Chapter I
Introduction and Review of Literature

This research addressed herbicide resistance in smooth pigweed (*Amaranthus hybridus* L.) and herbicide-based weed management programs in corn (*Zea mays* L.). Specific areas of research were: 1) whole-plant and molecular characterization of acetolactate synthase (ALS, EC 2.2.1.6 [formerly EC 4.1.3.18]) -inhibiting herbicide-resistant smooth pigweed and 2) evaluation of corn weed management programs utilizing mesotrione in combinations with ALS-inhibiting herbicides, atrazine, and S-metolachlor.

Characterization of ALS-Inhibiting Herbicide-Resistant Smooth Pigweed

Since the first report of triazine-resistant common groundsel (*Senecio vulgaris* L.) in the late 1960s, there has been an increase in the number of herbicide-resistant weed biotypes worldwide (Ryan 1970). Currently, 293 biotypes within 175 species have been confirmed resistant to one or more of 18 herbicide groups (Heap 2005). Herbicide-resistant redroot pigweed (*Amaranthus retroflexus* L.) biotypes are the second most widespread resistant species, existing in 15 countries. *Amaranthus* species are among the most herbicide resistance-prone annual broadleaf weeds due to high genetic variability, high production of rapidly germinating seed, and efficient pollen and seed distribution (Lovell et al. 1996). Herbicide resistance in *Amaranthus* species has been reported for bipyridilium, dinitroaniline, photosystem II inhibitors, and ALS-inhibiting herbicides (Heap 2005). Since 1993, *Amaranthus* species including redroot pigweed (Saari et al. 1994), Palmer amaranth [*Amaranthus palmeri* (S.) Wats.] (Gaeddert et al. 1997; Horak and Peterson 1995; Sprague et al. 1997), prostrate pigweed [*Amaranthus blitoides* (S.) Wats.] (Saari et al. 1994), common waterhemp (*Amaranthus rudis* Sauer) (Hinz and Owen 1997; Horak and Peterson 1995; Lovell et al. 1996; Sprague et al. 1997), livid amaranth (*Amaranthus lividus* L.) (Manley et al. 1996), and smooth pigweed (*Amaranthus hybridus* L.) (Manley et al. 1996; Poston et al. 2000) have been confirmed to be resistant to ALS-inhibiting herbicides. ALS inhibitor-resistant smooth pigweed, specifically, has been reported throughout the Mid-Atlantic region and Kentucky and is believed to occupy over 1,000 cropping acres (Heap 2003).
ALS-inhibiting herbicides have been widely used since the early 1980s and currently comprise the largest mode-of-action group in use. ALS-inhibiting herbicides control numerous weeds at low rates, provide excellent safety to numerous crops, and possess low mammalian toxicity (Beyer et al. 1988; Brown 1990; Devine and Shukla 2000; Powles et al. 1997; Saari et al. 1994). However, ALS-inhibiting herbicides target a specific mechanism of action, which is conducive to resistance selection with frequent or repeated applications (LeBaron and McFarland 1990). In fact, of the 18-herbicide groups to which weeds have developed resistance, the ALS-inhibiting herbicide group has the largest number of resistant species and crop hectareage affected (Heap 2003).

The mechanism of action of ALS-inhibiting herbicides is inhibition of the ALS enzyme, which is the first common enzyme in biosynthesis of the branched-chain amino acids, valine, leucine, and isoleucine (Durner et al. 1990). In the initial step of this pathway, ALS catalyzes two parallel reactions: 1) the condensation of one molecule of pyruvate to form $\alpha$-acetolactate and carbon dioxide, and 2) the condensation of one molecule of $\alpha$-ketobutyrate to form $\alpha$-aceto-$\alpha$-hydroxybutyrate and carbon dioxide (Schloss 1990). ALS requires two cofactors, thiamine pyrophosphate complexed to a divalent cation (Mn$^{2+}$ or Mg$^{2+}$), and flavine adenine dinucleotide. ALS is the target for five structurally unrelated herbicide chemistries that include the sulfonylurea (SU) (Chaleff and Muvais 1984), imidazolinone (IMI) (Shaner et al. 1984), pyrimidinylthiobenzoate (PTB) (Stidham 1991), triazolopyrimidine (TP) (Gerwick et al. 1990), and sulfonilaminocarbonyl-triazolinone families (Santel et al. 1999).

