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By

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ABSTRACT

REFINING POSTEMERGENCE WEED CONTROL IN CORN: HERBICIDE SELECTIVITY AND TIMING

By

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Nicosulfuron and primisulfuron are sulfonylurea herbicides that display differential selectivity despite similar chemical structures and a common site of action in susceptible plants. Corn is tolerant and johnsongrass is sensitive to both herbicides. Barnyardgrass and giant foxtail are sensitive to nicosulfuron and tolerant to primisulfuron. Eastern black nightshade is tolerant to nicosulfuron and sensitive to primisulfuron.

^{14}C-radiolabelled herbicides were used to determine if differences in whole plant responses to nicosulfuron and primisulfuron were due to differential herbicide absorption, translocation, or metabolism. Nicosulfuron and primisulfuron selectivity in corn, johnsongrass, barnyardgrass, and giant foxtail was primarily due to differential herbicide metabolism rate. Tolerant species metabolized the herbicide more rapidly and extensively than sensitive species. Differential herbicide absorption, translocation, or metabolism did not account for differential sensitivity of eastern black nightshade to nicosulfuron and primisulfuron.

Further experiments were conducted to determine if the difference in eastern black nightshade sensitivity to nicosulfuron and primisulfuron was due

to a difference at the herbicidal site of action: the acetolactate synthase (ALS) enzyme. Greater sensitivity of eastern black nightshade to primisulfuron was due to greater ALS inhibition by primisulfuron. Eastern black nightshade and johnsongrass ALS sensitivity to nicosulfuron were similar, despite differences in whole plant response. Eastern black nightshade tolerance to nicosulfuron was due to a combination of greater ALS level and less herbicide translocation.

Field studies were conducted in 1992 and 1993 to determine if weed interference prior to herbicide application reduces corn yield with a total postemergence herbicide program. Nicosulfuron plus bromoxynil plus nonionic surfactant applied to 5-, 10-, or 15-cm weeds provided nearly complete weed control. Weed interference did not reduce corn height or corn grain yield when postemergence applications were made to small weeds ($\leq$ 10 cm). Weed interference reduced corn height and grain yield in 1992 when applications were made to 15-cm weeds even though weed control was nearly complete. Weed control was incomplete and corn height and grain yield reduced both years when applications were delayed until weeds were 20 cm tall.

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REVIEW OF LITERATURE

INTRODUCTION

According to United Nations statistics, the global food supply must increase by 75% to feed the projected world population in the year AD 2000. To feed such numbers, agriculture must become even more efficient and productive than in the past. The judicious use of agrochemicals, including pesticides, is essential to insure optimum yields of high quality crops. To produce and use pesticides safely and effectively, it is crucial to understand how they interact with target species (31).

The importance of foliar applied (postemergence) herbicides has increased in the last decade. Numerous issues and production practices have driven this trend. Postemergence herbicides are commonly used in place of mechanical cultivation to control weeds in no-tillage (59,89,106). Atrazine [6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine] has historically been an economical, effective means of controlling weeds in corn (*Zea mays* L.). However, the continuing spread and development of triazine resistant weed species (88) and environmental concerns over the presence of atrazine in surface and groundwater have prompted corn producers and weed scientists to seek alternatives. Total postemergence programs are also attractive to promoters of

integrated weed management systems since they allow corn growers to determine the extent of the weed problem before making a herbicide application (45,104).

Nicosulfuron {2-[[[[(4,6-dimethoxy-2-pyrimidinyl)-amino]-carbonyl]-amino]sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide} and primisulfuron {2-[[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]-amino]sulfonyl]-benzoic acid} are sulfonylurea herbicides introduced to the corn market in 1990. Their introduction represented a major breakthrough in corn weed control. Nicosulfuron and primisulfuron provide selective control of emerged perennial and annual grasses and some broadleaf weeds at usage rates of 35 and 40 g a.i. ha^{-1}, respectively (1,2,45). Such low usage rates help solve handling, application and container-disposal issues, while reducing the amount of chemical applied to the field by a factor of 100-1000 over conventional herbicides (15). The sulfonylurea class of herbicides are also attractive from an environmental standpoint due to their low mammalian toxicity (3,4,45).

The combination of nicosulfuron or primisulfuron with postemergence broadleaf herbicides may provide single-application total postemergence weed control in corn (25,38,72). This type of weed control program was previously not available, and provides another option to the corn grower. However, the effects of early season weed competition with corn raise significant questions about this type of weed control program, since weeds are allowed to germinate and grow with the crop until the herbicides are applied.

The specificity and weed spectrum of the sulfonylureas is unpredictable (75). Although nicosulfuron and primisulfuron have similar chemical structures, usage rates, and a common site of action in susceptible plants, these herbicides display differential selectivity (3,4). An understanding of the basis for this differential selectivity might provide insight to improve herbicidal activity on tolerant weed species and to optimize their potential uses in weed control systems.

SULFONYLUREA HERBICIDES

Mode of herbicide action. Sulfonylurea herbicides are absorbed and translocated to their site of action where they inhibit the acetolactate synthase (ALS) enzyme in susceptible plants (8,27,28,55,64,80,82,83,87,102). Acetolactate synthase, also known as acetohydroxyacid synthase (AHAS), is a key enzyme in the branched-chain amino acid biosynthetic pathway of higher plants. ALS catalyzes the condensation of two pyruvate molecules to form CO_2 and α-acetolactate, which leads to valine and leucine synthesis, and the condensation of one molecule of pyruvate with α-ketobutyrate to form CO_2 and α-aceto-α-hydroxybutyrate, which leads to isoleucine formation (8). The ALS enzyme resides in the chloroplasts of plant cells (69).

Little is known about how inhibition of ALS results in plant death. Depletion of the branched-chain amino acid pool will contribute to inhibition of protein synthesis, but this may not be the primary pathway for inhibition of plant growth (30,84). The control of plant cell division has also been implicated as a possible contributor to plant death by sulfonylureas (8,10). Other research has implicated the buildup of the intermediate α-ketobutyrate as an important component of sulfonylurea herbicide action (65,87). α-Ketobutyrate has been shown to be toxic to bacteria cells, but little is known about the effects of high levels of this intermediate in higher plants (65,84,87).

Basis for selectivity. To exert toxicity, all postemergence herbicides must: 1) be absorbed into the foliage, and 2) move to the site of action. At the site of action the herbicide must be present in an adequate concentration and in the proper toxic form for sufficient duration of time (46,56). This sequence can fail in numerous places, thereby preventing adequate control of the weed (56).

Any factor which interrupts this sequence may contribute substantially to differential selectivity of postemergence herbicides between species, between species within the same genus, and different herbicides within the same species. Differential herbicide absorption, translocation, or metabolism are common contributing factors to differential selectivity (13,57,92,107). Properties of the ALS enzyme are also important factors which might contribute to the selectivity of herbicides whose mode of action is inhibition of this enzyme.

The specificity and weed spectrum of the sulfonylureas is unpredictable (75). Crop-selective sulfonylurea herbicides have been commercialized for use in wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), rice (*Oryza sativa* L.), corn, soybeans [*Glycine max* (L.) Merr.], and oilseed rape (*Brassica napus* L.), with additional crop-selective compounds in cotton (*Gossypium hirsutum* L.), potatoes (*Solanum tuberosum* L.), and sugarbeet (*Beta vulgaris* L.) (15).

Metabolism. The primary basis of selectivity for these herbicides is differential rate of herbicide metabolism. Tolerant species are able to rapidly detoxify sulfonylureas to herbicidally inactive products, while metabolism is much slower and less extensive in susceptible species (14,49,58,75,77,80,105,110). Metabolites of sulfonylurea herbicides have been shown to be inactive against plant ALS (14,16,58).

Metabolism is accomplished by a wide variety of processes. The relative importance of these processes, and the sites at which they occur, can differ significantly for the same compound in different species, or for close analogs in a given species (8). Pathways by which sulfonylurea herbicides are inactivated in plants include aryl and aliphatic hydroxylation followed by glucose conjugation, sulfonylurea bridge hydrolysis and sulfonamide bond cleavage, oxidative O-demethylation and direct conjugation with (homo)glutathione (15).

Absorption and translocation. Absorption and translocation of sulfonylurea herbicides is generally quite slow (5,6,17,23,42,66). Some research has correlated differences in absorption and translocation with plant sensitivity, but these processes were normally operating in conjunction with differences in metabolism (5,40,85,110). In some instances, translocation of sulfonylureas is greater in tolerant than in sensitive species (105). Generally, it is unlikely that species selectivity in this herbicide class results, primarily, from differential uptake and translocation (15,105).

ALS sensitivity. ALS enzyme sensitivity in sensitive plant species is similar to that in tolerant species, further emphasizing metabolism as the basis of selectivity (40,58,75,82,83,105). However, ALS sensitivity has been shown to be the basis for herbicide resistance in genetically engineered plants as well as weeds that have developed resistance through mutagenesis (7,27,28,47,52,53,54,67,81,91). This resistance results from production of an altered form of ALS that is enzymatically functional but much less sensitive than the normal enzyme to inhibition by sulfonylurea herbicides (27).

Although the vast majority of research has emphasized herbicide metabolism and de-emphasized ALS enzyme sensitivity as the basis for sulfonylurea selectivity, recent research has shown the level of tolerance to sulfonylureas may also be related to the amount or specific activity of ALS

present in the tissues (44). Elevated levels of a target enzyme may provide the basis for tolerance to certain herbicide molecules (39,44,73,96). Researchers found greater ALS content in roots than in shoots of certain corn inbreds. They concluded that the naturally occurring differences in ALS levels in the roots of the investigated inbred lines contribute largely to the differential in vivo response observed to chlorsulfuron {2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide} (44). Differential specific activity of ALS was correlated with in vivo tolerance to chlorsulfuron as well. In vivo corn tolerance to chlorsulfuron was greater with greater ALS specific activity (44).

Nicosulfuron and primisulfuron. Most research investigating absorption, translocation, metabolism, or ALS enzyme inhibition by nicosulfuron or primisulfuron has focused on corn, johnsongrass, or barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] (23,43,48,77).

Absorption of ^{14}C-nicosulfuron applied to a tolerant species (corn) or susceptible species (johnsongrass) was similar (about 40%). About 20-30% of the absorbed ^{14}C-nicosulfuron translocated beyond the treated leaf of rhizome johnsongrass at 21 days. Nicosulfuron was almost completely metabolized within 20 h in corn, while there was no perceptible metabolism in the treated leaves of johnsongrass even after 24 h (77).

Another study reported limited absorption of both primisulfuron and nicosulfuron by rhizome johnsongrass, but more nicosulfuron was absorbed than primisulfuron. Translocation out of the treated leaf was similar and less than 20% for both herbicides. Prevention of johnsongrass regrowth in the greenhouse by nicosulfuron but not by primisulfuron was attributed to differential absorption (23).

ALS from barnyardgrass and corn, and numerous weeds is similar in sensitivity to primisulfuron. Barnyardgrass is reported to be tolerant to primisulfuron because it can rapidly metabolize the herbicide into metabolites which do not inhibit ALS (75). Metabolism of primisulfuron in corn occurs by hydroxylation of the phenyl and pyrimidine rings followed by sugar conjugation. This process is catalyzed by a cytochrome P450-dependent monooxygenase system located in the microsomal membranes (43).

Other research has investigated corn hybrids with great differences in sensitivity to sulfonylurea herbicides. One study found ALS from normal and susceptible corn plants was equally inhibited by both nicosulfuron and primisulfuron. Herbicide uptake and initial translocation of nicosulfuron in both were also the same, but metabolism differed and was identified as the process responsible differential sensitivity (37). Variation in varietal response to nicosulfuron and primisulfuron was also due to metabolism (22).

Another study investigating corn inbreds and hybrids found greater than 40 000-fold differences in sensitivity to primisulfuron, despite similar ALS sensitivity (48). These results further support metabolism as the primary basis for differences in response. However, results from the same research found nicosulfuron was much less active than primisulfuron or thifensulfuron {3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylic acid} at the enzyme level in certain corn hybrids. These researchers concluded that the lower enzyme activity by nicosulfuron is probably the major factor in its greater corn tolerance. They also stated that the ALS enzyme in weeds is also less sensitive to nicosulfuron, but did not present the data (48).

Nicosulfuron and primisulfuron exhibit differential selectivity between species, and between varieties within species (1,2,22,37,48,75). Herbicide metabolism has been identified as the primary basis for these responses in many species (22,37,48,75,77), however, other factors have been implicated as possible contributors (23,48). A greater understanding of the interactions of nicosulfuron and primisulfuron with economically important weed species may lead to maximization of their potential uses for weed management in corn.

POSTEMERGENCE WEED CONTROL IN CORN

Nicosulfuron and primisulfuron. Nicosulfuron and primisulfuron were introduced to the corn herbicide market in 1990. Prior to their introduction, effective and reliable postemergence weed control in corn was limited to the use of selective herbicides which are effective against only broadleaf weeds, or directed sprays which require special application equipment to avoid crop interception of the herbicide during application (12).

Nicosulfuron and primisulfuron were the first selective postemergence herbicides for control of several grass weed species in corn (1,2). These herbicides also provided control of historically troublesome weeds in corn such as johnsongrass [*Sorghum halepense* (L.) Pers.] and quackgrass [*Elytrigia repens* (L.) Nevski] which were controlled by few preemergence herbicides and no selective postemergence herbicides (9,23,24,45,74,77). Nicosulfuron or primisulfuron in combination with postemergence broadleaf herbicides may provide single-application total postemergence weed control in corn (25,38,72).

Weed interference. The ability of weeds to interfere with the crop and reduce corn yields is well documented (51,61,62,63,71,76,98,99,100). Interference consists of 1) direct competition by weeds for light, water, and nutrients or 2) allelopathy which is the inhibition of plant growth through production of

biological toxins by the weeds (90). The degree to which weeds may interfere with a crop depends upon the weed species (19,20,21,29,35,79,86,94,97,103), weed density (11,19,20,21,26,29,32,34,35,36,62,71,93,98,103,111), weed growth and rate of development (29,50,60,70,95), environmental conditions (33,34,41,50,68,93,111), edaphic conditions (18,20,41,76,99,100,101), control of insects and diseases (18), and duration of interference (51,61,63,78,100). Perhaps the most significant effect of weed interference is crop yield reduction.

The term "critical period" has been used to describe the duration of weed control (51) or weed interference (109) which may occur without significant yield reduction as a result of weed interference. There are two recognized components of the critical period. The first component is the length of time the crop must remain weed-free to prevent crop yield loss. The second component is the length of time weeds can remain in the crop before they interfere with crop growth and ultimately reduce yield (104,108,109,112).

With a total postemergence weed control program in corn, we are concerned with the period of time the weeds interfere with the crop until the postemergence herbicide is applied. The longer application is delayed, the greater the duration of weed interference and the more likely the "critical period" will be exceeded and yields reduced.

Competitive effects of giant foxtail (*Setaria faberi* Herrm.) with corn are important early in the growing season (61). Increasing duration of giant foxtail

interference reduced yields gradually (61). Significant yield reductions occurred when giant foxtail was allowed to interfere with corn until weeds were 15-23 cm in height and corn was 41-61 cm in height with 9-10 leaves (61).

A study in Ontario, Canada found the critical period to occur from the 4-leaf to 14-leaf stage in corn. The critical period varied with environment, weed species, and weed density (51).

Herbicide application timing. Traditional studies investigating duration of weed interference in corn have employed hand pulling or hoeing as the method of weed removal to end the period of weed interference. This method ceases weed competition with the crop abruptly and completely. This is not an accurate approximation of herbicide action. A postemergence herbicide, even when completely effective, does not terminate weed competition in such a fashion.

Sulfonylurea herbicides in particular kill plants relatively slowly. Complete plant death in susceptible species occurs in 7-21 days (3) and 10-30 days (4) with nicosulfuron and primisulfuron respectively, depending upon growing conditions, weed species, and growth stage of the weeds. Weed interference could still occur with the crop after herbicide application. It would also seem possible that an effective herbicide treatment could completely control weeds after the critical period of weed interference has expired. Weed interference would have irreversibly reduced corn yield in such a situation.

