CHAPTER 1.0

INTRODUCTION AND LITERATURE REVIEW

1.1 CORN ACREAGE, IMPORTANCE, AND ECONOMICS IN VIRGINIA

The value of field corn harvested for grain represented the third most valuable field crop in Virginia, with an estimated value of production of $126,945,000, in 1996 (Anonymous 1997). Only hay and tobacco had higher annual values of production (Anonymous 1997). When both grain and silage use is considered, field corn had an estimated value of $185,504,000. In 1995, Virginia field corn (*Zea mays* L.) harvested for grain represented the fourth most valuable field crop, with an estimated annual value of production of $80,605,000 and 410,000 planted acres reported (Anonymous 1996). Only tobacco, peanuts, and soybeans had higher annual values of production (Anonymous 1996). When considered for grain and silage use, field corn had an estimated annual value of production of $139,503,000 and ranked second only to tobacco in Virginia (Anonymous 1996).

1.2 BERMUDAGRASS: INTRODUCTION, ADAPTATION, AND BIOLOGY

Bermudagrass (*Cynodon dactylon* [L.] Pers.) is an aggressive perennial grass weed species that is regarded as one of the world's worst weed problems (Holm et. al. 1972). Bermudagrass, a common name assigned to a number of species of the genus *Cynodon*, probably originated in southeast Africa (Burton and Hanna 1984; Gleason 1950). *C. dactylon* [L.] Pers., or common bermudagrass, is the only species described as a "ubiquitious, cosmopolitan weed" because of its worldwide distribution (Burton and Hanna 1984). Holm et al. (1972) suggested that it is a native of tropical Africa or the Indo-Malaysian area. One of the earliest records concerning the introduction of bermudagrass into the United States is found in the diary of Thomas Spalding (Burton and Hanna 1984), owner of Sapeloe Island, Georgia, and a prominent antebellum agriculturist. In his diary he made the following entry; "Bermudagrass was brought to Savannah in 1751 by Governor Henry Ellis. If ever this becomes a grazing country it must be through the instrumentality of this grass." This species has been referred to as one of the most important pasture grasses of the southern states (Hitchcock 1950).

C. dactylon [L.] Pers. is a long-lived, prostrate, fine-leaved perennial grass that spreads by strong, flat stolons and scaly rhizomes to form a dense turf (Burton and Hanna 1984). Turgeon (1980) reports lateral growth by both stolons and rhizomes and that the stolons root readily at the nodes. The leaf blades are 2 to 16 cm in length, 3 to 5 mm wide, and smooth or hairy on the upper surface. The distinguishing taxonomic characteristics are the ligule, which consists of a conspicuous ring of white hairs, the lemma, with a fringe of white hairs on its keel, and dull, gray-green foliage (Hitchcock 1950; Holm et al. 1972).

The inflorescence consists of three to seven green to purplish spikes in a single whorl arranged digitately (Hitchcock 1950; Holm et al. 1972).

Bermudagrass grows on open ground, grassland, fields, and waste places (Hitchcock 1950). Burton and Hanna (1984) describe the US distribution and adaptation as being in the states south of a line connecting the southern boundaries of Virginia and Kansas. Worldwide, it now grows throughout the tropical and subtropical areas of the world and extends into temperate zones along the coasts. In Eastern Africa it is distributed from sea level to 2200 m and in Hawaii, from sealevel to 1250 meters (Holm et al, 1972). It grows best when mean daily temperatures are above 24 C (Burton and Hanna 1984), and prospers where temperatures approach 38 C (Holm et al. 1972). Very little growth is made when temperatures drop to 6 to 9 C. Temperatures of -2 to -3 C usually kill above-ground portions of the plant (Burton and Hanna 1984).

1.3 BERMUDAGRASS AS A WEED IN CORN

The availability of water and plant nutrients is a limiting factor in corn production (Alley 1998; Pierre et al. 1964). Bermudagrass infestations reduce crop yield directly through competition for sunlight, water, and essential nutrients (McCormick 1977). Many common weeds accumulate greater quantities of nutrients than corn. Vengris et.al. (1955) listed seven weeds species that contained greater concentrations of nitrogen, phosphorous, potassium, calcium, and magnesium than corn on a per acre basis. Redroot pigweed and common lambsquarters utilized twice as much nitrogen and phosphorous and three times as much potash as corn. Five hundred pounds of common lambsquarters was shown to accumulate enough nutrients to produce approximately eight bushels of corn. Weed growth in cultivated corn often exceeds 500 pounds per acre, and substantial yield losses to competition occur (Pierre et al. 1964).

