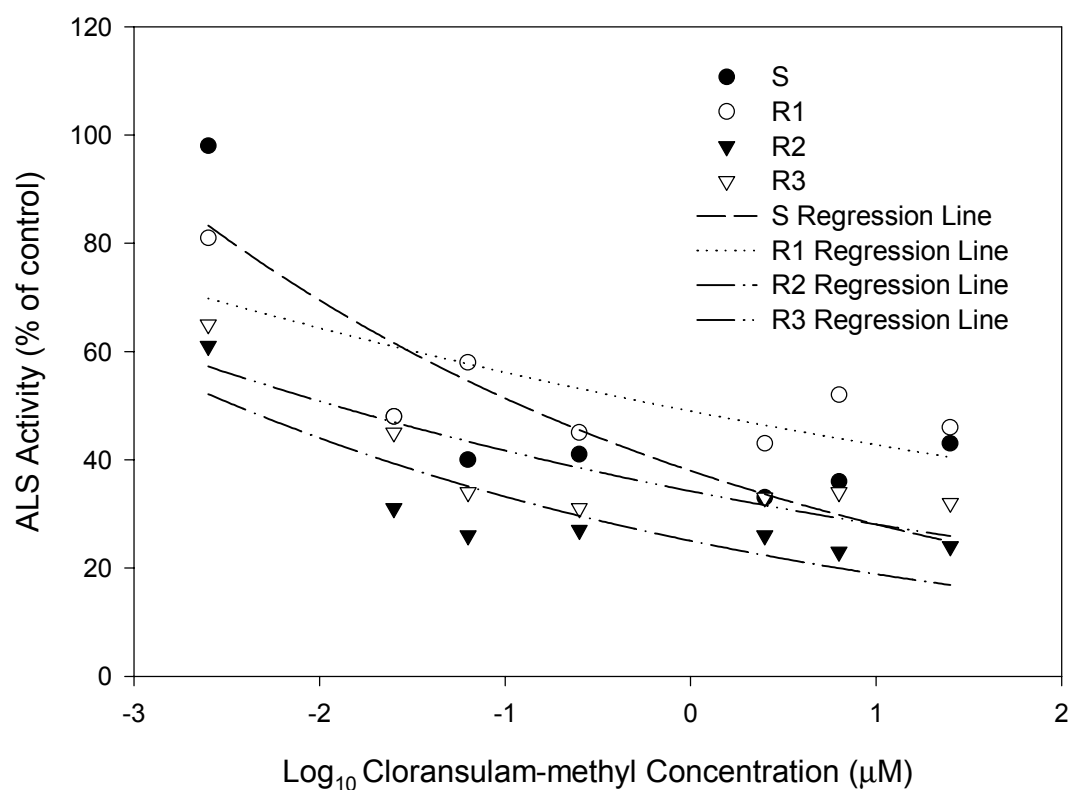
Chapter V

Growth and Development of One Imidazolinone-susceptible and Several –resistant

Smooth Pigweed (*Amaranthus hybridus*) Populations

Greenhouse and field studies were conducted in 1998 and 1999 to evaluate the growth of one imidazolinone-susceptible (S) and four –resistant (R1, R2, R3, and R4) smooth pigweed populations under noncompetitive and competitive conditions. Under noncompetitive conditions in the greenhouse, S produced 17, 23, 25, and 44 percent more biomass than R1, R2, R3, and R4 populations, respectively. S plants were also taller than R plants 17 and 21 d after planting (DAP) and displayed a faster initial rate of leaf area increase compared to all R populations. The net assimilation rate of S was significantly higher than R2 and R3 populations 24 wk after planting (WAP). R3 and R4 populations had significantly less chlorophyll per g of plant tissue compared to S, therefore reduced growth in some R populations compared to S may be linked to chlorosis that generally appears early in seedling development. Biomass production in the field under competitive conditions was similar for all populations using both monoculture and mixed populations. For this reason, the differences in growth observed in the greenhouse in the S population may not confer a competitive advantage over R populations in the field.

Nomenclature: Smooth pigweed, *Amaranthus hybridus* L. AMACH.

Key words: Acetolactate synthase; ALS; competition; herbicide resistance; imidazolinones, imidazolinone resistance; fitness.

More than 50 weed species resistant to acetolactate synthase (EC 4.1.3.18) (ALS)-inhibiting herbicides have been reported within the past 10 to 15 years with several *Amaranthus* species being among the more recent reported (Heap 1999). Biotypes of Palmer amaranth (*Amaranthus palmeri* S Wats.) (Horak and Peterson, 1995; Gaeddert et al. 1997; Sprague et al. 1997), redroot pigweed (*Amaranthus retroflexus* L.) (Saari et al. 1994), prostrate pigweed (*Amaranthus blitiodes* S Wats.) (Saari et al. 1994), common waterhemp (*Amaranthus rudis* Sauer) (Foes et al. 1998; Horak and Peterson 1995;

Sprague et al. 1997; Hinz and Owen 1997; Lovell et al. 1996), livid amaranth (*Amarnthus lividus* L.) (Manley et al. 1996), and smooth pigweed (Manley et al. 1996; Schmenk et al. 1997) resistant to ALS-inhibiting herbicides have been reported within the past 6 to 7 years. In all instances, repeated use of ALS-inhibiting herbicides was documented and resistance was due to an altered ALS.

Fitness is one of the most important factors affecting the appearance and persistence of a herbicide-resistant weed biotype (Gressel and Segel 1990; Maxwell et al. 1990). Fitness is defined as the ability of an organism to establish, survive, and reproduce successfully (Silvertown 1982). Under natural selection, weed biotypes that are most fit produce the most offspring and dominate in the gene pool. Selection pressure imposed on a wild population to select for a given trait generally results in a fitness penalty for selected individuals (Gressel and Segel 1982). Unnatural selection pressure imposed by repeated use of highly effective and persistent herbicides resulting in the selection of herbicide-resistant weed biotypes can be utilized as an example to illustrate this biological phenomenon. Ahrens and Stoller (1983) demonstrated that triazine-resistant smooth pigweed produced less shoot biomass and seed dry weight under competitive conditions, fixed less CO_2 under saturated light and CO_2 conditions, and exhibited a significantly lower relative growth rate (RGR) and net assimilation ratio (NAR) compared to a triazine-susceptible biotype. Conrad and Radosevich (1979) concluded that triazine-resistant redroot pigweed and common groundsel (*Senecio vulgaris* L.) were less fit than their respective wild types under both competitive and non-competitive conditions. Conrad and Radosevich (1979) attributed reduced competitiveness in the resistant biotype to photosynthetic inefficiency and concluded that the triazine resistance trait was only of benefit to the plant where triazine herbicides are repeatedly used. Gressel and Segel (1982) suggest that one possible result of reduced fitness in triazine-resistant weed biotypes is that the selected biotypes may only continue to exist in a population where herbicide selection pressure is great enough to kill the wild type. Based on this premise, reversion to a mostly susceptible population will likely occur over time in the absence of the herbicidal selection agent.

Interestingly, weed biotypes that have developed resistance to ALS inhibitors may not suffer fitness penalties as severe as those observed in triazine resistant weed biotype. Thompson et al. (1994) noted similar growth rates, seed production, and competitiveness in both sulfonylurea-susceptible and –resistant kochia (*Kochia scoparia* (L.) Schrad.). With sulfonylurea-resistant prickly lettuce (*Lactuca serriola* L.) reductions in biomass production compared to the wild type were observed under noncompetitive conditions, but the biotypes grew similarly in competition studies (Alcocer-Ruthling et al. 1992).

We have recently identified several imidazolinone-R smooth pigweed populations that appear to display differential growth rates in the greenhouse (Poston et al. 1998). The objectives of these studies were to evaluate the growth of S, R1, R2, R3, and R4 smooth pigweed populations under noncompetitive and competitive conditions.

Materials and Methods

Seed Sources

Smooth pigweed seed were collected in the fall of 1993 from four soybean fields in Worcester County, MD that had histories of repeated imazaquin (2-[4,5-dihydro-4-methyl-4-(methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolinecarboxylic acid) use. Seed were collected from several plants in each field that survived imazaquin applications. Seed were also collected from IMI-susceptible smooth pigweed plants from the Eastern Shore Agricultural Research and Extension Center near Painter, VA. Seed were threshed and stored under refrigeration until needed.

Growth Rate in the Greenhouse

Seed from S, R1, R2, R3, and R4 smooth pigweed populations were planted into 43- by 53-cm greenhouse flats[1] containing a commercial potting soil mix[2]. Flats were kept moist and placed on propagation mats[3] calibrated to maintain soil temperature at approximately 24 C. Seed were germinated and seedlings allowed to develop for several

days before being transplanted into 11 by 11 cm pots filled with potting soil. Four evenly sized cotyledon-stage seedlings were transplanted into each pot. Plants were maintained in the greenhouse under natural sunlight and sprinkler irrigation. Plants were fertilized[4] weekly to maintain active growth.