*Amaranthus* species with resistance to the SU and IMI chemistries of ALS-inhibiting herbicides have been confirmed in field-isolated biotypes that have been subjected to repeated herbicide applications. Manley et al. (1998) reported the selection of a smooth pigweed population with imazaquin (IMI) that exhibited high-level resistance to IMI herbicides, but low level cross-resistance to the SU herbicides chlorimuron and rimsulfuron. However, this biotype was effectively controlled by other SU herbicides and pyrithiobac, a PTB herbicide. In contrast, selection of Palmer amaranth (Gaeddert et al. 1997) and common waterhemp (Horak and Peterson 1995) biotypes with repeated use of imazethapyr, an IMI herbicide, exhibited high-level resistance to both SU and IMI herbicides. In all three cases an IMI chemistry was the selection agent, but different
levels of cross-resistance were observed. In addition, negative cross-resistance was reported for IMI-resistant smooth pigweed biotypes that were more sensitive to reduced rates of thifensulfuron, an SU herbicide, pyrithiobac, and cloransulam-methyl, a TP herbicide, compared with a susceptible biotype (Manley et al. 1998; Poston et al. 2000).

In most instances of ALS inhibitor-resistance, single or cross-resistance patterns observed on the whole-plant level were due to an altered ALS enzyme, but faster herbicide detoxification has also been reported to confer resistance in a few biotypes (Devine and Eberlein 1997; Saari et al. 1994). A single nucleotide mutation in the ALS DNA sequence that results in a single amino acid substitution is capable of conferring resistance to ALS-inhibiting herbicides. In total, seventeen different amino acid substitutions that confer herbicide resistance have been identified from intentionally selected plants, yeast, bacteria, and green algae or natural field-selected biotypes (Duggleby and Pang 2000). However, only five of these sites, Ala122, Pro197, Ala205, Trp574, and Ser653, have been confirmed in the field-selected weed biotypes investigated. In most instances, a single amino acid substitution results in target-site cross-resistance differences between ALS-inhibiting herbicide chemistries. A substitution reported in the ALS of common cocklebur (*Xanthium strumarium* L.) at Ala122 conferred resistance to IMI herbicides only (Bernasconi et al. 1995). In addition, an identical substitution was reported in a commercial field corn (*Zea mays* L.) hybrid, ICI 8532 IT, and sugar beet (*Beta vulgaris* (L.) line Sur), which are crops resistant to only IMI herbicides (Bernasconi et al. 1995; Wright et al. 1998). Substitutions at Pro197 conferred high-level resistance to SU herbicides with little or no resistance to IMI herbicides (Boutsalis et al. 1999; Guttieri et al. 1992; Guttieri et al. 1995). Substitution at Ser653 has conferred high-level resistance to IMI herbicides with low resistance to SU herbicides (Devine and Eberlein 1997). In contrast, amino acid substitutions at Ala205 and Trp574 have conferred cross-resistance to SU, IMI, PTB, and TP chemistries (Bernasconi et al. 1995; Woodworth et al. 1996).

Variable patterns of resistance to ALS-inhibiting herbicide chemistries indicate that a resistance assessment is necessary for each individual ALS inhibitor-resistant weed biotype.

ALS-inhibiting herbicides are an important component of weed management programs worldwide; however, development of resistance to this herbicide group has had
a negative impact on their use. Characterization of field-selected ALS inhibitor-resistant weed biotypes provides a broad-range of information useful in the development of herbicide-resistance management and prevention strategies, which are important for the preservation of ALS-inhibiting herbicides.

**Objectives:** The primary objective of this research was to investigate the characteristics of ALS-inhibiting herbicide-resistant smooth pigweed on the whole-plant and molecular levels. Specific objectives were: 1) to characterize the response of suspected ALS-resistant smooth pigweed biotypes collected from Virginia, Delaware, Maryland, and Pennsylvania to herbicide representatives of SU, IMI, PTB, and TP herbicide classes, 2) to determine the mechanism and molecular basis for resistance of these smooth pigweed biotypes, and 3) to confirm the molecular basis of resistance in a smooth pigweed biotype from Pennsylvania by the response of transgenic *Arabidopsis thaliana* expressing genes that encode an ALS enzyme from this resistant biotype and from an ALS-inhibitor-susceptible biotype to ALS-inhibiting herbicides.