The important weed species found in corn vary in morphology, taxonomic classification, and growth habit. Both broadleaf and grassy types are among the most serious weeds (Pierre et al. 1964). The important annual weeds are more prevalent than the important perennial weeds. However, when perennial weeds are present in corn, they compete more vigorously and usually cause greater yield losses than are caused by annuals (Pierre et al. 1964).

Bermudagrass has been recognized as a serious weed in Virginia in surveys conducted by the Southern Weed Science Society (Dusky 1997; Street 1994; Witt 1991). The survey, “The Southern States 10 Most Common and Troublesome Weeds in Corn”, is conducted every three years. The 1991 survey ranks bermudagrass as the tenth most

common and fifth most troublesome weed in Virginia corn (Witt 1991), the tenth most common and fourth most troublesome in 1994 (Street 1994), and the tenth most common and fourth most troublesome in 1997 (Dusky 1997).

In 1991, only two other states, South Carolina and Mississippi, listed bermudagrass as a problem. In that year, in Mississippi, it was the tenth most common and eighth most troublesome, while South Carolina ranked as the number one most troublesome. Since 1991 the importance of bermudagrass as a weed in corn has steadily increased. It was included in the rankings of three states in addition to Virginia in 1994; Georgia, Mississippi, and South Carolina (Street 1994). Georgia ranked it as the tenth most common and the eighth most troublesome. Mississippi included it as the ninth most common and the seventh most troublesome weed. South Carolina did not include bermudagrass as one of the top ten common weeds but did rank it as the first most troublesome. In 1997 North Carolina and Tennessee joined the states of Virginia, Georgia, Mississippi, and South Carolina listing bermudagrass as a problematic weed species (Dusky 1997). In that year Georgia included bermudagrass as the tenth most common and eighth most troublesome. Mississippi did not rank it in the ten most common weeds but listed it as the fourth most troublesome. North Carolina, South Carolina, and Tennessee did not rank bermudagrass in the ten most common weeds but as the sixth, first, and seventh most troublesome weed, respectively.

This perennial species has proliferated in no-till corn production, and is a problem weed in conventional corn production as well (Hagood 1996). Its potential as a weed is enhanced by early vegetative growth in the spring that supports competitive advantage

over subsequently planted crops (Vencill et al. 1992). Conservation tillage, especially no-tillage, favors growth of bermudagrass because perennial structures are not disturbed.

No research has been published concerning the effect of bermudagrass competition in corn. Studies of this nature with other weeds in corn have been conducted, however. Stoller et al. (1979) investigated yellow nutsedge (*Cyperus esculentus* L.) competition in corn and found that when no control was practiced, yields were reduced 17% in a moderate infestation (initially infested with 300 tubers/m^2) and 41% in a heavy infestation (initially infested with 1200 tubers/m^2). Young et al. (1984) conducted field studies to determine the competitive nature of quackgrass (*Agropyron repens*) in corn and found densities of 745 shoots/m^2 reduced corn yields an average of 37% and significantly reduced corn height and ear length. Giant foxtail (*Setaria faberi* Herrm.) has been reported to cause yield losses of as much as 26% in corn, and shattercane (*Sorghum bicolor* (L.) Moench.) has been shown to reduce yield in corn by 75% (Beckett et al. 1988).

1.4 BERMUDAGRASS CONTROL IN CORN

Preemergence herbicides including one or more of the symmetrical triazines, often in combination with other herbicides for broad spectrum grass and broadleaf weed control are applied at, or prior to corn emergence (E. S. Hagood, Personal Communication). In reduced tillage, nonselective herbicides are added for the control of vegetation existing at the time of planting. Implementation and success of no-till production involves controlling weeds, especially difficult to control weed species. In the absence of tillage, herbaceous perennial broadleaf species can proliferate. Perennial grass species can also be troublesome. Johnsongrass (*Sorghum halepense* [L.] Pers.) has been regarded as the most troublesome and difficult to control weed in Virginia corn (Dusky 1997; Street 1994; Witt 1991). Only partial control in conventional tillage is afforded by soil incorporated thiocarbamate herbicides (Stevens et al. 1986), and until recently, no selective control was available in no-till. The development of nicosulfuron and primisulfuron, however, allowed selective postemergence control of this species in corn, and has therefore allowed successful no-till production in fields infested with johnsongrass.