The grower has some level of control over the duration of weed interference with the crop through the choice of weed control methods and time of control. If a grower adopts a total postemergence weed control program in corn, the time of herbicide application is important not only to insure successful weed control, but to control the duration of weed interference and prevent crop yield reduction. Knowledge of the maximum duration of interference allowable before yield reduction occurs would be valuable information needed to effectively implement total postemergence herbicide programs in corn.

LITERATURE CITED

1. Anonymous. 1993. Accent® herbicide product label. E.I. duPont de Nemours and Co., Agricultural Products, Wilmington, DE 19898.

2. Anonymous. 1993. Beacon® herbicide product label. CIBA-GEIGY Corp., Agricultural Division, Greensboro, NC 27419.

3. Anonymous. 1990. Accent® Herbicide. Technical Bull. E.I. duPont de Nemours and Co., Agricultural Products, Wilmington, DE 19898. 12 p.

4. Anonymous. 1989. Beacon® Herbicide. Technical Release. Agricultural Division, CIBA-GEIGY Corp., Greensboro, NC 27419. 8 p.

5. Baird, J.H., J.W. Wilcut, G.R. Wehje, R. Dickens, and S. Sharpe. 1989. Absorption, translocation, and metabolism of sulfometuron in centipedegrass (*Eremochloa ophiuroides*) and bahiagrass (*Paspalum notatum*). Weed Sci. 37:42-46.

6. Beckett, T.H. and E.W. Stoller. 1991. Effects of methylammonium and urea ammonium nitrate uptake of thifensulfuron in velvetleaf (*Abutilon theophrasti*). Weed Sci. 39:333-338.

7. Bedbrook, J., R.S. Chaleff, S.C. Falco, B.J. Mazur, and N. Yadav. 1988. Nucleic Acid Fragment Encoding Herbicide Resistant Plant Acetolactate Synthase. Eur. Patent Appl. 0257993.

8. Beyer, E.M. Jr., M.J. Duffy, J.V. Hay, and D.D. Schleuter. 1988. Sulfonylureas. p. 117-189 *in* P.C. Kearney and D.D. Kaufman eds. Herbicides: Chemistry, Degradation and Mode of Action. 3rd ed., Marcel-Dekker, New York.

9. Bhowmik, P.C., B.M. O'Toole, and J. Andaloro. 1992. Effects of nicosulfuron on quackgrass (*Elytrigia repens*) control in corn (*Zea mays*). Weed Technol. 6:52-56.

10. Blair, A.M. and T.D. Martin. 1988. A review of the activity, fate and mode of action of sulfonylurea herbicides. Pest. Sci. 22:195-219.

11. Bloomberg, J.R., B.L. Kirkpatrick, and L.M. Wax. 1982. Competition of common cocklebur (*Xanthium pensylvanicum*) with soybean (*Glycine max*). Weed Sci. 30:507-513.

12. Bode, L.E. 1987. Spray application technology. p. 85-109 *in* C.G. McWhorter and M.R. Gebhardt, eds. Methods of Applying Herbicides. Weed Science Society of America, Champaign, IL.

13. Boldt, P.F. and A.R. Putnam. 1981. Selectivity mechanisms for foliar applications of diclofop-methyl. II. metabolism. Weed Sci. 29:237-241.

14. Brown, H.M. and S.M. Neighbors. 1987. Soybean metabolism of chlorimuron ethyl: physiological basis for soybean selectivity. Pestic. Biochem. Physiol. 29:112-120.

15. Brown, H.M. 1990. Mode of action, crop selectivity, and soil relations of the sulfonylurea herbicides. Pestic. Sci. 29:263-281.

16. Brown, H.M., V.A. Wittenbach, D.R. Forney, and S.D. Strachan. 1990. Basis for soybean tolerance to thifensulfuron methyl. Pestic. Biochem. Physiol. 37:303-313.

17. Bruce, J.A., D. Penner, and J.J. Kells. 1993. Absorption and activity of nicosulfuron and primisulfuron in quackgrass (*Elytrigia repens*) as affected by adjuvants. Weed Sci. 41:218-224.

18. Buchanan, G.A. 1981. Management of weeds in cotton. p. 215-242 *in* D. Pimental, ed. Handbook of Pest Management in Agriculture. Vol. III. CRC Press, Boca Raton, FL.

19. Buchanan, G.A. and E.R. Burns. 1971. Weed competition in cotton I. sicklepod and tall morningglory. Weed Sci. 19:576-579.

20. Buchanan, G.A. and E.R. Burns. 1971. Weed competition in cotton II. cocklebur and redroot pigweed. Weed Sci. 19:580-582.

21. Buchanan, G.A., R.H. Crowley, J.E. Street, and J.A. McGuire. 1980. Competition of sicklepod (*Cassia obtusifolia*) and redroot pigweed (*Amaranthus retroflexus*) with cotton (*Gossypium hirsutum*). Weed Sci. 28:258-262.

22. Burton, J.D., E.M. Maness, D.W. Monks, and D.K. Robinson. 1992. Differential herbicide tolerance of Landmark and Merit sweet corn is due to different rates of metabolism. Abstr. Weed Sci. Soc. Am. 32:91.

23. Camacho, R.F. and L.J. Moshier. 1991. Absorption, translocation, and activity of CGA-136872, DPX-V9360, and glyphosate in rhizome johnsongrass (*Sorghum halepense*). Weed Sci. 39:354-357.

24. Camacho, R.F., L.J. Moshier, D.W. Morishita, and D.L. Devlin. 1991. Rhizome johnsongrass(*Sorghum halepense*) control in corn (*Zea mays*) with primisulfuron and nicosulfuron. Weed Technol. 5:789-794.

25. Carey, J.B., P.B. Knoerr, and J.J. Kells. 1990. Herbicide interactions with nicosulfuron and primisulfuron in corn. Abstr. Weed Sci. Soc. Am. 32:12.

26. Carey, J.B. and M.S. DeFelice. 1991. Timing of chlorimuron and imazaquin application for weed control in no-till soybeans (*Glycine max*). Weed Sci. 39:232-237.

27. Chaleff, R.S. and C.J. Mauvais. 1984. Acetolactate synthase is the site of action of two sulfonylurea herbicides in higher plants. Science 224:1443-1445.

28. Chaleff, R.S. and T.B. Ray. 1984. Herbicide resistant mutants of tobacco cell cultures. Science 223:1148-1151.

29. Chandler, J.M. 1977. Competition of spurred anoda, velvetleaf, prickly sida, and Venice mallow in cotton. Weed Sci.25:151-158.

30. Cobb, A. 1992. The inhibition of amino acid biosynthesis. p. 131-143 *in* Herbicides and Plant Physiology. Chapman & Hall, New York, NY.

31. Cobb, A. 1992. Preface. p. xi-xii *in* Herbicides and Plant Physiology. Chapman & Hall, New York, NY.

32. Coble, H.D. and R.L. Ritter. 1978. Pennsylvania smartweed (*Polygonum pensylvanicum*) interference in soybeans (*Glycine max*). Weed Sci. 26:556-559.

33. Coble, H.D., F.M. Williams, and R.L. Ritter. 1981. Common ragweed (*Ambrosia artemisiifolia*) interference in soybeans (*Glycine max*). Weed Sci. 29:339-342.

34. Cordes, R.C. and T.T. Bauman. 1984. Field competition between ivyleaf morningglory (*Ipomoea hederacea*) and soybeans (*Glycine max*). Weed Sci. 32:364-370.

35. Crowley, R.H. and G.A. Buchanan. 1978. Competition of four morningglory (*Ipomoea spp.*) species with cotton (*Gossypium hirsutum*). Weed Sci. 26:484-488.

36. Diarra, A., R.J. Smith, Jr., and R.E. Talbert. 1985. Interference of red rice (*Oryza sativa*) with rice (*O. sativa*). Weed Sci. 33:644-649.

37. Diehl, K.E., H. Mukaida, R.A. Liebel, and E.W. Stoller. 1993. Sensitivity mechanism in an ALS-susceptible corn hybrid. Abstr. Weed Sci. Soc. Am. 33:191.

38. Dobbels, A.F. and G. Kapusta. 1992. Postemergence weed control in corn with nicosulfuron combinations. Proc. North Cent. Weed Sci. Soc. 47:47.

39. Donn, G., E. Tischer, J.A. Smith, and H.M. Goodman. 1984. Herbicide-resistant alfalfa cells: an example of gene amplification in plants. J. Mol. Appl. Genet. 2:621-635.

40. Eberlein, C.V., K.M. Rosow, J.L. Geadelmann, and S.J. Openshaw. 1989. Differential tolerance of corn genotypes to DPX-M6316. Weed Sci. 37:651-657.

41. Feltner, K.C., H.R. Hurst, and L.E. Anderson. 1969. Yellow foxtail competition in grain sorghum. Weed Sci. 17:211-213.

42. Fielding, R.J. and E.W. Stoller. 1990. Effects of additives on efficacy, uptake, and translocation of chlorimuron ethyl ester. Weed Technol. 4:264-271.

43. Fonnè-Pfister, R., J. Gaudin, K. Kreuz, K. Ramsteiner, and E. Ebert. 1990. Hydroxylation of primisulfuron by an inducible cytochrome P450-dependent monooxygenase system from maize. Pestic. Biochem. Physiol. 37:165-173.

44. Forlani, G., E. Nielsen, P. Landi, and R. Tuberosa. 1991. Chlorsulfuron tolerance and acetolactate synthase activity in corn (*Zea mays* L.) inbred lines. Weed Sci. 39:553-557.

45. Foy, C.L. and H.L. Witt. 1990. Johnsongrass control with DPX-V9360 and CGA-136872 in corn (*Zea mays*) in Virginia. Weed Technol. 4:615-619.

46. Freed, V.H. and R.O. Morris. 1967. eds. Environmental and other factors in the responses of plants to herbicides. Tech. Bull. 100, Oregon State Univ., Corvallis, OR.

47. Gebard, J.M., P.J. Charest, V.N. Iyer, and B.L. Miki. 1989. Cross-resistance to short residual sulfonylurea herbicides in transgenic tobacco plants. Plant Physiol. 91:574-580.

48. Green, J.M. and J.F. Ulrich. 1993. Response of corn (*Zea mays* L.) inbreds and hybrids to sulfonylurea herbicides. Weed Sci. 41:508-516.

49. Hageman, L.H. and R. Behrens. 1984. Basis for response differences of two broadleaf weeds to chlorsulfuron. Weed Sci. 32:162-167.

50. Hagood, E.S., Jr., T.T. Bauman, J.L. Williams, Jr., and M.M. Schreiber. 1981. Growth analysis of soybeans (*Glycine max*) in competition with jimsonweed (*Datura stramonium*). Weed Sci. 29:500-504.

51. Hall, M.R., C.J. Swanton, and G.W. Anderson. 1992. The critical period of weed control in grain corn (*Zea mays*). Weed Sci. 40:441-447.

52. Hart, S.E., J.W. Saunders, and D. Penner. 1993. Semidominant nature of monogenic sulfonylurea herbicide resistance in sugarbeet (*Beta vulgaris*). Weed Sci. 41:317-324.

53. Hart, S.E., J.W. Saunders, and D. Penner. 1992. Chlorsulfuron-resistant sugarbeet: cross-resistance and physiological basis of resistance. Weed Sci. 40:378-383.

54. Haughn, G.W., J. Smith, B. Mazur, and C. Somerville. 1988. Transformation with a mutant *Arabadopsis* acetolactate synthase gene renders tobacco resistant to sulfonylurea herbicides. Mol. Gen. Genet. 211:266-271.

55. Hay, J.V. 1990. Chemistry of sulfonylurea herbicides. Pestic. Sci. 29:247-261.

56. Hess, F.D. and R.H. Falk. 1990. Herbicide deposition on leaf surfaces. Weed Sci. 38:280-288.

57. Holly, K. 1976. Selectivity in relation to formulation and application methods. p. 249-275 *in* L.J. Audus, ed. Herbicides - Physiology, Biochemistry, Ecology. Vol. II. Academic Press, London.

58. Hutchison, J.M., R. Shapiro, and P.B. Sweetser. 1984. Metabolism of chlorsulfuron by tolerant broadleaves. Pestic. Biochem. Physiol. 22:243-247.

59. Kapusta, G. 1979. Seedbed tillage and herbicide influence on soybean (*Glycine max*) weed control and yield. Weed Sci. 27:520-526.

60. Keisling, T.C., L.R. Oliver, R.H. Crowley, and F.L. Baldwin. 1984. Potential use of response surface analyses for weed management in soybeans (*Glycine max*). Weed Sci. 32:552-557.

61. Knake, E.L. and F.W. Slife. 1969. Effect of time of giant foxtail removal from corn and soybeans. Weed Sci.17:281-283.

62. Knake, E.L. and F.W. Slife. 1962. Competition of *Setaria faberii* with corn and soybeans. Weeds 10:26-29.

63. Knake, E.L. and F.W. Slife. 1965. Giant foxtail seeded at various times in corn and soybeans. Weeds 13:331-334.

64. LaRossa, R.A. and S.C. Falco. 1984. Amino acid biosynthetic enzymes as targets of herbicide action. Trends Biotechnol. 2:158-161.

65. LaRossa, R.A., T.K. VanDyk, and D.R. Smulski. 1987. Toxic accumulation of α-ketobutyrate caused by inhibition of the branched-chain amino acid biosynthetic enzyme acetolactate synthase in *Salmonella typhimurium*. J. Bacteriol. 169-1372-1378.

66. Leys, A.R. and F.W. Slife. 1988. Absorption and translocation of ^{14}C-chlorsulfuron and ^{14}C-metsulfuron in wild garlic (*Allium vineale*). Weed Sci. 36:1-4.

67. Mallory-Smith, C.A., D.C. Thill, and M.J. Dial. 1990. Identification of sulfonylurea herbicide-resistant prickly lettuce (*Lactuca serriola*) Weed Technol. 4:163-168.

68. McWhorter, C.G. and E.E. Hartwig. 1972. Competition of johnsongrass and cocklebur with six soybean varieties. Weed Sci. 20:56-59.

69. Miflin, B. 1974. The localization of nitrate reductase and other enzymes related to amino acid biosynthesis in the plastids of root and leaves. Plant Physiol. 54:550.

70. Moiser, D.G. 1986. Common cocklebur (*Xanthium strumarium*) and entireleaf morningglory (*Ipomoea hederacea* var. *integriuscula*) interference on soybeans (*Glycine max*). Ph.D. dissertation, Univ. Arkansas, Fayetteville.

71. Moolani, M.K., E.L. Knake, and F.W. Slife. 1964. Competition of smooth pigweed with corn and soybeans. Weeds 12:126-128.

72. Moseley, C.M., M.G. Schnappinger, J. Cantwell, and S.W. Pruss. 1992. Primisulfuron combinations for broad spectrum weed control in corn. Proc. North Cent. Weed Sci. Soc. 47:39.

73. Nafziger, E.D., J.M. Widholm, H.C. Steinrücken, and J.L. Killmer. 1984. Isolation and characterization of a carrot cell line tolerant to glyphosate. Plant Physiol. 76:571-574.

74. Nalewaja, J.D., Z. Woznica, and F.A. Manthey. 1991. DPX-V9360 efficacy with adjuvants and environment. Weed Technol. 3:227-231.

75. Neighbors, S. and L.S. Privalle. 1990. Metabolism of primisulfuron by barnyard grass. Pestic. Biochem. Physiol. 37:145-153.

76. Nieto, J.H. and D.W. Staniforth. 1961. Corn-foxtail competition under various production conditions. Agron. J. 53:1-5.

77. Obrigawitch, T.T., W.H. Kenyon, and H. Kuratle. 1990. Effect of application timing on rhizome johnsongrass (*Sorghum halepense*) control with DPX-V9360. Weed Sci. 38:45-49.

