Occurrence and characterization of dicambre resistant Kochia (Kochia scoparia) in Montana
by Josette Lynn Hackett

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


Abstract:
Kochia is a summer annual belonging to the Chenopodiaceae family. It was found in Montana by the 1940's. Frequent use of sulfonylureas and dicamba for kochia control in small grain production has selected for resistant populations of kochia.

In 1992 and 1993, some percentage of chlorsulfuron resistant kochia occurred in most areas of kochia collection. Ninety-four percent and 96% of the cells had kochia plants resistant to chlorsulfuron, in 1992 and 1993, respectively. Agricultural districts with high selection intensity generally had a significantly higher percentage of chlorsulfuron resistant kochia.' Occurrence of dicamba resistant kochia in Montana was investigated. Kochia resistant to dicamba was found in eleven and seven cells, from kochia seed collected in 1992 and 1993, respectively. The percent of kochia resistant to dicamba was low. In 1992, the highest percentage of resistance was 5% of the sample from a cell. In 1993, two cells had about 12.5% dicamba resistance. Seven complaint fields were investigated, and all of the fields had some degree of resistance ranging from 2% to 20%.

Dose response to dicamba was evaluated for dicamba resistant and susceptible lines. GR50 values were estimated for the resistant and susceptible lines. The R/S ratio was 15.5. Resistant lines were then evaluated for multiple resistance to other herbicides. Dicamba resistant kochia lines were resistant to chlorsulfuron, metsulfuron, MCPA, and picloram and low rates of 2,4-D. They were not resistant to bromoxynil, fluroxypyr, or carfentrazone.

Uptake, translocation, and metabolism of 14C-dicamba were evaluated in dicamba resistant and susceptible lines. No significant differences explained the mechanism of resistance.

# OCCURRENCE AND CHARACTERIZATION OF DICAMBA RESISTANT KOCHIA (*Kochia scoparia*) IN MONTANA

by

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A thesis submitted in partial fulfillment
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in

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APPROVAL

of a thesis submitted by

Josette Lynn Hackett

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

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In 1992 and 1993, some percentage of chlorsulfuron resistant kochia occurred in most areas of kochia collection. Ninety-four percent and 96% of the cells had kochia plants resistant to chlorsulfuron, in 1992 and 1993, respectively. Agricultural districts with high selection intensity generally had a significantly higher percentage of chlorsulfuron resistant kochia.

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Uptake, translocation, and metabolism of $^{14}C$-dicamba were evaluated in dicamba resistant and susceptible lines. No significant differences explained the mechanism of resistance.

Chapter 1

# LITERATURE REVIEW

## Kochia

Kochia (*Kochia scoparia* L. Schrad) is a summer annual weed belonging to the Chenopodiaceae or goosefoot family. Kochia is often called fireweed in Montana and summer cypress, belvedere, Mexican fireweed, ironweed, and railroad weed in other areas of the world (Eberlein and Fore, 1984). The senescing leaves turn brilliant red late in the fall, hence the name fireweed. It was introduced to North America as an ornamental in the early eighteen nineties. Kochia escaped cultivation and has become widely distributed throughout the Great Plains.

Kochia was first reported in southeastern Wyoming in the eighteen nineties (Forcella, 1985). Kochia was found in central Montana by the 1940's (Figure 1). A decade later most Montana counties had infestations of kochia. In a survey conducted in 1980, kochia was found in 53% of the roadside plots, 45% of the grain fields, 17% of the rangeland, and 6% of the pastures surveyed. Kochia was not observed in forested areas (Forcella, 1985).

Leaves of kochia are linear-lancelolate or linear, alternate, hairy, sessile, 2.5 to 5.0 cm long, becoming gradually smaller toward the top of the plant. The flowers are sessile in the axils of the upper leaves, and they occur in terminal spikes. Kochia has a perfect flower that is very small, green, and bractless (Eberlein and Fore, 1984).

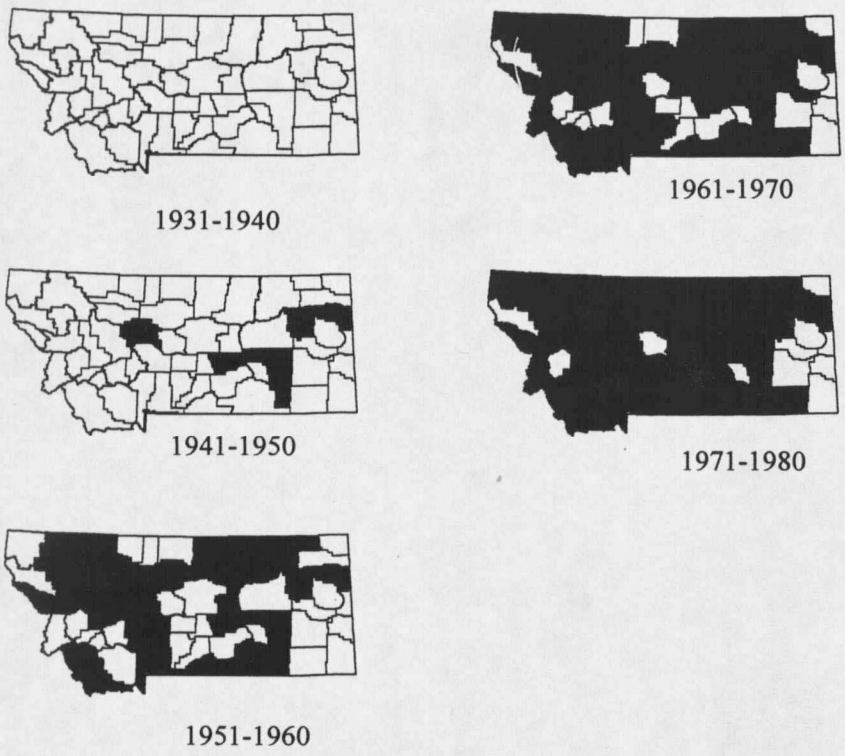


Figure 1. The spread of kochia documented by herbarium specimens collected from 1931-1980 (Forcella, 1985).

Bell et al. (1972) reported that kochia flower induction was triggered by a photoperiod shorter than a critical light period per 24-hour cycle. Flower induction by photoperiod varied from 13 to 15 hours among kochia selections. The period from emergence to flowering, varied among selections from 57 to 100 days. Kochia plants were estimated to produce from 14,600 to 23,350 seeds per plant (Stevens, 1932 and Nussbaum et al., 1985). Seeds are 2 to 3 mm long enclosed in a star-shaped pericap (Eberlein and Fore, 1984). Kochia seed germination in a ten year burial study in Nebraska was initially 40% and had 0 to 1% germination after three years (Burnside et al., 1981).

Kochia is a competitive weed in several crops including wheat (*Triticum* sp.) (Buhler et al., 1985), barley (*Hordeum vulgare* L.), flax (*Linum usitatisumum* L.), and

oats (*Avena sativa* L.) (Dexter, 1982). Four kochia plants per 30 meters caused an 8% yield loss in sugarbeets (*Beta vulgaris* L.) (Schweizer, 1973). A survey was conducted in 1984 of 371 growers in western Nebraska. Twenty-one percent of the growers reported kochia as a weed in wheat production, and 29% reported kochia as a problem in wheat stubble (Wicks et al., 1984). In Montana, kochia is a common weed in most crops and is a troublesome weed in wheat (Bridges, 1992).

Many factors contribute to the competitiveness of kochia including early germination in the spring (Stevens, 1965), tolerance to both dry soil conditions and a wide range in soil pH, and tolerance to salinity (Evetts and Burnside, 1972). Germination occurs early in the spring when average minimum soil temperature of $3^0$ C is reached at a depth of 5-cm (Nussbaum et al., 1985). Germination exceeded 60% when soil pH ranged from 2 to 8 (Evetts and Burnside, 1972). Davis et al. (1967) measured the root system of sorghum (*Sorghum bicolor* L. Moench) and seven weed species including kochia. In early growth stages kochia root systems develop slowly. Ten weeks after transplanting, root growth rapidly increased. The root system of a mature kochia plant covered an area 6.7 m wide and more than 2 m deep. The average plant was 170 cm tall and dry-matter production averaged 771 g (Davis et al. 1967). Weise and Vandiver (1970) tested kochia in three watering regimes, kochia produced increasing amounts of dry matter as soil moisture decreased.

Mature kochia plants distribute seeds effectively by wind dispersion. Stem flexibility gradually decreases during senescence, and the plant eventually breaks off. Stem brittleness contributes to fracturing of the abscission zone by wind (Becker, 1978).

The globe shaped plants tumble very effectively and distribute seed over long distances.

## Herbicide Resistance

The first report of herbicide resistance was reported in the 1960's and the occurrence of resistance has dramatically increased since the mid-1980's (Holt and LeBaron, 1990). More than 100 herbicide resistant weed species have been identified (Holt and LeBaron, 1990). Of these, the majority are resistant to one or more of the triazine herbicides.

There are three general plant responses to herbicides: susceptible (S), tolerant, and resistant (R). A plant is susceptible if it is damaged by the herbicide. Tolerance is traditionally described as the ability to withstand herbicide treatment that may be overcome by higher doses (Holt and LeBaron, 1990). Resistance is described as the ability of plants in a population to grow normally at the previously lethal herbicide dose (LaBaron and Gressel, 1982).

LeBaron and McFarland (1990) described the characteristics of herbicides that contributed to high risk of herbicide resistance. Resistance has a high probability of occurring if the herbicide has a single target site of action, a specific mode of action, a high frequency of use, high efficacy, and provides season long control. Examples of herbicides that meet some or all of these criteria are the bipyridiliums, dinitroanilines, diphenyl ethers, imidazolinones, sulfonylureas, triazines, uracils, and ureas. Low risk herbicides include amides, aliphatics, benzoics, carbamates, nitriles, organic arsenicals, phenoxys, and thiocarbamates (Holt and LeBaron, 1990). Newer herbicides, such as

tribenuron and thifensulfuron, appear to fit the criteria rapid evolution of herbicide resistance.

Several management practices have been proposed to delay or eliminate the selection of resistance. Rotation of herbicides with different modes of action and tank mixtures have been proposed to delay resistance (Gressel and Segel, 1990). Seeds and pollen are sources for spreading and maintaining resistance. Kochia pollen is viable 48 hrs after dehiscence. A Russian thistle (*Salsola iberica* Sennen & Pau) plant with similar dispersal mechanism as kochia distributed seed from a stubble and summer fallow site a maximum of 4069 m and 3438 m, respectively (Stallings et al. 1995). Limiting dispersal of seed would slow the spread of resistance. Crop rotations may extend the evolution of resistance by using alternative cultural management, rotation of mode of action and rate changes.

## Chlorsulfuron

Chlorsulfuron (2-chloro- N [[(4-methoxy-6-methyl-1, 3, 5-triazin-2-yl) amino) carbonyl] benzenesulfonamide) is a sulfonylurea herbicide (Figure 2). Chlorsulfuron has low mammalian toxicity, low application rates, excellent crop safety, is highly persistent in soils and provides excellent control of many species. Chlorsulfuron is applied as a pre- or post- herbicide in wheat, barley and oats for the control of many broadleaf weeds including kochia (WSSA Herbicide Handbook, 1994).

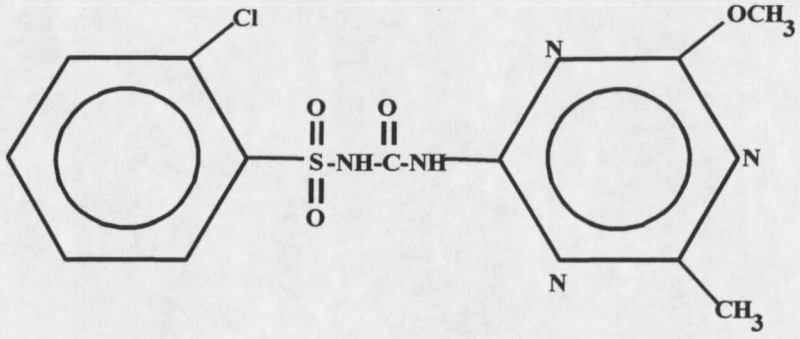


Figure 2. Chemical structure of chlorsulfuron.

Chlorsulfuron inhibits acetolactate synthase (ALS), an enzyme in the biosynthesis of branched-chain amino acids isoleucine, luecine, and valine. Plants treated with chlorsulfuron are inhibited within hours of application. Chlorotic symptoms are not observed for 1 to 2 weeks after application. Symptoms are observed in the meristematic regions initially, followed by the foliar region (WSSA Herbicide Handbook, 1994).

Mechanisms of tolerance or resistance to chlorsulfuron have been evaluated by several researchers (Saari et al., 1990; Sweetzer et al., 1982; Hatzios and Howe, 1982; Guttieri et al., 1995; Heap and Knight, 1986; Preston et al., 1996). Two distinct mechanisms have been identified for chlorsulfuron resistant weeds.

Preston et al. (1996) studied the mechanism for resistance to chlorsulfuron in a population of *Lolium rigidum* (Boiss. & Hohen) resistant to several herbicides with five different modes of action. The majority of the population exhibited the ability to detoxify

chlorsulfuron, however 4% of the population expressed an insensitive ALS.

Sweetzer et al. (1982) evaluated chlorsulfuron for selectivity in several species. Slight differences in translocation between tolerant and S species did not account for the large differences in tolerance. However, speed of metabolism correlated with sensitivity to chlorsulfuron. Wheat, barley, wild oat, and annual bluegrass had less than 10% $^{14}C$-chlorsulfuron in treated leaves 24 hours after treatment, however cotton, soybean, mustard, and sugar beet had greater than 80% of the labeled carbon. In a comparison of sugar beet and wheat, 24 hours after treatment wheat had converted 68% of absorbed $^{14}C$-chlorsulfuron to what the author refers to as metabolite A (Sweetzer et al. ,1982). Sugar beet, a very S species, exhibited no metabolism. Sweetzer et al. (1982) concluded that metabolite A was the major metabolite in the tolerant grass species.

**Chlorsulfuron Resistance**

Chlorsulfuron was registered in 1982. The first documented case of resistance was reported in 1987 where prickly lettuce (*Lactuca serriola*) was not controlled by a fall application of DPX-G8311, a 5:1 mixture of chlorsulfuron:metsulfuron, in winter wheat (Mallory-Smith et al., 1990). By 1990, 14 species exhibited resistance to one or more of nine sulfonylureas. Fifteen states, three Canadian provinces, Australia, Denmark, England, and Israel have confirmed resistance to one or more sulfonylurea herbicides (Saari et al., 1994).

## Chlorsulfuron Resistant Kochia

Saari et al. (1990) evaluated the mechanism for resistance in two kochia biotypes collected in Kansas. A post emergent rate of 350-fold chlorsulfuron was required to suppress growth in the resistant biotype. The author evaluated wheat and the two kochia biotypes 24 hours after treatment with 10 $\mu$l of $^{14}$C-chlorsulfuron and 0.25% (v/v) X-77. Wheat metabolized 100% of the $^{14}$C-chlorsulfuron while kochia biotypes metabolized less than 5%.

Lovell et al. (1996) evaluated two kochia biotypes from Montana at the whole plant level and in vivo ALS inhibition. At the whole plant level, biotypes were evaluated for phytotoxicity, the R biotype was 170-fold more resistant than the S biotype. Analysis of in vivo ALS activity showed a 73-fold resistance. The R biotype was also cross resistant to imazethapyr demonstrating a 5-fold resistance compared to the S biotype (Lovell et al., 1996).

## Dicamba

Dicamba (3,6-dichloro-2-methoxybenzoic acid) was discovered by S. B. Richter in 1958 and awarded U.S. patent 3013054 (Figure 3). It is used as a pre-emergence, post-emergence, or pre-plant herbicide for broadleaf weed control in small grains, corn, sorghum, asparagus, perennial seed grasses, turf, grassland, pasture, rangeland and non-crop land. Dicamba is a substituted benzoic acid that causes typical auxin type symptoms. It is thought to mimic indole-3-acetic acid. The stem and nodes of plants treated with dicamba swell and the plants exhibit epinasty, elongation, and downward leaf cupping. Chlorosis at the growing points, stunting, wilting, and necrosis follow initial symptoms.

The specific mechanism of action of dicamba remains unknown.

Scott and Norris (1990) exposed 48 hour old pea seedlings to 1 X $10^{-5}$ M dicamba solution for 4 hr. Seedlings were rinsed and planted into vermiculite. Symptoms were measured immediately, 9, 12, and 72 hrs after treatment. The peas ceased longitudinal growth immediately. Nine hours after the application of dicamba root primordia was initiated. Swelling behind the root tips occurred 12 hours after application. Seventy-two hours after treatment massive lateral root formation was observed. Dicamba significantly increased ethylene production from treated plants.

Malformation and yield loss from the application of dicamba to wheat and barley during actively growing periods have been reported (Friesen et al., 1964; Martin et al., 1988). Friesen et al. (1964) applied two rates of dicamba at eight growth stages at two locations. Dicamba applied before the four leaf stage of wheat caused bending of internodes on the main culm. Barley exhibited trapped heads and curled awns when dicamba was applied at the four leaf stage. Sterile wheat florets were found in 68% of the main culms sprayed with dicamba at the boot stage.

Dicamba is weakly adsorbed and mobile in soils. At 25° C, dicamba acid has a solubility in water of 4500 mg/L. Donald and Syrginnis (1995) collected samples of water, sediment and zooplankton from 19 large lakes in Saskatchewan during a period of severe drought conditions and analyzed the samples for several pesticides. Dicamba was detected in 10% of the water samples, but was not detected in the sediment or zooplankton samples.

Dicamba has a half life of less than 14 days in soil. Two *Pseudomonas* spp. and a *Moraxella* sp. are able to readily degrade dicamba (Fogarty and Tuovinen, 1995). Microbes transformed dicamba to 3,6 - dichlorosalicylate. Non-biological means of degradation were inconsequential (Krueger et al.1992; Fogarty and Tuovinen, 1995). With a half life of 269 days under xenon lamps, photodegradation of dicamba is slow (WSSA Handbook, 1994).