Bermudagrass is another perennial species that has proliferated in no-till corn production. It is the second most troublesome grass species in Virginia cornfields (Dusky 1997; Street 1994; Witt 1991), and, given the development of selective johnsongrass control discussed above, represents the primary grass species in corn for which adequate control measures are not available.

The only bermudagrass control measures have relied primarily on thiocarbamate herbicides, which are appropriate only to conventional tillage systems because

incorporation of these herbicides is required due to herbicide volatility of the thiocarbamates when placed on the soil surface (Stevens 1986). Since incorporation can not be practiced in no-till corn production, there are no available herbicides to control bermudagrass (Hagood et al. 1996; Hagood et al. 1998).

Bermudagrass is susceptible to systemic herbicides, such as glyphosate, N-(phosophonomethyl)glycine, which is used to eliminate existing vegetation prior to planting no-till. At the time of corn planting in Virginia, however, bermudagrass has not developed sufficiently to allow translocation of the herbicide from the foliage to the stolons (Hagood 1998). For this reason, use of non-selective herbicides for bermudagrass control prior to corn planting affords only suppression.

1.5 DEVELOPMENT OF GRAMINICIDES

In the last 15 years, several herbicides, members of the aryloxyphenoxy propionate and the cyclohexanedione families of chemistry, have been developed and registered. They provide selective, postemergence control of annual and perennial grass weeds in broadleaf crops (Ware 1994). These herbicides, which are collectively referred to as graminicides due to their specificity for grass weeds, act on the lipid synthesis pathway by inhibiting acetyl-CoA carboxylase (ACCase) (Ahrens 1996). Both classes of chemistry afford high activity against many grasses at economical rates, and can be applied to most broadleaf crops with little risk of injury (Meister 1996).

These chemicals represent a completely new alternative for controlling weeds in crops because previously registered postemergence grass herbicides such as dalapon and glyphosate had no selectivity (Ross and Lembi 1985). These new herbicides have no activity on broadleaf species or nongrass monocots, either crops or weeds. Interest in the recently developed herbicides revolves around their potential for use in a wide range of broadleaf crops and their potential for use in minimum tillage systems (Ross and Lembi 1985).

The common nucleus of the chemical structures for the aryloxyphenoxy propionate herbicides is 4-oxyphenoxypropanoic acid; with butyl, ethyl, or methyl ester substituent groups. The cyclohexanedione family differs from the aryloxyphenoxy propionates in the various aryl-derived groups bonded to the oxygen at the 4-position of the phenoxy ring and, in some cases, in the ester group (Anderson 1996).

Diclofop-methyl, fenoxaprop-ethyl, fluazifop-ethyl, and quizalofop-ethyl are racemic mixtures (Anderson 1996). A racemic mixture contains both *dextro* (+) and *levo* (-) enantiomers in equal proportions. The *levo* (-) form is nonherbicidal. Fluazifop-P-ethyl and quizalofop-P-ethyl are herbicidally active *dextro* enantiomers of the mixture.

The common nucleus of the cyclohexanedione herbicides is cyclohexene with an oxygen double-bonded to the ring at the 1-position, a hydroxy group bonded at the 3-position, and other substitutions at the 2- and 5-positions of the hexene ring (Anderson 1996b). The cyclohexanedione chemistry is markedly different from the aryloxyphenoxy propionates, but both groups have essentially the same activity in plants. Sethoxydim and clethodim are the most prominent members of this group.

Graminicides are active on annual grasses and certain perennial grasses, such as johnsongrass and bermudagrass. They are absorbed rapidly by plant tissue and move in the symplast to areas of active growth, where they inhibit meristematic activity (Ross and Lembi 1985). The first effect on the shoot is death of the growing point and the inner whorl of leaves. The outermost leaves may appear healthy for longer periods but eventually wither and die (Ahrens 1996; Ross and Childs 1995; Ross and Lembi 1985). Maximum control is observed 10-21 days after treatment.