78. Oliver, L.R. 1988. Principles of weed threshold research. Weed Technol. 2:398-403.

79. Patterson, D.T. and E.P. Flint. 1983. Comparative relations, photosynthesis, and growth of soybean (*Glycine max*) and seven associated weeds. Weed Sci. 31:318-323.

80. Petersen, P.J. and B.A. Swisher. 1985. Absorption, translocation, and metabolism of ^{14}C-chlorsulfuron in Canada thistle (*Cirsium arvense*). Weed Sci. 33:7-11.

81. Primiani, M.M., J.C. Cotterman, and L.L. Saari. 1990. Resistance of kochia (*Kochia scoparia*) to sulfonylurea and imidazolinone herbicides. Weed Technol. 4:169-172.

82. Ray, T.B. 1986. Sulfonylurea herbicides as inhibitors of amino acid biosynthesis in plants. TIBS 11:180-183.

83. Ray, T.B. 1984. Site of action of chlorsulfuron: inhibition of valine and isoleucine biosynthesis in plants. Plant Physiol. 75:827-831.

84. Ray, T.B. 1989. Herbicides as inhibitors of amino acid biosynthesis. p. 105-122 *in* P. Böger and G. Sandmann, eds. Target Sites of Herbicide Action. CRC Press, Boca Raton, FL.

85. Reddy, K.N. and L.E. Bendixen. 1988. Toxicity, absorption, translocation, and metabolism of foliar-applied chlorimuron in yellow and purple nutsedge (*Cyperus esculentus* and *C. rotundus*). Weed Sci. 36:707-712.

86. Reynolds, D.B. 1984. Johnsongrass (*Sorghum halepense*) interference with soybeans (*Glycine max*) and cotton (*Gossypium hirsutum*). M.S. thesis, Univ. Arkansas, Fayetteville.

87. Rhodes, D., A.L. Hogan, L. Deal, G.C. Jamieson, and P. Haworth. 1987. Amino acid metabolism of *Lemna minor* L. Plant Physiol. 84:775-780.

88. Ritter, R.L. 1989. Understanding Herbicide Resistance in Weeds. Sandoz Corp. Des Plaines. 35 p.

89. Robinson, E.L., G.W. Langdale, and J.A.Stuedemann. 1984. Effect of three weed control regimes on no-till and tilled soybeans (*Glycine max*). Weed Sci. 32:17-19.

90. Ross, M.A. and C.A. Lembi. 1985. Characteristics and importance of weeds. p.1-19 *in* Applied Weed Science. Burgess Publishing Co., Minneapolis, MN.

91. Saari, L.L., S.C. Cotterman, and M.M. Primiani. 1990.Mechanism of sulfonylurea resistance in the broadleaf weed, *Kochia scoparia*. Plant Physiol. 93:55-61.

92. Sargent, J.A. 1976. Relationship of selectivity to uptake and movement. p. 303-312 *in* L.J. Audus, ed. Herbicides - Physiology, Chemistry, Ecology, Vol. II. Academic Press, London.

93. Schweizer, E.E. 1983. Common lambsquarters (*Chenopodium album*) interference in sugarbeets (*Beta vulgaris*). Weed Sci. 31:5-8.

94. Shurtleff, J.L. and H.D. Coble. 1985. The interaction of soybean (*Glycine max*) and five weed species in the greenhouse. Weed Sci. 33:669-672.

95. Sims, B.D. 1986. Influence of johnsongrass (*Sorghum halepense*) and sicklepod (*Cassia obtusifolia*) on soybean (*Glycine max*) growth and yield. Ph.D. dissertation, Univ. Arkansas, Fayetteville.

96. Smart, C.C., D. Johanning, G. Muller, and N. Amrhein. 1985. Selective overproduction of 5-*enol*-pyruvylshikimic acid 3-phosphate synthase in a plant cell culture which tolerates high doses of the herbicide glyphosate. J. Biol. Chem. 260:16338-16346.

97. Snipes, C.E., G.A. Buchanan, J.E. Street, and J.A. McGuire. 1982. Competition of common cocklebur (*Xanthium pensylvanicum*) with cotton (*Gossypium hirsutum*). Weed Sci. 30:553-556.

98. Staniforth, D.W., W.G. Lovely, and F.B. Cady. 1965. Sampling procedures for estimates of weed yields in corn plots. Weeds 13:357-360.

99. Staniforth, D.W. 1961. Responses of corn hybrids to yellow foxtail competition. Weeds 9:132-136.

100. Staniforth, D.W. 1957. Effects of annual grass weeds on the yield of corn. Agron. J. 49:551-555.

101. Staniforth, D.W. 1962. Responses of soybean varieties to weed competition. Agron. J. 54:11-13.

102. Stidham, M.A. 1991. Herbicides that inhibit acetohydroxyacid synthase. Weed Sci. 39:428-434.

103. Street, J.E., G.A. Buchanan, R.H. Crowley, and J.A. McGuire. 1981. Influence of cotton (*Gossypium hirsutum*) densities on competitiveness of pigweed (*Amaranthus retroflexus*) and sicklepod (*Cassia obtusifolia*). Weed Sci. 29:253-256.

104. Swanton, C.J. and S.F. Weise. 1991. Integrated weed management: the rationale and approach. Weed Technol. 5:657-663.

105. Sweetser, P.B., G.S. Schow, and J.M. Hutchison. 1982. Metabolism of chlorsulfuron by plants: biological basis for selectivity of a new herbicide for cereals. Pestic. Biochem. Physiol. 17:18-23.

106. Triplett, G.B., Jr. 1978. Weed control for doublecrop soybeans planted with the no-tillage method following grain harvest. Agron. J. 70:577-581.

107. Wain, R.L. and M.S. Smith. 1976. Selectivity in relation to metabolism. p. 279-320 *in* L.J. Audus, ed. Herbicides - Physiology,Biochemistry, Ecology. Vol. II. Academic Press, London.

108. Weaver, S.E. 1984. Critical period of weed competition in three vegetable crops in relation to management practices. Weed Res. 24:317-325.

109. Weaver, S.E. and C.S. Tan. 1983. Critical period of weed interference in transplanted tomatoes (*Lycopersicon esculentum*): growth analysis. Weed Sci. 31:476-481.

110. Wilcut, J.W., G.R. Wehtje, M.G. Patterson, T.A. Cole, and T.V. Hicks. 1989. Absorption, translocation, and metabolism of foliar-applied chlorimuron in soybeans (*Glycine max*), peanuts (*Arachis hypogaea*), and selected weeds. Weed Sci. 37:175-180.

111. Young, F.L., D.L. Wyse, and R.J. Jones. 1984. Quackgrass (*Agropyron repens*) interference on corn (*Zea mays*). Weed Sci. 32:226-234.

112. Zimdahl, R.L. 1988. The concept and application of the critical weed-free period. p. 145-155 *in* M.A. Altieri and M. Liebman, eds. Weed Management in Agroecosystems: Ecological Approaches. CRC Press, Boca Raton, FL.

Physiological Basis For Nicosulfuron and Primisulfuron Selectivity in Five Plant Species[1]

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Abstract. Greenhouse and laboratory studies were conducted to determine the physiological basis for selectivity of nicosulfuron and primisulfuron in five plant species. Differential sensitivity of the species was quantified by determining GR_{50} values (herbicide rate required to reduce plant growth by 50%) for each species/herbicide combination. GR_{50} data indicated the following levels of sensitivity; corn - tolerant to both herbicides, seedling johnsongrass - sensitive to both herbicides, barnyardgrass - sensitive to nicosulfuron and tolerant to primisulfuron, giant foxtail - sensitive to nicosulfuron and tolerant to primisulfuron, and eastern black nightshade - tolerant to nicosulfuron and sensitive to primisulfuron. Studies utilizing ^{14}C-radiolabelled herbicides were conducted to determine if differential herbicide absorption, translocation, or metabolism contributed to whole plant responses. Nicosulfuron and primisulfuron selectivity in corn, johnsongrass, barnyardgrass, or giant foxtail

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was primarily due to differential herbicide metabolism rate. Tolerant species metabolized the herbicide more rapidly and extensively than sensitive species. Differential herbicide absorption, translocation, or metabolism did not explain differential sensitivity of eastern black nightshade to the herbicides. Further studies indicated that differential eastern black nightshade response to nicosulfuron and primisulfuron was due to differential ALS enzyme sensitivity. The ALS sensitivity of johnsongrass and eastern black nightshade was similar in the presence of nicosulfuron. A combination of ALS level and herbicide translocation are factors which contribute to differential selectivity of nicosulfuron for eastern black nightshade and johnsongrass. Nomenclature: Nicosulfuron, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]-sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide; primisulfuron, 2-[[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl]benzoic acid; barnyardgrass, *Echinochloa crus-galli* (L.) Beauv. #[3] ECHCG; eastern black nightshade, *Solanum ptycanthum* Dun. # SOLPT; giant foxtail, *Setaria faberi* Herrm. # SETFA; johnsongrass, *Sorghum halepense* (L.) Pers. # SORHA; corn, *Zea mays* L. 'Pioneer 3751'.

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

Additional index words. Absorption, acetolactate synthase, corn, GR_{50}, metabolism, sulfonylurea, tolerance, translocation, ECHCG, SETFA, SOLPT, SORHA.

INTRODUCTION

Nicosulfuron and primisulfuron are sulfonylurea herbicides introduced to the U.S. corn market in 1990. They were the first selective postemergence herbicides which would effectively control problem perennial and annual grasses as well as some broadleaf weeds in corn (3,4,17). As members of the sulfonylurea family of herbicides, nicosulfuron and primisulfuron have similar chemical structures, usage rates, and the same site of action (1,2,3,4,17). Sulfonylurea herbicides inhibit the acetolactate synthase (ALS)[4] enzyme in susceptible plants (5,12,13,23,26,34,36,37,38,42). Despite all the similarities, there is a difference in the selectivity of nicosulfuron and primisulfuron for species they will or will not control (3,4).

The specificity and weed spectrum of this family of herbicides is unpredictable (32). Sulfonylurea herbicides are used in wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), rice (*Oryza sativa* L.), corn, soybeans [*Glycine max* (L.) Merr.], and oilseed rape (*Brassica napus* L.), with additional crop-selective compounds in cotton (*Gossypium hirsutum* L.), potatoes (*Solanum tuberosum* L.), and sugarbeet (*Beta vulgaris* L.) (8).

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[4]Abbreviations: ALS, acetolactate synthase; PPFD, photosynthetic photon flux density; NIS, nonionic surfactant; GR_{50}, herbicide rate required to reduce plant growth by 50%; LSS, liquid scintillation spectrometry; TLC, thin-layer chromatography; FAD, flavin adenine dinucleotide; I_{80}, herbicide concentration required to inhibit enzyme activity by 80%; HAT, hours after treatment.

To exert toxicity, all postemergence herbicides must be absorbed into the foliage and move to the site of action where they must be present in an adequate concentration and proper toxic form for a sufficient duration of time (18,24). Any factor which does not allow this sequence to occur may account for differential selectivity of herbicides between species, or differential sensitivity of a species between herbicides. Properties of the ALS enzyme are also important factors which can affect the selectivity of herbicides whose mode of action is inhibition of this enzyme.

The primary basis for selectivity of sulfonylurea herbicides is differential rate of herbicide metabolism. Tolerant species are able to rapidly detoxify sulfonylureas to herbicidally inactive products, while metabolism is much slower and less extensive in susceptible species (7,20,25,32,33,34,43,45). Metabolites of sulfonylurea herbicides have been shown to be inactive against plant ALS (7,9,25,32). Nicosulfuron was rapidly metabolized within 20 h in corn (tolerant) while there was no perceptible metabolism in johnsongrass (sensitive) even after 24 h (33). Barnyardgrass is tolerant of primisulfuron because it can rapidly metabolize the herbicide into products which do not inhibit ALS (32).

ALS from tolerant and sensitive plants is normally similar in sensitivity to nicosulfuron and primisulfuron (14,19,32), further emphasizing metabolism as the primary basis for differences in response. However, nicosulfuron is much less active than primisulfuron at the enzyme level in certain corn hybrids and

unspecified weed species. This differential enzyme sensitivity is probably the major factor in the greater tolerance of these corn hybrids to nicosulfuron (19). The level of tolerance to sulfonylureas may also be related to the amount or specific activity of ALS present in tissues. Naturally occurring differences in ALS levels from the roots of certain corn inbreds contribute largely to the differential in vivo responses to chlorsulfuron (16).

Nicosulfuron and primisulfuron display differential selectivity between species, between varieties within species, and between herbicides within species (1,2,10,14,19,32). Herbicide metabolism has been identified as the primary basis for these responses (10,14,19,32,33), however, other factors have been implicated as possible contributors (11,19).

The objective of this research was to determine the physiological basis for nicosulfuron and primisulfuron selectivity in corn, johnsongrass, barnyardgrass, giant foxtail, and eastern black nightshade.

MATERIALS AND METHODS

Plant material. Barnyardgrass and giant foxtail seed were obtained from a commercial seed supplier[5]. Johnsongrass seed was collected in the state of

[5]F & J Seed Service, P.O. Box 82, Woodstock, IL 60098-0082.

Mississippi and eastern black nightshade was collected on the Michigan State University Crop and Soil Science Research Farm at East Lansing. The corn variety investigated was Pioneer 3751[6]. The same seed material was used for all studies.

Plants used for all experiments were grown in the greenhouse using 945-ml plastic pots with a commercial potting mix[7]. Environmental conditions were maintained at 24 $\pm$ 2 C in a 16-h photoperiod of natural and supplemental metal halide lighting with an average midday photosynthetic photon flux density (PPFD)[4] of 700 $\mu E\ m^{-2}\ s^{-1}$. Plants were watered and fertilized as needed to insure maximum growth. Plants were thinned after emergence to uniform numbers and growth stages.

Species sensitivity. Individual greenhouse experiments were conducted to compare the sensitivity of each plant species to nicosulfuron and primisulfuron. Plants were grown to the stages presented in Table 1. Growth stages were chosen as representative of those normally treated with a field application of either herbicide. Commercial formulations of nicosulfuron or primisulfuron were

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[6]Pioneer Hi-Bred International, Inc., Des Moines, IA.

[7]Baccto Professional Planting Mix. Michigan Peat Co., P.O. Box 980129, Houston, TX 77098

applied at a range of rates with nonionic surfactant[8] (NIS)[4] at a rate of 0.25% (v/v). All herbicides were applied with a laboratory sprayer fitted with a 8001E flat fan nozzle[9] delivering 234 L ha^{-1} at 170 kPa. Root uptake of herbicide was prevented by covering the soil surface with vermiculite before herbicide application. The vermiculite was removed after treatment.

Herbicide injury was rated visually at 10 to 12 d after treatment with 0 representing no visible injury and 100 representing plant death. Above ground biomass was harvested and dry weight determined immediately after visual ratings. Experiments were conducted and analyzed separately for each species. Nonlinear regression analysis was conducted [$Y = B(1) \times e^{(B(2) \times X)} + B(3)$] and GR_{50}[4] values (herbicide rates required to reduce plant growth by 50%) calculated based on both visual ratings and percent dry weight reduction. GR_{50} values (Table 1.) are means from two experiments each with four replications, with the exception of eastern black nightshade dry weight reduction which is from one experiment. Paired t-tests at $\alpha = 0.05$ were used to compare GR_{50} values for nicosulfuron and primisulfuron within a species. No test was conducted for corn since no GR_{50} was identified for either herbicide.

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[8]X-77®-Nonionic-type spreader and activator. Principle functioning agents: Alkylaryl polyoxyethylene, free fatty acids, glycols, isopropanol. Constituents effective as spray adjuvant-90%. Constituents ineffective as spray adjuvant-10%. Valent U.S.A. Corp., 1333 N. California Blvd., P.O. Box 8025, Walnut Creek, CA 94596-8025.

[9]Spraying Systems Co., North Avenue, Wheaton, IL 60188.

Absorption, translocation, and metabolism. A single experiment utilizing ^{14}C-radiolabelled herbicides was designed to compare absorption, translocation, and metabolism of nicosulfuron and primisulfuron among all five plant species.