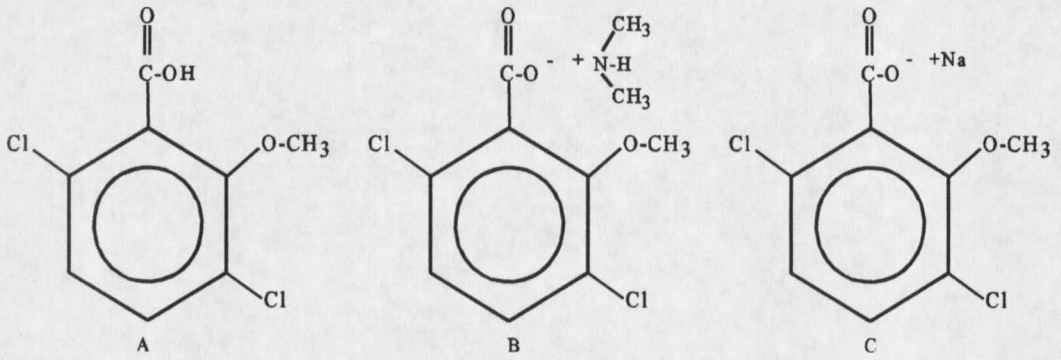


Figure 3. Chemical structure of dicamba free acid (A), dimethylamine salt of dicamba (B) and sodium salt of dicamba (C).

## Volatility

Richter and Malina (1972) reported that the dimethylamine (DMA) formulation of dicamba has a vapor pressure of $9.24 \times 10^{-6}$ mm Hg at 25 C and caused no injury from vapor drift. Berhrens and Lueschen (1979) did extensive studies with the sodium, dimethylamine, diethanolamine, and N-tallow-N, $N^1$, $N^1$-trimethyl-1, 3 diaminopropane salts of dicamba. They concluded that the DMA formulation caused injury from vapor drift.

In a closed chamber, soybeans (*Glycine max* L.) were exposed to dicamba-treated corn (*Zea mays* L.). Soybean injury varied greatly depending on the formulation used. Sodium, lithium, potassium, tallow amine, diethanolamine and triethanolamine caused little injury. Methylamine and dimethylamine formulated dicamba caused injury. In field experiments, soybeans exhibited less injury from the sodium and tallow salts than from the dimethylamine.

Rainfall also effects the degree of injury to soybeans from vapor drift. Dicamba treated corn was placed into glass jars at 30 C with soybeans followed by simulated rainfall, which eliminated soybean injury. Increased temperatures influenced injury from dicamba vapor. Soybean injury increased substantially when temperatures increased from 15 to 30 C. Gas chromatographic-mass spectrometric analysis of vapors emitted from a glass surface showed that the dimethylamine salt of dicamba was degraded to the free acid which caused vapor drift injury to soybeans (Behrens and Lueschen, 1979).

## Dicamba Antagonism

Dicamba is antagonistic to several herbicides (Flint and Barrett, 1989; Aguero-Alvarado et al., 1991; Hart et al., 1992; and Andrews, 1990). The addition of dicamba to primisulfuron reduced visual control of shattercane (*Sorghum bicolor* L. Moench) and giant foxtail (*Setaria faberi* Herrm.) in greenhouse studies.

Further studies showed $^{14}C$- primisulfuron absorption by shattercane was reduced when tank mixed with dicamba. However, the addition of ammonium sulfate reversed dicamba antagonism. Absorption of $^{14}C$-primisulfuron by giant foxtail was not affected by

addition of dicamba (Hart et al., 1992).

Aguero-Alvarado et al. (1991) treated tall fescue (*Festuca arundinacea* Schreb.) plants with several concentrations of haloxyfop-methyl alone or mixed with two concentrations of dicamba. Herbicidal activity of haloxyfop-methyl was reduced by the addition of dicamba. The tank mixture of haloxyfop-methyl and dicamba had an increased level of epinasty that led to the suggestion that haloxyfop-methyl may have increased dicamba uptake (Aguero-Alvarado et al.,1991). Antagonism was observed when a tank mix combination of diclofop-methyl and dicamba was used to treat common oat (*Avena sativa* L.) and wild oat (*Avena fatua* L.) (Andrews, 1990).

Glyphosate uptake and translocation were reduced by the addition of dicamba in Johnsongrass (*Sorghum halipinse* (L.) Pers.). The treated leaf had a higher percentage of $^{14}$C-glyphosate when dicamba was applied (Flint and Barrett, 1989).

**Dicamba Absorption, Translocation**

Wheat (*Triticum astevium* L.), barley (*Hordeum vulgare* L.), tartary buckwheat (*Fagopyrum tartaricum* (L.) Gaertn.) and wild mustard (*Brassica kaber* (DC.) Wheeler) were used to conduct uptake, translocation and metabolism studies with $^{14}$C-dicamba. Tartary buckwheat and wild mustard accumulated $^{14}$C-dicamba in young leaves and meristimatic regions. Wheat and barley had a more even distribution of $^{14}$C-dicamba. Plants treated by a foliar application or by root uptake using a nutrient solution application of $^{14}$C-dicamba showed similar distribution patterns. More radioactivity was found in the roots following nutrient solution application (Chang and Vander Born, 1971).

Ray and Wilcox (1969) treated purple nutsedge (*Cyperus rotundus* (L.)) plantlets that were planted in individual pots. Plantlets were connected to each other by rhizomes. They estimated 6% of the dicamba applied was detected in the indirectly treated plantlets.

Injury caused by a late application of dicamba to wheat and barley can be accounted for by a greater amount of dicamba found in the main culm of wheat. Quimby and Nalawaja (1971) reported more $^{14}C$ label in the main culm than the other tillers 22 days after application.

Root exudation followed by foliar application has been noted by several researchers (Chang and Vander Born, 1971; Linder et al., 1964; Quimby and Nalawaja, 1971; Petersen et al., 1985). Wheat and wild buckwheat excreted less than 2.5% $^{14}C$-dicamba 22 days after application (Quimby and Nalawaja, 1971). Chang and Vander Born (1971) estimated that approximately 0.5% foliar applied dicamba was exuded from the roots of Canada thistle (*Cirsium arvense* (L.) Scop.). Less than 1% of foliar applied dicamba was exuded by bean plants 24 hrs after application (Linder et al., 1964). Petersen et al. (1985) reported that 66% of absorbed $^{14}C$-dicamba was exuded out of soybean roots 60 hrs after application.

## Dicamba Metabolism

Several authors have reported the major metabolite of dicamba as 5-hydroxy-3,6-dichloro-*o*-anisic acid (5-OH) and the minor as 3,6-dichlorosalicylic acid (DCSA) (Robocker and Zamora, 1976; Chang and Vander Born, 1971; and Broadhurst et al., 1966) (Figure 4).

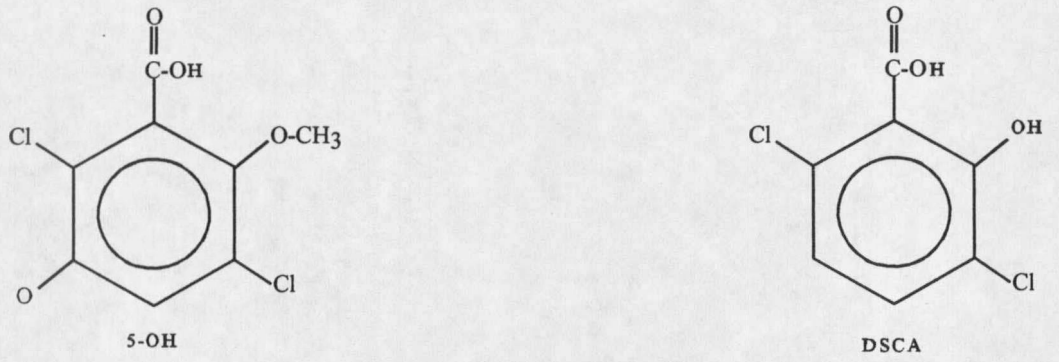


Figure 4. 5-hydroxy-3,6-dichloro-*o*-anisic acid (5-OH) and 3,6-dichlorosalicylic acid (DCSA) metabolites of dicamba.

Chang and Vander Born (1971) examined tartary buckwheat's ability to metabolize dicamba. Twenty days after treatment with 0.1 $\mu$c of $^{14}$C-dicamba, 10% of the recovered dicamba was in the form of metabolites. The major metabolite was 5-OH dicamba. Tartary buckwheat was then treated with 5-OH dicamba, and no injury resulted from the application. Dicamba and 5-OH dicamba both translocated in the plants. The 5-OH dicamba translocated slowly, while little dicamba remained in the treated leaf 13 days after application.

Twenty days after application of $^{14}$C-dicamba, 90% and 85% of total activity was metabolized, by wheat and barley, respectively (Chang and Vander Born, 1971). Quimby and Nalawaja (1971) reported a significant difference in the metabolism rates (76.1 and 21.6%) of main culms of wheat and wild buckwheat meristems, after 15 days. The $^{14}$C activity was reported as an unknown metabolite and/or conjugate.

Canada thistle metabolized $^{14}$C-dicamba very slowly, fifty-four days after application 36.9% of radioactivity was identified as a metabolite. Most of the metabolism

occurred in the treated leaf. The shoot above the treated leaf only accounted for 3% of radioactivity in the form of metabolites nine days after treatment. Purple nutsedge, which is susceptible to dicamba, exhibited no indication of metabolism 10 days after application (Magalhaes et al., 1968).

**Dicamba Resistance**

Auxin-type resistance in wild mustard was first reported in 1990 (Heap and Morrison, 1992). Resistance was observed following an application of a mixture of dicamba, MCPA and mecoprop was used in a field for 10 years. R wild mustard was 104 times more resistant to dicamba than the S biotype based on dose response experiments (Heap and Morrison, 1992).

Peniuk et al. (1993) conducted uptake, translocation and metabolism studies on wild mustard with radiolabelled 2,4-D, dicamba, and picloram. A time course of 12, 24 and 48 hours was used to evaluate differences between R and S biotypes of wild mustard. The only significant difference between the biotypes occurred at 48 hours in the foliage above the treated leaf. R and S biotypes had 71.8 and 81.4% of recovered radioactivity, respectively. Metabolism of dicamba in both biotypes exhibited no significant differences. Ethylene evolution was also evaluated. S biotypes produced more ethylene when treated with 2,4-D. A sixfold increase was observed at 44 hours. Differences may exist at target site(s) or auxin receptors based on differences in ethylene evolution (Peniuk et al., 1993). Inheritance of dicamba resistance in wild mustard was determined to be a single, completely dominant nuclear gene (Jasieniuk et al., 1996).

The removal of chlorsulfuron from Montana in 1992 led to speculation on how the level of kochia R to chlorsulfuron would change. The options for kochia control were to use another sulfonylurea, dicamba, or bromoxynil. Bromoxynil is the most expensive alternative. In 1995, there were several complaints in Montana about the efficacy of dicamba on kochia.

**Objectives**

The objectives of this study were to: 1) describe the distribution of chlorsulfuron R kochia during 1992 and 1993 in Montana, 2) describe the occurrence of dicamba R kochia in Montana, 3) evaluate dicamba R kochia for cross resistance, 4) determine the dose response of dicamba on R and S kochia biotypes, 5) determine the differences of uptake, translocation, and metabolism of dicamba R and S kochia biotypes.

Chapter 2

# DISTRIBUTION OF CHLORSULFURON RESISTANT KOCHIA IN SMALL GRAIN PRODUCING AREAS OF MONTANA

## Introduction

Kochia (*Kochia scoparia* L. Schrad) is a summer annual weed belonging to the Chenopodiaceae or goosefoot family. It was introduced to North America as an ornamental in the early-1890's. This weed escaped cultivation and has become widely distributed throughout the Great Plains. Kochia was found in central Montana in the 1940's. In Montana, kochia is considered one of the most troublesome weeds in wheat (Bridges, 1992).

In the United States, chlorsulfuron was registered in 1982. Chlorsulfuron was a highly effective herbicide used at low application rates in small grains for kochia control (Levitt et al., 1981). Primiani (1990) reported that in the United States more than 2.5 million ha of wheat were treated with chlorsulfuron. Within five years after registration, kochia had developed resistance to chlorsulfuron (Primiani et al. 1990). In 1992, chlorsulfuron was removed from Montana in an attempt to manage sulfonylurea resistance in kochia.

Other sulfonylurea herbicides are being used to replace chlorsulfuron for controlling kochia in small grains. It is important to know the distribution of chlorsulfuron resistance in order to implement sulfonylurea resistance management strategies. The objective of this study was to characterize the degree and distribution of resistant kochia in small grain production areas in Montana.

# Methods and Materials

## Plant Material Collection

Kochia seeds were collected from six of the seven agricultural statistic districts in Montana (Sands and Lund, 1997; Figure 5). The northwest district was excluded from this sampling because of the limited number of hectares in small grains and the limited infestations of kochia. Various cells (5 km x 5 km) were located within each district (Figures 6,7). Cell numbers were stratified randomly based on the number of hectares in small grain production. Those areas with more hectares of small grain received a greater number of samples. In 1992 and 1993, all seeds from 30 individual kochia plants were collected from 151 and 168 cells, respectively, to be used for determining the occurrence of chlorsulfuron resistance. Seeds were removed from plants and placed into individual seed packets (14 x 21 cm). Packets were placed in cold (5°C) seed storage.

A subsample of 15 $mm^3$ of seeds was removed from each of the 30 seed packets and cleaned by sifting through 2 mm mesh screens. Seeds were put into a composite seed packet. Approximately 75 seeds (1 cm apart) from each cell were sown into plastic flats (25 x 52 x 6 cm) containing a 1:1:1 mixture of Bozeman silt loam: washed sand: peat moss. Each flat had eight rows 5 cm apart. Seeds representing each cell were consecutively sown in 19 (151 cells/8 rows) flats in 1992 and 21 (168 cells/8 rows) flats in 1993. For example, the first flat consisted of cells 1-8. Flats were treated pre-emergence with 0.45 g ai/ha chlorsulfuron in a carrier volume of 71 liters $ha^{-1}$ using a belt sprayer. Each cell was replicated three times and flats were placed randomly in a greenhouse. The experiment was repeated twice for each year the seeds were collected.