Cyclohexanediones and aryloxyphenoxy propionates have been investigated for potential use in tank mixes with herbicides active on dicotyledonous plants. However, antagonistic effects on weed control may limit the use of graminicides and broadleaf herbicides in combination sprays (Hatzios and Penner 1985; Holshouser and Coble 1990; Jordan et al. 1993a; Jordan et al. 1993b; Meuller et al. 1989; Ross and Lembi 1985; Wanamarta et al. 1993).

Dicots are tolerant to graminicides, and grasses with few exceptions are susceptible (Butler and Appleby 1986; Hosaka et al. 1984). A number of studies have shown that this differential expression of herbicidal activity is not the result of differential herbicide uptake, translocation, or detoxification (Buhler 1985; Hendley et al. 1985; Kells et al. 1985; Swisher and Colson 1982), suggesting that selectivity is expressed at the site of action. A number of earlier studies indicated that fatty acid biosynthesis is a sensitive target site for cyclohexanedione and aryloxyphenoxypropionate herbicides in grasses (Burgstahler et al. 1986; Cho et al. 1986). Burton et al. (1989) reported that sethoxydim and haloxyfop are potent inhibitors of acetyl-CoA carboxylase (ACCase) in corn, a herbicide-susceptible species and that report was confirmed by Rendina and Felts (1988). Burton et al. (1989) also presented data that indicate acetyl-CoA carboxylase as a sensitive site of action of the cyclohexanedione and aryloxyphenoxypropionate herbicides in corn, a grass species susceptible to these herbicides, but not in pea, a dicot that is tolerant to these herbicide

1.6 SETHOXYDIM

Sethoxydim, 2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one, a cyclohexanedione graminicide, controls annual and perennial grasses in several broadleaf crops (Hagood et al. 1996; Hagood et al. 1998; Hosaka et al. 1984; Swisher and Colson 1982). It can be applied as follows: postemergence at 112-448 g ai/ha in soybeans and peanuts; postemergence at 112-560 g ai/ha in alfalfa, sugarbeets, sunflowers, and cotton; postemergence at 112-560 g ai/ha in flax; as a foliar applied preplant burndown at 112 g ai/ha for no-till soybeans; postemergence at 336-560 g ai/ha in many ornamental trees, shrubs, flowers, and ground covers, and postemergence at 1-1.5% v/v as a spray-to-wet application for spot treatment in soybeans and ornamentals. An oil adjuvant or nonionic surfactant is required for maximum efficacy (Ahrens 1996).

Visual symptoms of sethoxydim injury include a cessation in growth within 48 hours of application (Asare-Boamah and Fletcher 1983; Hosaka et al. 1984), chlorosis of younger expanding leaves, and increases in anthocyanin pigments followed by extensive leaf necrosis after 7 days (Campbell and Penner 1985). In bermudagrass, thylakoids and mitochondria of mesophyll cells are affected (Chandler and Paul 1982). Ahrens et al. (1996) describe this behavior as a cessation of growth within a few days of application, with young and actively growing tissues affected first, followed by leaf chlorosis and necrosis within 7-21 days after treatment (DAT). Leaf sheaths become brown in color, and deteriorate at and just above their point of attachment to the node (Ahrens 1996). Older leaves often turn purple, orange, or red before becoming necrotic. Ross and Childs (1995) describe the phenomenon of “grass meristem destroyers” as eliciting the same

symptoms on all grass species, which include discoloration and disintegration of meristematic tissue at and above the nodes, including nodes of rhizomes. Leaves yellow, redden and sometimes wilt. Seedling grasses tend to lodge by breaking over at the soil surface.

Sethoxydim is rapidly absorbed into leaves, particularly when applied with an adjuvant (Ahrens 1996). Sethoxydim generally is rainfast by 1 h after application, although herbicide efficacy has been shown to be diminished when rainfall occurs 2 h after application (Bryson 1988). Sethoxydim probably moves across the plasmalemma by passive diffusion (Hosaka and Takagi 1987). The relatively acid environment outside the cell presumably allows a significant proportion of sethoxydim to remain in the protonated form which readily diffuses across the plasmalemma and into the cell. Once inside the cell, sethoxydim dissociates in the relatively alkaline cytoplasm, trapping the sethoxydim anion inside the cell due to its inability to traverse the plasmalemma, a consequence of its negative charge and low lipophilicity. Thus, the ion trapping principle facilitates a build-up of sethoxydim in the symplasm (Ahrens 1996).

