AGRONOMIC, PHYSIOLOGICAL, AND GENETIC STUDIES INVOLVING SULFONYLUREA HERBICIDES

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ABSTRACT

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Chlorsulfuron {2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide} resistant sugarbeet (*Beta vulgaris* L.) (CR1-B) was evaluated in greenhouse and field studies for cross-resistance to other herbicides that inhibit acetolactate synthase (ALS). The physiological basis and genetic inheritance of the resistance trait was also determined. CR1-B was cross resistant to primisulfuron {2-[[[[[4,6-*bis*-difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl]benzoic acid} and thifensulfuron {3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylate} applied at or exceeding the field use rate. The ALS enzyme in CR1-B was less sensitive to sulfonylurea herbicide inhibition compared to ALS from sensitive sugarbeet.

Progeny segregation studies indicated that sulfonylurea resistance may be inherited in a semi-dominant fashion. Homozygous resistant sugarbeet was 2 to 3 times more resistant to foliar applications of sulfonylurea herbicides than heterozygous resistant sugarbeet. ALS enzyme analysis

was consistent with whole plant results. The inheritance of sulfonylurea resistance in sugarbeet is semi-dominant. Sulfonylurea resistance represents a potentially useful agronomic trait that would provide an economic benefit to sugarbeet growers.

The potential interactions between primisulfuron and other corn (*Zea mays* L.) herbicides was also evaluated. Atrazine, {6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine}, dicamba (3,6-dichloro-2-methoxybenzoic acid), and bentazon {3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide} antagonized primisulfuron control of giant foxtail (*Setaria faberi* Herrm.) and shattercane {*Sorghum bicolor* (L.) Moench.}. Reductions in foliar absorption and/or spray retention explained bentazon antagonism on both species and dicamba antagonism on shattercane. Adding ammonium sulfate or replacing non-ionic surfactant (NIS) or crop oil concentrate with methylated seed oil (MSO) or the organosilicone DC-X2-5394 reversed these antagonisms by restoring foliar absorption and/or spray retention to normal levels. MSO and DC-X2-5394 consistently increased primisulfuron control of giant foxtail by increasing foliar absorption and/or spray retention compared to NIS.

Applying primisulfuron in combination with atrazine reduced velvetleaf (*Abutilon theophrasti* Medicus) control. Atrazine had no effect on the foliar absorption and metabolism of ^{14}C-primisulfuron by velvetleaf and giant

foxtail. The translocated ^{14}C-primisulfuron was found above the treated leaves in velvetleaf and below the treated leaves in giant foxtail indicating phloem transport. Atrazine reduced the phloem transport of ^{14}C-primisulfuron in both species.

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INTRODUCTION

Sulfonylurea and imidazolinone herbicides are used to effectively control a broad spectrum of weed species in a variety of crops including corn *(Zea mays* L.), soybeans (*Glycine max* (L.) Merr), and small grains. These herbicides control weeds by inhibiting the enzyme acetolactate synthase (ALS[1], also referred to as acetohydroxy acid synthase) involved in the synthesis of the branched chain amino acids valine, leucine, and isoleucine. Weed control is obtained at very low use rates of these compounds. Combined with their low mammalian toxicity, these herbicides provide attractive weed control options from both the commercial and environmental standpoint.

Sulfonylurea and imidazolinone herbicides vary in soil persistence depending on the herbicide, soil, and environmental conditions. Persistence that provides season-long weed control is highly desirable, however, some of these herbicides have the potential to persist in alkaline soils for several years and subsequently injure sensitive rotational crops. Among these sensitive rotational crops are sugarbeet (*Beta vulgaris* L.) which are grown on highly

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[1]Abbreviations: ALS, acetolactate synthase; CR1-B, Chlorsulfuron resistant isolate 1-B; NIS, non-ionic surfactant; COC, crop oil concentrate.

alkaline soils in many regions of the United States.

The generation and development of sugarbeet cultivars that are resistant to sulfonylurea and imidazolinone herbicides is a potential solution to this carryover problem. Resistant cultivars could also provide more economical weed control options for sugarbeet growers. Currently, weed control in sugarbeet often requires multiple herbicide applications, several cultivations, and hand hoeing due in part to the wide spectrum of weeds and marginal crop tolerance to herbicides. Consequently, weed control production costs may be as much as $250 per ha.

The following studies were conducted to evaluate a chlorsulfuron {2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl]amino]carbonyl] benzenesulfonamide} resistant sugarbeet clone (CR1-B), generated via somatic cell selection in tissue culture, as a potential germplasm source for ALS inhibiting herbicide resistance in sugarbeet. The objectives of the following experiments were to identify herbicides to which CR1-B is cross-resistant, the magnitude of the resistance, and to determine the physiological basis for resistance in CR1-B. Genetic studies were also conducted to determine if the sulfonylurea resistance trait is inherited in a completely dominant or semi-dominant fashion.

Primisulfuron {2-[[[[[4,6-*bis*-difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl]benzoic acid} was recently registered for postemergence grass and broadleaf

weed control in corn. Broad spectrum weed control could potentially be obtained by tank-mixing primisulfuron with other herbicides. However, it is not known if tank-mixing primisulfuron will adversely affect grass control activity.

The objectives of this research were to determine the effect of atrazine {6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine}, dicamba {3,6-dichloro-2-methoxy benzoic acid}, and bentazon {3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide} on the efficacy, absorption, and foliar spray retention of primisulfuron applied with standard adjuvants such as non-ionic surfactant (NIS) or crop oil concentrate (COC) to giant foxtail (*Setaria faberi* Herrm.) and shattercane {*Sorghum bicolor* (L.) Moench.}. The effect of methylated seed oil (MSO), experimental adjuvant DC-X2-5394 or ammonium sulfate on the efficacy, absorption, and spray retention of primisulfuron applied alone or in a tank-mix with these herbicides was also evaluated. Physiological studies were also conducted to determine the effect of atrazine on the efficacy, absorption, translocation, and metabolism of ^{14}C-primisulfuron in giant foxtail and velvetleaf (*Abutilon theophrasti* Medic.).

Chapter 1

REVIEW OF LITERATURE

HERBICIDE RESISTANT CROPS FROM CELL SELECTION

Introduction

The development and utilization of herbicide resistant crops could potentially provide farmers with many economic advantages. Herbicide resistant crops would allow farmers to safely apply herbicides to crops which were normally sensitive to these herbicides. Chemical weed control options in minor agronomic crops such as canola (*Brassica napus* L.), tobacco (*Nicotiana tabacum* L.), and sugarbeet (*Beta vulgaris* L.) as well as numerous vegetable crops are limited. Herbicide resistant cultivars of these minor crops would increase the chemical weed control options for farmers. Crop safety could be further increased for crops considered tolerant to particular herbicides. Increasing the tolerance of these crops would allow the possibility of increasing herbicide at application rate to broaden weed control spectrum. Many crops cannot be safely rotated to fields that were previously treated with herbicides having a long persistence in the soil. Herbicide resistant crops could alleviate this problem. Herbicide resistant crops would also help to decrease the incidence of crop injury from herbicide spray drift.

The time and cost involved in developing new, effective, and environmentally sound herbicides to which crops are naturally tolerant is increasing. Agrochemical companies could realize an economic advantage by expanding the use of proven existing herbicides to herbicide resistant crops with a minimum of development costs.

The general public may also benefit if the use of environmentally sound herbicides was expanded by developing crops resistant to these herbicides. An indirect benefit of herbicide resistances in crops is their potential use as genetic markers in molecular biology. Herbicide resistances might also be used as a dominant selectable markers for classical breeding purposes. Herbicide resistance genes can be physically linked to other genes conferring agronomically useful traits that are difficult to monitor such as male sterility.

Breeding Methods

There are four potential methods to generate herbicide resistant crops; conventional plant breeding, mutation breeding, genetic transformation utilizing recombinant DNA technology, and *in vitro* somatic cell selection.

A conventional breeding method that has been successfully used to generate herbicide resistant crops are backcrosses with herbicide resistant weedy relatives. The occurrence of a large variety of weed species resistant to

triazine herbicides such as atrazine (6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine) has provided potential germplasm to generate triazine resistant crops. Resistant weeds are unaffected by triazine herbicides due to a change in the binding affinity of triazine herbicides for the plastoquinone B binding protein (Q_B protein) in photosystem II (23, 34). Triazine resistant canola (*Brassica napus* L.) was developed by backcrossing with triazine resistant birds rape (*Brassica campestris* L.). (5). The resistance trait was cytoplasmically inherited facilitating a simple and rapid backcross procedure due to the lack of progeny segregation. Resistant cultivars were unaffected by atrazine, cyanazine (2-[[4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl]amino]-2-methylpropanenitrile), and metribuzin (4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one) applied postemergence at 3.0, 3.0, and 0.6 kg ai ha^{-1}, respectively (5). However, agronomic evaluations determined that triazine resistant cultivars yielded 20% to 30% less when compared with reciprocal susceptible cultivars (4). This yield reduction was apparently due to a lower rate of photosynthesis associated with the change conferring triazine resistance on the Q_B protein (37, 75).

Mutation breeding, utilizing a chemical agent such as ethyl methane sulfonate (EMS), has been successfully used to generate a sulfonylurea resistant soybean line (58) and

imidazolinone tolerant corn[2]. Resistant soybean lines were generated by soaking the seeds in EMS, while imidazolinone resistant corn lines were generated by treating pollen with EMS. Individual seedlings were then screened for tolerance to herbicides of interest. Resistance in both species is due to an altered acetolactate synthase (ALS) binding site and inherited as a single dominant nuclear gene facilitating transfer into commercial soybean and corn varieties. In the case of resistant soybean the lines are being utilized to increase the tolerance of soybean to the sulfonylurea herbicides chlorimuron (2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoic acid) and thifensulfuron (3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylic acid) (59).

Conventional breeding methods and mutation breeding has been successfully used to generate triazine and sulfonylurea resistant crops. However, the development of triazine resistant crops is severely limited by the yield depression associated with the resistance trait and the lack of related resistant weeds to our crop plants. Mutation breeding appears promising but requires a large labor and space input for screening large numbers of seedlings. The utilization of EMS may also cause other potentially deleterious mutations in the plants that have become herbicide resistant.

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[2]Paul Kaylor, Personal Communication, ICI Seeds.

An alternative to conventional breeding approaches is to genetically engineer herbicide resistant crops utilizing recombinant DNA technology. A glyphosate (*N*-(phosphonomethyl)glycine) resistant allele, which was isolated and sequenced from a mutant strain of *Salmonella typhimurium*, was cloned in *Escherichia coli* (14, 68). The allele had a single base pair change resulting in a proline to serine amino acid substitution on the enzyme 5-enolpyruvylshikimate-3-phosphatase (EPSP), the primary site of action of glyphosate. This modification reduced the binding affinity of glyphosate for the EPSP enzyme by two to three fold. The gene was transferred to a tobacco cell line using *Agrobacterium tumefaciens* as a transfer vector. Regenerated tobacco plants from the transformed cell line had a 2-to 3-fold increase in glyphosate resistance as compared to untransformed plants (14).

Glyphosate resistant petunia (*Petunia hybrida* L.) was also successfully generated though recombinant DNA technology (60). Regenerated petunia plants were able to survive foliar applications of 1.1 kg ai ha^{-1} glyphosate. In this case the resistance was due to a 20- fold increase in the production of the EPSP enzyme (60). Delannay et al. reported that these techniques had also been used to develop glyphosate resistant tomato (*Lycopersicon esculentum* L.) and canola lines (17). However, in initial field evaluations, where resistant plants were sprayed with 0.6 and 1.1 kg ai

ha^{-1} of glyphosate, 30% and 100% yield reductions were observed in canola and tomato, respectively.

Although the initial successes of developing herbicide resistant crops though recombinant DNA technology appeared very promising, a major limitation to the technology is the limited number of crops that can be transformed with *Agrobacterium tumefaciens*. The regeneration of fertile whole plants from transformed somatic cells is also necessary.

A new method of transferring foreign genes into crop plants is microprojectile bombardment. This technique has been successfully used to generate glufosinate (2-amino-4-(hydroxymethylphosphinly)butanoic acid) resistant corn (*Zea mays* L.) plants by introducing a gene that codes for enzymes to metabolize glufosinate into embryonic cell cultures (29). This new method has tremendous potential to generate herbicide resistant crops because herbicide resistant genes can be introduced into a wide range of crop plants, especially those in the *Gramineae* family. The regeneration of transformed plants is facilitated because embryonic cell cultures can be utilized. However, the financial investment required for the purchase and maintenance of a "gene gun" precludes its use by many researchers.

Cell Selection

With the exception of mutation breeding, the development of herbicide resistant crops, through

conventional or recombinant DNA methods requires an existing source of herbicide resistant genes. In recombinant DNA technology these genes are usually obtained from mutant bacteria or plants selected for resistance in cell cultures. Therefore, a less expensive alternative to recombinant DNA technology is to directly generate herbicide resistant crops through direct cell selection where possible. The use of cell selection as an alternative to transformation requires less technical specilization and less specialized laboratory equipment further reducing cost. The use of cell cultures allows for the potential to generate resistant plants with varying resistance mechanisms, such as an altered herbicide target site or ability to detoxify the herbicide. Cell cultures also allow the potential to select recessive mutants through the use of haploid cell cultures. Finally Resistant crops generated though cell selection are not considered genetically transformed plants eliminating the regulatory constraints when field tests are conducted.

The utilization of cell selection to generate herbicide resistant crops is hampered by limitations in the number of crops that can be easily manipulated in tissue culture. The ability to obtain regenerated whole plants from cell cultures is currently not possible for many crops. However, as knowledge of physiological and genetic factors concerning cell tissue culture increase, the potential for whole plant regeneration will also increase.

The remainder of this review will focus on the generation and development of herbicide resistant crops obtained though cell selection. The review will discuss cell selection from a variety of sources, including suspension cultures, callus culture, protoplasts, and microspores. The paper will also discuss agronomic, physiological, and genetic studies involved with herbicide resistant crops obtained from cell cultures.

Cell selection for herbicide resistance from cultured plant cells relies occasional spontaneous mutants which differ from the rest of the cell population in any number of characteristics. Placing the herbicide in the test culture medium selects for these mutants that may be able to survive and grow on a lethal concentration of the herbicide.

Cell selections for herbicide resistance can include *en masse* stepwise selection or lethal single-step selection. In stepwise selection, herbicide concentrations are gradually increased over time to eliminate susceptible cells. Surviving cells are transferred to fresh culture medium (plus or minus the herbicide) to increase cell populations. Herbicide concentrations are increased and the process repeated. In single-step selection, cells are exposed to a herbicide concentration that is lethal to all cells in the population with the exception of possible mutant cells that are resistant to the herbicide.

The stepwise selection procedure is more labor

intensive and requires a longer period of time for obtaining herbicide resistant cell lines compared to the single-step selection procedure. Resistant cell lines that have gone through many selection cycles may also undergo other physiological and genetic changes reducing the potential to obtain regenerated resistant plants. However, the use of the multiple selection procedure rather than a single-step procedure may improve the chances of obtaining resistant cell lines as well as increase the possibility of obtaining mutant cell lines with differing resistance mechanisms.

Resistance to Non-Selective Herbicides

The broad weed control spectrum of the non-selective herbicides glyphosate, paraquat (1,1′-dimethyl-4,4′-bipyridinium ion), glufosinate, and amitrole (1*H*-1,2,4-triazol-3-amine) has led many researchers to attempt selection for resistant crops to these herbicides. Fifty-one glyphosate resistant cell lines were generated by treating haploid cell suspension cultures of tobacco with a single-step treatment of 1 mM glyphosate (65). Many of the resistant cells lines maintained resistance for 3 years with and without the presence of the herbicide. However, the resistance of the regenerated plants was low, and regenerated plants were not fertile (65). Dyer et al. (22) conducted similar selections using somatic cells of tobacco with a single-step selection on 3 mM glyphosate (22). Eleven

resistant cell lines were generated and one cell line, designated I7, was characterized further. The resistance of I7 cells has been stable for over a year with and without the presence of the herbicide. Regenerated plants were able to survive applications of glyphosate at 0.6 and 1.1 kg ai ha^{-1}. However, growth was severely retarded compared to untreated regenerated plants. Physiological studies found that I7 cells had twice as much EPSP synthase enzyme activity than susceptible cells (22). Additional cell lines have been selected from I7 using a step-wise selection procedure generating cell line I7/20 which is capable of growth on 20 mM glyphosate (28). I7/20 cells had more than 20 times the EPSP synthase activity than susceptible cells. The increase in EPSP synthase activity was the result of increased levels of the enzyme in resistant cells rather than a change in the activity of the enzyme. The increase in the level of the EPSP synthase enzyme was due to an increase in messenger RNA levels coding for the enzyme which was the result of amplification of two genes in resistant I7/20 cells (28).

Glyphosate resistant tomato cell lines have also been generated via single-step selection on 10 mM glyphosate (67). Resistant carrot (*Daucus carota* L.) cell lines have also been generated using step-wise selection procedure up to 25 mM glyphosate (43). However, attempts to regenerate fertile plants were not successful in either case. Analysis

of EPSP synthase found that both cell lines had elevated levels of enzyme activity compared to susceptible cell lines (43, 67). Resistant cell cultures of *Corydalis sempervirens* Pers. and *Catharanthus roseus* L. generated by stepwise selection were also found to have elevated levels of EPSP synthase activity compared to susceptible cell cultures (16, 66).

Paraquat resistant tobacco cell lines were successfully generated from both callus and suspension cultures using step-wise selection up to 10 mM paraquat (41). Utilization of X-ray treatment increased the proportion of stable resistant cell lines. Regenerated plants showed increased resistance to foliar applications of paraquat compared to susceptible plants (41). Resistant cell lines were also generated from callus cultures derived from tobacco protoplasts using stepwise selection (27). Resistant cell lines possessed a 14- to 159-fold increase in activity of the enzyme superoxide dismutase compared to susceptible cells (27). High levels of superoxide dismutase activity are believed to counteract the production of superoxide anion radicals produced by paraquat treatment. Nineteen paraquat resistant cell lines of tomato were generated using single-step selection (74). However, regenerated plants from only one cell line were slightly more resistant to foliar applications of paraquat.

Stepwise selection procedure has been used to generate

glufosinate resistant lines of tobacco cell suspension cultures. The resistant cell lines were stable, with resistance due to an overproduction of glutamine synthase, the primary site of action of glufosinate (35). Glufosinate resistant alfalfa cell lines were generated via a stepwise selection procedure. These resistant cell lines were also stable and resistance due to an overproduction of glutamine synthase (18).

Barg and Umiel conducted early work using stepwise selection to generate tobacco cell lines resistant to amitrole. Two cell lines were isolated that showed greater growth on 1 mM amitrole than unselected cell lines (2). Thirty-one resistant cell lines were generated using single-step selection of haploid tobacco cells in the presence of 1.9 mM amitrole (64). Seven cell lines retained tolerance for 3 years when cultured in the presence of the herbicide. However, none of the cell lines retained resistance when cultured without amitrole. In contrast, amitrole resistant cell lines generated from diploid tobacco suspension cultures were stable not only in cell culture but also through two sexual generations (64). An amitrole resistant tobacco cell line was also generated using stepwise selection up to 10 mM amitrole. The resistant cell line was stable and regenerated plants were resistant to foliar applied amitrole. The resistance trait was also successfully transmitted through one sexual generation (73).

The generation of resistant crop plants to non-selective herbicides has enjoyed success at the cellular level. When determined, the physiological basis of resistance was due to overproduction of the herbicide target enzyme or of an enzyme to counteract the deleterious effects of the herbicide. However, results at the whole plant level were not as successful for many reasons. In many cases resistant cell lines were not stable or regenerated plants were not obtained. In some cases many of the regenerated plants from resistant cell lines did not retain resistance at the whole plant level (64, 73). In other cases tolerance at the whole plant level was low; this was especially true for paraquat resistant cell lines (22, 65).

Amitrole resistant cell lines were stable in both culture and at the whole plant level but genetic analysis determined that the resistance trait was not inherited in a simple Mendelian fashion making transfer of the resistance to commercial tobacco cultivars difficult (64, 73).

Resistance to Growth Regulator Herbicides

Growth regulator herbicides such as 2,4-D {(2,4-dichlorophenoxy) acetic acid} have been extensively used to control broadleaf weeds in corn and small grains for over 40 years. 2,4-D was one of the first organic herbicides developed and one of the least expensive. Cell lines resistant to 2,4-D were generated using stepwise selection

of diploid tobacco cells (44). The cell line was also cross-resistant to picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid), another growth regulator herbicide. Physiological studies were unsuccessful in determining the resistance mechanism but eliminated uptake and 2,4-D detoxification as potential mechanisms (45). Resistant cell lines of white clover (*Trifolium repens* L.) were generated using single-step selection of suspension cultures to 2,4-D and 2,4,5-T {(2,4,5-trichlorophenoxy) acetic acid} (47). A 6 -to 7-fold increase in resistance was observed to these herbicides for selected cell lines compared to susceptible cell lines.

The generation of 2,4-D resistant birdsfoot-trefoil (*Lotus corniculatus* L.) has been attempted using single-step selection of cell suspension cultures (40, 72). Cell lines with increased tolerance to 2,4-D were obtained, but tolerance at the whole plant level was not evident (40) or only slightly higher than unselected plants (72). Reduced chromosome numbers and reduced pollen viability of some selected regenerated plants was also observed. Resistant cell suspension cultures of potato (*Solanum tuberosum* L.) have been generated using stepwise selection up to 50 mg l^{-1} of the auxin herbicide MCPA ((4-chloro-2-methylphenoxy) acetic acid). Tolerance was expressed in tests with regenerated shoots, but no observations were reported for whole plants (76).

Picloram resistant cell lines obtained from single-step selection of suspension cell cultures have been generated (11). Whole plants have been regenerated and genetic studies determined inheritance of the resistance trait as a single dominant nuclear gene (11). The physiological basis of resistance has not been determined, but reduced uptake or increased detoxification of picloram by resistant cells was not detected (9).

Resistance to Thiocarbamate Herbicides

Single-step selection of tobacco suspension cultures was used to generate vernolate (*S*-propyl-dipropyl carbamothioate) resistant cell cultures (25). Resistance was unstable in the absence of continued selection on vernolate, but regenerated shoots produced plants in which resistance was stable. However, many of the regenerated plants displayed morphological abnormalities. A potential problem of selecting resistant cells to a thiocarbamate herbicide such as vernolate is the low water solubility and high volatility of the herbicide. These physical properties may result in a lack of consistent selection pressure on all cells. To solve this problem a single-step selection was made using a non-volatile thiocarbamate analog R-14705 (*S*-3-methylpyridyl *N,N*-di-butyl-thiocarbamate) (24). Resistant cell lines were stable in the absence of continuous selection, and showed cross-resistance to commercial

thiocarbamate herbicides. However, whole plant observations were not reported.

Resistance to Triazine Herbicides

Triazine resistant cell lines of tobacco have been generated using a stepwise selection procedure with cell suspension cultures (55). Resistant cell lines were 200 times more resistant to atrazine than susceptible cells, and the resistance was stable in the absence of selection. Further studies revealed that the cells were resistant due to a new base pair change on the chloroplast *psbA* gene which codes for the Q_B protein (63). The base pair change resulted in an amino acid change on the Q_B protein from serine to threonine, whereas all other higher plants resistant to triazines have had a change to glycine.

The generation of resistant crops to a wide variety of herbicides has been successful at the cellular level. Success has been obtained with cell suspension cultures, callus, and protoplasts using single-step or stepwise selection procedures, although stepwise selection appears to generate cell lines with greater resistance. However, these previous attempts have fallen short of producing herbicide resistant crops for commercial production. There has been limited research on the generation of resistant crops to the previously mentioned herbicides outside of tobacco. Due to the limited acreage of tobacco and negative public opinion,

it is highly unlikely that commercial herbicide resistant cultivars will be developed. However, tobacco was widely utilized due to its easy manipulation in tissue culture. The research with tobacco has greatly enhanced our knowledge of selecting herbicide resistant mutants from cell cultures and the physiological and genetic factors involved in herbicide resistance. These earlier studies have also demonstrated that there is potential for developing herbicide resistant crops from cell cultures. If maintained, the resistant cell lines generated in these studies could also serve as potential sources for herbicide resistant genes that can be cloned and introduced into crop plants using recombinant DNA technology. A gene used to transform canola and tomato plants for glyphosate resistance was obtained from resistant cell cultures of petunia (69).

Resistance to Lipid Synthesis Inhibiting Herbicides

Herbicides in the aryloxyphenoxypropionate family such as haloxyfop (2-[4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid) and fluazifop ((±)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid) and cyclohexanedione family such as sethoxydim (2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) are widely used postemergence to control grass weeds in many dicot crops such as soybean. The primary mode of action of these herbicides is the inhibition of

acetyl-CoA carboxylase (ACCase), which is involved in the synthesis of fatty acids. (7, 52). Until recently, postemergence grass weed control options in corn were limited. This deficiency, combined with the single site of action of these herbicides, led researchers to attempt the generation of resistant corn cultivars.

Resistant corn cell lines were generated by selecting corn callus cultures for sethoxydim resistance using a stepwise selection procedure (49). Selection of corn callus cultures began at 5 *u*M sethoxydim and gradually increased to 100 *u*M. Resistant callus cultures were also cross-resistant to haloxyfop. ACCase activity analysis indicated that resistance was due to an overproduction of the ACCase enzyme (49). Selections conducted with other corn lines but using similar procedures generated resistant corn lines that possessed an altered ACCase enzyme that was less sensitive to sethoxydim inhibition compared to ACCase enzyme from susceptible callus cultures (50). Genetic studies determined inheritance as a single dominant nuclear gene. Whole plant studies have demonstrated that the sethoxydim resistant corn is cross-resistant to diclofop-methyl ((±)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid), fluazifop, and fenoxaprop ((±)-2-[4-[(6-chloro-2-benzoxazolyl)oxy] phenoxy]propanoic acid) (20). Field studies have shown that the corn line is resistant to sethoxydim applications at four times the field use rate, with no effect on corn yield

(19).

Tank-mix combinations of sethoxydim with other postemergence corn broadleaf herbicides would provide broad spectrum weed control if applied to sethoxydim tolerant corn cultivars (21). Unfortunately, the potential economic advantages for farmers has been lessened by the recent introduction of primisulfuron (2-[[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl]benzoic acid) and nicosulfuron (2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide) for postemergence control of grass weeds in corn. Sethoxydim tolerant corn cultivars would still provide farmers with increased chemical grass weed control options with a different mode of action from sulfonylurea herbicides.

Resistance to ALS Inhibiting Herbicides

Sulfonylurea and imidazolinone herbicides are used to effectively control a broad spectrum of weed species in a variety of crops including corn, soybeans, and small grains. These herbicides control weeds by inhibiting the enzyme acetolactate synthase (ALS, also referred to as acetohydroxy acid synthase) which catalyzes the synthesis of the branched chain amino acids valine, leucine, and isoleucine (51, 61). Weed control is obtained at very low use rates of these compounds. Combined with their low mammalian toxicity (39),

these herbicides provide attractive weed control options from both the commercial and environmental standpoint. These advantages, and the knowledge that sulfonylurea and imidazolinone herbicides most likely have a single target site of action, has led researchers to explore the possibilities of generating resistant crops to ALS inhibiting herbicides.

Chaleff and Ray conducted the first experiments to determine the potential of generating sulfonylurea resistant crops. Tobacco callus cultures were selected using a single-step procedure on 5.6 nM chlorsulfuron (2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5,-triazin-2-yl)amino]carbonyl] benzenesulfonamide) and sulfmeturon methyl (2-[[[[(4,6-dimethyl-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoic acid) (12). Resistant cultures were obtained with and without using the mutagen ethylnitrosourea. Resistance was stable in the absence of the herbicides and inherited sexually as a single dominant or semi-dominant gene in all cases. Whole plant studies showed that regenerated plants were at least 100 times more resistant to foliar applications of chlorsulfuron compared to regenerated plants from sensitive cell lines (12). The basis of resistance was due to an altered form of the ALS enzyme that was less sensitive to sulfonylurea inhibition compared to ALS enzyme from susceptible plants (10). The resistance of one of the mutant lines was increased by a second single-step selection

on 560 nM sulfmeturon-methyl (15). This resistant line was at least 500 times more resistant to sulfmeturon-methyl than susceptible cell lines. ALS enzyme activity of this double mutant was approximately 20 times more resistant to sulfmeturon-methyl and chlorsulfuron inhibition than ALS enzyme activity from sensitive plants. Resistant cell lines of tobacco have also been generated using a single-step procedure with primisulfuron (30).