## MONTANA AGRICULTURAL STATISTICS DISTRICTS

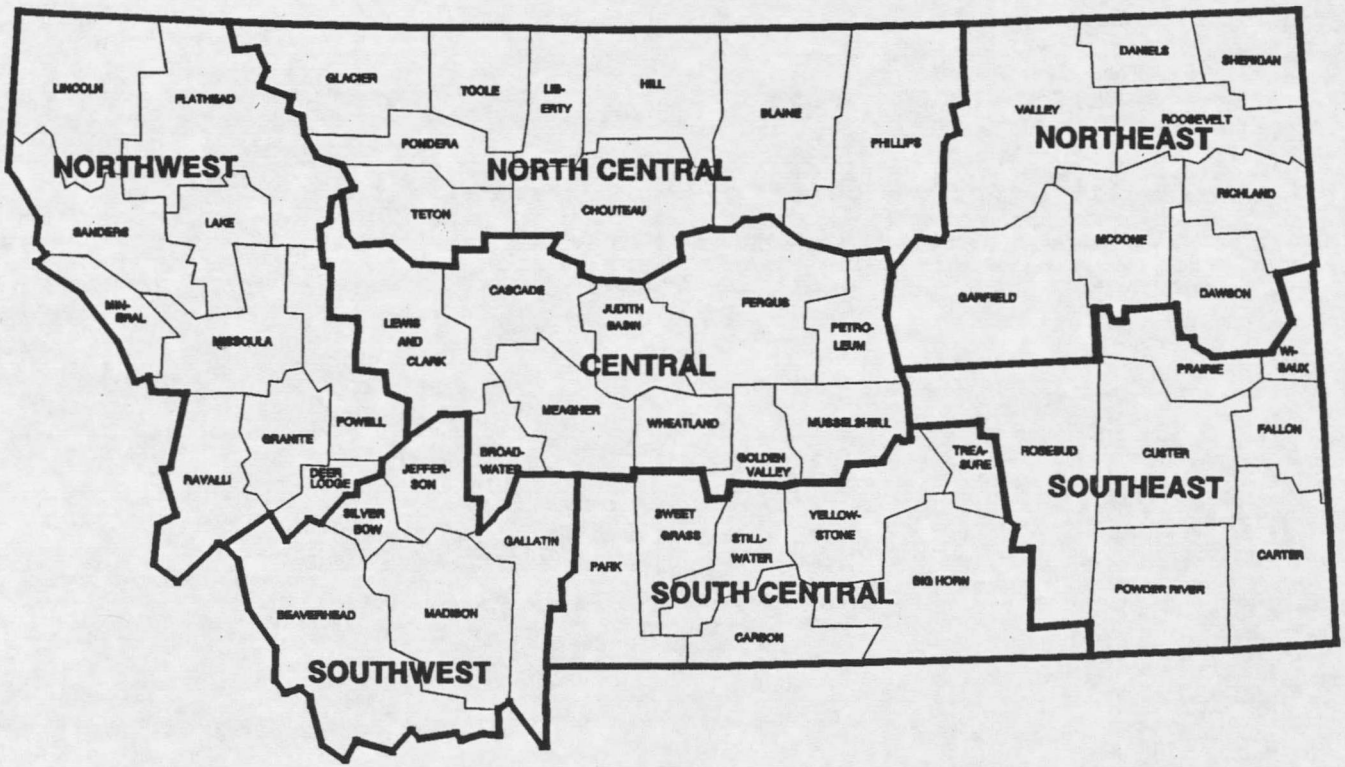


Figure 5. Agricultural districts in Montana

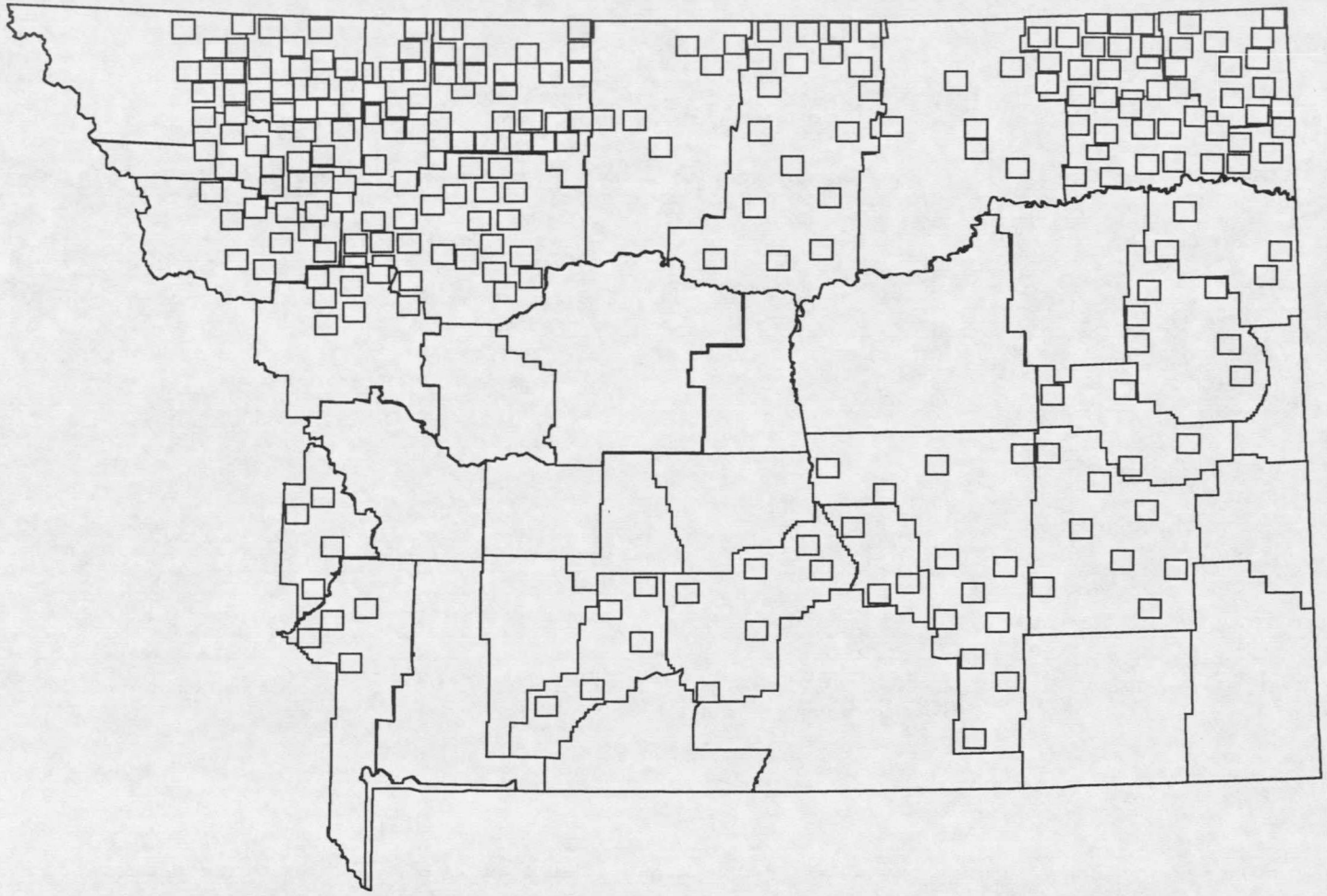

Figure 6. Stratification of cells in the small grain agricultural districts in Montana.

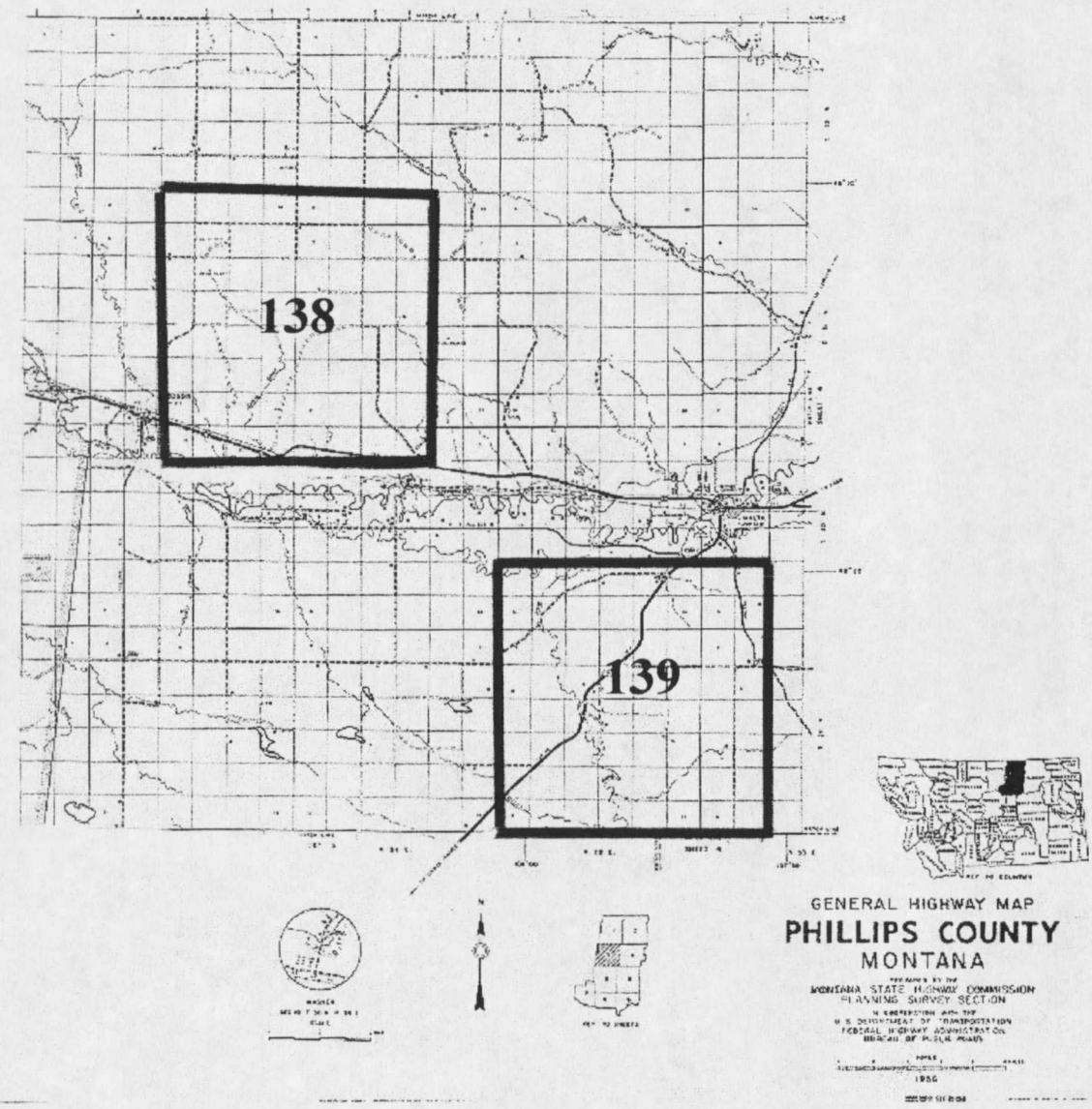


Figure 7. Example of map used to collect kochia samples.

Kochia plants were grown in a greenhouse with day/night temperatures of 22/16°C, respectively. Flats received 16 hrs of artificial light (600 umoles/m2/sec). Flats were treated post-emergence two weeks after seeding, just prior to true leaf formation, with 0.001 kg ai $ha^{-1}$ chlorsulfuron and 0.25% v/v nonionic surfactant in a carrier volume of 71 l/ha using a belt sprayer. Plants were visually rated for percent control two weeks after the post-emergence application. Susceptible kochia plants do not produce true leaves which makes them distinguishable from resistant plants.

**Analysis**

Data were analyzed as a split-block in time with cells as whole plots and year as subplots. Mean separations were made using standard error comparisons. Maps were created using the original highway maps and cell locations were transferred onto a computer generated map. Each map is one of six districts.

## Results

In 1992 and 1993, 151 and 168 cells were sampled for resistance, respectively. Of these, 108 cells were sampled in both years. Analysis of variance indicated that chlorsulfuron resistance was dependant upon the agricultural district and year collected (Table 1). Of the 108 cells sampled in 1992 and 1993, the average percent chlorsulfuron resistant kochia was 23% and 31%, respectively (Figures 1-6).

Table 1. Mean square generated from SAS® split-block analysis of kochia resistance.

| Source | DF | Mean Square [1] |
|---|---|---|
| Block | 5 | 581 |
| Year | 1 | 1568 |
| Year * Block (error a) | 5 | 56 |
| District | 5 | 37700 |
| District * Block | 25 | 117 |
| District * Year | 5 | 3124 |
| Year * District * Block (error b) | 25 | 152 |

[1] Model mean square error = 898

Central, North Central, and Northeast districts had about 32, 43, and 25 percent resistance in 1992, which was similar to that in 1993 (Table 2). In 1992, percent resistance was lowest in the southern districts. However, the Southeast and Southwest districts had similar percent resistance which was similar to that in the Northeast district. In 1992, the South Central and Southeast had the lowest resistance. Interestingly, the Southwest district had the highest percent resistance in 1993. The trend is similar in cells that were only collected in one year. Districts with high selection intensity had greater

percent resistance than districts with low selection intensity. Although the average percent resistance for the districts is relatively low, there was only seven of the 108 cells sampled in both years that did not exhibit any resistance. Of all the cells collected in 1992, 94% of them had some resistant plants in the tested population (Figures 8-13). In 1993, 96% of the cells contained resistant plants.

Table 2. Percent resistance of six districts in 1992 and 1993.

| | | 1992 | 1993 |
|---|---|---|---|
| District | # of Cells | -------% Resistance------ | |
| Central | 4 | 32.7 | 32.8 |
| North central | 42 | 42.6 | 43.4 |
| Northeast | 27 | 25.1 | 33.0 |
| South central | 10 | 7.3 | 11.4 |
| Southeast | 22 | 16.1 | 12.7 |
| Southwest | 3 | 15.9 | 52.9 |
| District LSD (0.05) = 14.67 | | Year LSD (0.05) = 8.91 | |

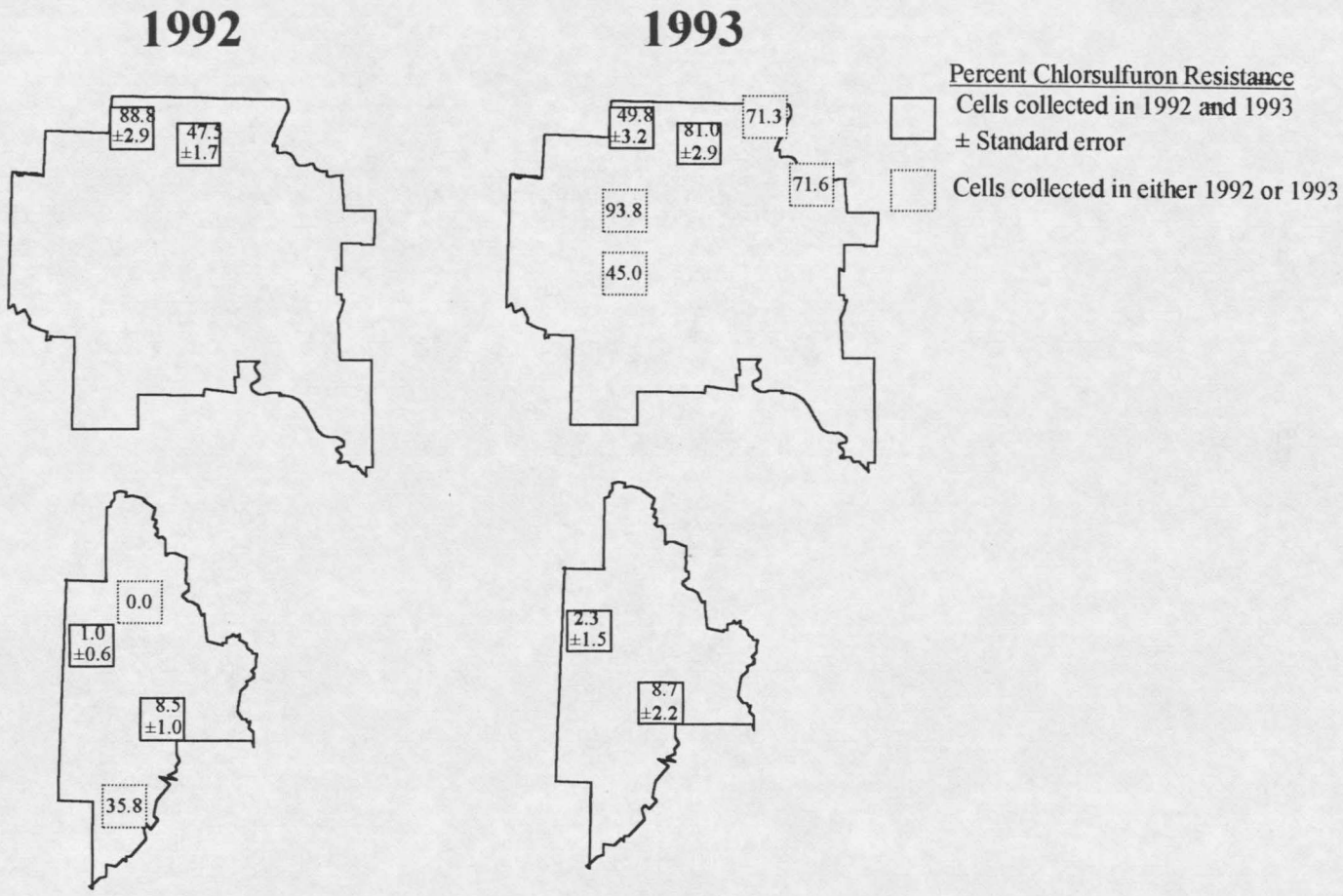


Figure 8. Distribution of kochia resistant to chlorsulfuron in the Central district in 1992 and 1993.

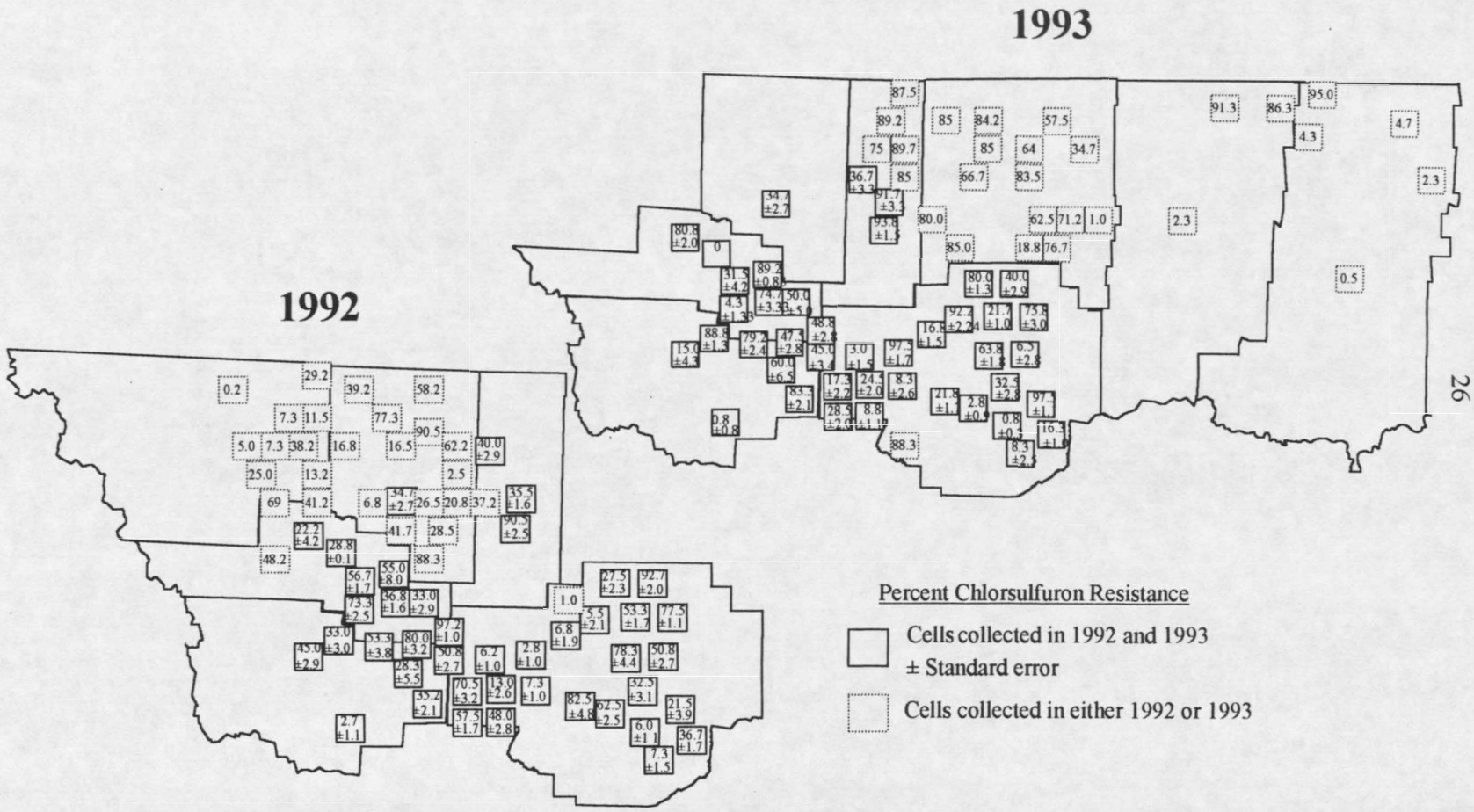


Figure 9. Distribution of kochia resistant to chlorsulfuron in the North Central district in 1992 and 1993.