Sethoxydim is systemic and translocates both in the phloem and xylem, although primarily in the phloem. It accumulates in meristematic areas of shoots and roots, but the rate of translocation out of treated leaves is low and the extent of translocation is limited (Harker and Dekker 1983; Wills 1984).

The mechanism of action of sethoxydim, as with other cyclohexanedione and aryloxyphenoxy propionate herbicides, is inhibition of ACCase, the enzyme catalyzing the first committed step in fatty acid synthesis (Burton et al. 1989; Rendina and Felts 1988). Inhibition of fatty acid synthesis presumably blocks the production of phosphoplipids used

in building new membranes required for cell growth. Broadleaf species are naturally resistant to cyclohexanedione and aryloxyphenoxy propionate herbicides because of an insensitive ACCase. Similarly, natural sethoxydim tolerance in red fescue appears to be due to a less sensitive ACCase (Stoltenberg et al. 1989). An alternative mechanism of action has been proposed, involving destruction of the electrochemical potential of the cell membrane, but this mechanism remains hypothetical (Stoltenberg et al. 1989).

Sethoxydim detoxification occurs rapidly in plants, particularly tolerant grasses. In quackgrass, barnyardgrass, alfalfa, and navy beans, 98% of applied sethoxydim was shown to be metabolized to at least nine by-products within 24 hours after application (Chandler and Paul 1982). Two of these metabolites were possibly photodegradation products. One of the two metabolites was identified as desethoxy-sethoxydim.

Sethoxydim is characterized by a low use rate for weed control (Young et al. 1996), low mammalian toxicity (Ahrens 1996), little movement in soil (Koskinen et al. 1993; Smith and Hsiao 1983), rapid degradation (Campbell and Penner 1985b; Shoaf and Carlson 1992), and as having a soil half-life of 2-13 days (Koskinen et al. 1993; Ross and Lembi 1985; Shoaf and Carlson 1992). These characteristics make sethoxydim a prime candidate for the development of sethoxydim-resistant monocotyledonous crops.

1.7 HERBICIDE TOLERANT CROPS: INTEREST AND DEVELOPMENT

Burnside (1992) states crop resistance is essential to have selective herbicides. Most modern herbicides exhibit selectivity to certain plant species rather than being non-selective. Generally, crop tolerance has been identified by evaluating a large number of chemical compounds against a limited number of major crops and weeds. Once a selective herbicide is identified, the environmental impact of that herbicide must be determined. Herbicides with potentially deleterious effects on the environment have been developed and registered. It could be more beneficial to identify herbicides that are environmentally benign and then identify resistant crop genotypes. This approach to identifying selective herbicides is the reverse of most past screening procedures, but has the potential of reducing many negative impacts of herbicides on the environment, facilitating more use of alternative weed control procedures, solving present and new weed control problems more expeditiously.

Knake (1992) suggests that recent interest in developing herbicide-tolerant crops has intensified for several reasons. In the field, farmers were faced with more carryover problems than anticipated from some new herbicides. Further concern about water quality has increased interest in exploring other approaches that might allow expanded use of some herbicides considered to have certain environmental advantages (Knake 1992). Interest in developing herbicide-tolerant crop cultivars, clones, or hybrids has been accelerated by the reduction in the rate of discovery of new herbicidal compounds, the rising expense of developing new herbicides, and the advent of biotechnological tools that have greatly increased our ability to develop herbicide tolerant genoytpes (Gresel 1979).

This scientific approach is particularly promising for minor crops for which new herbicide development is essentially lacking.

Potential benefits of developing herbicide tolerant crops include an increased margin of safety with respect to herbicide injury, reduced risk of crop damage from residual herbicides to rotational crops, and introduction of new herbicides for use on normally susceptible crops (Harrison 1992). Other potential advantages include the use of environmentally-sound herbicides, superior weed control for specific weed problems, better or more options for herbicide use in minor acreage crops, more weed control options and greater flexibility in both timing and in varying cropping systems, and improved management options for resistant weeds (Giaquinta 1992; Wyse 1992; Kishore et al. 1992).

