CHAPTER 1

Literature Review

1. General Overview

1.1. Italian ryegrass (*Lolium multiflorum* Lam.)

Introduced from Europe as a forage crop, Italian ryegrass is an annual, cool-season grass that grows vigorously in winter and early spring. Italian ryegrass seedlings have shiny, glabrous leaf blades and sheathes (Uva et al., 1997). Its flat leaf blades have short, membranous ligules. Italian ryegrass has small, scale-like auricles, which may be absent on young seedlings. Mature plants reach 1.2 m in height and have flat, terminal spikes alternate along the stem (Whitson et al., 1996). Awned lemmas and a minimum of 10 florets per spikelet help to differentiate Italian from perennial ryegrass (*Lolium rigidum* Gaudin) (Britton and Brown, 1970; Uva et al., 1997; Whitson et al., 1996).

When not grown for forage, Italian ryegrass had been found throughout the continental United States in meadows, grain fields, and waste places (Uva et al., 1997). As forage crops, ryegrasses have numerous desirable agronomic qualities and are among the most widely grown cool-season grasses in the world. Establishing quickly, they are high yielding under favorable environments, possess high nutrient content, and can be used for grazing, silage, or hay (Hall, 1996).

Despite being useful as a forage crop, Italian ryegrass has become a competitive weed in small grains, especially in northwestern and southeastern United States, affecting crop yields, contaminating grain, and interfering with harvest (Appleby and Brewster 1992; Mersie and Foy, 1985; Stone et al., 1998). In a 1988 weed survey conducted by the Southern Weed Science Society, Italian ryegrass was considered one of the 10 most troublesome weeds in 10 of 13 southern states (Elmore, 1988). Liebl and Worsham (1987) reported that wheat grain yields were

reduced an average of 4.2% for every 10 Italian ryegrass plants/m^2, primarily due to decreased crop tillering caused by Italian ryegrass competition. Similarly, an earlier report by Appleby et al. (1976) indicated a 61% reduction in wheat yield due to 93 Italian ryegrass plants/m^2. Italian ryegrass also causes severe lodging and may complicate harvesting by maturing later than a small grain crop (Ritter and Menbere, 2002).

In small grains, Italian ryegrass has generally been controlled with postemergence applications of diclofop, or diclofop-methyl, a member of aryloxyphenoxypropionate (APP) family of herbicides. Along with cyclohexanedione (CHD) herbicides, APP herbicides are classified as the inhibitors of lipid biosynthesis (Heap, 2002). Diclofop, a graminicide used to selectively control annual and perennial grasses in broadleaf crops, is formulated as an ester of the parent acid (Devine et al., 1993). In susceptible species, diclofop inhibits fatty acid biosynthesis by blocking the production of phospholipids used in building new membranes required for cell growth (WSSA, 1994).

1.2. Acetyl Co-A carboxylase (ACCase; EC 6.4.1.2.)

Biosynthesis of lipids and fatty acids is essential for plant growth and development. Lipids are involved in the biogenesis and function of various membranes, cellular signal transduction, and other physiological functions (Browse and Somerville, 1991). In this diverse group of chemicals, phospholipids, galactolipids, and sterols provide the hydrophobic barrier of cell membranes, while cuticular wax and esters provide a coating on the aerial surface of the plants preventing water loss and protecting plants from environmental and biological stresses (Schmid et al, 1997).

In plants, biosynthesis of fatty acids takes place in plastids, involving acetyl Co-A carboxylase and fatty acid synthetase (FAS). Formed by one multifunctional polypeptide chain, ACCase, a multifunctional, biotinylated enzyme, is located primarily in the chloroplasts where its enzymatic activity can be strongly increased by the light, catalyzing the following reaction (Devine et al., 1993; Harwood, 1988; Schmid et al, 1997):

$$\text{Acetyl Co-A} + HCO_3 + ATP \rightarrow \text{Malonyl Co-A} + ADP + Pi$$

In this first committed step of de novo fatty acid biosynthesis, ACCase functions in a three-step sequence catalyzing partial reactions involving a kinetic mechanism of the carboxybiotin. In an ATP-dependent reaction, ACCase initiates biotin carboxylation using CO_2 from bicarbonate, followed by the transfer of the activated carbon to the carboxyltransferase site of biotin, and then from biotin to acetyl-CoA to form malonyl-CoA (Somerville et al., 2000).

