Abstract:
Kochia is a summer annual weed introduced to North America as an ornamental plant. Early emergence, rapid growth, tolerance to both salinity and moisture stress, rapid biomass accumulation and prolific seed production offer competitive advantages to kochia. Frequent use of the sulfonylurea herbicides in wheat and barley fields has resulted in selection for sulfonylurea resistant populations of kochia. The appearance and spread of resistance in kochia has been rapid.

Seed production of self- and cross-pollinated branches of 12 plants was similar indicating kochia is self compatible. Differences in time of maturation of floral parts was observed. In some kochia plants the style emerged and was receptive to pollen for about a week before pollen of the same flower was shed. Pollen-mediated gene flow of resistance to chlorsulfuron from large resistant populations to small artificial populations was demonstrated. Percent resistance of progeny ranged from 0 to 13%. Gene flow of resistance averaged 4 to 4.5%. Thus, schemes for management of resistant kochia should consider pollen as a potential source of resistance.

Inheritance of resistance to chlorsulfuron was investigated using reciprocal crosses of resistant and susceptible genotypes of kochia. The level of resistance of the heterozygous F2 population was lower than the expected 75% indicating some heterozygous plants were killed. A portion of the progeny derived from homozygous resistant plants was also killed when treated with chlorsulfuron. The resistance trait could, therefore, be either dominant or semi-dominant, and appeared to be under the control of one gene.

The viability of kochia pollen was evaluated. Germination of pollen on agar media containing various ions, sugars, and hormones was extremely low. Maximum germination, which ranged from 2.9 to 17.8%, was recorded when pollen was incubated on a dry surface for two to three days at high relative humidity. Pollen longevity was influenced by temperature and humidity, and ranged from less than a day to twelve days depending upon treatment.

MANAGEMENT, INHERITANCE, AND GENE FLOW OF RESISTANCE

TO CHLORSULFURON IN KOCHIA SCOPARIA L. (SCHRAD)

by

Dawit Mulugeta

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ABSTRACT

Kochia is a summer annual weed introduced to North America as an ornamental plant. Early emergence, rapid growth, tolerance to both salinity and moisture stress, rapid biomass accumulation and prolific seed production offer competitive advantages to kochia. Frequent use of the sulfonylurea herbicides in wheat and barley fields has resulted in selection for sulfonylurea resistant populations of kochia. The appearance and spread of resistance in kochia has been rapid.

Seed production of self- and cross-pollinated branches of 12 plants was similar indicating kochia is self compatible. Differences in time of maturation of floral parts was observed. In some kochia plants the style emerged and was receptive to pollen for about a week before pollen of the same flower was shed. Pollen-mediated gene flow of resistance to chlorsulfuron from large resistant populations to small artificial populations was demonstrated. Percent resistance of progeny ranged from 0 to 13%. Gene flow of resistance averaged 4 to 4.5%. Thus, schemes for management of resistant kochia should consider pollen as a potential source of resistance.

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CHAPTER 1

LITERATURE REVIEW

Kochia

Kochia (<u>Kochia scoparia</u> L. Schrad), also known as burning bush, fireweed, belvedere, railroad weed, and ironweed, is an annual herbaceous dicot native to Eurasia that was introduced to North America in the early eighteen nineties as an ornamental plant because of its bright red autumnal color. Kochia quickly escaped cultivation and now infests cultivated fields, fallow land, roadsides, ditch banks, and open waste areas (Durham and Durham, 1979).

Kochia belongs to the Chenopodiaceae, a family with 100 genera and 1200 to 1500 species. To date, 45 species of kochia are known (Standley, 1916). Holm et al. (1979) limited the current world distribution of kochia to the US, Canada, Argentina and Afghanistan. However, workers in Europe and Russia have also studied kochia (Drost-Karbosuska, 1978; and Khamadamov et al., 1976).

Kochia is troublesome in sugarbeets (<u>Beta vulgaris</u> L.) (Weatherspoon and Schweizer, 1969), wheat (<u>Triticum</u> sp.) (Buhler et al.,1985), sunflower (<u>Helianthus annus</u> L.) (Durgan and Dexter, 1984), and in a number of other crops including barley (<u>Hordeum vulgare</u> L.), oats (<u>Avena sativa</u> L.), and flax (<u>Linum usitatisimum</u> L.) (Dexter, 1982). Kochia is common in

the great plains from Texas to Canada, and east as far as Mississippi. In Montana, Schweitzer et al. (1988) found kochia more abundant under conservation tillage regimes than in conventionally tilled fields. Similarly, the frequency of occurrence in sunflower fields was associated with reduced or no till cropping systems (Durgan and Dexter, 1984). In Nebraska, kochia was among the ten most common weeds of wheat and wheat stubble but not of fallow land (Wicks et al., 1984). While kochia is normally regarded as a troublesome weed, it has the potential to be a beneficial forage species (Erickson, 1947).