**Corn Weed Management Programs Utilizing Mesotrione in Combinations with Other Herbicides**

Conventional field corn (*Zea mays* L.) weed management programs have relied on preemergence (PRE) applications of a grass herbicide plus a broadleaf herbicide for residual season-long weed control. This standard PRE program usually includes a chloroacetamide herbicide in combination with atrazine for broad-spectrum PRE weed control (Ahrens 1994). In 2002, two chloroacetamides, metolachlor and acetochlor, were the primary PRE herbicides used for control of annual grasses and some small-seeded broadleaf weeds (Anonymous 2003). Atrazine, which can be applied as a PRE or postemergence (POST) herbicide for control of annual broadleaf weeds and some grasses, was applied on 75% of the U.S. corn hectareage in 2002. However, there are concerns with atrazine use, which include detection in surface and groundwater, residual activity, poor perennial weed activity, and the development of triazine resistant weeds (Ahrens 1994). Reductions in atrazine use have prompted a search for broadleaf weed control alternatives in corn.
Mesotrione is a PRE and POST herbicide that received EPA registration in 2001 for use in corn to control broadleaf weeds, including some species that are triazine- and ALS-resistant, and some grasses (Anonymous 2001; Sutton et al. 2002). Mesotrione inhibits the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD, EC 1.13.11.27), which indirectly affects phytoene desaturase activity by reducing the pool of available plastoquinone, leading to decreased carotenoid levels (Pallett et al. 1998). Without carotenoids, plants are unable to protect themselves from photooxidation, leading to bleaching symptoms (Norris et al. 1998). Plastoquinone is a key element in photosynthesis that is utilized as an electron carrier between the Qb site of photosystem II and Cyt b6/f, as well as in shuttling electrons through other photosynthetic processes (Ort 1986; Wise and Cook 1998). Inhibition of HPPD also blocks production of alpha-tocopherol, an antioxidant compound believed to strengthen membrane structures and scavenge free radicals within chloroplast membranes (Hess 1993; Pallet et al. 1998).

Mesotrione applied PRE has controlled smooth pigweed and common lambsquarters (Chenopodium album L.), but common ragweed (Ambrosia artemisiifolia L.) and morningglory species (Ipomoea spp.) control was inconsistent and dependent upon a timely rainfall following application (Armel et al. 2003a; Armel et al. 2003b; Ohmes et al. 2000). Whether applied PRE or POST, mesotrione is likely to be applied in a mixture for broad-spectrum weed control in corn. Armel et al. (2003a) reported improved weed control with tank mixtures of mesotrione with acetochlor and atrazine applied PRE over that of mesotrione alone. Commercial PRE mixtures currently available are mesotrione plus metolachlor (Camix®) and mesotrione plus metolachlor plus atrazine (Lumax® and Lexar®). However, mesotrione has been reported to be more effective on most weed species POST rather than PRE (Armel 2002; Johnson et al. 1999; Sprague et al. 1999; Young et al. 1999).

Mesotrione alone POST is primarily a broadleaf weed herbicide that controls common lambsquarters, pigweed species, jimsonweed (Datura stramonium L.), spurred anoda [Anoda cristata (L.) Schlecht], velvetleaf, wild radish (Raphanus raphanistrum L.), wild mustard [Brassica kaber (DC.) L.C. Wheeler], henbit (Lamium amplexicaule L.), and carpetweed (Mollugo verticillata L.) (Armel 2002). Two other important broadleaf weeds on the Delmarva Peninsula and Virginia, morningglory species and common
ragweed, were not always controlled by mesotrione applied POST. Mesotrione has activity on a few annual grasses including large crabgrass \textit{[Digitaria sanguinalis (L.) Scop.]}], red rice \textit{(Oryza sativa L.)}, and barnyardgrass \textit{[Echinochloa crus-galli (L.) Beauv.] (Armel 2002; Sutton et al. 1999)}. Giant foxtail \textit{(Setaria faberi Herrm.)} control with mesotrione alone POST has been reported as unacceptable (Bauman et al. 2000).