The success of obtaining stable cell cultures of tobacco that were highly resistant to sulfonylurea herbicides without the use of a chemical mutagen, and the knowledge that the resistance trait was inherited as a single dominant gene, has led to attempts to develop other crops resistant to ALS inhibiting herbicides.

Resistant callus cultures of flax (*Linum usitatissimum* L.) were generated using single-step selection on 100 nM chlorsulfuron. Fertile whole plants were regenerated and callus cells obtained from progeny of these plants exhibited resistance to chlorsulfuron (36). Microspores and protoplasts of canola have been used to conduct single-step selections for chlorsulfuron resistance (70). Regenerated plants were 10 to 100 times more resistant to foliar applications of chlorsulfuron than susceptible plants. The resistance mechanism in canola was also shown to be an altered ALS enzyme that was less sensitive to sulfonylurea inhibition. Similar methods were used to generate

imidazolinone resistant canola except that ethylnitosourea was used as a mutagen (71). Regenerated plants were resistant to field application rates of imazaquin (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolinecarboxylic), imazethapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid), and imazethabenz-methyl ((±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-4(and 5)-methylbenzoic acid (3:2)) in greenhouse and field trials (70).

A single-step selection procedure with 2.8 nM chlorsulfuron using sugarbeet cell suspension cultures was successful in generating resistant sugarbeet plants (56). Shoot tests showed that the resistant sugarbeet line was 300 to 1000 times more resistant than susceptible shoots. Resistance was inherited as a single gene trait (56). Chlorsulfuron resistant sugarbeet were highly cross-resistant to other sulfonylurea herbicides including chlorimuron, thifensulfuron, and primisulfuron applied at or exceeding field use rates (32). Cross-resistance to the imidazolinone herbicides was not detected. The physiological basis of resistance was an altered ALS enzyme less sensitive to sulfonylurea herbicide inhibition (32).

Imidazolinone resistant cell lines of corn were generated using a stepwise selection with callus cultures on imazaquin and imazethapyr. Three lines were resistant to

only imidazolinone herbicides while a fourth line was cross-resistant to chlorsulfuron (1). Resistance was due to an altered ALS enzyme and was stable following four to six backcrosses to corn inbred lines. Pioneer Hi-Bred International is currently backcrossing the imidazolinone resistance trait into more that 100 inbred lines of corn (46). Field studies have shown that imidazolinone resistant corn hybrids are resistant to field use rates of imazethapyr (8, 46, 54). Agronomic evaluations have indicated that there are no deleterious effects attributed to or linked to the herbicide resistance trait. Commercial imidazolinone resistant corn hybrids are expected to be widely available for the 1992 growing season. Imidazolinone resistant corn hybrids are also being marketed by the seed division of Imperial Chemicals Incorporated (ICI) that were generated via pollen mutagenesis. Yield studies have shown that resistant hybrids have the same yield potential as sensitive hybrids (13).

The generation and development of crop cultivars that are resistant to sulfonylurea and imidazolinone herbicides will provide more chemical weed control options for farmers especially in minor agronomic crops such as sugarbeet and canola, resulting in an economic advantage for farmers. Weed control in sugarbeet often requires multiple herbicide applications, several cultivations, and hand-hoeing due in part to the wide spectrum of weeds and marginal crop

tolerance to herbicides (62, 77). Consequently, weed control production costs may be as much as $250 per ha (57).

Heretofore, a disadvantage to the use of some sulfonylurea and imidazolinone herbicides has been that they may persist in alkaline soils for several years and subsequently injure sensitive rotational crops including corn and sugarbeets (3, 6, 26, 38, 42, 48). The generation of sulfonylurea and imidazolinone resistant crops may potentially solve this carryover problem. In initial field evaluations, sulfonylurea resistant sugarbeet grew normally in soil concentrations of chlorimuron and primisulfuron that killed sensitive sugarbeet (31).

The generation of crops resistant to ALS inhibiting herbicides has been very successful. Many new ALS inhibiting herbicides are currently under development and existing resistant crop cultivars may potentially be cross-resistant to these new herbicides. However, further research is needed to evaluate resistant crops, especially in the areas of the genetics of the herbicide resistance trait and agronomic performance of resistant crop cultivars. The initial reports on sulfonylurea and imidazolinone crops as well as sethoxydim resistant corn suggest no detrimental effect of the herbicide resistance trait on agronomic performance. However, comprehensive comparisons between herbicide resistant and susceptible crop cultivars have not been reported. Recent studies of sulfonylurea resistant sugarbeet

have shown that homozygous resistant lines possessed a two to three fold increase in resistance compared to heterozygous resistant lines (33). Differential tolerance to imazethapyr between homozygous and heterozygous resistant imidazolinone corn lines has been reported in field studies (8). These results suggest that the highest level of crop resistance in hybrid cultivars would be obtained by developing homozygous resistant crop cultivars. The commercial development of heterozygous resistant crop cultivars would be less costly and require less time than homozygous resistant cultivars. Extensive evaluations comparing heterozygous and homozygous resistant lines should be conducted to determine if heterozygous resistant cultivars can provide a commercially acceptable level of crop safety.

A large number of experiments have been conducted to determine the potential of generating herbicide resistant crops using cell selection techniques (Table 1). The generation and development of herbicide resistant crops has been successful for new, effective, and environmentally sound herbicides, especially in corn, sugarbeets, and canola. ALS inhibiting herbicides and postemergence graminicides are an integral part of our current chemical weed control practices and more herbicides in these chemical families are expected to be developed. Therefore, crops resistant to these herbicides will undoubtedly provide the

economic and environmental advantages discussed in the beginning of this paper.

The generation of herbicide resistant cell lines and subsequent regeneration of whole plants is only part of the breeding process required to obtain commercially available herbicide resistant crop cultivars. Breeding lines utilized in tissue culture are generally chosen and may be specifically bred for manipulation in tissue culture. As such, these breeding lines may not possess the same genetic potential for superior agronomic performance compared with breeding lines utilized to generated commercial crop cultivars. Herbicide resistant crop plants may also possess deleterious agronomic traits associated with other somaclonal variation aside from the herbicide resistance trait.

Therefore, it is necessary to incorporate the herbicide resistance trait into superior or elite breeding lines. Herbicide resistance traits inherited as a single dominant or semi-dominant nuclear gene could be incorporated into elite breeding lines utilizing conventional backcross breeding techniques. Backcross breeding would also eliminate undesirable somaclonal variation. However, this procedure usually requires five to seven backcross generations for resistance traits inherited in a completely dominant fashion. Herbicide resistance traits inherited in a semi-dominant fashion would require additional time and effort if

homozygous resistance breeding lines are desired. Herbicide resistance traits inherited in a recessive fashion require progeny tests for each backcrossed generation.

Conventional backcross breeding methods may be further slowed if deleterious somaclonal variation is tightly linked to the desired herbicide resistance trait. The utilization of restriction fragment length polymorphisms (RFLP's) reduce the number of sexual generations required for incorporation of the herbicide resistance trait into superior breeding lines. Young and Tanksley (78) conducted RFLP-assisted backcrossed breeding studies utilizing a high resolution map of RFLP's tightly linked to the *Tm-2* gene which codes for tobacco mosaic virus resistance. RFLP tightly linked to the resistance gene are identified and progeny in a segregating population identified that have retained the resistance gene, as well as a crossover event near the gene. RFLP assisted backcross breeding could also help select segregating progeny that had the least amount of unlinked DNA from the donor parent. Thereby allowing selection of individuals that were most like the agronomically superior recurrent parent.

As knowledge of cell tissue culture in other crops, in particular soybean and small grains continues to expand, the capability of regenerating whole plants through routine manipulation will increase. Cell and tissue culture is an excellent tool for the generation of new herbicide resistant

genes. Resistant cell lines can give rise to resistant plants in some cases or the resistant genes may also be cloned from these crops or cell lines and transferred to other crop plants using recombinant DNA technology. The generation of herbicide resistant crops from cell selection will continue to be successful in the future and will remain an integral part of the strategies employed to generate and develop herbicide resistant crop plants.

Table 1. Generation of herbicide resistant crops through cell selection.

Herbicide	Crop	Mutagen Used +/-	Stepwise or Singlestep	Regnerated Plants	Mendelian Inheritance Yes/No	Reference
Glyphosate	Tobacco	-	Singlestep	Yes	?	22, 65
	Tobacco	-	Stepwise	No		28
	Tomato	-	Singlestep	No		67
	Carrot	-	Stepwise	No		43
	Petunia	-	Singlestep	Yes	?	69
Paraquat	Tobacco	+	Stepwise	Yes	?	41
			Stepwise	No		27
	Tomato	-	Singlestep	Yes	?	74
Glufosinate	Tobacco	-	Stepwise	No		35
	Alfalfa	-	Stepwise	No		18
Amitrole	Tobacco	-	Singlestep	No		2
		-	Stepwise	Yes	No	64, 73

(Continued)

Table 1 (continued)

Herbicide	Crop	Mutagen Used +/-	Stepwise or Singlestep	Regnerated Plants	Mendelian Inheritance Yes/No	Reference
2,4-D	Tobacco	-	Stepwise	No		44, 45
	White clover	-	Singlestep	Yes	?	47
	Birdsfoot-trefoil		Singlestep	Yes		40, 72
2,4,5-T	White clover	-	Singlestep	Yes	?	47
MCPA	Potato	-	Singlestep	Yes	?	76
Picloram	Tobacco	-	Singlestep	Yes	Yes	11
Vernolate	Tobacco	-	Singlestep	Yes	?	25
Thiocarbamate	Tobacco	-	Singlestep	Yes	?	24
Atrazine	Tobacco	-	Stepwise	No		55, 63
Sethoxydim	Corn	-	Stepwise	Yes	Yes	49, 50
Chlorsulfuron	Tobacco	+	Singlestep	Yes	Yes	12, 10
	Flax	-	Singlestep	Yes	Yes	36
	Canola	-	Singlestep	Yes	?	70
	Sugarbeet	-	Singlestep	Yes	Yes	56

(Continued)

Table 1 (continued)

Herbicide	Crop	Mutagen Used +/-	Stepwise or Singlestep	Regnerated Plants	Mendelian Inheritance Yes/No	Reference
Sulfmeturon-methyl	Tobacco	-	Singlestep	Yes	Yes	10, 12, 15
Primisulfuron	Tobacco	-	Singlestep	No		30
Imazethapyr	Canola	+	Singlestep	Yes	?	71
	Corn	-	Stepwise	Yes	Yes	1
Imazapyr	Corn	-	Stepwise	Yes	Yes	1

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Chapter 2

Chlorsulfuron Resistant Sugarbeet: Cross-resistance and Physiological Basis of Resistance

ABSTRACT

Greenhouse and laboratory studies were conducted to determine the extent of cross-resistance of chlorsulfuron resistant sugarbeet (CR1-B) to other herbicides that inhibit acetolactate synthase (ALS) and to determine the physiological basis of resistance. Cross-resistance to metsulfuron, imazaquin, and imazethapyr was not evident, while only marginal cross-resistance was observed to triasulfuron, DPX-L5300, and nicosulfuron. CR1-B was moderately resistant to chlorsulfuron and chlorimuron and was highly cross-resistant to thifensulfuron and primisulfuron. Further greenhouse studies demonstrated that CR1-B was not significantly injured by thifensulfuron and primisulfuron applied at or exceeding the field use rate. Studies with ^{14}C-primisulfuron concluded that differential absorption or metabolism of primisulfuron could not account for the observed resistance. ALS enzyme assays showed that the CR1-B ALS enzyme activity was 66, 26, and 13 times less sensitive to chlorsulfuron, thifensulfuron, and primisulfuron inhibition, respectively, compared to ALS enzyme extracted from sensitive sugarbeets. An altered ALS

enzyme, which is less sensitive to sulfonylurea herbicide inhibition, appears to be the physiological basis of resistance. Nomenclature: Chlorimuron, [[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoic acid; chlorsulfuron, 2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide; DPX-L5300, methyl 2-[[[[*N*-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)methylamino]carbonyl]amino]sulfonyl]benzoate; imazaquin, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolinecarboxylic acid; imazethapyr, (±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid; metsulfuron, 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] carbonyl]amino]sulfonyl]benzoic acid; nicosulfuron, 2-[[[[(4,6-dimethoxy-pyrimidin-2-yl)amino]carbonyl]-amino]sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide monohydrate; primisulfuron, (2-[3-(4,6-*bis*-(difluoromethoxy)pyrimidin-2-yl)ureidosulfonyl]benzoic acid methylester; triasulfuron, 2-(2-chloroethoxy)-N-[[4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]-benzenesulfonamide;thifensulfuron, methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylate; sugarbeet, *Beta vulgaris* L. 'CR1-B', 'REL-1'

Additional index words. Chlorimuron, imidazolinone, primisulfuron, sulfonylurea, thifensulfuron. Acetolactate

synthase, tissue culture.

INTRODUCTION

Sulfonylurea and imidazolinone herbicides are used to effectively control a broad spectrum of weed species in a variety of crops including corn (*Zea mays* L.), soybeans (*Glycine max* (L.) Merr), and small grains. These herbicides control weeds by inhibiting the enzyme acetolactate synthase (ALS[3], also referred to as acetohydroxy acid synthase) which catalyzes the formation of the branched chain amino acids valine, leucine, and isoleucine (14, 20). Weed control is obtained at very low use rates of these compounds. Combined with their low mammalian toxicity (11), these herbicides provide attractive weed control options from both the commercial and environmental standpoint.

Sulfonylurea and imidazolinone herbicides vary in soil persistence dependent upon the herbicide, soil, and environmental conditions. Persistence that provide season-long weed control is highly desirable. However, some of these herbicides have the potential for persistence in alkaline soils for several years and potential injury to sensitive rotational crops (5). Sugarbeets are extremely

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[3]Abbreviations: ALS, acetolactate synthase; TLC, thin layer chromatography; FAD, flavin adenine dinucleotide; CR1-B, Chlorsulfuron resistant isolate 1-B; NIS, non-ionic surfactant; MSO methylated seed oil; REL-1, Regenerating East Lansing-1.

sensitive to these herbicides with severe stand and root yield reductions observed in fields treated 2 or 3 yr earlier with chlorsulfuron (2, 13). Renner and Powell (15) reported reduced sugarbeet root yields from fields that had received applications of imazaquin, imazethapyr, or chlorimuron 1 or 2 yr earlier.

The generation and development of sugarbeet cultivars that are resistant to sulfonylurea and imidazolinone herbicides is a potential solution to this carryover problem. Resistant cultivars could also provide more economical weed control options for sugarbeet growers. Currently, weed control in sugarbeets often requires multiple herbicide applications, several cultivations, and hand hoeing due in part to the wide spectrum of weeds and marginal crop tolerance to herbicides (21, 24). Consequently, weed control production costs may be as much as $250 per ha (19).

The development of resistant germplasm has been initiated by the USDA sugarbeet breeding group at Michigan State University. A chlorsulfuron resistant sugarbeet clone (CR1-B) has been successfully generated via somatic cell selection in tissue culture using susceptible regenerating East Lansing-1 (REL-1) as the source material (17). Resistance to chlorsulfuron has been confirmed in tissue culture shoot tests and whole plant greenhouse tests. However, the extent of cross-resistance in CR1-B to other

ALS inhibiting herbicides has not been evaluated. The physiological basis for resistance is also unknown.

The objectives of the following experiments were to identify herbicides to which CR1-B is cross-resistant and the magnitude of the resistance and to determine the physiological basis for resistance in CR1-B.

MATERIALS AND METHODS

Whole Plant Bioassay

CR1-B S_1 seeds (obtained by self pollinating resistant CR1-B ramets) and S_1 seeds of REL-1 were planted in 55 ml peat pots containing greenhouse potting soil. Plants were grown in the greenhouse at 24 ±2 C with supplemental lighting from high pressure sodium lights to provide 1200 μE m^{-2} s^{-1} for both supplemental and natural light. The day length was 18 h. Two wk after planting, individual sugarbeet plants were transplanted into 945 ml plastic pots. Plants were watered and fertilized as needed to insure maximum growth. Ten ALS inhibiting herbicides were tested on these sugarbeets at one-half the commercial use rate. All herbicide treatments were applied POST with a continuous link-belt sprayer at 170 Kpa and 230 L ha^{-1} spray pressure and volume respectively, when sugarbeet plants were in the two- to four-leaf growth stage.

The presence of susceptible S_1 segregates in the CR1-B

test population made detailed evaluations and statistical analysis of experimental results difficult. Therefore, an additional greenhouse experiment was conducted in which a tissue culture leaf disk test was employed to eliminate susceptible S_1 CR1-B segregates from the test population prior to herbicide application. Plants were grown and cultured as above except that the 3rd and 4th leaves were removed, surface sterilized for 20 min with 15% NaOCl solution, and cut into 10-mm diameter leaf disks. Two leaf disks were placed in 100 by 20 mm sterile petri dishes containing a solidified growth media developed by Doley and Saunders (4) for rapid sugarbeet leaf disk expansion and callus production. The growth media contained either 0 or 140 Nm chlorsulfuron and the plates were incubated in the dark for 7 d at 31 C. Plants whose leaf disks exhibited vigorous tissue expansion on 140 nM chlorsulfuron were chosen for the greenhouse experiment.

Herbicides to which cross-resistance was evident in the previous experiment were applied at or exceeding the commercial use rate with various adjuvant combinations to determine the magnitude of observed resistance. Herbicide treatments were applied as described above except that plants were in the three- to six-leaf growth stage. Dry weights of the above ground foliage were taken 20 d after treatment of plants that were not completely desiccated.

Both experiments were completely randomized with four

replications per treatment. An analysis of variance test was conducted as a two-factor (variety by herbicide treatment) factorial. Each experiment was conducted twice, and analysis of variance revealed that treatment means did vary significantly between experiments; therefore, the combined results are presented. Treatment means were separated by a least significant difference test at the 5% probability level.

Absorption and Metabolism

The absorption and metabolism of ^{14}C-primisulfuron (spec. act. = 1.88 x 10^6 Bq mg^{-1} URL) was examined on both S_1 CR1-B and REL-1 plants in the greenhouse. S_1 CR1-B plants were identified as resistant prior to the experiment by the tissue culture leaf disk test as described above. Plants were sprayed in the three- to six-leaf growth stage with 40 g ai ha^{-1} unlabeled primisulfuron plus non-ionic surfactant[4] (NIS). Immediately after application, five 1 μl drops, each containing 3.7 x 10^2 Bq, were applied to both the 1st and 2nd leaves. Spotting vials contained the ^{14}C-primisulfuron with appropriate amounts of formulation blank and NIS. Treated leaves were rinsed with 40 ml of 100% methanol 12 and 72 h after treatment to remove the unabsorbed ^{14}C-

[4]Non-ionic surfactant was X-77 which is a mixture of alklarylpoly-oxyethylene glycols, free fatty acids, and isopropanol. Chevron Chemical Co., Richmond, CA.

primisulfuron. Two 1-ml aliquots were sampled from the rinse solution and radioassayed by liquid scintillation spectrometry.

Absorbed ^{14}C-primisulfuron was extracted and analyzed by thin-layer-chromatography (TLC) with a modification of previously published methods for chlorimuron (23) and sulfometuron (2-[[[[(4,6-dimethyl-2-pyrimidinyl)amino]-carbonyl]amino]sulfonyl]-benzoic acid) (1). Treated leaves were ground in a tissue homogenizer[5] in 40 ml of 100% methanol. The homogenized mixture was centrifuged and the resulting pellet was resuspended in methanol and recentrifuged. The combined supernatants were concentrated under a stream of nitrogen to 1 ml and used directly for TLC analysis. The residue pellet was air dried and oxidized[6] to quantify unextracted radioactivity.

A 200 μl sample of extract were spotted onto 20 by 20 cm TLC silica gel plates and developed in a solvent system of methanol:benzene (2:1 v/v). Ten μl of ^{14}C-primisulfuron standard (1.8×10^2 Bq) was also spotted onto each plate. Radioactive spots were located with a radioactive plate scanner[7], removed, and quantified by liquid scintillation spectrometry.

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[5]Sorvall Omni-mixer. Sorvall Inc., Newton, Conn.

[6]Biological oxidizer. R.J. Harvey Inst. Corp., Hillsdale, N.J.

[7]Radioactive plate scanner. AMBIS Inc., San Diego, CA.

The experimental design was completely randomized with four replications per treatment. The experiment was conducted twice and the combined results presented. Data was subjected to an analysis of variance and means separated by the least significant difference test at the 5% probability level.

Acetolactate Synthase Activity

ALS activity levels were determined in the leaves of plants obtained from ramets of resistant CR1-B and susceptible REL-1 plants. Plants were grown in a growth chamber with day and night temperatures of 24 and 20 C, respectively. Supplemental lighting was provided by fluorescent and incandescent lamps at 250 μE m^{-2} s^{-1} with an 8 h d length.

ALS was extracted and enzyme activity levels measured in the presence of primisulfuron, thifensulfuron, and chlorsulfuron with a modification of methods outlined by Ray (14) and Shaner (20). All extraction, centrifugation, and column procedures were conducted at 4 C. Forty to 50 g of plant leaves were homogenized in a volume of cold homogenization buffer [0.1 M K_2HPO_4, Ph 7.5, 1 mM sodium pyruvate, 0.5 mM MgCl, 0.5 mM thiamine pyrophosphate, 10 μM flavin adenine dinucleotide (FAD), 10% v/v glycerol] twice the weight of the tissue. Polyvinylpolypyrrolidone (2.5 g) was also added for every 10 g of plant material homogenized.

The homogenate was filtered through eight layers of cheesecloth and then centrifuged at 27000 g for 20 min. Saturated cold $(NH_4)_2SO_4$ solution was added to the supernatant to bring the final $(NH_4)_2SO_4$ concentration to 50% saturated. The solution was centrifuged at 15000 g for 15 min and the pellet redissolved in resuspension buffer (0.1 M K_2HPO_4, Ph 7.5, 20 mM sodium pyruvate, 0.5 mM MgCl) and placed on a Sephadex G-25 PD-10[8] column. The desalted protein was immediately used for enzyme assays.

ALS enzyme activity was assayed by mixing 0.5 ml of enzyme preparation with 1 ml of reaction buffer (25 mM K_2HPO_4, pH 7.0, 0.625 mM MgCl, 25 mM sodium pyruvate, 0.625 mM thiamine pyrophosphate, 1.25 μM FAD) and incubated for 1 h at 35 C. Reaction tubes contained either 0, 5, 25, 50, 500, or 5000 nM of chlorsulfuron, thifensulfuron, or primisulfuron. The reaction was stopped by the addition of 50 μl of 6 N H_2SO_4 and the solutions were heated at 60 C for 15 min. Then 0.5 ml of 0.5% (w/v) creatine and 0.5 ml of 5% (w/v) α-naphthol freshly prepared in 2.5 N NaOH were added. The solutions were heated for an additional 15 min at 60 C and the acetoin content measured by the method of Westerfield (22). Protein concentration was determined by the method of Lowery (12).

The experiment was conducted twice with two

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[8]PD-10 column. Pharmacia, Inc., Piscataway, N.J.

replications of each herbicide concentration per experiment. ALS enzyme activity is presented as a percent of control assays and data subjected to a linear regression analysis using a $\log_{10}$ transformation of herbicide concentrations. Paired T-tests were conducted to determine if the slopes of the regression lines were significantly different. I_{50} values (herbicide concentration required to inhibit enzyme activity by 50%) were calculated from the regression analysis.

RESULTS AND DISCUSSION

Whole Plant Bioassay

All REL-1 plants were completely desiccated by 8 of 10 of the ALS inhibiting herbicides applied at one-half the commercial use rate (Table 1). Several REL-1 plants were able to survive applications of 20 g ha^{-1} primisulfuron and nicosulfuron. CR1-B was able to survive all herbicide treatments with the exception of metsulfuron, imazaquin, and imazethapyr. However, applications of triasulfuron, DPX-L5300, and nicosulfuron severely reduced CR1-B dry weights as compared to untreated controls. Factorial analysis of dry weight means revealed a highly significant variety ($p < .001$) and variety by herbicide interaction ($p < .001$). CR1-B exhibited a moderate degree of resistance to chlorsulfuron and chlorimuron. Shoot dry weights for CR1-B plants treated with primisulfuron were significantly higher compared to

Table 1. Dry weights of S_1 CR1-B and REL-1 sugarbeet shoots 20 days after postemergence herbicide treatment[a].

Treatment	Rate	Shoot dry weights	
		CR1-B	REL-1
	g ha^{-1}	- g/plant -	
Chlorsulfuron	15	1.8	0.0
Metsulfuron	5	0.0	0.0
Triasulfuron	8	0.4	0.0
DPX-L5300	8	0.5	0.0
Chlorimuron	10	2.1	0.0
Thifensulfuron	2	3.4	0.0
Primisulfuron	20	3.2	0.2
Nicosulfuron	20	0.6	0.1
Imazaquin	60	0.0	0.0
Imazethapyr	30	0.0	0.0
Untreated		3.8	4.0
	LSD (.05)	0.4	

[a]All herbicide treatments included NIS at 0.25% (v/v).

CR1-B plants treated with the other ALS inhibiting herbicides except thifensulfuron. Shoot dry weights for CR1-B plants treated with thifensulfuron were the same as untreated control plants. Although the response of CR1-B to the ALS inhibiting herbicides was variable, a high degree of cross-resistance was clearly evident to the sulfonylurea herbicides chlorimuron, thifensulfuron, and primisulfuron. These herbicides were selected for further whole plant studies.

Due to the presence of susceptible S_1 segregates in the CR1-B test population, a second whole plant study was conducted employing the tissue culture leaf disk test to eliminate the susceptible segregates prior to herbicide application. The leaf disk test helped to provide a more uniform population for the evaluation of the potential of CR1-B to tolerate selected sulfonylurea herbicides applied at or exceeding commercial use rates.

All S_1 CR1-B plants survived all herbicide treatments, indicating that the leaf disk test was completely successful in eliminating susceptible S_1 CR1-B segregates (Table 2). Factorial analysis of dry weight means revealed a highly significant variety ($p < .001$) and variety by herbicide treatment interaction ($p < .001$). All REL-1 plants were completely desiccated by all herbicide treatments. Therefore, REL-1 treatment means are not presented and CR1-B treatment means analyzed separately (Table 2). The CR1-B

Table 2. Dry weights of resistant S_1 CR1-B sugarbeet shoots 20 days after postemergence herbicide treatment.[a]

Treatment	Rate	Shoot dry weight
	g ha^{-1}	g/plant
Primisulfuron+NIS[b]	40	6.6
	80	6.8
	160	6.1
Primisulfuron+MSO[c]	40	6.2
Thifensulfuron+NIS	4	6.4
	8	6.2
Thifensulfuron+NIS+28% N[d]	4	5.9
	8	4.8
Thifensulfuron+MSO	4	5.7
Thifensulfuron+chlorimuron+NIS	4 + 2	6.6
	4 + 4	5.9
Thifensulfuron+chlorimuron+NIS+28% N	4 + 2	4.2
	4 + 4	3.4
Untreated		6.6
	LSD (.05)	0.6

[a]S_1 CR1-B plants identified as resistant by tissue culture leaf disk test prior to herbicide application.

[b]NIS applied at 0.25% (v/v).

[c]MSO was methylated seed oil applied at 0.75% v/v.

[d]28% N was ammonium nitrate and urea applied at 4.0% (v/v).

sugarbeets were resistant to all rates of primisulfuron and thifensulfuron applied with NIS, as indicated by the lack of an effect of these herbicides on CR1-B dry weights (Table 2). However, the addition of 28% N or replacing NIS with methylated seed oil (MSO)[9] increased the activity of the thifensulfuron treatments to the extent that injury to the CR1-B sugarbeets occurred. CR1-B sugarbeets were resistant to combinations of thifensulfuron and chlorimuron applied at 4 and 2 g ha^{-1} respectively, but significant injury occurred when the chlorimuron rate was increased to 4 g ha^{-1}. Addition of 28% N to both herbicide combinations had the most detrimental effect on the growth of CR1-B.

