

Management, inheritance, and gene flow of resistance to chlorsulfuron in Kochia scoparia L. (Schrad)
by Dawit Mulugeta

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
Montana State University
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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.

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.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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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 (Kochia scoparia 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 (Beta vulgaris L.) (Weatherspoon and Schweizer, 1969), wheat (Triticum sp.) (Buhler et al.,1985), sunflower (Helianthus annus L.) (Durgan and Dexter, 1984), and in a number of other crops including barley (Hordeum vulgare L.), oats (Avena sativa L.), and flax (Linum usitatisimum 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 Phythium deBaryum. 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 Nacobbus aberrans than sugar beet. Hinks et al. (1990) evaluated the feeding preference of a grasshopper (Melanoplus sarguimipes) 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 cyclohexylethylcarbamothioate) 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-(Triflurомethyl) 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 Arabidopsis thaliana, 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 Escherichia coli 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 (Nicotiana tabacum 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 (Saccharomyces cerevisiae) although a bacterial mutation in Escherichia coli 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

on stigma surfaces is a direct and more reliable technique than the use of mechanical pollen traps. However, the monitored pollen must have special morphological markers to permit identification. This condition is rare.

Movement of pollen in Erythrium grandiflorum was studied using dimorphic grain color characteristics present in some populations (Thomson and Thomson, 1989). Likewise, differences in pollen morphology have been used to study pollen transfer among taxa. Levin and Kerster (1967) demonstrated interspecific pollen movement between populations of Pilox pilosa and Pilox glaberrima using differences in pollen morphology.

Genetic markers have been used to measure pollen flow. Handel (1982) tested the dominant bitter gene of Cucumis sativus as a marker which conveys a distinctive, distasteful flavor to the cotyledons and leaves of the plant. Handel (1983) also used golden flower petals as a dominant marker to evaluate pollen flow into plants with a recessive pale yellow petal color.

Ellstrand et al. (1989) evaluated the pollen dispersal characteristics of wild radish (Raphanus sativus L.), an out-crossing species polymorphic for several isozyme loci that are expressed in both adult and seedling tissues. Similarly, gene flow in Carduus nutans L. was measured by observing the distribution of electrophoretic markers at two allozyme loci (Smyth and Hamrick, 1987).

The use of male sterile plant populations that act as pollen receiver was effectively employed to measure gene flow in Plantago lanceolata L. (Tonsor, 1985). Depending upon the specific characteristics of the study and the types of techniques used, various estimates of gene flow were made in several species. The range of gene flow is not uniform. Kirkpatrick and Wilson (1988) measured a range of 0 to 15% gene flow from Curcubita pepo L. cultivars to individual wild plants of C. texans Scheel & Gray isolated by distances of 450 to 1300 m. Similarly, Ellstrand (1988) measured rates of gene flow from 4.5% to almost 20% at isolation distances of 100 m to 1000 m in six different populations of wild radish.

Gene movement in some populations is restricted to a few meters. The average dispersal distance recorded for a marker allele in Carduus nutans was 5 m (Smyth and Hamrick, 1987). Gene flow between populations of Triticum dicoccoides separated by 10 m or more was minimal (Golenberg, 1987).

Ellstrand et al. (1989) demonstrated the importance of population size when measuring gene flow. Almost all of the gene flow observed in a small, synthetic population of wild radish originated from a large, natural population rather than from a nearby, small, synthetic population. On the other hand, William and Evans (1935) reported that gene flow into test populations that were established adjacent to a single population containing a marker allele was higher with small populations indicating that the rate of fertilization by

marker pollen decreased as the plant receptor population size increased. Therefore, density of plants and gene flow are closely related. Bateman (1947) evaluated the spread of a dominant marker allele to the surrounding plants that were established at different densities and found that the dominant gene travelled less distance in dense rather than in sparse populations.

In a given population, the spatial arrangement of plants relative to each other will influence the amount of pollen that flows within and between plants. Gleaves (1973) demonstrated that closely spaced plants resulted in reduced gene flow from outside populations because fertilization within the population was higher than for plants spaced farther apart. Differences in microclimate could also restrict gene flow due to variation in maturity. Manhall and Borman (1978) evaluated the effect of slope topography on gene flow and found significant differences in time of flowering with increasing elevation.

Pollen dispersal is also affected by the flying range of pollinators. Schmitt (1980) compared the foraging behavior of butterflies and bumblebees on three Senecio species. He found that pollen dispersal by bumblebees was localized. On the other hand, butterflies tend to bypass nearby plants and fly greater distances among plants. Pollen dispersal distance also varied within and among populations of the same species. Campbell and Waser (1989) measured stamen length in several

species that are pollinated by hummingbirds. They found that the shorter the stamen length, the farther pollen was disseminated.

Seed Dispersal

Most plants have several modes of seed dispersal which facilitate capture of new habitats and successful establishment (Howe and Smallwood, 1982). Wind mediated dispersal is aided by special seed structures such as wings, plumes and feathers (Beattie and Lyons, 1975). In wind dispersed species, wind velocity, propagule weight and height above the ground, and morphology of the dispersed structures are important factors regulating the distance of seed dispersal (Howe and Smallwood, 1982).

Animals may be attracted by nutritious fruit and aid in seed dispersal (Howe, 1980). Light weight, buoyant seeds are common features of plants growing near water (Ridley, 1930). Forceful dehiscence is another means of dispersal which enables seeds to move away from the mother plant (Beattie and Lyons, 1975).

The spatial arrangement of plants of a given species is influenced by the pattern of seed dispersal since adult distribution reflects seed distribution (Ridley, 1930). In some species the survival of seeds under the canopy of mother plants is low because of high seed density (Howe and Smallwood, 1982). In general, survival of seeds increases as

seeds are dispersed farther from the mother plant. However, Howe and Primark (1975) reported higher seedling establishment under fruiting trees of Casearia corymbosia than for seeds placed some distance away.

Some dispersal agents take seeds to places that are conducive for establishment and growth of seedlings. For instance, ant-assisted colonization often places seed in well drained, nutrient rich mounds (Davidson and Morton, 1981). The time and mode of seed dispersal are often interrelated. Several workers found that dry, windy habitats favor wind dispersal while wet sites optimize conditions for animal dispersal (Hilty, 1980; Braum, 1936).

Competition for dispersal agents is common among animal dispersed seeds. Some plant species produce limited amounts of fruit which contain large seeds in a rich, palatable pulp. This often limits dispersal to specialized birds which seek rare, bulky, highly nutritious food resources (Howe and Smallwood, 1982).

Measurement of seed dispersal depends on the habits of the dispersal agent. Seed traps have been used to assess seed dissemination by wind (Werner, 1975) or water (Skoglund, 1990). Westelaken and Maun (1985) painted seeds prior to dissemination to allow recovery after wind dispersal of Lithospermum caroliniense. Germination of seeds recovered from animal droppings has also been used to study animal dispersed seeds (Brunner et al., 1976). Dispersal has been

estimated for species which use explosive dehiscence using pod characteristics including pod length, firing angle, and initial velocity (Trapp, 1988). Artificial seeds such as colored beads have also been used to measure dispersal (Augspurger and Franson, 1987).

Pollen

Pollen represents the haploid phase of development in the life cycle of plants. Unlike other plant dispersal organs, pollen is not designed for long term survival therefore it usually germinates readily after deposition on stigmatic surfaces. Pollen is fragile and exposure to sudden and continuous changes in its immediate environment greatly affects the success of fertilization.

In many species, pollen grains are aborted, lose viability, or become shrunken even before shedding (Gwyn and Stelly, 1989). Other constraints following pollen release include: failure to germinate on the stigma, bursting of pollen tubes in the style, slow or no growth of germinated pollen tubes through the style, failure of the male gamete to fuse with the egg nucleus, or arrested embryo development after fertilization (Johri and Vasil, 1961).

Pollen and its Environment

The influence of environmental factors on pollen viability, and the influence of sugars, growth regulators,

chemicals and tissue extracts on in vitro pollen germination have been studied. Various stains have also been used to estimate pollen viability. Pollen viability has been indirectly estimated by measuring seed set after pollination. These studies have been largely confined to trees, horticultural plants, and annual crops. Little emphasis has been given to pollen of herbaceous weeds apart from morphological characterization for taxonomic purposes.

The effect of temperature and humidity on storability of pollen are well studied in several plant species. Maximum pollen longevity in different plant taxa is obtained at relative humidities ranging from 0 to 50%. The longevity of pollen, in general, is negatively correlated with storage temperature (Nebel, 1939). In contrast, the pollen of most Graminaeae species retains high viability for a short period of time when stored at 80 to 100% relative humidity (Reviewed in Johri and Vasil, 1961). The loss of viability is rapid following fluctuations in relative humidity which indicates the sensitivity of pollen to variation in its immediate environment (Bullock and Overly, 1949).

Khosh-Khui et al. (1976) measured significant interaction between temperature and humidity in six Rosa species. Optimal humidity conditions for pollen storage, as determined by staining, varied with temperature. Pollen exposure to high temperature (37 C) prior to low temperature storage (-20 C) produced fruit equal in quality to those

produced by fertilization with fresh pollen in oil palm (Elaeis sp.) (Ekaratne and Senathirajah, 1983).

Pollen has been used as a rapid assay to evaluate whole plant characteristics. For example, pollen from single plants can be tested against a wide range of stress conditions. In eight cultivars of tomato, pollen viability was used to screen for plant tolerance to high temperatures (Weaver, 1989). Mackill et al. (1982) also used pollen viability to screen for high temperature tolerance in rice cultivars.

Other environmental factors that affect pollen viability have been studied. In Pinus sp., the rate of pollen tube growth decreased in white light compared to dark, and increased in red light (Dhawan and Malik, 1981). Differences in atmospheric air pressure had variable effects on pollen viability. However, barley pollen remained viable longer at normal than at reduced pressure (Anthony and Harlan, 1920). Alternatively, reduced atmospheric pressure prolonged pollen viability in apple (Malus sp.) (reviewed in Johri and Vasil, 1961).

Methods to Measure Pollen Viability

There are several methods used for determining pollen viability and most involve routine staining procedures. Pollen grains with both an intact nucleus and cytoplasm tend to stain readily with iodine-based stains (Edwardson and Corbett, 1961) or acetocarmine (Pearson and Harney, 1984).

However, these stains often give false positive results of viability (Parfitt and Ganeshan, 1989).

Tetrazolium salts have a distinct advantage over other indicators since they only produce color when reduced (Oberle and Watson, 1953). Upon contact with viable tissue, the soluble, colorless triphenyl tetrazolium salt is reduced by reductases in living tissues giving a red or deep purple color which provides an estimate of viability.

Some reports indicate that pollen fertility is overestimated by the tetrazolium assay. Aslam et. al. (1964) evaluated seven tetrazolium salts in normal and translocation stocks of cotton and found that 2% tetrazolium chloride and 4% tetrazolium red effectively stained both normal and genetically deficient pollen grains whose fertility was doubtful. Similarly, Oberle and Watson (1953) found that tetrazolium stained sterile pollen incapable of germination and concluded that the chemical was of no value as an indicator of pollen viability in peaches (Prunus sp.), pears (Pyrus sp.), apples and grapes (Vitis sp.). Barrow (1983) has also reported tetrazolium to be a less useful indicator of pollen fertility in cotton.

Parfitt and Ganeshan (1989) compared pollen staining procedures with in vitro germination to estimate the viability of peach pollen. He found that pollen staining procedures were not reliable or consistent, and were not positively correlated with in vitro germination assays. In contrast,

some workers have found tetrazolium salt to be a good indicator of *in vitro* germinability in *Populus* species (Rajora and Zsuffa, 1986) and pine (Cook and Stanley, 1960). It appears that tetrazolium salt can identify pollen that is capable of oxidative metabolism but may not be able to germinate.

Alternative staining tests have also been used. The oxidation of benzidine by peroxidase in the presence of hydrogen peroxide gives distinctive color specific to pollen of a given species (Anon., 1960). This technique is not commonly used due to the carcinogenic properties of benzidine.

The fluorochrome reaction has been used to measure the integrity of the plasmalemma of the vegetative cell of microgametophytes. This reaction measures the presence and level of activity of several esterase enzymes essential for gametophyte function (Heslop-Harrison and Heslop-Harrison, 1970). Alexander (1980) developed a versatile stain consisting of malachite green and acid fuchsin which distinguishes between pollen grains with or without protoplasm. Following staining, aborted grains stain green while normal pollen becomes purple. Unfortunately, this stain failed to discriminate between live and dead pollen in five species of peach (Parfitt and Ganeshan, 1989). Appearance of different pollen types following treatment with IKI was also used to assess fertile and sterile pollen of petunia (*Petunia* sp.) (Edwardson and Corbett, 1961).

In vitro germination is commonly used both as an indicator of pollen fertility, and as an assessment of factors which influence metabolic processes in plants. At the time of shedding, pollen contains limited food reserves which are essential during the initial stages of germination (Brink, 1924a). Using C^{14}-labelled sugars, O'Kelly (1955) demonstrated the role of sources of sugars during pollen tube growth.

While most pollen grains germinate in media containing simple sugars (Johri and Vasil, 1961), the germination requirements of other species may be more complex. Using pollen from 80 plant species, Brewbaker and Kwack (1963) demonstrated that relatively few pollen grains germinated, and that many of those that germinated grew poorly without certain additives. They increased germination and rate of growth with the addition water extracts of specific plant tissue rich in calcium ion. Pollen germination and pollen tube growth of 13 species were stimulated by manganese sulphate concentrations ranging from 10^{-4} to 10^{-10} M (Loo and Hwang, 1944).

Pollen, in general, is often deficient in boron thus the requirement for boron exceeds that for hormones, vitamins or other chemicals (Johri and Vasil, 1961). Hormone addition is frequently used to increase germination and tube growth of pollen. Indole-3-acetic acid (IAA), gibberellic acid (GA), ethylene, abscissic acid, and cyclic AMP at low concentrations (1 to 10 mg/l) promoted germination and tube growth of *Pinus roxburghii* pollen (Dhawan and Malik, 1981). Similar results

were also found with IAA, GA, succinic acid and fumaric acid in Allium cepa (Kwan et al., 1969). Nevertheless, some reports indicate that some hormones have no effect on either pollen germination or pollen tube growth (Rietsema, 1961). Extracts from stigma and other floral parts had variable effects as germination stimulants (Brink, 1924b) and inhibitors (Sasaki, 1919 as cited in Johri and Vasil, 1961).