Morphology

The following are some morphological traits of kochia as described by Harrington (1964), and Davis (1952):

Kochia is a summer annual weed that emerges early in the spring, is highly variable in appearance, either bushy or erect with mature plants ranging from a few cm to 2 m . Growth is usually monopodial, indeterminate, and often highly branched with a taproot system. Foliage is dark green when young and turns brownish red with maturity. Leaves are simple, numerous, hairy, sessile, narrow and pointed, lanceolate and linear, 2.5 to 5.0 cm long and 0.8 to 8.0 mm wide. The stem is usually smooth but pubescent. Stem color varies from green or yellowish green to green streaked with red, and becomes purplish red in the fall. Kochia seed is

small (2 to 3 mm long), finely granular, dull grayish black, rough, flat, and ovate shaped with a fragile, shell-like hull (calyx) that encloses the seed.

Growth and Development

Early emergence and establishment coupled with rapid growth offers kochia distinct survival and competitive advantages especially where moisture stress is common (Evetts and Burnside, 1972). Since kochia emerges from cool soil early in the growing season, it is a troublesome weed in crops that are planted early. Kochia can also be a problem in a wide range of crops because emergence extends for a relatively long period following the onset of spring (Smith et al., 1975).

Kochia thrives in saline soils (Braidek et al., 1984). Surprisingly, kochia's growth rate is lower under wet conditions than in dry soils (Wiese and Vandiver, 1970). Evetts and Burnside (1972) found that seedling shoot and root growth were much faster than common milkweed (Asclepias syriaca L.) when grown under moisture stress conditions. Kochia root elongation, as with most plants, exceeded the rate of shoot growth (Wiese, 1968).

Davis et al. (1967) compared root profiles of seven weed species and sorghum (Sorghum bicolor L. Moench), and found that the root system of kochia was among the largest. Kochia roots can penetrate to a depth of 5 m and extend laterally 2.4

m (Phillips and Launshbaugh, 1958). Alternatively, the kochia root system was among the smallest of nine weeds studied by Davis et al. (1965) in Texas.

Water use efficiency of kochia was lower than Russian thistle (Salsola iberica Sennen & Pav.) and comparable to wild oat (Avena fatua L.) and redroot pigweed (Amaranthus retroflexus L.) (Baker, 1974). Kochia is drought tolerant (Pafford and Wiese, 1964) and has one third to one half the water requirement of cereal crops (Coxworth et al., 1969). Of the eight weeds compared by Nussbaum et al. (1985), kochia was among the three which grew tallest, produced the most dry matter, was the highest in water use efficiency, had the highest heat unit accumulation and seed production.
Kochia is very responsive to additions of nitrogen and other nutrients (Pafford and Wiese, 1964). Growth in kochia is indeterminate so biomass accumulation occurs during the entire growing season. Sherrod (1971) studied dry matter production under rainfed conditions and measured yields of 3.5, 8.7, and 11.3 T/ha at the prebloom, bloom, and postbloom growth stages, respectively. Yields of 12.5 T/ha have also been recorded when kochia was grown under irrigation (Rommann, 1983).

Bell et al. (1972) evaluated the flowering behavior of kochia ecotypes collected in the U.S. Flowering was induced when the photoperiod was shorter than 13 to 15 hours. When selected plants were self-pollinated for three generations, the time from emergence to flowering varied from 57 to 100

days among the progeny tested. Exposure to ultraviolet light reduced leaf blade and internode length, and increased leaf production of kochia (Barnes et al., 1990).

Kochia seedlings were attacked by a damping off organism, tentatively identified as <u>Phythium</u> <u>deBaryum</u>. In addition, a leaf spot organism caused stunted growth and gradual death in cool, rainy weather (Erickson, 1947). Inserra et al. (1984) found kochia to be a less favorable host and more tolerant to a nematode <u>Nacobbus</u> <u>aberrans</u> than sugar beet. Hinks et al. (1990) evaluated the feeding preference of a grasshopper (<u>Melanoplus</u> <u>sarguimipes</u>) among kochia, oat and wheat. Grasshoppers which were fed on kochia had the highest egg viability but biotic potential (including survival, development and reproduction) was highest when fed wheat and lowest in kochia. They predicted that kochia would have adverse effects on grasshoppers when it was the dominant plant species consumed.

Seed Biology

Kochia is a prolific seed producer. A single plant can produce from 14,600 (Stevens, 1932) to 23, 350 seeds (Nussbaum et al., 1985). Seed yields of 2.8 T/ha (Coxworth et al., 1969) and 1.8 T/ha (Erickson, 1947) were reported for kochia grown for forage.

The tumble weed habit exists in a number of taxonomic groups including the Chenopodiaceae, Amaranthaceae, and Poaceae. On a worldwide basis about twenty percent of the

tumble weed species are members of the Chenopodiaceae (Becker,1968).

Becker (1978) studied the anatomical, histochemical and mechanical aspects of stem abscission. In the fall, progressive desiccation of the plant is accompanied by the gradual loss of stem flexibility. The corresponding increase in rigidity and brittleness at the base of the stem causes the plant to eventually succumb to external forces and the stem breaks. There was significant reduction in the wind stress requirement to affect abscission over time due to the effect of a fungus that degrades the nonlignified wall of the abscission zone.