Plants were initially grown in the greenhouse under the conditions described previously. Plants were transferred to a uniform environment in growth chambers approximately 7 days before treatment. Environmental conditions in the growth chambers were maintained at 26/22 C day/night temperatures and 45/55 % day/night relative humidities. Lighting was provided by fluorescent and incandescent lamps at 500 $\mu E\ m^{-2}\ s^{-1}$ PPFD with a 16-h photoperiod. Plants were grown to the same stages as used in GR_{50} studies.

The youngest fully developed leaf of each species was chosen for treatment with ^{14}C-herbicide. This was the third true leaf of corn, johnsongrass, and barnyardgrass, and the fourth and fifth true leaf of giant foxtail and eastern black nightshade, respectively. To achieve sufficient uptake of radioactivity for metabolism research, a minimum of 3.7×10^3 Bq would need to be applied to the treated leaf. Preliminary spray retention studies were conducted to determine the amount of spray solution and herbicide dosage that the leaf chosen for ^{14}C treatment intercepts during a herbicide application. A version of the technique reported by Boldt and Putnam (6) was followed. Consideration of this information in conjunction with the specific activity of the radiolabelled herbicides allowed us to determine the concentration and amount of ^{14}C-spotting

solution to apply as the treatment. None of the species received a ^{14}C treatment which exceeded the normal herbicide dosage for the treated leaf by more than 1.5 X.

The leaf targeted for ^{14}C-herbicide application was covered with cellophane and the remainder of the plant treated with unlabelled nicosulfuron at 35 g ai ha^{-1} or primisulfuron at 40 g ai ha^{-1} and NIS at 0.25% v/v. Herbicides were applied with the same equipment and conditions as used for spray retention and GR_{50} studies. The cellophane was removed and ^{14}C-herbicides applied immediately thereafter to the youngest fully developed leaf.

The radiolabelled spotting solution contained pyrimidine-2-^{14}C-nicosulfuron (2.3×10^6 Bq mg^{-1} specific activity, 98.8% purity) or phenyl-^{14}C-primisulfuron (1.9×10^6 Bq mg^{-1} specific activity, 97.2% purity), with appropriate amounts of formulation blank, NIS, and water diluent to approximate a normal spray solution. Each treatment consisted of six droplets of solution at 2 μL each for a total of 3.7×10^3 Bq of radioactivity applied to the adaxial surface of the treated leaf. Pursuant to results of the spray retention studies, johnsongrass, barnyardgrass, and giant foxtail treatments were split between two plants by applying three droplets to each plant. Multiple plant parts were combined for analysis.

Plants were returned to growth chambers immediately after treatment. Treated leaves were excised from the plant at 12, 72, or 168 h after treatment.

The leaf was rinsed in methanol:distilled water (2:1, v/v) to remove unabsorbed herbicide. The rinse solution was radioassayed by liquid scintillation spectrometry (LSS)[4]. All plant parts were immediately frozen on dry ice and stored at -30 C until further analysis.

All plant parts excluding the treated leaf were oxidized in a biological sample oxidizer[10] using a mixture of carbon dioxide absorbent and scintillation fluid (1:2 ratio) to trap evolved CO_2. Samples were radioassayed by LSS.

Treated leaves were the only plant sections found to contain sufficient amounts of ^{14}C-herbicide for detection of metabolites, therefore metabolism was determined in the treated leaf only. Treated leaves were ground in a tissue homogenizer[11] with 20 ml of cold acetone:water (80:20, v/v). The homogenate was then vacuum filtered[12] and the residue rinsed with additional solvent. The rinsate volume was recorded and two 1-ml aliquots were radioassayed with LSS to determine total extractable ^{14}C. The residue along with the filter paper was air dried and oxidized to determine unextractable radioactivity.

The filtrate was evaporated to a volume of 1 to 5 ml with a rotary evaporator at 42 and 35 C for nicosulfuron and primisulfuron extracts, respectively. The solution was transferred to a test tube and brought back to

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[10]R.J. Harvey Instruments Corp., 123 Patterson St., Hillsdale, NJ 07642

[11]Sorvall Omni-mixer. Sorvall, Inc., Newton, CT.

[12]Whatman #1. Whatman International Ltd., Maidstone, England.

volume with acetonitrile. The sample was then concentrated under a stream of nitrogen and filtered again through a 0.2 μm millipore filter[13].

Twenty to fifty μL of the concentrated extract containing 15 to 50 Bq of radioactivity was spotted onto 20- by 20-cm silica gel thin layer chromatography (TLC)[4] plates for metabolite separation. Plates with nicosulfuron extracts[14] were developed to a 13-cm solvent front in dichloromethane:methanol:ammonium hydroxide (165:30:5, v/v/v). Plates with primisulfuron extracts[15] were first developed to a 13-cm solvent front in chloroform:methanol:formic acid:water (70:25:4:2, v/v/v/v). Plates were air dried and then rotated 90° counterclockwise and developed a second time to a 13-cm solvent front in chloroform:methanol: ammonium hydroxide:water (80:30:4:2, v/v/v/v). ^{14}C-nicosulfuron and primisulfuron standards were also spotted and chromatographed in the same manner as their respective extracts to determine the R_f values for the parent herbicides. Radioactive positions, proportions, and their corresponding R_f values were determined by scanning TLC plates with a radiochromatogram scanner[16]

Herbicide absorption was calculated as the total ^{14}C recovered in the plant divided by the ^{14}C applied. ^{14}C translocation out of the treated leaf was

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[13]Gelman Sciences Inc., Ann Arbor, MI 48106.

[14]Whatman® Linear-K Preadsorbent, Whatman International Ltd., Maidstone, England.

[15]Fisherbrand Redi/Plate®, Fisher Scientific, 711 Forbes Avenue, Pittsburgh, PA, 15219-4785.

[16]Ambis Systems, Inc., 3939 Ruffin Road, San Diego, CA 92123.

calculated as the ^{14}C recovered in plant parts excluding the treated leaf divided by the total ^{14}C recovered in the plant. Herbicide metabolism in the treated leaf was calculated by dividing the extractable ^{14}C remaining as intact herbicide by the total ^{14}C in the treated leaf.

A randomized complete block design with a two factor (herbicide by species) factorial arrangement of treatments and four replications was used. The experiment was conducted twice and the combined results presented. Data within each harvest time were subjected to analysis of variance and means separated using Fisher's Protected Least Significant Difference Test at $\alpha = 0.05$. ^{14}C recovery averaged over all species and harvest times was 89% for both nicosulfuron and primisulfuron (Table 2).

Acetolactate synthase activity. ALS activity levels were determined in the leaves of eastern black nightshade and johnsongrass grown to heights of 5 and 15 cm, respectively. Plant were grown in the greenhouse as previously described.

ALS was extracted and enzyme activity levels measured in the presence of nicosulfuron or primisulfuron with a modification of the methods outlined by Ray (37), Shaner (40), and Hart et al. (21,22). All extraction, centrifugation, and column separation procedures were conducted on ice or at 4 C. Two 10 g samples were taken from each species. Samples were a composite of newly

formed plant leaves excised from the apex of several plants. Each 10 g sample was homogenized in 40 ml of cold homogenization buffer [0.1 M K_2HPO_4, 1.0 mM sodium pyruvate, 0.23 mM $MgCl_2$, pH 7.5, 0.5 mM thiamine pyrophosphate, 10 μM flavin adenine dinucleotide (FAD)[4], 10% by vol glycerol] with 2.5 g of polyvinylpolypyrrolidone in the homogenization vessel. The homogenate was filtered through eight layers of cheesecloth and then centrifuged at 27 000 g for 20 min. The supernatant fraction was brought to 50% saturation with cold $(NH_4)_2SO_4$ and allowed to stand 1 h on ice. The mixture was then centrifuged at 18 000 g for 15 min. The supernatant was discarded and the precipitated pellet dissolved in resuspension buffer (0.1 M K_2HPO_4, 20 nM sodium pyruvate, 0.23 mM $MgCl_2$, pH 7.5). This solution was passed through a Sephadex® G-25M PD-10[17] column equilibrated with the same buffer. The desalted enzyme preparation was immediately used for enzyme assays.

ALS enzyme assays were carried out in a final volume of 1.5 ml containing the enzyme preparation, reaction buffer (25 mM K_2HPO_4, 25 mM sodium pyruvate, 0.29 mM $MgCl_2$, pH 7.0, 0.625 mM thiamine pyrophosphate, 1.25 μM FAD), and technical grade nicosulfuron or primisulfuron at 0, 5, 50, 500, or 5 000 nM concentrations. Reaction tubes were incubated for 1 h at 35 C when the reaction was stopped with the addition of 50 μL of 6 N H_2SO_4. The reaction tubes were then heated for 15 min at 60 C to facilitate

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[17]Pharmacia, 800 Centennial Avenue, Piscataway, NJ 08855-1327.

decarboxylation of acetolactate to acetoin. Then 0.5 ml of 0.5% wt by vol creatine in 2.5 N NaOH and 0.5 ml of 5% wt by vol α-naphthol freshly prepared in 2.5 N NaOH were added consecutively to each tube. The solutions were heated for an additional 15 min at 60 C. Acetoin content was then determined by spectrophotometric analysis as described by Westerfield (44). Protein concentration was determined by the method of Lowry (29). ALS activity is expressed as nM acetoin h^{-1} mg^{-1} protein.

The experiment was repeated with four replications of each herbicide concentration per experiment. ALS enzyme activity is presented as a percent of control assays using a $\log_{10}$ transformation of herbicide concentrations. Paired t-tests were conducted at $\alpha = 0.01$ to determine if means at each herbicide concentration were significantly different. Tests were conducted to determine if ALS activity in response to the log of the herbicide concentration was linear or quadratic. A quadratic model was fit to eastern black nightshade ALS activity in response to nicosulfuron concentration with a coefficient of determination of 0.88. A linear model was fit to johnsongrass ALS activity in response to nicosulfuron concentration with a coefficient of determination of 0.94. Quadratic models were fit to eastern black nightshade and johnsongrass ALS activity in response to primisulfuron concentration with coefficients of determination of 0.92 and 0.97, respectively. I_{80}[4] values (herbicide concentration required to inhibit enzyme activity by 80%) were calculated from these equations. Comparisons

between eastern black nightshade and johnsongrass were made in terms of ALS activity and protein level with paired t-tests at $\alpha = 0.01$.

RESULTS AND DISCUSSION

Species sensitivity. Plant species were chosen based on wide variation in species sensitivity to nicosulfuron and primisulfuron applications in field trials. Greenhouse experiments were conducted to quantify and validate the observations made in field trials.

Evaluation of GR_{50} values calculated from visual ratings or from percent dry weight reductions led to similar conclusions. GR_{50} values could not be obtained with several species/herbicide combinations with rates as high as 480 g ha^{-1}, while GR_{50} values for some combinations were less than 5 g ha^{-1}. The field application rates for nicosulfuron and primisulfuron are 35 and 40 g ha^{-1}, respectively (1,2).

Corn was tolerant to both herbicides (Table 1.). No comparisons between herbicides was made since GR_{50} values could not be determined for either herbicide. Johnsongrass was sensitive to both herbicides with GR_{50} values well below the field application rates for either herbicide. GR_{50} values calculated on

a percent dry weight reduction basis indicated no difference in sensitivity to the herbicides.

Barnyardgrass and giant foxtail were both more tolerant to primisulfuron. The difference in tolerance to the two herbicides was greater with barnyardgrass than with giant foxtail. Giant foxtail was not as tolerant to primisulfuron as barnyardgrass or corn. Eastern black nightshade was tolerant to nicosulfuron and sensitive to primisulfuron.

Foliar absorption. Foliar absorption of ^{14}C-nicosulfuron or ^{14}C-primisulfuron by johnsongrass was less than or equal to absorption of either herbicide by corn at 72 or 168 HAT[4], even though johnsongrass was sensitive and corn was tolerant to both herbicides (Tables 1 and 3).

Barnyardgrass and giant foxtail both absorbed more ^{14}C-primisulfuron than ^{14}C-nicosulfuron at each harvest time even though both species are tolerant to primisulfuron and sensitive to nicosulfuron. ^{14}C-nicosulfuron absorption was equal to or greater than ^{14}C-primisulfuron in eastern black nightshade at each harvest time even though the species is tolerant to nicosulfuron and sensitive to primisulfuron.

Eastern black nightshade is extremely tolerant to nicosulfuron yet absorbed more ^{14}C-nicosulfuron than an extremely sensitive species (johnsongrass) at each harvest time. Barnyardgrass and giant foxtail are tolerant to primisulfuron yet

each absorbed more ^{14}C-primisulfuron than an extremely sensitive species (johnsongrass) at each harvest time.

Foliar herbicide absorption by itself cannot account for differential selectivity of nicosulfuron or primisulfuron in any of the species investigated, or for differences in sensitivity to the herbicides by any of the species.

Translocation. Nicosulfuron and primisulfuron movement within the plant was approximated by measuring ^{14}C translocation out of the treated leaf. Greater ^{14}C translocation did not consistently correlate with greater sensitivity of any species to nicosulfuron or primisulfuron (Table 4). Corn treated with primisulfuron translocated more ^{14}C than corn treated with nicosulfuron at 12 or 72 HAT. ^{14}C translocation in nicosulfuron or primisulfuron treated corn was equivalent at 168 HAT. Johnsongrass treated with primisulfuron translocated more ^{14}C than johnsongrass treated with nicosulfuron at 12 HAT. At 72 or 168 HAT, johnsongrass treated with nicosulfuron translocated more ^{14}C than johnsongrass treated with primisulfuron. Neither corn nor johnsongrass displayed differential sensitivity between nicosulfuron and primisulfuron even though there were differences in translocation (Tables 1 and 4). Differences in ^{14}C translocation between barnyardgrass or giant foxtail plants treated with nicosulfuron and primisulfuron do not correlate with sensitivity or are not great enough to account for whole plant differences in sensitivity.

^{14}C translocation in nicosulfuron treated plants was greater in sensitive species (johnsongrass, barnyardgrass, and giant foxtail) than in tolerant species (corn and eastern black nightshade) (Table 4). The greatest ^{14}C translocation (> 20%) occurred in johnsongrass plants treated with nicosulfuron at 72 and 168 HAT. The least ^{14}C translocation ($\leq$ 3%) occurred in eastern black nightshade treated with nicosulfuron. This difference in translocation could contribute to the difference in johnsongrass and eastern black nightshade sensitivity to nicosulfuron.

Plants tolerant to primisulfuron (corn, barnyardgrass, and giant foxtail) translocated similar or greater amounts of ^{14}C than plants sensitive to primisulfuron (johnsongrass and eastern black nightshade). Translocation does not appear to contribute to selectivity of primisulfuron in the species investigated.

Metabolism. Metabolism of both herbicides occurred in each species by 12 HAT (Table 5). Three distinct metabolites of ^{14}C-nicosulfuron were separated from the parent herbicide. Their R_f values in order of appearance over time were 0.88, 0, and 0.20. ^{14}C-nicosulfuron had an R_f value of 0.40. The relative abundance and rate of formation of the metabolites was specific to each species, however, the order of appearance of the metabolites was the same for each species (data not shown).

Numerous ^{14}C-primisulfuron metabolites were formed by the species investigated. ^{14}C-primisulfuron had R_f values of 0.87 and 0.74 for the first and second developments, respectively. The presence, relative abundance, and order of primisulfuron metabolite formation was species specific. The most common primisulfuron metabolites among species had first/second development R_f values of 0.77/0.58, 0.90/0.59, and 0.59/0.17. As many as 10 distinct primisulfuron metabolites were formed in giant foxtail alone (data not shown). The same sulfonylurea herbicide may be metabolized by different pathways in different plants (25,32,43). No attempt was made to identify the metabolites of either herbicide in this research.