The structure and subunit mass of plant ACCase is not fully known. At this time, the majority of research maintains that in almost all plants ACCase may exist as a combination of two types, a heteromeric form in the plastid and the homodimeric form in cytosol. In view of a continuing discussion regarding the structure of ACCase subunits, multiple genes may encode ACCase enzymes (Incledon and Hall, 1997).

As an exception, plants from the grass family (Poaceae) were found to have similar homodimeric ACCase isoforms in plastids as well as in cytosol (Egli et al, 1993). This unique trait had been shown to be the basis for selectivity for APP herbicides in susceptible species (Gronwald et al., 1992; Herbert et al., 1997). This herbicide-enzyme relationship is highly specific and any chemical modification of the herbicide or the enzyme can negate herbicidal activity (Burton et al., 1989; Devine, 1997; Devine and Shimabukuro, 1994; Gronwald et al., 1992; Herbert et al., 1997; Incledon and Hall, 1997).

2. Resistance to ACCase-inhibiting Herbicides

2.1. APP and CHD herbicides: chemistry and use in agriculture

Aryloxyphenoxypropionate (APP) and cyclohexanediones (CHD) herbicides differ chemically but share common structural elements that enable them to be highly selective in controlling grass weeds in cereal and dicot crops. Both families of herbicides were discovered and introduced in

the late 1970s to early 1980s. Found to be very effective for grass weed control, they quickly gained wide acceptance, accounting for over five percent of global herbicide sales (Devine and Shimabukuro, 1994; Heap, 1997). APP and CHD herbicides are active primarily on annual grass weeds and some are effective on perennial grasses as well. The majority of the APP and CHD herbicides are characterized by postemergence application, low concentrations required to achieve phytotoxicity, and low toxicity to non-target species (Somers, 1996).

In general, APP herbicides contain two aryl groups with an isopropyl group in the "1" position (Figure 1.1, A). This group contains a chiral center resulting in two possible enantiomers, designated as R (+) and S (-), with the R enantiomer having herbicidal properties (Devine and Shimabukuro, 1994; Gerwick et al., 1988). There is a considerable variability of substitutions in the second aryl group, in part accounting for differential sensitivity in susceptible species. The APP herbicides are usually formulated as butyl, ethyl, or methyl esters of the parent acid. Herbicides in this group could be either racemic mixtures containing both (+) and (-) enantiomers in equal proportions (diclofop, fenoxaprop-ethyl, and quizalofop-ethyl) or herbicidally active (+) enantiomers of the mixtures (fluazifop-P-ethyl and quizalofop-P-ethyl). Ester properties facilitate penetration of the leaf cuticle, allowing herbicides to be rapidly absorbed by plant foliage (WSSA, 1994). Once inside the plant, the ester is converted by carboxylesterase activity to release the respective parent acid, which then is readily translocated to plant meristematic tissues (Anderson, 1996; Devine et al., 1993). Thus, APP herbicides form weak acids in plant tissue. However, because APP herbicides are lipophilic, their movement in the phloem is more restricted than with other weak acid herbicides (Devine and Shimabukuro, 1994).

Unlike the APP herbicides, the common nucleus of CHD herbicides is cyclohexene with oxygen double-bonded to the ring in the "1" position, a hydroxy group bonded at the "3" position, and a variety of substitutions at the "2" and "5" positions of the hexene ring (Figure 1.1, B) (Devine and Shimabukuro, 1994). The hydroxy group in the "3" position can deprotonate, conferring the weak acid characteristics of CHD herbicides (Maier et al., 1994). Similar to APP

herbicides, CHD herbicides are also readily absorbed by the plant's foliage and translocated throughout the plant, where they accumulate in meristems (Anderson, 1996).

2.2. Mechanisms of action of APP and CHD herbicides

While it is generally accepted that APP and CHD herbicides have similar modes of action, inhibition of *de novo* fatty acid biosynthesis, their mechanism of action requires further investigation. Several mechanisms of action and target sites have been suggested including antagonism to auxin-mediated processes through depolarization of cellular membranes (De Prado et al., 1999; Devine et al., 1993; Devine and Shimabukuro, 1994; Ratterman and Balke, 1988; Wright, 1994) and inhibition of ACCase (Burton et al., 1989; De Prado et al., 2000; Devine et al., 1993; Devine and Shimabukuro, 1994; Egli et al, 1993; Gronwald et al., 1992; Somers, 1996).