Data were collected over the course of two weeks beginning approximately 2 wk after seeding and 1 wk following transplanting. Four pots from each population were randomly selected every 3 to 4 d and used for nondestructive and destructive measurements. Data collected from each pot included shoot height, leaf area, and shoot dry weight. A completely randomized design with 4 replications was used and the study was repeated. Population by test interactions were not detected, therefore all data were combined across tests. Non-linear regression analysis techniques similar to those employed by Chism et al. (1992) were used to generate growth curves and to compare growth in different smooth pigweed populations. Shoot dry weight and height data were regressed to fit the exponential function given here as:

$$y = a*e^{b*x} \qquad\qquad [1]$$

where y is the shoot dry weight in g or height in mm, x is DAP, a is a magnitude constant, and b is a rate constant. Leaf area data were regressed to fit the sigmoidal model:

$$y = a / (1 + e^{(-(x-c)/b)}) \qquad\qquad [2]$$

where y is leaf area in cm^2, x is DAP, a is a magnitude constant, b is a rate constant, and c is the y intercept. Because a general nonlinear model was used to generate growth curves for all populations; pairwise comparisons between regression coefficients could be made using techniques described by Chism et al. (1992) to establish significant differences between regression lines. Pseudo R^2 values were calculated to assess goodness of fit for individual regression equations. Relative growth rates (RGR's) were calculated for each population from data collected over the first 10-d of observations using the formula:

$$RGR = (\ln W_2 - \ln W_1) / (T_2 - T_1) \qquad\qquad [3]$$

where W_2 is shoot dry weight in g at the end of the observation period, W_1 is shoot dry weight in g at the beginning of the observation period, T_2 is time in d at the end of the observation period, and T_1 is time in d at the beginning of the observation period. Net assimilation rates (NAR's) were also calculated for all populations over the course of 10-

d during which a linear relationship between leaf area and shoot dry weight existed using the formula:

$$NAR = (W_2 - W_1)/ (T_2 - T_1) * (\ln L_{A2} - \ln L_{A1}) / (L_{A2} - L_{A1}) \qquad [4]$$

where W_2 is shoot dry weight in g at the end of the observation period, W_1 is shoot dry weight in g at the beginning of the observation period, T_2 is time in d at the end of the observation period, T_1 is time in d at the beginning of the observation period, L_{A2} is leaf area in cm^2 at the end of the observation period, and L_{A1} is leaf area in cm^2 at the beginning of the observation period. Means for RGR and NAR were separated using Fisher's LSD at the alpha = 0.05 level.

Chlorophyll Extraction and Quantification

Chlorophyll was extracted from shoots of 4- to 5-leaf smooth pigweed seedlings to quantify the chlorosis observed in some R smooth pigweed populations during early development compared to S. Chlorophyll was extracted using the method of Hiscox and Israelstram (1979). Excised shoots from S, R1, R2, R3, and R4 seedlings were weighed and soaked in 10-ml of dimethyl sulphoxide (DMSO) overnight to extract chlorophyll. A 1-ml aliquot of DMSO/chlorophyll solution was diluted 10-fold and absorbance was measured at 645- and 663-nm. Chlorophyll content was determined using the equation of Arnon (1949):

$$Chlorophyll (g/L) = (0.0202 A_{645} + 0.00802 A_{663}) \text{ x dilution factor} \qquad [5]$$

where A_{645} and A_{663} represent absorbance at 645- and 663-nm, respectively. Extinction coefficients for acetone were used for all calculations. Chlorophyll content in the leaf tissue was expressed as mg chlorophyll/g plant tissue and determined by dividing the calculated chlorophyll concentration in the sample by the weight of the pigweed shoot from which it was extracted.

Leaf Emergence and Elongation in the Greenhouse

Seedlings were established as previously described. Four evenly sized cotyledon-stage seedlings were transplanted into 11 by 11 cm pots filled with a soil mix containing

Profile[5] and topsoil in a 3:1 ratio. Plants were maintained in the greenhouse under natural sunlight supplemented with metal halide lamps to deliver a photoperiod of 14 hours d^{-1}. Plants were watered as needed and fertilized[4] weekly to maintain active growth. A completely random design with 3 replications was utilized and the study was repeated. Plant height, leaf number, and leaf length were determined on 2 d intervals for every plant in each of 3 pots representing S, R1, R2, R3, and R4 populations. The observation period began approximately 8 DAP and coincided with the emergence of the first true leaf on most plants. Observations spanned a period of 16 d ending 24 DAP. Measurements were taken only on leaves that were at least 5 mm long. Leaf emergence rates (LER), days to 5-leaf stage (DT5), and days to 8-leaf stage (DT8) were calculated for each population. LER and DT8 data means were combined across studies because no population by study interaction was detected (Pr>F = 0.0659 and 0.1564 for LER and DT8 data sets, respectively). Population by study interactions were detected for DT5 data (Pr>F = 0.0049); therefore data are presented independently for each study. LER, DT5, and DT8 data means were separated using Fisher's LSD at the alpha = 0.05 level. Regression analysis was used to compare the development of leaves 1 through 5 over time in S, R1, R2, R3 and R4 populations. Leaf length data were plotted against time and regressed to fit the sigmoidal curves described by equation 2. For leaf length data regressed to fit equation 2, y is leaf length in mm, x is DAP, c is the y intercept, and both a and b are constants. Differences in regression coefficients were established using methods described previously.

Competitive and Noncompetitive Growth in the Field

Seed from S, R1, R2, R3, and R4 smooth pigweed populations were planted and germinated as previously described. Evenly sized cotyledon-stage seedlings were transplanted into 5.7-cm round peat cups filled with potting soil and grown in the greenhouse under natural sunlight and sprinkler irrigation to approximately 7- to 10-cm tall before being transplanted into the field. For noncompetitive growth studies, individual plants from all populations were planted 1-m apart in the field using a completely random design. Three plants from each population were randomly selected

and harvested once weekly for 10 wk. Plant heights and dry weights were determined at each harvest interval and RGR's for each population were determined using equation 3 listed previously. Monoculture and mixed population plots were used to assess intra- and inter-population competition, respectively. All plots had 64 plants (8 x 8 plants) spaced approximately 6 cm apart creating plots with dimensions approximately 0.5 by 0.5 m ($0.25m^2$). Therefore, plots were representative of a 256 plants m^{-2} density. Monoculture plots were established for S, R1, R2, R3, and R4 smooth pigweed populations. Mixed plots were comprised of 32 plants from each of two populations placed in alternating fashion throughout the plots. Mixed treatments were S/R1, S/R2, S/R3, and S/R4. Competitive effects of R populations on each other were not assessed and alternating densities were not established. Shoot dry weights were determined following approximately 10 wk of growth in the field by harvesting entire plots and separating respective populations. Plots were arranged in a randomized complete block design with three replications. R to S biomass ratios were calculated for monoculture and mixed treatments. Noncompetitive and competitive growth studies were repeated in location. A population by location interaction was detected in noncompetitive growth studies. Therefore data are presented individually for each location. All populations behaved similarly over locations in competition studies and data were combined across locations. All means were separated using Fisher's LSD at the alpha = 0.05 level.

Results and Discussion

Growth Rate in the Greenhouse

In the greenhouse, shoot dry weight increased exponentially in all populations during the period of 14 to 28 DAP (Figure 1A). Dry weight data regressed to fit equation 1 resulted in R^2 values of 0.95, 0.91, 0.94, 0.94, and 0.88 for S, R1, R2, R3, and R4 data, respectively. Shoot dry weights were 0.06, 0.05, 0.04, 0.05, and 0.03 g for S, R1, R2, R3, and R4 populations, respectively at the beginning of the observation period (14 DAP). At 28 DAP, S had produced 17, 23, 25, and 44 percent more shoot biomass than R1, R2, R3, and R4 populations, respectively. Based on pairwise comparisons of regression

coefficients (Table 1), the S population had significantly more biomass during the course of the experiment compared to R2, R3, and R4 populations. However, R2 and R3 had significantly higher rates of biomass accumulation during this 14 d interval compared to S. Therefore, biomass production in R2 and R3 populations under noncompetitive conditions may approach levels similar to the S population given enough time. Both the level of biomass production and the rate of biomass production were similar for S and R1 populations. This supports visual observations made in the greenhouse.

Plant height also increased exponentially in all populations over the 14 d observation period and data regressed to fit equation 1 resulted in R^2 values of 0.78, 0.91, 0.85, 0.87, and 0.82 for S, R1, R2, R3, and R4 data, respectively (Figure 1B). Plant heights 14 DAP were 33, 30, 25, 26, and 25 mm for S, R1, R2, R3, and R4 populations, respectively. Based on pairwise comparisons of regression coefficients (Table 1), S plants were significantly taller than plants from R populations at least during most of the observation period. However, the rate at which plants increased in height was significantly greater in R populations during this time interval compared to S plants. In fact, S plants were taller than R plants 14, 17, 21, and 24 DAP. However, by 28 DAP R1 plants were actually taller than S plants. Therefore, plants from some R populations may eventually grow taller than S plants given enough time under noncompetitive conditions.

Leaf area data plotted against time resulted in sigmoidal-shaped growth curves for all populations (Figure 1C). Excellent fits ($R^2 > 0.86$) were observed for leaf area data from all populations fit to equation 2. Leaf area of S was significantly greater that R4 14 and 17 DAP, significantly greater than all R populations 21 DAP, and significantly greater than R2 and R4 populations 24 DAP (data not presented). However, by 28 DAP leaf areas in all populations were similar. Pairwise comparisons of regression coefficients revealed a significantly smaller predicted y intercept (coefficient c) for S compared to all R populations (Table 1). This reflects the higher rate of leaf area increase observed in the S population during the first 7 d of observations compared to all R populations.