Corn metabolized both herbicides more rapidly and extensively than any other species by 12 HAT (Table 5). There were no differences in the amount of nicosulfuron or primisulfuron remaining in the treated corn leaf, nor was there greater than 20% parent herbicide remaining at any harvest time. Johnsongrass did not metabolize either herbicide as rapidly or extensively as corn. Greater than 50% of both herbicides remained as parent at 12 HAT, compared to less than 20% for corn. The percentage of parent nicosulfuron or primisulfuron in johnsongrass decreased over time but there was no difference between the percentage of parent herbicides remaining at any harvest.

Both barnyardgrass and giant foxtail metabolized primisulfuron more rapidly than nicosulfuron. More nicosulfuron remained in both species at 12 and

72 HAT. Both species continued to metabolize each herbicide over time with the difference between the percentage of parent herbicides remaining diminishing in barnyardgrass by 168 HAT. At 72 HAT the percentage of primisulfuron remaining in barnyardgrass and corn was equivalent.

The rate of herbicide metabolism is a major factor which determines whether corn, johnsongrass, barnyardgrass, or giant foxtail is tolerant or sensitive to nicosulfuron or primisulfuron. Species tolerant to a herbicide were able to metabolize it more rapidly and extensively than sensitive species. Previous researchers have identified herbicide metabolism as the primary basis for differential selectivity of nicosulfuron and primisulfuron (10,14,19,32,33).

Eastern black nightshade did not metabolize either herbicide as quickly or extensively as any other species. The percentage of parent nicosulfuron in the treated leaf of eastern black nightshade was greater than or equal to that in species sensitive to nicosulfuron (johnsongrass, barnyardgrass, and giant foxtail) at each harvest. The percentage of parent nicosulfuron remaining in eastern black nightshade was greater than or equal to the percentage of parent primisulfuron at each harvest, even though this species is tolerant to nicosulfuron and sensitive to primisulfuron. Differential foliar herbicide absorption, translocation, or metabolism cannot account for the differential tolerance of eastern black nightshade to nicosulfuron and primisulfuron. Differential foliar absorption or metabolism cannot account for differential selectivity of

nicosulfuron in the species investigated. Differential translocation of nicosulfuron may contribute, but by itself cannot totally account for the selectivity of nicosulfuron in the species investigated.

ALS enzyme properties. ALS activity was studied in eastern black nightshade to determine if differential enzyme sensitivity could account for differential whole plant response to nicosulfuron and primisulfuron. ALS activity in johnsongrass was used for comparison since this species is sensitive to both herbicides and the ALS enzyme would be sensitive to inhibition by both herbicides as well.

ALS from both eastern black nightshade and johnsongrass was more sensitive to inhibition by primisulfuron than nicosulfuron at 50 and 500 nM concentrations (Figures 1 and 2). Calculated I_{80} values demonstrate the differential sensitivity in both species as well (Table 6). Other researchers have found nicosulfuron to be much less active than primisulfuron at the enzyme level in certain corn hybrids and unspecified weed species (19). They concluded that the lower enzyme activity is probably the major factor in the greater tolerance of the corn hybrids to nicosulfuron. In the bacteria *Salmonella typhimurium* and *Escherichia coli* there are several isozymes of ALS each encoded by a separate gene (5). Two of these isozymes have been shown to be sensitive to the sulfonylurea sulfometuron methyl {methyl 2-[[[[(4,6-dimethyl-2-

pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoate}, while a third isozyme is not (27,28). Weeds that have developed resistance through mutagenesis have an altered form of ALS that is enzymatically functional but much less sensitive than the normal enzyme to inhibition by sulfonylurea herbicides (30,35,39). Consideration of the variability of ALS forms and sensitivity in bacteria and plants makes it reasonable to assume that variations in ALS sensitivity among species is likely and could contribute to differential sensitivity to herbicides.

In this research, the lack of differences in herbicide absorption, translocation, or metabolism emphasize the difference in enzyme sensitivity as the basis for differential tolerance of eastern black nightshade to nicosulfuron and primisulfuron.

Comparisons of eastern black nightshade and johnsongrass ALS sensitivity in the presence of nicosulfuron or primisulfuron revealed very similar enzyme inhibition patterns (Figures 3 and 4). Eastern black nightshade and johnsongrass ALS sensitivity to nicosulfuron was roughly equivalent at the enzyme level even though whole plant response is drastically different (Table 1).

The specific activity of the ALS from johnsongrass was nearly three times that of eastern black nightshade ALS (Table 6). However, eastern black nightshade contained nearly three times as much protein per mg of tissue fresh weight as johnsongrass. ALS activity on a unit of tissue fresh weight basis was equivalent in the two species. This indicates that differences in specific activity

and protein level in eastern black nightshade and johnsongrass offset each other and the ratio of protein content is a valid estimation of the ratio of ALS content. Consequently, the tissue of eastern black nightshade had nearly three times as much ALS per unit of tissue as johnsongrass. In addition, less nicosulfuron translocation occurred in eastern black nightshade (3%) compared to johnsongrass (> 20%) at 72 or 168 HAT (Table 4). Other research determined that in johnsongrass plants treated with ^{14}C-nicosulfuron, a large portion of translocated ^{14}C accumulated in the growing point of the shoot (33). Metabolites of sulfonylurea herbicides have been shown to be inactive against plant ALS (7,9,25), suggesting that accumulation of ^{14}C in the growing points represents translocated nicosulfuron. The combination of less ALS and greater translocation resulted in greater concentration of nicosulfuron per unit of ALS at the growing point in johnsongrass compared to eastern black nightshade. This would account for differential selectivity of nicosulfuron in these two species.

Elevated levels of a target enzyme may provide the basis for tolerance to certain herbicide molecules (15,31,41). A recent study concluded that naturally occurring differences in ALS levels from roots of certain corn inbreds contributed largely to differential in vivo response to the sulfonylurea herbicide chlorsulfuron (16).

Herbicide metabolism rate was the major factor which contributed to the tolerance of corn, barnyardgrass, and giant foxtail to nicosulfuron or

primisulfuron (Table 7). Johnsongrass did not display tolerance to either herbicide (Table 1). Differential ALS enzyme sensitivity was the major contributing factor which explained the differential tolerance of eastern black nightshade to nicosulfuron and primisulfuron on the whole plant level (Table 7). The combination of ALS level and herbicide translocation rate are factors which contribute to differential selectivity of nicosulfuron in eastern black nightshade and johnsongrass.

The results of this research indicate that tolerance to sulfonylurea herbicides is not always a function of herbicide metabolism rate. Other factors can contribute to or be responsible for selectivity. A complex interaction of several factors may determine the degree of sensitivity of a particular plant species to any one sulfonylurea herbicide.

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LITERATURE CITED

1. Anonymous. 1993. Accent® herbicide product label. E.I. du Pont de Nemours and Co., Agricultural Products, Wilmington, DE 19898.

2. Anonymous. 1993. Beacon® herbicide product label. CIBA-GEIGY Corp., Agricultural Division, Greensboro, NC 27419.

3. Anonymous. 1990. Accent® Herbicide. Technical Bull. E.I. duPont de Nemours and Co., Agricultural Products, Wilmington, DE 19898. 12p.

4. Anonymous. 1989. Beacon® Herbicide. Technical Release. CIBA-GEIGY Corp., Agricultural Division, Greensboro, NC 27419. 8 p.

5. Beyer, E.M. Jr., M.J. Duffy, J.V. Hay, and D.D. Schleuter. 1988. Sulfonylureas. p. 117-189 *in* P.C. Kearney and D.D. Kaufman eds. Herbicides: Chemistry, Degradation and Mode of Action. 3rd ed., Marcel-Dekker, New York.

6. Boldt, P.F. and A.R. Putnam. 1980. Selectivity mechanisms for foliar applications of diclofop-methyl. I. Retention, absorption, translocation, and volatility. Weed Sci. 28:474-477.

7. Brown, H.M. and S.M. Neighbors. 1987. Soybean metabolism of chlorimuron ethyl: physiological basis for soybean selectivity. Pestic. Biochem. Physiol. 29:112-120.

8. Brown, H.M. 1990. Mode of action, crop selectivity, and soil relations of the sulfonylurea herbicides. Pestic. Sci. 29:263-281.

9. Brown, H.M., V.A. Wittenbach, D.R. Forney, and S.D. Strachan. 1990. Basis for soybean tolerance to thifensulfuron methyl. Pestic. Biochem. Physiol. 37:303-313.

10. Burton, J.D., E.M. Maness, D.W. Monks, and D.K. Robinson. 1992. Differential herbicide tolerance of Landmark and Merit sweet corn is due to different rates of metabolism. Abstr. Weed Sci. Soc. Am. 32:91.

11. Camacho, R.F. and L.J. Moshier. 1991. Absorption, translocation, and activity of CGA-136872, DPX-V9360, and glyphosate in rhizome johnsongrass (*Sorghum halepense*). Weed Sci. 39:354-357.

12. Chaleff, R.S. and C.J. Mauvais. 1984. Acetolactate synthase is the site of action of two sulfonylurea herbicides in higher plants. Science 224:1443-1445.

13. Chaleff, R.S. and T.B. Ray. 1984. Herbicide resistant mutants of tobacco cell cultures. Science 223:1148-1151.

14. Diehl, K.E., H. Mukaida, R.A. Liebel, and E.W. Stoller. 1993. Sensitivity mechanism in an ALS-susceptible corn hybrid. Abstr. Weed Sci. Soc. Am. 33:191.

15. Donn, G., E. Tischer, J.A. Smith, and H.M. Goodman. 1984. Herbicide-resistant alfalfa cells: an example of gene amplification in plants. J. Mol. Appl. Genet. 2:621-635.

16. Forlani, G., E. Nielsen, P. Landi, and R. Tuberosa. 1991. Chlorsulfuron tolerance and acetolactate synthase activity in corn (*Zea mays* L.) inbred lines. Weed Sci. 39:553-557.

17. Foy, C.L. and H.L. Witt. 1990. Johnsongrass control with DPX-V9360 and CGA-136872 in corn (*Zea mays*) in Virginia. Weed Technol. 4:615-619.

18. Freed, V.H. and R.O. Morris. 1967. eds. Environmental and other factors in the responses of plants to herbicides. Tech. Bull. 100, Oregon State Univ., Corvallis, OR.

19. Green, J.M. and J.F. Ulrich. 1993. Response of corn (*Zea mays* L.) inbreds and hybrids to sulfonylurea herbicides. Weed Sci. 41:508-516.

20. Hageman, L.H. and R. Behrens. 1984. Basis for response differences of two broadleaf weeds to chlorsulfuron. Weed Sci. 32:162-167.

21. Hart, S.E., J.W. Saunders, and D. Penner. 1993. Semidominant nature of monogenic sulfonylurea herbicide resistance in sugarbeet (*Beta vulgaris*). Weed Sci. 41:317-324.

22. Hart, S.E., J.W. Saunders, and D. Penner. 1992. Chlorsulfuron-resistant sugarbeet: cross-resistance and physiological basis of resistance. Weed Sci. 40:378-383.

23. Hay, J.V. 1990. Chemistry of sulfonylurea herbicides. Pestic. Sci. 29:247-261.

24. Hess, F.D. and R.H. Falk. 1990. Herbicide deposition on leaf surfaces. Weed Sci. 38:280-288.

25. Hutchison, J.M., R. Shapiro, and P.B. Sweetser. 1984. Metabolism of chlorsulfuron by tolerant broadleaves. Pestic. Biochem. Physiol. 22:243-247.

26. LaRossa, R.A. and S.C. Falco. 1984. Amino acid biosynthetic enzymes as targets of herbicide action. Trends Biotechnol. 2:158-161.

27. LaRossa, R.A. and J.V. Schloss. 1984. The sulfonylurea herbicide sulfometuron methyl is an extremely potent and selective inhibitor of acetolactate synthase in *Salmonella typhimurium*. J. Biol. Chem. 259:8753-8757.

28. LaRossa, R.A. and D.R. Smulski. 1984. *ilvB*-encoded acetolactate synthase is resistant to the herbicide sulfometuron methyl. J. Bacteriol. 160:391-394.

29. Lowry, O.H, N.S. Rosebrough, A.L. Farr, and R.S. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275.

30. Mallory-Smith, C.A., D.C. Thill, and M.J. Dial. 1990. Identification of sulfonylurea herbicide-resistant prickly lettuce (*Lactuca serriola*) Weed Technol. 4:163-168.

31. Nafziger, E.D., J.M. Widholm, H.C. Steinrücken, and J.L. Killmer. 1984. Isolation and characterization of a carrot cell line tolerant to glyphosate. Plant Physiol. 76:571-574.

32. Neighbors, S. and L.S. Privalle. 1990. Metabolism of primisulfuron by barnyard grass. Pestic. Biochem. Physiol. 37:145-153.

33. Obrigawitch, T.T., W.H. Kenyon, and H. Kuratle. 1990. Effect of application timing on rhizome johnsongrass (*Sorghum halepense*) control with DPX-V9360. Weed Sci. 38:45-49.

34. Petersen, P.J. and B.A. Swisher. 1985. Absorption, translocation, and metabolism of ^{14}C-chlorsulfuron in Canada thistle (*Cirsium arvense*). Weed Sci. 33:7-11.

35. Primiani, M.M., J.C. Cotterman, and L.L. Saari. 1990. Resistance of kochia (*Kochia scoparia*) to sulfonylurea and imidazolinone herbicides. Weed Technol. 4:169-172.

36. Ray, T.B. 1986. Sulfonylurea herbicides as inhibitors of amino acid biosynthesis in plants. TIBS 11:180-183.

37. Ray, T.B. 1984. Site of action of chlorsulfuron: inhibition of valine and isoleucine biosynthesis in plants. Plant Physiol. 75:827-831.

38. Rhodes, D., A.L. Hogan, L. Deal, G.C. Jamieson, and P. Haworth. 1987. Amino acid metabolism of *Lemna minor* L. Plant Physiol. 84:775-780.

39. Saari, L.L., S.C. Cotterman, and M.M. Primiani. 1990.Mechanism of sulfonylurea resistance in the broadleaf weed, *Kochia scoparia*. Plant Physiol. 93:55-61.

40. Shaner, D.L. P.C. Anderson, and M.A. Stidham. 1984. Imidazolinones, potent inhibitors of acetohydroxy acid synthase. Plant Physiol. 76:545-546.

41. Smart, C.C., D. Johanning, G. Muller, and N. Amrhein. 1985. Selective overproduction of 5-*enol*-pyruvylshikimic acid 3-phosphate synthase in a plant cell culture which tolerates high doses of the herbicide glyphosate. J. Biol. Chem. 260:16338-16346.

42. Stidham, M.A. 1991. Herbicides that inhibit acetohydroxyacid synthase. Weed Sci. 39:428-434.

43. Sweetser, P.B., G.S. Schow, and J.M. Hutchison. 1982. Metabolism of chlorsulfuron by plants: biological basis for selectivity of a new herbicide for cereals. Pestic. Biochem. Physiol. 17:18-23.

44. Westerfield, W.W. 1945. A colorimetric determination of blood acetoin. J. Biol. Chem. 161:495-502.

45. Wilcut, J.W., G.R. Wehtje, M.G. Patterson, T.A. Cole, and T.V. Hicks. 1989. Absorption, translocation, and metabolism of foliar-applied chlorimuron in soybeans (*Glycine max*), peanuts (*Arachis hypogaea*), and selected weeds. Weed Sci. 37:175-180.

Table 1. Plant sensitivity to nicosulfuron and primisulfuron.[a]

Species	Height	Growth Stage	GR_{50}[b]					
			Visual Basis			Dry Weight Basis		
			Nicosulfuron		Primisulfuron	Nicosulfuron		Primisulfuron
	cm	true leaves	g ha^{-1}					
Corn	18	3	>480	——	>480	>480	——	>480
Johnsongrass	13	3	8	*	5	3	n.s.	2
Barnyardgrass	15	3	22	*	>480	7	*	>480
Giant Foxtail	10	4	10	*	40	7	*	32
Eastern Black Nightshade	5	5-6	>480	*	5	>480	*	4

[a]GR_{50} values with * between them are different according to paired t-test at $\alpha = 0.05$, n.s = nonsignificant, —— = no test conducted. Means cannot be compared between species or between visual basis and dry weight basis.