The lack of cross-resistance in CR1-B to all the ALS inhibiting herbicides tested in this experiment was not surprising. The response of chlorsulfuron resistant weeds and transgenetic plants to other ALS inhibiting herbicides has been reported to be variable. A high degree of cross-resistance to thifensulfuron has been observed in resistant kochia [*Kochia scoparia* (L.) Schrad] (16), and tobacco (*Nicotiana tabacum* L.) (6) which had been transformed with the *csr-1* gene coding for chlorsulfuron resistance (9). However, the same kochia was only slightly more tolerant to metsulfuron (16), while cross-resistance to imidazolinone herbicides in plants containing the *csr-1* gene has not been

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[9]Methylated seed oil was SCOIL, from Agsco Inc., Grand Forks, N.D.

observed (6, 8).

CR1-B exhibited a high degree of tolerance to primisulfuron and thifensulfuron applied with a standard adjuvant at or exceeding the commercial use rates of these compounds. The resistance in CR1-B appears adequate for potential direct use of these herbicides for weed control in resistant sugarbeet varieties. Although no direct evaluations of CR1-B were made to date for ALS inhibiting herbicides applied PRE to a field soil, it is reasonable to assume that CR1-B would have a high potential to tolerate residues of thifensulfuron, primisulfuron, chlorimuron, and possibly even chlorsulfuron remaining in the soil from applications in previous years.

Absorption and Metabolism.

Both resistant CR1-B and susceptible REL-1 absorbed ^{14}C-primisulfuron with values ranging between 36 and 44% at 12 and 72 h, respectively (Table 3). Differences in foliar absorption could not account for the resistance mechanism. Total % recovery of ^{14}C-primisulfuron averaged 86 and 84% at both sampling times (translocation losses were not measured). Extraction of absorbed ^{14}C-primisulfuron averaged 94 and 91% at 12 and 72 h, respectively.

TLC analysis of alcohol-soluble ^{14}C-primisulfuron revealed two radioactive spots. One with an R_f of 0.89 which co-chromatographed with the ^{14}C-primisulfuron standard and

Table 3. Absorption and metabolism of ^{14}C-primisulfuron in resistant S_1 CR1-B[a] and susceptible REL-1 sugarbeet plants.

	Absorption		Metabolism	
Sugarbeet Line	12 h	72 h	12 h	72 h
	-- % of applied --		- % of extracted -	
CR1-B	30	43	6	10
REL-1	42	44	5	12
LSD (.05)	NS	NS	NS	NS

[a]S_1 CR1-B plants identified as resistant by tissue culture leaf disk test prior to herbicide application.

another that remained at the origin. There were no differences in the percentage of radioactivity remaining at the origin in either species. Metabolism of ^{14}C-primisulfuron increased from 6% at 12 h to 11% at 72 h. Although differences in metabolism could not account for the mechanism of resistance (Table 3), metabolism of ^{14}C-primisulfuron was detectable in both species and may help explain the tolerance of sugarbeets to primisulfuron as compared to other ALS inhibiting herbicides.

ALS enzyme assay

ALS enzyme activity was assayed in leaves obtained from plants derived from resistant CR1-B and susceptible REL-1 ramets. Activity was measured in the presence of primisulfuron, thifensulfuron, and chlorsulfuron concentrations ranging from 5 to 5000 nM (Figures 1-3). ALS activity in control assays averaged 118-124 and 122-132 nM acetoin h^{-1} mg^{-2} protein for CR1-B and REL-1, respectively. Paired t-tests to determine if the slopes of the linear regression lines were different were highly significant ($p < .001$) for all three herbicides. CR1-B still retained 35 to 45% of the activity of the control assays, while REL-1 activity was reduced to less than 10% at 5000 nM of all three herbicides (Figures 1-3).

Figure 1. CR1-B and REL-1 ALS activity in the presence of primisulfuron.

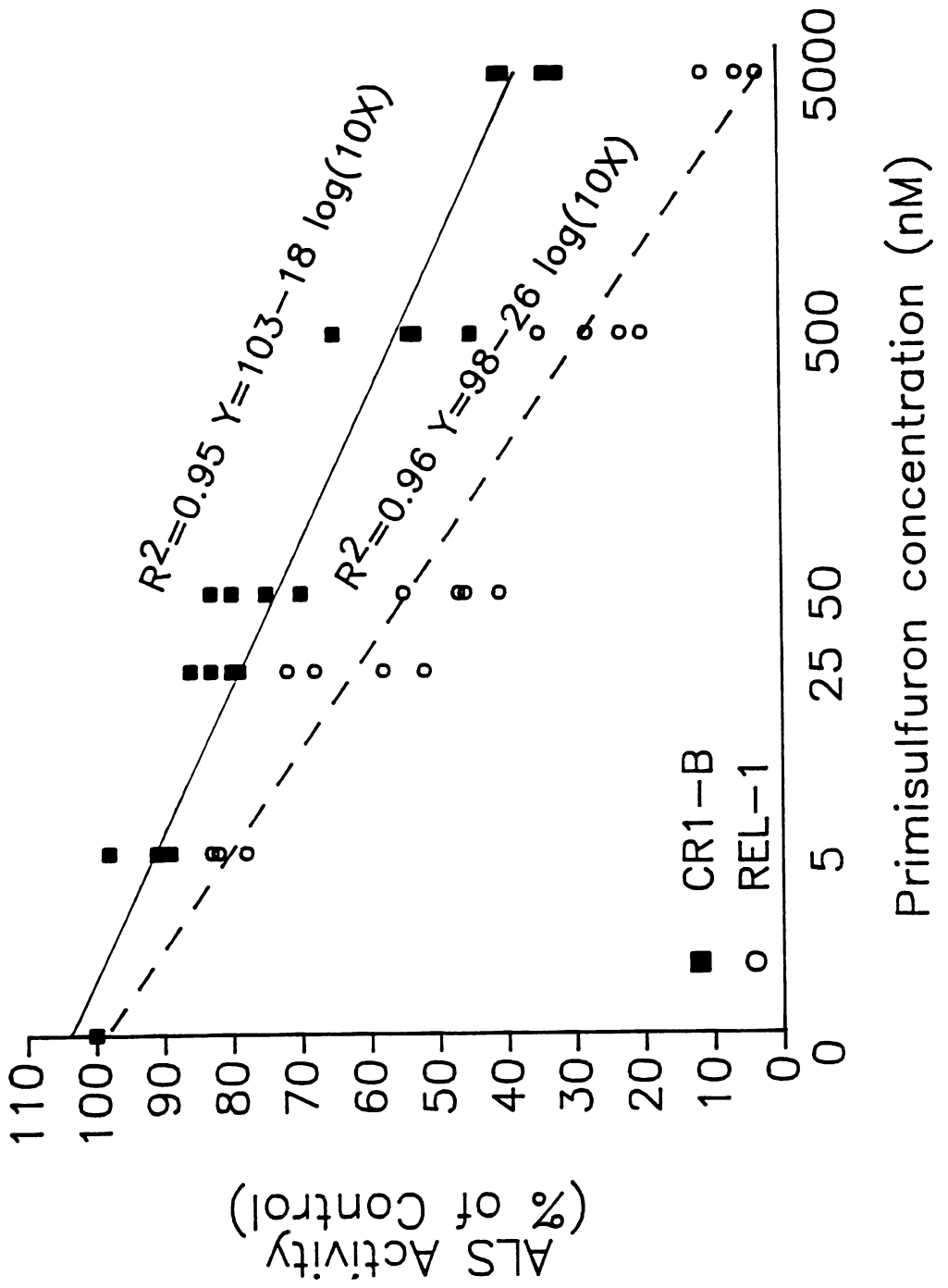

ALS Activity
(% of Control)
110
100
90
80
70
60
50
40
30
20
10
0
0
5
25 50
500
5000
Primisulfuron concentration (nM)
CR1-B
REL-1
R2=0.95 Y=103-18 log(10x)
R2=0.96 Y=98-26 log(10x)

Figure 2. CR1-B and REL-1 ALS activity in the presence of thifensulfuron.

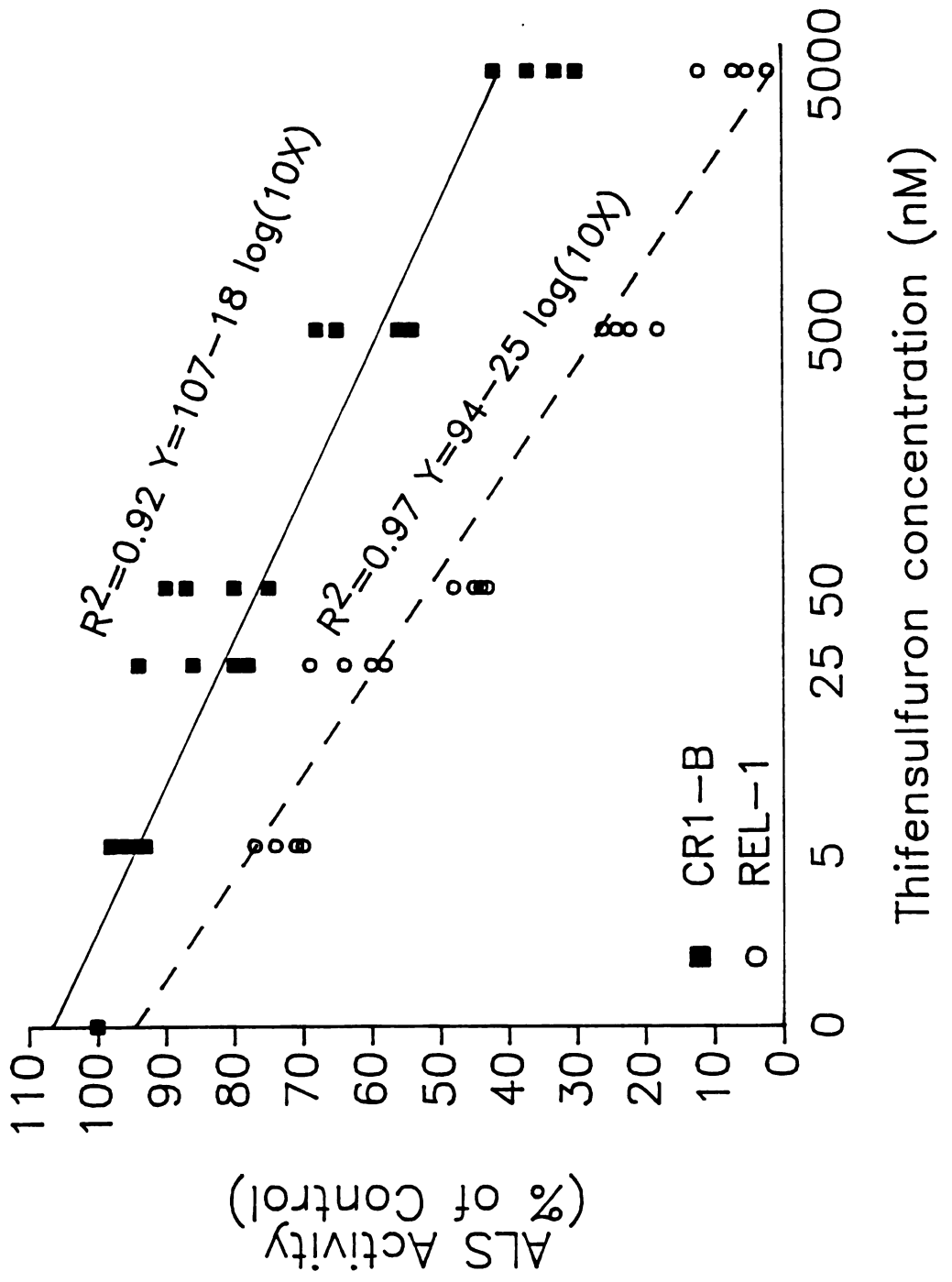
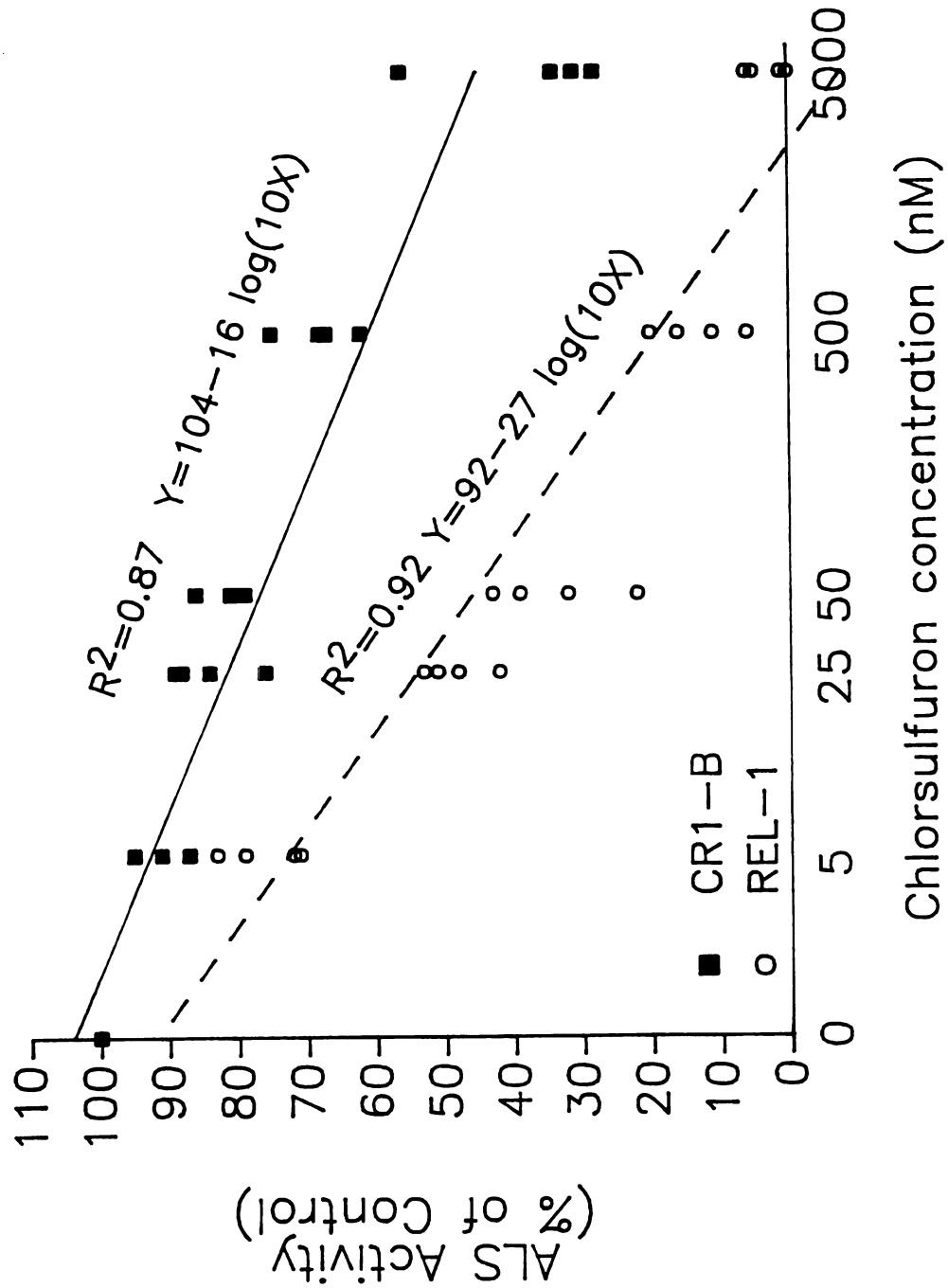

ALS Activity
(% of Control)
110
100
90
80
70
60
50
40
30
20
10
0
0
5
25 50
500
5000
Thifensulfuron concentration (nM)
CR1-B
REL-1
R2=0.92 Y=107-18 log(10x)
R2=0.97 Y=94-25 log(10x)

Figure 3. CR1-B and REL-1 ALS activity in the presence of chlorsulfuron.


ALS Activity
(% of Control)
110
100
90
80
70
60
50
40
30
20
10
0
0
5
25 50
500
5000
Chlorsulfuron concentration (nM)
CR1-B
REL-1
R2=0.87 Y=104-16 log(10X)
R2=0.92 Y=92-27 log(10X)

Calculated I_{50} values (herbicide concentration required to inhibit enzyme activity by 50%) obtained from the linear regression line equations show large differences between CR1-B and REL-1 in the sensitivity of the ALS enzymes to herbicide inhibition (Table 4). The greater activity of the ALS enzyme in CR1-B in the presence of primisulfuron, thifensulfuron, and chlorsulfuron may explain the tolerance of CR1-B to these herbicides on the whole plant level. These differences between the ALS enzyme activity in CR1-B and REL-1 appear to be the primary physiological basis for the differential tolerance of CR1-B to these herbicides.

REL-1 I_{50} values are in close agreement with previously reported values for a wide variety of plants susceptible to chlorsulfuron (8, 14, 16) and thifensulfuron (6, 16). Values for resistant species ranged widely and varied depending on whether the resistance occurred naturally (7, 16), was obtained by transforming plants with the *csr-1* gene (6, 8, 9), or was selected for in tissue culture (18). Several researchers have determined that several different amino acid changes in the ALS enzyme can confer resistance to sulfonylurea herbicides (3, 10, 25). Therefore, it is not possible to compare the values obtained for CR1-B to values obtained from other resistant sources.

The resistance of CR1-B to various sulfonylurea herbicides represents a potentially useful agronomic trait. The resistance trait in CR1-B, if incorporated into

Table 4. Herbicide concentrations (nM) required to inhibit ALS enzyme activity 50% in leaves of resistant CR1-B and susceptible REL-1 sugarbeet plants.

	Sugarbeet Clone		
Herbicide	CR1-B	REL-1	Ratio (R/S)
Primisulfuron	880	70	13
Thifensulfuron	1468	56	26
Chlorsulfuron	2371	36	66

commercial sugarbeet varieties, may potentially increase rotational flexibility for growers using sulfonylurea herbicides in other crops by decreasing, or possibly eliminating, the rotational restrictions of sugarbeets for the long soil residual sulfonylurea herbicides. Development of the resistance trait may also provide new and effective chemical weed control options for sugarbeet growers and help to decrease weed control costs.

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Chapter 3

Semi-dominant Nature of Monogenic Sulfonylurea Herbicide Resistance in Sugarbeet

Abstract

Greenhouse and laboratory studies were conducted to determine the degree of dominance of the monogenic sulfonylurea herbicide resistance trait in diploid sugarbeet by comparing the response of homozygous and heterozygous resistant sugarbeet to primisulfuron, thifensulfuron, and chlorimuron on the whole plant and acetolactate synthase (ALS) enzyme level. Progeny tests suggested that the monogenic sulfonylurea herbicide resistance was semi-dominant. Subsequently, heterozygous resistant (R-1) and homozygous resistant (R-2) sugarbeet lines were sprayed with increasing rates of primisulfuron, thifensulfuron, and chlorimuron, and herbicide rates required for 50% growth reduction (GR_{50}) determined. GR_{50} values were also determined for susceptible counterpart sugarbeet lines (S-1 and S-2). GR_{50} values indicated that the R-2 sugarbeet was 377, 269, and 144 times more resistant to primisulfuron, thifensulfuron, and chlorimuron, respectively, than the susceptible counterpart S-2 sugarbeet. In contrast, R-1 sugarbeet was only 107, 76, and 57 times more resistant to primisulfuron, thifensulfuron, and chlorimuron,

respectively, than the counterpart S-1 sugarbeet, indicating at least a two-fold difference in the magnitude of resistance between homozygous resistant and heterozygous resistant sugarbeet lines. ALS enzyme activity analysis were consistent with whole plant results. Thus, based on these two criteria, the monogenetic sulfonylurea herbicide resistance trait is semi-dominant in nature, indicating that maximum crop resistance can be obtained by developing homozygous resistant cultivars. Nomenclature: Chlorimuron, 2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl] amino]sulfonyl]benzoic acid; primisulfuron, (2-[[[[[4,6-*bis*(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino] sulfonyl]ureidosulfonyl]benzoic acid; thifensulfuron 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] carbonyl]amino]sulfonyl]-2-thiophenecarboxylate; sugarbeet, *Beta vulgaris* L. 'CR1-B', 'REL-1'.

Additional index words. Chlorimuron, primisulfuron, thifensulfuron, Acetolactate synthase.

INTRODUCTION

Sulfonylurea herbicides are used at very low use rates to effectively control a broad spectrum of weed species in a variety of crops by inhibiting the enzyme acetolactate synthase (ALS[10], also referred to as acetohydroxy acid synthase) (15). Combined with their low mammalian toxicity (11), these herbicides provide attractive weed control options from both the commercial and environmental standpoint. Consequently, the development of sulfonylurea herbicide resistant crops has become a research goal in recent years.

A chlorsulfuron resistant sugarbeet clone (CR1-B) was successfully generated via somatic cell selection in tissue culture using susceptible self-fertile clone REL-1 as the immediate source material (18). Preliminary genetic analysis determined inheritance via a single dominant nuclear gene, heterozygous in CR1-B. Further studies indicated that CR1-B is cross-resistant to postemergence applications at or exceeding field use rates of primisulfuron and thifensulfuron and that the physiological basis of resistance is an ALS enzyme less sensitive to sulfonylurea

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[10]Abbreviations: ALS, acetolactate synthase; CMS, cytoplasmic male-sterile; FAD, flavin adenine dinucleotide; CR1-B, chlorsulfuron resistant isolate 1-B; REL-1, Regenerating East Lansing-1; NIS, non-ionic surfactant. S_1, generation produced by self pollination.

herbicide inhibition (5). The successful incorporation of the sulfonylurea herbicide resistance trait into commercial sugarbeet varieties would not only increase the herbicide options for weed control in sugarbeet, but would also provide growers with increased sugarbeet rotational flexibility when sulfonylurea herbicides having a long soil persistence are used in other crops.

Genetic analysis of sulfonylurea herbicide resistance in soybean {*Glycine max* (L.) Merr.} (19) and *Lactuca* spp. (13), as well as imidazolinone herbicide resistance in corn (*Zea mays* L.) (14) has indicated that the resistance trait in these species is inherited as a single nuclear gene in semi-dominant fashion. Consequently, heterozygous resistant plants may display a lower level of resistance than homozygous resistant plants. Recent field evaluations of imidazolinone herbicide resistant corn demonstrated that in some instances homozygous resistant lines showed less injury when treated with field use rates of imazethapyr {(±)-2-{4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolinecarboxylic acid} than the heterozygous resistant lines (1, 16). Therefore, the development of crop cultivars with the highest level of resistance may require that all parents be homozygous resistant. Most modern sugarbeet hybrid seed production consists of a 3-way cross [A X B] X C (21); therefore, the inheritance of the sulfonylurea herbicide resistance trait and the differential

tolerance between homozygotes and heterozygotes is of crucial importance to sugarbeet breeders.

The objective of this study was to determine if the monogenic sulfonylurea herbicide resistance trait is inherited in a completely or semi-dominant fashion in sugarbeet. The differential tolerance between homozygous and heterozygous resistant lines to primisulfuron, thifensulfuron, and chlorimuron was also determined on a whole plant and ALS enzyme basis.

MATERIALS AND METHODS

Progeny Evaluation

CR1-B S_1 seeds (obtained by self-pollinating heterozygous resistant CR1-B ramets) were planted in 55-ml peat pots containing greenhouse potting soil. Plants were grown in the greenhouse at 24 $\pm$ 2 C with supplemental lighting from high pressure sodium lights to provide a maximum 1200 $\mu E\ m^{-2}\ s^{-1}$ for both supplemental and sunlight. The day length was 18 h. Two weeks after planting, individual sugarbeet plants were transplanted into 945-ml plastic pots. Plants were watered and fertilized as needed to insure maximum growth. Blocks of 70 to 80 S_1 plants of CR1-B were sprayed POST with 320 g ai

ha^{-1} primisulfuron plus non-ionic surfactant (NIS)[11] with a continuous link-belt sprayer at 170 Kpa in 230 L ha^{-1}. Sugarbeet plants were at the two- to four-leaf stage at time of application.

Ten DAT plants were evaluated for visual injury by comparing with untreated control plants. Plants showing less than 25% injury, 25% to 75% injury, and 75% or greater injury were classified homozygous resistant, heterozygous resistant, and susceptible, respectively. A separate block of 70 to 80 S_1 CR1-B Plants were also evaluated under the same conditions except that dry weights were recorded at 10 DAT. Dry weight values were compared to the average dry weight of 12 untreated control plants. Dry weight was converted to a percent of control. Plants showing less than 25% dry weight reduction, 25% to 75% dry weight reduction, and greater than 75% dry weight reduction were classified homozygous resistant, heterozygous resistant, and susceptible, respectively.

Six plants classified homozygous resistant and four plants classified heterozygous resistant were randomly selected from the visual injury experiment and test-crossed in paper bags with susceptible CMS line LO3. F_1 test-cross progeny and S_2 progeny were sprayed with 80 g ha^{-1}

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[11]The Non-ionic surfactant was X-77 which is a mixture of alklarylpolyoxyethylene glycols, free fatty acids, and isopropanol. Chevron Chemical Co., Richmond, CA.

primisulfuron plus NIS as described above to determine if the selections were true breeding (homozygous resistant) or segregating (heterozygous resistant).

The visual injury and biomass experiments were repeated with random plant selections taken from both visual injury experiments. All progeny segregation ratios were analyzed by the "goodness of fit" Chi square test at the 5% probability level.

Whole Plant Bioassay

Further whole plant studies were conducted to compare the response of F_1 heterozygous resistant (R-1)[12], CR1-B S_2 homozygous resistant (R-2), F_1 susceptible (S-1), and REL-1 S_2 susceptible (S-2) sugarbeet to POST applications of primisulfuron, thifensulfuron, and chlorimuron. Primisulfuron was applied at rates ranging from 40 to 1280 g ha^{-1} to R-1 sugarbeet, 80 to 2560 g ha^{-1} to R-2 sugarbeet, and 0.6 to 20 g ha^{-1} to both S-1 and S-2 sugarbeet lines. Thifensulfuron was applied at rates ranging from 1 to 32 g ha^{-1}, 2 to 64 g ha^{-1}, and 0.02 to 0.5 g ha^{-1} to R-1, R-2, and both susceptible sugarbeet lines, respectively. Chlorimuron was applied at rates ranging from 2.8 to 88 g ha^{-1}, 5.5 to

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[12]R-1, F_1 progeny of susceptible CMS LO3 X homozygous resistant S_1 CR1-B plants; R-2, S_1 progeny of homozygous resistant S_1 CR1-B plants; S-1, F_1 progeny of susceptible CMS LO3 X susceptible S_1 REL-1 plants; S-2, S_1 progeny of susceptible S_1 REL-1 plants.

176 g ha^{-1}, and 0.04 to 1.38 g ha^{-1} to R-1, R-2, and both susceptible sugarbeet lines, respectively. All treatments were applied when sugarbeets were in the two- to four-leaf growth stage and included a NIS at 0.25% (v/v). Shoot dry weights were recorded at 20 DAT and converted to a percentage of untreated control plants.

The experiments were conducted and analyzed separately for each herbicide. The experiments were repeated with four replications per treatment. Treatment means were subjected to an analysis of variance and separated by the least significant difference test at the 5% probability level. Data was also subjected to a curve linear regression analysis ($Y = B(0) + B(1) * X + B(2) * X^2$) and GR_{50} values (herbicide rates required to reduce shoot dry weight by 50%) calculated from the regression analysis.

Acetolactate synthase activity

ALS activity levels were determined in the leaves of R-1, R-2, S-1, and S-2 sugarbeet plants. Plants were grown in a growth chamber with day and night temperatures of 24 and 20 C, respectively. Supplemental lighting was provided by fluorescent and incandescent lamps at 250 $\mu E\ m^{-2}\ s^{-1}$ with an 8 h day length.