CHAPTER 2

GENE FLOW OF RESISTANCE TO CHLORSULFURON BY POLLEN IN KOCHIA

Introduction

The sulfonylurea herbicides control a broad spectrum of weeds and have good crop selectivity at extremely low rates of application (Brown, 1990). Chlorsulfuron, the first commercialized sulfonylurea herbicide, was used extensively on large acreages of wheat and barley in the United States and Canada during the 1980's. Repeated use of such persistent sulfonylurea herbicides led to the appearance of resistant populations of kochia (Primiani et al., 1990), prickly lettuce (Lactuca serriola) (Mallory-Smith et al., 1990), Russian thistle (Anon.,1990) and common chickweed (Stellaria media) (Hall and Devlin, 1989). Results of a recent survey (personal communication with, Dupont Co. personnel, 1991) indicate that the number of states with sulfonylurea resistant weed populations has increased from three in 1987 to ten in 1990. Resistant kochia accounted for more than 85% of the reported sites in North America.

The observed variation in the appearance and spread of the resistance trait among the naturally occurring populations of resistant weeds can be explained by the biological characteristics that influence the processes of ecological fitness and gene flow (Maxwell et al.,1990). The sexual transfer of genes among different populations of the same

species (Campbell and Waser,1989; Smyth and Hamrick, 1987) and even among populations of different species (Kirkpatrick and Wilson, 1988) is common.

The normal range of gene flow is a function of several parameters including the breeding system of the species (Ellstrand and Hoffman, 1990), the population structure (Gleaves, 1973), the size of the pollen source (Ellstrand et al., 1989) or recipient population (William and Evans, 1935), the foraging behavior of pollinators (Schmitt, 1980) and the microclimate (Jackson, 1966). Various estimates of pollen-mediated gene flow have been made among populations of several species (Ellstrand et al., 1989; Smyth and Hamrick, 1987; Handel, 1982; and Handel, 1983b).

Resistance to the sulfonylurea herbicides in kochia (See Chapter 3), and other species (Chaleff and Ray, 1984; Chaleff and Bascomb, 1987; and Haughn and Somerville, 1986) was demonstrated to be a dominant or semi- dominant trait. The objective of this study was to determine if pollen-mediated gene flow of resistance to chlorsulfuron was occurring among populations of kochia under field conditions.

Materials and Methods

Gene Flow by Pollen

Field Experiment Pollen-mediated gene flow from a large, resistant natural population into a small, artificial

population of susceptible kochia was studied at Great Falls and Conrad, Montana. Both sites were selected in early June, 1990 and had a 4 to 5 year history of continuous chlorsulfuron use. They were planted either with barley or wheat. The kochia populations in both fields were confirmed to be resistant to chlorsulfuron by making field observations and measuring seedling response to chlorsulfuron in the greenhouse in the fall of 1989.

The distribution pattern of resistant kochia plants was similar in both fields. Typically, there were numerous patches of densely growing plants (30 to 120 per sq. meter) covering an area of 800 to 1000 m by 100 to 200 m in wet, saline areas at the edges of each field. Kochia plants were also growing sparsely throughout the cultivated field. Small populations of kochia were also present along the roads, ditchbanks, and waste places in the area surrounding the cropped field.

In June, 1990, susceptible kochia plants were obtained from two areas with no history of chlorsulfuron use located at least 25 km from either experimental site. Susceptible plants were dug and transplanted into 20 and 14 sites in Conrad and Great Falls, respectively, around the large resistant populations at distances ranging from 0 to 4.3 km away (Figure 1). The susceptible plants were transplanted into pots when they were 20 to 25 cm tall, and replanted the same day. After establishment, and prior to flowering, one to eight branches

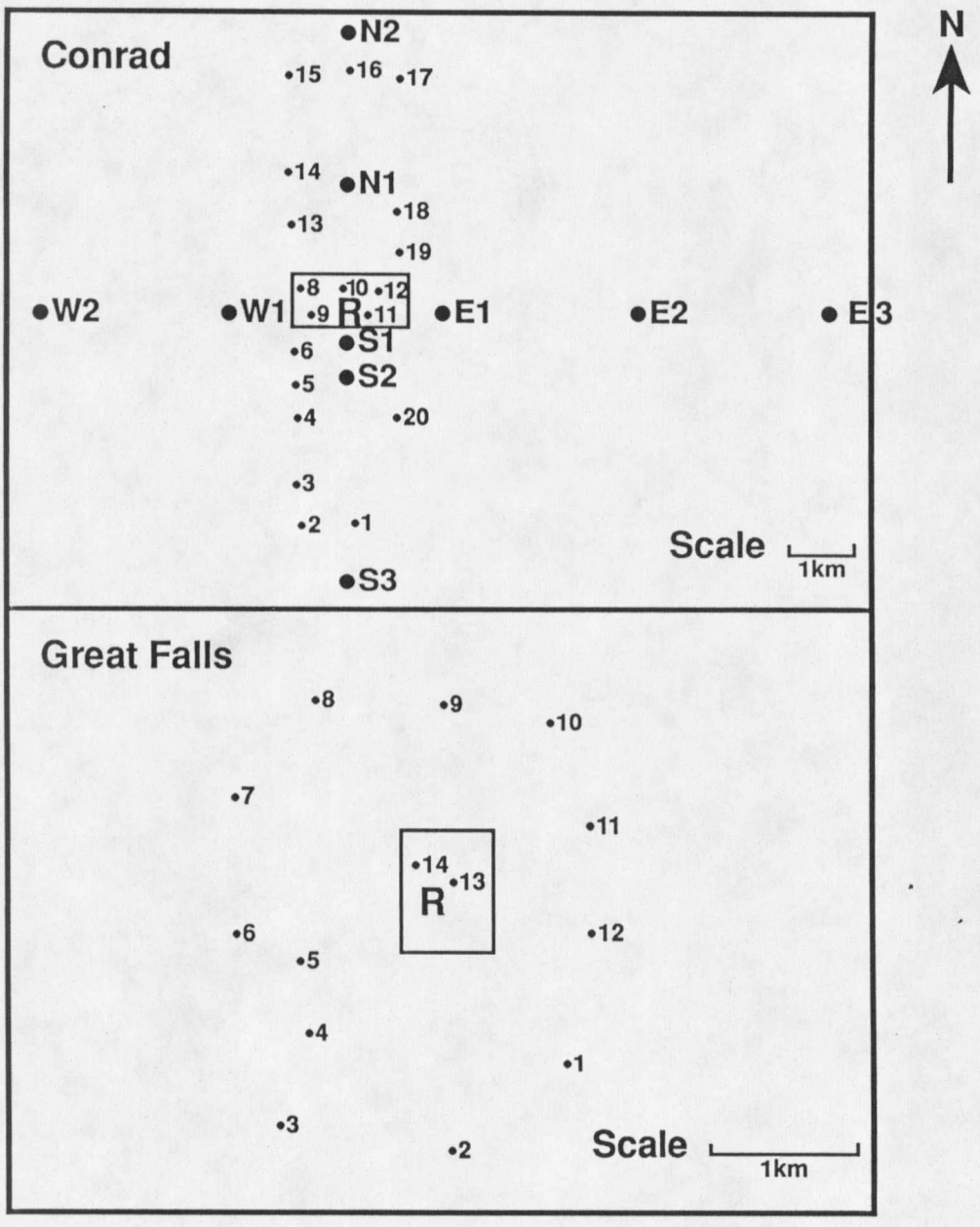


Figure 1 A Diagram of the Field Site where Experiment on Gene Flow of Resistance to Chlorsulfuron was Conducted in Kochia.

Key - Numbers indicate the location of chlorsulfuron suseptible artificial population of kochia.

- N_1 and N_2 (north), W_1 and W_2 (west)..are the location of crop fields with different history of chlorsulfuron use at the Conrad site..

- Large natural population of clorsulfuron resistant kochia are indicated by R.

from each susceptible plant were bagged with 5 cm by 25 cm paper bags to prevent out-crossing. An equal number of branches of susceptible plants were left open to permit out-crossing. Periodic visits were made to water the transplanted susceptible plants, and to evaluate flowering behavior. In early October, 1990, seeds were collected from bagged and open pollinated branches of each susceptible plant in artificial populations, from 50 to 60 plants in the large resistant populations, and from 10 to 20 plants that were growing within a 50 m radius of the artificial, susceptible population at both sites. Seeds were also collected from 10 to 20 plants of ten crop fields surrounding the experimental site in Conrad, each with varying histories of chlorsulfuron use.

Greenhouse Study

Seeds collected from the field were planted in 60 X 40 X 10 cm flats filled with peat moss and fine sand (2:1). Seeds were planted 0.5 to 1.0 cm deep and seedlings emerged two to three days later. Plants were watered with tap water as needed and grown under a 14 hour photoperiod maintained by metal arc halide lamps to supplement natural sunlight. Day and night temperatures were 24 and 20 C, respectively. Chlorsulfuron was applied twice at 144 g/ha two and three weeks after emergence. This split application procedure coupled with the high rate of application provided better plant coverage and ensured that only resistant plants would survive treatment. Application was made when seedlings were

3 to 5 cm tall, and one week later in a spray volume of 87 l/ha using a moving nozzle laboratory sprayer equipped with flat fan (8002E) nozzle. Nonionic surfactant (0.25% v/v) was added to the herbicide mixture. The number of plants established just before spraying (A), and those (B) which survived three weeks after the second treatment, were recorded, and percent resistance [(B/A) X 100] was computed. Plant damage was visually rated using a scale of 0 to 100%, 0 being no effect, and 100% representing complete kill.

Compatibility Study

Field Experiment

Seed production of self-pollinated and open-pollinated branches was measured to determine if kochia is self-compatible. In early May, 1990, kochia seeds were sown in twelve 5 X 5 m plots at the Arthur Post Research Farm, Bozeman, Montana. The number of kochia plants established in each plot ranged from 10 to 20 per square meter. The average size of plants at maturity, and the number of branches per plant were 80 cm and 50, respectively. Single plants were selected from the center of each plot and 8 to 10 branches were bagged as described above prior to flowering. The remaining branches from the plant were left unbagged so that cross-pollination with the surrounding plants could take place. When the plants reached senescence, self-pollinated and open-pollinated branches were collected, their length was

measured, and the number of seeds per unit length of branch was determined. Seed production of self pollinated and open-pollinated branches was determined for branches on 12 plants.

Greenhouse Study

Twenty four plants were grown in 15 cm pots in the greenhouse under the growing conditions described above. All flower buds except one were removed from each plant before flowering, and each plant was bagged. The success or failure to produce a viable seed from each bagged plant was determined.

Results and Discussion

Gene Flow by Pollen

Kochia plants were established with ease following transplanting. Fifty-five and 90% of the transplanted susceptible kochia plants survived, flowered, and produced seed in Conrad and Great Falls, respectively. Flowering in the field started in early August and extended until mid-September. Individual plants produced flowers for more than two weeks. This long period of pollen production might have facilitated out-crossing due to the variability in wind direction over time.

The response to chlorsulfuron of seedlings from seed produced by open pollinated flowers of susceptible plants that were established around the large resistant populations was tested (Table 1). Extreme variation in both seed production

Table 1. Effect of Chlorsulfuron on Seedlings from Seed Produced on Open-Pollinated Branches of Susceptible, Artificial Populations of Kochia at Two Locations in Montana in 1990.

Conrad				Great Falls			
Susceptible Plant Location	Susceptible Mother Plants Present	Seedlings Treated	Resistance	Susceptible Plant Location	Susceptible Mother Plants Present	Seedlings Treated	Resistance
	No.	No.	-%-		No.	No.	-%-
1	1	41	2.4	1	2	57	2.1(±3.0)
2	2	208	4.1(±3.6)	2	1	27	7.4
3	2	101	-0-	3	1	2	-0-
4	1	62	9.6	4	1	123	6.5
5	2	32	3.7(±5.2)	5	3	546	3.9(±3.3
6	5	502	5.4(±5.9)	6	1	48	4.2
7	2	80	-0-	7	2	106	6.3(±3.8)
8	1	51	-0-	8	1	144	-0-
9	5	527	4.4(±2.5)	9	1	37	8.1
10	2	117	9.1(±1.2)	10	3	639	2.7(±2.8)
11	4	5	2.2(±2.2)	11	3	357	2.2(±2.0)
12	1	71	4.2	12	1	336	2.7
13	1	124	4.8	13	2	102	6.0(±2.1)
14	4	154	2.7(±3.4)	14	1	250	7.6
15	4	242	2.6(±3.2)				
16	1	18	5.6	–	–	–	–
17	1	49	4.1	–	–	–	–
18	1	37	-0-	–	–	–	–
19	1	58	1.7	–	–	–	–
20	2	141	3.3(±1.7)	–	–	–	–
Total	43	2620	Mean 4.5		23	2774	Mean 4.0

and seedling establishment was observed among kochia plants.

Three weeks after chlorsulfuron treatment all of the progeny from self-pollinated branches of susceptible kochia plants at both sites showed typical symptoms of chlorsulfuron damage which led to eventual death. In contrast, progeny from open branches of susceptible plants displayed varying levels of resistance. The percent resistant seedling plants obtained from a single susceptible plant location ranged from 0 to 8.9% in Great Falls and up to 13.3% in Conrad. The maximum resistance obtained for a seedling population obtained from individual susceptible locations at Great Falls and Conrad was 8.1 and 9.6%, respectively.

The source of resistant pollen could not be identified in this study. However, the effect of chlorsulfuron on seedlings obtained from kochia plants growing within 50 m of the susceptible, artificial population was tested (Table 2). In Conrad, all kochia plants growing in proximity to each susceptible plant location possessed varying degrees of resistance except near locations 14 and 17. More than 75% of the susceptible plant locations were established along roads, ditches and waste areas, locations not expected to harbor resistant plants since those areas and plants were probably never treated with sulfonylurea herbicides. Apparently gene flow occurred from resistant plants in nearby fields either by pollen and/or seed dispersal.