[b]GR_{50} values were determined by visual injury ratings and by percent dry weight reduction using the nonlinear regression analysis equation $Y = B(1) \times e^{(B(2) \times X)} + B(3)$.

Table 2. ^{14}C recovery[a].

Species	Recovery[b]	
	Nicosulfuron	Primisulfuron
	%	
12 hours after treatment		
Corn	93 d	99 a
Johnsongrass	92 d	96 ab
Barnyardgrass	88 e	93 cd
Giant Foxtail	94 bcd	96 abc
Eastern Black Nightshade	92 d	96 abc
72 hours after treatment		
Corn	90 a	90 a
Johnsongrass	89 a	90 a
Barnyardgrass	88 ab	83 d
Giant Foxtail	89 a	85 cd
Eastern Black Nightshade	86 bc	90 a
168 hours after treatment		
Corn	85 bc	79 f
Johnsongrass	90 a	85 bcd
Barnyardgrass	86 bc	81 ef
Giant Foxtail	88 ab	82 def
Eastern Black Nightshade	83 cde	87 ab

[a]Means may be compared within or across columns within harvest times. Means followed by the same letter are not significantly different according to Fisher's Protected LSD Test ($\alpha = 0.05$).

[b]Recovery expressed as % of total ^{14}C recovered from all processes divided by ^{14}C applied.

Table 3. Nicosulfuron and primisulfuron absorption in five plant species[a].

Species	Absorption	
	Nicosulfuron	Primisulfuron
	% of applied ^{14}C	
12 hours after treatment		
Corn	8 g	3 h
Johnsongrass	10 f	11 ef
Barnyardgrass	12 ef	22 a
Giant Foxtail	16 c	19 b
Eastern Black Nightshade	13 de	14 cd
72 hours after treatment		
Corn	12 e	12 e
Johnsongrass	12 e	14 e
Barnyardgrass	15 de	28 b
Giant Foxtail	18 d	23 c
Eastern Black Nightshade	33 a	32 ab
168 hours after treatment		
Corn	18 e	23 cd
Johnsongrass	15 e	16 e
Barnyardgrass	15 e	26 c
Giant Foxtail	17 e	22 d
Eastern Black Nightshade	38 a	33 b

[a]Means may be compared within or across columns within harvest times. Means followed by the same letter are not significantly different according to Fisher's Protected LSD Test ($\alpha = 0.05$).

Table 4. Nicosulfuron and primisulfuron translocation in five plant species[a].

Species	Translocation[b]	
	Nicosulfuron	Primisulfuron
	% of absorbed ^{14}C	
12 hours after treatment		
Corn	4 ef	13 abc
Johnsongrass	12 c	15 a
Barnyardgrass	9 d	12 bc
Giant Foxtail	12 bc	14 ab
Eastern Black Nightshade	2 f	5 e
72 hours after treatment		
Corn	7 d	15 bc
Johnsongrass	23 a	18 bc
Barnyardgrass	16 bc	16 bc
Giant Foxtail	18 b	14 c
Eastern Black Nightshade	3 e	4 de
168 hours after treatment		
Corn	6 ef	8 e
Johnsongrass	21 a	16 bc
Barnyardgrass	19 ab	13 cd
Giant Foxtail	19 ab	16 bc
Eastern Black Nightshade	3 f	10 de

[a]Means may be compared within or across columns within harvest times. Means followed by the same letter are not significantly different according to Fisher's Protected LSD Test ($\alpha = 0.05$).

[b]Translocation of ^{14}C out of the treated leaf.

Table 5. Nicosulfuron and primisulfuron metabolism in five plant species[a].

Species	Metabolism[b]	
	Nicosulfuron	Primisulfuron
	% parent	
12 hours after treatment		
Corn	18 e	16 e
Johnsongrass	51 c	55 bc
Barnyardgrass	58 bc	33 d
Giant Foxtail	69 a	54 bc
Eastern Black Nightshade	69 a	64 ab
72 hours after treatment		
Corn	13 g	19 fg
Johnsongrass	39 d	48 cd
Barnyardgrass	60 b	24 ef
Giant Foxtail	59 b	29 e
Eastern Black Nightshade	70 a	54 bc
168 hours after treatment		
Corn	12 e	20 de
Johnsongrass	38 ab	31 bcd
Barnyardgrass	32 bc	22 cde
Giant Foxtail	46 a	31 bcd
Eastern Black Nightshade	48 a	50 a

[a]Means may be compared within or across columns within harvest times. Means followed by the same letter are not significantly different according to Fisher's Protected LSD Test ($\alpha = 0.05$).

[b]Metabolism expressed as % of ^{14}C within the treated leaf remaining as intact parent.

Table 6. ALS activity, protein levels, and I_{80} values for eastern black nightshade and johnsongrass.

Species	Specific Activity	Protein Level	Enzyme Activity	I_{80}[a]	
				Nicosulfuron	Primisulfuron
	nM acetoin h^{-1} mg^{-1} protein	µg protein mg^{-1} fresh weight	nM acetoin h^{-1} mg^{-1} fresh weight	nM	
Eastern Black Nightshade	117	6.97	0.827	333	28
Johnsongrass	301	2.60	0.806	988	70
t-test ($\alpha = 0.01$)[b]	*	*	NS		

[a] I_{80} values determined from the appropriate regression analysis equations.

[b] * = means within column are different according to paired t-test at $\alpha = 0.01$, NS = nonsignificant.

Table 7. Factors which contribute to plant tolerance to nicosulfuron or primisulfuron.

	Tolerance		Factors[a]				
			Herbicide:			ALS Enzyme:	
Species	Nicosulfuron	Primisulfuron	Absorption	Translocation	Metabolism	Level	Sensitivity
Corn	Yes	Yes	—	—	*	?	?
Johnsongrass	No	No	—	—	—	—	—
Barnyardgrass	No	Yes	—	—	*	?	?
Giant Foxtail	No	Yes	—	—	*	?	?
E. B. Nightshade	Yes	No	—	*	—	*	*

[a] * = contributes significantly, —— = does not contribute significantly, ? = not investigated.

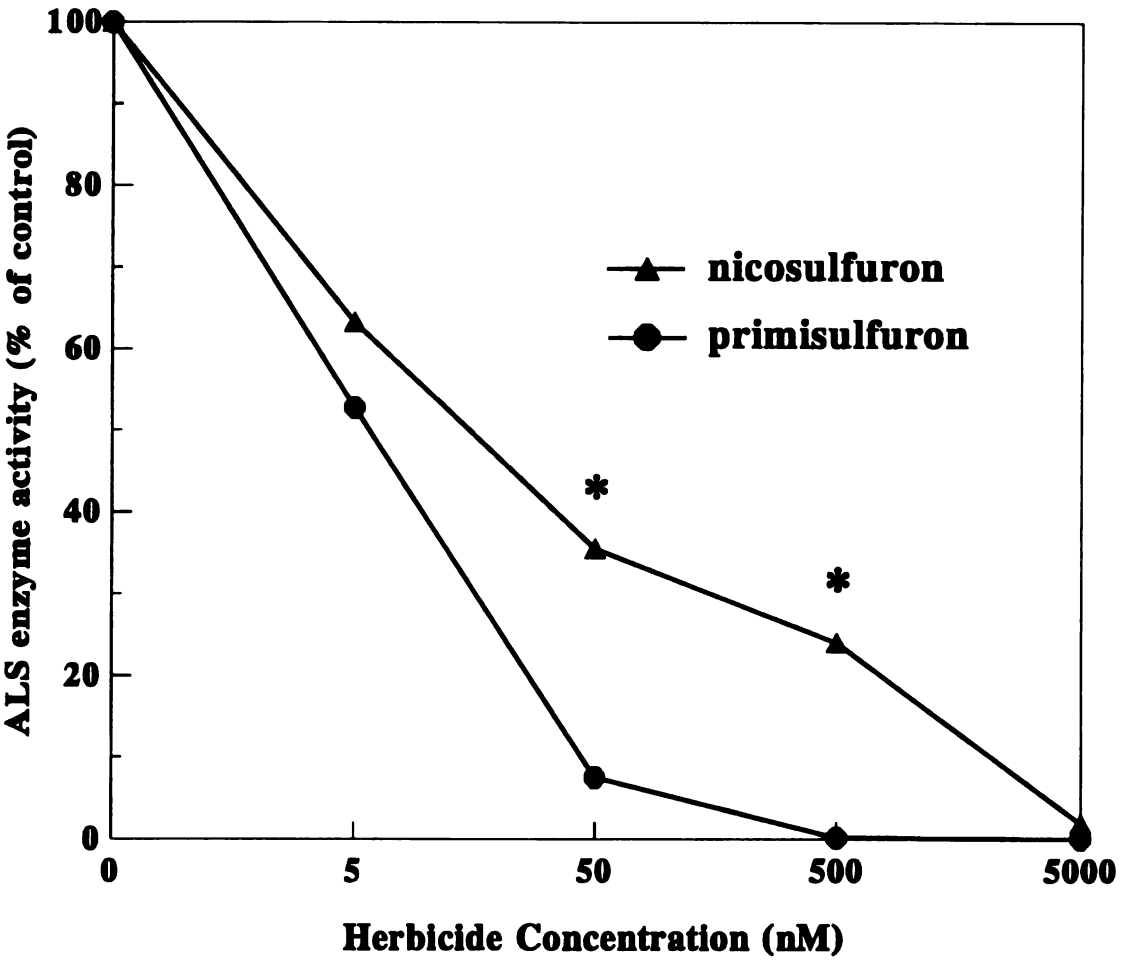

Figure 1. Inhibition of eastern black nightshade ALS activity. Means with a * are different at the corresponding herbicide concentration according to paired t-test at $\alpha = 0.01$

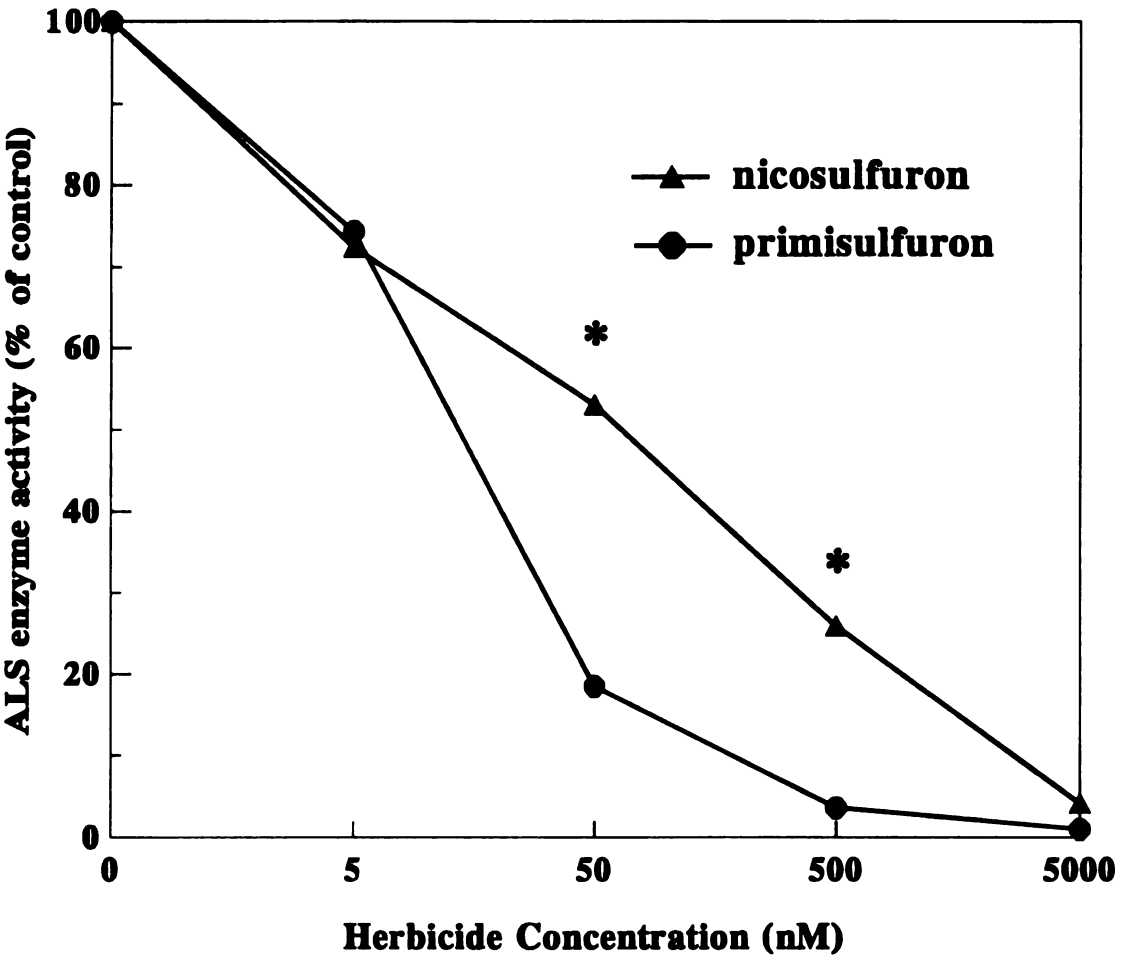

Figure 2. Inhibition of johnsongrass ALS activity. Means with a * are different at the corresponding herbicide concentration according to paired t-test at $\alpha = 0.01$

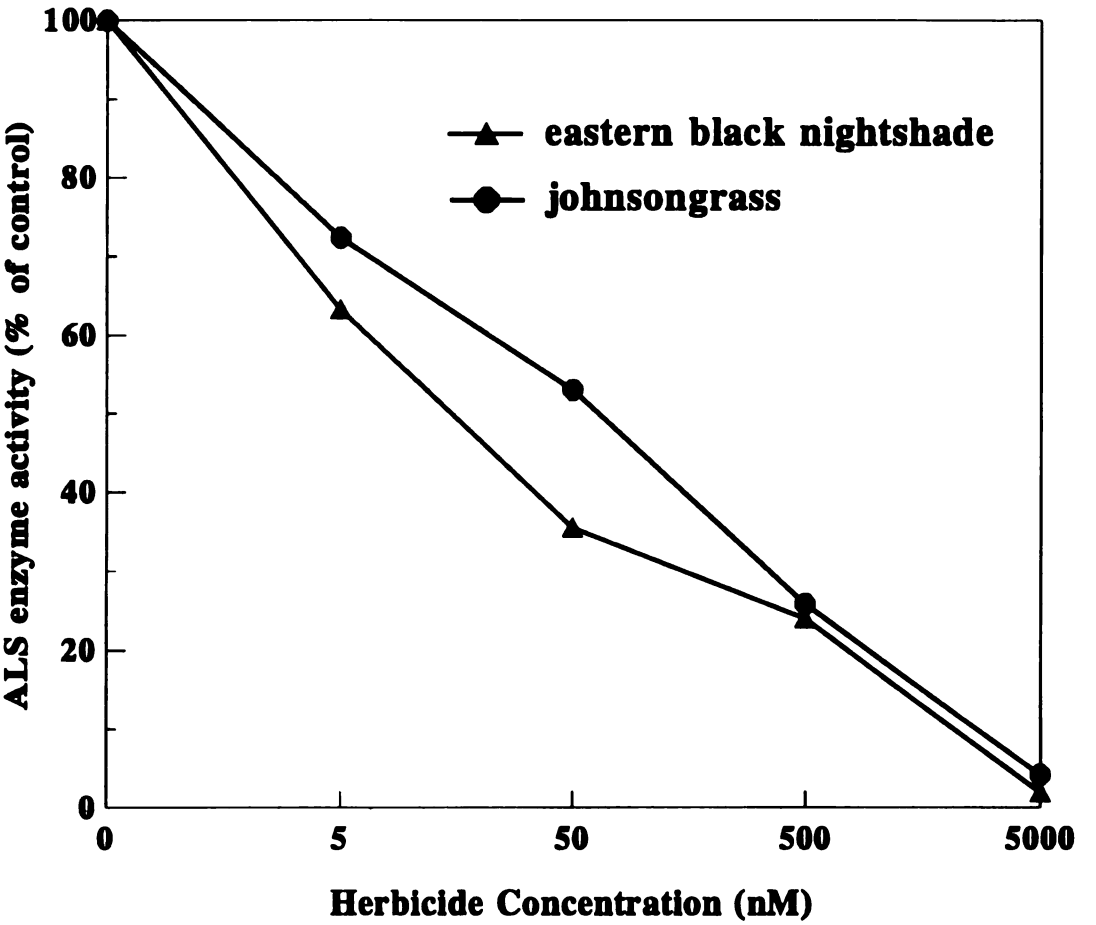

Figure 3. Inhibition of ALS activity by nicosulfuron. Means are not different at each herbicide concentration according to paired t-test at $\alpha = 0.01$