ALS was extracted, and enzyme activity levels then measured in the presence of primisulfuron, thifensulfuron, and chlorimuron with a modification of methods outlined by

Ray (15) and Shaner (20). All extraction, centrifugation, and column chromatography clean-up procedures were conducted at 4 C. Twenty to 30 g of plant leaves were homogenized in a volume of cold homogenization buffer [0.1 M K_2HPO_4, pH 7.5, 1 mM sodium pyruvate, 0.5 mM $MgCl_2$, 0.5 mM thiamine pyrophosphate, 10 μM flavin adenine dinucleotide (FAD), 10% v/v glycerol] twice the weight of the tissue. Polyvinylpolypyrrolidone (2.5 g) was also added for every 10 g of plant material homogenized. The homogenate was filtered through eight layers of cheesecloth and then centrifuged at 27000 g for 20 min. Saturated cold $(NH_4)_2SO_4$ solution was added to the supernatant to bring the final $(NH_4)_2SO_4$ concentration to 50% saturated. The solution was centrifuged at 15000 g for 15 min and the pellet redissolved in resuspension buffer (0.1 M K_2HPO_4, pH 7.5, 20 mM sodium pyruvate, 0.5 mM $MgCl_2$) and placed on a Sephadex G-25 PD-10[13] column. The desalted enzyme preparation was immediately used for the enzyme assays.

ALS enzyme activity was assayed by mixing 0.2 ml of enzyme preparation with 0.8 ml of reaction buffer (25 mM K_2HPO_4, pH 7.0, 0.625 mM $MgCl_2$, 25 mM sodium pyruvate, 0.625 mM thiamine pyrophosphate, 1.25 μM FAD) and incubated for 1 h at 35 C. Reaction tubes contained either 0, 10, 100, 1000, or 10000 nM of primisulfuron, thifensulfuron, or

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[13]PD-10 column. Pharmacia, Inc., Piscataway, N.J.

chlorimuron. The reaction was stopped by the addition of 50 µl of 6 N H_2SO_4 and the solutions were heated at 60 C for 15 min. Then 0.5 ml of 0.5% (w/v) creatine and 0.5 ml of 5% (w/v) α-naphthol freshly prepared in 2.5 N NaOH were added. The solutions were heated for an additional 15 min at 60 C and the acetoin content measured by the method of Westerfield (22). Protein concentration was determined by the method of Lowry (12).

The experiment was repeated with three replications of each herbicide concentration per experiment. ALS enzyme activity is presented as a percent of control assays and data subjected to a curve linear regression analysis ($Y = B(0) + B(1) * X + B(2) * X^2$) using a $\log_{10}$ transformation of herbicide concentrations. I_{50} values (herbicide concentration required to inhibit enzyme activity by 50%) were calculated from the regression analysis.

RESULTS AND DISCUSSION

Progeny Evaluation

The response of CR1-B S_1 progeny to 320 g ha^{-1} of primisulfuron ranged from little to no injury to complete desiccation at 10 DAT (Table 1). The majority of plants exhibited moderate injury (25% to 75%) that consisted of stunting and leaf chlorosis. These results confirm our previous observations of the presence of susceptible

Table 1. Progeny distribution and chi-square analysis of S_1 CR1-B generation based on visual injury evaluation.

Class	Expected Ratio	Expected[a]	Observed[b]	x^2
Susceptible	1	38.75	24	5.61
Heterozygous resistant	2	77.50	85	0.73
Homozygous resistant	1	38.75	46	1.36
				$x^2 = 7.70$
				$0.01 < P < 0.05$

[a]The number of plants expected in each class.

[b]The number of plants observed in each class.

segregates in a CR1-B S_1 population (5). However, chi-square analysis of the S_1 progeny distribution did not support the 1 : 2 : 1 (homozygous resistant : heterozygous resistant : susceptible) segregation model at the 5% confidence level. This appeared to be due to the large discrepancy between the lack of observed verses expected susceptible CR1-B S_1 progeny (Table 1). A similar progeny distribution was observed when CR1-B S_1 plants were evaluated based on dry weights (Table 2). In contrast to the visual injury evaluation, chi-square analysis supported the 1 : 2 : 1 segregation model. The preset numerical boundaries of the three classes were arbitrary and are dependent on the amount of herbicide applied. These boundaries were chosen based on past experience evaluating the response of CR1-B S_1 progeny to high application rates of primisulfuron. This type of progeny evaluation based on quantitative data, as opposed to qualitative phenotypic traits, does not lend itself well to strict mendelian segregation analysis.

A complete S_1 progeny segregation analysis could not be conducted for all randomly selected heterozygous resistant plants due to poor seed set and/or poor germination by several of the heterozygous selections. The S_1 progeny exhibited two distinct responses when sprayed with 80 g ha^{-1} of primisulfuron: little to no injury (< 25%) and severe injury (75% to complete dessication) 10 DAT. The total S_2 progeny segregation was 78 : 16 (resistant : susceptible)

Table 2. Progeny distribution and chi-square analysis of S_1 CR1-B generation based on dry weight evaluation.

Class	Expected Ratio	Expected[a]	Observed[b]	x^2
Susceptible	1	38.25	30	1.78
Heterozygous resistant	2	76.50	82	0.40
Homozygous resistant	1	38.25	41	0.20
				$x^2 = 2.38$
				$0.05 < P < 0.25$

[a]The number of plants expected in each class.

[b]The number of plants observed in each class.

(data not shown). Chi-square analysis for a 3 : 1 segregation model was 2.97 ($0.10 > P > 0.05$). Although the analysis supports the 3 : 1 segregation model the high chi-square value was again due to a lack of observed susceptible segregates.

Test-cross progeny from randomly selected heterozygous resistant plants also exhibited two distinct responses to 80 g ha^{-1} of primisulfuron: little to no injury and severe injury. In contrast to progeny segregation ratios from self pollinated plants, the progeny segregation ratios for these out-crossed progeny all closely fit the 1 : 1 (resistant : susceptible) segregation model (Table 3). The reasons for the differences in progeny distribution between selfed and out-crossed progeny is not known. However, CR1-B is a direct regenerate from callus, and is probably carrying other somaclonal variation besides the sulfonylurea herbicide resistance. Selfing (but not outcrossing) would expose detrimental recessive traits. Any such traits linked to the resistance locus could effect the ratio of resistant to susceptible plants.

Overall, 7 of 8 CR1-B S_1 plants provisionally classified as heterozygous resistant produced segregating progeny. Eleven of 12 of the homozygous resistant CR1-B S_1 selections produced resistant progeny only. These results suggest that a very high level of success can be achieved (90%) by visually selecting between homozygous and

Table 3. Test cross progeny distribution ratios and chi-square analysis for heterozygous resistant plants from S_1 CR1-B population.

Plant No.	Progeny Distribution	x^2	P value
	Resistant:Susceptible	1:1	
1	13:11	0.38	>0.50
2	13:12	0	>0.99
3	19:20	0	>0.99
4	11:10	0	>0.99
5	14:11	0.16	>0.50
6	25:21	0.23	>0.50
7	10:14	0.38	>0.50
8	13:14	0	>0.99

heterozygous resistant plants based on a subjective injury scale.

The results of these studies confirm initial findings that the sulfonylurea resistance trait in sugarbeet is inherited as a single dominant nuclear gene (18). The high degree of success in visually delineating between homozygous and heterozygous resistant plants based on the extent of herbicidal injury suggests that the sulfonylurea herbicide resistance trait is inherited in a semi-dominant fashion.

Sulfonylurea resistance in microorganisisms such as the *bacterium Salmonella typhimurium* (10), the yeast Saccharomyces cerevisiae (3) and the green algae *Chlamydomonas reinhardtii* (6) has been determined to be due to a dominant mutation. Genetic analysis of sulfonylurea resistance in plants has determined that inheritance in arabidopsis {*Arabidopsis thaliana* (L.) Heynh} (7) and flax (*Linum usitatissimum* L.) (9) appeared to be as a single dominant nuclear gene. Tobacco (*Nicotiana tabacum* L.) plants transformed with the sulfonylurea resistance gene from arabidopsis also had similar inheritance patterns (8).

The results obtained from the sugarbeet progeny tests are similar to those observed by Chaleff and Ray (2) which showed S_1 progeny of resistant tobacco segregated in a 1 : 2 : 1 ratio. Similar studies using test-cross progeny segregation data in *Lactuca* spp. (13) and soybean (19) determined that plants exhibiting a lower level of

resistance were heterozygous resistant.

Whole plant bioassay

Further studies were conducted to compare the whole plant response of heterozygous and homozygous resistant as well as their susceptible counterparts to rate ranges of three sulfonylurea herbicides. The response of S-1 and S-2 sugarbeet was similar for all application rates of primisulfuron (Figure 1) and chlorimuron (Figure 3). Shoot dry weights of S-1 resistant were significantly higher than S-2 sugarbeet when thifensulfuron was applied at rates ranging from 0.03 to 0.25 g ha^{-1} (Figure 2). However, the trend in the shoot dry weight reduction curves were similar for both susceptible sugarbeet lines.

The use of higher rate structures were necessary to generate GR_{50} values for R-2 sugarbeet (Figures 4 to 6). The whole plant response of R-1 and R-2 sugarbeets did not differ significantly at rates of primisulfuron (Figure 4) ranging from 80 to 160 g ha^{-1} and thifensulfuron (Figure 5) ranging from 2 to 4 g ha^{-1}. However R-2 sugarbeet exhibited a higher degree of resistance as the rates of primisulfuron and thifensulfuron increased. In contrast, R-2 sugarbeet was more resistant than R-1 sugarbeet at all application rates of chlorimuron (Figure 6).

Curve linear regression analysis further substantiates that there was little difference between the whole plant

Figure 1. Response of susceptible sugarbeets to primisulfuron.

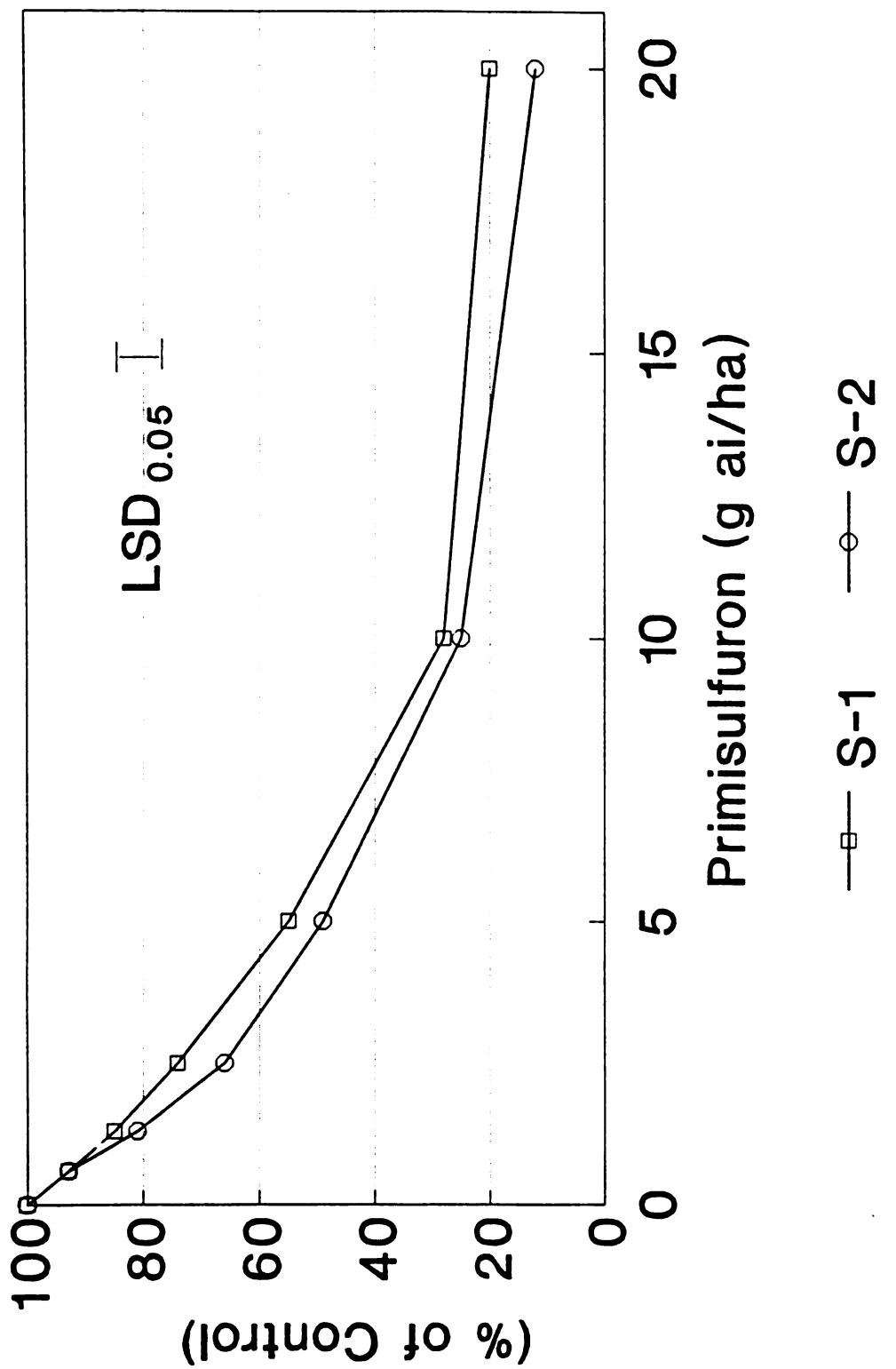

100
80
60
40
20
0
(% of Control)
0
5
10
15
20
Primisulfuron (g ai/ha)
LSD0.05
S-1
S-2

Figure 2. Response of susceptible sugarbeets to thifensulfuron.

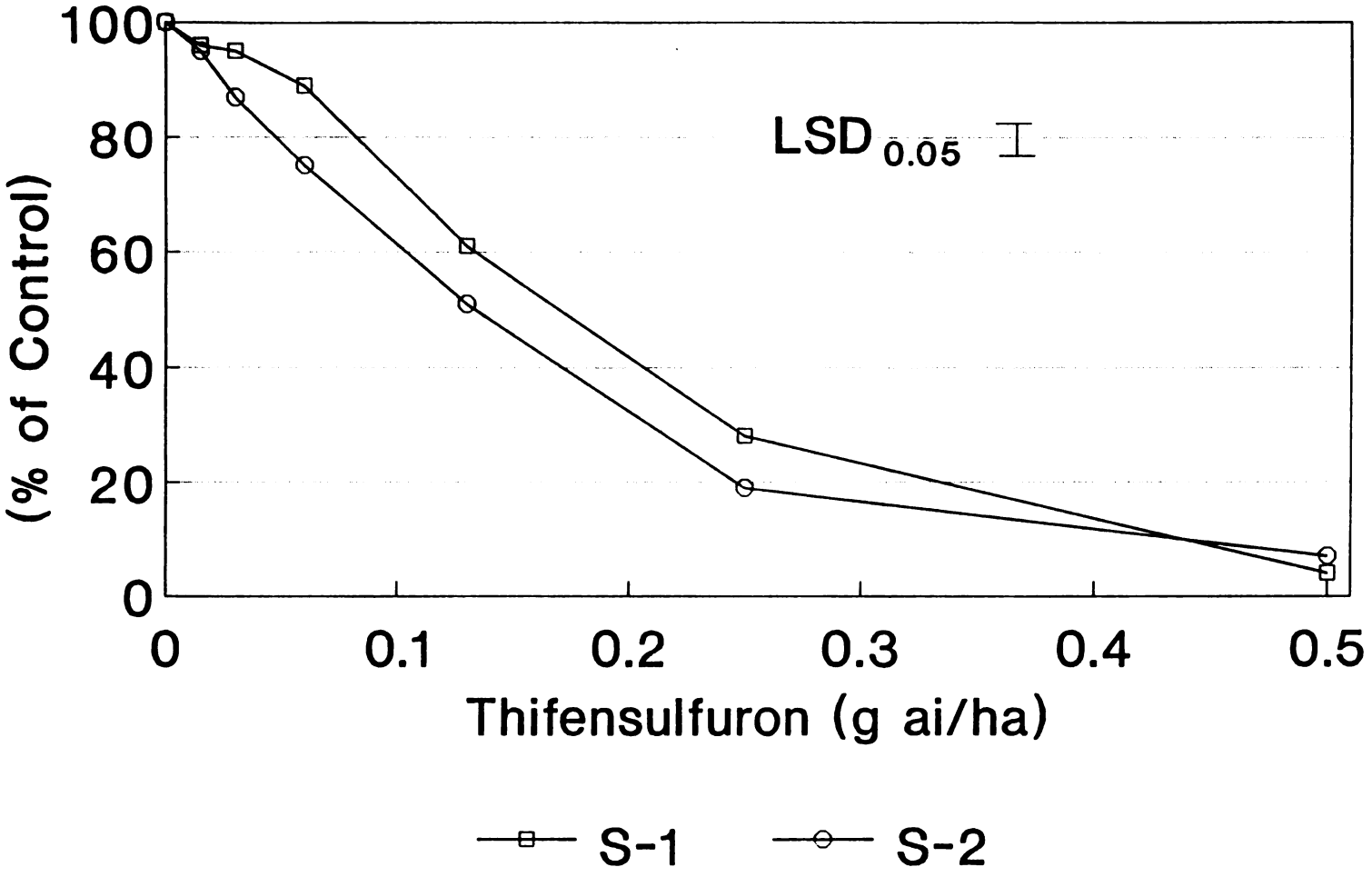

100
80
60
40
20
0
(% of Control)
LSD 0.05
0
0.1
0.2
0.3
0.4
0.5
Thifensulfuron (g ai/ha)
S-1
S-2

Figure 3. Response of susceptible sugarbeets to chlorimuron.

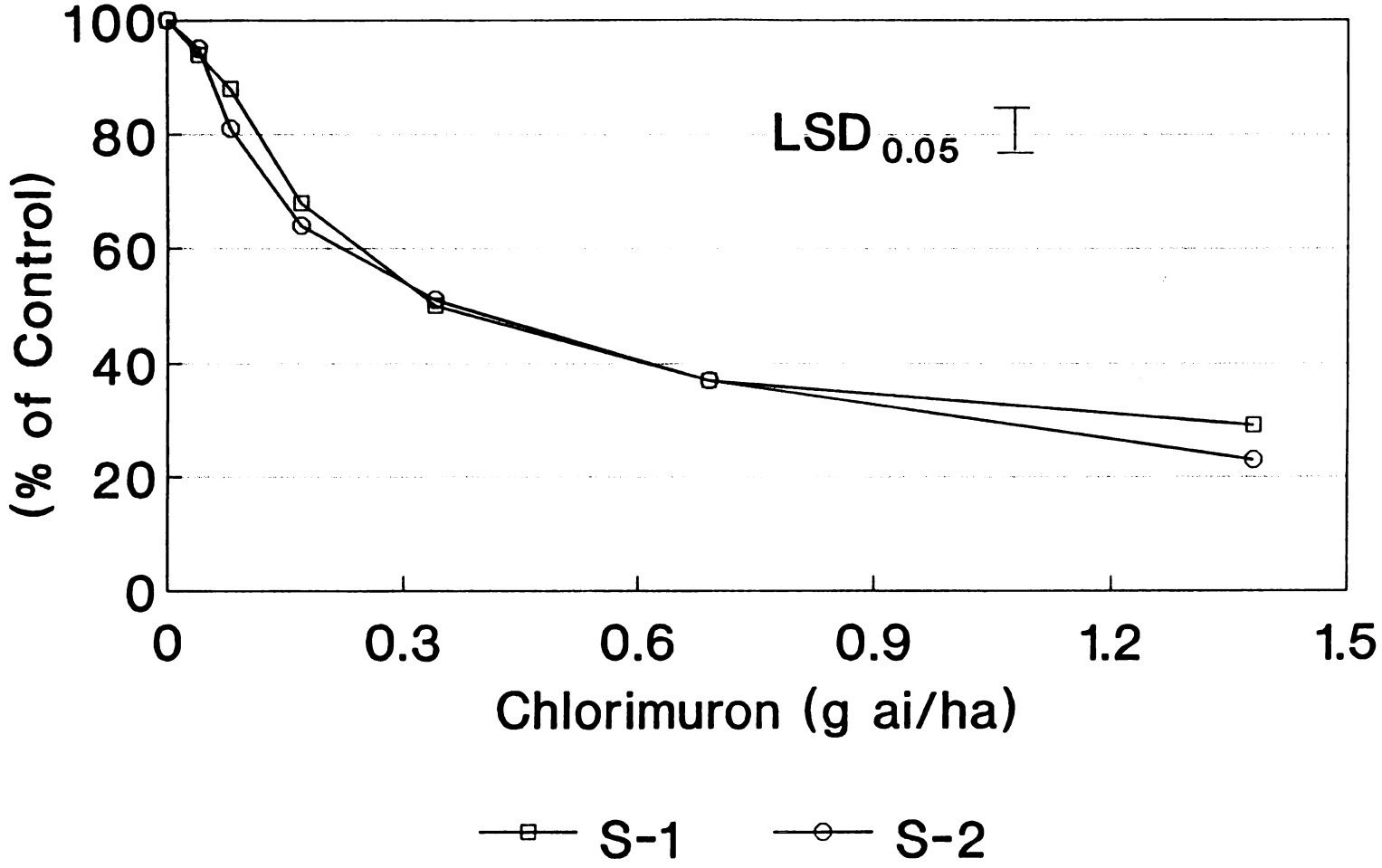

100
80
60
40
20
0
(% of Control)
LSD 0.05
0
0.3
0.6
0.9
1.2
1.5
Chlorimuron (g ai/ha)
S-1
S-2

Figure 4. Response of resistant sugarbeets to primisulfuron.

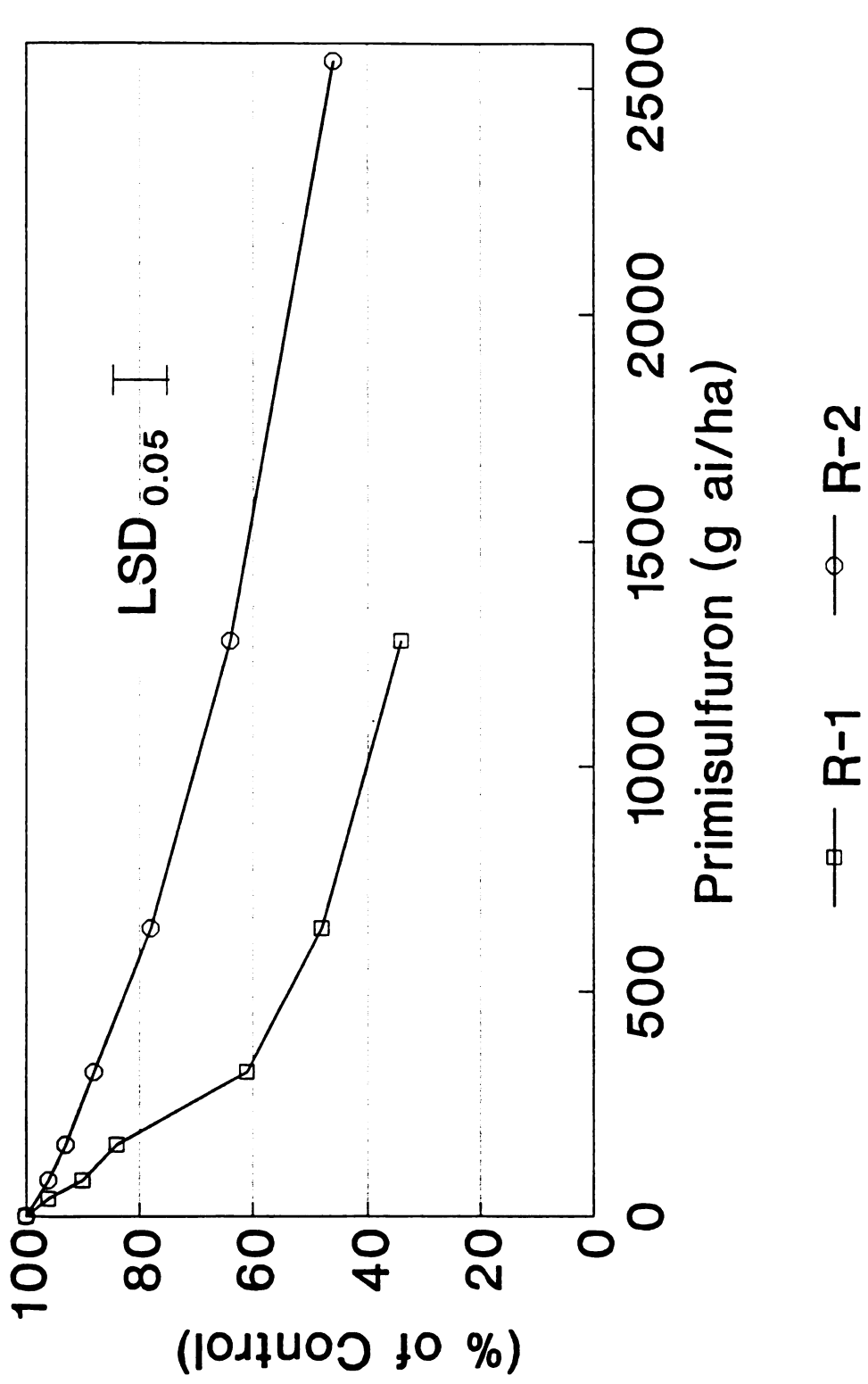

100
80
60
40
20
0
(% of Control)
0
500
1000
1500
2000
2500
Primisulfuron (g ai/ha)
LSD 0.05
R-1
R-2

Figure 5. Response of resistant sugarbeets to thifensulfuron.