Researchers have reported that a mixture of mesotrione and a low rate of atrazine POST increased control of common ragweed, morningglory species, prickly sida \textit{(Sida spinosa L.)}, sicklepod \textit{(Cassia obtusifolia L.)}, yellow foxtail \textit{[Setaria glauca (L.) Beauv.]}, and broadleaf signalgrass \textit{[Brachiaria platyphylla (Griseb.) Nash]} over mesotrione alone (Armel 2002; Johnson et al. 1999). This combination also improved control of perennial weeds including horsernettle \textit{(Solanum carolinense L.)}, Canada thistle \textit{[Cirsium arvense (L.) Scop.]}, yellow nutsedge \textit{(Cyperus esculentus L.)}, purple nutsedge \textit{(Cyperus rotundus L.)}, and common mugwort \textit{(Artemisia vulgaris L.)} over mesotrione alone (Armel 2002). This POST combination also provided needed residual control with early application timings and improved control of larger weeds with later application timings (Johnson et al. 2002).

In the past decade, more interest has developed in utilizing total POST programs for weed control in corn. Besides the concerns with atrazine, other reasons for increased interest include the introduction of effective grass and broadleaf POST herbicides, the need by farmers to spread the work load, and the use of non-selective POST herbicides on transgenic crops. Total POST weed management programs allow farmers to assess weed species and densities present in each field so herbicides can be selected, rates adjusted, and programs customized. A further advantage is that POST herbicides do not rely on rainfall for activation, unlike PRE herbicides. Frequently, a POST herbicide application is required following a PRE application due to inadequate PRE weed control.

Examples of commercial POST herbicides for weed control in non-transgenic field corn that can be applied following a standard PRE herbicide application include nicosulfuron plus rimsulfuron plus atrazine (Basis Gold®), nicosulfuron plus rimsulfuron (Steadfast®), and rimsulfuron plus thifensulfuron (Basis®). All of these active ingredients, excluding atrazine, are representatives of the SU class of herbicides that inhibit the ALS enzyme and are used for control of selected annual broadleaf and grass
weeds (Anonymous 2003c). Basis Gold® has become a standard POST herbicide on the Delmarva Peninsula for weed control following a PRE application. Basis Gold® controls common cocklebur, jimsonweed, common lambsquarters, morningglory species, eastern black nightshade \textit{(Solanum ptycanthum} Dun.), pigweed species, common ragweed, velvetleaf \textit{(Abutilon theophrasti} Medicus), common waterhemp, large crabgrass \textit{[Digitaria sanguinalis} (L.) Scop.], barnyardgrass, foxtail species \textit{(Setaria} spp.), fall panicum \textit{(Panicum dichotomiflorum} Michx), shattercane \textit{[Sorghum bicolor} (L.) Moench], and broadleaf signalgrass (Anonymous 2003b). Steadfast® is similar to Basis Gold®, however, it does not contain atrazine. Steadfast® controls jimsonweed, morningglory species, pigweed species, large crabgrass, barnyardgrass, foxtail species, fall panicum, Texas panicum \textit{(Panicum texanum} Buckl.), shattercane, and broadleaf signalgrass (Anonymous 2003d). Weeds suppressed by Steadfast® include common cocklebur, common lambsquarters, velvetleaf, and common waterhemp. Basis® is another commercial mixture containing rimsulfuron. Basis® controls common lambsquarters, pigweed species, velvetleaf, large crabgrass, foxtail species, fall panicum, and shattercane (Anonymous 2003a). Before the development of nicosulfuron and rimsulfuron, POST alternatives for grass weed control in field corn were limited. However, as with atrazine, numerous weed species have developed resistance to ALS-inhibiting herbicides (Heap 2003).

Use of mesotrione as a POST herbicide alone will require a standard PRE application of an annual grass herbicide, or a tank-mix combination of mesotrione plus an annual grass herbicide. PRE applications that include mesotrione in mixtures with metolachlor and atrazine may improve weed control and may be effective to provide season-long residual weed control. Mesotrione in mixtures with other herbicides in a total POST weed management program may be effective for weed control and may eliminate the need for PRE applications. Limited information is available on mesotrione applied in mixtures with atrazine and metolachlor PRE or on POST applications of mesotrione in combination with other herbicides in a total POST weed management program in corn.

**Objectives:** The primary objective of this research was to evaluate mesotrione for use in Virginia corn weed management programs. Specific objectives were: 1) to evaluate weed control and corn response to mesotrione applied PRE alone and in mixtures with S-
metolachlor and atrazine and 2) to evaluate weed control and corn response from a total POST weed management program that included mesotrione alone and in mixtures with nicosulfuron plus rimsulfuron, nicosulfuron plus rimsulfuron plus atrazine, rimsulfuron plus thifensulfuron, and atrazine.
Literature Cited


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