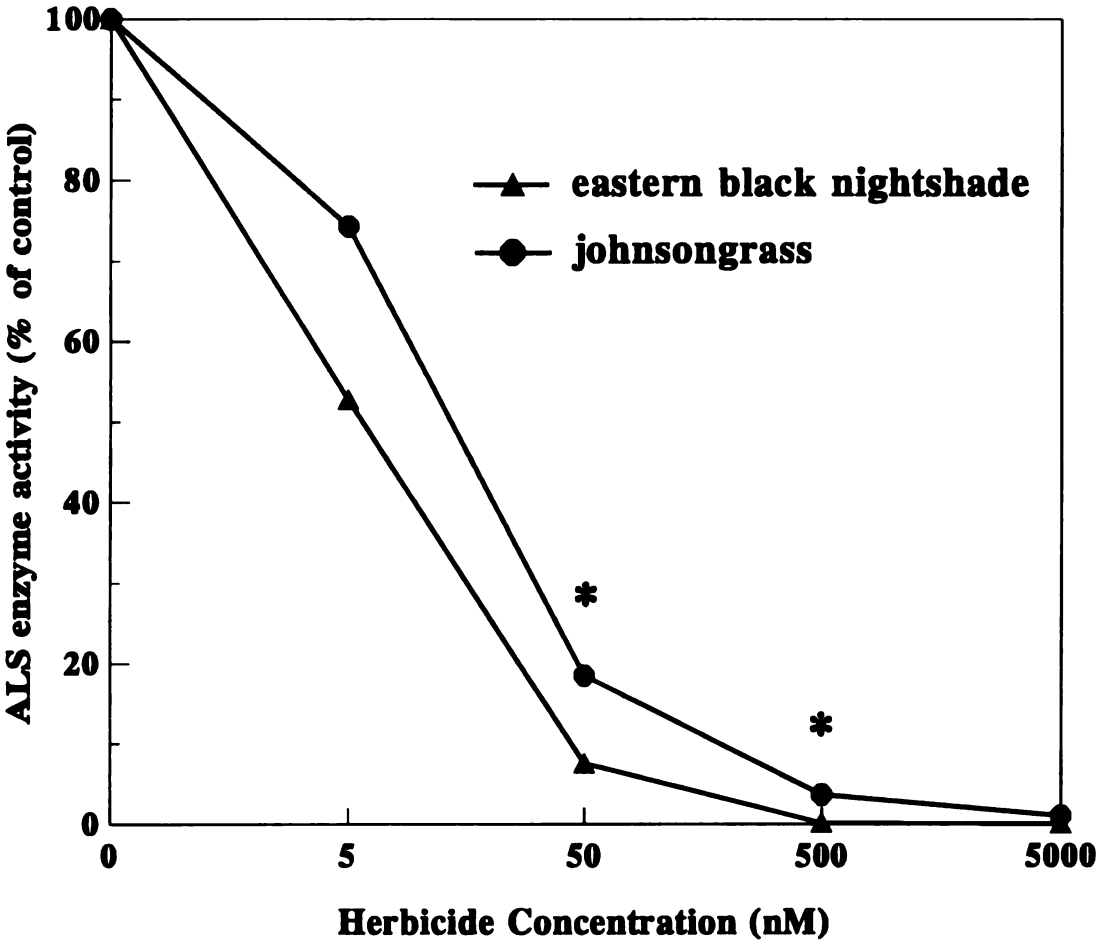

Figure 4. Inhibition of ALS activity by primisulfuron. Means with a * are different at the corresponding herbicide concentration according to paired t-test at $\alpha = 0.01$

Timing of Total Postemergence Herbicide Applications for Weed Control and Corn Yield[1]

J. BOYD CAREY and JAMES J. KELLS[2]

Abstract. Postemergence grass and broadleaf herbicides are available for effective, single-application total postemergence weed control in corn. Field experiments were conducted in 1992 and 1993 to determine the effects of weed interference prior to herbicide application on corn yield. Nicosulfuron plus bromoxynil was applied at 5, 10, 15, or 20-cm weed canopy heights in plots with or without weed interference. Both experiments were conducted on sites with extremely heavy natural weed infestations. Crop injury was more severe when herbicides were applied to smaller corn. Injury was temporary and did not reduce corn yield. Herbicide applications made to 5-, 10-, or 15-cm weeds provided nearly complete weed control. Weed interference did not reduce corn height or grain yield when postemergence applications were made to small weeds

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(≤ 10 cm). Weed interference reduced corn height and grain yield in 1992 when applications were made to 15-cm weeds even though weed control was nearly complete. Weed control was incomplete and corn height and grain yield were reduced when applications were delayed until weeds were 20 cm tall. **Nomenclature:** nicosulfuron, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)-amino]carbonyl]amino]sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide; bromoxynil, 3,5-dibromo-4-hydroxybenzonitrile; corn, *Zea mays* L. 'Pioneer 3753'.

Additional index words: Bromoxynil, nicosulfuron, interference, weed biomass, ABUTH, AMARE, AMBEL, CHEAL, SETFA.

INTRODUCTION

Nicosulfuron and primisulfuron are sulfonylurea herbicides which provide selective postemergence control of many grass and some broadleaf weed species in corn (1,2). Prior to their introduction, effective and reliable postemergence weed control in corn was limited to the use of selective herbicides which controlled only broadleaf weeds, or directed sprays which require special application equipment to avoid crop interception of the herbicide during application (6).

Tank-mixing nicosulfuron with postemergence broadleaf herbicides can broaden the spectrum of weed control and provide single-application total postemergence weed control in corn (10,13). This type of program provides an effective alternative to the traditional practice of using soil applied herbicides as a preventative approach to weed control. Total postemergence programs allow the corn grower to assess the extent of the weed problem before making a herbicide application (14,22).

However, since weeds are allowed to germinate and grow with the crop until the herbicide application is made, significant questions are raised about the effect of early season weed interference on the corn. The competitive effects of weeds on corn are important early in the growing season (16,24). Significant

yield reductions occurred when giant foxtail (*Setaria faberi* Herrm. #[3] SETFA) was allowed to interfere with corn until weeds were 15-23 cm in height and corn was 41-61 cm in height with 9-10 leaves (16).

Researchers have attempted to identify what is called a "critical period" for corn (15). In terms of total postemergence programs, the critical period is the period of time weeds may interfere with a crop before significant yield reduction will occur (23). Studies show that the critical period for weed competition in corn is difficult to define, and varies with environment, weed species, and weed density (15,24).

Traditional studies investigating duration of weed interference in corn have employed hand pulling or hoeing as the method of removal to end the period of weed interference (15,16). This method ceases weed competition with the crop abruptly and completely. Most postemergence herbicides, even when completely effective, do not terminate weed competition in such a fashion. Sulfonylurea herbicides in particular kill plants relatively slowly. Complete plant death in susceptible species occurs in 7 to 21 days with nicosulfuron, depending upon growing conditions, weed species, and growth stage of the weeds (4). Weed interference could still occur with the crop after herbicide application. It would also seem possible that an effective herbicide treatment could completely control

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

weeds after the critical period had expired, having already allowed irreversible interference and subsequent yield reduction.

The corn grower has some level of control over the duration of weed interference with the crop by choosing the time and method of weed control. If a grower adopts a total postemergence weed control program in corn, the time of herbicide application is important not only to insure successful weed control, but to control the duration of weed interference and prevent crop yield reduction. Knowledge of the duration of interference allowable before yield reduction occurs would be valuable to effectively implement total postemergence weed control programs in corn.

The objective of this research was to determine if weed interference prior to herbicide application reduces corn yield with a total postemergence herbicide program.

MATERIALS AND METHODS

Experiments were conducted in 1992 and 1993 at two separate locations on the Michigan State University Crop and Soil Science Research Farm at East Lansing, MI to evaluate total postemergence herbicide application timing in conventional tillage corn. Both studies were conducted on a Capac (fine-loamy,

mixed, mesic, Aeric Ochraquolfs) soil with a pH of 7.2 and 3.1% organic matter in 1992, and a pH of 6.6 and 3.3% organic matter in 1993.

Tillage at the sites consisted of moldboard plowing corn stalks the fall prior to disking and field cultivation the spring of 1992, and chisel plowing soybean stubble followed by disking and cultivation the spring of 1993. Pioneer 3753 corn was planted at 60 540 seeds ha^{-1} on May 14, 1992 and May 17, 1993. Prior to spring field cultivation 305 kg ha^{-1} of 46-0-0 was applied broadcast and 365 kg ha^{-1} of 6-24-24 was applied as a banded treatment 5 cm below and 5 cm beside the corn seed at planting. Tefluthrin [(2,3,5,6-tetrafluoro-4-methylphenyl)methyl-(1α,3α)-(Z-(±)-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate] insecticide was applied at 1.22 kg ai ha^{-1} at planting in 1992.

Plots consisted of four rows spaced 76 cm apart with lengths of 10.7 and 9.1 m in 1992 and 1993, respectively. A randomized complete block design was used with a factorial arrangement of treatments replicated six times. The first factor consisted of plots with (weedy) or without (weed-free) weed interference. Weed-free plots received a preemergence application of atrazine [6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine] at 1.12 kg ai ha^{-1} plus metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide] at 2.24 kg ai ha^{-1} and handweeding as necessary to prevent any weed interference with the crop throughout the growing season.

Weedy plots received a postemergence application of bromoxynil at 0.42 kg ai ha^{-1} plus nicosulfuron at 0.035 kg ai ha^{-1} plus nonionic surfactant[4] at 0.25% (v/v) applied at application timings of 5, 10, 15, or 20-cm measured weed canopy heights. Two additional treatments not included in the factorial arrangement were a handweeded treatment with no herbicides, and a preemergence application of atrazine plus metolachlor applied at the same rates used in the weed-free plots but without handweeding. All herbicides were applied with a tractor-mounted compressed-air sprayer calibrated to deliver 206 L ha^{-1} at 207 kPa using 8003 flat-fan nozzles[5]. Postemergence herbicide application information is presented in Table 1.

Weed density by species was determined at each application timing by counting all weeds within a 929 cm^{-2} quadrant at four random locations within each plot to be treated. Weed biomass was harvested at each application timing in each plot to be treated, and at 30 days after the last treatment (DALT)[6] in all treated plots. At each harvest, all weeds within a single 0.25 by 2.3 m quadrant were cut at ground level, oven dried at 49 °C for 7 days, and weighed. Harvests

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[4]X-77® -Nonionic-type spreader and activator. Principle functioning agents: Alkylaryl polyoxyethylene, free fatty acids, glycols, isopropanol. Constituents effective as spray adjuvant-90%. Constituents ineffective as spray adjuvant-10%. Valent U.S.A. Corp., 1333 N. California Blvd., P.O. Box 8025, Walnut Creek,CA 94596-8025.

[5]Spraying Systems Co., North Avenue, Wheaton, IL 60188.

[6]Abbreviations: DALT, days after the last treatment; DAT, days after treatment.

at application and at 30 DALT were taken at fixed locations 1 and 1.4 m from the back of the plot, respectively.

Weed control by species and corn injury were evaluated visually in weedy plots with 0 representing no visible injury and 100 representing complete plant death. Corn injury was evaluated 4 days after treatment (DAT)[6] and 14 DALT. Weed control was evaluated at 14 DALT.

Corn height was determined 30 DALT by measuring five randomly chosen plants within each plot. Corn grain yield was determined by harvesting the center two rows of each plot with a plot combine. Seed weight was corrected to 15.5% moisture.

Weed densities at each application are presented in Table 2. Nonlinear regression analysis was performed on weed biomass data taken at application and the best fit models are presented in Figure 1. All other data were subjected to analysis of variance and means separated using Fisher's Protected Least Significant Difference Test at $\alpha = 0.05$. Weed biomass data obtained 30 DALT were transformed to the square root and visual weed control data transformed to the arcsin before analysis of variance and mean separation. Original means are presented. Results are presented by year due to year by factor interactions.

RESULTS AND DISCUSSION

Crop injury. Corn injury at 4 DAT was primarily leaf burn typical of bromoxynil injury (3). The injury was much more severe when applied to smaller corn at the 5- and 10-cm application timings (Table 3). Corn grew rapidly and injury symptoms were much less evident by 14 DALT. Applications at 5 or 10-cm weed canopies were made to 20-cm or smaller corn with 3 or fewer fully collared leaves (Table 1). Bromoxynil is more injurious to smaller corn and tank mixing bromoxynil with nicosulfuron requires addition of a nonionic surfactant which may also result in increased initial crop leaf burn (3).

Effects of herbicide injury can be assessed independent of weed interference. The factorial arrangement of treatments allows corn height and grain yield comparisons between application timings within weed-free plots (Table 4).

Herbicide injury reduced corn height at 30 DALT in weed-free plots when applications were made to 5 or 10-cm weeds in 1992, and to 5,10, or 15-cm weeds in 1993 (Table 4). However, corn grain yield was not reduced by herbicide injury from any application in 1993, and in 1992 herbicide injury reduced yields in the 5 and 10-cm plots when compared to the untreated weed-free plots. A severe frost injured corn 3 days after corn emergence and 9 days prior to the 5-cm application in 1992. The combination of environmental stress

and herbicide injury to small corn may explain why yield reduction occurred in 5 and 10-cm treatments in 1992 but not 1993.

The effects of the atrazine plus metolachlor portion of the weed-free plots on corn were assessed by analyzing all treatments as a randomized complete block. The atrazine plus metolachlor plus handweeding treatment provided corn heights and corn yields equivalent to the handweeded treatment (data not shown). From these results, we concluded that the methods used to maintain plots free from weed interference did not have any adverse effects on corn growth or yield.

Weed density. Weed densities were extremely high both years of the study (Table 2). Giant foxtail was the most predominant weed species present both years. More broadleaf weeds were present in 1993, in particular common lambsquarters (*Chenopodium album* L. # CHEAL) and redroot pigweed (*Amaranthus retroflexus* L. # AMARE). Relatively small populations of common ragweed (*Ambrosia artemisiifolia* L. # AMBEL) and velvetleaf (*Abutilon theophrasti* Medik. # ABUTH) were present in both years.

Weed biomass increased exponentially over the time period between planting and the last application timing both years (Figure 1). Between the first and last application timings, 19 and 14 days elapsed in 1992 and 1993, respectively (Table 1). Weed biomass increased by factors of 26 and 17, respectively over these time periods (Figure 1).

Weed control. Weeds were controlled at 14 DALT by postemergence applications of bromoxynil plus nicosulfuron plus nonionic surfactant made at 5, 10, or 15-cm canopy heights (Table 5). Giant foxtail was not controlled completely either year when weed canopies reached 20 cm before application. Giant foxtail control was less in the 5-cm treatment than in the 10 or 15-cm treatment in 1992. Weeds present in the 5-cm treatment at 30 DALT germinated after the initial population was controlled by the herbicide treatment. Neither bromoxynil nor nicosulfuron have residual soil activity (1,3). Greater weed biomass in the 5-cm plots at 30 DALT in 1992 was primarily a result of giant foxtail germination after the postemergence application since this was the most abundant species and the other weeds were controlled. The 20-cm treatment reduced weed biomass less than any of the other treatments in 1992, due primarily to incomplete giant foxtail control at the time of application.