Unlike other tumble weeds, stem abscission in kochia is not related to development of a distinct abscission layer, or to chemical dissolution of pectic material (Becker, 1968). In a related shrub species, Kochia indica, an increase in ethylene evolution and cellulase activity was measured at the site of abscission in the transition region between root and stem (Zeroni et al.,1978).

Following abscission, the entire plant, with a portion of the seeds intact, may be blown for many kilometers, dispersing thousands of seeds enroute. The influence of seed invasion from the area surrounding a strip mine reclamation site was studied. High numbers of kochia seeds were introduced as a result of tumbling (Archibold, 1980). Kochia tumbling is an effective means of seed dispersal.

The response of kochia seeds to different environmental factors is well documented. Chepil (1946) analyzed survival of more than fifty weed species and concluded that kochia seeds did not persist for two years in soil. Everitt et al. (1983) also concluded that kochia had no seed dormancy. Burnside et al. (1981) compared germination of exhumed seeds of 12 weed species in Nebraska. Kochia seeds, unlike most of the other weeds studied, lost viability rapidly. At a low rainfall site, few seeds survived ten years of burial however complete loss of viability occurred at a high rainfall site after just one year of burial.

In Colorado, dormant and nondormant seeds were buried for three years at depths ranging from 1 to 30 cm. Seeds were recovered and germination tests were conducted. The results showed that viability loss from the initially nondormant population was significant at burial depths of 10 cm or less. Dormant and nondormant seeds buried 10 to 30 cm deep had 2 to 3 percent viability after three years (Zorner et al., 1984).

Zorner et al. (1984) observed that nearly all kochia germination occurred before herbicides were normally applied thus he concluded that chemical control would provide effective control if the appropriate herbicides were employed. Short seed longevity and effective chemical control means that kochia biotypes would change rapidly in response to changes in both control practices and crop production systems (Burnside et al., 1981).

Seed germination was not inhibited by the chloride salts of Ca, K, Na, and Mg, or the sulfate forms of Na or Mg at conductances up to 20 mmho. Moreover, germination was only slightly reduced when soil pH was as low as 2 and as high as 12, and was only decreased by moisture stress when osmotic potential reached 8 bars. In addition light was not required for kochia germination (Everitt et al, 1983). Evetts and Burnside (1972) also detected similar responses to moisture stress. About half of the stressed seeds were able to germinate at 13.2 bars. Radicle and hypocotyl growth was normal at salt concentrations up to 1000 ppm. The optimum range of pH recorded for germination was 2 to 8. Although kochia seedling mortality was high, the ability of seeds to germinate under extremes of moisture tension, pH and salinity indicate that the species is adapted to a wide range of soil conditions.

Romo and Haferkamp (1987) suggested that Kochia prostrata, a shrub related to kochia with moderate tolerance to NaCl and KCl may have potential for regeneration of salt-affected soil in the intermountain range lands of the U.S. Kochia could probably serve the same purpose.

The Forage Value of Kochia

Much attention has been given to the potential feed value and nutritional composition of kochia for use as a forage crop. Evaluation of the forage value began with extensive field and laboratory research in South Dakota

(Erickson, 1947). Erickson found kochia hay to be palatable and nutritionally comparable with alfalfa in terms of digestible proteins, fat, and fiber if harvested when 60 to 75 cm tall. In addition, kochia had abundant leaf growth, a high level of drought tolerance, grasshopper resistance, and good hay aroma.

Sherrod (1971, 1973) analyzed macro and micro-nutrients, crude fiber, and protein levels. He concluded that the high nutritive value of kochia, especially at the earlier stages of growth, made it a good forage candidate for livestock. He reported crude protein and crude fiber value ranged from 13.2 to 25.0% and 17.9 to 37.0%, respectively. Research in Saskatchewan, Canada, confirmed that the protein level of kochia was higher than that of native grasses, and comparable to the best of the introduced forage species (Bell et al., 1952). Alfalfa (Medicago sativa L.) and kochia were found to be nutritionally comparable (Kiesling et al., 1984).

Although kochia hay contains similar amounts of digestible nutrients as other forage crops, and the plant survived under stress when most grass species died, it can be toxic. In Oklahoma, forage yields as high as 12.5 T/ha were obtained under irrigation. Despite high yields, it is not recommended for use as a forage by some because of the high oxalate content, and low palatability at the end of the season (Rommann, 1983). The digestibility of kochia in a sheep ration increased as the kochia to alfalfa ratio increased,

however, nitrogen retention by the animals was generally low (Sherrod, 1973).

The palatability of kochia seeds was studied by Coxworth et al. (1969). In a fourteen day feeding study, they measured a 6.7 to 9.5 g weight reduction in mice when fed a ration which contained 28 to 35% kochia seeds due to excessive nitrate concentrations. Nitrates, if consumed in large quantities, will interfere with animal health and can cause death (Kingsbury, 1964).