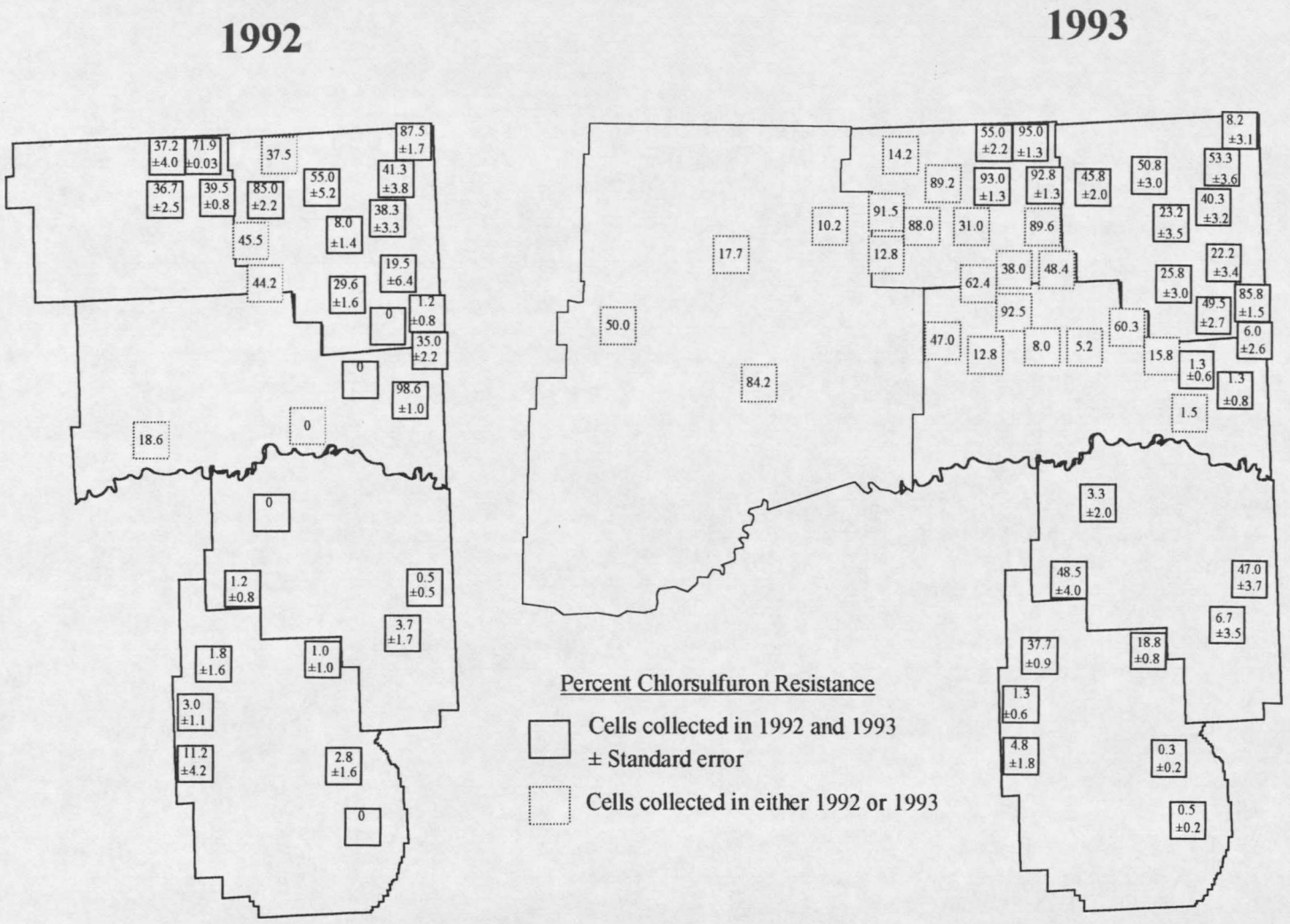


Figure 10. Distribution of kochia resistant to chlorsulfuron in the Northeast district in 1992 and 1993.

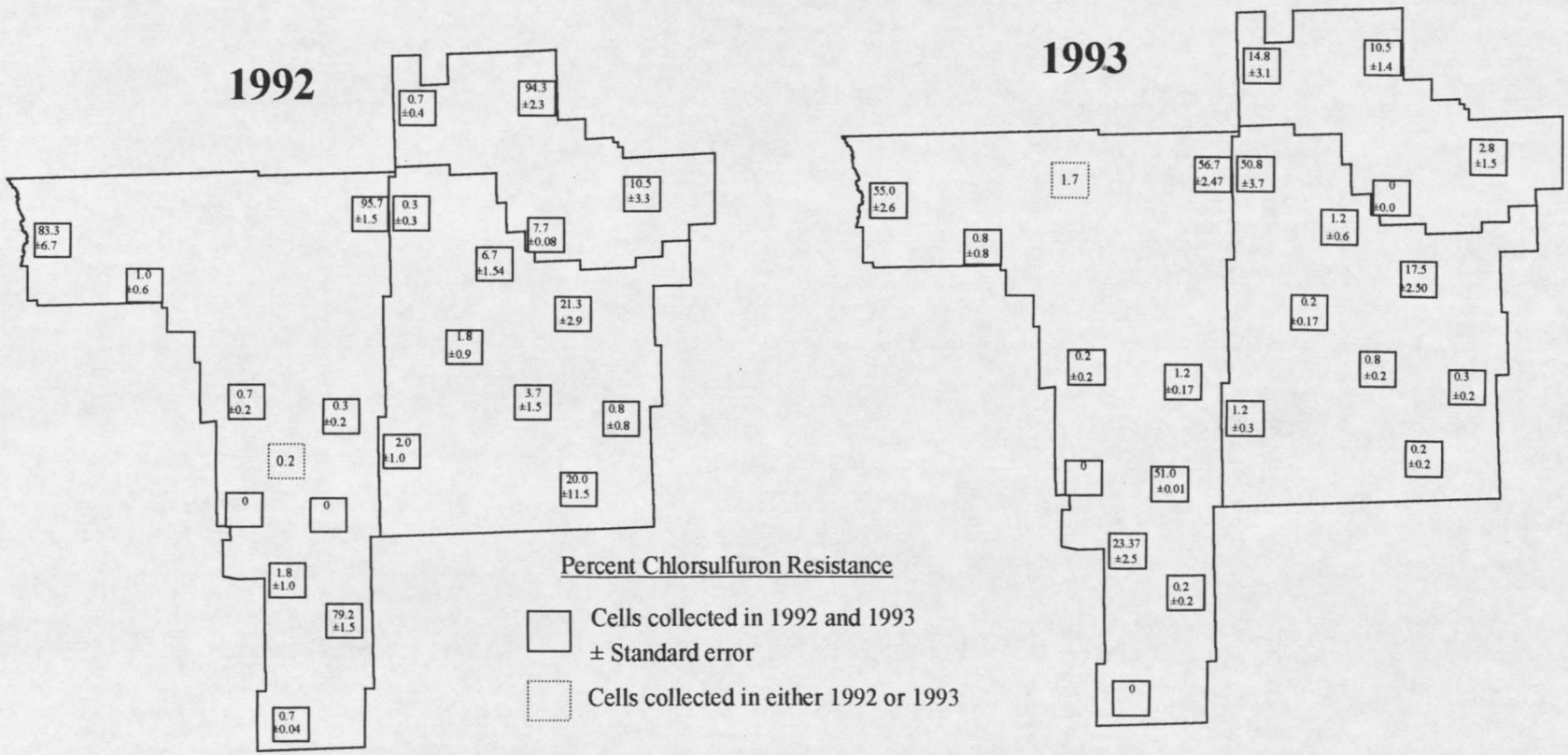


Figure 11. Distribution of kochia resistant to chlorsulfuron in the Southeast district in 1992 and 1993.

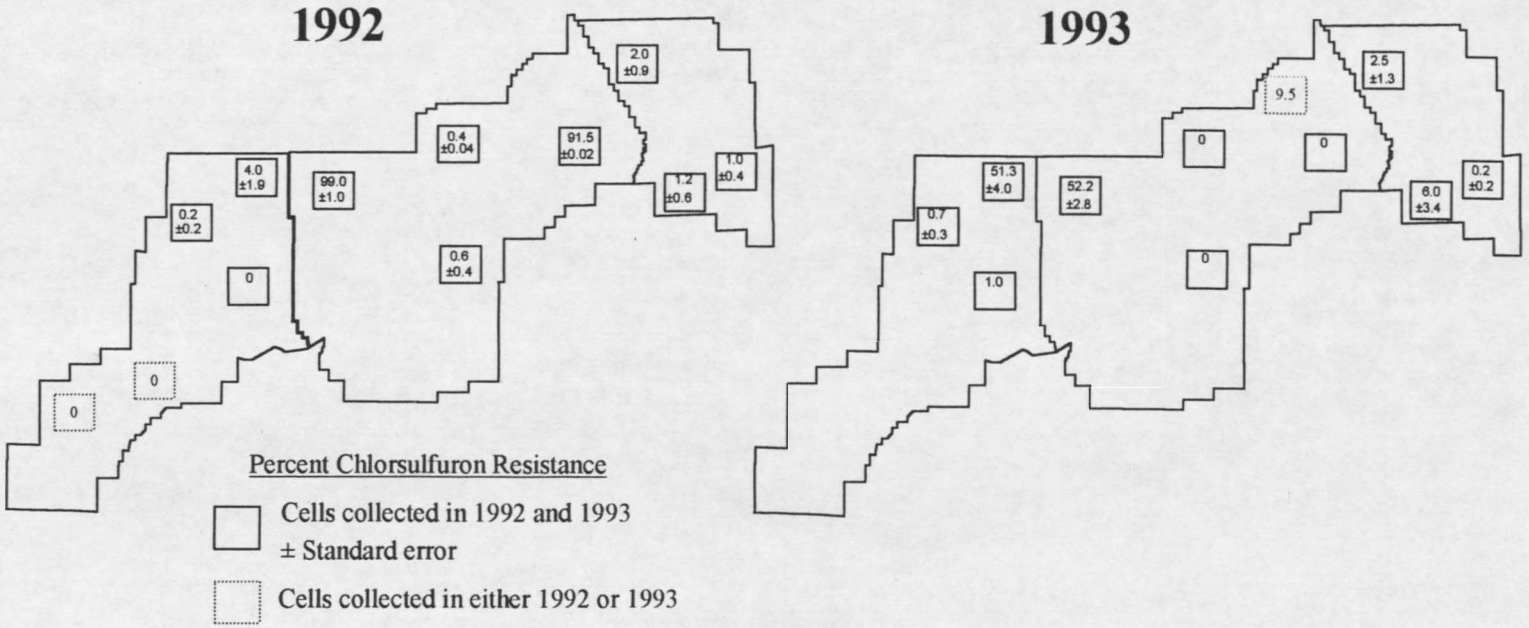


Figure 12. Distribution of kochia resistant to chlorsulfuron in the South Central district in 1992 and 1993.

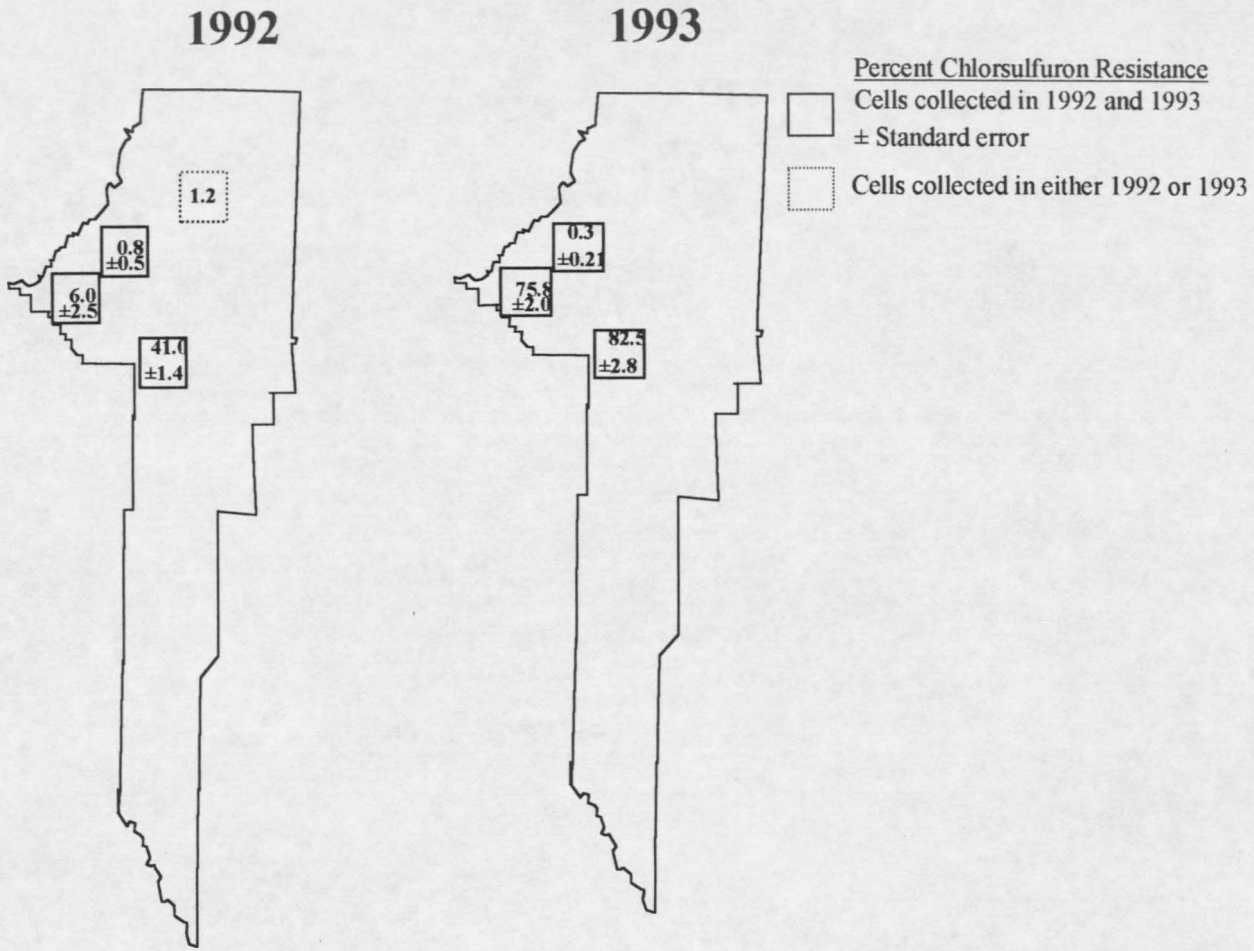


Figure 13. Distribution of kochia resistant to chlorsulfuron in the Southwest district in 1992 and 1993.

## Discussion

Kochia resistant to ALS-inhibitors has been documented in Alberta, Manitoba, Saskatchewan, Canada, Colorado, Idaho, Kansas, Nebraska, New Mexico, North Dakota, Oklahoma, and South Dakota (Saari et al., 1994). In 1992, the South Central district was significantly lower in percent resistance than Central, North Central, and Northeast districts (Table 2). There was less selection intensity in the South Central and Southeast districts because of row crop production, such as sugar beets. The South Central district had 11,420 hectares planted to sugar beets (Montana Agricultural Statistics, 1994). In areas with large acreage of sugar beet production, chlorsulfuron was not generally used because of plant back restrictions. In support of this contention, Richland and Dawson counties in the Northeast district have numerous hectares of sugar beet production and the percent resistance tends to be lower in these two counties than the other three counties in that district (Table 3).

Table 3. Sugar beet production in the Northeast district in relation to kochia resistance.

| | Sugar beet production | | Kochia | |
|---|---|---|---|---|
| | harvested ha [1] | | % Resistance | |
| Northeast District | 1992 | 1993 | 1992 | 1993 |
| Richland and Dawson | 6580 | 6333 | 2.5 | 16.9 |
| Daniel, Sheridan and Roosevelt | ---- | 316 | 42.9 | 41.5 |

[1] Montana Agricultural Statistics, 1994. Source: The National Weather Service, Great Falls, Montana, Montana-NOAA.

The Southwest district had three cells that were sampled in both 1992 and 1993 (Figure 13). Of these, two cells had significant increases in percent resistance. The decline in available kochia plants in the area might explain the increase. In some areas, kochia was difficult to find in 1993. Weise and Vandiver (1970) found that kochia dry matter increased as soil moisture decreased. In the six districts studied in this experiment, the annual precipitation in 1992 and 1993 was 11 and 15 inches, respectively. Average annual precipitation was 10 inches for the period of 1951-1980[2].

Management of resistance is important for all areas of the state, including districts with low levels of resistance. Resistant kochia plants were found in greater than 90% of the cells. Several species resistant to one ALS inhibitor have been reported to be cross resistant to other sulfonylurea herbicides (Mallory-Smith et al., 1990; Primiani et al., 1990; and Saari et al., 1992). Sulfonylurea herbicides are continuing to be used in all areas of the state, alone or as tank-mix partners. In the areas that had low selection intensity, new herbicides, such as tribenuron and thifensulfuron, are being used in sugar beet areas. The continued use of sulfonylurea herbicides will contribute to selecting for and maintaining resistant populations of kochia.

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2

Montana Agricultural Statistics, 1994. Source: The National Weather Service, Great Falls, Montana, Montana-NOAA.

Chapter 3

# OCCURRENCE, DISTRIBUTION, AND CROSS RESISTANCE OF DICAMBA RESISTANT KOCHIA

## Introduction

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is an auxin-like herbicide used for broadleaf weed control in small grains and chemical fallow in Montana. Malformation and yield loss has been reported following an application of dicamba to small grains during actively growing periods (Friesen et al., 1964; Martin et al., 1988). For this reason, dicamba had a reputation for causing "sleepy" wheat when applied after the five leaf stage.

The introduction of chlorsulfuron in 1982 provided a desirable alternative to dicamba because of residual broad spectrum weed control and crop safety. During the following ten years, kochia (*Kochia scoparia* L.), a major weed in small grains, developed resistance to chlorsulfuron. In 1992, chlorsulfuron was removed from the market in an effort to manage the spread of chlorsulfuron resistance in the state. Therefore, dicamba was often used for controlling kochia in small grains. Currently, dicamba is used with 2,4-D (2-4 dichlorophenoxyacetic acid) or in a three-way tank mix with a sulfonylurea herbicide.