RGR expresses dry weight accumulation over a specified time interval and takes into consideration the initial weight at the start of the time interval (Gardner et al. 1985). During the interval of 14 to 17 DAP, RGR's were 0.447, 0.350, 0.287, 0.357, and 0.368 for S, R1, R2, R3, and R4 populations, respectively (Table 2). R2 was the only population displaying a RGR significantly lower than S. During the interval of 14 to 24 DAP, R3 was the only population with a RGR less than S. It should also be noted that R4 had the highest RGR during this interval despite accumulating the least biomass of all populations over the course of the experiment. This is due in part to the fact that R4 was the smallest population at the beginning of the observation period. In fact R4 was 50% smaller than S 14 DAP. RGR's calculated 21 and 28 DAP were similar for all populations. Therefore, all populations may have performed equally as well if plant sizes had been equal 14 DAP. If S does possess a growth advantage over R populations then the advantage is likely to occur very early in the development of plants. That S had already established a size advantage over R populations during the interval of 0 to 14 DAP adds support to this idea and should be taken into consideration. Growth analyses from seedling emergence to the 1-leaf stage may be beneficial in establishing differences in growth that may exist between these populations very early in development.

NAR is the net gain of assimilate per unit of leaf area and time (Gardner et al. 1985). Therefore, NAR provides a limited estimate of photosynthetic efficiency by measuring how well a plant uses available leaf area to produce biomass. NARs calculated during the interval of 14 to 17 DAP were similar for all populations (Table 2). The NAR of the S population was significantly higher than R4 for the interval 14 to 21 DAP and significantly higher than R3 for the interval 14- to 24-DAP. Because NAR is a crude estimate of photosynthetic efficiency, it could be speculated that reduced growth in some R populations compared to S might be due to reductions in photosynthetic efficiency. One would not expect photosynthetic efficiency to play a significant role in growth differences that may exist between ALS inhibitor-S and –R weed biotypes unless the weed biotypes displayed multiple resistance to both ALS inhibitors and triazine herbicides. Conrad and Radosevich (1979) attributed reduced competitiveness in triazine-

resistant weed biotypes to photosynthetic inefficiency. Interestingly, all of our populations were controlled with atrazine in greenhouse trials (data not presented).

Chlorophyll Extraction and Quantification

Chlorophyll was extracted from shoots of 4- to 5-leaf smooth pigweed seedlings to quantify the chlorosis observed in some R smooth pigweed populations during early development compared to S. Chlorophyll concentrations per g of fresh weight were generally higher for S and R1 than for R2, R3, and R4 (Figure 2). Both R3 and R4 had concentrations significantly lower than S based on Fisher's LSD test conducted at the alpha = 0.05 level. Therefore, the lower NAR's displayed by R3 and R4 compared to S at 24 and 21 DAP, respectively may be partially explained by lower chlorophyll concentrations.

Leaf Emergence and Elongation in the Greenhouse

Separate greenhouse studies were conducted to investigate leaf emergence and leaf expansion in S, R1, R2, R3, and R4 smooth pigweed populations. Days required to produce five true leaves were statistically similar for all populations in one repetition of the study, but S and R1 required a significantly shorter interval than R2, R3, and R4 to reach the 5-leaf stage in the second repetition (Table 3). S and R1 also required the shortest interval to produce eight true leaves with R2 and R3 requiring significantly longer to reach the same developmental stage compared to both S and R1. LER's were 0.39, 0.38, 0.35, 0.36, and 0.36 leaves d^{-1} for S, R1, R2, R3, and R4 populations, respectively with LER's for R2, R3, and R4 being significantly lower than S.

Development of the first five true leaves was also monitored for each population during the period of 10 to 24 DAP. Leaf length was plotted against time and growth curves were regressed to fit equation 2 (Figure 3). The first true leaf of S was 11, 20, 24, and 25 percent longer than R1, R2, R3, and R4 populations at the beginning of the observation period (10 DAP) (Figure 3a). Pairwise comparison of regression coefficients

revealed significant differences in predicted *y* intercepts (regression coefficient c) for all populations reflecting the differences in leaf size that existed at the onset of the observations period (Table 4). Predicted *y* intercepts of all populations were significantly different for all populations with leaf 3 (Figure 3c), leaf 4 (Figure 3d), and leaf 5 (Figure 3e). With leaf 2 (Figure 3b), predicted *y* intercepts for S and R1 were similar reflecting the similar growth habits of S and R1 that have been detected in several studies. Early size advantages for both S and R1 populations were very apparent with leaf 5 (Figure 3e). Throughout the course of the experiment, leaves of all populations tended to expand for approximately 10 to 12 d following emergence before reaching their maximum length (Figure 3). R2 was the only population that tended to deviate from this pattern. Regression coefficient a associated with leaf 2 (Figure 3b), leaf 3 (Figure 3c) and leaf 4 (Figure 3d) for the R2 population were significantly different than S reflecting the fact that R2 leaves were still expanding at the end of each observation period while the leaves of other populations had already reached their maximum lengths. This may partially explain the fact that R2 had the smallest LER of all populations (Table3). It would appear that R2 expends energy expanding existing leaves while other populations are producing new leaves. This may reflect a competitive advantage of other populations over R2 keeping in mind that these tests were conducted under noncompetitive conditions.

Competitive and Noncompetitive Growth in the Field

Field studies using larger plants (5 to 7 cm tall) were conducted as a follow-up to greenhouse studies to compare growth of S, R1, R2, R3, and R4 populations under noncompetitive and competitive conditions. At location one in the field, R1 and R4 generally displayed the fastest and slowest rates of growth, respectively (Table 5) with the RGR of R1 being significantly larger than R4 at 2, 4, 5, 6, and 9 WAP. RGR in R2, R3, and R4 were generally similar to S except at 4 and 9 WAP. At location 2, S generally displayed the fastest rate of growth and R1 grew the slowest with R1 RGR being significantly smaller than S at 2, 3, 6, 7, and 9 WAP. These data conflict directly with the findings at location 1 and may suggest that location played a significant role in the development of individual populations. Based on these findings, it is unlikely that

consistent growth rate differences exist between S, R1, R2, R3, and R4 smooth pigweed populations in the field under noncompetitive conditions using plants larger than those used in earlier greenhouse studies. Under competitive conditions in the field, plants grown in monoculture averaged 7.3, 8.9, 7.0, 7.8, and 8.8 g plant^{-1} for S, R1, R2, R3, and R4 populations, respectively (Figure 4a). Biomass per plant in the S population was similar to all R populations. However, biomass production in R1 was significantly greater per plant than with R2. Ratios of R/S biomass under interpopulation competition were 1.12, 1.0, 1.23, and 0.82 for R1/S, R2/S, R3/S, and R4/S mixtures, respectively (Figure 4b). With all mixtures, however, no significant differences in biomass between S and R populations was detected suggesting little if any competitive advantage of S over R populations when tests are initiated using 5 to 7 cm tall plants. In the greenhouse under noncompetitive conditions, growth differences between populations were generally detected very early in the development of seedlings. Therefore, future studies investigating potential growth and competition differences between S and R populations should likely be conducted using seedlings smaller than those used in our studies.

Concluding Remarks

Under noncompetitive conditions in the greenhouse, the S population produced significantly more biomass, displayed a faster rate of leaf emergence and expansion, and had a significantly higher RGR and NAR compared to some R populations. Differences tended to be more apparent during very early stages of development. Differences in RGR were not as apparent in the field under noncompetitive conditions when studies were conducted using plants much larger than those used in the greenhouse. These findings are similar to those with sulfonylurea-resistant prickly lettuce where reductions in biomass production compared to the wild type were observed under noncompetitive conditions but not under competitive conditions (Alcocer-Ruthling et al. 1992). Increased growth of S in the greenhouse may or may not confer a fitness

advantage over R populations. Growth of the S population in the field under competitive conditions revealed little if any differences between S and R populations. Gressel and Segel (1982) remind us that many factors like: (a) the proportion of seeds germinating at a given time, (b) the rate of germination, (c) success in establishment following thinning, (d) any physiological character resulting in differences in growth rate, (e) Parkinsonian plasticity, and (f) seed size and yield per flower and per plant collectively determine whether a wild-type population is more fit than a selected population. We have concluded that differences in growth rate during early stages of development exist between the S population and some R populations. However, further studies investigating seed production and germination characteristics as well as competition studies using very small seedlings are needed before estimates of relative fitness between S and R populations can be established. Studies to address possible differences in photosynthetic rates that may exist between S and R populations should also be conducted.

Sources of Materials

[1] Sutton universal greenhouse flat. Inside dimensions 51 cm x 40 cm x 5.7 cm. Wetzel, Inc., 1345 Diamond Springs Road, Virginia Beach, VA 23455.

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

[3] Heat-A-Matic propagation mats. E. C. Geiger, Inc., 1104 W. Roslyn Rd., Colonial Heights, VA 23834.

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

[5] Profile™, a granular porous ceramic for use as a soil modifier. Applied Materials Corp., Buffalo Grove, IL 60089.

Acknowledgments

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TABLE 1. Pairwise comparisons of nonlinear regression coefficients for growth curves established using shoot dry weight, shoot height, and leaf area data collected in the greenhouse 14 to 28 d after planting from one-imidazolinone-susceptible (S) and four –resistant (R1, R2, R3, and R4) smooth pigweed populations.[a]

| | | Regression Coefficients | | | | | | |
| | | Shoot Dry Weight[b] | | Shoot Height[b] | | Leaf Area[c] | | |
Population		a	b	a	b	a	b	c
S vs	R1	ns	ns	*	*	ns	ns	*
	R2	*	*	*	*	ns	ns	*
	R3	*	*	*	*	ns	ns	*
	R4	*	ns	*	*	ns	ns	*
R1 vs	R2	ns	ns	ns	ns	*	ns	*
	R3	ns	ns	ns	ns	ns	ns	*
	R4	ns	ns	ns	ns	ns	ns	*
R2 vs	R3	ns	ns	ns	ns	ns	ns	ns
	R4	ns	ns	ns	ns	ns	ns	*
R3 vs	R4	ns	ns	ns	ns	ns	ns	ns

[a] Comparisons between populations followed by an * indicate significant differences as determined by comparing the 95% confidence intervals of the difference between two regression coefficients.