Interference with Crop Growth

Early emergence, rapid growth, prolonged presence during the growing period of crops in the field, and adaptation to stress conditions are the major characteristics that offer kochia a competitive advantage over crops and other weeds (Nussbaum et al., 1985). Estimates of yield losses incurred due to kochia competition vary with density, growth stage, period of competition, and location.

In a two year study, sugarbeet root yield was reduced 95% when kochia was allowed to compete for the entire season. When kochia was controlled for the first 3 to 4 weeks of crop growth, sugarbeet yield was not reduced (Weatherspoon and Schweizer, 1969). One kochia plant per 8 m of row reduced the average sugarbeet yield by 2.6 T/ha and lowered sucrose content in the roots by more than 1 T/ha (Weatherspoon and Schweizer, 1971). Using these and other data, Schweizer

(1973) produced a model designed to predict the reduction in root yield of sugarbeet caused by specific densities of kochia. The accuracy of his model decreased as the density of kochia increased.

Arp (1969) measured the relative light intensity reaching sugarbeet plants growing under a kochia canopy. Kochia spaced 60 to 75 cm apart reduced light intensity by 60 to 80%. When kochia and wild oat were grown individually or together with sunflower for two weeks, sunflower achene yield was reduced 20% (Durgan and Dexter, 1984). Yield reduction was less than additive with mixed wild oat and kochia infestations than with either species alone. In Nebraska, a weed infestation consisting of 54% redroot pigweed, 21% kochia and 25% annual grass weeds growing in a band in onion (Allium cepa L.) rows for 4, 5, and 8 weeks reduced yield 20, 40 and 65%, respectively (Wicks et al., 1973). Competitive ability is also affected by differential response of kochia biotypes to herbicides. Salhoff and Martin (1985) reported reduced competitive ability of atrazine resistant kochia biotypes.

The allelopathic effects of kochia on crop plants have been studied. Spowles (1981) reported that kochia was the dominant pioneer species in denuded areas of the southeastern United States. Following the first year, kochia density decreased dramatically in successional stands. Wali and Inverson (1978) recorded an average kochia height of 1 m in pure stands. The following year, kochia seedlings occurred in

very high density and the resulting plant height was only 3 to 6 cm. They speculated that the total disappearance of kochia after 3 to 4 years was due to the autoallelopathic nature of decaying leaves and roots.

Sugarbeet emergence was reduced by germinating kochia seeds at densities greater than one seedling per square centimeter when the fungus Rhizopus was present. Interactive effects of the fungus with unidentified compounds from kochia were believed to cause reduced emergence (Wiley et al., 1985).

Lodhi (1979) evaluated the autotoxic properties of kochia phytotoxins on germination, radicle, and seedling growth. Germination was not inhibited and reached nearly 100% in 24 hours when tested against different phenolics and flavinoids including caffeic acid, chlorogenic acid, ferulic acid, myricein and quercetin. There was a pronounced effect on radicle growth which supports earlier observations that high seedling density drastically reduced growth of kochia in the second season on reclaimed mine soil. These compounds are also known to reduce the quality and palatibility of forages (Martem, 1973). Aqueous extracts of stem and leaves of kochia affected radicle and shoot growth of blue grama (Bouteloua gracilis [H. B. K.] Lag.), but had no effect on seed germination (Karachi and Pieper, 1987). Kochia leaf extracts reduced seedling growth and water potential of sorghum and soybean (Glycine max L.) (Einhellig and Schon, 1982).

Chemical Control

Kochia is controlled by numerous herbicides in a variety of crops. The competitive ability of kochia often necessitates the use of herbicides for optimum crop yields. Bell et al.(1972b) compared the response of thirteen selections of kochia to 2,4-D ((2, 4-dichlorophenoxy) acetic acid), dicamba (3, 6-dichloro-2-methoxybenzoic acid), and picloram (4-amino-3, 5, 6-trichloro-2-pyridinecarboxylic acid). All selections were tolerant to picloram, but there was wide variation in injury, growth and seed production following treatment with 2,4-D and dicamba. Response differences were attributed to physiological differences among kochia selections. Response of selections to dicamba was generally independent of their response to 2,4-D.

Control increased when herbicides were tank mixed. A tank mixture of cycloate (S-ethyl cyclohexylethylcarbamothi oate) plus R-11913 applied as a preplant treatment reduced the stand of kochia by 89% compared to 19% with cycloate alone (Schweizer, 1973b).

It has been difficult to control kochia in sugarbeets because both belong to the same plant family. While benzadox provided good control of kochia in sugarbeets, the activity was temperature dependent (Weatherspoon and Schweizer, 1970). Phenmedipham (3-[(methoxycarbonyl) amino] phenyl (3-methylphenyl) carbamate) has given satisfactory control of kochia without injuring sugarbeet (Smith et al., 1975).