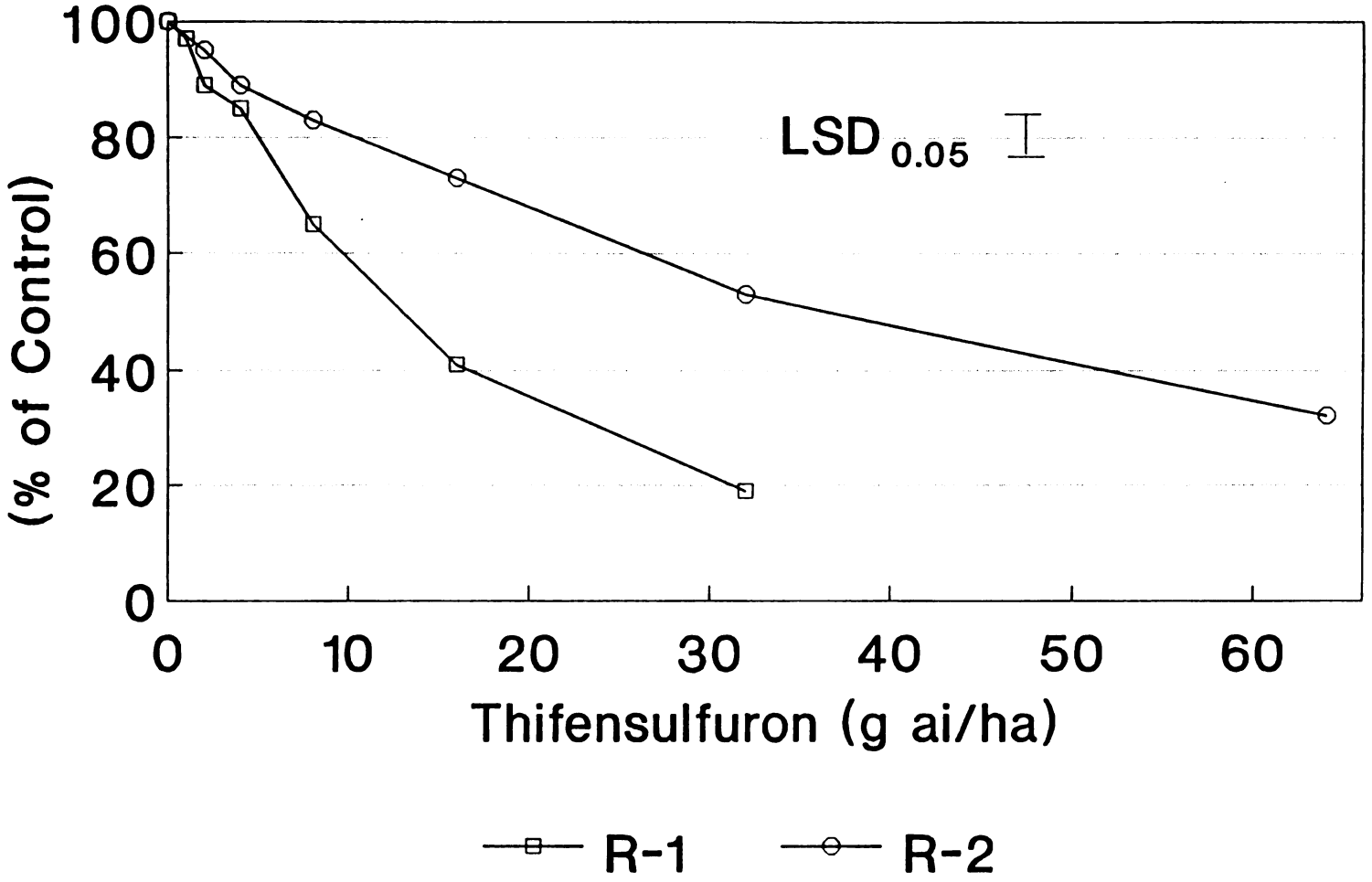
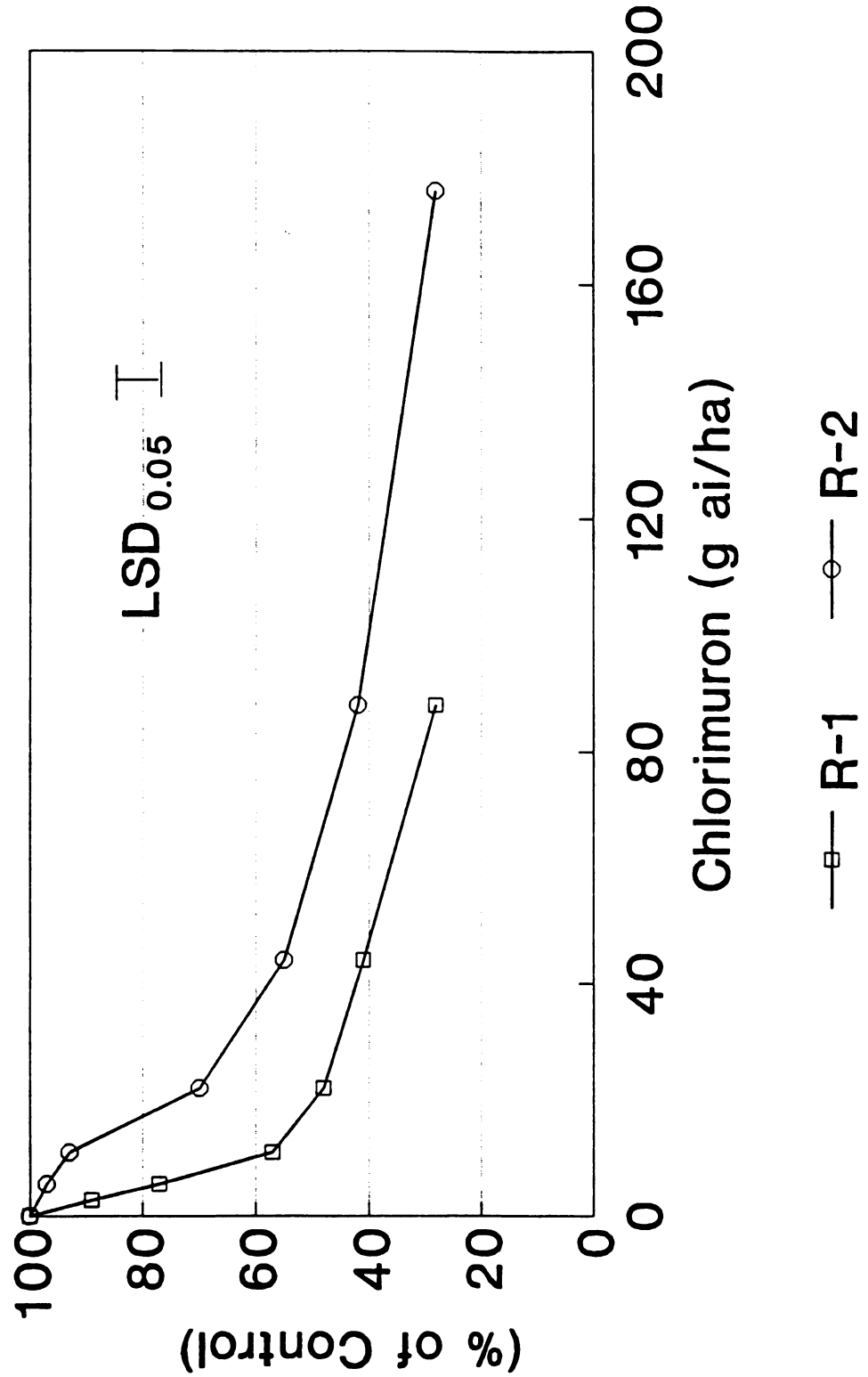

100
80
60
40
20
0
(% of Control)
LSD 0.05
0
10
20
30
40
50
60
Thifensulfuron (g ai/ha)
R-1
R-2

Figure 6. Response of resistant sugarbeets to chlorimuron.


100
80
60
40
20
0
(% of Control)
0
40
80
120
160
200
Chlorimuron (g ai/ha)
LSD 0.05
R-1
R-2

response of S-1 and S-2 sugarbeet to primisulfuron, thifensulfuron, and chlorimuron (Table 4). In contrast R-2 sugarbeet had GR_{50} values that were at least twice as high or greater than R-1 sugarbeet for all three herbicides. Comparison of GR_{50} ratio values between R-1 and R-2 sugarbeet showed a similar increase in the magnitude of resistance for R-2 sugarbeet ranging from 2.5 for chlorimuron to 3.5 for primisulfuron and thifensulfuron.

The ALS activity in control assays averaged 192-227 and 196-234 Nm acetoin h^{-1} mg^{-2} protein for S-1 and S-2 sugarbeet, respectively (data not shown). The ALS activity in the control assays for resistant sugarbeet were slightly lower averaging 180-196 and 188-212 Nm acetoin h^{-1} mg^{-2} for R-1 and R-2 sugarbeet, respectively.

The inhibition of ALS enzyme activity by primisulfuron, thifensulfuron, and chlorimuron was similar for both S-1 and S-2 sugarbeet lines (Figures 7 to 9). These results appear to explain the similar response of the susceptible sugarbeet lines on the whole plant level (Figures 1 to 3). ALS activity in R-2 sugarbeet was significantly higher compared to R-1 sugarbeet at concentrations of primisulfuron and chlorimuron ranging from 10 to 1000 nmol and at all concentrations of thifensulfuron (Figures 7 to 9). Although the ALS activity of R-2 and R-1 sugarbeet was similar at 10000 nmol of primisulfuron and chlorimuron it is clearly evident that the ALS enzyme extracted from the leaves of R-1

Table 4. Curve linear regression analysis of the response of sugarbeet to selected sulfonylurea herbicides.

Herbicide	r^2	Susceptible	r^2	Resistant	Ratio R/S	Ratio (R-2/R-1)
		kg ha^{-1} x 10^{-5}		kg ha^{-1} x 10^{-3}		
Primisulfuron	0.96	51 (S-1)	0.96	544 (R-1)	107	
	0.97	56 (S-2)	0.91	2111 (R-2)	377	3.5
Thifensulfuron	0.97	17 (S-1)	0.97	13 (R-1)	76	
	0.98	13 (S-2)	0.93	35 (R-2)	269	3.5
Chlorimuron	0.88	42 (S-1)	0.84	24 (R-1)	57	
	0.87	41 (S-2)	0.94	59 (R-2)	144	2.5

[a]GR_{50} values determined using curve linear regression analysis equation ($Y = B(0) + B(1) * X + B(2) * X^2$).

Figure 7. Sugarbeet ALS enzyme activity in the presence of primisulfuron.

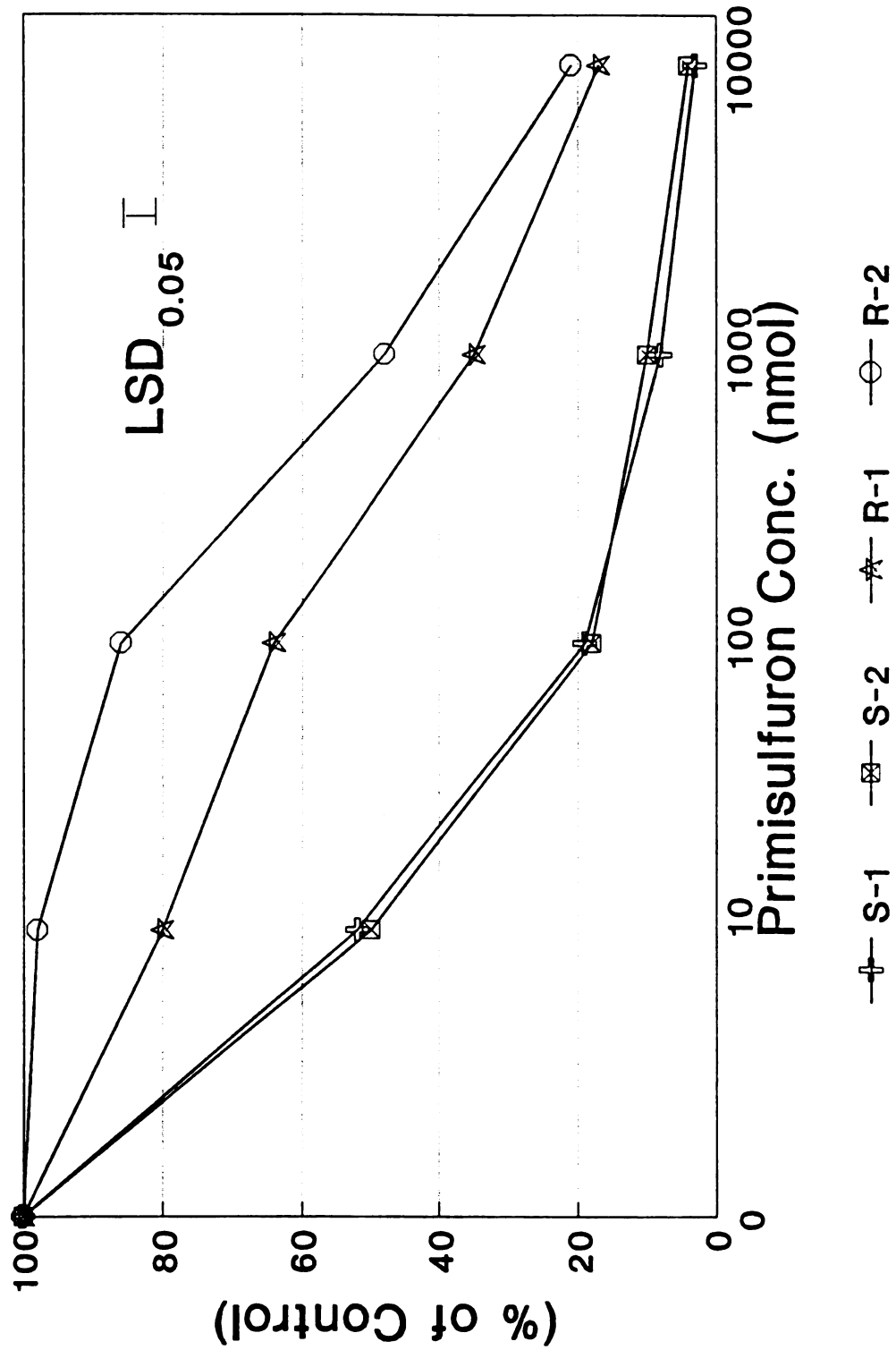

LSD0.05
(% of Control)
100
80
60
40
20
0
0
10
100
1000
10000
Primisulfuron Conc. (nmol)
S-1
S-2
R-1
R-2

Figure 8. Sugarbeet ALS enzyme activity in the presence of thifensulfuron.

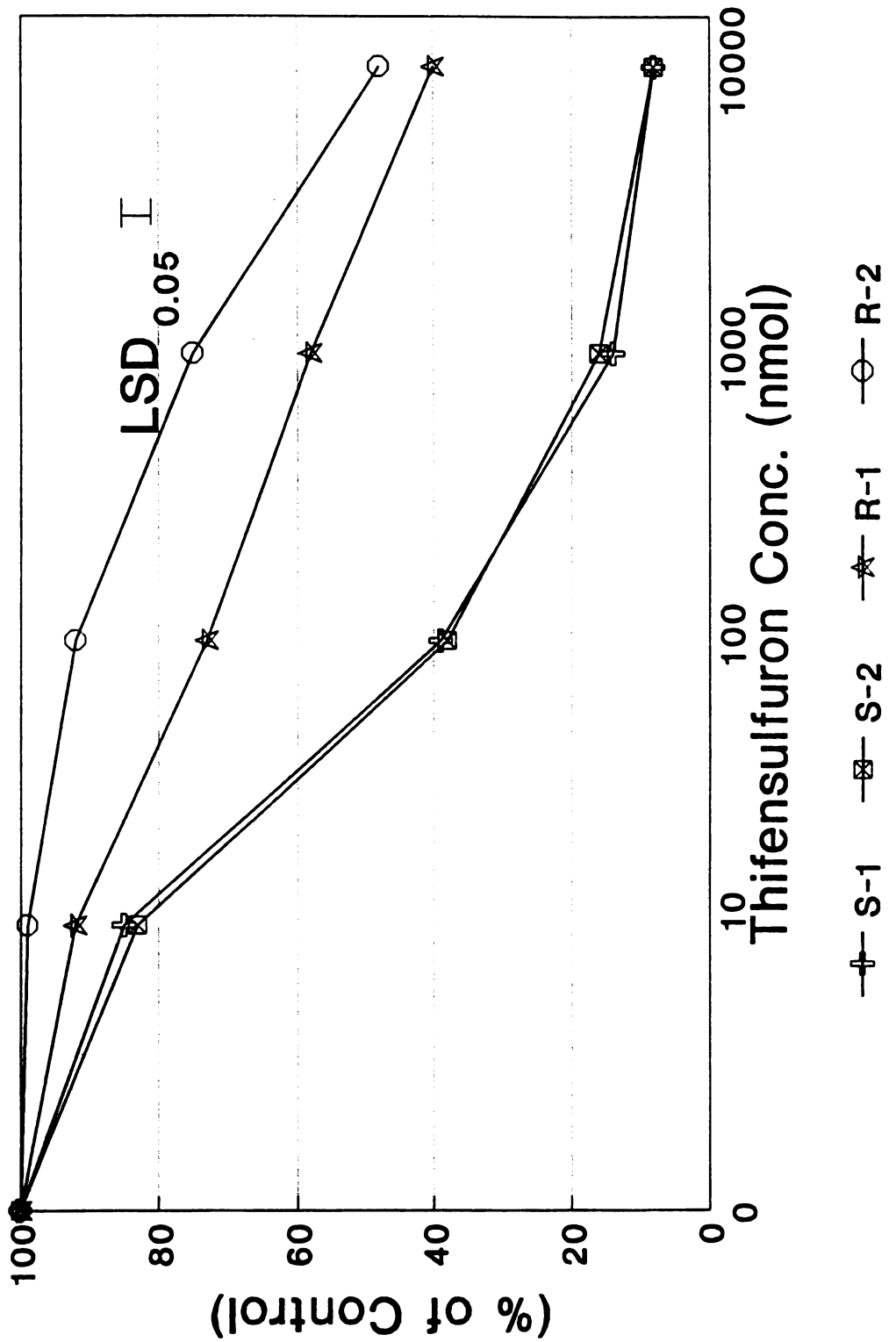
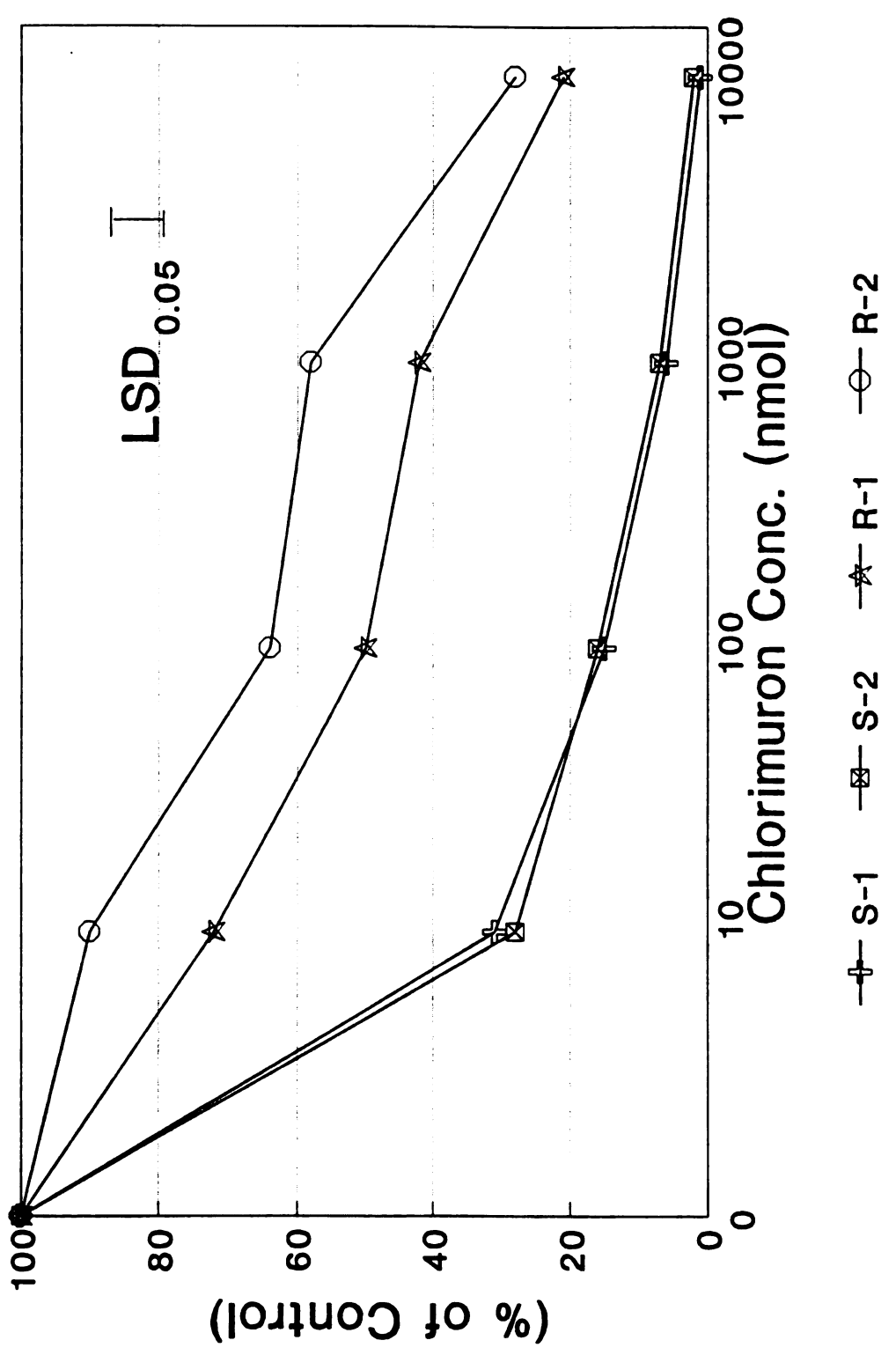

(% of Control)
100
80
60
40
20
0
0
10
100
1000
10000
Thifensulfuron Conc. (nmol)
LSD 0.05
S-1
S-2
R-1
R-2

Figure 9. Sugarbeet ALS enzyme activity in the presence of chlorimuron.


LSD0.05
100
80
60
40
20
0
(% of Control)
0
10
100
1000
10000
Chlorimuron Conc. (nmol)
S-1
S-2
R-1
R-2

sugarbeet has a lower level of resistance then R-2 sugarbeet.

Curve linear regression analysis also demonstrate that ALS enzyme activity in the shoots of both susceptible sugarbeet lines was similar for primisulfuron, thifensulfuron, and chlorimuron (Table 5). I_{50} values for R-2 sugarbeet were at least three times greater than R-1 sugarbeet. There was a wider range in the magnitude of resistance values between R-1 and R-2 sugarbeet compared to the values obtained from whole plant data (Table 4). However, ratio values differed by a factor less than two, ranging from 3.9 for thifensulfuron to 7.5 for chlorimuron (Table 5). These results may explain the difference in the magnitude of resistance between R-1 and R-2 on the whole plant level.

Calculated I_{50} values for the susceptible sugarbeet lines are in close agreement with previously reported values for thifensulfuron in tobacco (4) and thifensulfuron and chlorimuron in *kochia scoparia* (L.) Schrad.} (17). These values were also similar to values we have previously reported for susceptible REL-1 ramets (5).

The results of these studies indicate that the monogenetic sulfonylurea resistance trait in sugarbeets is semidominant in nature. Homozygous resistant sugarbeet was more resistant to both growth and ALS activity inhibition by primisulfuron, thifensulfuron, and chlorimuron as compared

Table 5. Herbicide concentrations for 50% inhibition of ALS activity of susceptible and resistant sugarbeet leaves (I_{50})[a].

Herbicide	r^2	Susceptible	r^2	Resistant	GR_{50} Ratio or R/S	GR_{50} Ratio (R-2/R-1)
		nmol		nmol		
Primisulfuron	0.99	11 (S-1)	0.98	309 (R-1)	28	
	0.99	11 (S-2)	0.97	1288 (r-2)	117	4.2
Thifensulfuron	0.96	56 (S-1)	0.96	1995 (R-1)	36	
	0.96	54 (S-2)	0.95	7586 (R-2)	140	3.9
Chlorimuron	0.95	7 (S-1)	0.97	146 (R-1)	21	
	0.93	7 (S-2)	0.96	1097 (R-2)	157	7.5

[a]I_{50} values determined using curve linear regression analysis equation ($Y = B(0) + B(1) * X + B(2) * X^2$).

to heterozygous resistant sugarbeet. The response of both susceptible sugarbeet lines to growth and ALS enzyme activity inhibition by these herbicides was nearly identical, indicating that the introduction of CMS LO3 germplasm did not alter the response of REL-1 to sulfonylurea herbicides. Thus, direct comparisons between the response of self pollinated homozygous resistant progeny and heterozygous resistant progeny obtained by outcrossing appear to be valid.

These findings represent the most conclusive evidence that the monogenetic sulfonylurea resistance trait is semi-dominant in nature. Previous studies concluding that the monogenetic sulfonylurea resistant trait was semi-dominant in nature were based purely on progeny segregation studies using subjective visual injury rating system (13), progeny segregation studies that lacked correlation between whole plant response and ALS enzyme activity (19), or comparison of heterozygous and homozygous resistant cell cultures to growth inhibition (2). To our knowledge, these studies represent the first comprehensive direct comparison of homozygous and heterozygous resistant plants to sulfonylurea herbicide inhibition on both a whole plant and ALS enzyme activity basis. This was made possible, in part, by the utilization of susceptible CMS LO3 germplasm to generate ample amounts of pure F_1 heterozygous resistant seed.

The response of heterozygous resistant sugarbeet did

not differ from homozygous resistant sugarbeet at four times the field application rate of primisulfuron in corn and at the field application rate for thifensulfuron in soybean. Therefore, heterozygous resistant sugarbeet appear to have a high enough level of resistance for direct postemergence use of primisulfuron and thifensulfuron for weed control. However, homozygous resistant sugarbeet cultivars would undoubtedly provide a greater margin of crop safety to postemergence use of these sulfonylurea herbicides.

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Chapter 4

Initial Field Evaluation of Sulfonylurea Resistant Sugarbeet.

Abstract

Field studies were conducted to compare the yield, sugar content, and processing purity of sulfonylurea herbicide resistant and counterpart susceptible sugarbeet germplasm. Other studies evaluated the response of sulfonylurea resistant sugarbeet to simulated carryover residues as well as postemergence (POST) applications of selected sulfonylurea herbicides. In the absence of herbicides there were no differences between resistant and susceptible sugarbeets for root yield, sugar content, and clear juice purity at both locations. Nicosulfuron preplant incorporated (PPI) at 9 g ai ha^{-1} had no effect on the growth of resistant or susceptible MONO-HY E-4 sugarbeets. Primisulfuron and chlorimuron PPI at 10 and 3 g ai ha^{-1}, respectively, caused over 95% visual injury to the susceptible E-4 sugarbeet 6 weeks after treatment but had no adverse effect on the growth of resistant sugarbeet. POST applications of primisulfuron at 40 and 80 g ai ha^{-1}, the field use rate and twice the field use rate for corn, respectively, and thifensulfuron at 4 and 8 g ha^{-1} caused only slight visual injury (< 15%) to the resistant sugarbeet

4 weeks after treatment, while causing severe injury to susceptible E-4 sugarbeet. The sulfonylurea resistant sugarbeet was tolerant to POST applications of primisulfuron at four times and thifensulfuron at two times the field use rate. This magnitude of resistance is great enough for primisulfuron and thifensulfuron application for weed control in sulfonylurea resistant sugarbeet. Nomenclature: Chlorimuron, 2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino] carbonyl]amino]sulfonyl] benzoic acid; nicosulfuron, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino] sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide; primisulfuron, 2-[[[[[4,6-*bis*(diflouromethoxy)-2-pyrimidinyl]amino] carbonyl]amino]sulfonyl] benzoic acid;thifensulfuron, 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl] amino]sulfonyl]-2-thiophenecarboxylic acid; sugarbeet, *Beta vulgaris* L. 'CR1-B', 'MONO-HY E-4', 'TR-504'.

Additional index words. Herbicide carryover.

INTRODUCTION

A major disadvantage to sulfonylurea herbicide use in sugarbeet production areas is the potential for residues to persist in alkaline soils and injure sensitive crops planted in rotation (1, 7). The development of sulfonylurea resistant sugarbeet cultivars is a potential solution to this carryover problem. Resistant cultivars could also

increase the chemical weed control options for farmers. A sulfonylurea resistant sugarbeet clone (CR1-B) has been successfully generated via somatic cell selection against chlorsulfuron in tissue culture (9). Greenhouse studies determined that (CR1-B) was resistant to primisulfuron and thifensulfuron applied POST at rates exceeding field use rates, and slight cross-resistant was observed to nicosulfuron and chlorimuron (5). However, the response of resistant sugarbeet to herbicide residues in soil and postemergence applications of sulfonylurea herbicides in the field has not been evaluated. The effect of the sulfonylurea resistance trait on the agronomic performance of sugarbeet in the absence of herbicides also has not been determined. The objectives of the research were to assess the effect of the sulfonylurea resistance trait on the agronomic performance of sugarbeet, and to evaluate the response of sulfonylurea resistant sugarbeet to herbicide residues in soil, and to POST applications of primisulfuron and thifensulfuron at or exceeding field use rates.

MATERIALS AND METHODS

Plant Material

To obtain a sufficient amount of resistant and susceptible seed for field studies, initial crosses were made in the greenhouse between the susceptible biennial

breeding line TR-504 and CR1-B ramets from tissue culture, heterozygous for both resistance and annualism. F_1 progeny was grown in the greenhouse and resistant or susceptible segregates identified using a non-destructive tissue culture leaf disk test. The 5th and 6th leaves were removed, surface sterilized for 20 min with 15% NaOCl solution, and cut into 10-mm diameter leaf disks. Two leaf disks were placed in each 100 by 20 mm petri dish containing an agar-solidified growth medium for rapid sugarbeet leaf disk expansion and callus (4). The growth medium contained either 0 or 140 nM chlorsulfuron and the plates were incubated in the dark for 14 d at 31 C. Plants whose leaf disks exhibited vigorous tissue expansion on 140 nM were considered to be resistant F_1 segregates. The ratio of resistant to susceptible segregates was 54 : 46 and in close agreement with the 1 : 1 progeny segregation ratio expected ($X^2 = 0.52$).