Giant foxtail, common lambsquarters, and redroot pigweed control was inadequate with the 20-cm treatment in 1993 and contributed to greater weed biomass than in any of the other treatments (Table 5). The 5 and 10-cm applications provided the greatest control of giant foxtail and redroot pigweed and the greatest reduction of weed biomass.

Common ragweed and velvetleaf control was generally adequate both years (Table 5). Their populations were relatively low and appeared to contribute relatively little to total weed biomass when compared to giant foxtail,

common lambsquarters, or redroot pigweed (Table 2). Weed biomass was reduced by all postemergence treatments when compared to the untreated plots (Table 5).

Weed interference with corn. Effects of weed interference can be assessed independent of crop injury. The factorial arrangement of treatments allows corn height and corn yield comparisons between weedy and weed-free plots in which each received the postemergence herbicide application at the same timing (Table 4).

Corn height reduction at 30 DALT was closely correlated with yield reduction from weed interference (Table 4). Other research has demonstrated corn height reduction from increasing weed density (17). Weed interference reduced corn height at 30 DALT and corn grain yield at harvest both years in untreated plots. Weed interference reduced corn height and corn grain yield in the 20-cm treatment both years. Weed interference reduced corn height and grain yield in the 15-cm treatment only in 1992. Late season germination of weeds after effective initial control with the 5- and 10-cm applications in 1992 did not reduce corn yields. These results are similar to previous findings in which significant yield reduction occurred when giant foxtail was allowed to interfere with corn until weeds were 15 to 23 cm in height, and late season competition effects were not as great as early season (16).

Corn height and grain yield reduction due to weed interference in the 20-cm treatments can be explained by the inability to control weeds at that advanced growth stage, thereby allowing weed interference to continue throughout the season (Tables 4 and 5). However, the reduction in corn height and corn yield caused by weed interference in the 15-cm treatment in 1992 occurred even though weed control from the postemergence treatment was complete (Table 5).

The critical period is defined as the duration of weed interference with a crop that is allowable before yield reduction will occur (15,24). Previous research has shown that the critical period for weed interference in corn is difficult to define (15,24), and that it varies with environment, weed species, and weed density (15). In our study, no attempt was made to define a critical period. However, our results suggest that the critical period was exceeded when the postemergence application was made to 15-cm weeds in 1992.

This study was not intended to define the mechanisms by which yield reductions occur. However, differences in relative proportions of weed species at the 1992 and 1993 sites may help explain why yield reduction with the 15-cm treatment occurred in 1992 but not 1993. Weed densities were extremely high both years, but the 1993 site had a greater proportion of broadleaf weeds than the 1992 site which was infested almost entirely by giant foxtail (Table 2). Weed species differ in their ability to interfere with crops (7,8,9,11,12,18,19,20,21). Significant allelopathic effects from giant foxtail on corn have also been

demonstrated (5). The likely contribution of environmental and other uncontrolled factors is also recognized.

This study suggests that weed interference will not reduce corn yields under normal environmental conditions if weeds are controlled in a timely manner with postemergence herbicides. Even with intense weed pressure, no yield reductions from weed interference occurred when bromoxynil plus nicosulfuron plus nonionic surfactant was applied to weeds at growth stages recommended by the respective herbicide labels (1,3). Delaying postemergence herbicide applications increases the possibility of inadequate weed control and yield reduction. With intense weed pressure, later applications (>10-cm weeds) may result in yield reductions even though weed control is complete.

LITERATURE CITED

1. Anonymous. 1993. Accent® herbicide product label. E.I. duPont de Nemours and Co., Agricultural Products, Wilmington, DE 19898.

2. Anonymous. 1993. Beacon® herbicide product label. CIBA-GEIGY Corp., Agricultural Division, Greensboro, NC 27419.

3. Anonymous. 1993. Bromoxynil® Product Label. Rhône-Poulenc Ag Co., Research Triangle Park, NC 27709.

4. Anonymous. 1990. Accent® Herbicide. Technical Bull. E.I. duPont de Nemours and Co., Agricultural Products, Wilmington, DE 19898. 12 p.

5. Bell, D.T., and D.E. Koeppe. 1972. Noncompetitive effects of giant foxtail on growth of corn. Agron. J. 64:321-325.

6. Bode, L.E. 1987. Spray application technology. p. 85-109 *in* C.G. McWhorter and M.R. Gebhardt, eds. Methods of Applying Herbicides. Weed Science Society of America, Champaign, IL.

7. Buchanan, G.A. and E.R. Burns. 1971. Weed competition in cotton I. sicklepod and tall morningglory. Weed Sci. 19:576-579.

8. Buchanan, G.A. and E.R. Burns. 1971. Weed competition in cotton II. cocklebur and redroot pigweed. Weed Sci. 19:580-582.

9. Buchanan, G.A., R.H. Crowley, J.E. Street, and J.A. McGuire. 1980. Competition of sicklepod (*Cassia obtusifolia*) and redroot pigweed (*Amaranthus retroflexus*) with cotton (*Gossypium hirsutum*). Weed Sci. 28:258-262.

10. Carey, J.B., P.B. Knoerr, and J.J. Kells. 1990. Herbicide interactions with nicosulfuron and primisulfuron in corn. Abstr. Weed Sci. Soc. Am. 32:12.

11. Chandler, J.M. 1977. Competition of spurred anoda, velvetleaf, prickly sida, and Venice mallow in cotton. Weed Sci.25:151-158.

12. Crowley, R.H. and G.A. Buchanan. 1978. Competition of four morningglory (*Ipomoea spp.*) species with cotton (*Gossypium hirsutum*). Weed Sci. 26:484-488.

13. Dobbels, A.F. and G. Kapusta. 1992. Postemergence weed control in corn with nicosulfuron combinations. Proc. North Cent. Weed Sci. Soc. 47:47.

14. Foy, C.L. and H.L. Witt. 1990. Johnsongrass control with DPX-V9360 and CGA-136872 in corn (*Zea mays*) in Virginia. Weed Technol. 4:615-619.

15. Hall, M.R., C.J. Swanton, and G.W. Anderson. 1992. The critical period of weed control in grain corn (*Zea mays*). Weed Sci. 40:441-447.

16. Knake, E.L. and F.W. Slife. 1969. Effect of time of giant foxtail removal from corn and soybeans. Weed Sci.17:281-283.

17. Moolani, M.K., E.L. Knake, and F.W. Slife. 1964. Competition of smooth pigweed with corn and soybeans. Weeds 12:126-128.

18. Patterson, D.T. and E.P. Flint. 1983. Comparative relations, photosynthesis, and growth of soybean (*Glycine max*) and seven associated weeds. Weed Sci. 31:318-323.

19. Shurtleff, J.L. and H.D. Coble. 1985. The interaction of soybean (*Glycine max*) and five weed species in the greenhouse. Weed Sci. 33:669-672.

20. Snipes, C.E., G.A. Buchanan, J.E. Street, and J.A. McGuire. 1982. Competition of common cocklebur (*Xanthium pensylvanicum*) with cotton (*Gossypium hirsutum*). Weed Sci. 30:553-556.

21. Street, J.E., G.A. Buchanan, R.H. Crowley, and J.A. McGuire. 1981. Influence of cotton (*Gossypium hirsutum*) densities on competitiveness of pigweed (*Amaranthus retroflexus*) and sicklepod (*Cassia obtusifolia*). Weed Sci. 29:253-256.

22. Swanton, C.J. and S.F. Weise. 1991. Integrated weed management: the rationale and approach. Weed Technol. 5:657-663.

23. Weaver, S.E. and C.S. Tan. 1983. Critical period of weed interference in transplanted tomatoes (*Lycopersicon esculentum*): growth analysis. Weed Sci. 31:476-481.

24. Zimdahl, R.L. 1988. The concept and application of the critical weed-free period. p. 145-155 *in* M.A. Altieri and M. Liebman, eds. Weed Management in Agroecosystems: Ecological Approaches. CRC Press, Boca Raton, FL.

Table 1. Postemergence herbicide application information.

			Corn Growth Stage[a]			
	Date		Height		Collared Leaves	
Treatment[b]	1992	1993	1992	1993	1992	1993
cm			— cm —		— no. —	
5	6-3	6-10	8	10	2	2
10	6-9	6-16	15	20	3	3
15	6-16	6-21	30	38	5	4
20	6-22	6-24	46	46	6	4

[a]Corn planted 5-14-92, and 5-17-93.

[b]Weed canopy height at postemergence application of bromoxynil at 0.42 kg ha^{-1} plus nicosulfuron at 0.035 kg ha^{-1} plus nonionic surfactant at 0.25% (v/v).

Table 2. Weed densities at herbicide application.

	Weed Density									
	SETFA		CHEAL		AMARE		AMBEL		ABUTH	
Treatment[a]	1992	1993	1992	1993	1992	1993	1992	1993	1992	1993
cm	no. m^{-2}									
5	500	436	22	288	8	122	0	11	4	4
10	587	395	66	369	11	138	13	22	4	11
15	464	318	61	172	27	109	15	22	5	31
20	588	292	94	178	28	102	15	23	11	12

[a]Weed canopy height at postemergence application of bromoxynil at 0.42 kg ha^{-1} plus nicosulfuron at 0.035 kg ha^{-1} plus nonionic surfactant at 0.25% (v/v).

Table 3. Corn injury from bromoxynil plus nicosulfuron[a].

	Corn Injury			
	4 DAT[b]		14 DALT[b]	
Treatment[c]	1992	1993	1992	1993
cm	%			
5	33 b	38 b	3 a	9 a
10	35 a	43 a	2 ab	10 a
15	7 c	19 c	2 abc	6 b
20	8 c	7 d	1 bc	5 b
Untreated	0 d	0 e	0 c	0 c

[a]Means within a year followed by the same letter are not significantly different according to Fisher's Protected LSD Test ($\alpha = 0.05$). Means cannot be compared across years.

[b]DAT = days after treatment, DALT = days after the last treatment.

[c]Weed canopy height at postemergence application of bromoxynil at 0.42 kg ha^{-1} plus nicosulfuron at 0.035 kg ha^{-1} plus nonionic surfactant at 0.25% (v/v).

Table 4. Influence of herbicide application timing on corn height and corn grain yield[a].

	Corn Height[b]				Corn Grain Yield			
	1992		1993		1992		1993	
Treatment[c]	Weed-free[d]	Weedy	Weed-free	Weedy	Weed-free	Weedy	Weed-free	Weedy
cm	cm				kg ha^{-1}			
5	181 c	185 bc	225 e	232 cd	11090 b	11190 ab	11840 a	12240 a
10	184 c	182 c	233 cd	232 cd	11030 bc	11500 ab	11920 a	11780 a
15	193 ab	167 d	234 cd	236 bc	11650 ab	10380 c	11900 a	11830 a
20	193 ab	150 e	241 ab	228 de	11690 ab	9355 d	12110 a	11190 b
Untreated	194 a	137 f	243 a	199 f	11830 a	3770 e	12300 a	6321 c

[a]Means within a year followed by the same letter are not significantly different according to Fisher's Protected LSD Test ($\alpha = 0.05$). Means cannot be compared across years.

[b]Corn height recorded at 30 days after the last treatment.

[c]Weed canopy height at postemergence application of bromoxynil at 0.42 kg ha^{-1} plus nicosulfuron at 0.035 kg ha^{-1} plus nonionic surfactant at 0.25% (v/v).

[d]Weed-free plots received a preemergence application of atrazine at 1.12 kg ha^{-1} plus metolachlor at 2.24 kg ha^{-1} and handweeding as necessary to prevent any weed interference with the crop throughout the growing season.

Table 5. Influence of weed canopy height on weed control with bromoxynil plus nicosulfuron[a].

	Weed Control[b]										Total Weed Biomass[d]	
	SETFA		CHEAL		AMARE		AMBEL		ABUTH			
Treatment[c]	1992	1993	1992	1993	1992	1993	1992	1993	1992	1993	1992	1993
cm	%										g m^{-2}	
5	86 c	95 b	89 b	94 ab	100 a	99 a	86 b	87 a	87 a	81 ab	14 c	12 cd
10	93 b	99 a	92 b	96 a	95 b	100 a	92 b	94 a	96 a	86 a	4 d	4 d
15	95 a	92 c	99 a	92 b	99 ab	92 b	98 a	94 a	81 b	76 ab	7 cd	22 c
20	60 d	72 d	94 b	78 c	85 c	66 c	95 ab	93 a	70 b	71 b	121 b	46 b
Untreated	0 e	0 e	0 c	0 d	0 d	0 d	0 c	0 b	0 c	0 c	444 a	659 a

[a]Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD Test ($\alpha = 0.05$). Means cannot be compared across years.

[b]Percent control evaluated at 14 days after the last treatment.

[c]Weed canopy height at postemergence application of bromoxynil at 0.42 kg ha^{-1} plus nicosulfuron at 0.035 kg ha^{-1} plus nonionic surfactant at 0.25% (v/v).

[d]Biomass = Above ground dry weight harvested at 30 days after the last treatment.

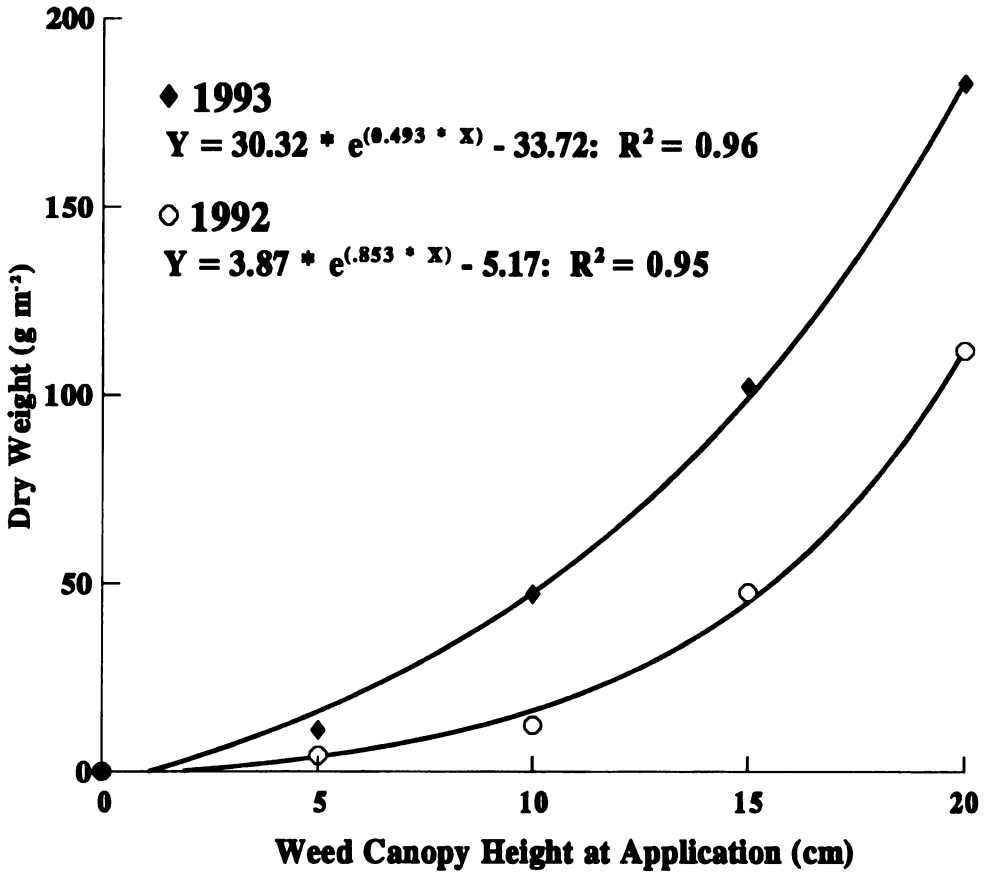

Figure 1. Weed biomass accumulation at postemergence application timings in corn.