Auxinic herbicide resistance has been identified in several species (Barnwell et al., 1989; Bourdot et al., 1989; Lutman and Snow, 1978; and Migo et al., 1986). Heap and Morrison (1992) and Peniuk et al. (1993) described a biotype of resistant wild mustard (*Sinapsis arvensis* L.) that is resistant to dicamba, picloram (4-amino-3,5,6-trichloropicolinic acid), MCPP((±)-2-(4-chloro-2-methylphenoxy) propionate), and

MCPA ((4-chloro-2-methylphenoxy)acetate).

Resistance to more than one chemical has been identified in wild mustard (Peniuk et al., 1993), kochia (Friesen et al., 1993), and especially in a population of *Lolium rigidum*. *Lolium rigidum* is resistant to 12 classes of herbicide families, including 7 modes of action (Hall et al., 1994). Friesen et al. (1993) identified kochia resistant to chlorsulfuron that was also resistant to one imidazolinone and five sulfonylureas herbicides.

The objectives of these studies were to: 1) identify the occurrence of dicamba resistance kochia, 2) evaluate dicamba resistant kochia for cross resistance, and 3) determine the dose response of R and S kochia to dicamba.

# Materials and Methods

## Plant Material Collection

Kochia seeds were collected from six of the seven agricultural statistic districts in Montana (Sands and Lund, 1997; Figure 14). The Northwest district was excluded from this sampling because of the limited number of hectares in small grains and the limited infestations of kochia. Various cells (5 km x 5 km) were located within each district (Figures 15,16). Cells numbers were stratified randomly based on the number of hectares in small grain production. Those areas with more hectares of small grain received greater number of samples. In 1992 and 1993, all seeds from 30 individual kochia plants were collected from 151 and 168 cells, respectively, to be used for determining the occurrence of chlorsulfuron resistence. Seeds were removed from plants and placed into individual seed packets (14 x 21 cm). Packets were placed in cold (5°C) seed storage. After Dr. Peter Fay received grower complaints about lack of kochia control with dicamba in 1995, I used a subsample of these previously collected seeds for this study.

## Occurrence and Distribution of Dicamba Resistance

A subsample of 15 $mm^3$ of seeds were removed from each of the 30 seed packets and cleaned by sifting through 2 mm mesh screens. Seeds were put into a composite seed packet. Approximately 25 seeds (1 cm apart) from each cell were sown into plastic flats (25 x 52 x 6 cm) containing a 1:1:1 mixture of Bozeman silt loam: washed sand: peat moss. Each flat had eight rows 5 cm apart. Seeds representing each cell were consecutively sown in 19 (151 cells/8 rows) flats in 1992 and 21 (168 cells/8 rows) flats in

1993. For example, the first flat consisted of cells 1-8. Each cell was replicated three times and flats were placed randomly in a greenhouse. The experiment was repeated twice for each year the seeds were collected.

Kochia plants were grown in a greenhouse with day/night temperatures of 22/16°C, respectively. Flats received 16 hrs of artificial light (600 umoles/m2/sec). Flats were treated with 0.07 kg ai $ha^{-1}$ dicamba in a carrier volume of 71 liters $ha^{-1}$ using a belt sprayer. Surviving kochia plants were counted two weeks after application.

**Occurrence and Distribution Analysis**

Since the objective of this portion of the study was to identify the occurrence and distribution of dicamba resistant kochia, I used non-parametric rank test (Mann-Whitney)(Snedecor and Cochran, 1989) to identify those cells having survivorship greater than zero. The total number of surviving kochia plants in each cell are presented on maps.

**Complaint Fields**

As a response to complaints about the lack of kochia control using dicamba in 1995, seven fields were sampled and investigated that year. In this experiment, obtaining seeds required sampling kochia plant material, rather than seeds, because they were going to be controlled prior to seed production. Five branches were cut from 25 randomly selected kochia plants in each field. Cuttings were packed in a wet wrap and placed in an iced cooler for transport. Lower leaves were removed from all branches. Branches were re-cut at an angle to enhance uptake and dipped into 15 $mm^3$ of rooting hormone (Rootone® (1-Napthaleneacetamide: 0.20%; tetramethyl thiuramdisulfide:4.04%)

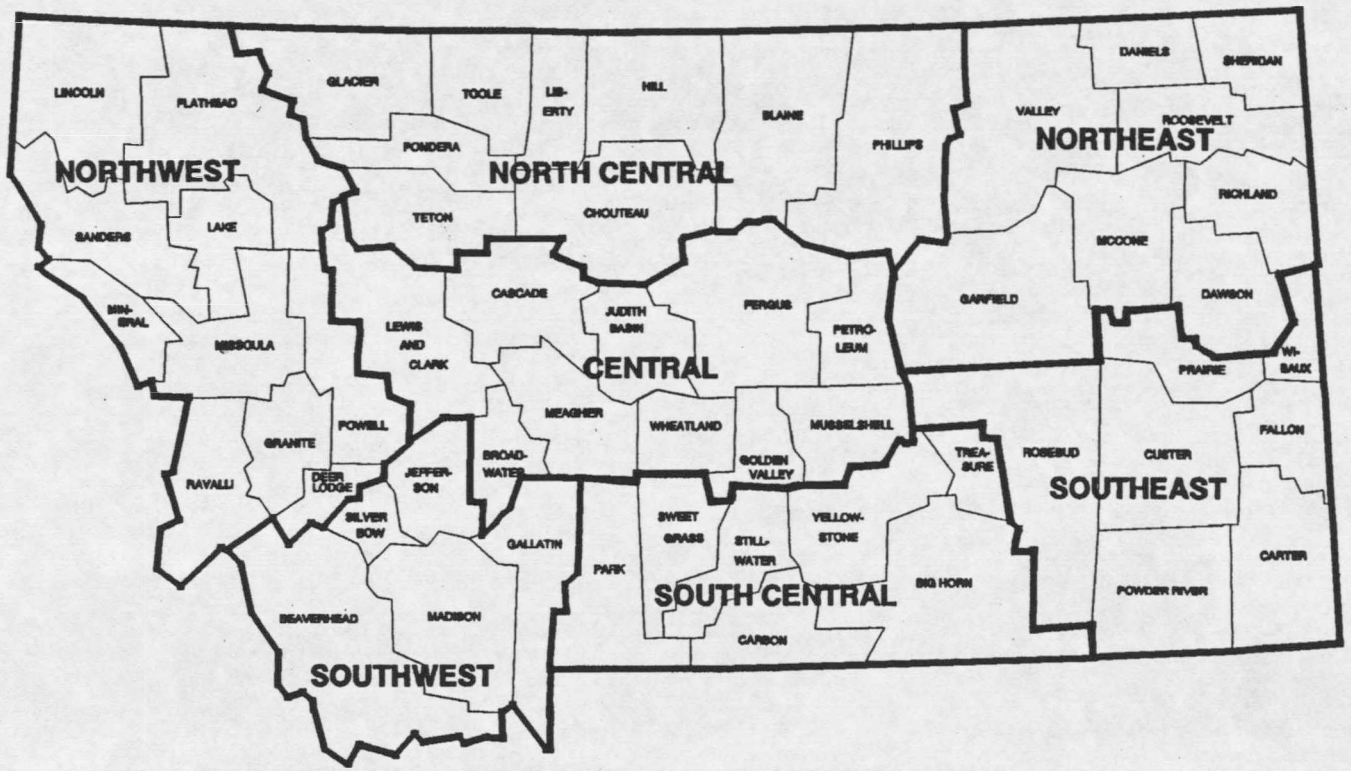



Figure 14. Agricultural districts in Montana

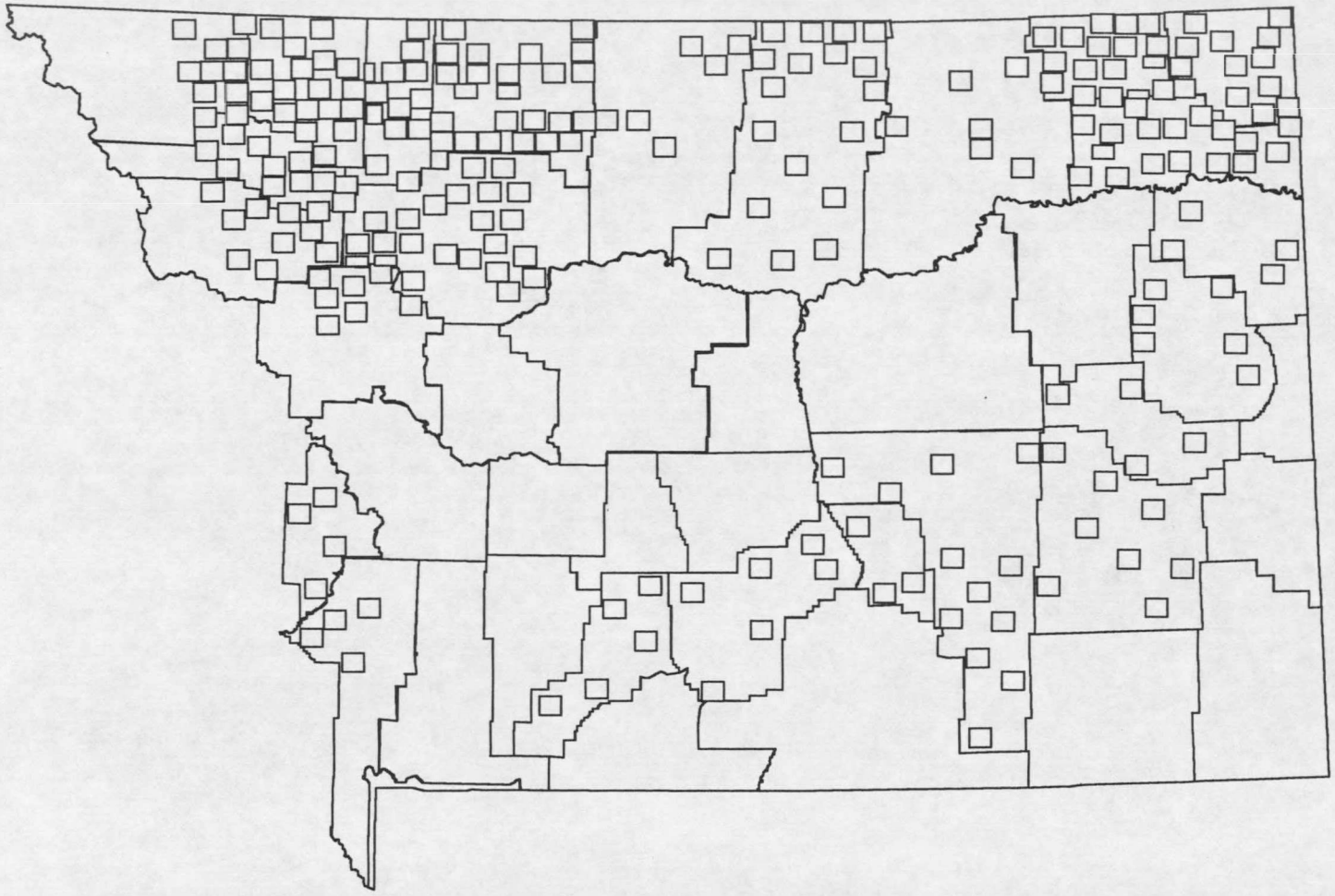


Figure 15. Stratification of cells in the small grain agricultural districts in Montana.

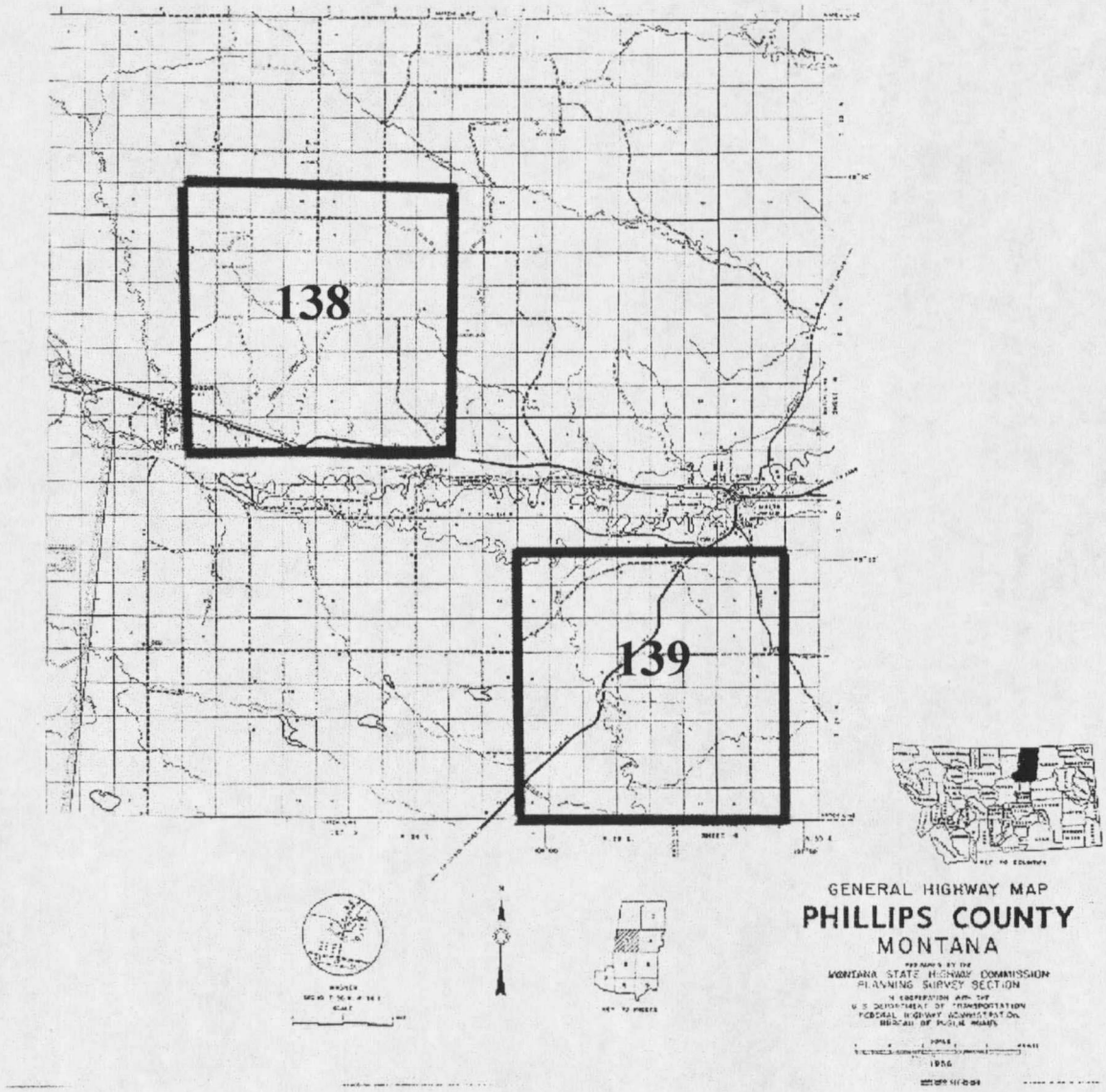



Figure 16. Example of map used to collect kochia samples.

(Hartmann and Kester, 1983). Each branch was planted in circular pots (78 $cm^2$ :surface area; 10 cm deep) filled with the same soil described in the previous study. Pots were placed in a greenhouse with day/night temperatures of 22/16 °C and 16 hrs of light. This light regime was used to prevent flowering and initiate root development and enough biomass to produce maximum number of seeds. Three months later, the light period was change to 12 hrs to induce flower production. Branches were isolated to prevent cross pollination and allowed to grow until seed set. Seeds were harvested from each branch, and stored at 5°C.

Each of three flats (25 x 52 x 6 cm) was filled with the same soil as described above and divided into 9 rows 2.5 cm apart. Fifteen cubic centimeters of seeds from each field (7), a known susceptible (S) line, and resistant (R) line designated as 254 and 99, respectively, were seeded in randomly selected rows. In the previous experiment, 12% of the plants tested survived treatment with 0.07 kg $ha^{-1}$ of dicamba in cell 99. Eighteen of these plants were transplanted to 15 cm pots for seed production, and were isolated to prevent pollen flow from other plants in the greenhouse. Seeds from these plants were collected and the seedlings treated with 0.07 kg $ha^{-1}$ dicamba. Seeds were collected from the surviving plants (about 20 out of 30 plants sprayed) and used for the following studies. Susceptible seeds were from cell 254, and was found to be 100% susceptible to 0.07 kg $ha^{-1}$ dicamba. The experiment was replicated six times and arranged in a randomized-complete-block (flat) design.

Kochia plants were grown for about 4 weeks (4 to 7 cm tall). Flats were treated with 0.07 kg ai/ha dicamba in a carrier volume of 71 liters/ha using a belt sprayer. Dead

and surviving kochia plants were counted two weeks after application.

**Complaint Field Analysis**

Percent of surviving plants was calculated by dividing the number of surviving plants by the total number of emerged plants. Data were analyzed using analysis of variance. Replication and field or line were included in the model. Mean separations were made using Fisher's protected LSD comparisons (Peterson, 1985).

**Dose Response**

Kochia seeds from R (99) and S (254) lines, described above, were sown into 12 (8 x 15 x 8 cm) flats filled with the same soil as described above. Five seeds from either lines 254 or 99 were sown into a row in each flat. Flats were randomly placed in a greenhouse with day/night temperatures of 22/16°C and 16 hrs of artificial light (600 umoles/m2/sec). Kochia plants were allowed to grow for about 4 weeks (4 to 7 cm tall). Two flats were treated with one of twelve rates of dicamba. were applied at 0.001, 0.004, 0.008, 0.018, 0.035, 0.070, 0.140, 0.028, 0.56, 1.12, 2.24, and 4.48 kg ai $ha^{-1}$ using a belt sprayer in a total volume of 71 liters $ha^{-1}$. The plants were evaluated based on an ocular estimate of percent control four weeks after treatment.