F_1 segregates with annual characteristics were eliminated after exposing the sugarbeet plants to continuous incandescent light for 30 d after plants had reached the 4- to 8-leaf growth stage.

Biennial F_1 segregates were kept in a controlled growth room at 4 C for 10 weeks and then placed in the greenhouse and exposed to continuous incandescent light to induce flowering. Resistant and susceptible F_1 segregates were isolated in two locations and allowed to self and cross freely among themselves. F_2 Seed from resistant and

susceptible plants was bulked seperately, and used for field studies.

Agronomic Evaluation

Field studies were conducted in 1991 at Saginaw (Clay, organic matter was 3.2%, and pH was 8.1) and Bay City (Sandy Clay Loam, organic matter was 2.5%, and soil pH was 8.0), Michigan. Plots were 3 by 8.5 m, with a 76 cm row spacing and consisted of two MONO-HY E-4 border rows and two middle rows of the variety under evaluation. F_2 seed was mutigerm. Sugarbeets were hand-planted and thinned to a population of one plant per 20 cm of row after emergence, with the exception that one resistant variety evaluation was treated prior to thinning with 10 g ha^{-1} primisulfuron and non-ionic surfactant (NIS)[14][15] POST, to eliminate susceptible segregates. Plots were hand-weeded throughout the growing season and harvested at maturity for root yields. Clear juice purity, and percentage sugar were measured from a random sample of 20 sugarbeets per plot (3). Each plot was replicated four times. Treatment means were subjected to an analysis of variance as a two factor factorial (location by variety) and treatment means

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[14]Abbreviation: NIS, non-ionic surfactant; WAT, weeks after treatment.

[15]Non-ionic surfactant was X-77 which is a mixture of alklarylpolyoxyethylene glycols, free fatty acids, and isopropanol. Chevron Chemical Co., Richmond, CA.

separated by the least significant difference test at the 5% probability level.

Herbicide Evaluations

Studies were conducted at the Saginaw site with the same soil parameters as described above. Plot size was 3 by 3.6 m, with a 76 cm row spacing. Each plot consisted of two MONO-HY E-4 border rows, a F_2 resistant row, and another MONO-HY E-4 row for evaluation purposes. The susceptible F_2 line was not planted.

All herbicide treatments were applied with a tractor mounted compressed air sprayer in 205 L ha^{-1} at 200 kPa. Nicosulfuron, chlorimuron, and primisulfuron were applied at 9, 3, and 10 g ha^{-1}, respectively, and incorporated to a depth of 8 to 10 cm with a Danish S tine field cultivator. Primisulfuron and thifensulfuron were applied POST at rates of 40 to 160 g ha^{-1} and 4 to 8 g ha^{-1} when sugarbeets were in the 2nd leaf pair. All treatments included a non-ionic surfactant at 0.25% (v/v). PPI and POST treatments were evaluated for visual sugarbeet injury at 6 and 4 weeks after treatment, respectively. Treatment means were subjected to a two factor factorial analysis of variance (variety by herbicide treatment) and separated by the least significant difference test at the 5% probability level.

RESULTS AND DISCUSSION

Agronomic Evaluation

A significant location by variety interaction ($p \leq 0.001$) was observed for sugarbeet root yield and sugar content (Table 1). This interaction was due to differentially lower root yields and sugar content at the Bay City location caused by agronomic factors.

Root yield and sugar content of MONO-HY E-4 was greater than susceptible and resistant F_2 sugarbeets at both locations. Root yield, sugar content, and clear juice purity did not differ among the susceptible and resistant F_2 at Saginaw (Table 1). However, root yield of resistant progeny treated with primisulfuron was greater than susceptible progeny at Bay City (Table 1), and resistant progeny treated with primisulfuron had a lower sugar content then non-treated resistant progeny at Bay City.

These results suggest that sulfonylurea resistant sugarbeets possess the same yield potential and sugar content as susceptible sugarbeets with similar genetic background. Imidazolinone resistant corn hybrids, were found to have the same yield potential as susceptible corn hybrids in the absence of herbicides (6).

The F_2 "resistant" line from F_1 resistant segregates, in theory contained 25% susceptible segregates as well as 25% homozygous resistant segregates. The primisulfuron treatment

Table 1. Agronomic performance of sulfonylurea resistant and susceptible sugarbeet lines in the absence of herbicides.

Location		Sugarbeet line	Yield	Sugar	C.J.P.[a]
			kg/ha	---- % -----	
Saginaw	Susceptible	MONO HY E4	40200	18.5	95
		TR504 X CR1-B progeny	35600	17.0	93
	Resistant	Non-Treated	33400	16.9	94
		10 g ha^{-1} primisulfuron[b]	35000	16.5	93
	LSD (0.05)		3800	0.9	2
Bay City	Susceptible	MONO HY E4	21500	15.2	95
(Nematode Infested Site)		TR504 X CR1-B progeny	11500	14.0	93
	Resistant	Non-treated	12700	14.3	94
		10 g ha^{-1} primisulfuron	14600	13.9	94
	LSD (0.05)		2600	0.4	2

[a]C.J.P. = Clear juice purity

[b]Plots sprayed with 10 g ha^{-1} of primisulfuron prior to thinning to eliminate susceptible segregates.

of the "resistant" F_2 line should have eliminated the susceptible segregates and provided a 100% resistant entry. While the comparison of resistant and susceptible lines does not enjoy the benefits of near isogeniaty, it represents a comparible pair of genetic backgrounds assuming no linkage between the monogenic sulfonylurea resistant gene and other major genes affecting agronomic characters. We know of no linkages to the resistant locus including the B locus conferring annual vs. biennial behaviors.

Herbicide Evaluations

MONO-HY E-4 and the resistant F_2 sugarbeet line were unaffected by nicosulfuron PPI (Table 2). In contrast, MONO-HY E-4 was severely injured by PPI primisulfuron and chlorimuron (98% or greater), while the resistant line was not injured. Susceptible sugarbeet germinated but failed to develop beyond the cotyledon growth stage.

The susceptible MONO-HY E-4 was severely injured (95% or greater) by all POST applications of primisulfuron and thifensulfuron (Table 3). Sulfonylurea resistant sugarbeets were unaffected by primisulfuron at 40 and 80 g ha^{-1} 4 WAT, and only slightly injured by thifensulfuron applied at 4 and 8 g ha^{-1}. Although the resistant sugarbeet showed 21% visual injury from primisulfuron at 160 g ha^{-1} 4 WAT, visual injury was not evident at 8 WAT.

These results indicate that the sulfonylurea resistance

Table 2. Response of sulfonylurea resistant and susceptible (MONO-HY E-4) sugarbeet to PPI treatments of sulfonylurea herbicides.

		Visual Injury (6 WAT)[a]	
Treatment	Rate	MONO-HY E4	Resistant
	g ha^{-1}	---------- % ----------	
Nicosulfuron	9	0	0
Primisulfuron	10	98	0
Chlorimuron	3	100	5
LSD (0.05)		---------- 6 ----------	

[a]WAT = weeks after treatment.

Table 3. Response of resistant and susceptible (MONO-HY E-4) sugarbeet to post applications of primisulfuron and thifensulfuron.

		Visual injury (4 WAT)[a]	
Treatment[b]	Rate	MONO-HY E-4	Resistant
	g ha^{-1}	---------- % ----------	
Primisulfuron	40	95	0
	80	100	5
	160	100	21
Thifensulfuron	4	99	10
	8	100	14
LSD (0.05)		---------- 8 ----------	

[a]WAT = weeks after treatment.

[b]All herbicides applied with NIS at 0.25% (v/v).

trait had no adverse effect on agronomic performance of sugarbeet in the absence of the herbicides. Field performance of sulfonylurea resistant sugarbeet was similar to that observed with imidazolinone resistant corn (2, 6, 8). The PPI herbicide study demonstrated that sulfonylurea resistant sugarbeet were resistant to primisulfuron and chlorimuron soil residues that killed the susceptible sugarbeet. Sulfonylurea resistant sugarbeet was resistant to POST applications of primisulfuron and thifensulfuron at field use rates, raising the possibility of direct use of these herbicides for weed control in resistant sugarbeet.

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Chapter 5

REVIEW OF LITERATURE

POSTEMERGENCE TANK-MIX ANTAGONISM

Many new herbicides have been recently introduced for postemergence weed control in major agronomic crops such as soybean {*Glycine max* (L.) Merr.}, small grains, and corn (*Zea mays* L.). With postemergence herbicides, growers are able to target their herbicide selections for control of the weed spectrum present. Many postemergence herbicides have a higher specific activity and persist for a shorter period of time in the soil compared to soil applied herbicides. Many soil applied herbicides must also be incorporated into the soil to maximize activity, which severely limits there use in reduced and no-tillage production systems.

There are however several disadvantages in using postemergence herbicides. Timliness is critical to control young weed seedlings that are actively growing. Inclement weather, mechanical failure, and lack of skilled labor can limit the ability of the grower to apply postemergence herbicides in a timely fashion.

Postemergence herbicides may have a more limited weed control spectrum compared to soil applied herbicides. For example the sodium salt of acifluorfen (Na-acifluorfen) {5-[2-chloro-4-(triflouromethyl) phenoxy]-2-nitrobenzoic acid}

and dicamba {3,6-dichloro-2-methoxybenzoic acid} are applied postemergence to control broadleaf weeds, while metolachlor {2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide} and alachlor {2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide} are soil applied herbicides that control many grass and some broadleaf weed species.

Recently several herbicides have been introduced that have a broader postemergence weed control spectrum. Imazethapyr {2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid}, controls many broadleaf weeds and shattercane {*Sorghum bicolor* (L.) Moench}. Nicosulfuron {2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-*N*,*N*-dimethyl-3-pyridinecarboxamide} controls many grass species and also controls redroot pigweed (*Amaranthus retroflexus* L.). Primisulfuron has a very broad spectrum of activity on both grass and broadleaf weeds. However, the weed control spectrum of these herbicides is not complete. Imazethapyr and primisulfuron will not control giant foxtail, while nicosulfuron will not control large seeded broadleaf weeds. Therefore, broad spectrum postemergence weed control may require combining two or more herbicides. This practice has worked very well with preemergence herbicides, and a wide variety of package mixes of two or more herbicides are available for preemergence broad spectrum weed control in

corn and soybeans.

Two or more herbicides applied in postemergence tank-mix combination may act independently and the weed spectrum controlled predicted from the performance of each herbicide applied alone. Hatzios and Penner describes the combined independent effect of two chemicals as additive (18). However, in many cases, the weed spectrum controlled by two or more herbicides applied in postemergence tank-mix combination can not be predicted from the performance of each chemical applied independently. Hatzios and Penner (18) described an interaction that results in a decrease in biological activity compared to the performance of each herbicide applied alone as antagonistic. Conversely, an enhancement of biological activity is described as synergistic.

A well documented example of synergism is the enhancement of pitted morningglory {*Ipmoea lacunosa* L.} control by the addition of imazapyr {(±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-pyridinecarboxylic acid} to imazaquin or imazethapyr. Wills and McWhorter observed a 23% and 49% increase in pitted morningglory control by imazaquin or imazethapyr when imazapyr was applied in combination with either of these herbicides (52). Riley and Shaw also observed significant increases in pitted morningglory and johnsongrass {*Sorghum halepense* (L.) Pers.} control by adding imazapyr to

imazaquin or imazethapyr (43). Imazapyr alone had no activity on pitted morningglory or johnsongrass in either study. This synergistic interaction was independent of temperature and relative humidity (23).

Herbicide synergism as described above is very desirable. Pitted morningglory is a difficult and expensive weed to control (8). Adding as little as 4 g ai ha^{-1} of imazapyr has potential to increase the weed control spectrum of imazaquin and imazethapyr and increase the consistency of control of other labeled weed species. However, the documented cases of postemergence herbicide tank-mix synergism are rare. Postemergence tank-mix antagonisms are more frequently observed. The remainder or this review will document these observed antagonisms, describe the physiological basis for these antagonisms where possible, and discuss proven, as well as, potential solutions to postemergence herbicide tank-mix antagonism problems.

The availability of a wide range of postemergence broadleaf herbicides and graminacides that are selective in soybean has made broad spectrum postemergence weed control in soybean a possibility. However, many researchers have observed reductions in grass weed control with numerous broadleaf and grass herbicide combinations. The antagonistic interaction between the sodium salt of bentazon (Na-bentazon) {3-(1-methylethyl)-(1*H*)-2,1,3,-benzothiadiazin-4(3*H*)-one 2,2-dioxide} and sethoxydim {2-[1-

(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one} has been studied in the greatest detail.

In early evaluations of sethoxydim and Na-bentazon tank-mixtures, barnyardgrass {*Echinochloa crus-galli* (L.) Beauv.} control was reduced by Na-bentazon when sethoxydim was applied at 0.03 kg ai ha^{-1}, a very low use rate (3). When sethoxydim was increased to 0.22 kg ha^{-1} in the greenhouse and 0.56 kg ha^{-1} in the field no antagonism of barnyardgrass control was observed. In Greenhouse studies, Na-bentazon at 0.84 and 1.12 kg ai ha^{-1} decreased control of large crabgrass {*Digiteria sanguinalis* (L.) Scop.} by as much as 60% when sethoxydim was added at 0.14 kg ha^{-1} (17). However, increasing the sethoxydim rate to 0.28 and 0.56 kg ha^{-1} overcame the Na-bentazon antagonism. Delaying the sethoxydim application for as little as 20 min after applying Na-bentazon partially overcame the antagonism. Delaying the sethoxydim application for 48 h completely overcame the antagonism problem.

Results from field studies were similar to those observed in the greenhouse. Tank-mixing Na-bentazon at 0.84 kg ha^{-1} antagonized sethoxydim control of broadleaf signalgrass {*Brachiaria platyphylla* (Griseb.) Nasn.}, fall panicum {*Panicum dichotomiflorum* Michx.}, and large crabgrass (41). Higher sethoxydim application rates or application of the sethoxydim following Na-bentazon reduced or completely overcame the antagonism.

The prevention of Na-bentazon antagonism by delaying sethoxydim application indicated that the antagonism could be caused by a chemical incompatibility that reduced sethoxydim absorption. This hypothesis was confirmed by Rhodes and Coble who observed that the absorption of ^{14}C-sethoxydim by goosegrass {*Eleusine indica* (L.) Gaertn.} 6 h after treatment was reduced to 27% when applied with Na-bentazon, while absorption was 83% when sethoxydim was applied alone (42). These researchers also determined that the sodium salt of bentazon was reducing absorption and not the formulation blank. Wanamarta et al. also observed a similar decrease in ^{14}C-sethoxydim absorption by quackgrass {*Elytrigia repens* (L.) evski} when applied with Na-bentazon (48). These researchers also conducted extensive absorption studies utilizing various cation salts and organic acids and determined that sodium salts such as sodium acetate reduced ^{14}C-sethoxydim absorption to a similar extent as Na-bentazon. However, the absorption could be restored by adding organic acids to serve as complexing agents of divalent and polyvalent cations. From these results the researchers hypothesized that the sodium from the Na-bentazon exchanged with the hydrogen ions of the hydroxyl group of sethoxydim and formed the sodium salt of sethoxydim. Consequently, the absorption of the sodium salt of sethoxydim was reduced since it was more polar than sethoxydim.

The antagonistic effect of Na-bentazon on sethoxydim

grass control can be alleviated by increasing the rate of sethoxydim or delaying application of the sethoxydim. Neither of these solutions is desirable due to the increased cost of increasing sethoxydim rates or both the increased cost and time involved with multiple herbicide applications. A simple method to increase the absorption of the sethoxydim when applied with Na-bentazon would be a far less costly alternative.

The addition of 1.1 kg ha^{-1} of ammonium sulfate to the sethoxydim-Na-bentazon tank-mix restored the foliar absorption of ^{14}C-sethoxydim by quackgrass to normal levels (48). Restoration of ^{14}C-sethoxydim absorption by ammonium sulfate was also observed on large crabgrass (21). Addition of ammonium sulfate significantly increased the control of corn and large crabgrass with the sethoxydim-Na-bentazon combination in field studies (18). However, control of these species was still lower compared to sethoxydim applied alone. Studies were also conducted to determine the effect of replacing crop oil concentrate with the adjuvant DASH[16], on the absorption and efficacy of sethoxydim applied with bentazon. DASH not only restored foliar absorption of ^{14}C-sethoxydim by quackgrass to normal levels but completely overcame the antagonistic effect of Na-bentazon on sethoxydim control of quackgrass in field studies (48). DASH

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[16]DASH is a proprietary product of the BASF Corperation, P.O. Box 181, Parsippany, NJ 07054.

also increased the control of corn and large crabgrass in field studies but did not completly overcome Na-bentazon antagonism at all locations (20).

Reduction in the grass control with sethoxydim when applied in tank-mix combination with Na-aciflourfen have also been observed (13, 19, 50). A reduction in foliar ^{14}C-sethoxydim absorption by large crabgrass appeared to be the primary cause for the Na-aciflourfen antagonism (19). Acifluorfen is also forulated as the sodium salt, therefore, it is very possible that Na-aciflourfen is exerting a influence on sethoxydim absorption similar to Na-bentazon. Studies have also shown that tank-mixing sethoxydim with imazaquin (19, 33) or chlorimuron {2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino] sulfonyl]benzoic acid} (19) will antagonize grass weed control. In contrast to the basis for antagonism with bentazon and aciflourfen, reductions in ^{14}C-sethoxydim absorption could not explain the observed antagonism caused by chlorimuron or imazaquin (19).

Other postemergence grass herbicides are also available for use in soybeans such as fluazifop {(±)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid} and quizalofop {(±)-2-[4-[(6-chloro-2-quinoxalinyl)oxy] phenoxy]propanoic acid}. Although not registered for use in the United States, haloxyfop {2-[4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid} is used widely in other parts of the world for grass weed

control in soybeans.

The potential for antagonistic interactions between these herbicides and postemergence broadleaf weed herbicides has not been studied to the same extent as the interaction between sethoxydim and Na-bentazon.

Several researchers have reported reduced grass weed control by fluazifop when tank mixed with Na-aciflourfen (33, 32), imazaquin and chlorimuron (7, 32, 33), Na-bentazon (32, 33), and fomesafen {5-[2-chloro-4-(trifluoromethyl) phenoxy]-2-nitrobenzoic acid}, and lactofen {(±)-2-ethoxy-1-methyl-2-oxoethyl 5-[2-chloro-4-(triflouromethyl)phenoxy]-2-nitrobenzoate} (33). The physiological basis for these antagonisms has not been determined.

Imazaquin and chlorimuron (7, 32, 33) as well as Na-bentazon (32, 33) and Na-aciflourfen (32) antagonize the grass weed control of haloxyfop. Na-bentazon reduced the foliar uptake of ^{14}C-haloxyfop by yellow foxtail {*Setaria glauca* (L.) Beauv.} by 37% (10). Foliar absorption was reduced by 20% and 50% in yellow foxtail when Na-bentazon was applied at 0.84 and 1.68 kg ha^{-1} with ^{14}C-haloxyfop, respectively (31). Tank-mixtures of chlorimuron (12, 32) and imazaquin (32), as well as Na-bentazon (12), have also been shown to antagonize grass weed control by quizalofop. Wilhm et al. observed significant decreases in ^{14}C-quizalofop absorption by quackgrass when applied in combination with Na-bentazon (51). Cantwell et al. reported reductions in

giant foxtail (*Setaria faberi* Herrm.) control when imazethapyr was applied in combination with Na-bentazon or Na-acifluorfen (4).

Tank-mixing broadleaf weed herbicides and graminicides to obtain broad spectrum postemergence weed control in soybeans is severely limited by the antagonistic interactions between many of these herbicide combinations. However, in some cases antagonism is only observed when the graminacide is applied at lower than field use rates (3, 7, 12, 17, 41,). There are also many other potential tank-mix combinations that have not been extensively evaluated. Tank-mixtures with the graminacide clethodim {(*E*,*E*)-(±)-2-[1-[[3-chloro-2-propenyl)oxy]imino]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one} have only been evaluated in two studies (32, 33) and found to be compatible with most broadleaf herbicides with the exception of imazaquin. Recently, many new postemergence broadleaf weed herbicides have been introduced in soybean; including thifensulfuron, {3-[[[[(4-methoxy-6-methyl-1,3,5triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylic acid} imazethapyr, and lactofen. Their potential for antagonistic interactions with soybean graminicides has been evaluated to only a limited extent. We must continue to explore new herbicide combinations that have minimal potential for antagonistic interactions at labeled use rates to obtain broad spectrum postemergence weed control in

soybean.

Diclofop-methyl {(±)-2-[4-(2,4-dichlorophenoxy) phenoxy]propanoic acid} is a postemergence herbicide which is mainly used to selectively control many annual grass weeds in small grains. Broadleaf weed herbicides such as 2,4-D {(2,4-dichlorophenoxy)acetic acid}, MCPA {(4-chloro-2-methylphenoxy)acetic acid}, and chlorsulfuron {2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl] benezenesulfonamide} could potentially be tank-mixed with diclofop-methyl for broad spectrum postemergence weed control.

Todd and Stobbe found that tank-mixing diclofop-methyl with 2,4-D reduced wild oat {*Avena fatua* L.} control by as much as 30% (46). Hall et al. also observed similar reductions on diclofop-methyl activity on cultivated oats when applied in combination with 2,4-D (14). In physiological studies, 2,4-D appeared to reduce the amount of ^{14}C-diclofop-methyl translocated to the meristematic regions of wild oat plants (46). In contrast, other reports indicate 2,4-D had no effect on ^{14}C-diclofop-methyl translocation in both wild and cultivated oats {*Avena sativa* L.} (15, 22). Reduction in ^{14}C-diclofop-methyl absorption by 2,4-D was not observed.

The auxin herbicides, MCPA and dicamba, may also reduce the control of wild oat by diclofop-methyl (35). Studies conducted to determine the basis of the antagonistic

interaction of MCPA on the activity of diclofop-methyl found that MCPA reduced basipetal transport of ^{14}C-diclofop-methyl to meristematic regions in wild oat (36). In contrast, MCPA reduced the foliar absorption of ^{14}C-diclofop-methyl by wild oat by 10% one day after treatment (38). These researchers also found that MCPA reduced the rate of conversion of diclofop-methyl to the free acid diclofop which plays an important role in the herbicidal activity of diclofop-methyl on wild oat (47).

The lack of consistent results concerning the physiological basis of auxin herbicide antagonism of diclofop-methyl activity has led several researchers to propose that the antagonism may be due to a physiological reversal of the herbicidal effect of diclofop-methyl by auxin herbicides. Shimabukuro et al. demonstrated that there was reciprocal physiological antagonism between diclofop-methyl and 2,4-D in corn and soybean tissue cultures and that the extent of antagonism was concentration dependent for both herbicides (45). The use of plant tissue culture eliminates physiological factors such as long range transport. It has been proposed that diclofop-methyl exerts herbicidal activity by increasing the membrane permeability to protons thereby dissipating the electrochemical proton gradient (30, 53). Auxin type herbicides may counteract this effect. Hatzios and Penner define this as a physiological antagonism (18). However, other researchers have found no

effect of 2,4-D on diclofop-methyl activity *in vitro* and proposed that 2,4-D may adversely effect the translocation of diclofop-methyl (9). The physiological antagonism hypotheses is also not substantiated by recent reports that show the primary mode of action of diclofop-methyl to be the inhibition of the enzyme, acetyl-CoA carboxylase (ACCase), involved in the synthesis of fatty acids (2, 25, 40). These researchers also determined that only the R(+) enantiomer was effective in inhibiting ACCase and that the S(-) enantiomer was inactive. However, the most recent work by Shimabukuro showed that The R(+) enantiomer inhibited ACCase and depolarized the electrochemical proton gradient, while the S(-) enantiomer only depolarized the electrochemical proton gradient (44). 2,4-D reversed the growth inhibition effects of both S(-) and R(+) enantiomers.

The physiological basis for the antagonism of diclofop-methyl by auxin type herbicides remains unclear. The basis of antagonism may be due to several of these previously mentioned factors. The complex nature of the antagonistic interaction reduces the potential for finding a simple solution to overcome the antagonism.

The recent introduction of sulfonylurea herbicides for broadleaf weed control in small grains has provided new tank-mix possibilities with diclofop-methyl for broad spectrum weed control. However, early evaluations of diclofop-methyl and chlorsulfuron tank-mixes determined that

chlorsulfuron antagonized the control of cultivated oats when diclofop-methyl was applied at 0.25 kg ai ha^{-1}. Increasing the rate of diclofop-methyl to labeled use rates overcame the antagonism (14). However, O'Sullivan and Kirkland reported that chlorsulfuron consistently antagonized the activity of diclofop-methyl applied at 0.70 kg ha^{-1} in field studies (37). Chow also observed reduced wild oat control in field and greenhouse studies from diclofop-methyl when chlorsulfuron was applied. He attributed the antagonism to reduction in the foliar absorption and translocation of ^{14}C-diclofop-methyl (6). Chlorsulfuron antagonism of diclofop-methyl control of Italian ryegrass {*Lolium multiflorum* Lam.} has also been reported (28). However, in this study chlorsulfuron did not appear to effect the absorption, translocation, and molecular fate of ^{14}C-diclofop-methyl in Italian ryegrass (29). DPX-L5300 has also been shown to antagonize the activity of diclofop-methyl on wild oat (1). The DPX-L5300 had no effect on the absorption, translocation, and metabolism of ^{14}C-diclofop-methyl.

It appears that the majority of postemergence broadleaf weed compounds for use in small grains have the potential to antagonize the grass weed control activity of diclofop-methyl. However, it has been shown that bromoxynil {3,5-dibromo-4-hydroxybenzonitrile} had no effect on the activity of diclofop-methyl on wild oat (35). Green demonstrated that

the thifensulfuron had a far less adverse effect on the control of wild oat by diclofop-methyl compared to chlorsulfuron and metsulfuron {2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoic acid} (12). Tank-mix combinations of chlorsulfuron with difenzoquat {1,2-dimethyl-3,5-diphenyl-1*H*-pyrazolium}, another herbicide for annual grass control in small grains, were less antagonistic compared to chlorsulfuron tank-mixed with diclofop (6, 37).

These results strongly suggest that additional work is necessary to evaluate the potential for broad spectrum postemergence weed control in small grains, especially combinations with bromoxynil and sulfonylurea herbicides.

The sulfonylurea herbicides nicosulfuron and primisulfuron were recently introduced for postemergence weed control of many grass and some broadleaf weed species in corn. Tank-mixtures of these herbicides with other postemergence broadleaf weed herbicides could potentially provide broad spectrum postemergence weed control in corn. Antagonism of grass weed control with primisulfuron when applied in tank-mix combination with bentazon, atrazine {6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazin-2,4-diamine}, 2,4-D, and bromoxynil has been reported (5, 11). Atrazine, dicamba, and bentazon antagonized primisulfuron control of shattercane in greenhouse studies and giant foxtail in greenhouse and field studies (16). Na-bentazon

reduced ^{14}C-primisulfuron absorption by shattercane and giant foxtail whereas dicamba reduced ^{14}C-primisulfuron absorption by only shattercane. These antagonistic interactions were overcome by restoring foliar absorption to normal levels by either adding ammonium sulfate to the spray solution or replacing non-ionic surfactant or crop oil concentrate with SCOIL[17] (16).

Tank-mixtures of nicosulfuron with Na-bentazon, dicamba, 2,4-D, cyanazine, {2-[[4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl]amino]-2-methylpropanenenitrile} thifensulfuron, or atrazine have resulted in reduced grass control under low soil moisture conditions (34, 39). However, other researchers have obtained excellent broad spectrum weed control with tank-mixtures of nicosulfuron and a wide variety of broadleaf weed herbicides (24, 26, 27, 49).

Further research is needed to evaluate the potential for broadleaf weed herbicides to antagonize the grass control activity of primisulfuron and nicosulfuron. Initial research demonstrates that tank-mix combinations with nicosulfuron have a tremendous potential for broad spectrum postemergence weed control in corn. New adjuvants such as SCOIL or spray tank additions such as ammonium sulfate should be evaluated for their potential to increase the

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[17]SCOIL is a product of Agsco Inc., Grand Forks, ND 58201.

consistency of nicosulfuron tank-mixtures with broadleaf herbicides.