2.2.1. Depolarization of cellular membranes

The electrochemical potential of cell membranes is an important component of metabolizing cells (Douce et al., 1997). Electrochemical potential gradient is generated by proton-pumping ATP synthases (ATPases) and consists of electrical potential and concentration gradients (Staehelin and Newcomb, 2000). The electrochemical potential is reversible, can be formed across many membranes, and is regulated by an array of mechanisms (Sanders and Bethke, 2000; Wright, 1994). In the plant, the ATPase energy transduction mechanism regulates a number of processes including active transport of organic and inorganic solutes across the membrane (Staehelin and Newcomb, 2000), control of intracellular pH (Sanders and Bethke, 2000), and hormonal control of plant growth (Devine and Shimabukuro, 1994; Douce et al., 1997).

As proposed by Ratterman and Balke (1988), APP and CHD herbicides may act as antagonists to auxin-mediated processes. In their study, graminicides would solubilize in the cell membranes and, once embedded, begin to act as protonophores. These protonophores lead to rapid destabilization of the cell membrane, shifting the electrochemical balance that facilitates auxin-

mediated functions (Ratterman and Balke, 1988). To eliminate the indirect effects of other biosynthetic processes, these experiments were conducted with an *in vitro* system of isolated membrane vesicles. Diclofop and fluazifop-butyl significantly disrupted the proton gradient formation, but only fluazifop-butyl significantly affected ATPase activity. Such differences suggest that these herbicides affect proton permeability rather than proton pumping. However, the effects were not correlated with dose response. Ratterman and Balke (1988) concluded that determining the role of proton gradient disruption of in herbicide mode of action requires the evaluation of dose response *in vivo*.

De Prado et al. (1999) proposed that lethal actions of APP herbicides are due to oxidative stress involving ethylene induction and generation of different reactive oxygen species. The oxidative stress mechanism, initiated by various factors, creates a signal that is perceived and transduced at the plasmalemma. Depolarization of membrane potentials of susceptible species varied among biotypes and across application rates.

Lethal effects of APP herbicides have been reversed *in vitro* by auxinic compounds (lipoxygenase inhibitors and free radical scavengers) that may prevent peroxidation of free polyunsaturated fatty acids to reactive oxygen species by lypoxygenase (Banas et al., 1993; Shimabukuro and Hoffer, 1996). De Prado et al. (1999) found that membrane depolarization results in decreased intracellular pH, increased Ca^{2+}, and increased ethylene evolution. This cascade of events leads to senescence due to the production of lipid peroxidases and free radicals. De Prado et al. (1999) suggested membrane depolarization eventually leads to plant death and is the primary mode of action of APP herbicides. However, interspecific differences in plant response to APP herbicides are not understood (De Prado et al., 1999).

In summary, APP herbicides do not dissipate the proton gradient by inhibiting the plasma membrane ATPase. Inhibition of the electrochemical balance appears to be the result of a specific herbicide-receptor interaction at the plasma membrane that causes increased permeability to protons (Devine and Shimabukuro, 1994). This increased permeability leads to an oxidative

stress resulting in the formation of reactive oxygen species (ROS), thus becoming the most likely lethal mechanism of action of APP herbicides (De Prado et al., 1999). Lethal effects of APP herbicides were found to be reversible by lipoxygenase inhibitors, free radical scavengers, and auxinic compounds by interrupting the formation of ROS (Banas et al., 1993; De Prado et al., 1999; Shimabukuro and Hoffer, 1996). Antagonism of auxin-mediated growth occurs through proton gradient dissipation that eliminates net acidification of the apoplast without affecting the proton pump (De Prado et al., 1999; Devine and Shimabukuro, 1994; Wright, 1994).