**Dose Response Analysis**

Dose response was determined by using PROC NLIN in the SAS® package. A log-logistic curve is fit through the data to produce the dose response for each of the dicamba R and S line (Seefelt et al.,1995). The model was of the following form:

$$C + \frac{D-C}{1+ \exp [b(\log (x) - \log (GR_{50}))]};$$

where C is the lower limit, D is the upper limit, $GR_{50}$ is the dose giving 50% response, and b is the slope at the $GR_{50}$. A resistance ratio was determined ($GR_{50}$ of R line/$GR_{50}$ of S line). When the $GR_{50}$ was fit the 95% confidence interval was calculated and used to determine significance of differences between the R and S lines at the $GR_{50}$.

**Multiple Resistance**

Kochia seeds from R (99) and S (254) lines were sown into seven 8 x 15 x 8 cm flats filled with the same soil as described above. Flats were divided into two rows 5 cm apart. Five seeds from each line were randomly sown in each row. Flats were randomly placed in a greenhouse with day/night temperatures of 22/16°C and 16 hrs of artificial light (600 umoles/m2/sec). The experiment was replicated three times. Kochia plants were allowed to grow for about 4 weeks (4 to 7 cm tall). Each of the six flats were sprayed with a different herbicide. Herbicide were carfentrazone (α,2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzeneprop anoic acid) , dicamba, bromoxynil (3,5-dibromo-4-hydroxybenzonitrile), fluroxypyr ([(4-amino-3,5-dichloro- 6-fluoro-2-pyridinyl)oxy]acetic acid ), metsulfuron (2[[[[4-methoxy-6-methyl-1,3,5-triazine-2-yl)amio]carbinyl]amino]sulfonyl]benzoate), and chlorsulfuron(2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazine-2-yl)aminocarbonyl]

benzenesulfonamide) applied at 0.05, 0.07, 0.42, 0.005, 0.004, and 0.08 kg ai $ha^{-1}$, respectively. A seventh flat was untreated. Plants were visually rated for percent control two weeks after treatment.

**Multiple Resistance Analysis**

This experiment was analyzed as a one-way analysis of variance blocked by herbicide. Mean separations were made using Fisher's protected LSD comparisons (Peterson, 1985).

**Auxin-Like Resistance**

Kochia seeds from R (99) and S (254) lines were sown into ten 8 x 15 x 8 cm flats filled with the same soil as described above. Flats were divided into two rows 5 cm apart. Five seeds from each line were randomly sown in each row. Flats were randomly placed in a greenhouse with day/night temperatures of 22/16°C and 16 hrs of artificial light (600 umoles/m2/sec). The experiment was replicated three times. Kochia plants were allowed to grow for about 4 weeks (4 to 7 cm tall). Each of the nine flats were treated with a different herbicide and/or rates. MCPA or 2,4-D were applied at 0.56, 0.84, or 1.12 kg ai $ha^{-1}$. Picloram was applied at 0.53, 1.3, or 2.6 kg ai $ha^{-1}$. A tenth flat was untreated.

**Sampling**

Kochia plants were visually rated for percent control two weeks after treatment. Plants were allowed to continue growing for an additional four weeks. At that time, above ground biomass was harvested by clipping plants to the soil surface. Plants were immediately weighed.

**Auxin-Like Resistance Analysis**

Data were analyzed as a split-plot with S and R lines as whole plots and herbicides as subplots. Mean separations were made using Fisher's protected LSD comparisons (Peterson, 1985).

# Results

## Occurrence and Distribution of Dicamba Resistance

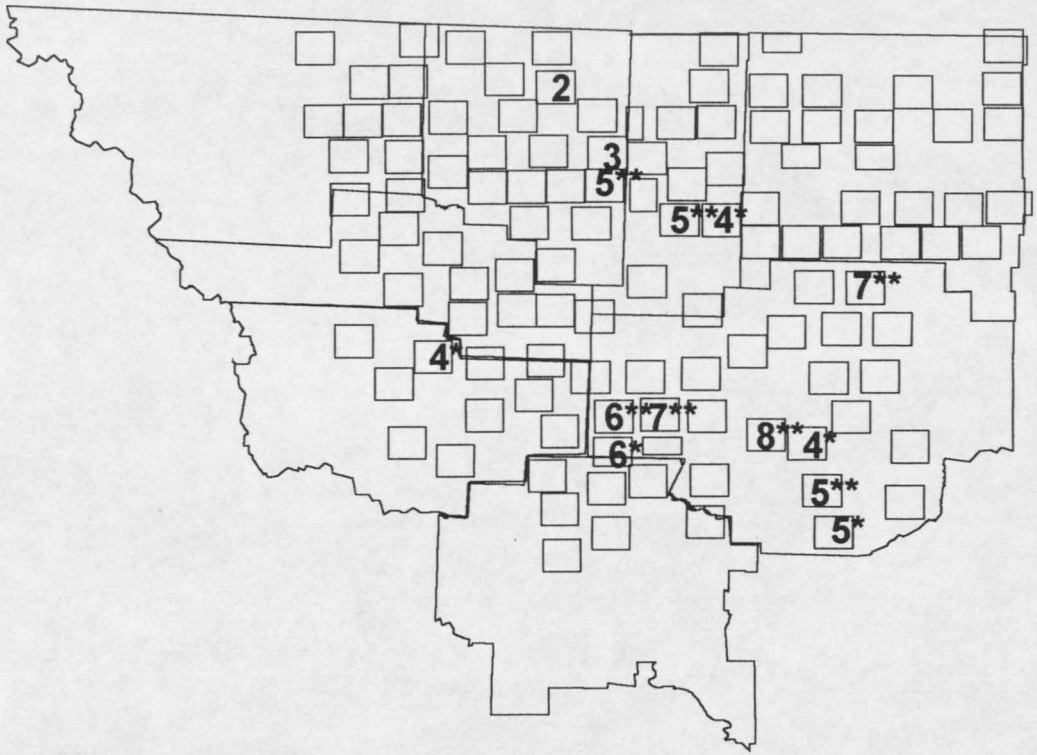
Based on Mann-Whitney rank test, seven of the 151 cells had a significant ($P \leq 0.05$) number of kochia plants that survived the dicamba applied at a rate of 0.07 kg ai $ha^{-1}$ in 1992 (Figure 17). Kochia collected from five other cells had significant survivorship at $P \leq 0.1$. Cell 104, located in Chouteau County had highest percentage of resistance (5% of the sample). In 1993, four and three cells out of 168 had significantly increased numbers of surviving kochia at $P \leq 0.05$ and $P \leq 0.01$, respectively(Figure 18). About 12.5% of the samples collected from kochia plants in cells 99 and 113, survived, both of which were from Chouteau County.

## Complaint Fields

Based on analysis of variance, 71% of the kochia plants from line 99 were resistant to dicamba (Table 4). None of the complaint fields were resistant at the same level as the resistant line. All of the seven fields expressed some degree of resistance, ranging from 2% to 20%.

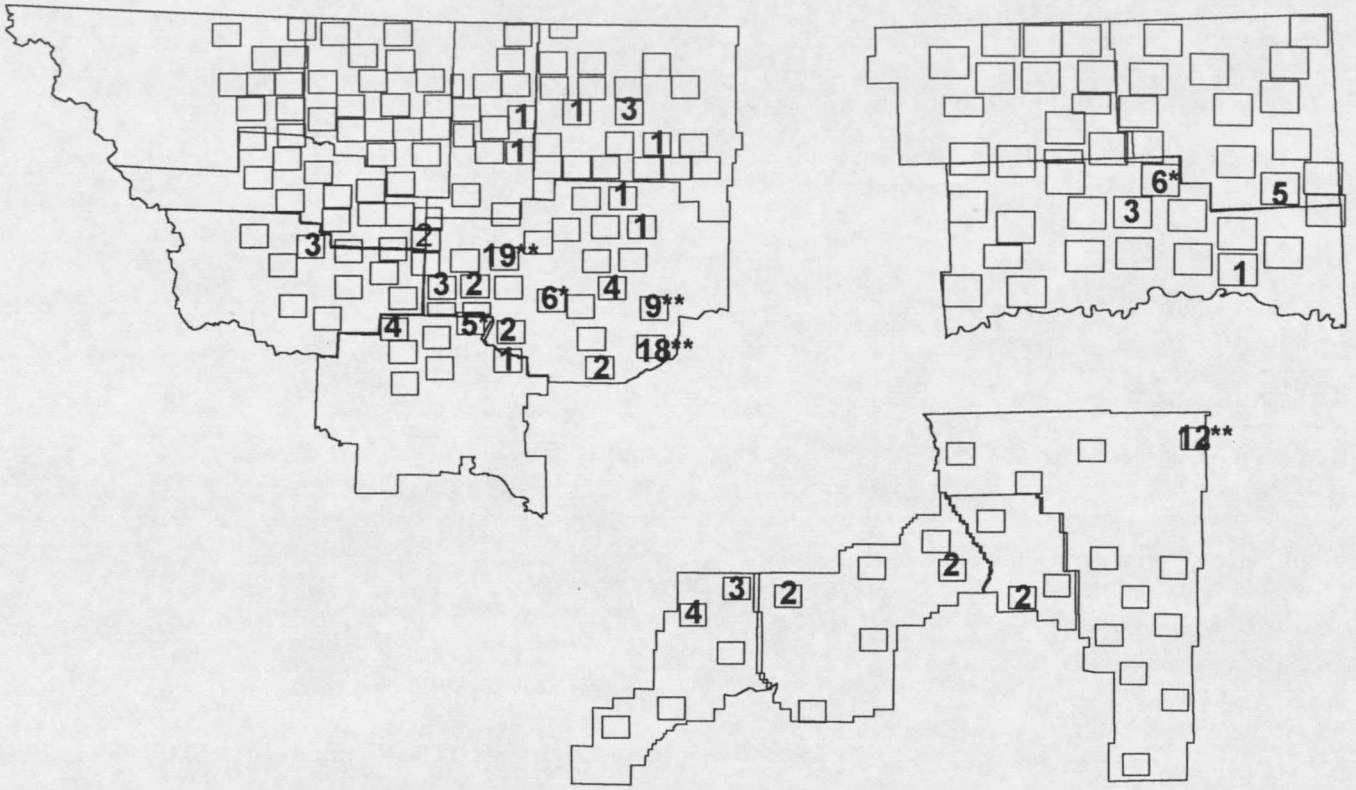
## Dose response

$GR_{50}$ for the R line (99) was estimated to be 0.63 kg $ha^{-1}$, which is nine times greater than the field rate (0.07 kg $ha^{-1}$) (Table 5 ; Figure 19). The $GR_{50}$ for the S line (254) was estimated to be 0.04 kg $ha^{-1}$. The R/S (R $GR_{50}$/S $GR_{50}$) response ratio is 15.5 when comparing lines 99 and 254.




** = significantly different than zero at (P<0.05).
* = significantly different than zero at (P<0.05).

Figure 17. Total number of kochia plants surviving 0.07 kg $ha^{-1}$ in 1992.




** = significantly different than zero at (P<0.05).
* = significantly different than zero at (P<0.05).

Figure 18. Total number of kochia plants surviving 0.07 kg $ha^{-1}$ in 1993.

Table 4. Dicamba resistant kochia in complaint fields in Montana.

| Field | Location | % R |
|---|---|---|
| 2 | Angela | 20 c[1] |
| 1 | Angela | 10 abc |
| 4 | Angela | 2 ab |
| 5 | Loma | 15 bc |
| 6 | Loma | 11 ab |
| 7 | Big Sandy | 8 ab |
| 8 | Big Sandy | 5 ab |
| 99[1] | Big Sandy | 71d |

[1] Means followed by the same letter are not significantly different at $p<0.05$ .

[2] 99 = R line

Table 5. Parameter estimates for dicamba R and S dose response curves.

| Parameter | Estimate | Asymptotic standard error | 95% Confidence Interval Lower | Upper |
|---|---|---|---|---|
| $GR_{50}$ (R) | 0.6305 | 0.0173 | 0.596 | 0.665 |
| B (R) | 7.8790 | 1.7190 | 4.475 | 11.283 |
| C (R) | 2.3975 | 0.7124 | 0.987 | 3.808 |
| $GR_{50}$ (S) | 0.0408 | 0.0008 | 0.039 | 0.042 |
| B (R) | 8.3746 | 0.9937 | 6.412 | 10.337 |
| C (R) | 1.5935 | 0.3150 | 0.971 | 2.215 |

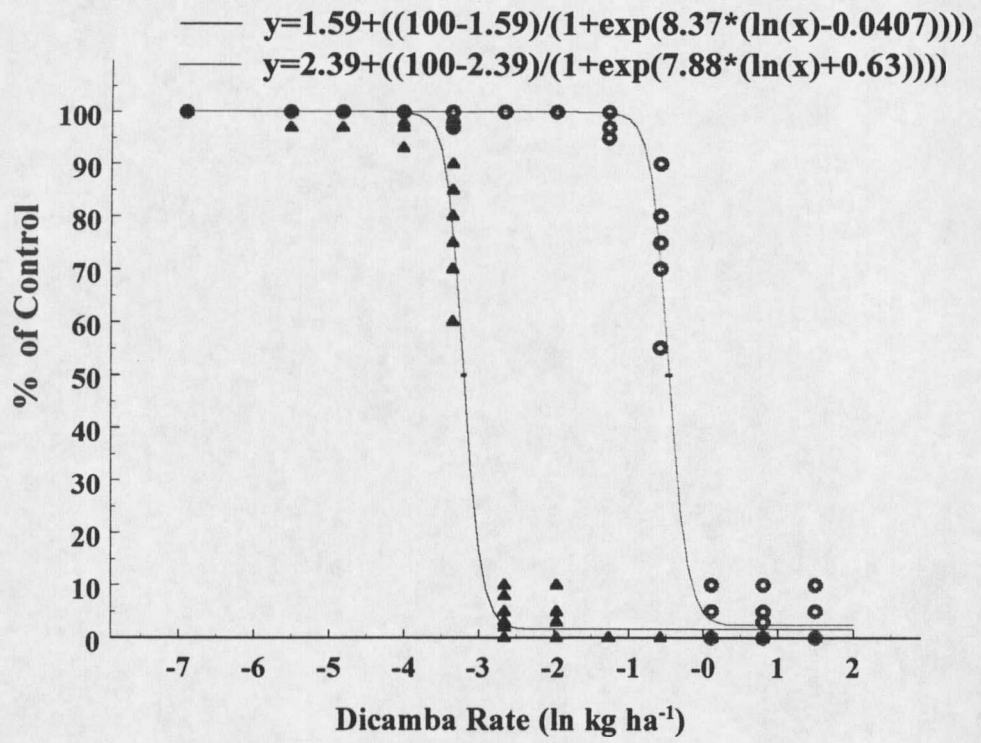



Figure 19. Dose response of dicamba R and S kochia. Log-logistic curves fit through growth inhibition means of R (circles) and S (triangles).

## Multiple Resistance

In this experiment, dicamba provided 3% and 90% control for the R and S lines, respectively (Table 6). The R line (99) was resistant to the two sulfonylurea herbicides metsulfuron and chlorsulfuron. Metsulfuron and chlorsulfuron provided 22% and 7% control, respectively. Bromoxynil, fluroxypyr, and carfentrazone all controlled the resistant lines with greater than 97% control.

Table 6. Evaluating R and S kochia with different herbicide families for cross resistance.

| | | Visual Control (%) | |
|---|---|---|---|
| Treatment | kg ha$^{-1}$ | 99 (R) | 254 (S) |
| Carfentrazone | 0.05 | 99 a [1] | 98 a |
| Dicamba | 0.07 | 3 b | 90 a |
| Bromoxynil | 0.42 | 100 a | 100 a |
| Fluroxypyr | 0.005 | 97 a | 96 a |
| Metsulfuron | 0.004 | 22 b | 99 a |
| Chlorsulfuron | 0.1 | 7 b | 99 a |

[1] Means within the same treatment followed by the same letter are not significantly different at $p<0.05$ .

**Auxin-Like Resistance**

MCPA and 2,4-D at all rates provided 93% or greater control of kochia for the S line (254) (Table 7). MCPA and picloram provided less control than the R line at all of the rates two weeks after application. However, 1.12 kg ai ha$^{-1}$ MCPA only provided 34% control of kochia. Conversely, the R line treated with 0.84 or 1.12 kg ha$^{-1}$ 2,4-D provided 93% and 100% control, respectively. Picloram is not used for kochia in small grains, but was added to the study because of its auxin-like properties. Picloram did not control R kochia, and tended to provide the poorest control of S kochia.

Table 7. Effect of MCPA, 2,4-D, and picloram on the percent control of dicamba R and S kochia.

| | | Percent Control | |
|---|---|---|---|
| **Treatment** | **kg ha$^{-1}$** | **99 (R)** | **254 (S)** |
| MCPA | 0.56 | 7 | 100 |
| MCPA | 0.84 | 28 | 93 |
| MCPA | 1.12 | 34 | 100 |
| 2,4-D | 0.56 | 42 | 100 |
| 2,4-D | 0.84 | 100 | 100 |
| 2,4-D | 1.12 | 93 | 100 |
| Picloram | 0.53 | 7 | 45 |
| Picloram | 1.30 | 10 | 77 |
| Picloram | 2.60 | 8 | 83 |
| Trt LSD (0.05) = 16.13 | | Type LSD (0.05) = 17.23 | |

Fresh weight was harvested four weeks after visual control was rated. The resistant line had more fresh weight for all treatments for all rates (Figures 20, 21, 22 ).