[b] General equation: $y = a*e^{b*x}$ where y is the shoot dry weight in g or height in mm, x is DAP, a is a magnitude constant, and b is a rate constant.

[c] General equation: $y = a / (1 + e^{(-(x-c)/b)})$ where y is leaf area in cm^2, x is DAP, a is a magnitude constant, b is a rate constant, and c is the y intercept.

TABLE 2. Relative growth rates (RGR) and net assimilation rates (NAR) for one imidazolinone-susceptible (S) and four –resistant (R1, R2, R3, and R4) smooth pigweed populations as determined by shoot dry weight accumulation under noncompetitive conditions over time in the greenhouse.[a]

Population	RGR[b]				NAR[c]		
	17 DAP	21 DAP	24 DAP	28 DAP	17 DAP	21 DAP	24 DAP
	$g\,g^{-1}\,d^{-1}$				$g\,cm^{2-1}\,d^{-1}$		
S	0.447	0.367	0.328	0.284	0.0011	0.0010	0.0011
R1	0.350	0.323	0.315	0.286	0.0009	0.0009	0.0010
R2	0.287	0.324	0.300	0.292	0.0008	0.0009	0.0009
R3	0.357	0.313	0.288	0.281	0.0010	0.0009	0.0008
R4	0.368	0.312	0.334	0.293	0.0009	0.0008	0.0010
LSD (0.05)	0.150	ns	0.032	ns	ns	0.0002	0.0002

[a] Means represent the averages of values taken from a total of 24 plants and were separated using Fisher's LSD test at the alpha = 0.05 level.

[b] RGR = $(\ln W_2 - \ln W_1) / (T_2 - T_1)$ where W_2 is shoot dry weight in g at the end of the observation period, W_1 is shoot dry weight in g at the beginning of the observation period, T_2 is time in d at the end of the observation period, and T_1 is time in d at the beginning of the observation period. T_1 was 14 DAP for all RGR calculations.

[c] NAR = $(W_2 - W_1)/ (T_2 - T_1) * (\ln L_{A2} - \ln L_{A1}) / (L_{A2} - L_{A1})$ where W_2 is shoot dry weight in g at the end of the observation period, W_1 is shoot dry weight in g at the beginning of the observation period, T_2 is time in d at the end of the observation period, T_1 is time in d at the beginning of the observation period, L_{A2} is leaf area in cm^2 at the end of the observation period, and L_{A1} is leaf area in cm^2 at the beginning of the observation period. NAR was calculated for all populations over the course of 10 d (14 to 24 DAP) during which a linear relationship between leaf area and shoot dry weight existed using the formula. T_1 was 14 DAP for all NAR calculations.

Table 3. Days to five-leaf stage (DT5), days to eight-leaf stage (DT8), and leaf emergence rates (LER) for one imidazolinone-susceptible (S) and four –resistant (R1, R2, R3, and R4) smooth pigweed populations under noncompetitive conditions in the greenhouse.[a]

Population	DT5[b]		DT8[c]	LER[c, d]
	Test 1	Test 2		
	———————— d ————————			–leaves d^{-1}–
S	8.8	7.0	20.2	0.39
R1	8.8	6.5	20.3	0.38
R2	9.2	8.0	21.4	0.35
R3	9.5	7.7	21.3	0.36
R4	8.8	8.2	20.9	0.36
LSD (0.05)	ns	0.6	0.8	0.02

[a] All means were separated using Fisher's LSD test at the alpha = 0.05 level.

[b] Means represent the average of measurements taken on 12 individual plants from each population.

[c] Means represent the average of measurements taken on 24 individual plants from each population.

[d] LER was determined 24 d after planting by dividing the number of leaves present by 24.

TABLE 4. Pairwise comparisons of nonlinear regression coefficients associated with growth curves of leaves one, two, three, four, and five from one imidazolinone-susceptible (S) and four –resistant (R1, R2, R3, and R4) smooth pigweed populations.[a, b]

Population		Leaf 1			Leaf 2			Leaf 3			Leaf 4			Leaf 5		
		a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
S vs	R1	ns	ns	*	ns	ns	ns	ns	ns	*	ns	ns	*	ns	ns	*
	R2	ns	ns	*	*	ns	*	*	ns	*	*	ns	*	ns	ns	*
	R3	ns	ns	*	ns	ns	*	ns	ns	*	ns	ns	*	ns	ns	*
	R4	ns	ns	*	ns	ns	*	ns	ns	*	ns	ns	*	ns	ns	*
R1 vs	R2	ns	ns	*	*	ns	*	*	ns	*	*	ns	*	ns	ns	*
	R3	ns	ns	*	*	ns	*	ns	ns	*	ns	ns	*	ns	ns	*
	R4	ns	ns	*	*	ns	*	ns	ns	*	ns	ns	*	ns	ns	*
R2 vs	R3	ns	ns	*	ns	ns	*	ns	ns	*	*	ns	*	ns	ns	ns
	R4	ns	ns	*	ns	ns	*	ns	ns	*	*	ns	*	ns	ns	ns
R3 vs	R4	ns	ns	*	ns	ns	*	ns	ns	*	ns	ns	ns	ns	ns	ns

[a] General equation: $y = a / (1 + e^{(-(x-c)/b)})$.

[b] Comparisons between populations followed by an * indicate significant differences as determined by comparing the 95% confidence intervals of the difference between two regression coefficients.
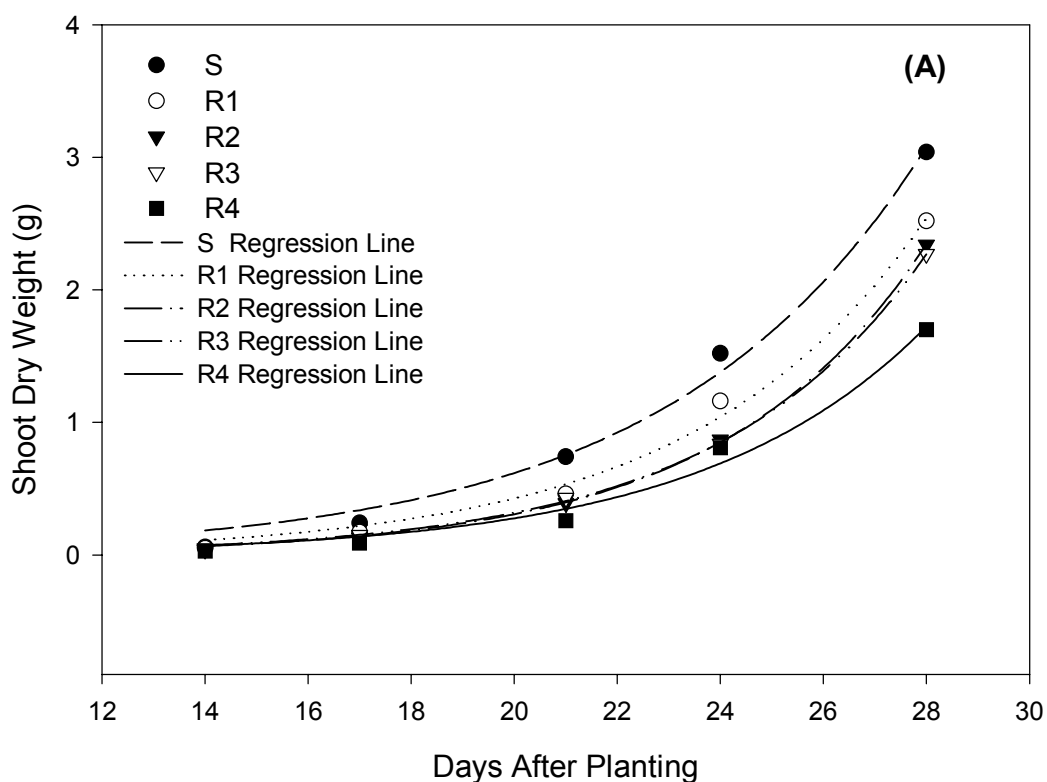
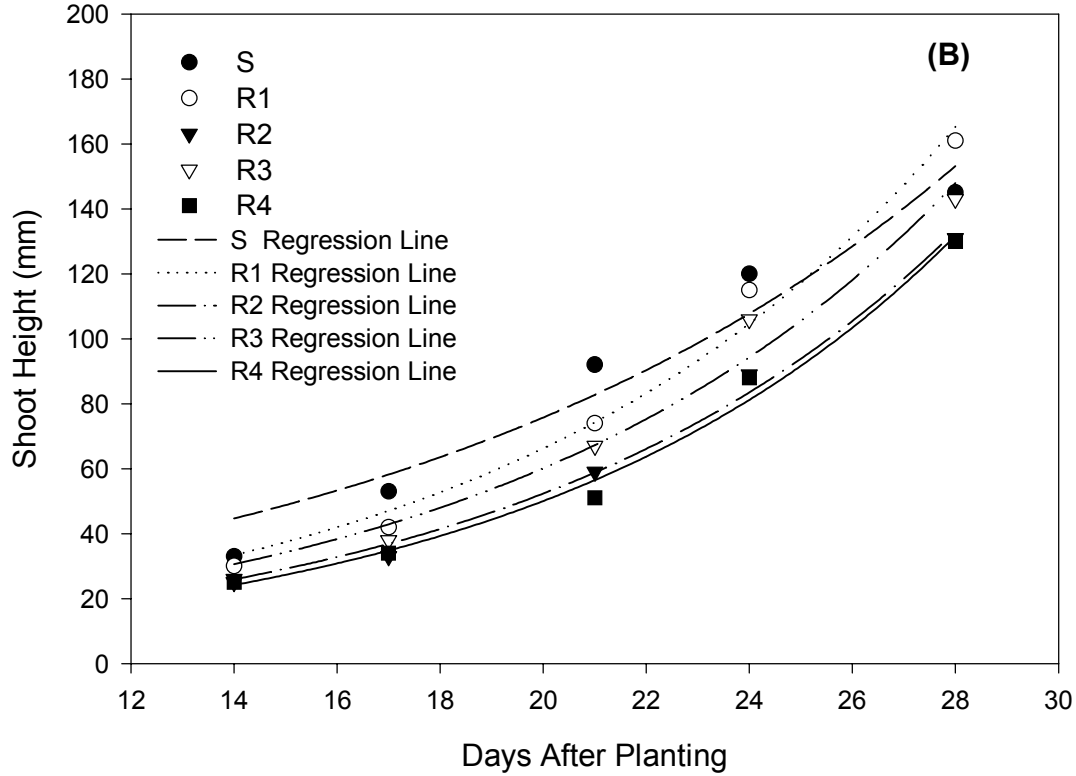FIGURE 1A. Shoot dry weight over time in the greenhouse 14 to 28 d after planting for one imidazolinone-susceptible (S) and four –resistant (R1, R2, R3, and R4) smooth pigweed populations. Shoot dry weight data were regressed to fit the exponential model y = $a*e^{b*x}$ where y is the shoot dry weight in g or plant height in mm, x is d after planting, a is a magnitude constant, and b is a rate constant. Regression equations for dry weight data are $y = 0.0113*e^{0.2003*x}$, $R^2 = 0.95$, $y = 0.0048*e^{0.2238*x}$, $R^2 = 0.91$, $y = 0.0020*e^{0.2530*x}$, R^2 = 0.94, $y = 0.0023*e^{0.2465*x}$, $R^2 = 0.94$, and $y = 0.0028*e^{0.2295*x}$, $R^2 = 0.88$, for S, R1, R2, R3, and R4 populations, respectively.