A large number of antagonistic interactions between numerous herbicides from many differenent chemical families has been documented (Table 1). These interaction are complex and may be due to a wide array of chemical, physiological, and biochemical interactions. Although the exact chemical interaction between herbicides formulated as sodium salts such as bentazon with a wide variety of graminicides is not completely understood, it is evident that these broadleaf herbicides are overall antagonists by reducing the foliar absorption of a wide variety of herbicides from many different chemical families (10). Fortunately these antagonistic interactions can be overcome by adding ammonium sulfate to the spray solution or by utilizing a different adjuvant. (16, 20, 21, 48). These relatively simple and inexpensive solutions have provided growers with the possibility of obtaining consistent broad spectrum weed control.

The antagonistic interactions between auxin type herbicides and diclofop-methyl are not clearly understood and appear to be very complex. It is possible that there are multiple reasons for this antagonistic interaction. It has been shown that increasing the rate of diclofop-methyl or delaying application of diclofop-methyl for several days are potential solutions to solve the antagonistic interaction

Table 1. Summary of documented postemergence tank-mix antagonisms.

Antagonizing herbicide	Herbicide antagonized	Plant species	Reference
Na-bentazon	sethoxydim	barnyardgrass	3
		broadleaf signalgrass	41
		corn	20
		fall panicum	41
		goosegrass	42
		large crabgrass	17, 41, 20
	fluazifop-butyl	barnyardgrass	32
		red rice	33
	haloxyfop	barnyardgrass	32
		red rice	33
		yellow foxtail	10, 31
	quizalofop	barnyardgrass	12
		quackgrass	51
	primisulfuron	?	11
		giant foxtail	5, 16
		shattercane	16
	nicosulfuron	giant foxtail	39
	imazethapyr	giant foxtail	4
Na-acifluorfen	sethoxydim	Johnsongrass	50
		fall panicum	19
		large crabgrass	19
		goosegrass	19
		southern crabgrass	13
		Texas panicum	13

(Continued)

Table 1 (continued)

Antagonizing herbicide	Herbicide antagonized	Plant species	Reference
Na-acifluorfen	fluazifop-butyl	barnyardgrass	32
		red rice	33
	haloxyfop	barnyardgrass	32
	imazethapyr	giant foxtail	4
imazaquin	sethoxydim	fall panicum	19
		large crabgrass	19
		goosegrass	19
		red rice	33
	fluazifop-butyl	barnyardgrass	32
		red rice	33
		sorghum	7
	haloxyfop	barnyardgrass	32
		red rice	33
		sorghum	7
	clethodim	barnyardgrass	32
		red rice	33
chlorimuron	sethoxydim	fall panicum	19
		large crabgrass	19
		goosegrass	19
	fluazifop-butyl	barnyardgrass	32
		red rice	33
		sorghum	7
	haloxyfop	barnyardgrass	32
		red rice	33
		sorghum	7

(Continued)

Table 1 (continued)

Antagonizing herbicide	Herbicide antagonized	Plant species	Reference
chlorimuron	quizalofop	barnyardgrass	12, 32
formesafen	fluazifop-butyl	redrice	33
lactofen	fluazifop-butyl	red rice	33
DPX-L5300	diclofop-methyl	wild oat	1
chlorsulfuron	diclofop-methyl	wild oat	6, 37
		oat	14
		Italian ryegrass	28, 29
metsulfuron	diclofop-methyl	wild oat	12
MCPA	diclofop-methyl	wild oat	35, 36, 38
2,4-D	diclofop-methyl	wild oat	32, 46
		oat	14, 15
	primisulfuron	giant foxtail	5
	nicosulfuron	giant foxtail	39
dicamba	diclofop-methyl	wild oat	35
	primisulfuron	giant foxtail	16
		shattercane	16
	nicosulfuron	giant foxtail	36, 39
atrazine	primisulfuron	giant foxtail	5, 16
		shattercane	16
		?	11
	nicosulfuron	giant foxtail	39

(Continued)

Table 1 (continued)

Antagonizing herbicide	Herbicide antagonized	Plant species	Reference
cyanazine	nicosulfuron	giant foxtail	36
bromoxynil	primisulfuron	giant foxtail	5
thifensulfuron	nicosulfuron	giant foxtail	36

(23, 25). However, these solutions may not be economically feasible, and due to the complex nature of the interaction the potential to find a simple and inexpensive solution to overcome the antagonistic interaction is low.

There appears to be a very high potential for ALS inhibiting herbicides, both sulfonylureas and imidazolinones, to antagonize the grass weed control of graminicides in the aryloxyphenoxypropionic acid or cyclohexanedione families. This interaction is extremely unfortunate due to the large role these herbicides play for postemergence weed control, especially in soybeans and small grains. The limited number of studies conducted to determine the basis of the antagonistic interaction are inconclusive. Further studies are needed to determine the basis of these interactions and to determine if there are potential solutions.

Postemergence herbicide mixtures will be increasenly used in the future to increase weed control efficiency, potentially reduce costs, and to reduce the environmental impact of herbicides. As the number of postemergence herbicides increase we must continue to evaluate potential tank-mix combinations for broad spectrum weed control in our major agronomic crops. There has been very little work in identifying weed species where antagonism is most likely to occur for various herbicide mixtures. This information could help growers choose the best herbicide combination to reduce

the chance for antagonistic interactions based on the weed population in the field. Very little work has been done on the effect of weed growth stage on the potential for antagonistic interactions with herbicide tank-mix combinations. We have seen that we can easily overcome the antagonist interactions in tank-mix combinations with Na-bentazon. We must continue to asses the potential of new adjuvants to reduce and reverse antagonistic interactions between postemergence herbicides.

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Chapter 6

Influence of Adjuvants on the Efficacy, Absorption, and Spray Retention of Primisulfuron

ABSTRACT

The effect of methylated seed oil (MSO), the organosilicone adjuvant DC-X2-5394, and ammonium sulfate on the efficacy, absorption, and spray retention of primisulfuron applied alone or with atrazine, dicamba, and bentazon to shattercane and giant foxtail was evaluated. Primisulfuron efficacy on both species was reduced by the three tank-mix combinations. Atrazine antagonism was not explained by decreases in foliar absorption and/or spray retention. Reductions in primisulfuron absorption and/or foliar spray retention appeared to cause for bentazon antagonism on both weeds and dicamba antagonism on shattercane. MSO, DC-X2-5394, and ammonium sulfate completely reversed dicamba and bentazon antagonism on shattercane and partially reversed bentazon antagonism on giant foxtail by increasing foliar absorption and/or spray retention. Compared with non-ionic surfactant MSO and DC-X2-5394 consistently increased giant foxtail control with primisulfuron by increasing foliar absorption and/or spray retention. Nomenclature: Atrazine, 6-chloro-*N*-ethyl-*N'*-(1-

methylethyl)-1,3,5-triazine-2,4-diamine; bentazon, 3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide; dicamba, 3,6-dichloro-2-methoxybenzoic acid; primisulfuron, 2-[[[[[4,6-*bis*-difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl]benzoic acid; giant foxtail, *Setaria faberi* Herrm. #[18] SETFA; shattercane, *Sorghum bicolor* L. Moench # SORVU.

Additional index words: Herbicide interaction, atrazine, bentazon, dicamba.

INTRODUCTION

Primisulfuron was recently registered for POST grass and broadleaf weed control in corn (*Zea mays* L.). Broad spectrum weed control could potentially be obtained by tank-mixing primisulfuron with other POST broadleaf weed herbicides. However, it is not known if tank-mixing primisulfuron with broadleaf weed herbicides will adversely affect grass control activity.

Reductions in grass control have been reported for many POST herbicide tank-mix combinations. The antagonism of sethoxydim [2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)

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[18]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Revised 1989. Available from WSSA, 309 W. Clark St., Champaign, IL 61820.

propyl]-3-hydroxy-2-cyclohexen-1-one] activity on grasses by tank-mixing with bentazon has been well documented (4, 10, 15, 27). Antagonism of sethoxydim activity in tank-mix combinations with acifluorfen [5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid] (1), as well as chlorimuron [2-[[[[4-chloro-6-methoxy-2-pyrimidinyl) amino]carbonyl]amino]-sulfonyl] benzoic acid] and imazaquin {2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolinecarboxylic acid} (11), have also been observed. Antagonism of haloxyfop {2-[4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid}, fluazifop-p {(*R*)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid}, and quizalofop {(±)-2-[4[(6-chloro-2-quinoxalinyl)oxy]phenoxy] propanoic acid} activity on grasses have also been reported in tank-mix combinations with bentazon, imazaquin, and chlorimuron (6, 8, 18, 19). Annual grass control with diclofop {(±)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid} decreased when tank-mixed with MCPA [(4-chloro-2-methylphenoxy)acetic acid] (23, 26), 2,4-D [(2,4-dichlorophenoxy)acetic acid] (9), or chlorsulfuron {2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide} (16, 25).

Reduced foliar absorption of sethoxydim is the physiological basis for bentazon antagonism (13, 28, 35). However, adding ammonium sulfate (13, 35) or replacing crop

oil concentrate (COC)[19] with DASH[20] (35) reversed bentazon antagonism by restoring the foliar absorption of sethoxydim to normal levels.

Replacing standard adjuvants such as COC or non-ionic surfactant (NIS) with superior adjuvants or adding of ammonium sulfate to herbicide tank-mix combinations may solve herbicide antagonism problems by increasing foliar absorption. The objectives of this research were to determine the effect of atrazine, dicamba, and bentazon on the efficacy, absorption, and foliar spray retention of primisulfuron applied with standard adjuvants such as NIS or COC. The effect of methylated seed oil (MSO)[21], experimental adjuvant DC-X2-5394[22] or ammonium sulfate on the efficacy, absorption, and spray retention of primisulfuron applied alone or in tank-mix combination with these herbicides was also evaluated.

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[19]Abbreviations: COC, crop oil concentrate; MSO, methylated seed oil; NIS, non-ionic surfactant; DAT, days after treatment.

[20]DASH is a proprietary product of BASF Corporation, P.O. Box 181, Parsippany, NJ 07054

[21]Methylated seed oil was SCOIL from Agsco Inc., Grand Forks, ND 58201.

[22]DC-X2-5394 is a proprietary material of Dow Corning Corp., Midland, MI 48686.

MATERIALS AND METHODS

Greenhouse studies

Shattercane and giant foxtail[23] were planted in BACCTO[24] greenhouse potting soil in 945-ml plastic pots. After emergence, the plants were thinned to three per pot. The plants were grown at 24 C ± 2 C with supplemental lighting from high pressure sodium lights to provide a midday light intensity of 1200 $\mu E\ m^{-2}\ s^{-1}$ for both supplemental and natural light. The day length was 18 h. All herbicide treatments were applied POST with a continuous link-belt sprayer at 170 kPa and 230 L ha^{-1} spray pressure and volume. Shattercane and giant foxtail plants were treated at the two- to three-leaf growth stage in all studies.

NIS[25] and COC[26] were used as standard adjuvants for comparison to MSO and DC-X2-5394. Separate studies were conducted to evaluate 0.6 kg ha^{-1} ammonium sulfate as a spray solution additive. NIS was used at 0.25% (v/v) with

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[23]F&J Seed Service Inc., Woodstock, IL 60098.

[24]BACCTO is a product of Michigan Peat CO. Houston, TX 77098.

[25]X-77 nonionic surfactant is a mixture of alkylarylpolyoxy-ethylene glycols, free fatty acids, and isopropanol marketed by Chevron Chemical Co., Richmond, CA 90412.

[26]Herbimax, a crop oil concentrate product of Loveland, Inc., Greeley, CO 80632.

primisulfuron applied alone and with dicamba. COC was used at 1.0% (v/v) with primisulfuron applied with atrazine or bentazon. Application rates for MSO and DC-X2-5394 were 0.75% and 0.5% (v/v), respectively. Application rates for all adjuvants and ammonium sulfate were kept constant throughout the greenhouse experiments.

Primisulfuron was applied at 0.01 kg ai ha^{-1} to shattercane in both efficacy studies whereas application rates were 0.03 and 0.04 kg ha^{-1} for giant foxtail in the adjuvant and ammonium sulfate study, respectively. The primisulfuron rate for ^{14}C-absorption and spray retention studies was 0.02 kg ha^{-1} for both weed species. Application rates for atrazine, the dimethylamine salt of dicamba and the sodium salt of bentazon were 1.7, 0.6, and 0.8 kg ai ha^{-1}, respectively, in all greenhouse experiments.

The effect of the various adjuvants or ammonium sulfate on the foliar absorption of ^{14}C-primisulfuron (Spec. act. = 1880 k Bq mg^{-1} uniformly ring-labelled) applied alone or tank-mixed with atrazine, dicamba, or bentazon was also evaluated in the greenhouse. An initial experiment was conducted to determine the absorption of ^{14}C-primisulfuron applied with NIS at 0.25% (v/v) to shattercane and giant foxtail plants at sampling times ranging from 1 to 12 h. Immediately following application of non-radiolabled spray solution to both weed species, a 2-μl drop containing 0.37 k Bq of ^{14}C-primisulfuron was applied to the center of the

adaxial surface of the second leaf. The spotting solution contained the ^{14}C-primisulfuron with appropriate amounts of formulation blank, unlabeled herbicide, adjuvant, and water to simulate a spray volume of 230 L ha^{-1}.

Treated leaves were rinsed in a 20-ml glass scintillation vial containing 3 ml of methanol:H_2O (1:1 v/v), and were gently swirled for 45 s. This washoff procedure successfully removed 99% of the applied ^{14}C-primisulfuron 15 s after treatment in preliminary studies. Unabsorbed ^{14}C-primisulfuron was quantified by liquid scintillation spectrometry and absorption determined by subtracting the amount of ^{14}C-primisulfuron recovered in the washoff solution from the amount of ^{14}C-primisulfuron applied.

^{14}C-Primisulfuron absorption studies were then conducted for each herbicide and adjuvant combination using the same procedures as described above except that ^{14}C-primisulfuron absorption was measured 8 h after application.

Foliar spray retention experiments were conducted using a similar procedure conducted by Boldt and Putnam (3) and Stolenberg and Wyse (31) to determine the effect of the various adjuvants or the addition of ammonium sulfate on the foliar spray retention of the various herbicide spray solutions. Chicago sky blue dye[27] (2.5 g L^{-1}) was added to

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[27]Sigma Chemical Co., St. Louis, MO 63187.

each mixture of herbicide and adjuvant combinations. Treatments were applied to both weed species as described earlier. Immediately after application, the second leaf was removed and rinsed with 4 to 6 ml distilled water containing NIS at 0.25% (v/v) and the rinsate was collected into graduated test tubes. All tubes were brought to a constant volume and the absorbance of the rinsate determined spectrophotometrically (625 nm). Leaf areas were measured and data presented as μg of dye retained per cm^2 of leaf area.

For greenhouse efficacy studies injury was visually estimated 16 DAT on a scale of 0 (no injury) to 100 (complete desiccation). A completely randomized design was used for all greenhouse experiments and the data reported are the means of two experiments with four replications each. An analysis of variance test was conducted as a two factor (herbicide treatment by adjuvant) factorial and means were separated by the LSD test at the 0.05 probability level for all experiments except the ^{14}C-primisulfuron time course absorption study. The ^{14}C-primisulfuron time course study was subjected to a curvilinear regression analysis ($Y = B(0) + B(1) * X + B(2) * X^2$) and coefficient of determination values determined. Analysis of variance test was conducted as a two factor (weed species by sampling time) factorial and means separated by the LSD test at the 0.05 probability level.

Field studies

Field studies were conducted in 1990 and 1991 in East Lansing, Michigan (Marlette fine sandy loam; soil organic matter was 3.2%, and pH was 7.0 each year) to examine the effect of MSO, DC-X2-5394, and ammonium sulfate on giant foxtail control with primisulfuron applied alone or with atrazine, dicamba, or bentazon. Giant foxtail seed was spread onto the field at 8 kg ha^{-1} and shallowly incorporated into the soil prior to planting corn ('Great Lakes 482') to insure adequate weed pressure throughout the field. Herbicide treatments were applied POST to plots 3 m wide by 6.5 m long with a tractor mounted compressed air sprayer delivering 206 L ha^{-1} of spray volume at a pressure of 207 kPa. Giant foxtail was in the two- to three-leaf growth stage when treated. Primisulfuron was applied at 0.04 kg ha^{-1} in all treatments. Application rates for the other herbicides and the adjuvants were the same as in the greenhouse efficacy studies.

Weed control was visually estimated 4 wk after treatment on a scale of 0 (no control) to 100 (complete control). In addition, two 0.5 m^2 areas were selected at random in each plot wherein the percentage of giant foxtail plants that were completely desiccated or that showed no visible signs of regrowth 20 DAT was visually determined. The experimental design was a randomized complete block with four replications per treatment. An analysis of variance was

conducted as a two factor (herbicide treatment by adjuvant) factorial and means were separated by the LSD test at the 0.05 probability level.

RESULTS AND DISCUSSION

Greenhouse studies

Replacing the standard NIS with MSO or DC-X2-5394 increased the efficacy of primisulfuron applied alone to giant foxtail (Table 1). Shattercane control was 89% with primisulfuron applied with NIS; thus, increased efficacy with MSO or DC-X2-5394 was not readily detectable.

The foliar absorption of ^{14}C-primisulfuron by giant foxtail (R^2 = 0.92) was greater than shattercane (R^2 = 0.96) at the earlier sampling times ranging from 1 h to 4 h (Figure 1). However, absorption of ^{14}C-primisulfuron by both weed species was not different at 6 h and 8 h. The ^{14}C-primisulfuron absorption rate by either weed species declined after 8 h, therefore 8 h was selected as the sampling time for the subsequent ^{14}C-primisulfuron absorption studies.

MSO enhanced the absorption of ^{14}C-primisulfuron more than DC-X2-5394; whereas, foliar spray retention was increased more by DC-X2-5394 than by MSO (Table 1).

Table 1. Effect of MSO and DC-X2-5394 on efficacy, absorption, and dye retention of primisulfuron applied alone or with other herbicides.

Treatment	Rate	Visual injury (16 DAT)[a]			^{14}C-Primisulfuron absorption (8 h)			Dye retention		
		NIS/COC[b]	MSO[c]	DC-X2-5394[d]	NIS/COC	MSO	DC-X2-5394	NIS/COC	MSO	DC-X2-5394
	kg ha^{-1}	%			% of applied			μg cm^{-2}		
Shattercane										
Primisulfuron	0.01[e]	89	95	96	25	76	42	8	9	17
+atrazine	1.70	75	94	83	37	79	31	8	11	12
+dicamba	0.60	73	91	91	12	76	35	10	11	17
+bentazon	0.80	73	93	92	5	25	3	5	11	15
LSD (.05)		9			5			3		

(Continued)

Table 1 (continued)

Treatment	Rate	Visual injury (16 DAT)[a]			^{14}C-Primisulfuron absorption (8 h)			Dye retention		
		NIS/COC[b]	MSO[c]	DC-X2-5394[d]	NIS/COC	MSO	DC-X2-5394	NIS/COC	MSO	DC-X2-5394
	kg ha^{-1}	%			% of applied			μg cm^{-2}		
Giant foxtail										
Primisulfuron	0.03[e]	49	73	83	24	73	67	12	12	15
+atrazine	1.70	54	73	53	51	81	44	10	12	14
+dicamba	0.60	36	48	50	33	72	45	9	12	14
+bentazon	0.80	35	53	48	9	27	11	8	11	14
LSD (0.05)		10			4			4		

[a]Abbreviations: COC, crop oil concentrate; DAT, days after treatment, MSO, methylated seed oil; NIS, non-ionic surfactant.

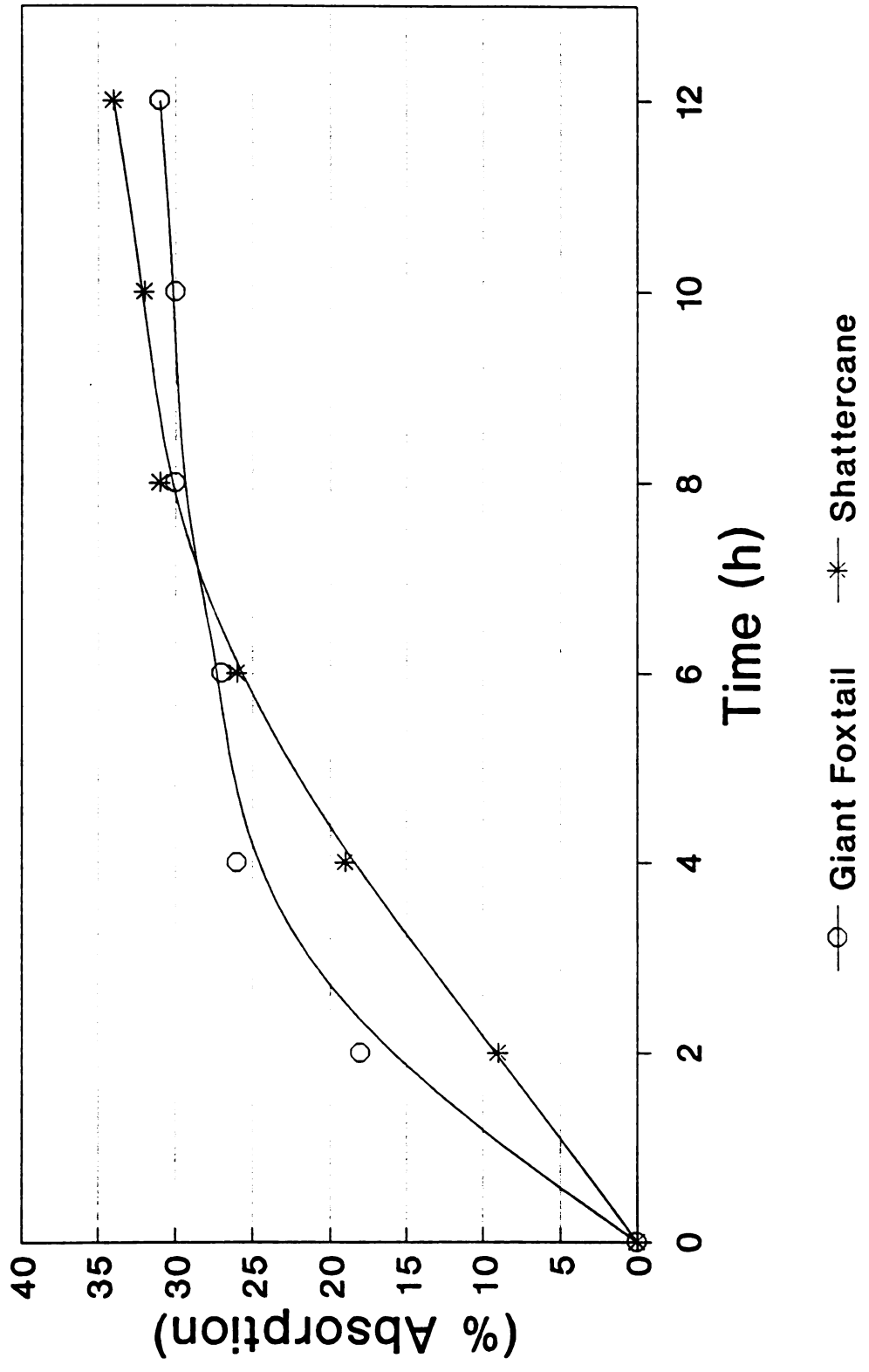
[b]NIS applied at 0.25% (v/v) with primisulfuron and primisulfuron tank-mix with dicamba. COC applied at 1.0% (v/v) with primisulfuron tank-mixed with atrazine and bentazon.

[c]MSO applied at 0.75% (v/v)

[d]DC-X2-5394 applied at 0.5% (v/v).

[e]This rate was 0.02 kg ha^{-1} for the ^{14}C-primisulfuron absorption and the dye retention studies.

Figure 1. Foliar absorption of ^{14}C-primisulfuron by giant foxtail and shattercane over time.


(% Absorption)
0
5
10
15
20
25
30
35
40
0
2
4
6
8
10
12
Time (h)
Giant Foxtail
Shattercane

Ammonium sulfate had no effect on the efficacy, absorption (Table 2), or foliar spray retention (data not shown) of primisulfuron applied with NIS. The substantial increase of giant foxtail control observed with primisulfuron supported previous studies wherein MSO substantially increased the activity of a wide variety of graminicides (20), and nicosulfuron {2-[[[[(4,6-dimethoxy-2-pyrimidinyl) amino]carbonyl]amino]sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide} (22), compared with NIS or petroleum oils. Further studies found that, compared with petroleum oil, MSO increased foliar absorption of fluazifop and sethoxydim (21).

Organosilicone based adjuvants may enhance the activity of a wide variety of herbicides (7, 12, 29). Increases in glyphosate [*N*-(phosphonomethyl)glycine] activity with ammonium sulfate have been documented (2, 24, 32). However, increases in herbicide activity with ammonium sulfate may vary depending on environmental conditions (14, 30), herbicides evaluated (34), and weed species (36).

Primisulfuron-atrazine tank-mix

The control of shattercane but not giant foxtail was reduced when primisulfuron was applied with atrazine in both greenhouse studies (Tables 1 and 2) as compared with primisulfuron applied alone. Decreased ^{14}C-primisulfuron absorption or foliar spray retention could not explain the

Table 2. Efficacy and absorption of primisulfuron and primisulfuron applied in tank-mix combinations as influenced by ammonium sulfate in greenhouse studies.

Treatment	Rate	Visual injury (16 DAT)[a]		^{14}C-Primisulfuron absorption (8 h)	
		NIS/COC[b]	NIS/COC + Am. sulfate[c]	NIS/COC	NIS/COC + Am. sulfate
	kg h^{-1}	%		% of applied	
Shattercane					
Primisulfuron	0.01[d]	94	91	30	34
+ atrazine	1.70	81	81	36	40
+ dicamba	0.60	73	90	14	36
+ bentazon	0.80	66	89	4	21
LSD (0.05)		6		5	
Giant foxtail					
Primisulfuron	0.04[d]	85	86	31	30
+ atrazine	1.70	86	89	57	46
+ dicamba	0.60	69	71	30	38
+ bentazon	0.80	76	90	12	36

(Continued)

Table 2 (continued)

Treatment	Rate	Visual injury (16 DAT)[a]		^{14}C-Primisulfuron absorption (8 h)	
		NIS/COC[b]	NIS/COC + Am. sulfate[c]	NIS/COC	NIS/COC + Am. sulfate
	kg h^{-1}	%		% of applied	
LSD (0.05)		8		5	

[a]Abbreviations: COC, crop oil concentrate; DAT, days after treatment, MSO, methylated seed oil; NIS, non-ionic surfactant.

[b]NIS applied at 0.25% (v/v) with primisulfuron and primisulfuron tank-mix with dicamba. COC applied at 1.0% (v/v) with primisulfuron tank-mixed with atrazine and bentazon.

[c]Ammonium sulfate applied at 0.6 kg ha^{-1}.

[d]This rate was 0.02 kg ha^{-1} for the ^{14}C-primisulfuron absorption and dye retention studies.

observed atrazine antagonism on shattercane. Replacing COC with MSO greatly increased the absorption of ^{14}C-primisulfuron by both weed species. Consequently, significant increases in the efficacy of primisulfuron applied with atrazine were observed on both weed species. Although DC-X2-5394 increased foliar spray retention of this tank-mix by both weed species, the absorption of ^{14}C-primisulfuron was lower when compared to COC and increased efficacy was not observed (Table 1). There was no beneficial effect of adding ammonium sulfate to the atrazine-primisulfuron tank-mix combination (Table 2).

Primisulfuron-dicamba tank-mix

Dicamba reduced the efficacy of primisulfuron on both species (Tables 1 and 2). Dicamba reduced ^{14}C-primisulfuron absorption by shattercane but not giant foxtail compared with primisulfuron applied alone. MSO or DC-X2-5394 increased the efficacy of the dicamba-primisulfuron tank-mix by increasing ^{14}C-primisulfuron absorption and/or foliar spray retention. However, giant foxtail control by this tank-mix combination with MSO or DC-X2-5394 was substantially lower than with primisulfuron applied alone with these two adjuvants. Ammonium sulfate appeared to completely reverse dicamba antagonism of shattercane control by restoring ^{14}C-primisulfuron absorption to normal levels (Table 2). Although ammonium sulfate increased ^{14}C-

primisulfuron absorption by giant foxtail, no increase in control was observed.