Table 2. Effect of Chlorsulfuron on Seedlings from Seed Produced by Plants Growing within 50 m of the Radius where Each Artificial, Susceptible Kochia Population was Located.

Conrad			Great Falls		
Susceptible Location	Seedlings Treated	Resistant Seedlings	Susceptible Location	Seedlings Treated	Resistant Seedlings
	-- No.--	--- % ---		-- No.--	--- % ---
1	315	90	2	120	40
2	197	91	3	139	50
3	270	81	4	143	89
4	225	93	5	138	33
5	163	68	6	8	75
6	196	92	7	197	29
7	187	81	8	34	56
9to13[a]	282	94	9	117	41
14	150	0	10	23	56
17	128	0	11	182	52
18	64	75	12	146	77
20	138	0	13and14[a]	430	87

[a]Susceptible plant locations located in the cultivated area of the field which contained the resistant kochia population

No kochia was found growing in the immediate vicinity of the susceptible artificial populations at locations 15, 16, and 19 in Conrad. However, some of the progeny of the susceptible plants were resistant (Table 1) therefore the source of resistant pollen must have been from resistant plants growing more than 50 m away.

Kochia plants located in crop fields surrounding the experimental site in Conrad were evaluated for their response to chlorsulfuron (Table 3). The experimental site at Conrad was the first location in Montana where chlorsulfuron failed to control kochia following repeated use (personal communication with DuPont Co. personnel).

Table 3. Effect of Chlorsulfuron on Seedlings from Seed Produced by Kochia Plants Collected in other Cultivated Fields Surrounding the Resistant Kochia Location in Conrad.

Field Location from the Resistant Kochia Site		Chlorsulfuron History		Resistant Seedlings
Direction	Distance	Years of Use	Dates of Use	
	(Km)	(No.)		-- % --
North	1.6	0	- 0 -	3
North	4.0	0	- 0 -	2
South	0.8	0	- 0 -	98
South	1.6	0	- 0 -	85
South	4.8	0	- 0 -	70
East	1.6	2	1988-89	97
East	4.8	1	1987	80
East	8.0	1	1989	94
West	1.6	0	- 0 -	72
West	4.8	0	- 0 -	71
Resistant Site		5	1985-89	97

Crops growing in the surrounding fields were wheat or barley, and the production practices, including time and method of land preparation and crop rotation, were similar. Chlorsulfuron use in the sampled fields varied from none to five continuous years. High levels of resistance, from 70 to 98%, were measured in five crop fields with no previous history of chlorsulfuron use indicating the resistance gene had moved into those fields (Table 3). The kochia populations in two crop fields located north of the resistant site had no history of chlorsulfuron use and were almost entirely susceptible.

The presence of resistant populations to the south and east can probably be attributed to pollen-mediated gene flow since the prevailing wind direction during kochia flowering

was from the northwest. Although three sites east of the experimental site had a history of only one or two years of chlorsulfuron use, a high degree of resistance was found in those populations. The high frequency of resistant populations in those fields can probably be attributed to gene flow acting in concert with the selection pressure imposed by chlorsulfuron use.

Compatibility Study

There was no difference in seed production in plants with self- and open-pollinated flowers indicating kochia is self-compatible on a whole plant level (Table 4). When individual flowers on 24 plants were self-pollinated, 17 plants produced viable seeds. The maturation time of floral parts in kochia is not identical. In some plants, the stigmas emerged up to one week before pollen was shed. During this

Table 4. Seed Production of Self-Pollinated and Open-Pollinated Flowers of Kochia.

Source of Seed	Kochia Branches Tested per Plant	Seeds Produced per cm of Branch
	No.	No.
Self Pollinated Flowers	8.6	31.3
Open Pollinated Flowers	7.6	29.7

period the stigma may have been receptive to foreign pollen. In other plants, by the time pollen was shed, the stigma appeared aged and may have been unreceptive to pollen from the same flower; this variability in maturation period of floral parts ensures gametic exchange among plants and among flowers

of the same plant.

The objective of this study was to evaluate the effect of distance and direction of susceptible populations from large resistant populations on the patterns of gene flow. Surprisingly, the distribution of resistant kochia plants was not limited to the cultivated field as expected. Instead, resistant plants were found growing abundantly along fences, ditches, waste areas and in crop fields that, most likely, had never been treated with chlorsulfuron. While this observation alone provides direct evidence for the existence of gene flow, the source of pollen which gave rise to resistant progeny is unknown. The most likely plants to serve as a source of resistant pollen are those growing in the immediate vicinity of the susceptible plants. Similar studies have shown that gene flow by pollen increased as the distance between the pollen source and receptor population decreased (Tonsor, 1987 ; Handel, 1983). Despite this, the large resistant population must have been the source for at least some of the resistance since kochia pollen is dispersed in large quantities during the peak flowering period. A portion of that pollen is capable of maintaining its viability for a few days under high temperature and low humidity (See Chapter 5).

Variability in the percent resistant progeny produced from susceptible parent plants was high, both among and within susceptible populations at both sites. This could be attributed to differences in flowering period which is a

function of plant size. The variation in pollen flow within and among populations could be influenced by differences in flowering time of the pollen donor and receiver plants (Schmitt, 1983), style length (Waser and Price, 1984) and whether pollen grains are clumping (Tonsor, 1985).

Herbicide resistance could be a valuable genetic marker to evaluate gametic exchange among populations. The allelic frequencies of the resistant gene would have to be known before herbicide resistance could be used to measure out-crossing among populations. If the plant of interest is diploid like kochia (Cooper,1935), and if herbicide resistance is a dominant trait controlled by a single gene, as in kochia (See Chapter 3), single pollen grains shed by plants heterozygous for the trait could carry either the resistant or susceptible trait. In field populations, susceptible plants would occur in varying proportions in resistant populations depending upon the level of selection pressure imposed. In this situation, pollen carrying either the resistant or susceptible gene would be dispersed and effect fertilization. While the success of fertilization by pollen carrying the resistant gene could be easily quantified by testing progeny with herbicide treatment, this procedure cannot detect the flow of genes by susceptible plants fathered by susceptible pollen since they would be killed by herbicide treatment. Therefore, estimation of gene flow under natural conditions will always be underestimated.

Models that explain the appearance, rate of increase, and management tactics required to prevent, delay, or reduce herbicide resistant weeds have been developed (Gressel, 1986; Maxwell et al., 1990). According to these models, the rate of evolution of resistance is a function of the initial frequency of the resistance gene among other things. The rate of increase, spread, and other dynamics of resistant weed populations are affected by factors related to ecological fitness including seed and pollen production, competitive ability, fertility, and gene flow. The appearance and spread of resistance to the sulfonylurea herbicides was rapid in kochia compared to other weed species. By June 1991, more than 200 sites contained resistant kochia populations in North America (personal communication with DuPont Co. personnel, 1991).

Comparison of the relative fitness of resistant and susceptible biotypes of kochia showed that biomass production and rate of growth of the resistant biotypes were comparable to susceptible biotypes (Christoffoleti and Westra, 1991). Alcocer-Ruthling and Thill (1991) on the other hand, found substantial differences in most of the growth parameters studied in susceptible and resistant biotypes of prickly lettuce.

Biochemical evidence that support these findings are available. Yadav et al. (1986) found a mutation in Escherichia coli that resulted in reduced level of ALS

activity and ALS sensitivity to valine. Similarly, Subramanian et al., (1990) demonstrated the presence of different mutations in suspension cultures of tobacco and cotton, with altered properties of the ALS to feed back regulation by branched chain aminoacids. Nevertheless, in kochia no difference in ALS has been found between mutant and normal biotypes (Saari et al, 1990).

The collective evidence suggests that the comparative fitness of mutants could be influenced by the mutation type that occurred in the ALS gene. Since several populations of kochia are reported to develop resistance to the sulfonylurea herbicides independently, it is reasonable to suspect the presence of several different mutations, therefore the relative role of ecological fitness and gene flow in influencing the dynamics of resistance should depend on the nature of each individual mutant population. In spite of these assumptions, gene flow seems to be more important than ecological fitness in kochia as all evidence obtained to date supports the notion that mutant biotypes are as equally fit as wild types.

Based on these data, gene flow in kochia appears to be extensive. Even when no kochia plants were growing within a 50 m radius, up to 5.6% of seedling progeny from susceptible artificial populations of individual kochia plants were resistant to chlorsulfuron. Estimation of mating rates of populations isolated by distance depends on the breeding

behavior of each species. Four to 15% gene flow was recorded for populations of Pseudostsuga menziesii isolated by a distance of 2000 m (reviewed in Ellstrand and Hoffman, 1990). However, gametic exchange of Triticum dicoccoides was minimal between populations separated by only 10 m because the plant is largely self-pollinated (Golenberg, 1987). Though kochia is self-compatible, the sequential maturation of male and female floral parts undoubtedly plays a key role in promoting gene flow among populations.

While gene flow via pollen is an important factor, seed dispersal may be just as important a component of gene flow in kochia since viable seeds can travel long distances when detached plants tumble (See Chapter 3).

CHAPTER 3
SEED PRODUCTION AND SEED MEDIATED GENE FLOW IN KOCHIA

Introduction

The tumbling habit, which occurs in several plant taxa (Becker, 1968), makes kochia a successful colonizer of new habitats. Many seeds are dispersed during tumbling. However, exact information on the amount of seed dispersal, and the distance of seed dispersal is lacking.

Seed dispersal has been studied in several plant species (Werner, 1975; Skoglund, 1990; Westelaken and Maun, 1985; Brunner et al., 1976; Trapp, 1988; and Augaspurger, 1987). Following dispersal, portions of a seed brood may die, fail to germinate or be consumed by animals (Howe and Smallwood, 1982). Thus, seed dispersal does not imply successful establishment of progeny. Therefore, most seed dispersal measurements overestimate gene flow.

The purposes of this study were to evaluate the effectiveness of cutting as a management technique, to study the growth characteristics of kochia that influence seed production and tumbling, and to determine the seed dispersal and seed mediated gene flow patterns of kochia under field c conditions.

Materials and Methods

Season 1

Plant growth, dry matter accumulation, and seed production of kochia were studied during the summer and fall of 1990 at the Arthur Post Research Farm, Bozeman, Montana. A 2.4 ha (185 m by 130 m) cultivated field devoid of kochia was clean cultivated and left unplanted (Figure 2). Following cultivation, kochia seeds were hand sown on a 30 m X 50 m area on May 20, 1990. There were 13 treatments arranged in a randomized complete block design with four replications. Treatments included six dates of clipping two weeks apart. Plot size was 3.3 m by 2.4 m. Plants were clipped at the soil surface or at the midpoint of the shoot. At each clipping time, plant height, canopy width, and number of branches per plant were recorded from ten randomly selected plants per plot. In addition, biomass production and seed yield were determined for each treatment from a one sq. meter area periodically and at maturity, respectively. Samples of 20 to 50 seeds each from ten plants were used to estimate the average weight of a single seed. The remaining plants were left undisturbed for the rest of the season. Assessment of the effect of wind direction, the amount and type of plants detached and blown by wind, and the percent of seeds dispersed were made. Analysis of variance was computed for all the data, and correlation coefficients among all growth parameters were also calculated (Lund,1987).

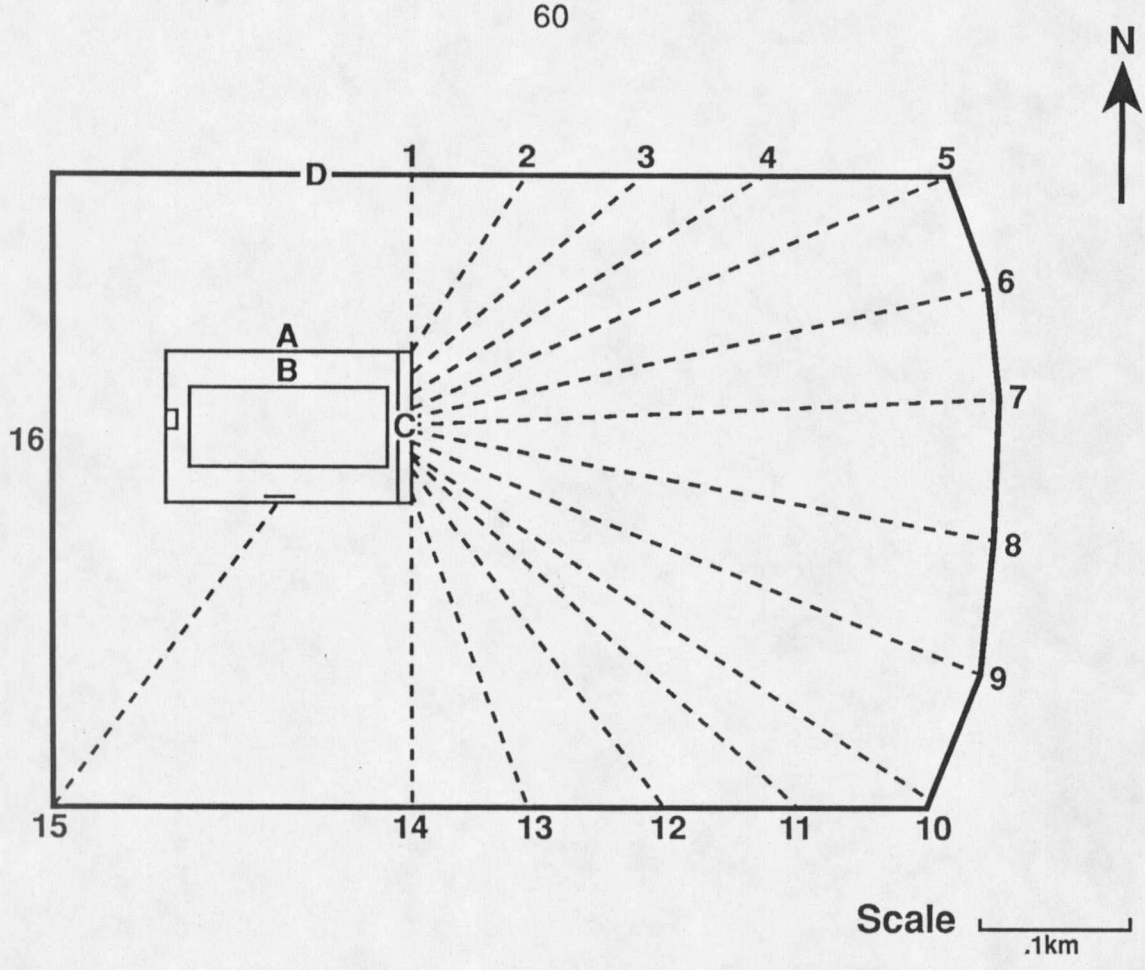


Figure 2 A Diagram of the Experimental Site where Kochia Seed Production, Seed Dispersal, and Establishment of Seedlings were Measured.