FIGURE 1B. Plant height over time in the greenhouse 14 to 28 d after planting for one imidazolinone-susceptible (S) and four –resistant (R1, R2, R3, and R4) smooth pigweed populations. Plant height data were regressed to fit the exponential model $y = a*e^{b*x}$ where y is the shoot dry weight in g or plant height in mm, x is d after planting, a is a magnitude constant, and b is a rate constant. Regression equations for plant height data are $y = 13.03*e^{0.088*x}$, $R^2 = 0.78$, $y = 6.77*e^{0.114*x}$, $R^2 = 0.91$, $y = 4.93*e^{0.118*x}$, $R^2 = 0.85$, $y = 6.29*e^{0.113*x}$, $R^2 = 0.87$, and $y = 4.52*e^{0.120*x}$, $R^2 = 0.82$, for S, R1, R2, R3, and R4 populations, respectively.
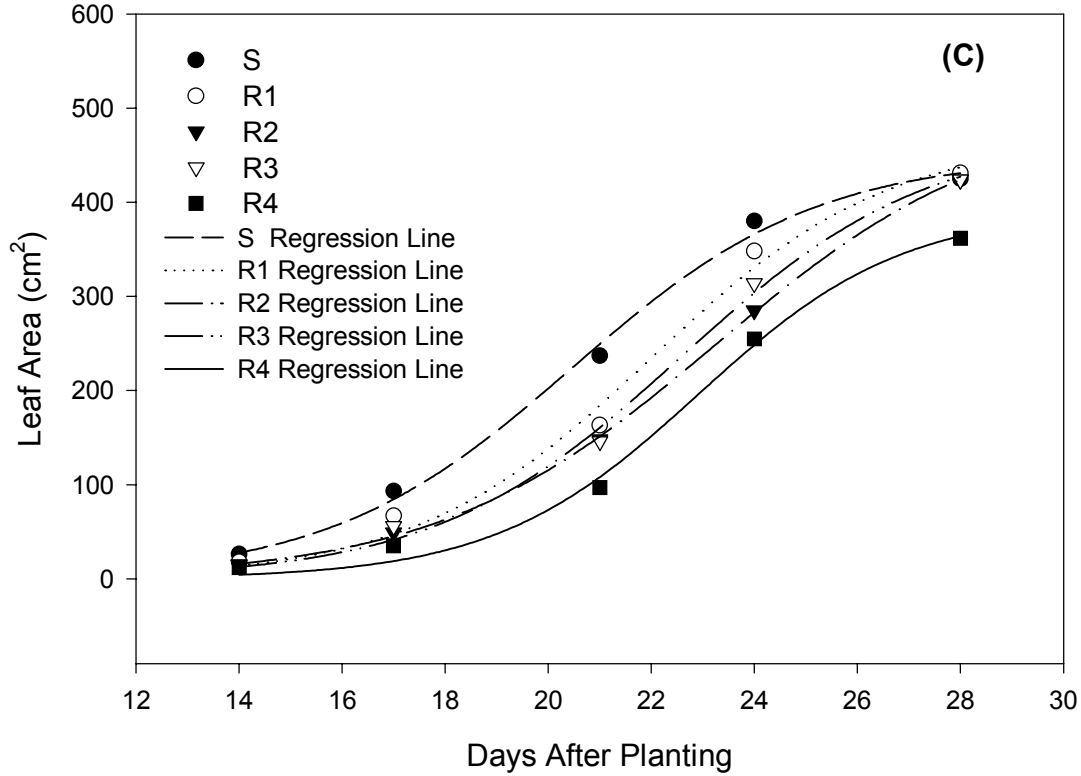
FIGURE 1C. Leaf area over time in the greenhouse 14 to 28 d after planting for one imidazolinone-susceptible (S) and four –resistant (R1, R2, R3, and R4) smooth pigweed populations. Leaf area data were regressed to fit the sigmoidal model $y = a / (1 + e^{(-(x-c)/b)})$ where y is leaf area in cm^2, x is d after planting, a is a magnitude constant, b is a rate constant, and c is the y intercept. Regression equations for leaf area data are $y = 449 / (1 + e^{(-(x-20.5)/2.38)})$, $R^2 = 0.96$, $y = 469 / (1 + e^{(-(x-22.0)/2.28)})$, $R^2 = 0.95$, $y = 500 / (1 + e^{(-(x-23.3)/2.73)})$, $R^2 = 0.93$, $y = 471 / (1 + e^{(-(x-22.6)/2.39)})$, $R^2 = 0.94$, and $y = 392 / (1 + e^{(-(x-22.9)/1.97)})$, $R^2 = 0.87$, for S, R1, R2, R3, and R4 populations, respectively.
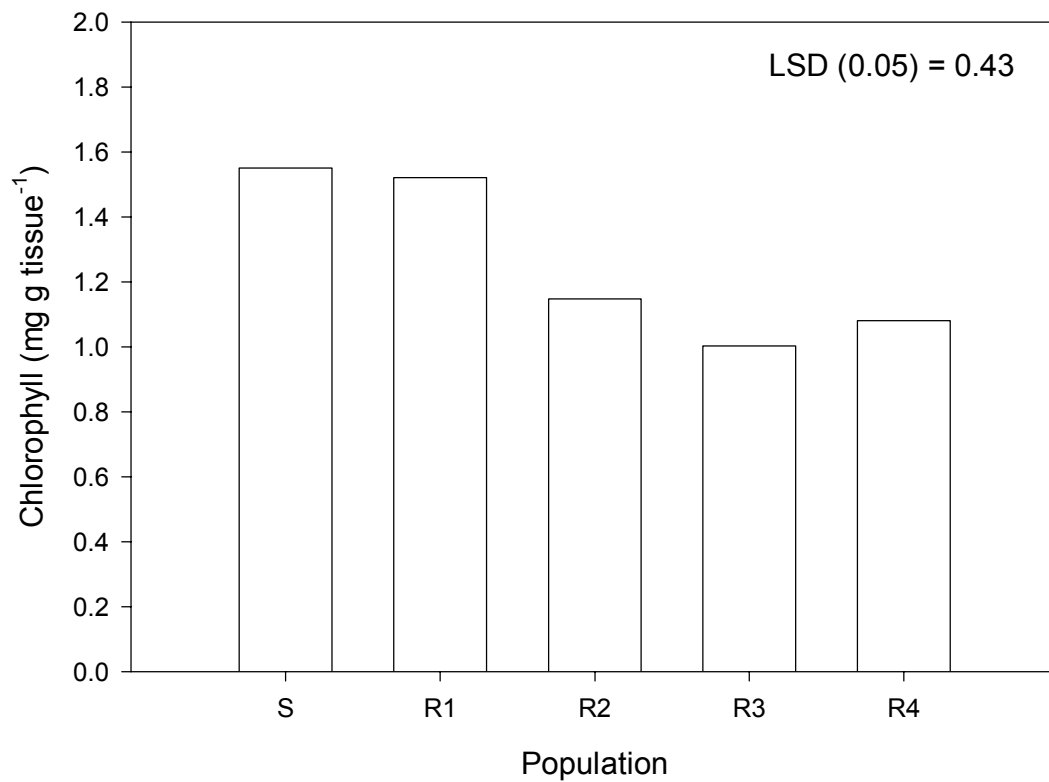
FIGURE 2. Chlorophyll concentrations in one imidazolinone-susceptible (S) and four – resistant smooth pigweed populations determined by extracting chlorophyll from the shoots of 4- to 5-leaf seedlings.
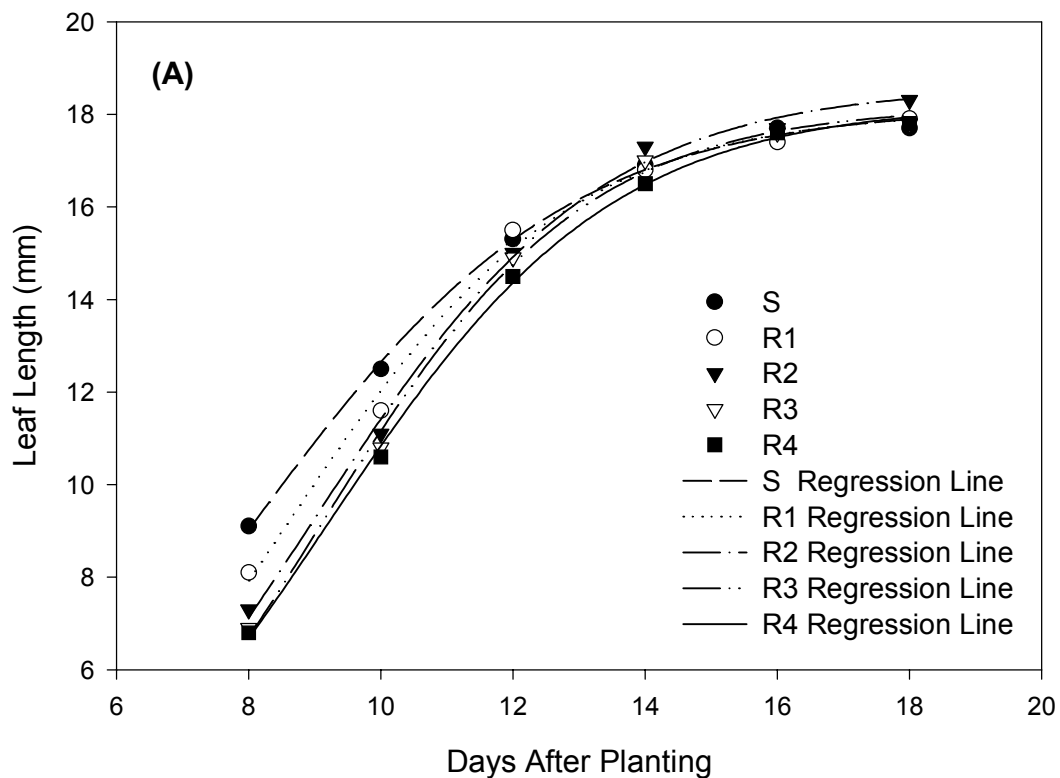
FIGURE 3A. Elongation of leaf number one in imidazolinone-susceptible (S) and –
resistant (R1, R2, R3, and R4) smooth pigweed populations over time in the greenhouse.
Leaf length data were plotted against time and regressed to fit the sigmoidal equation: $y =$
$a / (1 + e^{(-(x-c)/b)})$ where y is leaf length in mm, x is d after planting, c is the y intercept,
and both a and b are constants. Regression equations are $y = 18.09 / (1 + e^{(-(x-8.02)/2.34)})$,
$R^2 = 0.77$, $y = 18.12 / (1 + e^{(-(x-8.55)/2.16)})$, $R^2 = 0.84$, $y = 18.58 / (1 + e^{(-(x-9.03)/2.12)})$, $R^2 =$
0.87, $y = 18.15 / (1 + e^{(-(x-9.07)/1.98)})$, $R^2 = 0.86$, and $y = 18.20 / (1 + e^{(-(x-9.17)/2.13)})$, $R^2 =$
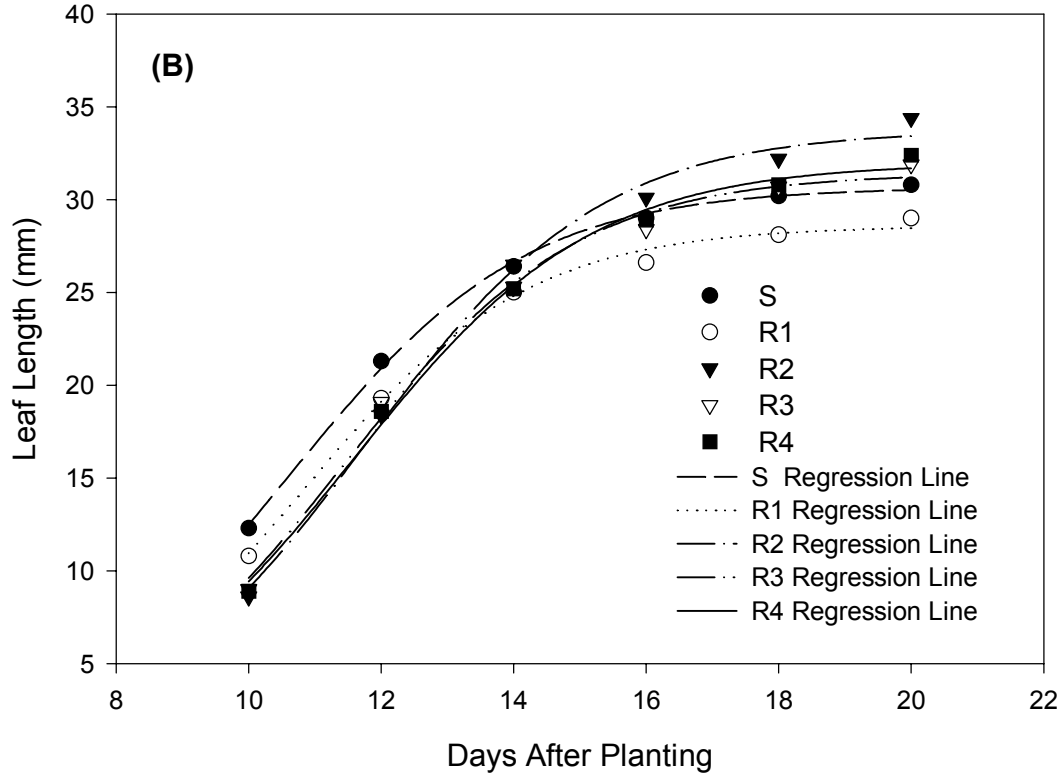0.80 for S, R1, R2, R3, and R4 populations, respectively.