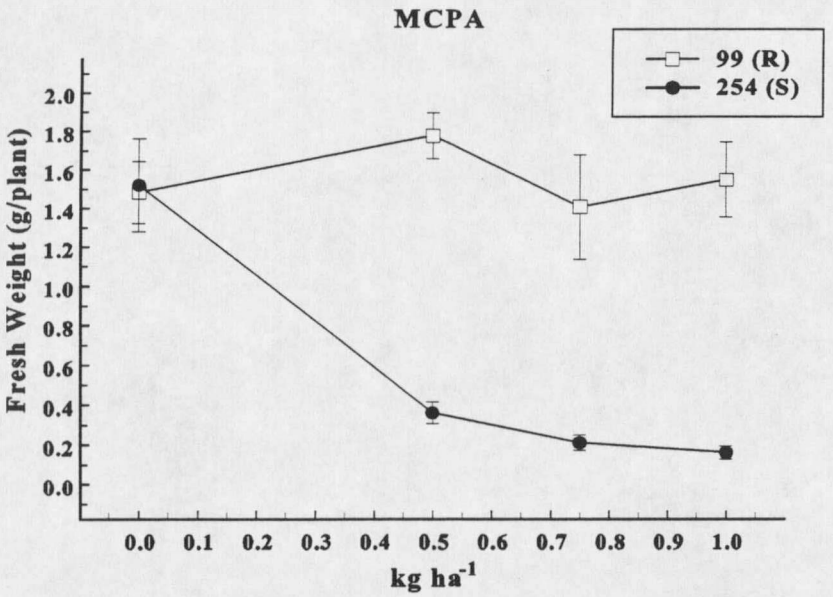



Figure 20. Effects of MCPA rates on dicamba R and S kochia. Means and standard errors of R (boxes) and S(circles).

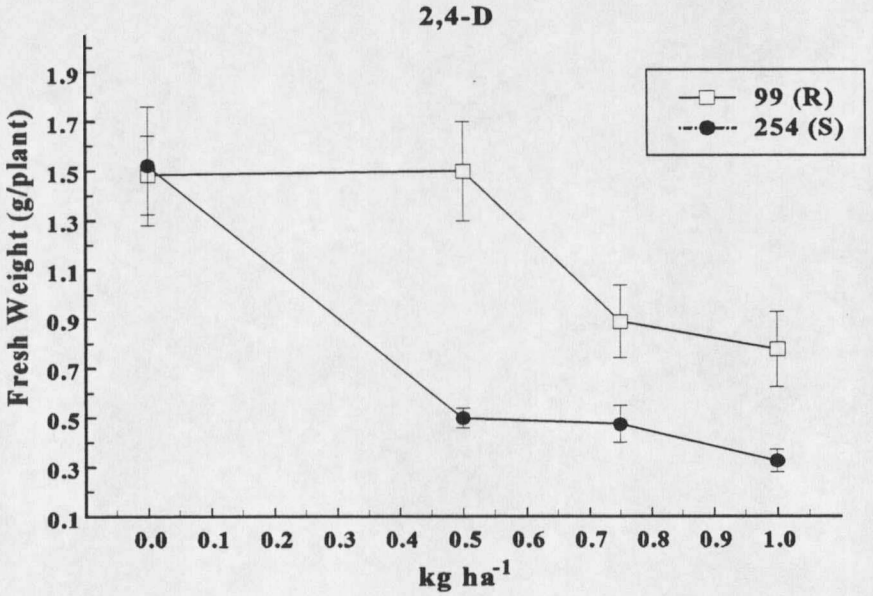



Figure 21. Effects of 2,4-D rates on dicamba R and S kochia. Means and standard errors of R (boxes) and S(circles).

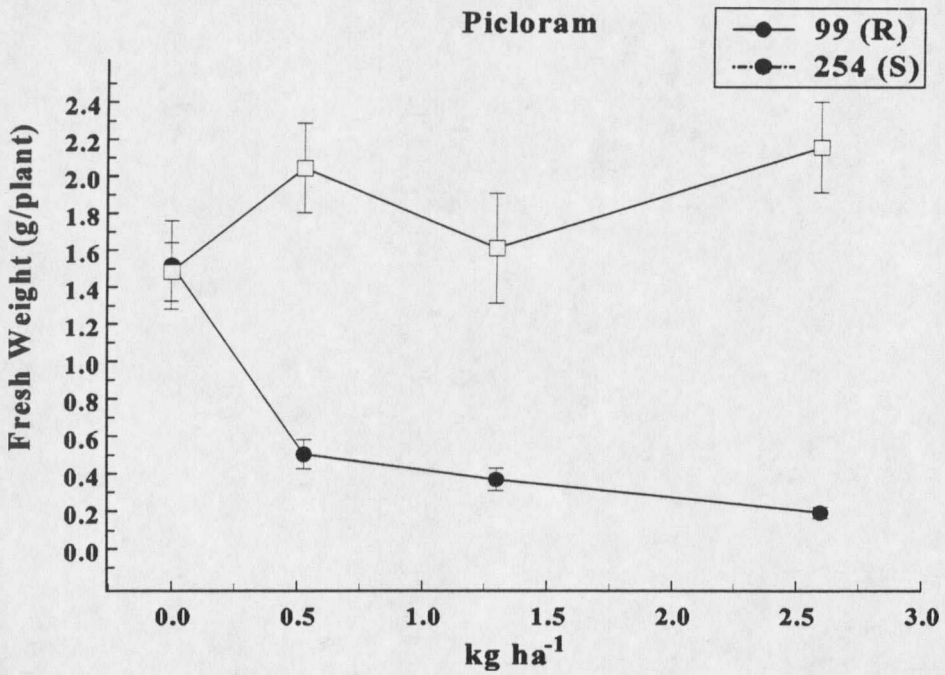



Figure 22. Effects of picloram rates on dicamba R and S kochia. Means and standard errors of R (boxes) and S(circles).

## Discussion

Kochia resistant to dicamba was found in eleven and seven cells in 1992 and 1993, respectively. Although less cells were found in 1993, the percent of dicamba resistance in those cells were higher. For both years, the number of cells and percent of kochia that were dicamba resistant were at low levels. Gressel and Segel (1978) noted that resistance would not be noticed until the year that resistance reaches 30%. Perhaps because of the heightened awareness of resistance, producers are noticing resistance earlier than predicted. Resistance detected in complaint fields 5-8 ranged from 5% to 15%. Theses fields are in the same county as cells 99 and 113. In the experiment with the complaint fields, I compared the fields to the bred line 99(R), however, it is interesting to note that samples 99 and 113 collected in 1993, had about 12.5% survival similar to that of the complaint fields.

Dicamba resistance has also been found in northwestern Manitoba in a population of wild mustard (Heap and Morrison, 1992). The resistant wild mustard population exhibits similar uptake, translocation, metabolism and cross resistance to that of the dicamba resistant kochia. Heap and Morrison (1992) tested wild mustard for cross resistance. In that study, wild mustard was not cross resistant to bromoxynil. Similarly, our study showed that dicamba resistant kochia was not resistant to bromoxynil. I also tested carfentrazone and fluroxypyr which provided 97% and 99% control of the resistant kochia, respectively. These three herbicides could be used as alternatives for kochia control. Several authors have suggested rotation of herbicides with different chemistries and modes of action (Holt and LeBaron, 1990; Powles and Howat, 1990).

The magnitude of the dicamba resistant wild mustard is greater than the kochia line tested. The $GR_{50}$ R/S ratio for the wild mustard population was 104 for dicmaba, whereas the kochia R/S ratio was 15.5.

Both the wild mustard (Heap and Morrison, 1992) and kochia are cross resistant to other auxin herbicides. The $GR_{50}$ R/S ratios for the wild mustard were 18 for 2,4-D and 10 for MCPA. Dicmaba resistant kochia responded differently to the application of 0.84 kg $ha^{-1}$. MCPA and 2,4-D provided 28 and 100 percent visual control, respectively. However, resistant kochia fresh weight was greater for 2,4-D applied at 0.84 and 1.12 kg $ha^{-1}$ than the susceptible. Although percent visual control was rated high for the 2,4-D applications, the R kochia may have succumbed to the herbicide application slower than the S kochia.

Commonly used tankmixes, such as 2,4-D and dicamba or 2,4-D, dicamba and a sulfonylurea were used to manage chlorsulfuron resistant kochia. However, they may not have solved the chlorsulfuron resistant problem, but have selected for kochia that is not controlled by chlorsulfuron, metsulfuron, dicamba, MCPA, 2,4-D or picloram.

Strategies for managing kochia resistance should be based on kochia biology. Matthews (1994) summarized the need for "planned use of nonselective herbicides, delayed planting or out of season planting, judicious cultivation, and more complexity in crop rotations may be long-term solutions to the problem of herbicide resistance". Kochia has a short lived seed bank of three years or less (Burnside et al., 1981). Kochia seed production occurs late in the season and some years at post-harvest. Control measures should be used to limit seed production, especially in fields where resistance is suspected.

Kochia management on CRP, field edges, and farm areas should be considered as they have the potential to harbor resistant genes.

Chapter 4

# UPTAKE, TRANSLOCATION AND METABOLISM OF $^{14}$C-DICAMBA IN RESISTANT AND SUSCEPTIBLE KOCHIA

## Introduction

Uptake, translocation, and metabolism of dicamba have been investigated in several species in order to determine the basis for natural tolerance and susceptibility to the herbicide. Resistant plants could avoid injury by taking up less of the herbicide, by failing to translocate it to the target site(s), or by metabolizing it before it can exert toxic effects.

Chang and Vanden Born (1971b) conducted uptake, translocation and metabolism studies on wheat, barley, tartary buckwheat and wild mustard. Tartary buckwheat and wild mustard, both sensitive species, accumulated $^{14}$C-dicamba in young leaves and meristematic regions. About 40% of the applied $^{14}$C-dicamba remained on the leaf surface of the tolerant wheat and barley plants, while less than 12% was found on wild mustard and tartary buckwheat leaves, indicating that the herbicide was absorbed more quickly in tolerant species one day after application. All species accumulated greater than 70% of the applied $^{14}$C-dicamba in plant parts other than the treated leaf.

Several authors have shown that the major metabolite of dicamba is 5-hydroxy-3,6-dichloro-*o*-anisic acid (5-OH dicamba) and the second most abundant metabolite is 3,6-dichlorosalicylic acid (DCSA) (Robocker and Zamora, 1976; Chang and Vanden

Born, 1971; and Broadhurst et al., 1966). Twenty days after treatment with 0.1 $\mu$Ci $^{14}$C-dicamba, 10% of the recovered herbicide had been metabolized to 5-OH dicamba in tartary buckwheat. To confirm that 5-OH dicamba was not phytotoxic, the authors showed that tartary buckwheat was not injured when treated with this metabolite. Dicamba and 5-OH dicamba were both translocated within plants; however, 5-OH dicamba was translocated more slowly while most dicamba was transported out of the treated leaf by 13 days after application in tartary buckwheat (Chang and Vanden Born, 1971a).

Canada thistle, a sensitive species, metabolized $^{14}$C-dicamba very slowly, since only 37% of the applied radioactivity was identified as a metabolite 54 days after application. Most metabolism occurred in the treated leaf, and the shoot above the treated leaf only contained 3% of the recovered dicamba metabolites 9 days after treatment. Purple nutsedge, a sensitive species, showed no indication of metabolism 10 days after application (Magalhaes et al., 1966). Wheat, a tolerant species, metabolized 75% of the applied $^{14}$C-dicamba by 10 days after treatment (Chang and Vanden Born, 1971b). Therefore, it appears that the basis for natural tolerance to dicamba in various species is a differential rate of metabolism.

Petersen et al. (1985) reported that 66% of absorbed $^{14}$C-dicamba was exuded from soybean roots 60 hrs after a foliar application. In contrast, several researchers (Chang and Vanden Born, 1968; Linder et al., 1964, Quimby and Nalawaja, 1971) showed that only 2.5% or less of the applied dicamba was exuded from roots of several species .

Because of the results reported above, I hypothesized that roots of resistant (R)

and susceptible (S) kochia might exude dicamba at different rates, and that soil residues of exuded dicamba could affect control of other weeds or induce crop injury. Therefore, an experiment was conducted to evaluate the effect of dicamba applied to the soil, to the foliage, or both soil and foliage of R and S plants.

The objectives of these studies were to: 1) compare the responses of R and S kochia to foliar, soil, and combination applications of dicamba, 2) compare rates of $^{14}$C-dicamba uptake and translocation in R and S kochia, and 3) compare rates of $^{14}$C-dicamba metabolism in R and S kochia.

## Methods and Methods

### Plant Material

After Dr. Peter Fay received grower complaints about lack of kochia control with dicamba in 1995, I decided to evaluate the seeds previously collected for the chlorsulfuron screen to detect the presence of kochia plants resistant to dicamba. In an area designated as 99 (Fort Benton, MT), 12% of the plants tested survived treatment with 0.07 kg $ha^{-1}$ of dicamba. Eighteen of these plants were transplanted to 15 cm pots for seeds production, and were isolated to prevent pollen flow from other plants in the greenhouse. Seeds from these plants were collected and the seedlings treated with 0.07 kg $ha^{-1}$ dicamba. Seeds was collected from the surviving plants (about 20 out of 30 plants sprayed) and used for the following studies. Susceptible seeds was from an area in the chlorsulfuron screen designated 254 (Bozeman, MT), and was found to be 100% susceptible to 0.07 kg $ha^{-1}$ dicamba.

Seeds from lines 254 and 99 were sown 1 cm deep in 10 x 15 x 5 cm flats containing greenhouse soil mix (Bozeman silt loam: washed sand: peat moss [1:1:1]), grown for 2 weeks, and the seedlings were transplanted into 12 cm tall conetainers. Preliminary experiments used sand and vermiculite for kochia germination and growth because several authors (Chang and Vanden Born, 1968; Chang and Vanden Born, 1971; Linder et al., 1964; Quimby and Nalawaja, 1971; Petersen et al., 1985) reported that $^{14}C$-dicamba was exuded from root systems, and I wanted to avoid generating $^{14}C$-contaminated soil . However, these media were not successful for kochia growth for the two following reasons. First, media to root contact after transplanting was poor, and

second, it was very difficult to maintain desirable water levels for the seedlings. Conetainer racks were either put into a tub of water (subirrigated) daily or watered daily, but neither method provided good kochia growth.

**Foliar vs. Soil Applied Dicamba**

An experiment was conducted to evaluate the response of R and S kochia to soil and foliar applications of dicamba. Seeds from lines 99 and 254 were planted in rows in 10 x 15 cm plastic flats. Twelve days after planting, seedlings were transplanted into 10 cm pots and sprayed when they were 4 cm tall. Dicamba was applied to: 1) foliage only, 2) foliage and soil, and 3) soil only. For the foliage only treatment, vermiculite was used to cover the soil approximately two centimeters deep and was removed after spraying. For the soil only treatment, aluminum foil was used to cover the plant during spraying. The foliage only and soil and foliage treatments were treated with 0.07 kg $ha^{-1}$ dicamba, and the soil only treatment was sprayed with 0.14 kg $ha^{-1}$ in a carrier volume of 94 liters/ha using a belt sprayer. The aluminum foil and vermiculite were removed 4 hrs after treatment. The soil only treatment was subirrigated and the other two treatments were watered daily. There were 3 replicate plants per treatment and the experiment was evaluated 2, 4, 7, and 17 days after spraying using a rating system of 0-4, where 0= no symptoms, 1 = < 10% curling and yellowing, 2 = 10-30% curling, yellowing, and slight stunting, 3 = 30-60% curling, yellowing, and stunting, and 4 = > 60% curling, yellowing and severe stunting.

**Dicamba Uptake and Translocation**

Kochia plants were grown in the greenhouse until 4 to 7 cm tall (about 4 weeks) and one leaf approximately midway on the stem was covered with aluminum foil. The plants were treated with 0.07 kg $ha^{-1}$ dicamba in a carrier volume of 71 liters $ha^{-1}$ using a belt sprayer. The plants were allowed to dry for approximately 1 hr and the aluminum foil was removed. $^{14}$C-dicamba ($7.05 \times 10^6$ Bq. $mg^{-1}$) spiked with technical grade dicamba (to a final concentration equal to the spray solution) was applied in three 1 $\mu$l drops along the midrib of the covered leaf. I initially added X-77 surfactant at 0.25% v/v to the treated solution. However, this concentration caused the treated leaf to turn black and droop down. Therefore, the surfactant concentration was reduced to the point so that $^{14}$C-dicamba droplets would not roll off the leaf (0.001% v/v). Plants were placed in a growth chamber under conditions identical to the greenhouse for 24, 48, 60, 96, or 168 hrs and harvested by dissecting the samples into: 1) treated leaf, 2) shoot above treated leaf, 3) shoot below treated leaf, 4) roots, and 5) soil. The treated leaf was vortexed for 30 sec in 500 $\mu$l of 0.25% X-77 solution in a 1.5-ml microcentrifuge tube. $^{14}$C-dicamba uptake was determined by liquid scintillation counting (LSC)[3] of the leaf wash (250 $\mu$l) and subtracting the amount recovered from the amount applied. Berhrens and Lueschen (1979) reported that dicamba volatility caused injury to adjacent plants so I corrected for volatility from the leaf surface at each time point during uptake and translocation experiments. Volatility was quantified by the application of $7.05 \times 10^6$ Bq. $mg^{-1}$ $^{14}$C-dicamba in 3 $\mu$l on three glass cover slips for each time point. The cover slips were

[3] Model Minimax $\beta$ Tri-carb ® 4000 series.

allowed to dry, placed in the growth chamber with the treated plants, washed with 750 $\mu$l 0.25% v/v X-77, and a subsample (250 $\mu$l) was counted at each time point. Volatility was determined by subtracting the total $^{14}C$ recovered from the total applied to the cover slip. Three or more replicate R and S seedlings were harvested at each time point and the experiment was repeated twice. The two experiments were combined after analyzing the data with Bartlett's test for homogeneity of variances. PROC GLM (SAS®) was used for analysis of variance and means separated by standard error.

Dicamba translocation was determined by individually oxidizing the dissected plant parts obtained as described above. The samples were oxidized in a biological sample oxidizer[4], liberated $^{14}CO_2$ trapped in basic scintillation cocktail and quantified using LSC as above.