Reductions in herbicide absorption may not always be the physiological basis for observed tank-mix antagonisms (11, 23, 33). Reductions in foliar absorption, caused by tank-mixing with other herbicides, may also be dependent on weed species. Chlorsulfuron added to diclofop decreased diclofop absorption by wild oat (*Avena fatua* L.) by one-half (5). However, diclofop absorption by Italian ryegrass (*Lolium multiflorum* L. Lam.) was not affected by added chlorsulfuron (17).

Atrazine antagonism of primisulfuron control of shattercane and dicamba antagonism on giant foxtail observed in these studies may involve other physiological factors such as decreased translocation or increased metabolism of primisulfuron.

Primisulfuron-bentazon tank-mix

Bentazon reduced the efficacy of primisulfuron on both species (Tables 1 and 2). The antagonism of primisulfuron activity can be related to reductions in absorption and foliar spray retention by both species. Replacing COC with MSO or DC-X2-5394 or the addition of ammonium sulfate increased the efficacy of the primisulfuron-bentazon tank-mix applied to both species. The increase in activity with MSO or ammonium sulfate could be

partially attributed to increases in ^{14}C-primisulfuron absorption and/or increases in foliar spray retention by both weed species. Although DC-X2-5394 did not increase ^{14}C-primisulfuron absorption, increases in foliar spray retention may explain the increased activity by the DC-X2-5394.

Reductions in herbicide absorption have been documented as the primary basis for antagonism in numerous tank-mix combinations with bentazon (8, 11, 13, 28, 35). It appears that bentazon is exerting a similar effect on primisulfuron absorption. Previous studies have demonstrated that bentazon antagonism of sethoxydim activity could be reversed by adding ammonium sulfate to the spray solution or replacing COC with a superior adjuvant (13, 35). Bentazon antagonism of primisulfuron activity was reversed in this study by similar means.

Field studies

Visual weed control ratings and quantitative evaluation of the percentage of dead or non-regrowing plants did not differ significantly between years. Therefore, the results of the field experiments were combined over years for presentation.

Dicamba and bentazon reduced giant foxtail control with primisulfuron by 18% and 13%, respectively, when NIS or COC were used (Table 3). This antagonism was also evident in

the lower percentages of dead or non-regrowing plants. Unlike the greenhouse study, atrazine plus primisulfuron controlled less giant foxtail than primisulfuron alone. Replacing NIS with MSO and DC-X2-5394 significantly increased control with primisulfuron applied alone, especially in the percentage of dead or non-regrowing plants. Tank-mixes of primisulfuron with MSO or DC-X2-5394 controlled giant foxtail better the same herbicide combinations applied with NIS or COC.

Giant foxtail control with all herbicide combinations applied with MSO and DC-X2-5394 was equal to that obtained with primisulfuron applied alone with NIS, except when DC-X2-5394 was applied with primisulfuron plus dicamba. However, as in the greenhouse studies, giant foxtail control with the primisulfuron tank-mix combinations applied with MSO or DC-X2-5394 was less than control with primisulfuron applied alone with these two adjuvants.

Ammonium sulfate increased giant foxtail control only with primisulfuron plus bentazon. MSO, DC-X2-5394, and ammonium sulfate were more beneficial when bentazon was mixed with primisulfuron than when atrazine or dicamba were.

Tank-mixing atrazine, dicamba, or bentazon with primisulfuron may potentially reduce grass weed control. The physiological mechanisms of the bentazon antagonism could be related to reduced foliar absorption and/or spray retention. However, this was not true for atrazine, and reductions in

Table 3. Response of giant foxtail to primisulfuron and primisulfuron tank-mix combinations applied with various adjuvants in field studies in 1990 and 1991.

Treatment	Rate	Giant foxtail control (28 DAT)[a]				Dead or non-regrowing plants (20 DAT)			
		NIS/COC[b]	MSO[c]	DC-X2-5594[d]	NIS/COC + Am. sulfate[e]	NIS/COC	MSO	DC-X2-5394	NIS/COC + Am. sulfate
	kg ha^{-1}	%				%			
Primisulfuron	0.04	79	90	89	70	57	80	77	49
+ atrazine	1.70	68	74	74	60	51	56	51	44
+ dicamba	0.60	61	78	69	57	39	53	43	30
+ bentazon	0.80	66	83	81	74	46	67	62	57
LSD (0.05)		6				10			

[a]Abbreviations: COC, crop oil concentrate; DAT, days after treatment, MSO, methylated seed oil; NIS, non-ionic surfactant.

[b]NIS applied at 0.25% (v/v) with primisulfuron and primisulfuron tank-mix with dicamba. COC applied at 1.0% (v/v) with primisulfuron tank-mixed with atrazine and bentazon.

[c]MSO applied at 0.75% (v/v).

[d]DC-X2-5394 applied at 0.5% (v/v).

[e]Ammonium sulfate applied at 0.6 kg ha^{-1}.

foliar absorption caused by dicamba were dependent upon weed species. Antagonism of primisulfuron control of sensitive weed species such as shattercane could be easily reversed with MSO for all tank-mixes and with DC-X2-5394 or ammonium sulfate for the dicamba or bentazon tank-mixes. Reversal of antagonism appeared related to increased foliar absorption of primisulfuron and/or spray retention. Antagonism by other herbicides of primisulfuron activity on a more tolerant species such as giant foxtail could not be completely reversed by any of the adjuvants tested. However, the most complete reversal was observed for the primisulfuron-bentazon combination. The use of MSO and DC-X2-5394 consistently increased giant foxtail control with primisulfuron in greenhouse and field experiments. These adjuvants may potentially broaden the weed control spectrum of primisulfuron.

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Chapter 7

Atrazine Reduces Primisulfuron Transport to Meristems of Giant Foxtail (*Setaria faberi*) and Velvetleaf (*Abutilon theophrasti*)

Abstract

Greenhouse and laboratory studies were conducted to determine the effect of atrazine on the efficacy, absorption, translocation, and metabolism of primisulfuron applied to velvetleaf and giant foxtail. The efficacy of primisulfuron was reduced by 18% and 22% when applied at 20 and 40 g ai ha^{-1}, respectively, in combination with 1.7 kg ai ha^{-1} atrazine to velvetleaf. The efficacy of primisulfuron was reduced by 15% and 16% when applied at 30 or 60 g ai ha^{-1}, respectively, in combination with 1.7 kg ai ha^{-1} atrazine to giant foxtail. The foliar absorption of ^{14}C-primisulfuron by either weed species was not affected by the addition of atrazine to the treatment solution. Atrazine had no effect on the metabolism of ^{14}C-primisulfuron by either weed species. In the absence of atrazine, translocation of absorbed ^{14}C from primisulfuron out of the treated leaves of velvetleaf and giant foxtail averaged 19% and 29%, respectively, across sampling times. These values were reduced to an average of 9% and 16% in velvetleaf and giant

foxtail, respectively, when ^{14}C-primisulfuron was applied in combination with atrazine. The majority of the translocated ^{14}C from primisulfuron was transported acropetally in velvetleaf and basipetally in giant foxtail. Atrazine significantly reduced ^{14}C translocation from primisulfuron to these meristematic sinks in both weed species. The reduced translocation was positively correlated with reduced control of these weeds when primisulfuron was tank-mixed with atrazine. Nomenclature: Atrazine, 6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine; primisulfuron, 2-[[[[[4,6-*bis*(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl]benzoic acid; giant foxtail, *Setaria faberi* Herrm. #[28] SETFA; velvetleaf, *Abutilon theophrasti* Medicus. # ABUTH.

Additional index words. Tank-mixture, herbicide interaction, foliar absorption, translocation, metabolism.

INTRODUCTION

Primisulfuron is a recently introduced herbicide applied postemergence in corn to control many grass and some broadleaf weed species. The broadleaf weed control spectrum of primisulfuron could be potentially increased by tank-

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[28]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Revised 1989. Available from WSSA, 309 W. Clark St., Champaign, IL 61820.

mixing primisulfuron with other postemergence herbicides for broadleaf weed control. However, we have previously observed that tank-mixing primisulfuron with atrazine, dicamba (3,6-dichloro-2-methoxybenzoic acid), or bentazon [3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide) may reduce control of shattercane [*Sorghum bicolor* (L.) Moench] and giant foxtail (17, 18). ^{14}C-Primisulfuron absorption and foliar spray retention studies indicated that bentazon antagonism on both species, and dicamba antagonism on shattercane could be explained by reductions in foliar absorption and/or spray retention. However, similar reductions in foliar absorption and spray retention were not observed when primisulfuron was tank-mixed with atrazine (17, 18).

Although reduced foliar absorption has been well documented as the primary basis for antagonism in numerous tank-mix combinations with bentazon (15, 20, 21, 32, 35), other researchers have demonstrated that antagonism of diclofop [(±)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid] grass control by 2,4-D [(2,4-dichlorophenoxy)acetic acid] (34), MCPA [(4-chloro-2-methylphenoxy)acetic acid] (30) and chlorsulfuron [2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide] (24) could not be attributed to reduced diclofop absorption. Research has also demonstrated that reduced herbicide absorption could not explain the antagonism of sethoxydim

[2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one] (20) or haloxyfop-methyl [2-[4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid] (7) grass control by imazaquin [2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolinecarboxylic acid] or chlorimuron [2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoic acid].

The primary physiological basis of atrazine antagonism on grass control with primisulfuron is unknown. Information is also limited on the potential for atrazine to reduce broadleaf weed control with primisulfuron. The following studies were conducted to determine the effect of atrazine on the efficacy, absorption, translocation, and metabolism of primisulfuron by giant foxtail and velvetleaf.

MATERIALS AND METHODS

Whole plant bioassay.

Giant foxtail and velvetleaf seeds[29] were planted in greenhouse potting soil in 945-ml plastic pots. After emergence, giant foxtail plants were thinned to three plants per pot and velvetleaf to one plant per pot. The plants were grown at 24 C ±2 C with supplemental lighting from high pressure sodium lights which produced an irradiance of 600

[29]F&J Seed Service Inc., Woodstock, IL 60098.

$\mu E\ m^{-2}\ s^{-1}$ to provide a total of 1200 $\mu E\ m^{-2}\ s^{-1}$ for both supplemental and natural light. The day length was 16 h. All herbicide treatments were applied postemergence with a continuous link-belt sprayer at 170 kPa and 230 L ha^{-1} spray pressure and volume, respectively. Primisulfuron was applied at 20 or 40 g ai ha^{-1} to velvetleaf and 30 or 60 g ha^{-1} to giant foxtail. Atrazine was applied as the liquid formulation at 1.7 kg ha^{-1}. Crop oil concentrate[30] (COC)[31] was applied at 1.0% v/v. Giant foxtail and velvetleaf plants were treated in the two- to three and four- to five-leaf growth stage, respectively. Dry weights of the above ground portion of the plants were taken 16 days after treatment (DAT).

Absorption, translocation, and metabolism studies.

Giant foxtail and velvetleaf plants were grown in the greenhouse as described above except that greenhouse potting soil was replaced with quartz builders sand and plants were fertilized every other day with full strength Hoagland's solution (19). Prior to herbicide application, the surface of each pot was covered with 1.5 cm of vermiculite to prevent soil absorption. Velvetleaf and giant foxtail

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[30]Herbimax, a crop oil concentrate product of Loveland, Inc., Greeley, CO 80632.

[31]Abbreviations: COC, crop oil concentrate; DAT, days after treatment; NIS, non-ionic surfactant; TLC, thin-layer chromatography.

plants were sprayed with the herbicide treatments solutions as described above except that both species were in the three- to four-leaf growth stage and the treatment rate for primisulfuron was 20 g ha^{-1}.

Immediately after spraying, two 1-μl drops, each containing 1.2 x 10^2 Bq of ^{14}C-primisulfuron (Spec. act. = 1.88 x 10^6 Bq mg^{-1} URL) were applied to the adaxial surface of the second leaf of giant foxtail. Three 1-μl drops, were also applied to the adaxial surface of the third leaf. The radiolabeled spotting solution contained the ^{14}C-primisulfuron with the formulation blank, unlabeled herbicide, COC, and water to simulate the contents of a spray solution at 230 L ha^{-1}.

^{14}C-Primisulfuron was applied to velvetleaf in the same way as with giant foxtail except that the second and third leaves each received five 1-μl drops each containing 1.8 x 10^2 Bq. Six giant foxtail or two velvetleaf plants constituted one replication of a particular herbicide treatment. Utilization of multiple plants per replication allowed a closer simulation of a field application rate of primisulfuron to each plant, while providing a total of 3.6 x 10^3 Bq of ^{14}C-primisulfuron for translocation and metabolism analysis. Immediately following ^{14}C-primisulfuron treatment, plants were placed in a growth chamber with day and night temperatures of 24 and 20 C, respectively. Lighting was provided by high pressure sodium lamps with an

irradiance of 650 $\mu E\ m^{-1}\ s^{-2}$. The day length was 12 h.

At 1, 3, and 7 DAT, the treated leaves were excised and rinsed with approximately 4 to 5 ml of methanol to remove unabsorbed ^{14}C-primisulfuron. The volume of the entire rinsate was recorded and two 1-ml aliquots were sampled from the rinse solution and radioassayed by liquid scintillation spectrometry. The excised treated leaves were frozen with dry ice and stored at -30 C for metabolism analysis. The sand was washed from the roots of the remainder of the plant and the plant sectioned into three parts: above the treated leaves, below the treated leaves, and roots. Fresh weights of the various plant parts were recorded and combusted in a biological oxidizer[32]. Radioactivity quantified by liquid scintillation spectrometry.

Absorbed ^{14}C-primisulfuron was extracted from the treated leaves with a modification of previously published methods (12, 27). The treated leaves were homogenized in 20-ml of acetone for 2 min in a stainless steel Sorvall Omnimixer[33]. The inside of the vessel and blades of the homogenizer were washed with 10 ml of 80% acetone and the leaves were then homogenized for an additional 4 minutes. The homogenate was filtered under vacuum through Whatman #1 filter paper. The homogenizing vessel was rinsed with two 20-ml portions of

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[32]Biological oxidizer, model OX-300. R.J. Harvey Inst. Corp., Hillsdale, N.J.

[33]Sorvall Omni-mixer. Sorvall Inc., Newton, CN.

80% acetone and the rinsate passed through the filter paper. The entire volume of the filtrate was recorded and two 1-ml aliquots were sampled from the filtrate and radioassayed by liquid scintillation spectrometry. The filter paper and extracted tissue was allowed to air dry and oxidized to determine unextracted radioactivity. The filtrate was transferred to a 250-ml round bottom flask and concentrated to 1 to 5 ml *in vacuo* at 35 C using a rotary evaporator. The flask was washed with 5 ml acetonitrile and combined with the aqueous plant extract. Following evaporation of the acetonitrile under a stream of nitrogen, the remaining aqueous extract was filtered through a polysulfone membrane filter[34] with a mean pore size of 0.45-μm. The filtrate was then evaporated to a final volume of 1 ml under a stream of nitrogen, and 0.1-ml samples were chromatographed on thin-layer chromatography (TLC) silica gel (250-μm-thick) plates[35]. Ten μl of ^{14}C-primisulfuron standard (1.8×10^2 Bq) were also placed on each plate. TLC plates were developed in a solvent system of methanol:benzene (2:1 by vol.). Radioactive spots were located, and R_f values recorded with a radioactive plate scanner[36]. The radioactive spots were then removed and radioassayed by

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[34]Supor 200 membrane filter. Gelman Sciences Inc., Ann Arbor, MI 48106.

[35]Uniplate silica-gel GF. Analtech, Newark, DE 19711.

[36]Radioactive plate scanner. AMBIS inc., San Diego, CA.

liquid scintillation spectrometry.

Statistical analysis.

All experiments were conducted separately on each species. A completely randomized design was used in all experiments with four replications per treatment. All experiments were conducted twice and the combined results presented. Data from the whole plant bioassay experiment were subjected to an analysis of variance, and means were separated by the least significant difference test at the 5% probability level. The absorption, translocation, and metabolism studies were analyzed as a two factor (treatment by day) factorial. Following an analysis of variance treatment means were separated by the least significant difference test at the 5% probability level.

RESULTS AND DISCUSSION

Whole plant bioassay.

Shoot dry weights of velvetleaf treated with 20 and 40 g ha^{-1} of primisulfuron were 26% and 19% respectively, of untreated control plants (Table 1). Shoot dry weights of giant foxtail plants treated with 30 and 60 g ha^{-1} of primisulfuron were 16% and 9%, respectively, of untreated control plants. The addition of atrazine significantly reduced the efficacy of primisulfuron applied at all rates

Table 1. Response of velvetleaf and giant foxtail to primisulfuron-atrazine tank-mix combinations.

Weed species	Treatment[1]	Rate	Dry weight (16 DAT)[2]	Dry weight
		(g ai ha^{-1})	(g/pot)	(% of untreated)
Velvetleaf	Primisulfuron	20	0.7	26
	+ atrazine[3]		1.2	44
	Primisulfuron	40	0.5	19
	+ atrazine		1.1	41
	Untreated	--	2.7	
	LSD (0.05)		0.2	8
Giant Foxtail	Primisulfuron	30	0.5	16
	+ atrazine		1.0	31
	Primisulfuron	60	0.3	9
	+ atrazine		0.8	25
	Untreated		3.2	
	LSD (0.05)		0.4	11

[1]Crop oil concentrate applied with all treatments at 1.0% (v/v).

[2]DAT = Days After Treatment.

[3]Atrazine applied at 1.7 kg ha^{-1}.

on both weed species.

Wettable powder formulations of atrazine applied in tank-mix combination with glyphosate have been reported to reduce glyphosate activity as compared to glyphosate applied alone (1, 33). Tank-mixing primisulfuron with atrazine may reduce grass weed control (16, 22). The results of the velvetleaf bioassay study demonstrated that atrazine may also reduce primisulfuron efficacy on broadleaf weed species. These results are supported by a recent report that velvetleaf control was reduced when primisulfuron was tank-mixed with atrazine (4).

Absorption, translocation, and metabolism studies.

Total recovery of ^{14}C-primisulfuron was greater than 92% from both weed species at all sampling times. Atrazine had no effect on total ^{14}C-primisulfuron recovery from either species (data not shown). ^{14}C-Primisulfuron was absorbed by the leaves of both weed species and absorption increased over time (Table 2). However, most of the ^{14}C-primisulfuron was absorbed within the first day by both weed species. The addition of atrazine had no effect on the absorption of ^{14}C-primisulfuron by either weed species at any sampling time. These results support previous reports that atrazine antagonism of weed control with primisulfuron could not be explained by reductions in ^{14}C-primisulfuron foliar absorption (17, 18). ^{14}C-Primisulfuron absorption values

Table 2. Effect of atrazine on the absorption and translocation of ^{14}C-primisulfuron by velvetleaf and giant foxtail.

DAT[1]	Treatment	Absorption	Distribution of radioactivity			
			Treated Leaf	Above treated leaf	Below treated leaf	Root
		(% of applied)	------ (% of absorbed) ------			
Velvetleaf						
1	Primisulfuron[2] + COC[1]	23	79	16	3	2
	+ Atrazine[3]	22	92	7	1	<1
3	Primisulfuron + COC	28	80	14	4	2
	+ Atrazine	27	89	9	1	1
7	Primisulfuron + COC	42	83	12	4	1
	+ Atrazine	38	92	6	1	1
	LSD (0.05)	4	5	3	1	1
Giant foxtail						
1	Primisulfuron + COC	42	75	7	13	5
	+ Atrazine	40	83	6	9	2
3	Primisulfuron + COC	50	71	11	13	5
	+ Atrazine	50	84	10	5	1
7	Primisulfuron + COC	59	70	14	10	6
	+ Atrazine	60	86	11	2	1
	LSD (0.05)	5	6	3	2	1

[1]COC = Crop Oil Concentrate (applied at 1.0% v/v), DAT = Days After Treatment.

[2]Primisulfuron applied at 20 g ai ha^{-1}.

[3]Atrazine applied at 1.7 kg ha^{-1}.

were much higher in the present experiments than values previously reported by Camacho and Moshier (3) for johnsongrass [*Sorghum halepense* (L.) Pers.]. They reported that johnsongrass absorbed only 3% of the applied ^{14}C-primisulfuron at 1 DAT. These differences suggest that ^{14}C-primisulfuron absorption may differ among weed species.

Translocation out of the treated leaves of velvetleaf and giant foxtail of absorbed ^{14}C from primisulfuron, was 21% and 25%, respectively, at 1 DAT (Table 2). Total ^{14}C translocation from primisulfuron did not increase with time. Most of the translocated ^{14}C from primisulfuron in velvetleaf was found above the treated leaves. Acropetal translocation of absorbed ^{14}C from primisulfuron in velvetleaf was 16% at 1 DAT, but decreased to 12% by 7 DAT. Translocation of the absorbed ^{14}C from primisulfuron below the treated leaf of giant foxtail was 13% at 1 and 3 DAT, and decreased to 10% at 7 DAT (Table 2). The reduction in the amount of ^{14}C from primisulfuron found below the treated leaves correlated with increased ^{14}C from primisulfuron found above the treated leaves.

Herbicides that are translocated primarily in the phloem will accumulate at active growth sites (6, 8, 10, 11). The active growth sites of developing annual weed seedlings are shoot apical meristems and developing immature leaves (31). In dicot weeds growth is initiated from apical meristems found at the top of the plant, while new leaves in monocot

weeds emerge from the base of the plant. The translocation patterns of primisulfuron observed in these studies, combined with the differences between dicot and monocot anatomy suggests that ^{14}C from primisulfuron is primarily translocated to actively growing sites in both weed species. Thus, the translocation of foliar absorbed ^{14}C from primisulfuron appears to be mainly via the phloem.

Although annual weed species were utilized in the present studies, the apparent phloem mobility of ^{14}C from primisulfuron observed may explain the control of perennial grass weeds (2, 13, 26, 36) with primisulfuron. Camacho and Moshier (3) found that the majority of translocated ^{14}C-primisulfuron was transported basipetally in rhizome johnsongrass. Translocation studies with other sulfonylurea herbicides have als shown transport to areas of high meristematic activity. Leys and Slife (23) observed that the majority of the translocated ^{14}C-metsulfuron {2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl] benzoic acid} and ^{14}C-chlorsulfuron was concentrated in young shoot tissues in wild garlic (*Allium vineale* L.). Devine and Vanden Born (10) observed that the majority of foliar applied ^{14}C-chlorsulfuron was transported to the upper shoot in both Canada thistle [*Cirsium arvense* (L.) Scop.] and perennial sowthistle (*Sonchus arvensis* L.). Obrigawitch et al. (28) observed that ^{14}C-nicosulfuron translocated throughout the

shoot and rhizomes of johnsongrass and autoradiographs showed a high concentration of radioactivity at the growing point of the shoot.

Tank-mixing primisulfuron with atrazine reduced the amount of ^{14}C from primisulfuron translocated out of the treated leaves of velvetleaf and giant foxtail by 13% and 8%, respectively 1 DAT (Table 2). These reductions constitute a 69% and 32% decrease in the amount of ^{14}C from primisulfuron translocated in velvetleaf and giant foxtail, respectively, as compared to primisulfuron applied alone. At 3 and 7 DAT translocation was reduced by 45 and 53%, respectively for both species, as compared to primisulfuron applied alone. Atrazine-induced reduction of ^{14}C-primisulfuron translocation was most evident in basipetal translocation by giant foxtail and acropetal translocation by velvetleaf (Table 2).

The reduction of ^{14}C-primisulfuron translocation to active growing sites by tank-mixing with atrazine may be a major physiological factor accounting for the antagonism observed ot the whole plant level. Translocation of phloem mobile herbicides such as chlorsulfuron may be inhibited when the production and translocation of photoassimilate is also inhibited (9). Thus, the inhibitory effect of the herbicides on photoassimilate production and translocation limits herbicide translocation in the phloem. It has also been shown that chlorsulfuron inhibited the translocation of

clopyralid (3,6-dichloro-2-pyridinecarboxylic acid) in the phloem (9). It is well established that atrazine inhibits electron transport in photosystem II (14). Therefore, it is reasonable to assume that atrazine will reduce photoassimilate production thereby reducing the phloem transport of primisulfuron.

Decreased translocation of specific herbicides has been observed following application in tank-mix combination. Reduced diclofop translocation appeared to explain MCPA (30) and chlorsulfuron (5) antagonism. Reduced translocation of ^{14}C-glyphosate (29) and ^{14}C-haloxyfop-methyl (25) appeared to contribute to the antagonism by 2,4-D. Imazaquin and chlorimuron antagonism of grass weed control by haloxyfop-methyl also may be attributed to reduced herbicide translocation (7).

Extraction of absorbed ^{14}C-primisulfuron averaged 93% or greater from the treated leaves of both weed species at all sampling times. There was no effect of atrazine on the amount of ^{14}C-primisulfuron in the treated leaves of both weed species that was insoluble in 80% acetone (data not shown). TLC analysis of extracted ^{14}C-primisulfuron revealed two radioactive spots that were similar in both weed species. One co-chromatographed with the ^{14}C-primisulfuron standard (R_f = 0.87) and the other remained at the origin, indicating a polar metabolite of ^{14}C-primisulfuron. In a separate study velvetleaf and giant foxtail plants were

grown and treated as previously described, except that plants were immediately harvested and extracted and the extract spotted onto TLC plates along with the ^{14}C-primisulfuron standard. Over 99% of the radioactivity co-chromatographed with the ^{14}C-primisulfuron standard. In an additional separate study untreated plant extract was spiked with ^{14}C-primisulfuron and over 99% of the radioactivity co-chromatographed with the ^{14}C-primisulfuron standard.

Metabolism of ^{14}C-primisulfuron in velvetleaf was 10% 1 DAT and did not increase as sampling time increased (Table 3). Metabolism of ^{14}C-primisulfuron in giant foxtail was 14% 1 DAT and increased as sampling time increased to 33% at 7 DAT (Table 3). The addition of atrazine did not effect the metabolism of ^{14}C-primisulfuron in giant foxtail at any sampling time. The addition of atrazine did not stimulate the metabolism of ^{14}C-primisulfuron in velvetleaf, and metabolism was lower at 7 DAT as compared to primisulfuron applied alone. A comparison of ^{14}C-primisulfuron metabolism between species may explain the differential tolerance of velvetleaf and giant foxtail at the whole plant level to postemergence applications of primisulfuron (Table 1), especially considering the differences in application rates and species growth stage at time of treatment.

Tank-mixing primisulfuron with atrazine can reduce the weed control activity of primisulfuron applied postemergence to both grass and broadleaf weed species. Decreases in ^{14}C-

Table 3. Effect of atrazine on the metabolism of ^{14}C-primisulfuron by velvetleaf and giant foxtail.

		Metabolism of ^{14}C-primisulfuron		
		Days after treatment		
Weed Species	Treatment	1	3	7
		------- (% of extracted) --------		
Velvetleaf	Primisulfuron[1] + COC[2]	10	11	12
	+ Atrazine[3]	9	10	8
	LSD (0.05)		---------- 4 ----------	
Giant Foxtail	Primisulfuron + COC	14	21	33
	+ Atrazine	12	19	28
	LSD (0.05)		---------- 5 ----------	

[1]Primisulfuron applied at 20 g ai ha^{-1}.

[2]COC = Crop Oil Concentrate, applied at 1.0% v/v.

[3]Atrazine applied at 1.7 kg ha^{-1}.

primisulfuron absorption or increases in ^{14}C-primisulfuron metabolism were not observed and therefore could not explain the observed atrazine antagonism. Results of the translocation study suggest that a primary factor in the herbicidal activity of primisulfuron is related to its translocation to meristematic regions. Tank-mixing primisulfuron with atrazine reduced the translocation of primisulfuron to these meristematic regions, and this appears to be the basis for the antagonism.

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