Key

- A = Site where kochia were established in season 1.
- B = Seed production study site.
- C = Spot from which counting of established kochia seedlings began.
- D = Cultivated field with no kochia history.
- Numbers and broken lines indicate directions at which counting of kochia seedlings was made.

Season 2

Germination of dispersed seeds and establishment of seedlings in the surrounding cultivated field were evaluated. On May 15, 1991 sixteen permanent transects were established by driving 1.5 cm diameter X 1 m metal rods 0.98 m into the soil in the configuration shown in Figure 1. A central rod was placed in the center of the 30 m X 50 m kochia planting described above. A tape measure, anchored at the central rod, was stretched tightly to each rod. Kochia seedlings per 0.09 sq. meter were counted at 4 locations at the central rod, and at 6.3 m intervals along each transect. At the 30 m X 50 m plot where kochia was established the preceding season, 10 permanent plots, assigned to low (600 to 800 plants per sq.meter) , medium (2600 to 3000 plants per sq. meter) and high seedling density (26000 to 30000 plants per sq. meter) were established to evaluate the effect of initial kochia plant density on subsequent growth, biomass, and seed production of the surviving kochia plants.

Results and Discussion

Season 1

Growth parameters measured during the growing season are summarized in Table 5. Plant height, number of branches per plant, canopy width, and biomass accumulation increased with time. Plant density decreased over time. When measurements on plant height, density, biomass production,

Table 5. Growth Characteristics of Kochia during the Growing Period.[a]

Time of Cutting (Weeks After Emergence)	Plant Density	Plant Canopy Width	Branches per Plant	Plant Height	Dry Matter Production of Plants	
					Fullcut	Halfcut
	No./m^2	(cm)	(No.)	(cm)	(T/ha)	
4	21	18 a	5 a	16 a	0.05 a	0.14 a
6	15	51 b	25 b	60 b	0.72 a	1.56 a
8	18	54 b	39 c	93 c	1.91 ab	3.75 ab
10	14	85 c	50 d	144 d	2.92 bc	6.46 bc
12	11	82 c	54 e	149 ed	4.39 c	7.64 c
14	14	101 d	55 e	155 e	4.37 c	9.62 c

[a] Means within a column followed by the same letter are not significantly different using Duncan's Multiple Range Test.

and seed yield were made at harvest, the highest values were obtained for plants that were cut early (Table 6). Regrowth of plants cut during the first four weeks after emergence was rapid. Therefore, biomass accumulation and seed production were significantly higher than for later cutting treatments.

Seed production was reduced significantly when kochia plants were cut at the base of the stem six weeks after emergence (Table 6). When plants were cut at the midpoint of the stem, seed production was much higher than for plants cut at the base. Therefore, the regrowth potential of half cut kochia plants was much higher than for the full cut plants.

Seed yield of kochia from uncut plants was more than 2.9 T/ha. When kochia plants were cut at the base 4 and 14 weeks after emergence, seed yield was reduced 39 and 100% respectively. Similarly, when kochia plants were cut at the midpoint 4 and 14 weeks after emergence, seed yields were reduced 37% and 79%., respectively. Earlier studies reported seed yields ranging from 1.8 T/ha (Erickson, 1947) to 2.9 T/ha (Coxworth et al., 1989).

As kochia plants mature, the base of the stem loses flexibility. Wind stress eventually causes the stem to break at the base and the tumbling plant scatters seeds (Becker, 1979). The degree of wind stress is directly related to plant size, growth form, plant density, and the growing conditions which occurred during plant development.

Table 6. Effect of Cutting Time and Cutting Intensity on Plant Height, Density, Dry Biomass Production and Seed Yield of Kochia Regrowth at Maturity.[a]

Time of Cutting (Weeks After Emergence)	Cutting Intensity (Full or Halfcut)	Plant Density	Plant Height	Dry Matter Accumulation	Seed Yield
		(No./M^2)	(cm)	(T/ha)	(T/ha)
4	F	8	118 fg	12.96 d	1.83 ecd
	H	4	111 f	11.27 cd	1.89 ed
6	F	8	37 bc	3.38 ab	0.47 abc
	H	15	80 ec	8.42 bcd	1.66 ecd
8	F	10	28 ab	1.60 a	0.46 abc
	H	9	66 de	6.42 abc	1.07 abcd
10	F	4	18 ab	0.93 a	0.20 ab
	H	17	56 dc	4.98 abc	1.53 bcd
12	F	1	10 a	0.24 a	0.01 a
	H	15	70 de	4.10 ab	0.62 abcd
14	F[b]	0	0	0	0
	H	15	76 de	6.40 abc	0.67 abcd
Check	-	16	140 g	14.21 d	2.99 e

[a] Means within a column followed by the same letter are not significantly different at P = 0.05 using Duncan's Multiple Range Test.

[b] Excluded from statistical analysis.

Plants grown under high densities or those found inside each plot had a narrow canopy width and were less exposed to wind stress. The bushy plants found at the edge of plots accumulated more biomass and detached readily.

Kochia plant tumbling started in early October before some of the plants were fully senescent and extended throughout the winter. The first plants to tumble were the most globose which were usually growing in isolation or at the edge of each plot. Very few seeds had dehisced from these plants when tumbling began so much of the seed dispersed in the surrounding area was attributed to them. The majority of plants found inside each plot stayed in place and did not tumble. Some were detached by wind long after their seeds had fallen to the ground.

Plants grown in dense infestations had more flexible stems and were less vulnerable to detachment by wind stress. Becker (1979) noted that environmental conditions and other factors including light intensity, plant density during the growing period, and shade influenced both plant shape and basal stem anatomy in kochia.

Extreme variation in nearly all of the growth characteristics measured was observed among kochia plants in each plot, although all seeds were obtained from a single location. Over all, 30% of kochia plants were estimated to be dispersed by wind. Few of the plants that were cut at the midpoint six weeks after emergence tumbled because of the

reduction in wind stress from low biomass accumulation. Therefore, mowing before pollination could be used to reduce both pollen and seed dispersal.

Except plant density, the growth characteristics of kochia during the growing season were positively correlated (Table 7). Plant density was negatively correlated to plant height, branches per plant, canopy width, and biomass production. The density of kochia plants declined during the season due to competition for light, nutrients, and water. The regrowth potential of kochia plants cut prior to the first six weeks was high and as a result cutting plants early in the season had minimal effect on seed production. Cutting at the base of the stem after six weeks but before the onset of seed production was most effective in reducing seed yield.

Seed yield was strongly correlated with biomass production, plant height and plant density at maturity

Table 7. Correlations Among Some Growth Characteristics of Kochia During the Growing Season of 1990.

	Correlations among Growth Characteristics				
Growth Character-istics	Plant Density (no./sq.m)	Plant Height (cm)	Branch no./Plant	Canopy Width (cm)	Shoot Dry Weight (gm/Plant)
Density		-0.74	-0.75	-0.78	-0.71[a]
Height			0.99	0.94	0.95
Branch				0.94	0.99
Canopy					0.97

[a] Not significantly correlated at P = 0.05

(Table 8). Tall, globose shaped plants produced more seed and were most vulnerable to wind stress. Therefore, plant height

and biomass production led not only to increased seed production per plant but also led to increased tumbling.

Table 8. Correlations Among Some Growth Parameters of Kochia at Harvest

	Correlations among Growth Characteristics			
Growth Characteristics	Plant Height (cm)	Plant Density (no./plant)	Shoot Dry Weight (gm/plant)	Seed Yield (gm/plant)
Height		0.85*	0.69	0.94
Density			0.66	0.74
Biomass				0.97

[a] All correlation values are significant at P = 0.05.

Individual kochia plants produced substantial amounts of seed. The average weight of a single seed was 9.5 mg. The number of seeds per plant ranged from 84 when kochia was cut at the soil surface twelve weeks after emergence to 5108 seeds per plant when plants were cut early and grew in plots which had low kochia densities. The uncut plants produced 1968 seeds per plant. These values are much lower than published reports due to the plant densities which occurred in this study. The absolute potential of seed production by single plants ranged from 14,600 (Stevens, 1932) to 23,350 seeds (Nussbaum et al., 1987). Seed production in this study ranged from 0.84 million to more than 314.7 million seeds per hectare for the lowest and highest yielding plots, respectively.

The number of kochia plants at maturity in the experimental site was estimated to be 16,500. Of these only

6,120 were found within the portion of the experimental plots assigned for the seed production study. The remaining were found outside these plots but within the 30 m X 50 m perimeter (Figure 2). The total number of seeds produced in the experimental area was approximately 20 million. Approximately 30% of the kochia plants tumbled, starting in early October and continuing through the winter. If 40 to 50% of the seeds from these plants were dispersed by wind, then 2.5 to 3.0 million seeds were dispersed to new areas from plants produced in an area covering only 1500 sq. meter.

Season 2

The number of seedlings found at various distances and directions from the 1990 experimental site was measured in 1991 (Figure 2). The seedling count reached 27,469 per sq. meter at the point of seed production in early May, 1991. The chances that a seedling would reach maturity was strongly influenced by plant density. Only 4% of the seedlings growing under the highest density reached maturity. Up to 44% reached maturity and produced seed at the lowest initial densities (Table 9). The variation was attributed to the intensity of competition among plants. Irrespective of plant density either at the early stage of growth or at harvest, there was little variation in biomass production and seed yield per hectare (Table 9). This indicates there was no reproductive penalty as a result of the excessive seedling stand in 1991.

Individual plants growing in the low density plots accumulated more than five times the biomass as plants from the dense population. Seed production per plant was strongly dependent upon population density.

The reduction in population density from the time of emergence until maturity measured in season 2 may be partially attributed to autoallelopathic effects from kochia plant residues from the preceding season. Earlier studies detected significant reductions in kochia density in the second season on strip mines. Allelopathic effects of kochia residues from the previous year's stand were thought to be responsible for the reduction in kochia density. (Spowles, 1989; Wali and Inverson, 1978).

The distribution of seedlings in the surrounding area was a reflection of the prevailing wind from the southwest. Though kochia seedling density was measured along sixteen transects (Figure 2), the pattern of dispersal in direction 1 through 12 was similar therefore only the seedling densities along transects 5, 7, 10, 13, 15, and 16 are presented (Figure 3). There was a sharp decline in the number of seedlings found in the first twenty meters from the point where the seed was produced. Seedlings density decreased less precipitously in the northern and easterly directions. Seedlings were found at every point along the transects to the boundary of the cultivated field, a distance of 30 m in transect 1 to over 120 m in several transects such as 7 and 10. The number of

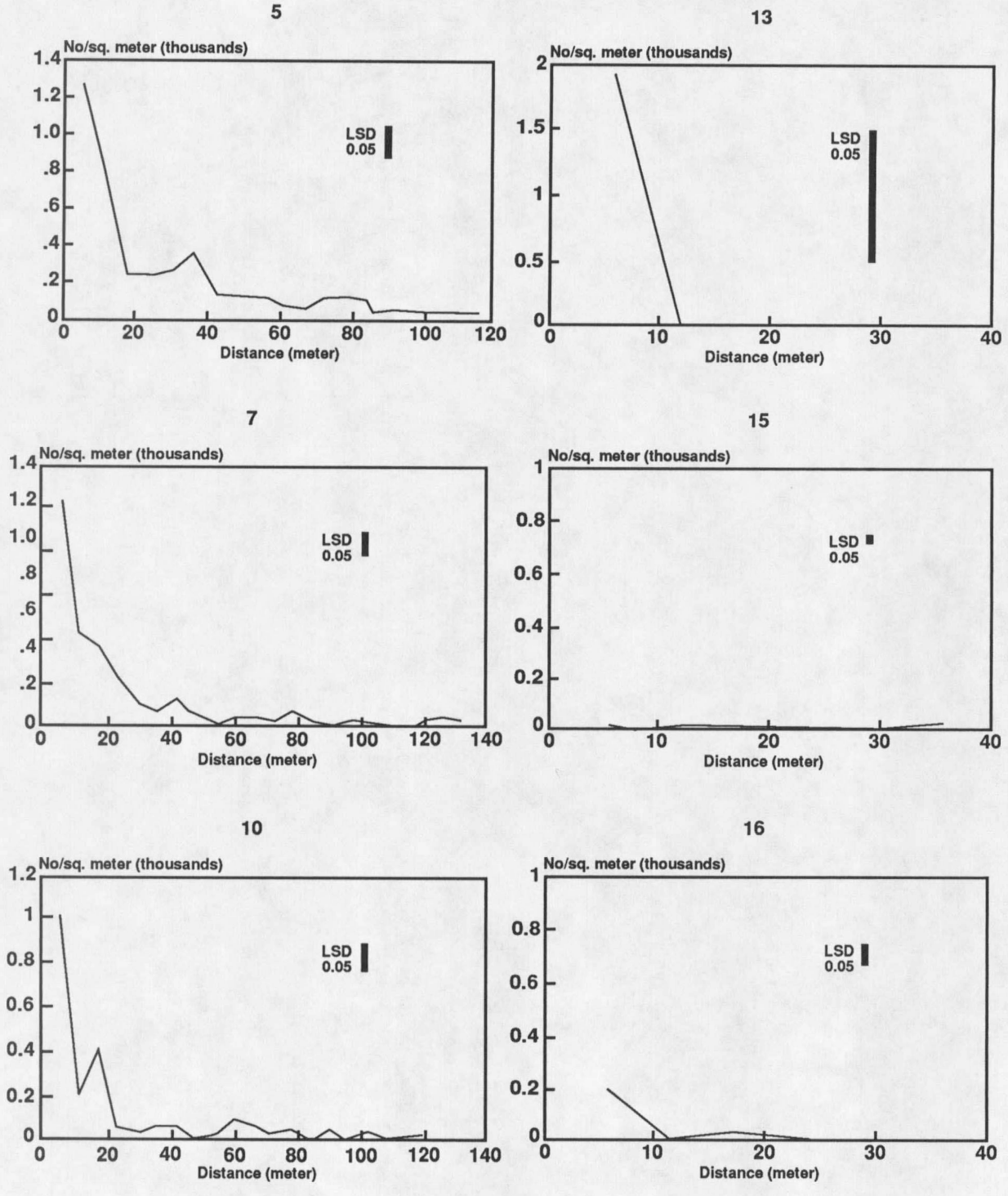


Figure 3 The Number of Seedlings Established Per Square Meter Along Transects shown in Figure 2 the Season After Seed Dispersal.