Burnside and Carlson (1983) compared several early preplant foliar and soil applied herbicides for no-till production of soybean in Nebraska. Kochia was effectively controlled by metribuzin (4-amino-6-6 (1, 1-dimethylethyl) - (methylthio)-1, 2, 4-triazin-5 (4H)-ow), diuron(N'-(3, 4-dichlorophenyl)-N, N-dimethylurea) , and tank mixed treatments of metribuzin with metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide), and prodiamine with oryzalin (4-(dipropylamino)-3, 5-dinitrobenzenesulfonamide), all applied at normal field application rates.

Best control was often obtained when herbicide use was integrated with optimum production practices. In Nebraska, cycloate or ethofumesate ((±)-2-ethoxy-2, 3-dihydro-3, 3-dimethyl-5-benzofuranyl methanesulfonate) plus trifluralin (2, 6-dinitro-N, N-dipropyl-4-(Trifluromethyl) benzenzmide) were injurious when applied to direct seeded sugarbeet, and kochia control was poor. However, when applied to transplanted sugarbeets, crop injury was minimized and control was much improved (Wilson et al., 1987).

Nonselective herbicides commonly used for conservation tillage provided good control of kochia. These included glyphosate (N-(phosphonomethyl) glycine), HOE-39866, and paraquat (1,1'-dimethyl-4, 4'-dipyridinum ion) (Blackshaw, 1989). Preemergence treatment of cyanazine (2-[[4-chloro-6-(ethylamino)-1, 3, 5-triazin-2-yl] amino]-2-methylpropane

nitrile) and oryzalin have also provided excellent control (Flake and Ahrens, 1987).

Crop safety is a priority in any weed control program. While benazolin gave adequate control of kochia, it was injurious to soybean. Nevertheless, Nord and Gillespie (1984) established the optimum dosage and soybean growth stage for satisfactory control of kochia.

The development of herbicide resistance is associated with continuous use of herbicides possessing the same or similar mode of action (Gressel and Segel, 1982). Extensive triazine herbicide use along railroad right of ways has resulted in triazine resistant kochia populations (Johnston and Wood, 1976; Burnside et al., 1979). The selected plants have a high degree of cross resistance to all of the commercial s-triazine herbicides (Burnside et al., 1979).

Recently, triazine resistant kochia populations were found in cultivated fields and waste areas in at least eleven western states (Bandeen et al., 1982). The first observation of sulfonylurea resistant kochia was made in a wheat field in Kansas treated with chlorsulfuron (2-chloro-N [[(4-methoxy-6-methyl-1, 3, 5-triazin-2-yl) amino] carbonyl] benzenesulfonamide) for five consecutive years (Primiani et al., 1990). Sulfonylurea resistant kochia populations have recently been reported in ten states and two Canadian provinces (DuPont Co., unpublished information).

The Sulfonylurea Herbicides

The sulfonylurea herbicides were discovered in the mid-1970's. This family represents a major advancement in agricultural chemistry because of low application rates, low mammalian toxicity, excellent crop safety, and flexibility of application timing (Levitt et al., 1981). By May, 1989, more than 375 sulfonylurea herbicides had been patented, most of them by the DuPont company (Brown, 1990).

Crop Use

In the early to mid-1980's, extensive studies were conducted on weed control and crop tolerance to chlorsulfuron. Brewster and Appleby (1983) reported that chlorsulfuron at rates up to 140 g/ha did not reduce wheat grain yield, however soil residues following application rates of 35 g/ha injured snap bean (Phaseolus vulgaris L.), alfalfa (Medicago sativa L.), sweet corn (Zea mays L.), sugarbeet and rape (Brassica campestris L.) one year after application. Phytotoxic levels of the herbicide were present 10 to 20 cm deep in a silt loam soil 168 days after application.

In a similar study conducted at several locations in Montana, Burkhart et al. (1984) determined that the dry weight of pinto bean, safflower, corn, and sugarbeet was reduced two years following chlorsulfuron application at rates of 35, 70 and 140 g/ha. Variation in susceptibility to sulfonylureas was shown to exist not only among different crop species but

also among cultivars of the same species (Hageman and Behrens, 1981).

One outstanding feature of the sulfonylureas is the wide spectrum of weeds controlled. Apart from the common annual weeds like kochia, the mustard species, and Russian thistle, the spectrum of control extends to perennial plants including Canada thistle [Cirsium arvense L. (Scop.)] (Donald, 1987; Dyer, 1983) and woody perennial plants like Texas white brush (Alaysia gratissima Gillies and Hook) and Macartney rose (Rosa bracteata J. C. Wendl.) (Meyer and Bovey, 1990).

The potential use of chlorsulfuron in susceptible crop plants has been studied. Parker (1980) showed increased tolerance of corn, rice (Oryza sativa L.) and sorghum to chlorsulfuron when applied with safeners, 1,8 napthalic anhydride or R-25788. They suggested the possibility of controlling itchgrass [Rottboellia exaltata (L.) T.F.(Rooex)] and red rice (Oryza sativa L.) in maize and rice, weeds which are difficult to control in those crops. BAS-145-138 mixed with chlorsulfuron also reduced corn injury (Devlin and Zbiec, 1990).