FIGURE 3B. Elongation of leaf number two in imidazolinone-susceptible (S) and –
resistant (R1, R2, R3, and R4) smooth pigweed populations over time in the greenhouse.
Leaf length data were plotted against time and regressed to fit the sigmoidal equation: $y =$
$a / (1 + e^{(-(x-c)/b)})$ where y is leaf length in mm, x is d after planting, c is the y intercept,
and both a and b are constants. Regression equations are $y = 30.64 / (1 + e^{(-(x-10.66)/1.74)})$,
$R^2 = 0.84$, $y = 28.58 / (1 + e^{(-(x-10.81)/1.69)})$, $R^2 = 0.80$, $y = 33.74 / (1 + e^{(-(x-11.77)/1.76)})$, $R^2 =$
0.84, $y = 31.41 / (1 + e^{(-(x-11.43)/1.75)})$, $R^2 = 0.84$, and $y = 31.96 / (1 + e^{(-(x-11.57)/1.81)})$, $R^2 =$
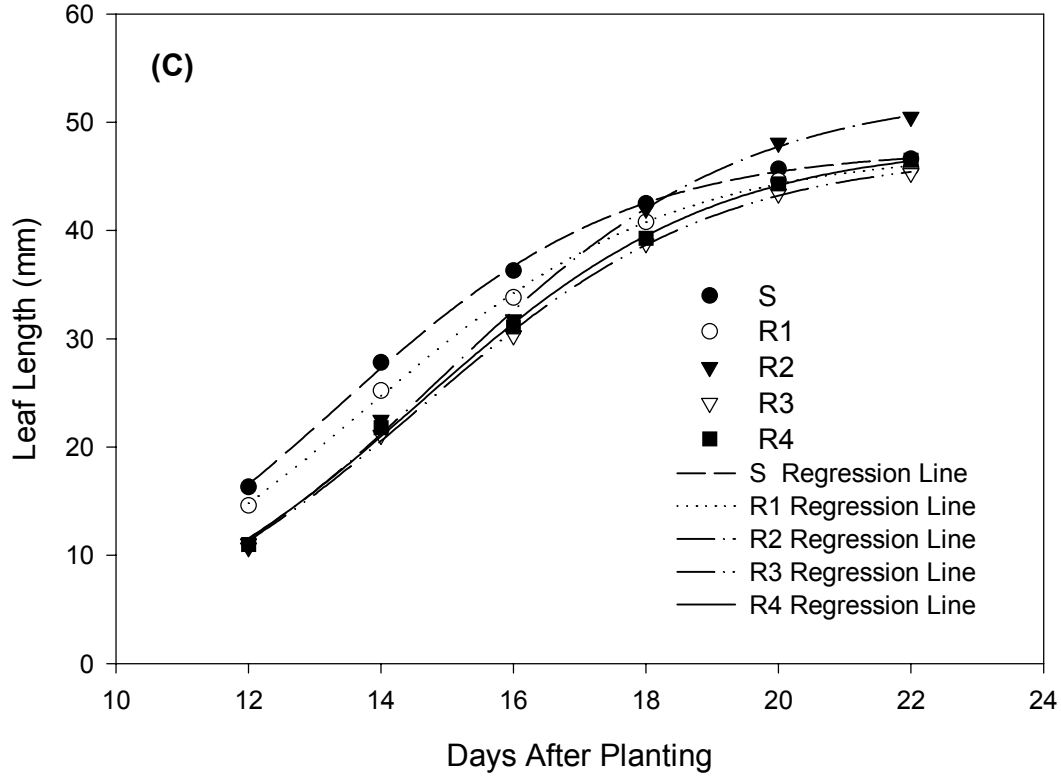0.90 for S, R1, R2, R3, and R4 populations, respectively.