Table 9. Biomass and Seed Production of Kochia Plants from Low, Medium and High Density Populations from Seeds Dispersed to a Place where Kochia Plants were Grown in 1990[a].

Kochia Density	Seedlings Established	Plants Harvested	Stand Reduction	Shoot Biomass Production		Seed yield	
	5-15-91	9-24-91					
	------No.per m^2------		---%---	gm/plant	T/ha	gm/plant	T/ha
Low	697 a	307 a	56	6.5 c	15.6 a	1.26 c	2.87 a
Medium	2963 b	679 a	77	2.2 b	14.9 a	0.32 b	2.11 a
High	27469 c	1152 b	96	1.3 a	14.0 a	0.19 a	2.29 a

[a] Means within a column followed by the same letter do not differ at the p=0.05 level according to Duncan's Multiple Range Test.

seedlings to the south and west was low and no plants were found more than 10 to 20 m from point zero indicating wind is a very important means of dispersal for kochia seeds. In areas with strong prevailing wind directions, plant tumbling and seed dispersal may be partly managed by erecting fences to capture tumbling plants.

The tumbling and seed dispersal patterns of kochia plants are affected by the condition of the soil surface. The pattern of seedling distribution observed in this study probably would have been different in rougher terrain. In this study, the soil surface was flat and very smooth due to clean cultivation performed in the fall, 1990. And yet, remains of very detached kochia plants were found along the transects. Where kochia plants did come to final rest in the experimental area, seedling density was high indicating that kochia plants disseminate seed at the site of seed production, during tumbling, and at the point where plants reach final rest. Seedling density at the point of production and at the final resting place were very high.

Kochia plants tumbled at least one to two kilometers to the north and east after detachment. An area 120 m starting northeast of the experimental site was covered for a distance of 100 m with a dense perennial grass sod followed by a cultivated area planted to winter wheat. While kochia plant remains were found in the perennial grass area, no seedling emergence was observed. There is no doubt that seed was

dispersed in these areas, but they did not establish because of the absence of a suitable seed bed.

Kochia seed germination has been studied extensively. Burnside et al. (1981) evaluated the germination responses of exhumed seeds of 12 weed species in Nebraska and found kochia seeds experienced the most rapid loss of viability especially at a high rainfall site. Dormant and nondormant kochia seeds buried 10 to 30 cm deep had 3% or less viability after three years (Zorner et al., 1984). Chepil (1946) concluded that kochia seeds did not survive in the soil for more than two years. Zorner et al. (1984) noted that most kochia seeds germinated during the period before herbicide application which they felt should facilitate nearly complete kochia control.

The short period of seed longevity in conjunction with the effectiveness of chemical control measures indicates that kochia can change biotypes rapidly with changes in control practices and production systems (Burnside et. al., 1981). The efficiency of kochia seed dispersal permits the plant to capture new sites routinely by tumbling which permits a constant flux of biotype penetration into areas that would otherwise be free from kochia. While the actual quantity of seed dispersed per area was not measured, the success of establishment in an area where kochia had not been present serves as a useful but indirect indicator of gene flow.

Various techniques have been utilized to measure gene flow. Genetic markers of various kinds (Handel, 1982; Handel, 1983b; Ellstrand et al., 1989) such as dimorphic pollen grains (Thomson and Thomson, 1989), the use of male sterile plants as a receptor population (Tonsor, 1985), and herbicide resistance (see Chapter 1) have been used to study the pollen-mediated gene flow characteristics of plant populations.

It is easy to determine seed-mediated gene flow among populations of different taxa since it doesn't require mating like pollen-mediated gene flow. If it happens among different populations of the same species the use of dimorphic markers or differences in time of germination, maturity, etc. between the incoming progeny and the recipient population would be essential.

The dispersal of the seeds from kochia measured in this study would probably have remained the same had there been a resident kochia population in the cultivated trap area. However, it would have been difficult to discriminate between the new and the resident population. It is therefore logical to assume that extensive, and continuous seed-mediated gene flow exists in areas where kochia is commonly found. Therefore, plant growth habit, and the amount and direction of wind stress are important factors. The artificial design of this study prevented the direct measurement of both the actual range of seed dispersal and gene flow because the area available for seed capture was limited. Had the trap area

been a large cultivated field, seedling establishment would have been measured for much further distances.

The extremely low seed dormancy in kochia simplified the measurement of seed mediated gene flow. If a high proportion of the seeds remained dormant, and germination had occurred over an extended period of time, measuring the success of establishment of the incoming propagules would have been impossible. The use of seed traps and other indirect approaches to measure dispersal elucidates little about the gene flow characteristics of a species.

Monitoring the germination of nondormant, newly dispersed seeds into an area where seeds of the species were absent provided a realistic measurement of gene flow into nearby areas. Prerequisites for the use of natural traps include the avoidance of seed influx from nondesignated sources, and the presence of a soil surface that favors germination of seeds after dispersal.

Seed production and dispersal characteristics of kochia are interrelated processes affected by a number of factors (Figure 4). While seed yield and the process of stem detachment are associated with factors related to growing conditions, wind (degree of stress and direction) and terrain have decisive roles in the regulation of the distance of seed dispersal. Tumbling is an efficient mechanism that offers reliable seed dispersal and plant establishment to new sites and ensures perpetuation of the species.

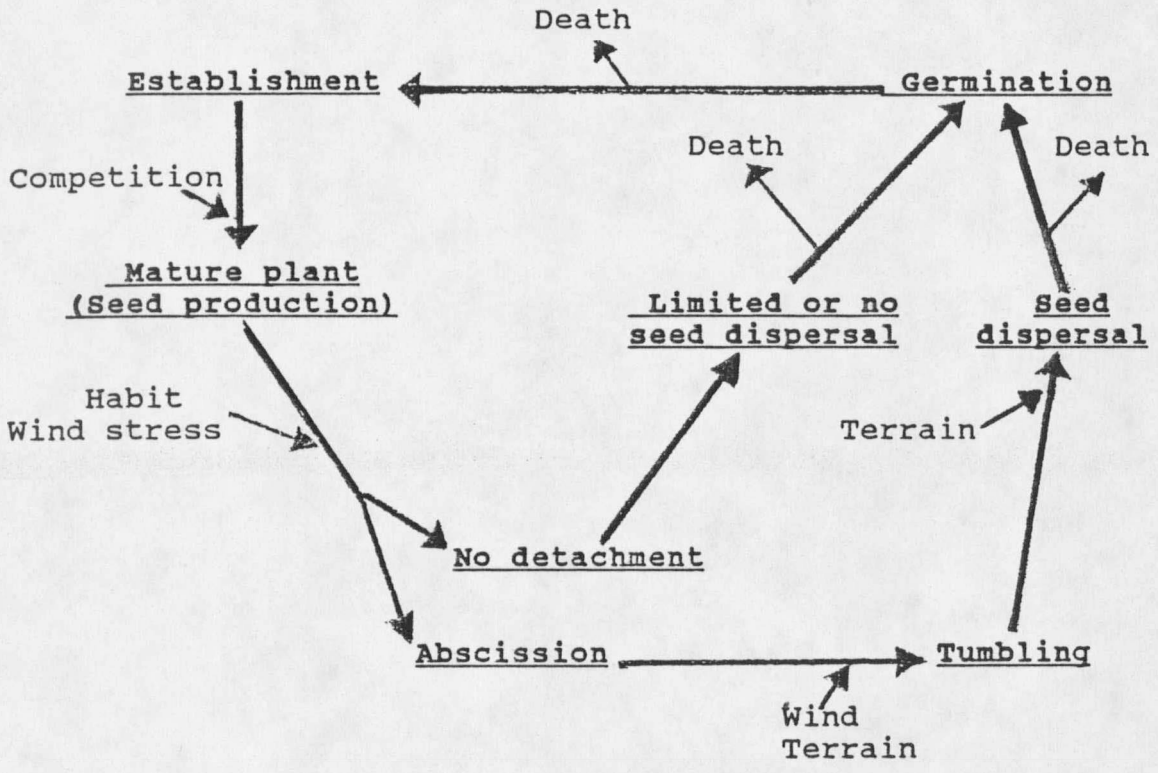


Figure 4. Factors Influencing the Process of Seed Dispersal in Kochia

CHAPTER 4

INHERITANCE OF RESISTANCE TO THE SULFONYLUREA HERBICIDE CHLORSULFURON IN KOCHIA

Introduction

The repeated use of chlorsulfuron and metsulfuron methyl in wheat and barley fields led to the selection of resistant biotypes of kochia in Nebraska (Primiani et al., 1990). A subsequent survey revealed that several more weed species had also developed resistance under field conditions (Hall and Devine, 1989; Mallory-Smith et al.,1990; Powles and Howat, 1990). The appearance and spread of resistance in kochia, however, has far exceeded that of all other species combined.

While the spread of the resistance trait is highly influenced by the gene flow characteristics of a given population, the speed of appearance of the gene under natural conditions is a function of the initial gene frequency within that population, the selection pressure exerted, and the modes of inheritance of the trait (Gressel, 1986).

The genetic basis of herbicide resistance has been studied in several plant species. In some, resistance or susceptibility to a given herbicide is under the control of a single gene. The susceptibility of maize to atrazine and simazine (Grogan et al., 1963), and soybean to metribuzin

(Edwards et al., 1976) are each determined by a single recessive gene. Similarly, resistance of barley grass (Hordeum glaucum) to paraquat (Islam and Powles, 1985) and tobacco to picloram (Chaleff and Parsons, 1987) were shown to be inherited as a single semidominant and dominant gene, respectively. On the other hand, the tolerance of flax to triazines (Comstock and Andersen, 1988), maize to HOE 23408 (Geadelmann and Andersen, 1977), and wild oat to diallate (Jacobson and Anderson, 1968) have been shown to be controlled by two or more genes. Inheritance of resistance to the triazines has also been studied in Chenopodium album (Warwick and Black, 1980), rape seed (Brassica campestris) (Souza-Machado et al., 1979) and Senecio vulgaris (Scott and Putwain, 1981) and was found to be uniparentally inherited through the female line.

Biochemical and genetic studies on mutants of bacteria, yeast, and higher plants have demonstrated several facets of resistance to the ALS inhibiting herbicides. The gene that encodes ALS from chlorsulfuron-resistant Arabidopsis thaliana (Haughn et al., 1988), yeast, and bacteria (Yadav et al., 1986) was cloned and sequenced. The DNA sequence of the mutant gene was found to differ from the wild type by a single base pair substitution. Resistance to chlorsulfuron and

sulfometron methyl was shown to result from a single semidominant mutation at either of the two loci, named SurA and SurB, in the nuclear genome of tobacco (Chaleff and Ray, 1984 ; Chaleff and Bascomb, 1987). In Arabidopsis, resistance segregated as a single dominant nuclear mutation and co-segregated with chlorsulfuron resistant ALS activity (Haughn and Somerville, 1986).

While the mechanism of resistance (Saari et al., 1990) and the response of plants to diverse groups of ALS inhibiting herbicides (Primiani et al., 1990) have been studied in kochia, information on the inheritance of resistance in plants is lacking.

The purpose of this study was to determine the variation in response, and inheritance of resistance to the sulfonylurea herbicides using the whole plant response to chlorsulfuron as a genetic marker in kochia.

Materials and Methods

Response of Resistant and Susceptible Collections to Several Sulfonylurea Herbicides

Seeds of chlorsulfuron-resistant kochia were collected in the Fall of 1989 at Chester and Conrad, MT from fields where chlorsulfuron was used for at least three consecutive years and where the population of kochia was confirmed to be resistant by DuPont Company personnel. Seeds of susceptible

kochia were obtained by handstripping seeds from plants grown in Bozeman. The response of each collection to several doses of chlorsulfuron ranging from the field use rate, 17.5, to 720.0 g/ha was evaluated in the greenhouse.

Seeds collected from the field were planted in rows by biotype at a depth of 0.5 to 1.0 cm in 60 X 40 X 10 cm flats filled with peat moss and fine sand (2:1). Following emergence, seedlings were thinned to 20 to 30 per row. Chlorsulfuron was applied 16 days after emergence when kochia plants were 3 to 4 cm tall using a moving nozzle laboratory sprayer equipped with a flat fan (8002E) nozzle. The spray volume was 87 l/ha, and nonionic surfactant (0.25 % V/V) was added to the herbicide mixture. Plants were watered daily and grown under a 14 hour photoperiod maintained by metalarc halide lamps to supplement natural light. Day and night temperatures were 24 and 20 C, respectively. Number of plants before treatment (A), and at harvest (B) were recorded and percent resistance [(B/A) X 100] was computed. Shoot biomass of individual plants was harvested and dried at 70 C for 48 hours to determine shoot dry weight production. The experiment was carried out two times in a randomized complete block design with four replications.