APP herbicides act as ionophores, an uncoupler that allows a free movement of protons across the plasma membrane, either by forming a proton selective pore or by being a lipophilic weak acid (Wright, 1994). An increase in proton permeability of the cell membrane changes the pH gradient and separates the proton pump from control of the electrochemical gradient (Devine and Shimabukuro, 1994; Dotray et al., 1993; Wright, 1994). Proton gradient repolarization occurs in all resistant but not all susceptible biotypes when the herbicide is removed from the solution (De Prado et al., 1999). Depolarization of membrane potential is rate-dependent and varies among plant species (De Prado et al., 1999). The herbicide concentration required for membrane depolarization is higher than that required for ACCase inhibition (Wright, 1994).

2.2.2. Inhibition of ACCase

The majority of research suggests that in most susceptible species, APP herbicides achieve phytotoxicity through inhibition of acetyl Co-A carboxylase (Burton et al., 1989; De Prado et al., 2000; Devine et al., 1993; Devine and Shimabukuro, 1994; Egli et al., 1993; Gronwald et al., 1992; Menendez and De Prado, 1999; Somers, 1996). Additional information can be obtained from comprehensive reviews and articles by Burton et al. (1989), Devine (1997), Devine and Shimabukuro (1994), Herbert et al. (1997), Incledon and Hall (1997), Rendina and Felts (1988), Somers (1996).

Burton et al. (1989) observed that the incorporation rate of [^{14}C]acetate into fatty acids in corn chloroplasts was linear and strongly dependent on the addition of exogenous Co-A. Addition of either sethoxydim or haloxyfop inhibited incorporation of acetate into fatty acids by corn chloroplasts in a concentration-dependent manner. Effects of these herbicides on fatty acid synthetase (FAS) activity were evaluated by measuring the incorporation of [^{14}C]malonyl-CoA into fatty acids in a lysed corn chloroplast fraction. Both sethoxydim and haloxyfop stimulated the incorporation of [^{14}C]malonyl-CoA, indicating the inhibitory effect must take place prior to herbicide involvement with FAS for phytotoxicity to occur.

Similarly, other researchers have found that in susceptible and resistant biotypes of the same plant species partially purified ACCase had significantly different affinity for binding with ACCase-inhibiting herbicides (Gronwald et al., 1992). However, the differential response to herbicides was not due to differential herbicide uptake, translocation, or metabolism (Gronwald et al., 1992; Rendina and Felts, 1988). Kinetic analyses strongly suggest that both APP and CHD herbicides act as reversible, linear inhibitors of ACCase (De Prado et al. 2000). Herbicide molecules appeared competitive with acetyl-CoA for ACCase binding, thus affecting the formation of malonyl-CoA (De Prado et al., 2000). It has been proposed that inhibitory effects of the herbicides take place during either biotin carboxylation or transcarboxylation reactions of the ACCase turnover. The majority of available data strongly supports ACCase as the target site for APP/CHD herbicides, although the precise mechanism of herbicidal action has not yet been fully characterized.

The inhibition of ACCase activity and the destabilization of transmembrane proton gradient may occur simultaneously, independently, or successively as a part of the total phytotoxic effect (Ratterman and Balke, 1988). The disruption of the ACCase function results in alteration of fatty acid incorporation into the cell membrane, shifting its electrochemical properties (Dotray et al., 1993; De Prado 1999). The alteration of transmembrane proton gradient may be a secondary event to ACCase inhibition and needs further research. Nevertheless, a plant's ability to

repolarize its membrane proton flux should be considered as a factor in the resistance to APP and CHD herbicides. An unknown relationship may exist between inhibition of ACCase and membrane effects (Wright, 1994).

2.3. Resistance to ACCase-inhibiting herbicides

One of the first instances of APP/CHD herbicide resistance was documented in 1987 (Stanger and Appleby, 1989). Diclofop-tolerant biotypes of Italian ryegrass were found in wheat fields that had been treated annually with diclofop for at least seven years. Diclofop GR50 (rate required to reduce shoot weight by 50%) values varied among tolerant biotypes and were 400 to 980 times higher than for susceptible biotypes (Stanger and Appleby, 1989). Continued use of these herbicides has resulted in an increasing number of resistant weed species. Currently over 25 species throughout the world have been confirmed to be resistant to ACCase-inhibiting herbicides (Heap, 2002). In most instances, the resistance to ACCase inhibitors was found to be due to the presence of an altered ACCase. Enzyme sensitivity differs among species, each conferring different patterns and levels of resistance. A plant sensitive to one APP/CHD herbicide may not be as sensitive to another APP or CHD herbicide, and not all tissues within the same plant are equally sensitive to the same herbicide (Egli et al., 1993; Nikolau et al., 1984).