FIGURE 3C. Elongation of leaf number three in imidazolinone-susceptible (S) and –
resistant (R1, R2, R3, and R4) smooth pigweed populations over time in the greenhouse.
Leaf length data were plotted against time and regressed to fit the sigmoidal equation: $y =$
$a / (1 + e^{(-(x-c)/b)})$ where y is leaf length in mm, x is d after planting, c is the y intercept,
and both a and b are constants. Regression equations are $y = 47.53 / (1 + e^{(-(x-13.35)/2.17)})$,
$R^2 = 0.90$, $y = 47.22 / (1 + e^{(-(x-13.78)/2.29)})$, $R^2 = 0.89$, $y = 52.86 / (1 + e^{(-(x-14.92)/2.28)})$, $R^2 =$
0.94, $y = 47.02 / (1 + e^{(-(x-14.56)/2.24)})$, $R^2 = 0.89$, and $y = 48.15 / (1 + e^{(-(x-14.58)/2.24)})$, $R^2 =$
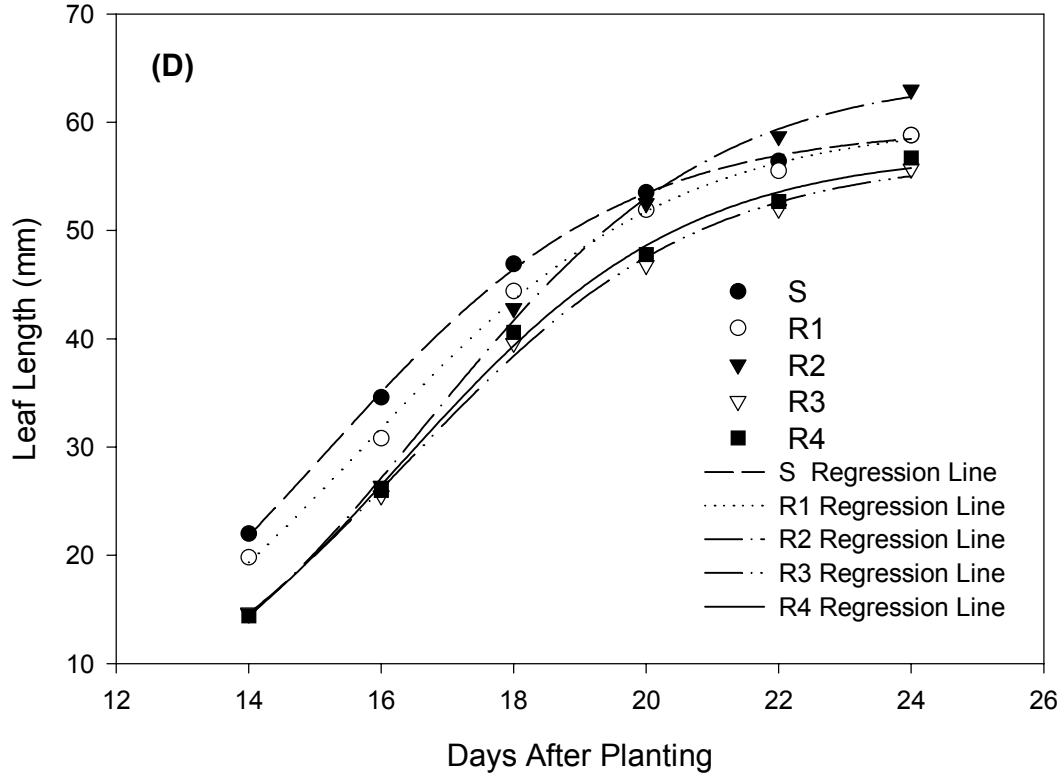0.90 for S, R1, R2, R3, and R4 populations, respectively.

FIGURE 3D. Elongation of leaf number four in imidazolinone-susceptible (S) and –
resistant (R1, R2, R3, and R4) smooth pigweed populations over time in the greenhouse.
Leaf length data were plotted against time and regressed to fit the sigmoidal equation: $y =$
$a / (1 + e^{(-(x-c)/b)})$ where y is leaf length in mm, x is d after planting, c is the y intercept,
and both a and b are constants. Regression equations are $y = 59.57 / (1 + e^{(-(x-15.21)/2.21)})$,
$R^2 = 0.88$, $y = 60.12 / (1 + e^{(-(x-15.74)/2.34)})$, $R^2 = 0.85$, $y = 64.45 / (1 + e^{(-(x-16.69)/2.16)})$, $R^2 =$
0.92, $y = 56.79 / (1 + e^{(-(x-16.36)/2.22)})$, $R^2 = 0.85$, and $y = 57.32 / (1 + e^{(-(x-16.32)/2.14)})$, $R^2 =$
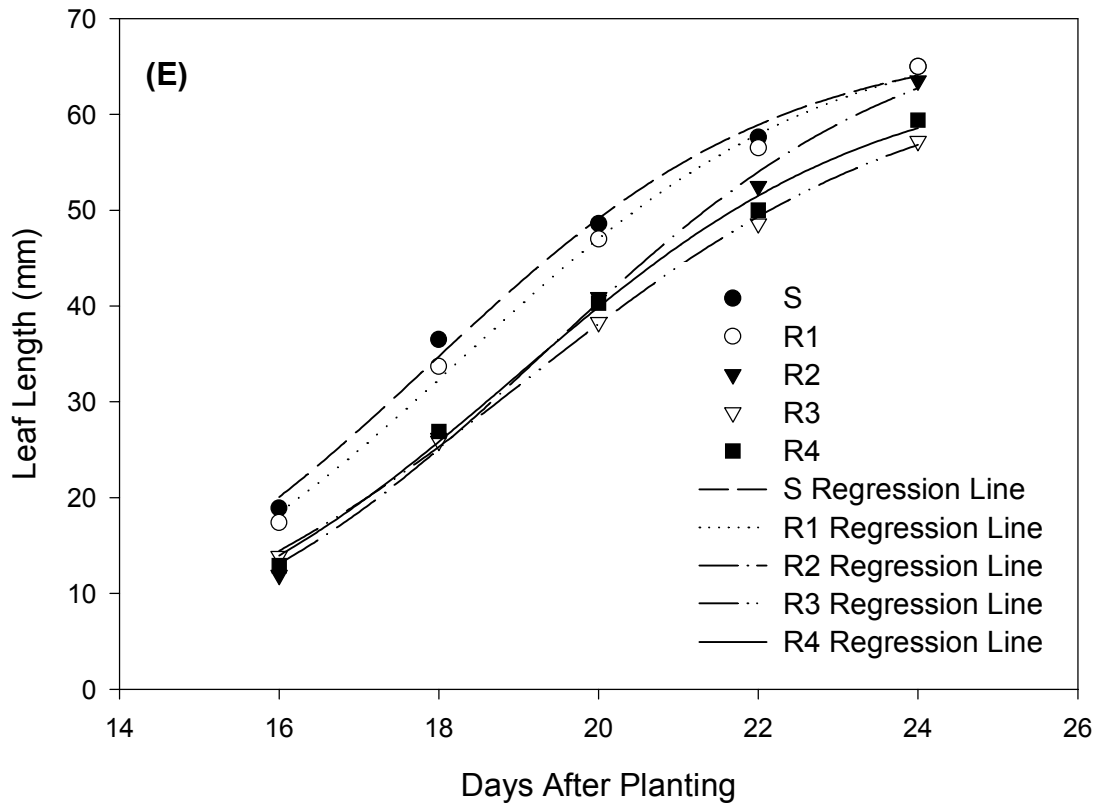0.85 for S, R1, R2, R3, and R4 populations, respectively.