The introduction of herbicide-resistant row crops could have far reaching consequences on crop production as a whole, and on weed control in particular (Shaw 1995). Giaquinta (1992) suggests that just because herbicide-tolerant crop technology is available does not mean it will be readily adopted. The adoption of herbicide resistant crops into agriculture and the concomitant market success will be determined by value added weed control, environmental and safety attributes of the herbicide, quality and acceptibility of germplasm which carries the herbicide-tolerant trait, availability of seed, economic benefit, regulatory framework, management of product and environmental stewardship issues, and public acceptance.

The molecular basis for natural herbicide selectivity can be most often traced to one of three possible biochemical mechanisms. These are herbicide detoxification, target enzyme insensitivity, and lack of herbicide uptake or translocation (Kishore 1992). Duke

(1996) describes the three mechanisms as metabolic detoxification, resistance at the site of action, and prevention of the herbicide from reaching the site of action. These mechanisms are operative during germplasm selection either by screening or mutational breeding. In many respects, the techniques offer an alternative to gene transfer technologies for generation of herbicide-tolerant crops (Kishore 1992).

The first mechanism is generally due to an alteration in the herbicide target site that prevents the herbicide from binding to that site and inhibiting the process mediated by the target site. An uncommon variation on this mechanism is for the plant to have many more copies of the target site than are found in susceptible plants, therefore it takes much more herbicide to effectively block the process for which the target molecule is required (Duke 1996).

Metabolic detoxification generally means that the plant can degrade the herbicide faster than the herbicide can cause irreversible damage to the plant. The speed of enzyme degradation can vary with plant growth stage, weather, and other factors (Duke et al. 1991). Thus, the same herbicide application rate might be phytotoxic to a species under some circumstances and not damaging under others. The degradation products of herbicides generally have little or no phytotoxicity, except in the case of pro-herbicides, which must be metabolically activated by the target plant (Duke et al. 1991). Some herbicides exert their effects through production of metabolites in the plant. Resistance to these herbicides can sometimes be attributed to metabolic detoxification of these toxic compounds (Duke et al. 1996).

The third mechanism for resistance occurs when the herbicide fails to reach its molecular target site. The molecular target site might be far removed from the site of

herbicide application (Duke et al. 1991). For example, the herbicide may need to be translocated from leaves to apical meristems to be effective. Thus, blockage of movement to the site of action, which can occur at the plant surface (leaf cuticle or root epidermis), just inside the plant (cell wall or plasma membrane), or in vascular tissues in which translocation occurs, would result in resistance (Duke et al. 1991). Sequestration of the herbicide in a metabolically inactive site is a variation on reducing movement of the herbicide to the site of action. Just as plants sequester phytotoxins which they produce themselves, herbicides sometimes can be partitioned into metabolically inactive sites. Sequestration has been used to explain the natural resistance of cotton to some herbicides and evolved paraquat resistance in some weeds (Duke et al. 1991).

Differential herbicide tolerance has been demonstrated for many crops and almost all classes of herbicides (Harrison 1992). Tolerant crop genotypes have arisen from insertion of genes conferring tolerance (Horsch et al. 1988), selection of tolerant mutants within a cultivar at the cell to whole plant level (Horsch et al. 1988; Sebastian and Chaleff 1987), and selection from naturally varying populations within a crop species (Barentine et al. 1976). In many cases where the genetic mechanisms have been studied, herbicide tolerance was simply inherited (Frey and Harrison 1990; Sebastian et al. 1989). For herbicide tolerance to be most useful, it should be transferable between cultivars through conventional plant breeding methods (Frey and Harrison 1990; Sebastian et al. 1989).

The ability and capacity to introduce and express genes in plants has vastly increased during the last 10 years. Since the generation of the first transgenic tobacco (*Nicotiana tabacum* L.) and petunia (*Petunia hybrida* Vilm.) plants, the science of gene introduction and expression in plants has seen rapid growth. To date, nearly 50 plant

species spanning both monocot and dicot families of plants have been transformed (Frey and Harrison 1990).