The majority of published reviews on the mechanisms of resistance to ACCase-inhibitors focus on four main possibilities: (1) resistance based on enhanced herbicide metabolism (Maneechote et al., 1997; McFadden et al., 1989; Hidayat and Preston, 1997; Shimabukuro et al., 1979); (2) resistance based on reduced ACCase sensitivity (De Prado et al., 2000; Devine and Shimabukuro, 1994; Egli et al, 1993; Gronwald et al., 1992); and (3) resistance based on alterations in cell wall membrane (DiTomaso, 1993; Wright, 1994); and (4) overproduction of ACCase (Bradley et al., 2001).

2.3.1. Metabolism-based resistance to ACCase inhibitors

In several weed biotypes and some cereal crops, resistance to ACCase inhibitors is conferred by enhanced herbicide metabolism, primarily through over-expression of cytochrome P450 monooxygenase activity (McFadden et al., 1989; Zimmerlin and Durst, 1992). This mechanism may be involved in the development of cross-resistance to other herbicide classes with different modes of action (Preston et al., 1996). For example, wheat (*Triticum aestivum* L. cv Etoile de Choisy) microsomes were found to catalyze the metabolism of chlorsulfuron, chlortoluron, and 2,4-dichlorophenoxyacetic acid (Zimmerlin and Durst, 1992).

Some APP herbicides may be metabolized through nonezymatic conjugation (Hidayat and Preston, 1997). In 1995, Tal et al. indicated that nonezymatic conjugation of fenoxaprop-ethyl with glutathione could be the mechanism for tolerance of wheat (*Triticum aestivum* L., cv. Fredrick), barley (*Hordeum vulgare* L., cv. Leger) and triticale (*Triticum* X *Secale* cv. OAC Trillium).

Metabolism-based resistance is not a wide spread phenomenon, as only a few grass species possess the natural ability to tolerate APP/CHD herbicide treatments. Furthermore, an ability to metabolize a particular APP/CHD herbicide does not confer resistance to other representatives of this herbicide class. For example, wheat (*Triticum* spp.) ACCase is susceptible to inhibition by APP/CHD herbicides but has a natural ability to rapidly detoxify diclofop (McFadden et al., 1989; Shimabukuro et al., 1987). Other species with the natural ability to rapidly metabolize certain APP/CHD herbicides include barley (*Hordeum* spp.), red fescue (*Festuca rubra* L.), and corn (*Zea mays* L.) (Dotray et al., 1993; Duke 1996).

Enhanced metabolism is attributed to resistance in several weeds, including various species of wild oats (*Avena* spp.), ryegrasses (*Lolium* spp.), and crabgrasses (*Digitaria* spp.) (Hidayat and Preston, 1997; Holtum et al., 1991; Shimabukuro et al., 1987). For example, a five-fold increase in the rate of diclofop metabolism was observed in a resistant biotype of wild oats compared to

the susceptible biotype (Maneechote et al., 1997). However, precise mechanisms of metabolism-based resistance to APP/CHD herbicides have not been fully described.

2.3.2. Resistance based on an alteration of ACCase

The resistance to APP/CHD herbicides in most weeds has been correlated with reduced ACCase sensitivity and confirmed by several studies involving dose-response evaluations and enzyme activity assays. Gronwald et al. (1992) reported that diclofop concentration that inhibited ACCase activity by 50% in a resistant Italian ryegrass biotype was 28-fold greater than for the ACCase from a susceptible biotype. Similarly, haloxyfop and quizalofop concentrations that inhibited ACCase activity by 50% in resistant Italian ryegrass were 9- and 10-times greater, respectively, when compared to concentrations achieving 50% enzyme inhibition in the susceptible biotype (Gronwald et al., 1992).

The majority of researchers supporting alteration of ACCase as the mechanism of resistance base their assumptions on significant differences in enzyme sensitivity among susceptible and resistant biotypes of the same plant species (Burton et al., 1989; De Prado et al., 2000; Devine and Shimabukuro, 1994; Egli et al, 1993). The evidence seems compelling since differences in absorption, retention, translocation, or metabolism in the tested species are negligible among resistant and susceptible biotypes.