FIGURE 3E. Elongation of leaf number five in imidazolinone-susceptible (S) and −
resistant (R1, R2, R3, and R4) smooth pigweed populations over time in the greenhouse.
Leaf length data were plotted against time and regressed to fit the sigmoidal equation: $y =$
$a / (1 + e^{(-(x-c)/b)})$ where y is leaf length in mm, x is d after planting, c is the y intercept,
and both a and b are constants. Regression equations are $y = 67.79 / (1 + e^{(-(x-17.89)/2.18)})$,
$R^2 = 0.86$, $y = 69.32 / (1 + e^{(-(x-18.30)/2.27)})$, $R^2 = 0.82$, $y = 70.73 / (1 + e^{(-(x-19.35)/2.26)})$, $R^2 =$
0.89, $y = 64.82 / (1 + e^{(-(x-19.11)/2.49)})$, $R^2 = 0.74$, and $y = 64.94 / (1 + e^{(-(x-18.94)/2.28)})$, $R^2 =$
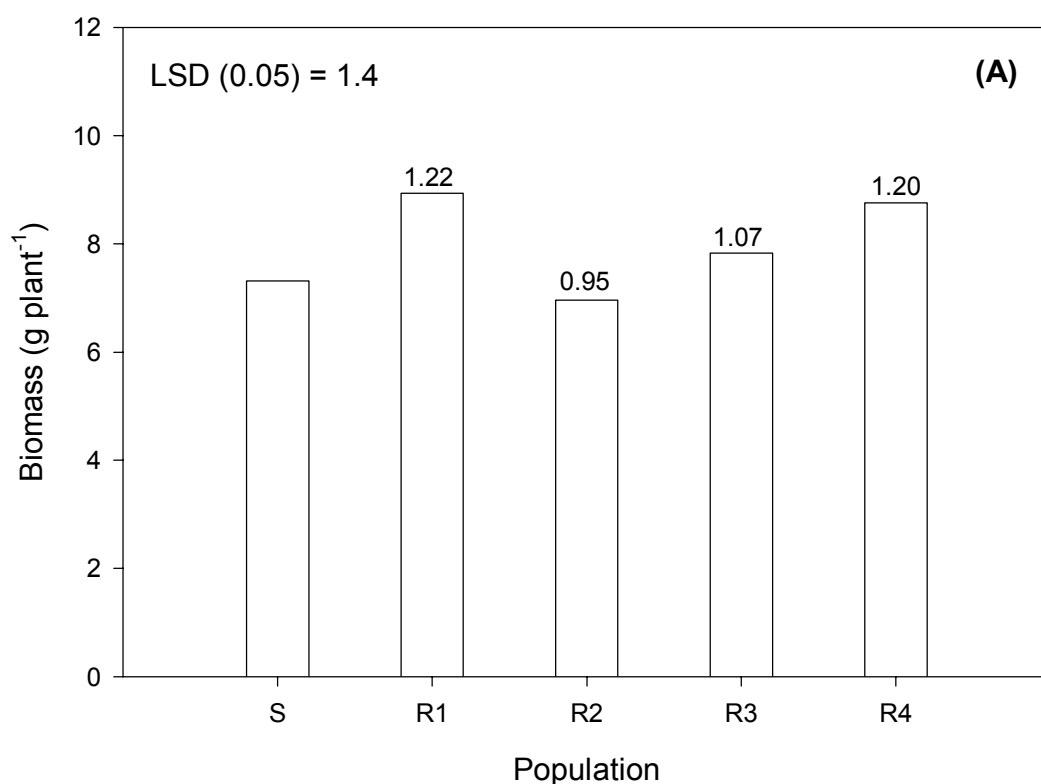0.76 for S, R1, R2, R3, and R4 populations, respectively.

FIGURE 4A. Mean shoot biomass production for imidazolinone-susceptible (S) and –
resistant (R1, R2, R3, and R4) smooth pigweed populations as influenced by
intrapopulation competition. Monoculture plots were established using 64 plants from
individual populations. All plants were spaced approximately 6 cm apart in a 0.5 by 0.5
m plot representing a final density of 256 plants m^{-2} for all plots. The numbers above the
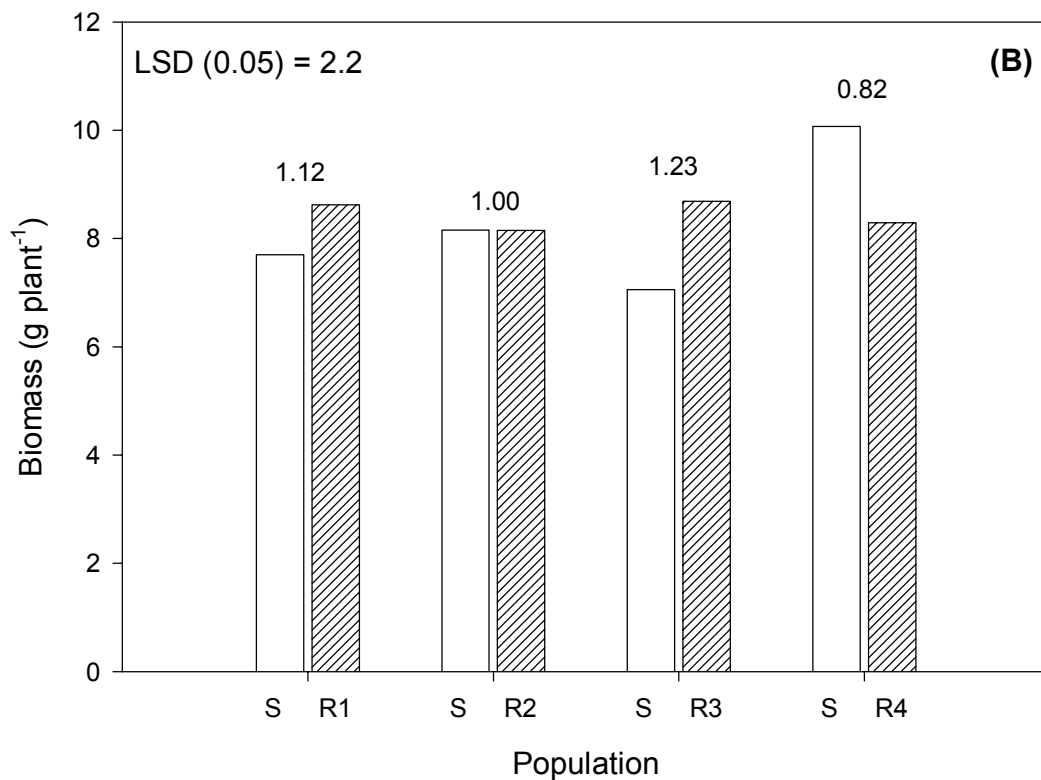bars are the ratios of resistant to susceptible biomass.

FIGURE 4B. Mean shoot biomass production for imidazolinone-susceptible (S) and –
resistant (R1, R2, R3, and R4) smooth pigweed populations as influenced by
interpopulation competition. Mixed plots contained 32 plants from each of two
populations planted in an alternating fashion. All plants were spaced approximately 6 cm
apart in a 0.5 by 0.5 m plot representing a final density of 256 plants m^{-2} for all plots. The
numbers above the bars are the ratios of resistant to susceptible biomass.

Chapter VI
Summary and Conclusions

Seed were collected from four different smooth pigweed populations (R1, R2, R3, and R4) in Worcester County, MD where repeated imazaquin use had selected for smooth pigweed that could no longer be controlled by imidazolinone herbicides. Seed were also collected from smooth pigweed plants near Painter, VA that were known to be imidazolinone-susceptible. Using field and greenhouse studies, imidazolinone resistance was confirmed in all R populations. Smooth pigweed control with chlorimuron, thifensulfuron, and pyrithiobac applied at commercial rates in the greenhouse was similar in all populations indicating no practical level of cross-resistance to sulfonylurea and pyrimidinylthiobenzoate herbicides. However, control differences between S and R populations were observed in the greenhouse using rates lower than commercially recommended. R populations were generally more tolerant to chlorimuron and more sensitive to thifensulfuron and pyrithiobac in the greenhouse. R populations, therefore, displayed low-level cross-resistance to chlorimuron and negative cross-resistance to thifensulfuron and pyrithiobac. At the enzyme level, R populations were generally more sensitive to chlorimuron. These data conflicted directly with the findings at the whole-plant level. Consequently, low-level cross-resistance to chlorimuron was not explained by target site sensitivity differences. Negative cross-resistance to thifensulfuron in the R3 population and to pyrithiobac in R1, R2, and R3 populations may be due to increased target site sensitivity.

R populations were generally more sensitive to cloransulam-methyl in the greenhouse. Unlike with thifensulfuron and pyrithiobac, significant control differences were observed at the commercially recommended use rate. R2 was particularly sensitive to cloransulam-methyl compared to S. Increased sensitivity to cloransulam-methyl in the R2 population could not be attributed to differences in absorption, translocation, and metabolism of cloransulam-methyl between S and R2 populations. Interestingly, R2 ALS was 25-fold more sensitive to cloransulam-methyl compared to S ALS. Therefore, differences in target site sensitivity between S and R2 populations may partially explain the whole-plant responses observed in the greenhouse.

Slower growth and development was observed in some R populations compared to S indicating that a significant fitness penalty may be associated with imidazolinone resistance in some populations. During the interval of 14 to 28 d after planting, S produced more biomass, was initially taller, and generally had more leaf area than R2, R3, and R4 populations. In contrast, S and R1 often grew similarly. Leaves generally emerged faster in the S population compared to R2, R3, and R4 populations. Leaf emergence rates were similar in S and R populations, however. Relative growth rates of 5- to 7-leaf seedlings in the field under noncompetitive conditions were often similar in S and R populations. This suggests that growth differences between S and some R populations may only occur very early in development. Growth under competitive conditions was also similar in S and R populations. Therefore, growth advantages in the S population under noncompetitive conditions may not correlate into a competitive advantage in the field.

In the greenhouse, R populations often became chlorotic during early development. This chlorosis was quantified by extracting chlorophyll from the shoots of young seedlings. R3 and R4 had significantly lower chlorophyll concentrations per gram of tissue compared to S. Net assimilation rates for S were also significantly higher than R4 21 d after planting and significantly higher than R2 and R3 24 d after planting. Therefore, chlorosis and possible photosynthetic inefficiency during the early stages of development in some R populations may be linked to growth reductions.

VITA

Daniel H. Poston was born the second of three sons to Mr. and Mrs. R. J. Poston, Jr. on November 30,1966 in Marion County, South Carolina. He grew up on a small row crop farm in Gresham, SC and graduated from Britton's Neck High School in 1985. He received a Bachelor of Science degreed in Agricultural Education and a Master of Science degree in Agronomy from Clemson University in 1989 and 1991, respectively. Daniel accepted a position as an Agricultural Education instructor at Marion High School in Marion, SC in the summer of 1991 and spent four years building a successful Agricultural Education program that offered courses in Agricultural Science, Farm/Business Management, Ornamental Horticulture, and Environmental and Natural Resources Education. He returned to college in January 1996 to pursue a degree in Weed Science at Virginia Polytechnic Institute and State University and completed his requirements for a Doctor of Philosophy degree in September of 1999.