Surfactants increase the herbicidal activity of the sulfonylureas. Chow and Taylor (1980) evaluated the influence of nonionic surfactants on the level of chlorsulfuron toxicity in oilseed rape and found a high correlation between increased activity and spray retention with surfactant use. Tankmixing the sulfonylureas with other herbicides increased control and

permitted use of lower rates which would lead to decreased soil persistence (Anon., 1989).

Chlorsulfuron tankmixed with difenzoquat (1, 2 dimethyl-3, 5 diphenyl-1H-pyrazolium) or flamprop (N-benzoyl-N-(3-chloro-4-fluorophenyl-DL-alanine) reduced wild oat control up to 35%. The antagonistic effect of chlorsulfuron was overcome by increasing the rate of the wild oat herbicide in the mixture (O'Sullivan and Kirkland, 1984). The extent of the antagonistic interaction was affected by the application method. Gillespie and Nalewaja (1989) found greater antagonism to triallate (S-2 (2, 3, 3-trichloro-2-propenyl) bis-(1-methylethyl) carbamothioate) when chlorsulfuron was incorporated before planting compared to a preemergence surface application following triallate incorporation. Using Anthemis cotula as a bioassay species, Howard and Whitesides (1984) found synergistic interaction between chlorsulfuron and bromoxynil (3, 5 dibromo-4-hydroxybenzonitrile).

Soil Relations

All sulfonylurea herbicides are subject to chemical hydrolysis and microbial degradation, and do not accumulate in non-target organisms (Brown, 1990). Joshi et al. (1985) found that chlorsulfuron did not degrade in sterilized soil. Aspergillus, Penicillium, and Streptomyces degraded chlorsulfuron in pure culture. Other soil microorganisms have also been isolated which can degrade sulfonylurea herbicides

in pure culture (Cited in Brown, 1990).

The effects of soil pH, organic matter, and clay content on uptake, degradation and movement in soil were evaluated (Fredrickson and Shea, 1984; Mersie and Foy, 1985; Walker et al., 1989). In several studies, organic matter was the only variable strongly correlated with phytotoxicity. Phytotoxicity to plants decreased as organic matter increased, and soil pH decreased. Degradation rate, in general, decreased with increasing soil depth and was negatively correlated with pH.

Microbial activity and bridge hydrolysis were responsible for the degradation of the sulfonylureas in soil. Depending upon the specific compound and type of soil, these chemical and microbial processes result in a typical half life of one to six weeks (Brown, 1990). Some sulfonylurea herbicides including chlorsulfuron, metsulfuron methyl (Methyl 2-[[[[(4-methoxy-6methyl-1,3,5-triazin-2-yl)-amino]carbonyl] -amino]sulfonyl] benzoate) and chlorimuron ethyl (Ethyl 2-[[[[(4-chloro-6-methoxypyrimidin-2-yl) amino] carbonyl] amino] sulfonyl] benzoate) persist for long periods of time in alkaline soils, and crop damage one or two seasons following application is not uncommon. Degradation of the sulfonylureas in soil depends largely on chemical hydrolysis, the rate of which is controlled by soil pH. Thus in alkaline soils chemical hydrolysis is minimal, and even small amounts of herbicide can injure sensitive rotational crops (Burkhart et al., 1984).

Selectivity

The causes of variation in tolerance to the sulfonylureas among crop and weed species were studied. Brown (1990) measured leaf uptake of thifensulfuron methyl (3-[[[[(4-methoxy-6-methyl-1, 3, 5-triazin-2-yl) amino] carbonyl] amino] sulfonyl]-2-thiophenecarboxylic acid methyl) in a tolerant crop, soybean, and in sensitive broadleaved weeds and found no correlation to tolerance. Sweetser et al. (1982) compared the uptake and translocation of chlorsulfuron in several sensitive and tolerant crop plants. They found small differences in leaf uptake which were poorly correlated with tolerance. Tolerant plants such as wheat, barley and oats rapidly metabolize chlorsulfuron to nonpolar compounds. In wheat plants, the metabolite was identified as an o-glycoside of chlorsulfuron in which the phenyl ring under went hydroxylation followed by conjugation with carbohydrate residues (Sweetser, et al, 1982). Metsulfuron methyl is metabolized by the same metabolic pathway in wheat as chlorsulfuron (Anderson et al., 1989).

The mode of metabolic inactivation in tolerant crops varies widely with different sulfonylurea herbicides. In wheat, thifensulfuron methyl is metabolized by three major routes: urea bridge cleavage, deesterification, and sulfonamide bond cleavage. In soybean, deesterification and conjugation with glucose are responsible for deactivation of chlorimuron ethyl (Brown et al.,1987).

The time span to metabolize the sulfonylureas varies widely in tolerant and sensitive species. Wheat plants metabolize chlorsulfuron rapidly with a half life of 1 to 3 hours. The half life for sensitive plants is often in the range of 24 to 28 hours (Sweester et al., 1982). While chlorimuron ethyl was metabolized very slowly in sensitive species such as redroot pigweed and cocklebur (Xanthium strumarium L.), the metabolic half life in soybean was 1 to 3 hours (Brown et al., 1987). Variation in rates of metabolism was also reported to account for the differential tolerance of inbred lines of corn to DPX-M6316 (Eberlin et al., 1989).