A second experiment was established to determine the response of each biotype to field use (1X) and 10X rates of metsulfuron methyl (Ally), triasulfuron (Amber), DPX-L5300 (Express), DPX-L5300 + DPX-M6316 (Harmony Extra), DPX-V9360

(Accent) and ARS-8498 (Beacon). The experiment was established as described above.

Inheritance Study

Twenty three resistant (R) and 12 susceptible (S) plants, grown in the greenhouse in 15 cm diameter pots, were self- pollinated by bagging as described below. Seeds were collected from each plant four months after planting. Similarly, 25 R and 25 S kochia plants were established at Arthur Post Research Farm, Bozeman, in the summer of 1990 with individual R and S plants grown adjacent to one another. Seven to ten branches from each R and S plant were bagged together in a 5 cm X 25 cm paper bag prior to flowering. The bags were agitated mechanically between 9 and 11 am three to four times a week until flowering was completed in order to facilitate cross-pollination. The same number of branches from the same plants were bagged individually to ensure self-pollination.

The bagging procedure described above was used for cross and self pollination on only 11 of the R and S plant since the flowering period of the other 14 pairs was not synchronized. Seeds produced by both the cross- and self-pollinated plants were planted in the greenhouse in flats and grown under conditions as described above.

Seedlings were treated with a high rate of chlorsulfuron (288 g/ha) to ensure that all susceptible plants were killed. Twenty seven plants obtained from seed produced by S plants

crossed with R plants as well as 42 plants which proved to be homozygous for the trait were self pollinated to produce an F_2 generation. The whole plant response of the S_2 generation seedlings was tested using the same rate of chlorsulfuron. Chi square analysis (Lund, 1987) of the segregation ratio of the S_1 resistant selfed and the F_2 heterozygous resistant selfed progeny was computed for individual plants. Visual scoring of the response of kochia seedlings to chlorsulfuron, and the number of plants which survived were recorded three weeks after herbicide treatment.

Results and Discussion

Response of Resistant and Susceptible Collections to Several Sulfonylurea Herbicides

The influence of increasing dosage level of chlorsulfuron on shoot dry weight production is presented (Table 10). Two of the collections from Chester and Conrad displayed a high level of resistance to all of the rates applied. However, the growth and biomass accumulation varied between the two R collections indicating that the collections were different.

The response of each resistant collection to chlorsulfuron was unpredictable. In several cases, plants produced more biomass following treatment with higher rates of the herbicide than with lower rates of application. Despite the variation, biomass production decreased as application rate increased. The field use rate of chlorsulfuron in

Montana prior to cancellation of the label ranged from 17 to 34 g/ha. The rates evaluated in this study were as high as twenty to forty times the labeled field rates. In general, the susceptible collection produced less shoot dry matter than the resistant collection without herbicide treatment. In addition, growth of S biotype seedlings was distinctly reduced compared to R collection when grown in wet soil. It is not clear how this difference in adaptation could be related to factors associated with their response to chlorsulfuron, or to variations in branched chain amino acid metabolism.

At the rates of chlorsulfuron tested, the resistant collections from Chester and Conrad produced 6 to 52 and 3 to 40 times higher biomass, respectively than the Bozeman collection. This comparison may not be meaningful since the biomass acccumulation of the Bozeman collection even in the untreated check was much lower than the R collections.

The lowest rate of chlorsulfuron used reduced shoot biomass production of susceptible seedlings by more than 50%. Under greenhouse conditions, even susceptible kochia plants may continue to grow and produce seeds after treatment with 17 and 35 g/ha of chlorsulfuron, rates which provide complete control of susceptible kochia plants under field conditions. Following application of higher rates, biomass production of the susceptible collection decreased dramatically followed by cessation of growth and eventual

Table 10. Shoot Dry Weight Production of Kochia Collections from Bozeman, Chester, and Conrad Following Post Emergence Application of Chlorsulfuron [a]

Rate of Chlorsulfuron Application	Kochia Collections					
	Bozeman		Chester		Conrad	
gm/ha	mg/Plant	% of Control	mg/Plant	% of Control	mg/Plant	% of Control
0 (Control)	33.8 d	100	92.6 ab	100	49.7 a	100
17.5	17.1 c	51	95.9 ab	104	70.4 ab	142
36.0	10.7 abc	32	78.8 ab	85	45.6 a	92
53.5	16.1 bc	48	97.8 ab	106	50.2 a	101
72.0	6.1 ab	18	111.5 b	120	48.5 a	98
144.0	4.5 a	13	97.7 ab	106	84.4 b	170
288.0	4.0 a	12	86.9 ab	94	54.5 ab	110
432.0	3.8 a	11	93.4 ab	101	47.5 a	96
576.0	1.5 a	4	66.4 b	72	59.6 ab	120
720.0	1.5 a	4	78.2 ab	84	50.4 a	101

[a] Means within a column followed by a different letter are significantly different at P = 0.05 according to Duncans Multiple Range Test.

death in about three weeks. The number of resistant collection plants killed with chlorsulfuron applied at rates as high as 40 times the field use rate did not exceed 25%.

The response of the three collections to six sulfonylurea herbicides was evaluated (Table 11). Each herbicide was applied at two rates of application, the field use rate (1X) and the 10X rate. When no herbicide was applied, the R collections again produced more biomass than the S collection. All collections produced higher shoot biomass at the 1X than at 10X rate in each of the herbicide applied. When the rate was increased ten fold, the reduction in biomass production varied among collections. The R collection from Chester was more resistant to all of the sulfonylureas than the other R collection. Chlorsulfuron resistant collections displayed varying levels of cross resistance to the other sulfonylureas studied. There were obvious differences in the growth and subsequent shoot dry weight production of individual resistant plants after treatment. In some cases, triasulfuron, metsulfuron methyl, DPX-L5300, and DPX-L5300+DPX-M6316 caused reduced plant stand than chlorsulfuron indicating that individual plants of the R biotype had low level of cross resistance. Cross resistance between chlorsulfuron and sulfometron methyl was demonstrated in tobacco cell culture (Chaleff and Ray, 1984). Saari et al (1990) compared both whole plant and ALS response of a single resistant kochia collection to three classes of ALS

Table 11. The Effect of Six Sulfonylurea Herbicides on Shoot Dry Weight Production of Kochia Collections from Bozeman, Chester, and Conrad[a] .

Herbicide	Rate of Application	Bozeman		Chester		Conrad	
	gm/ha	mg/ Plant	% of Control	mg/Plant	% of Control	mg/Plant	%of Control
Control		35.9 e	100	45.5 ab	100	50.6 d	100
DPX-V9360	45.0	17.1 d	48	58.4 b	128	39.1 dc	77
DPX-V9360	450.0	14.5 cd	40	43.2 ab	95	27.9 abc	55
DPX-L5300	8.8	13.9 cd	39	42.7 ab	94	37.0 bc	73
DPX-L5300	88.0	7.5 abc	21	33.3 a	73	20.8 a	41
DPX-L5300 + DPX-M6316	10.7	7.5 abc	21	46.6 ab	102	24.5 ab	48
DPX-L5300 + DPX-M6316	107.0	5.5 abc	15	34.5 ab	76	16.8 a	33
ARS-8498	67.3	19.4 d	54	43.3 ab	95	36.7 bc	73
ARS-8498	673.0	12.5 bcd	35	39.9 ab	88	23.3 a	46
Triasulfuron	33.6	7.5 abc	21	41.2 ab	91	25.4 ab	50
Triasulfuron	336.0	3.6 ab	10	34.9 ab	77	24.3 ab	48
Metsulfuron Methyl	7.0	14.5 cd	40	37.3 ab	82	21.8 a	43
Metsulfuron Methyl	70.0	3.0 a	8	36.5 ab	80	16.0 a	32

[a] Means within a column followed by different letter were significantly different at P = 0.05 using Duncans Multiple Range Test.

inhibiting herbicides: sulfonylureas, imidazolinones, and sulfonanilides, and found various levels of cross resistance. Similar observations were made with Arabidopsis thaliana when treated with chlorsulfuron and imazapyr (Haughn and Somerville, 1986). Cross resistance to the different classes of ALS inhibitors was also shown in Datura innoxia (Saxena and King, 1988), however there was no cross resistance in one line of Chlamydomonas reinharditi (Winder and Spalding, 1988). Cross resistance to several sulfonylurea herbicides was demonstrated in transgenic tobacco plants (Gabard et al., 1989). Saxena and King (1988), and Hall and Devine (1989) suggested that differences in the degree of cross resistance among herbicide families, and within the same family of ALS inhibitors (and in some cases, a lack of cross resistance) may be due to slight differences in the herbicide binding site on the ALS molecule.

More than 300 sites located in ten states of the United States and several provinces of Canada have resistant populations of kochia (personal communication DuPont Co. personnel, 1991). It is most likely that each population had developed resistance independently hence the presence of more than one mutation type is highly probable. If this is the case, the response of kochia collections to the ALS inhibiting herbicides should be expected to vary accordingly.

Inheritance Study

While controlled crossing of individual flowers on R and S plants was attempted, it was difficult because kochia flowers are very small, are produced in clusters, and produce only one seed per flower. For this reason, the branch bagging system was employed.

The response of kochia seedlings to chlorsulfuron was used as a screening marker instead of the other sulfonylureas because all of the R collections were resistant to chlorsulfuron. This is logical since all of the R collections were selected for by the repeated application of chlorsulfuron.

In most kochia plants, the male and female floral parts matured sequentially. The stigma appeared receptive to pollen for a few days, and in some cases, for more than a week prior to pollen shedding from the anthers of the same flower. This flowering behavior favors out-crossing so it was possible to make reciprocal crosses between R and S plants (Table 12).

While all progeny from seed produced on susceptible, self pollinated branches were killed by chlorsulfuron, 83 to 100% of the progeny from the resistant, selfed branches were resistant. The response to chlorsulfuron of the progeny of R and S branches bagged together was different from the progeny

produced on self pollinated branches from the same plants. From 0 to 31% resistant seedlings were produced from bagged crosses where the S plants were the female parent. This value indicates that the potential for pollen from the R plants to fertilize flowers of the S plants could range, in theory from 0 (if no crossing takes place) to 100%. While data shows that self and cross pollination occurred in kochia (Chapter 2) it appears that self pollination occurs more frequently when branches of two plants were bagged together (Table 12).

The growth habit of kochia offered several distinct advantages for conducting this study. Large kochia plants could be transplanted with ease. The survival of large plants dug and transplanted within half a day averaged more than 80%. Also the kochia plants produced large strong branches ladened with flowers which facilitated bagging.

Flowers were produced over a long period of time which permitted bringing plants together for bagging which facilitated successful crossing. All progeny produced on self pollinated branches of S plants were killed by chlorsulfuron. Some progeny from branches on the same S plant that were bagged with R plants were resistant which clearly demonstrates that cross pollination occurred, and that the R trait is expressed in the F_1 and is therefore dominant. It also

appears to be semi-dominant as will be explained later. The work of others has shown that resistance is inherited as a dominant or semi-dominant trait in tobacco (Keil and Chaleff, 1983; Chaleff and Ray, 1984), Arabidopsis thaliana (Haughn and Somerville, 1986) and Chlamydomonas reinhardtii (Hartnett et al., 1987).

All progeny from five of the R plants, Chester 3 and Chester 9 (Table 12) and Conrad 5, Conrad 6, and Conrad 7 (Table 13) were resistant to chlorsulfuron applied at 280 g/ha thus they must be homozygous for the resistant trait. Some individual seedling progeny of the other R plants were susceptible. Three to 14% of the seedling progeny were susceptible to chlorsulfuron. The segregation ratio of most resistant plants did not fit the expected Mendelian inheritance ratios for a single gene. There are three possible explanations for this. First, the plants might have been homozygous for the R trait but even R seedlings may be killed by the high rate of chlorsulfuron used which was approximately sixteen times the field use rate. This could also occur if the trait was semi-dominant instead of dominant. A second explanation might be that more than a single gene was involved in resistance however this is less probable based on earlier findings in other species. The third and least likely option is that the specific R biotype, from Chester, used in this study might have been selected for by another

Table 12. Response of Progeny Resulting from Self- and Cross-Pollination of Susceptible and Resistant Kochia Plants to Chlorsulfuron Applied at a Rate of 288 g/ha.[a]

	Self-Pollinated				Cross-Pollinated			
	Susceptible Parent Plant		Resistant Parent Plant		Susceptible Female Parent		Resistant Female Parent	
Family[b]	S_1 Seedlings Tested	Resistance	S_1 Seedlings Tested	Resistance	F_1 Seedlings Tested	Resistance	F_1 Seedlings Tested	Resistance
	--No.--	--%--	--No.--	--%--	--No.--	--%--	--No.--	--%--
1	86	0	195	89	195	28	26	58
2	179	0	132	83	266	6	53	76
3	453	0	164	100	577	31	271	96
4	-	-	400	85	42	26	336	71
5	277	0	218	97	238	31	388	77
6	218	0	54	89	270	22	228	78
7	58	0	-	-	265	7	97	56
8	528	0	-	-	177	10	37	60
9	8	0	307	100	4	0	36	100
10	197	0	-	-	88	17	668	64
11	497	0	157	86	307	10	20	95
Mean	250	0	203	91	221	20	196	74

[a] All resistant plants were from Chester, MT.

[b] Family refers to the original susceptible and resistant plants whose branches were bagged together to produce the F_1 progeny.

sulfonylurea herbicide such as metsulfuron methyl. If this is the case, the herbicide binding site on ALS may be less sensitive to the other sulfonylurea but not to chlorsulfuron. Consequently kochia plants, even if they were homozygous, would be killed by chlorsulfuron. Approximately 25% of the seedlings should have been killed if the trait was under the control of a single dominant gene.

The reaction of F_2 progeny of 42 self pollinated homozygous R plants to chlorsulfuron was variable (Table 14). While 24 plants produced completely resistant F_2 seedlings, the level of resistance in the remainder ranged from 82 to 99% which could be an indication that some homozygous R plants were killed by high rates of chlorsulfuron. The F_2 progeny from homozygous self pollinated R plants were less vigorous, shorter, and had reduced leaf size compared to F_1 and parental plants. In addition they flowered more quickly than the F_1 generation therefore inbreeding depression from sucessive self pollination may have caused the change in response of some of homozygous plants to chlorsulfuron.