2.3.3. Cell wall membrane and other mechanisms of resistance

In 1993, DiTomaso proposed plasma membrane alteration in the presence of diclofop as the basis for graminicide resistance. Acting as weak acids, APP/CHD herbicides can protonate, become lipophilic, and penetrate susceptible plant cell walls. Upon herbicide penetration, the cell wall becomes deprotonated and destabilized, due to the protonophoric nature of the herbicide-cell wall interaction. Furthermore, the herbicide molecule can be hydrolyzed to release parent acid again, thus prolonging the presence of the active toxic compound. This depolarization of the

membrane has been shown to be reversible in resistant plants and irreversible in susceptible plants (Wright, 1994).

The mechanism through which herbicides cause destabilization of the cell wall membrane is not fully understood. Actively metabolizing cells possess a differential electrochemical gradient generated by proton-channeling ATPases across their membranes. In addition to membrane permeability, other factors (transport processes, energy metabolism, cytoplasmic pH) could potentially have an effect on the shifts of the transmembrane proton gradient. The ability of resistant plants to re-polarize membranes to withstand herbicide toxicity may be part of more complex interactions not yet described as opposed to a single mechanism of herbicide resistance (Incledon and Hall, 1997).

2.3.4. Overproduction of ACCase

In studies evaluating ACCase-resistant and -susceptible biotypes of johnsongrass (*Sorghum halepense*), Bradley and Hagood (2001) suggest a mechanism of resistance other than an insensitive ACCase in a New Kent County, Virginia johnsongrass biotype. Their field and greenhouse dose-response experiments indicated that the New Kent biotype was 17 times more resistant to quizalofop-P, 5.7 times more resistant to sethoxydim, and 29.5 times more resistant to fluazifop-P than the susceptible biotype. However, both resistant and susceptible johnsongrass biotypes were equally sensitive to clethodim.

In a follow-up study the researchers found that there were no significant differences between the resistant (New Kent) and susceptible biotypes in the translocation of [^{14}C]quizalofop-ethyl, and only marginal differences between biotypes in herbicide absorption and metabolism (Bradley et al., 2001). Furthermore, ACCase from the resistant and susceptible johnsongrass biotypes was equally sensitive to quizalofop-P, clethodim, and sethoxydim, indicating no significant differences in the rate of inhibition (I_{50}) of ACCase activity between the biotypes in response to herbicide treatments. However, when quizalofop-P, clethodim, and sethoxydim were not

introduced into the ACCase assay mixture, ACCase specific activity of the resistant biotype was significantly higher than that of the susceptible biotype, ranging from 146-283 pmol/mg/min to 71.4-94.9 pmol/mg/min, respectively.

Nearly identical I_{50} values of the resistant and susceptible biotype ACCases indicate that both biotypes possess ACCase sensitive to APP and CHD herbicides. However, two- to three-times higher levels of ACCase specific activity suggest that an overproduction of ACCase in resistant johnsongrass biotypes is the mechanism of resistance to quizalopfop-P and sethoxydim (Bradley et al., 2001).

3. Resistance to Diclofop in Italian Ryegrass

In small grains, Italian ryegrass is selectively controlled by postemergence applications of diclofop. Introduced in 1979, diclofop became widely used in wheat-producing areas of Oregon and other northwestern states to control annual and perennial grasses in broadleaf crops, (Devine et al., 1993). Applied postemergence at 500 to 1500 g/ha, diclofop controlled susceptible Italian ryegrass 80 to 100% and increased wheat yields 20 to 60% (Griffin 1986, Khodayari et al., 1983, Shaw and Wesley, 1991).

As with a number of other herbicides, the extensive and repeated use of the product alone and in combination with other herbicides targeting the same site of action has lead to the development of herbicide resistance in weed populations. The first occurrence of diclofop-resistant Italian ryegrass was reported by 1987 (Stanger and Appleby, 1989). Currently, diclofop-resistance in Italian ryegrass has been documented in many US states and worldwide and is rapidly becoming a problem in many agro-ecosystems (Heap, 1997). In most cases diclofop resistance has been attributed to the presence of insensitive diclofop-resistant ACCase (Devine and Shimabukuro, 1994; Evenson et al., 1994; Evenson et al., 1997). Although mutations and cross-resistance

patterns may vary among the biotypes, resistance development is attributed to the changes in the same gene locus and may be encoded by a single, dominant nuclear gene (Murray et al., 1996).