Peterson and Sweetser (1984) observed deactivation of chlorsulfuron by Canada thistle when the herbicide was added to nutrient solution. On the other hand when nutrient solution was acidified it increased uptake of chlorsulfuron in leaf and root tissues of velvetleaf suggesting a decrease in selectivity between susceptible weeds and tolerant crops by enhancing the level of phytotoxicity (Mersie and Foy, 1987).

Mode of Action

The sulfonylurea herbicides inhibit the activity of acetolactate synthase (ALS) (also called acetohydroxy acid synthase [AHAS]), the first enzyme common to the synthesis of valine, leucine, and isoleucine (Ray, 1982). The forms, distribution, regulation, kinetic properties, chemical composition and mode of interaction of acetolactate synthase

with the sulfonylurea herbicides have been studied by numerous workers. Three major ALS isozymes, each with large and small subunits, were isolated in bacteria (Reviewed in Scholass, 1990). They differed in their sensitivity to both herbicide inhibition, and feedback inhibition by branched chain amino acids.

The inhibition of pea (Pisum sativum) root growth (Ray, 1984), and mitotic division in root tips (Rost and Reynold, 1985) caused by sulfonylureas was completely reversed by the addition of valine and isoleucine to the growth medium. Likewise, Scheel and Casida (1985) demonstrated partial reversal of chlorsulfuron-induced growth inhibition by leucine, valine, or 2-ketoisovalerate. Alternatively, Giardina et al. (1987) found no reversal of inhibition by chlorsulfuron with the addition of valine and isoleucine to corn and pea seedlings.

The manner in which ALS is regulated in higher plants is highly variable. Miflin and Cave (1972) demonstrated the presence of cooperative feedback regulation of ALS by leucine and valine in a range of higher plants. The enzyme from developing pea seed is inhibited by valine, but no evidence for possible multivalent control was found (Davies, 1964). Contrary to these findings, ALS from Phaseolus radiatus was not subject to feedback regulation (Satyanarayana and Radakrishran, 1963).

Mg^{++} or Mn^{++}, thiamine pyrophosphate, and FAD are co-factors of ALS (Durner and Boger, 1990). ALS activity is entirely localized in chloroplasts (Jones et al., 1985) or in the case of yeast, in mitochondria (Ryan and Kohlhaw, 1974). ALS from a wide range of plant species was very sensitive to the sulfonylureas (Ray, 1984). ALS is also sensitive to structurally unrelated groups of compounds: the imidazolinones (Shaner et al., 1984), the triazole pyrimidines, and the sulphonanilides (Durner and Boger, 1990). Since none of the herbicides that act on ALS have structural similarity to either the substrates, cofactors or allosteric effectors they are unusual enzyme inhibitors (Scholass et al., 1988).

Other than ALS inhibition, several other effects on treated plants were noted. Hatzios and Koch (1982) observed increased oxygen transport and reduced CO_2 fixation in chlorsulfuron-treated fababean (Vicia faba L.) where chlorsulfuron uncoupled photophosphorylation. However, the amount of chlorsulfuron required to inhibit photosynthesis in pea was 10,000 fold greater than the amount needed to inhibit growth (Ray, 1984). Inhibitory effect on cell cycle progression from G2 to mitosis and subsequent inhibition of DNA and RNA synthesis was also observed (Rost, 1984).

Resistance

Resistance to the sulfonylurea herbicides was reported to be due to a less sensitive ALS enzyme (Chaleff and Mauvais,

1984). Causes for reduced sensitivity of the enzyme are well documented. In <u>Arabidopsis thaliana</u>, the DNA sequence of the mutant gene was compared with that of the wild type. A single base substitution was found where cytosine was changed to thiamine (Mazur et al., 1987). Likewise, a single base mutation was detected in <u>Escherichia coli</u> ALS gene which changed alanine to valine resulting in an enzyme with resistance to sulfometuron methyl (Yadav et al., 1986). On the other hand, Muhitch et al. (1987) have found the existence of one or two base changes in a mutant ALS tobacco (<u>Nicotiana tabacum</u> L.) gene which did not confer resistance. Such changes may or may not be accompanied by altered enzymatic activity of ALS.

Yadav et al. (1986) found unaltered levels of activity in mutant yeast (<u>Saccharomyces cerevisiae</u>) although a bacterial mutation in <u>Escherichia coli</u> resulted in reduced levels of activity. Saari et al. (1990) found no difference in the ALS specific activity between mutant and wild type kochia plants. Studies in suspension cultures of tobacco and cotton showed the presence of different mutations with altered properties of the ALS such as loss of feedback regulation and lower affinity for pyruvate (Subramanian et al, 1990). If similar change that influence the rate and amount of branched chain amino acid synthesis occur in field selected resistant biotypes, variation in the level of vigor and fitness of resistant and susceptible plants should be expected.