The frequency of resistance in unchallenged, susceptible field populations of kochia is not known. In a field study conducted in Minot, ND., the frequency of resistance in a large field population of kochia was estimated to be one resistant plant for every 7300 susceptible plants (John Nalewaja, personal communication, North Dakota State University, 1990). Though the possibility of gene flow of

Table 13. Effect of 288 g/ha Chlorsulfuron on S_1 Progeny of Susceptible and Resistant Kochia Plants

S Parents			R Parents					
Family[a]	S_1 Seedlings Tested	Resistance	Family[b]	S_1 Seedlings Tested	Resistance	Family[a]	S_1 Seedlings Tested	Resistance
	--No.--	--%--		--No.--	--%--		--No.--	--%--
1	11	0	1	18	89	10	34	85
2	28	0	2	45	83	11	123	93
3	13	0	3	26	92	12	172	97
4	7	0	4	28	86	13	141	96
5	39	0	5	53	100	14	59	100
6	57	0	6	19	100	15	68	100
7	17	0	7	40	100	16	82	85
8	16	0	8	108	94	-	-	-
9	23	0	9	129	89	-	-	-

[a] The S parents were from Fessenden, ND (1 to 4) and Bokema, MT (4 to 9).

[b] All R plants were from Conrad.

resistance either by pollen or seed may not be zero, the frequency is high enough to pose a major problem with repeated use of the sulfonylureas in the field.

In spite of the relatively high frequency of resistance, the likelihood of resistance being detected in susceptible plants which had no access to pollen from resistant plants is too low to account for the observed variation.

The level of resistance of seedlings produced by branches on R plants that were bagged with S plants was 16% less than progeny from self pollinated R plants. This reduction in resistance was attributed to gene flow via pollination by S plants. The decrease can be attributed to two possible scenarios: First, if an R plant was homozygous for the R trait, and only one gene is involved, cross pollination with S plants would produce all heterozygous plants which would be killed upon exposure to a high rate of chlorsulfuron. This occurrence is in accord with the view that the level of response of R plants is a function of gene zygosity, hence dominance under the heterozygous condition could not be complete.

The second scenario is based on the assumption that the trait is conferred by a single gene. If the R parent was heterozygous for resistance, some of the progeny from crosses would be susceptible as a result of cross pollination. In either case, there is potential for susceptible pollen to reduce resistance under field conditions since kochia is both

Table 14. Effect of 288 g/ha Chlorsulfuron on S_2 Progeny of 42 Homozygous Resistant Kochia Plants from Conrad.

Mother plants								
Conrad - 5			Conrad - 6					
F_1 Parent	Progeny Treated	Resistance	F_1 Parent	Progeny Treated	Resistance	F_1 Parent	Progeny Treated	Resistance
	--No.--	--%--		--No.--	--%--		--No.--	--%--
1	82	100	1	25	96	18	64	100
2	117	98	2	39	85	19	108	100
3	100	95	3	63	100	20	79	100
4	63	98	4	11	100	21	180	98
5	187	82	5	29	93	22	29	100
6	91	99	6	10	100	23	20	100
7	40	100	7	33	100	24	23	100
8	42	88	8	21	100	25	6	100
9	35	100	9	23	100		Conrad - 7	
10	26	88	10	99	100	1	19	95
11	18	99	11	62	100	2	19	85
12	33	100	12	77	95	3	20	100
13	15	100	13	8	100	4	9	100
			14	31	90			
			15	205	97			
			16	102	100			
			17	65	95			

self compatible and an out-crossing (Chapter 2), diploid species. This information can serve as the foundation of an inexpensive and effective strategy for resistance management. Namely, the introduction and maintenance of massive amounts of susceptible pollen to dilute resistance. This strategy has been discussed in detail from a theoretical point of view (Maxwell et al., 1990).

The F_1 survivor progeny obtained following the application of chlorsulfuron that were derived from S female plants that were bagged with R male parents are assumed to be heterozygous for the R trait. Of 477 such seedlings, (Table 12) 27 were randomly selected and self pollinated for one generation. The F_2 progeny from these plants were then challenged with the same rate of chlorsulfuron used with the F_1 progeny.

The response of the F_2 plants was classified in distinct categories: Those that were killed were sensitive, while those that survived (albeit with varying levels of injury) were resistant. If independent random assortment of a single gene for resistance is assumed, then a 3 : 1 ratio of R to S is expected. In fact, the level of resistance observed was lower than the expected 75% (Table 15). Obviously, some of the heterozygous progeny were killed. It is reasonable to conclude that some homozygous R plants were killed based on the results discussed earlier with self pollinated plants. Some of the surviving plants had no damage from chlorsulfuron

application. The remaining survivors were moderately to severely injured. Those that were uninjured were assumed to be homozygous for the R trait. The remainder were, we believe, heterozygous. Such a wide range of reaction among a resistant, heterozygous population ranging from little or no tolerance to a moderate reaction to a high rate of chlorsulfuron indicates a nonuniform pattern of response of ALS among plants.

Sixty-five percent of the progeny from known heterozygous resistant plants were resistant to a high rate of chlorsulfuron. If a single gene controls the trait, this value is 10% lower than expected. The average level of resistance in progeny from sixteen field collected, selfed resistant kochia plants was 90.6% (Table 13). None of the progeny from these 16 plants fit the 3 R :1 S ratio of segregation since the proportion of survivors for each plant following treatment ranged from 83 to 100%. Twenty-five percent would have been killed if the plants were heterozygous and a single gene was involved. It is possible that the resistance gene confer little or no resistance in certain genetic backgrounds. The resistance gene may sometime confer no or little resistance in certain genetic backgrounds. There is also a likelihood occurrence of inbreeding depression after one generation of selfing which caused the death of some of the resistant plants. In any case, up to 9% of the resistant progeny from homozygous plants were killed by

Table 15. Effect of 288 g/ha of Chlorsulfuron on F_2 Progeny of Heterozygous Plants.

Family	Seedlings Established	Resistant Observed (Expected)	Susceptible Observed (Expected)	--P--	Total Resistance ----%----	Relative Damage Level within the Resistant Progeny ---------%---------- 0-10	11-49	50-80
1	423	265 (320.5)	162 (106.8)	0	62	22	32	46
2	193	130 (144.8)	63 (48.3)	0.018	67	21	34	45
3	10	5 (7.5)	5 (2.5)	0.144	50	50	0	80
4	84	54 (63)	30 (21)	0.032	64	6	41	33
5	28	13 (21)	15 (7)	0.001	46	23	46	31
6	8	6 (6)	2 (2)	1.000	75	0	13	87
7	19	13 (14.3)	6 (4.8)	0.691	68	31	38	31
8	54	43 (40.5)	11 (13.5)	0.529	80	60	23	17
9	27	16 (20.3)	11 (6.75)	0.096	59	25	31	44
10	32	23 (24)	9 (8)	0.838	72	39	30	31
11	59	43 (44.3)	16 (14.7)	0.822	73	44	37	19
12	107	54 (80.3)	53 (26.7)	0	50	26	46	28
13	175	144 (131.3)	31 (41.7)	0.325	82	36	40	24
14	22	15 (16.5)	7 (5.5)	0.623	83	0	60	40
15	20	16 (15)	4 (5)	0.796	80	63	25	12
16	160	96 (120)	64 (40)	0	60	37	28	35
17	27	18 (20.3)	9 (6.7)	0.437	67	22	17	61
18	160	98 (120)	62 (40)	0	61	10	54	36

Table 15 - Continued

Family	Seedlings Established	Resistant Observed (Expected)	Susceptible Observed (Expected)	--P--	Total Resistance --%--	Relative Damage Level within the Resistant Progeny ---------%--------- 0-10	11-49	50-89
19	25	15 (18.7)	10 (6.3)	0.133	60	20	60	20
20	10	8 (7.5)	2 (2.5)	1.000	80	88	12	0
21	33	17 (24.7)	16 (8.3)	0.004	52	24	47	29
22	81	54 (60.7)	27 (20.3)	0.109	67	19	29	52
23	238	154 (178.5)	84 (59.5)	0	65	29	38	33
24	29	25 (21.7)	4 (7.3)	0.238	86	92	4	4
25	38	19 (28.5)	19 (9.5)	0.001	50	32	26	42
26	36	15 (27)	21 (9)	0	42	7	7	86
27	136	83 (102)	53 (34)	0	61	28	44	28
Total	2244	1447(1683)	797(561)	0	65	29	35	36

chlorsulfuron. It is also possible that a comparable number of heterozygous plants were killed either due to the semi-dominant nature of the resistance trait or to the decrease in plant vigour following one generation of selfing. Had it not been for these proposed causes, the percent of resistance in progeny of the known heterozygous kochia plants may have ranged from 74 to 75 %.

CHAPTER 5

EFFECTS OF TEMPERATURE AND HUMIDITY ON THE VIABILITY OF KOCHIA POLLEN

Introduction

Kochia is both self-compatible and cross-pollinated (see Chapter 2). The main pollinating agent is wind, and pollen-mediated transfer of traits like herbicide resistance has been observed (see Chapter 1 and Chapter 3). The extent of pollen-mediated spread of herbicide resistance will be influenced by pollen longevity.

The longer the period of viability, the higher the potential for pollen to travel long distances. The duration and exposure period of wind blown pollen to various climatic factors influence the longevity of viability. In many plant species, pollen viability is influenced by temperature and relative humidity (Vasil, 1961, cited in Johri and Vasil, 1961), light (Dhawan and Malik, 1981) and air pressure (Kellerman, 1915). Variability in pollen longevity caused by differential responses to temperature and relative humidity has been recorded among several plant species (reviewed in Johri and Vasil, 1961).

Two techniques commonly used to estimate the viability of pollen are staining and germination on artificial media. In vitro germinability of pollen is influenced by the presence of hormones, various ions, plant tissue extracts, and simple

sugars (Asif et al., 1983; Brewbaker and Kwack, 1963; Brink, 1924a; O'Kelly, 1955). Variation in pollen viability has been observed among different pollen sources by the use of several staining procedures. In the presence of viable pollen, the colorless TTC is converted to the insoluble red colored triphenyl formazan by reductases present in living tissue (Oberle and Watson, 1953). With Alexander stain, aborted pollen grains stain green while nonaborted pollen turns crimson red (Alexander, 1980). Acetocarmine is specific for the nucleus and stains nonaborted pollen pink (Pearson and Harney, 1984). The level of pollen sterility can also be determined by using IKI (Edwardson and Corbett, 1961).

The purposes of this study were to compare various procedures to estimate pollen viability, and to evaluate the effects of temperature and relative humidity on the longevity of kochia pollen.

Materials and Methods

Pollen Staining

Kochia seeds, collected in October of 1990 from the Arthur Post Research Farm, Bozeman were planted 0.75 cm deep in six rows 5 cm apart in 60 x 40 x 10 cm deep flats containing peat moss and sand (1:1). Seedlings were thinned to 30 to 40 plants per flat and watered daily. Plants were grown in a greenhouse with a 14 hour photoperiod under natural sunlight supplemented with metalarc halide lamps. Day and

night temperatures were 24 and 20C, respectively. Plants began to flower nine to ten weeks after emergence and, depending upon plant size, produced pollen for five to ten days. Pollen was collected daily from 9 to 10 am from several plants at anthesis by shaking pollen in petridishes. Two, 3, 5-tetrazolium chloride (TTC) (200 mg TTC and 10 g sucrose in 20 ml water) (Aslam, et al., 1964), Alexander stain (20 ml of 95% ethanol, 2 ml of 95% ethanol containing 1% malachite green, 50 ml of distilled water, 40 ml of glycerol, 10 ml of distilled water containing 1% acid fuchsin, 5 g phenol, and 1 to 6 ml of lactic acid,) (Alexander, 1980), acetocarmine (1 g acetocarmine in 20 ml glacial acetic acid) (Pearson and Harney, 1984), and IKI (100 ml of distilled water containing 1 g of both KI and I) (Edwardson and Corbett, 1961) were compared.

Prior to staining preliminary trials were conducted to evaluate pollen media formulations. Fresh pollen was dusted onto glass microscope slides with a camel hair brush followed by the addition of four to five drops (0.1 to 0.125 ml) of stain. Immediately after the stain was applied, the pollen-stain mixture was covered with a coverslip that was tightly sealed around the edges with fingernail polish. Pollen reaction was measured after ten minutes for all stains except TTC which was examined after two to three hours. After color development occurred, the color reaction of 300 to 500 pollen grains per slide was counted with a compound microscope under

low power (10X). The same staining and counting procedures were performed for control pollen that was killed before staining by incubating fresh pollen at 70 C for 48 hours. Treatments were arranged in a completely randomized design with three replications and the experiment was carried out twice using separate pollen harvests.

Pollen Germination

Pollen germination was measured in several media using the pollen sources described above. Each of the germination medium tested was prepared in 1.1% defacto bacto agar which was autoclaved and poured into 5 cm diameter petridishes. Pollen was dusted on each media surface. The media evaluated were indol-3-acetic acid (IAA), and gibberellic acid (GA) at 20 and 40 ppm; 5, 10, 15, 20, and 30% sucrose, plant extracts, and complex nutrient medium (10-20% sucrose; H_3 BO_3, 100 ppm; $Ca(NO_3)_2.4(H_2O)$, 300 ppm; $MgSO_4.7(H_2O)$, 200 ppm; KNO_3, 100 ppm) (Brewbaker and Kwack, 1963). Plant extract media containing flower buds and leaves of kochia was prepared by homogenizing 5 g of plant material in 20 ml of water in a Waring blender followed by filtering through several layers of cheesecloth.