**Dicamba Metabolism**

The metabolism experiments were done together with Ms. Erica Miller, who did the majority of the high performance liquid chromatography (HPLC) analysis. Metabolism of dicamba was determined using the same plant material and harvest times as described above. Plants were dissected into: 1) treated leaf, and 2) remainder of the plant above the soil surface. Three R or S leaves or plants were combined for each sample. Treated leaves were vortexed in 750 $\mu$l 0.25% X-77 for 10 sec to remove unabsorbed $^{14}C$-dicamba. Treated leaves and the remainder of the plant were then homogenized separately in 500 $\mu$l of 55:45 methanol:distilled water (v/v) in a Broeck tissue homogenizer. Extracts were

[4] OX-300, R.J. Harvey Instrument Corp., Hillsdale, N.J. 07642

transferred to a 1.5 ml centrifuge tube, centrifuged at 16,000 x g at 4°C for 10 min, the supernatant transferred to a 0.45 um microspin centrifuge filter, and the extract filtered by centrifuging at 5000 x g at 4°C for 5 min. The resulting filtrate was subjected to HPLC using an Alltech Lichrosorb C18 reverse-phase column (particle size 10 $\mu$m) in a 4.6 x 250 mm format. The mobile phase consisted of 55:45 (v/v) methanol:30 mM tetrabutyl ammonium dihydogen phosphate as an ion-pair reagent at a flow rate of 1.0 ml/min. Radiolabeled dicamba and its metabolites were detected using an in-line scintillation counter and quantified using a dedicated integrator. Identification of dicamba and known metabolites was based on coelution with authentic standards kindly provided by Sandoz Agro., Inc. Rf values for dicamba and 5-OH dicamba were 11 and 4.5 min, respectively. DCSA was not detected at any time point tested.

## Results and Discussion

### Foliar vs. Soil Applied Dicamba

At all times after treatment, S plants treated on the foliage or on foliage and soil showed slight to moderate injury symptoms, and ratings were not significantly different between the two treatments (Table 8). The soil only treatment did not injure S kochia two days after treatment, but injury ratings were the same as for the foliar treatment at all later time points. The combination (foliar and soil) treatment caused significantly greater injury to S plants than the soil only treatments at all time points. In contrast, line 99 (R) was not injured by any of the treatments at any time after spraying.

The hypothesis that R and S kochia exuded dicamba at different rates could not be adequately tested with this experiment. Similar injury symptoms in S plants from foliar and soil treatments indicated both apoplastic and symplastic movement of dicamba, as reported by Chang and Vanden Born (1968) in Canada thistle. Further, the fact that R plants were not injured by soil applications of dicamba suggests that their resistance mechanism is equally effective in roots and shoots.

Table 8. Effects of foliar and soil applications of dicamba on R (99) and S (254) kochia.

| | | Average Rating [a] | |
|---|---|---|---|
| **DAT [b]** | **Treatment** | **99** | **254** |
| 2 | Foliar and Soil | 0.0 e [c] | 1.3 d |
| 2 | Foliar | 0.0 e | 1.7 cd |
| 2 | Soil | 0.0 e | 0.0 e |
| 4 | Foliar and Soil | 0.0 e | 2.7 ab |
| 4 | Foliar | 0.3 e | 2.0 bcd |
| 4 | Soil | 0.0 e | 1.3 d |
| 7 | Foliar and Soil | 0.3 e | 3.3 a |
| 7 | Foliar | 0.3 e | 2.7 ab |
| 7 | Soil | 0.0 e | 2.0 bcd |
| 17 | Foliar and Soil | 0.3 e | 3.3 a |
| 17 | Foliar | 0.3 e | 2.7 ab |
| 17 | Soil | 0.0 e | 2.3 bc |
| 17 | Control | 0.0 e | 0.0 e |

[a] A rating system of 0-4 was used where 0= no symptoms, 1 = < 10% curling and yellowing, 2 = 10-30% curling, yellowing, and slight stunting, 3 = 30-60% curling, yellowing, and stunting, 4 = > 60% curling, yellowing and severe stunting.
[b] DAT; Days after treatment
[c] Means followed by the same letter are not different at p = 0.05 as determined by Duncan's Multiple Range test.

**Dicamba Uptake and Translocation**

Twenty-four hrs after treatment, R and S kochia had absorbed 17% and 16% of the applied $^{14}$C-dicamba, respectively (Figure 23). At 48 and 60 hrs after treatment, R kochia absorbed significantly less and more, respectively, of the applied $^{14}$C-dicamba than S kochia. However, uptake was equal in R and S plants by 96 and 168 hrs after application. About 55% of the applied $^{14}$C-dicamba was absorbed by 168 hrs after treatment as compared to 95% absorption in 12 hr in wild mustard (Peniuk, 1993) and 75% absorption in 10 days in wheat (Chang and Vanden Born, 1971b). The fact that leaf epinasty was apparent on S plants by 24 hrs after treatment strongly suggests that sufficient amounts of herbicide had entered both R and S plants to induce injury symptoms. Thus, even though there were differences in $^{14}$C-dicamba uptake between R and S at some of the time points, I feel that a lack of uptake could not be responsible for the mechanism of resistance.

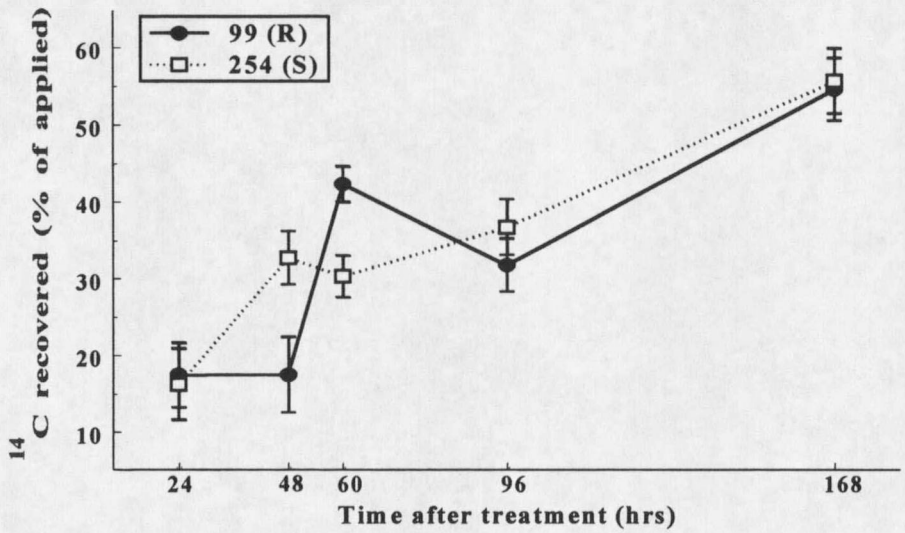



Figure 23. Uptake of $^{14}$C-dicamba in line 99 (R) and 254 (S) kochia.

Hall et al. (1995) reported that S wild mustard leaves became epinastic by 24 hrs after dicamba application. Auxinic herbicide resistance in wild mustard was also shown not to be due to differences in herbicide uptake.

Translocation values within the treated leaf and the shoot above the treated leaf were the same in R and S kochia at all times after treatment (Table 9). Less than 6.2% of the $^{14}C$ was translocated to the shoot below the treated leaf, with minor differences between R and S plants at 60, 96, and 168 hrs after treatment. Only negligible amounts of radioactivity were recovered from plant roots or soil. Most of the absorbed radioactivity was translocated to the shoot above the treated leaf, as reported by Peniuk et al. (1993) in wild mustard. Peniuk et al. (1993) also reported no differences in dicamba translocation between R and S wild mustard up to 48 hrs after application.

Table 9. Translocation of $^{14}$C-dicamba in resistant (R) and susceptible (S) kochia.

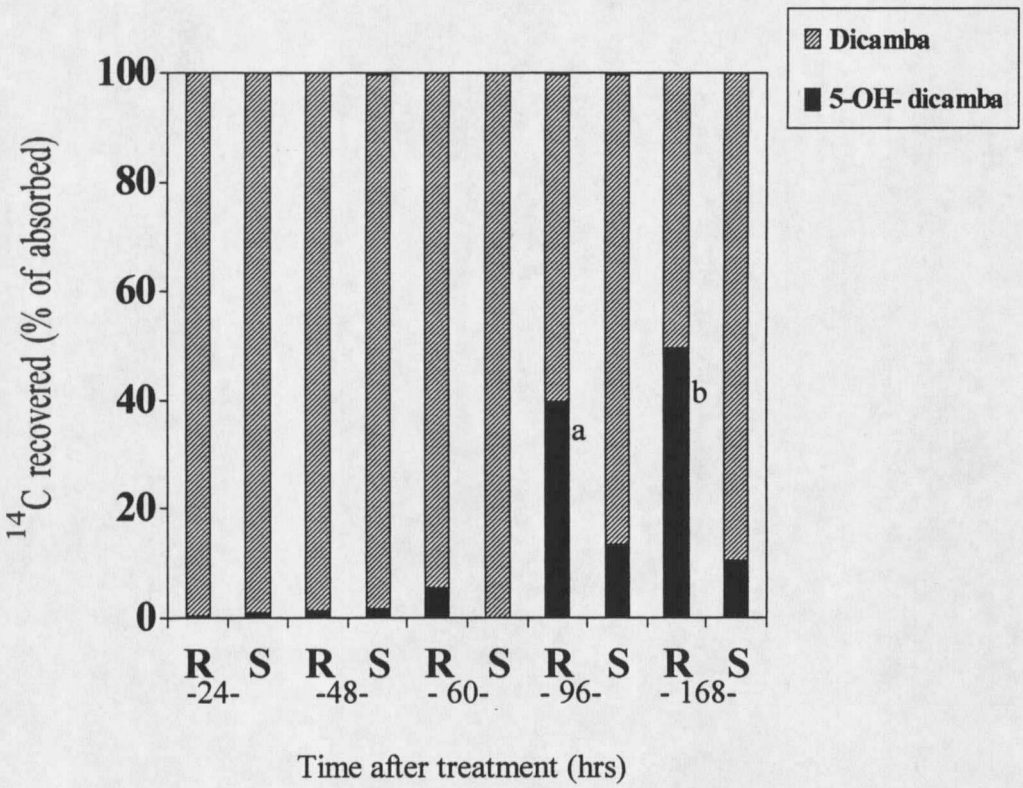
| Time after treatment | Plant Type | Treated leaf | Above TL [a] | Below TL | Root | Soil |
|---|---|---|---|---|---|---|
| (hrs) | | | ----------$^{14}$C (% of absorbed)---------- | | | |
| 24 | R | 29.3 | 64.7 | 5.9 | n/a [b] | n/a |
| | S | 18.9 | 75.5 | 5.6 | n/a | n/a |
| 48 | R | 13.0 | 83.6 | 5.2 | n/a | n/a |
| | S | 14.4 | 79.9 | 5.6 | n/a | n/a |
| 60 | R | 10.0 | 88.0 | 2.0* | n/a | n/a |
| | S | 11.2 | 84.9 | 3.9 | n/a | n/a |
| 96 | R | 11.4 | 84.8 | 2.6* | 0.6 | 0.5 |
| | S | 7.0 | 85.7 | 6.2 | 0.5 | 0.5 |
| 168 | R | 5.2 | 91.5 | 2.7* | 0.3 | 0.2 |
| | S | 5.5 | 88.3 | 5.5 | 0.4 | 0.3 |

[a] Abbreviations: TL treated leaf , R resistant , S susceptible. [b] n/a = data not taken
* = significant difference between R and S within tissue and time after treatment at $P < 0.05$.

**Dicamba Metabolism**

Within the treated leaf, very low levels of dicamba metabolism to 5-OH dicamba were detected in R and S kochia for the first 60 hrs after treatment (Figure 24). However by 96 hours after application R and S kochia had metabolized 39.4% and 13.6%, respectively, of the $^{14}$C-dicamba to 5-OH dicamba. This trend continued in that R treated leaves contained 49.2% 5-OH dicamba vs. only 10.9% 5-OH dicamba in S treated leaves by 168 hrs after treatment. Although these differences were significant, I do not believe that they adequately explain the mechanism of resistance, primarily because injury symptoms appear as soon as 24 hrs after treatment of S plants. In the remainder of the plant there were no significant differences in the amounts of dicamba or 5-OH dicamba at any time after treatment (Figure 25). Peniuk et al. (1993) similarly found no significant

differences in dicamba metabolism between R and S wild mustard by 96 hours after treatment.




a = means within the same hour after treatment significantly different at P< 0.1.
b = means within the same hour after treatment significantly different at P< 0.05.

Figure 24. Metabolism of $^{14}$C-dicamba in the treated leaf of R and S kochia.

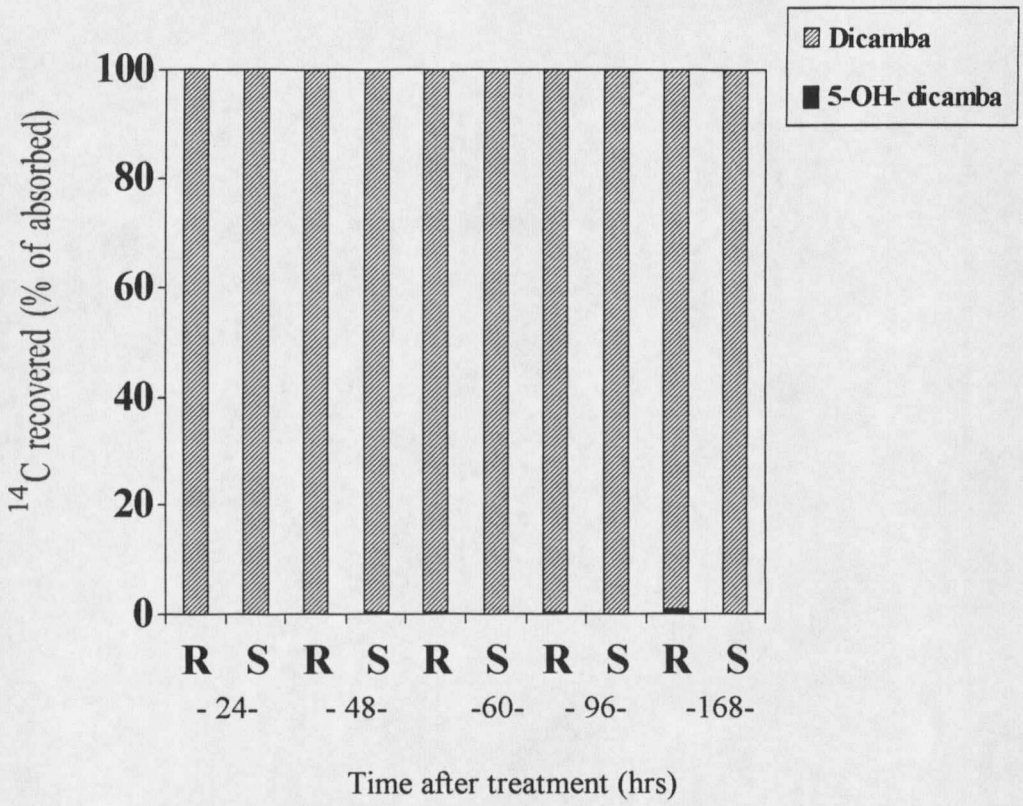



Figure 25. Metabolism of $^{14}$C-dicamba in the remainder of the treated plants of R and S kochia

Differential rates of metabolism appear to at least partially explain the natural tolerance of some plant species to dicamba. For example, wheat metabolized 11.7% of $^{14}$C-dicamba to an unknown metabolite by 3 days after treatment (Quimby and Nalawaja, 1971). Chang and Vanden Born (1971) reported that wheat metabolized 60% of applied $^{14}$C-dicamba 4 days after treatment, while the sensitive species tartary buckwheat only metabolized 3% of the herbicide. Quimby and Nalawaja (1971) concluded that partial resistance to dicamba was conferred by inactivating the herbicide.

The mechanism of resistance to auxinic herbicides in other species is not well understood, although several studies have been conducted on specific effects of the herbicides. Peniuk et al. (1993) reported that S wild mustard produced significantly more ethylene than the R biotype following an application of 75 g a.e. ha$^{-1}$ dicamba. Webb and Hall (1995) used auxin binding protein (ABP) preparations to examine the effects of auxinic herbicides on specific [$^3$H]IAA binding in wild mustard. In competition with dicamba, Webb and Hall (1995) reported a five-fold increase in the inhibition of [$^3$H]IAA binding ($IC_{50}$) in R vs. S wild mustard. They concluded that differences in ABP binding may partially account for resistance to auxinic herbicides.

I believe that the same mechanism of resistance as described for wild mustard may also be responsible for resistance in kochia and that it occurs within the first hours after application. It is important to understand the mechanism of dicamba resistance, it will help our current understanding of how auxinic herbicides and IAA affect plant cells. The understanding of IAA-induced signal transduction in plant cells has drastically changed in the past decade. Trewavas and Malho′ (1997) showed a 1990 model depicting our

understanding of signal transduction which included signals, $Ca^{2+}$, calmodulin, protein kinase, and a response. The 1997 version resembles the World Wide Web: a network of $Ca^{2+}$, protein kinases, second messengers, osmotic motors, channels, transcription factors, kinase cascades, cytoskeleton, metabolism, GTPases, and responses. There are several places where resistance could occur within this model. Deshpande and Hall (1996) hypothesized that disruption of normal $Ca^{2+}$ transport is important to herbicidal activity. In wild mustard R and S whole protoplasts, a difference in volume change was detected when comparing auxin herbicides and IAA. This indicates that there are different biochemical/physiological responses between biotypes.

Understanding the inheritance of dicamba resistance in kochia may demonstrate a correlation with the findings of Jasieniuk et al. (1995) and Estelle and Sommerville (1987). Jasieniuk et al. (1995) described the inheritance of dicamba resistance in wild mustard as a single, dominant nuclear gene. Estelle and Sommerville (1987) isolated mutant lines of *Arabidopsis thaliana* that were resistant to 2,4-D due to a mutation at the *axr*1 locus. They hypothesized that the mutant *axr*1 may encode an altered form of the auxin receptor and resistance was due to a change in affinity for 2,4-D and IAA. A differential response in dicamba resistant kochia to other auxinic herbicides may be due to a change in an auxin receptor and its affinity for each of the herbicides. It is also possible that there are several types of auxin receptors. Picloram and dicamba may be recognized by the same receptor while other auxinic herbicides may be recognized by another.

## LITERATURE CITED

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