4. Objectives

In Virginia, the first diclofop-resistant Italian ryegrass biotype was reported in 1993 in Mecklenburg County, where diclofop was used for ryegrass control for a number of consecutive growing seasons (Hagood[1], 2004). The potential spread of diclofop-resistant Italian ryegrass prompted the search for alternative control methods. Our general objective was to identify effective control measures of Italian ryegrass that has become increasingly prevalent in Virginia small grains. Our specific goals were to evaluate the efficacy of various herbicides for Italian ryegrass control, including confirmation of the presence of the diclofop-resistant biotypes of Italian ryegrass in various Virginia locations, to conduct greenhouse and field evaluation of possible alternative postemergence and preemergence herbicides for management of diclofop-resistant biotypes of Italian ryegrass in applicable cropping situations, and to evaluate whether the resistance to diclofop in the selected biotypes was due to the presence of insensitive ACCase.

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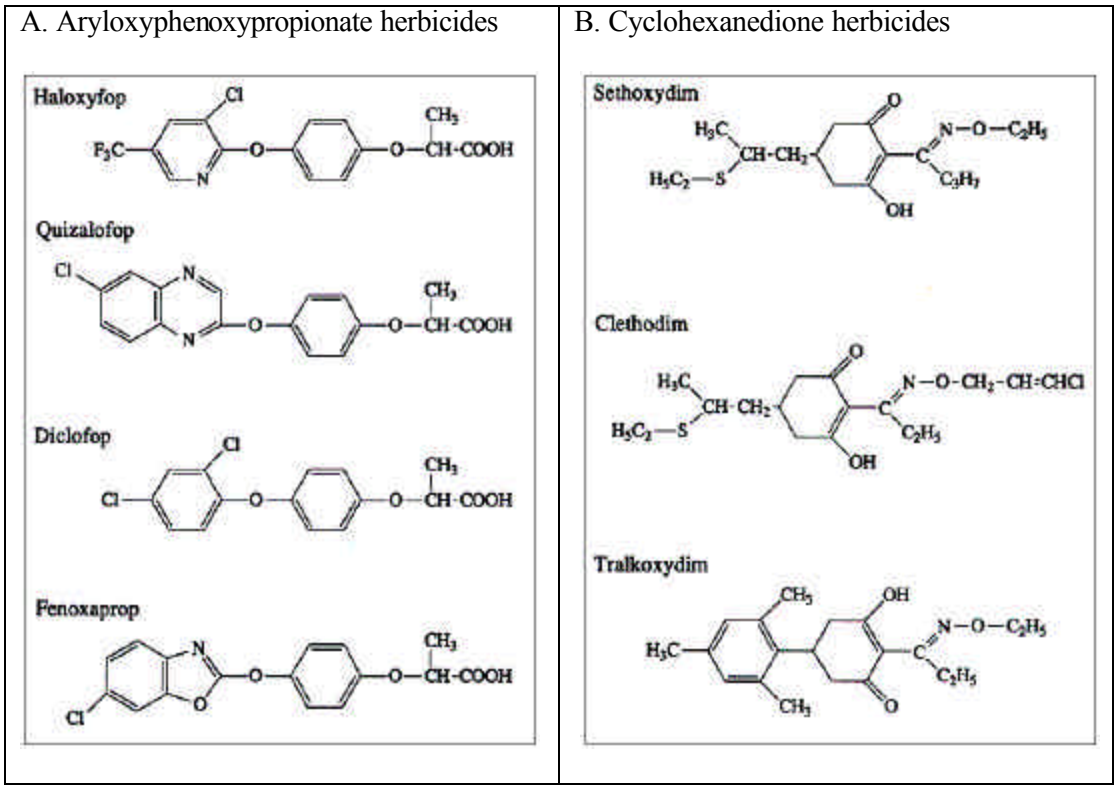

Figure 1.1. Chemical structures of most common representatives of ACCase-inhibiting herbicides (WSSA, 1994).