1.8 CHARACTERIZATION OF SETHOXYDIM-TOLERANT CORN

Sethoxydim-tolerant corn plants have been regenerated from tissue cultures selected for callus growth in the presence of sethoxydim. The plants were heterozygous for a single, partially dominant allele that conferred tolerance to sethoxydim (Parker et al. 1990a). Herbicide resistance originates from a partially dominant, allelic, nuclear mutation resulting in an altered form of the acetyl-coenzyme A carboxylase (ACCase) enzyme (Marshall et al. 1992). Initial laboratory data supports that these plants were more than 40 times more tolerant to sethoxydim than plants regenerated from tissue cultures not exposed to sethoxydim (Parker et al. 1990a).

Parker et al. (1990a; 1990b) used tissue culture selection to isolate sethoxydim and haloxyfop tolerant maize tissue cultures. Herbicide-tolerant corn plants were regenerated from three tissue cultures surviving sethoxydim treatment. These selections were labeled S1, S2, and S3, and two selections surviving haloxyfop treatment were H1 and H2. Segregation for tolerance in the progeny of each mutant indicated that the tissue cultures were heterozygous for partially dominant, nuclear mutations conferring herbicide tolerance (Parker et al. 1990a; Marshall et al. 1992). Decreased ACCase activity was evident in both sethoxydim and haloxyfop treated cultures as compared to the wildtype, which the naturally ocurring ACCase.

Somers et al. (1990) also selected corn tissue cultures for tolerance to sethoxydim and haloxyfop. Plants were regenerated from six different herbicide -tolerant tissue cultures. In progeny of all six selections, herbicide tolerance was inherited as single, partially dominant mutations (Somers et al. 1990). Of five herbicide -tolerant lines

characterized at the time the research was conducted, all appear to be alleles of the same locus. Homozygous herbicide-tolerant plants derived from two selections were characterized because they represent the extremes in herbicide-tolerant phenotype (Somers et al. 1990). It was reported that S2 plants are tolerant to both sethoxydim and haloxyfop whereas H1 is only tolerant to haloxyfop. Somers et al. (1990) reported that ACCase activity levels extracted from homozygous S2 and H1 seedlings were similar to the ACCase activity extracted from wildtype susceptible corn. However, the ACCase activity of S2 plants was less susceptible to inhibition by sethoxydim and haloxyfop compared to the wildtype ACCase, indicating that herbicide tolerance was conferred by altered ACCase.

Somers et al. (1990) reported that plants regenerated from the herbicide-tolerant tissue cultures appeared normal in all aspects of plant development compared with plants regenerated from the nonselected tissue cultures. Parker et al. (1990a) and Somers et al. (1990) sought to further charactertize the mutant phenotypes by determining the lethal rate of sethoxydim to wildtype, homozygous, and heterozygous tolerant seedlings. Sethoxydim was lethal to wildtype plants when applied at 0.05 kg/ha. Plants generated from S2 tissue survived all rates of sethoxydim applied. Homozygous seedlings of S2 and S3 exhibited only slight bleaching and some reduction in plant height, but were not killed, at rates of 7 kg/ha. This represents a rate that is 140 times the lethal rate for wildtypes. An intermediate phenotype was recognized, which was the heterozygote mutant derived from crosses with S lines and wildtytpes. It exhibited a tolerance that was intermediate between the wildtype and the homozygote.

Inheritance of the herbicide tolerance trait was determined by herbicide treatment of progeny of self-pollinated, regenerated plants, and reciprocal testcrosses of regenerated plants to wildtype plants (Somers et al. 1990). The segregation ratio of selfed progeny approximated a 1:2:1 segregation ratio for susceptible/injured/tolerant plants. Progeny of reciprocal test crosses of regenerated plants to wildtype plants segregated 1:1 for susceptible/injured phenotypes. These results further substantiate the fact that the regenerated plants were heterozygous for a partially dominant, nuclear mutation conferring herbicide tolerance.

In field studies, homozygous sethoxydim-tolerant corn was treated with sethoxydim applied at rates up to 0.88 kg ai/ha, which is over four times the normal use rate (Dotray 1993). The homozygous plants (Marshall 1990) that were treated with sethoxydim were produced from the self-progeny of plants generated from callous culture (Parker 1990). These treatments were applied to 3- and 7-leaf sethoxydim-tolerant corn and did not produce any visible symptoms, apparently non-injurious to the sethoxydim-tolerant corn line; but was lethal to the parental line that was not sethoxydim tolerant. The results of these experiments allow the broadcast postemergence applications of sethoxydim for weed control (Dotray et al. 1993).

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