In addition, pollen germination in 100% relative humidity on a dry surface was measured for four days. Pollen was dusted onto four centimeter diameter petridishes that were incubated in a sealed 15 X 15 X 3 cm plastic box. Each petirdish was placed on water saturated blotting paper which created 100% relative humidity atmosphere. This system was

maintained in a growth chamber at 22 and 28 C. Germination was examined after 24 hours by determining percent germination of 300 to 500 pollen grains per dish. Pollen was considered to have germinated when the length of the pollen tube exceeded the maximum width of the pollen grain. The experiment was conducted twice and treatments were arranged in a completely randomized design with three replications.

Effect of Temperature and Humidity on Pollen Viability

The influence of three temperature and five humidity regimes on pollen viability was evaluated over a 15 day period. Pollen was dusted into 4 cm diameter petridishes which were individually incubated in sealed 15 x 15 x 3 cm plastic boxes. Each box contained a distinct, constant humidity using a modified procedure of Ferrari et al. (1983). The humidities tested were 7, 32, 55, 75, 100%. Boxes were incubated at 4, 22, and 28 C. Pollen viability was measured with TTC after 0, 1, 2, 3, 5, 7, 9, 12, and 15 days by dusting pollen onto microscope slides as described above. Two hundred to 300 pollen grains per slide were counted. There were three replications per treatment which were arranged in a completely randomized design. The study was conducted twice. In the first experiment, pollen was obtained from greenhouse-grown plants. In the second, pollen was collected from kochia plants grown in the field in the summer of 1991. Statistical analysis of percent viability as determined by TTC test was conducted and means were separated using Duncan's Multiple Range Test.

Results and Discussion

Pollen Staining

Kochia pollen stainability in the four stains was determined (Table 16). Only TTC did not stain heat killed pollen. IKI, acetocarmine, and Alexander stain did not differentiate between dead and fresh pollen. Alexander stain was tested in 1 to 6 ml of lactic acid to measure percentage of aborted pollen (Alexander, 1980).

While differential staining was achieved when the stain was mixed with 2 ml of lactic acid, the green color, an indicator of aborted pollen, did not persist. Five to ten minutes after staining, all pollen including grains which turned green, turned red. Examination of pollen 2 to 3 minutes after staining initially revealed that green and red pollen was present in both the heat killed and fresh live pollen. With acetocarmine stain, a pink color was observed immediately for 97% of the pollen indicating kochia pollen was not aborted during shedding. Results with Alexander stain and acetocarmine were also unreliable with pollen of five Prunus species (Parfitt and Ganeshan, 1989).

To determine the optimum concentration of TTC needed for staining, 0.5, 1, 2, and 4% w/v TTC each in 50% w/v sucrose solutions were evaluated. Marked differences in staining efficiency occurred among the TTC concentrations. Best results were obtained with 1% TTC. During staining with TTC, the cover slip over the pollen needed to be sealed tightly for

staining to occur. The presence of air bubbles under the

Table 16. The Percentage of Fresh Live and Heat Killed Kochia Pollen Stained by Four Staining Techniques.[a]

Stain	Pollen Stained	
	Fresh Pollen	Heat-Killed Pollen
	----------%----------	
5% Acetocarmine	98 b	98 b
Alexander Stain	99 b	97 b
IKI	99 b	98 b
TTC	77 a	0 a

[a] Means within a column followed by a different letter were significantly different at P = 0.05 using Duncan's Multiple Range Test.

cover slip also reduced pollen staining. Intensity of color development was low near the edge of the cover slip even when completely sealed with nail polish. The importance of sealing the pollen-TTC mixture was reported by Oberle and Watson (1953) who found slow color development of pollen from several cultivars of peach, apple, pear, and grape if cover slips were not used.

While color development occurred within one hour, reliable results required two to three hours. The level of color intensity of staining with TTC is a function of pollen viability (Oberle and Watson, 1953) and ranged from deep red to light pink. In this study, all colors from light pink to deep red were considered to indicate that the pollen was viable. It was assumed that the higher the red color intensity, the better was the capacity of pollen to reduce TTC and hence the higher the pollen vigor. On the other hand, in

cotton, the longer the slides were kept in TTC, the greater was the color uniformity (Aslam et al., 1964).

Pollen Germination

Kochia pollen did not germinate in any of the agar media tested. Very little germination (up to 0.5 %) occurred in some of the media (Table 17). When germination occurred, pollen tube emergence and growth began after five to ten hours of incubation. Pollen incubation on a dry surface in petridishes maintained at 100% relative humidity for four days resulted in 17.8% and 11.3% germination at 22 and 28 C, respectively. Apparently, the presence of moist air in the vicinity of pollen grains favored germination and elongation of pollen tubes.

There was an increase in germination with prolonged incubation. Gradual absorption of moisture from air over a few days time appeared to stimulate more germination than sudden exposure of pollen to moisture in the agar media treatments tested. Considerable amounts of pollen burst when kept in agar media at 28 C. Also, media with sugar concentrations of 5% sucrose and below, and plant tissue extracts contained the most burst pollen grains.

Bursting was not restricted to specific directions in each pollen grain but occurred in random locations probably because of the structure of kochia pollen. Individual pollen grains contain an average of 60 to 80 pores which function as weak points to facilitate pollen tube emergence (Lewis et al.,

1983). Pollen incubation for three to four days made assessment of germination difficult because of fungal growth,

Table 17. Germination of Kochia Pollen in Media Maintained at 20 C or 28 C[a].

Treatments	Pollen Germination Temperature (C)	
	22C	28C
	----------%----------	
5% Sucrose Media	0 a	0.1 a
10% Sucrose Media	0 a	0 a
20% Sucrose Media	0 a	0.1 a
Complex Nutrient Media	0 a	0.5 a
Plant Extract Media	0 a	0 a
Incubation for 1 Day	0 a	0.7 ab
Incubation for 2 Days	2.9 ab	7.2 bc
Incubation for 3 Days	8.9 b	15.1 d
Incubation for 4 Days	17.8 c	11.3 cd

[a] Means in a column followed by a different letter differed significantly at P = 0.05 using Duncan's Multiple Range Test.

similar to the findings of Khosh-Khui et al., (1976) with six Rosa spp. It appears that in vitro germination requirements of kochia pollen are simple however the level of germination obtained was low.

Occasionally, erratic patterns of germination were observed. In one preliminary experiment, over 50% germination was observed with fresh pollen incubated for three days at high humidity and temperature. Despite repeated attempts, these results were never observed again, even with pollen from the same source.

Pollen tube growth varied from slight protrusions where pollen tube length was equal to the diameter of the pollen grain, to extensive pollen tube elongation. Some kochia

pollen grains produced two to three pollen tubes simultaneously from one or two locations on the pollen grain surface. It was impossible to follow subsequent growth of multiple pollen tubes because of eventual interference from fungal contamination.

Aggregation of pollen appeared to stimulate germination since individual pollen grains rarely germinated. The amount of aggregation depended on how much pollen was applied to the media, and the amount of clustering that occurred. Aggregation ranged from five to ten, to as many as several hundred pollen grains per clump, and was a source of much variability in the results obtained. In 86 species of flowering plants, the effect of aggregation on pollen germination was noted. (Brewbaker and Kwack, 1963). The failure of isolated pollen or small aggregations to germinate was overcome by the use of media containing plant tissue extracts which are known to be rich in Ca^{++} (Brewbaker and Kwack, 1963). Kochia pollen, on the other hand, did not germinate in agar media that contained complex nutrients including Ca^{++} or kochia tissue extracts so it appears that exposure to humidity is more important for pollen germination than Ca^{++} or plant extracts.

Effects of Temperature and Humidity on Viability of Pollen

Viability was estimated with TTC, the most reliable procedure tested (Table 16). The response of pollen to the temperature and relative humidity regimes tested varied for

the pollen collected from greenhouse and field grown plants. Pollen from greenhouse grown plants remained viable for one week, while pollen from field grown plants was alive for as long as 12 days when incubated at 4 C.

There were significant differences in viability for the humidity treatments (Table 18). There was a sharp contrast in the longevity of viability between 7 and 32% relative humidity, and 55% humidity. The period of field grown pollen viability was shorter than greenhouse grown pollen viability at the lower relative humidities tested.

Low humidity and high temperatures were detrimental to pollen viability and the effect was most pronounced with pollen from field grown plants. Irrespective of pollen source, the longevity of kochia pollen was low.

The intensity of red color development decreased with each day of incubation indicating gradual loss of activity of the enzymes that reduce TTC. In general, the higher the temperature, and the lower the relative humidity, the lower was the longevity of pollen viability.

Kochia produces considerable amounts of pollen for at least one week which makes it a useful plant for pollen study. Low <u>in vitro</u> germination may be due to the number of nuclei in pollen at the time of shedding. Brewbaker (1967) evaluated pollen of nearly 2000 species and found one third of the plants, including kochia, shed their pollen as a trinucleate cell. Brewbaker and Majumder (1959) have also shown that

cell. Brewbaker and Majumder (1959) have also shown that

Table 18. The Viability of Greenhouse Grown (G) and Field Grown (F) Kochia Pollen Incubated for 15 Days at Three Temperature and Five Relative Humidity Regimes.

Days of Incubation	Percent Pollen Viability[a]									
	Relative Humidity									
	7%		32%		55%		75%		100%	
	G	F	G	F	G	F	G	F	G	F
	4 C									
0	86c	65b	86d	65b	86c	65c	86d	65c	86b	65d
1	86c	5a	39bc	22a	75c	17ab	72c	31b	84b	12abc
2	86c	0a	56dc	9a	80c	15ab	94d	17ab	80b	38bcd
3	83c	-	86d	3a	84c	27b	92d	31b	87b	20abc
5	9b	-	28ab	0a	43b	15ab	14b	3a	19a	28abcd
7	3ab	-	7ab	-	6a	10ab	7ab	16ab	0a	34abcd
9	0a	-	0a	-	-	26b	0a	24b	-	46cd
12	-	-	-	-	-	5ab	-	1ab	-	7ab
15	-	-	-	-	-	0a	-	0a	-	0a
	22 C									
0	86c	65b	86d	65b	86c	65d	86d	65b	86c	65b
1	38b	0a	41b	12a	49b	18ab	51b	24a	73c	35ab
2	56b	-	68c	4a	80c	49dc	64b	18a	49b	43b
3	42b	-	46b	0a	76c	30bc	63b	26a	74c	43b
5	0a	-	0a	-	9a	7ab	0a	0a	17a	0a
7	-	-	-	-	0a	15ab	-	-	0a	-
9	-	-	-	-	-	0ab	-	-	-	-
	28 C									
0	86c	65b	86c	65b	86c	65d	86d	65c	86c	65c
1	5ab	0a	34b	7a	72c	41c	40b	22b	65cb	31b
2	9b	-	21ab	0a	35b	12ab	69c	5ab	53b	50cb
3	0a	-	0a	-	67c	30bc	64c	21b	70cb	33b
5	-	-	-	-	3a	10ab	7a	0a	0a	0a
7	-	-	-	-	0a	6a	0a	-	-	-

[a]-Means within a column followed by a different letter are significantly different from each other at the p = 0.05 level according to Duncan's Multiple Range Test.

pollen, generally, is low, and viability is lost shortly after dehiscence from anthers.

In this study, the only significant amount of germination occurred when pollen was incubated at 22 or 29 C in a chamber where the relative humidity was maintained at a high level for several days. Germination records during the incubation study were additive in that the value obtained at the second, third, and fourth day all included the germination of pollen that had occurred in the previous days. The exact duration of incubation required for pollen to initiate germination therefore was not known. It appears that a prerequisite of germination involves a gradual, not abrupt exposure to moisture for several hours.

The viability of greenhouse pollen, estimated by TTC staining, decreased slowly for three days followed by a sharp decline at several relative humidities and temperatures. With field grown pollen, the same trend occurred but in a single day. Variation in the growing conditions of the pollen source plants, and the inherent differences associated with greenhouse and field grown plants may account for the differences in reaction to temperature and humidity.

Kochia is well adapted to areas which experience moisture stress (Coxworth et al., 1969). In fact, moist habitats are less favorable for growth (Wiese and Vandiver, 1970). It is uncommon for high levels of humidity to exist even for short durations during the peak flowering season of kochia.

Brewbaker and Kwack (1963) found that the *in vivo* and *in vitro* germination requirements of pollen for several species were not similar. It is possible then, that germination of pollen on the surface of a kochia stigma may not require the conditions, especially high humidity, that were observed in this study.

Measurements of viability with TTC grossly overestimated pollen viability as assessed by germination. TTC was also an unreliable indicator of pollen viability in cotton (Barrow, 1983), *Prunus* sp. (Parfitt, 1989) and some fruit tree species (Oberle and Watson, 1953), however it was a reliable indicator of *in vitro* germinability in *Populus* sp. (Rajora and Zsuffa, 1986) and *Pinus* sp. (Cook and Stanley, 1960). Most species with trinucleate pollen, like kochia probably give higher viability values with TTC than with a germination assay. TTC, however does identify pollen capable of performing oxidation which may or may not correlate with the ability to complete fertilization.

The period of kochia pollen viability was strongly influenced by temperature when it was incubated under low humidity conditions. Some viability was measured for one to three days at the highest temperature and lowest humidities which indicates the potential for some pollen to remain functional under harsh conditions. It is assumed that a small proportion of the viable pollen would germinate. This possibility , coupled with the copious amounts of pollen that

are produced by kochia would permit long range transfer of pollen-mediated traits like herbicide resistance among populations. Maintenance of pollen viability for half a day, or for just a few hours at 22 to 28 C, typical temperature ranges for areas where kochia is adapted, would be sufficient time for wind-borne pollen to travel long distances. In addition to pollen longevity, pollen-mediated genetic transfer is influenced by the size of the pollen source (Ellstrand et al., 1989), the size of the recipient population (Willams and Evans, 1935), the density of plants in both populations (Bateman, 1947), and the variation in the immediate environment of spatially separated populations (Manhall and Bormann, 1978).

Since pollen viability can be lost rapidly with fluctuations in humidity (Bullock and Snyder, 1946), the viability of pollen under field conditions where temperature and humidity conditions are not constant over a period of hours could be lower than what was found in this study. The production of large amounts of pollen for an extended period of time would be an efficient survival strategy for species to offset such limitations imposed by the environment.

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