In some plants, altered ALS did not account for high levels of tolerance (Sebastian and Chaleff, 1987). They selected mutant soybean lines with increased tolerance to chlorsulfuron and chlorimuron ethyl. Tolerance was linked to a single recessive gene although the mutants contained normal ALS.

Biotypes of rigid ryegrass (Lolium rigidium L.) developed resistance to several groups of herbicides including the sulfonylureas following frequent exposure to diclofop methyl under field conditions (Heap and Knight, 1986). Metabolic detoxification of the herbicides is believed to account for the wide range of cross resistance observed (Powles and Howat,1990).

Naturally occurring populations of resistant kochia (Primiani et al., 1990), Russian thistle (DuPont Co., unpublished), prickly lettuce (Lactuca serriola L.) (Mallory-Smith et al., 1990) and common chickweed (Stellaria media L.) (Hall and Devine, 1989) were reported following repeated applications of chlorsulfuron or metsulfuron methyl. Unicellular organisms resistant to the sulfonylureas include mutants within Saccharomyces cerevisiae, Chlamydomonas reinhardtii (Hartnett et al.,1987), Escherchia coli (Yadav et al.,1986) and Salmonella typhimurium (LaRossa and Scholass, 1984). Resistant mutants were also isolated from tissue culture of Arabidopsis thaliana (Haughn et al., 1988), tobacco (Chaleff and Bascomb, 1987) and haploid suspension cultures of

Datura innoxia Mill. (Saxena and King, 1988) following mutagenesis. Inheritance studies conducted with tobacco (Chaleff and Ray, 1984; Chaleff and Bascomb, 1987; Creason and Chaleff, 1988), _Chlamydomonas reinhardtii_ (Hartnett et al., 1987) and soybean (Sebastin and Chaleff, 1989) showed that resistance is inherited as a single dominant or semidominant mutation which resides in one or two loci of the nuclear genome.

Gene Flow

Gene flow is the movement of gene by pollen, seed, or adult individuals from one point to another with subsequent establishment in the gene pool of the new locality (Levin and Kerster, 1974). Gene flow is a powerful evolutionary process that counteracts the diversifying effects of local or directional selection or genetic drift, and significantly influences the spatial distribution of genetic variation (Saltkin, 1973).

Several studies (Turner et al., 1982; Antonovics, 1968) showed that extensive gene movement leads to genetically similar populations over a wide range of spatial distribution while limited gene flow results in the genetic substructuring of populations. Knowledge of pollen and seed mediated gene movement is needed to understand the patterns of variation among populations and to assist in predicting the dynamics of a population over time.

Pollen and seed movement are influenced by a number of factors including wind, the ballistics of animal mediated seed dispersal, the plant reproductive system, pollinator behavior, the physical properties of seed and pollen, the effects of the surrounding environment, and the spatial distribution of individuals (Levin and Kerster, 1974). The direct measurement of gene flow is not easy since the movement of seed, pollen, or individuals does not necessarily imply reproductive success or establishment (Endler, 1973). In spite of these constraints, various direct and indirect techniques have been employed to estimate gene movement.

Pollen Movement

Several approaches have been used to measure the flow of pollen among populations. Tracking the movement of dyes (Thies, 1953) or radiolabelled powder (Schlisling and Turpin, 1971) following a period of pollination activity were used. Waser and Price (1982) reported a high correlation between movement of powder and pollen for Ipomopsis agregata (Pursh) V. Grant visited by humming birds. Similarly, Handel (1983b) found fluorescent dyes useful for predicting the distance and direction of pollen flow. However, fluorescent dyes and colored powders offer little help when studying the pollination dynamics in a population.

Studies have been conducted which extrapolate the pattern of pollen flow from pollinator movement alone (Handel,

1983b). Schaal (1980) studied bumblebee pollination in _Lupinus_ _texensis_ Hook. using the distribution of isozyme markers, and found that the movement of the marker allele was restricted to the range of bumblebee flight. They concluded that pollen migration was important when _L._ _texensis_ was pollinated by bumblebees. Campbell (1985) demonstrated that pollinators which forage indiscriminately transfer pollen from one species to another which reduces the amount of pollen that reaches conspecific flowers.

Mean pollinator movement can be a poor indicator of gene movement if flower fertility is low (Handel and Mishkin, 1984). Variation in out-crossing rates could influence pollen mediated gene flow distance since strictly self-pollinated plants have no gene flow distance (Handel, 1983). Gene flow by pollen is affected by pollinator activity over a wide range of plant spacing. Beattie (1976) showed that flight distances of pollinators in _Viola_ sp. were directly proportional to spacing parameters while frequency of interplant flights and percent pollination were inversely related to spacing distances.

Direct research approaches that provide accurate estimates of pollen dispersal, success of fertilization, and production of viable seeds are available. There are artificial pollen samplers that are often used to assess dispersal of pollen and other air-borne particles that cause public health problems (Raynor, 1970). Inspection of pollen