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Potential Interactions of Clomazone with Metribuzin, Linuron, and Atrazine in Soybean (Glycine max) and Common Cocklebur (Xanthium Strumarium)

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POTENTIAL INTERACTIONS OF CLOMAZONE WITH METRIBUZIN, LINURON, AND ATRAZINE IN SOYBEAN (GLYCINE MAX) AND COMMON COCKLEBUR (XANTHIUM STRUMARIUM)

By

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ABSTRACT

POTENTIAL INTERACTIONS OF
CLOMAZONE WITH METRIBUZIN, LINURON, AND ATRAZINE IN
SOYBEAN (GLYCINE MAX) AND COMMON COCKLEBUR (XANTHIUM STRUMARIUM)

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Observations in the field indicated a synergistic interaction between clomazone plus metribuzin and clomazone plus linuron in soybean. Experiments were conducted to determine if these herbicide combinations injured soybean and resulted in yield reduction compared to combinations of alachlor plus metribuzin and alachlor plus linuron, and if atrazine residues influenced these interactions. Further experiments were conducted to determine if temperature and rainfall after herbicide application were influencing these interactions and what rates of clomazone plus metribuzin would cause a synergistic interaction in soybean, common cocklebur, and redroot pigweed. The effect of clomazone plus metribuzin and clomazone plus linuron was also studied to determine if one herbicide was affecting the uptake, partitioning, and/or metabolism of the other. Field experiments indicated that combinations of clomazone plus metribuzin and clomazone plus linuron increased soybean injury, and reduced leaf area, shoot weight, and root weight. Yield was reduced 19% by combinations of clomazone plus linuron in one year. Injury was most severe in soils with low organic matter and clay content, which reduced the amount of metribuzin absorbed, allowing for increased plant uptake. The interactions of clomazone plus linuron and clomazone plus metribuzin

were not changed when soybean was germinated under cool and warm temperature regimes. Soybean shoot dry weight was reduced an average of 89% from combinations of clomazone plus metribuzin. Placement of herbicide-treated soil in the same zone as the soybean seed increased injury from clomazone plus metribuzin 31% but did not increase from clomazone plus metribuzin, compared to when treated soil was placed above or below the seed. Combinations of clomazone plus metribuzin were synergistic and reduced soybean and common cocklebur shoot weights in the greenhouse. Equivalent rates of clomazone and metribuzin reduced soybean shoot dry weight more on a 2.5% organic matter loam soil compared to a 4.4% organic matter loam soil. Studies with ^{14}C-herbicides indicated that parent metribuzin levels averaged 9% higher in soybean roots and shoots, and averaged 15% higher in common cocklebur roots and shoots when clomazone was present. Parent linuron levels were 19% higher in soybean roots. There were no differences in the uptake or partitioning of clomazone, metribuzin, or linuron by the addition of metribuzin or linuron to clomazone, or clomazone to metribuzin or linuron.

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INTRODUCTION

Clomazone, a soil-applied herbicide for weed control in soybean, has a limited weed control spectrum making it desirable to apply it with other herbicides to increase the species of weeds controlled. In 1986, it was noted that combinations of clomazone plus metribuzin and clomazone plus linuron interacted synergistically in soybeans resulting in severe injury, stand and yield reductions. Research on these potential synergistic interactions could provide several benefits. Environmental influences on these interactions could be documented and potential explanations formulated. Research on the rates of clomazone and metribuzin that interact synergistically in soybean and common cocklebur could lead to changes in recommendations that would provide increased control of common cocklebur, yet still be safe to soybean. An understanding of the uptake, translocation, and metabolism of each herbicide would not only supply answers to why the synergism was occurring, but could also provide information on the degradation of clomazone in soybean and common cocklebur. At this time, the degradation pathway of clomazone is unknown, though hypotheses have been formulated. A broad overview of the problem of synergism would allow for an opportunity to tie field observations to experiments in more controlled environments or to laboratory studies in order to better understand the synergism of clomazone plus metribuzin and clomazone plus linuron.

This research was conducted to determine: (a) if combinations clomazone plus metribuzin and clomazone plus linuron were synergistic and to compare the effects of these combinations to combinations of alachlor plus metribuzin and alachlor plus linuron; (b) if atrazine residues were influencing the synergism of clomazone plus metribuzin and clomazone plus linuron; (c) if either temperature or herbicide placement in the soil influenced soybean response to these herbicide combinations; (d) if the range of application rates at which soybean demonstrated the synergistic interaction of clomazone plus metribuzin and determine if the synergistic interaction could be exploited in common cocklebur; and (d) if the basis for the synergism in soybean and common cocklebur to clomazone plus metribuzin and clomazone plus linuron is due to differences in uptake, partitioning, and/or metabolism.

CHAPTER 1

INTERACTIONS AND HERBICIDAL EFFECTS ON PLANT PROCESSES

A REVIEW OF THE LITERATURE

INTERACTIONS

With the realization that chemicals could be used to selectively control unwanted plants growing alongside desirable plants, scientists began to combine two or more herbicides that had limited weed spectrums individually in an attempt to control a wider range of weed species. However, the results of these herbicide combinations was sometimes unexpected. In some instances two chemicals that when applied separately resulted in death and/or severe injury to individual weeds did not appear to have the same effect when applied together. Alternatively, some combinations of chemicals resulted in more severe injury to the weed and/or crop than was anticipated from the action of each individually.

The most accepted term to describe an unespected plant response to a herbicide combination is interaction. Research involving herbicide interactions has been conducted for some time, but there has not been general agreement on describing chemical interactions in terms of plant response. It should be noted that chemical interactions are not the same as statistical interactions. Interaction in a strict statistical sense in an analysis of variance has been defined as a measure of the departure of the simple effects from an additive law or model based on main effects only (10). In a factorial experiment this means the difference in response between the levels of one factor are not the same at all levels of the other factor (6). Drury (3) noted that a statistical interaction is actually rooted in calculus and is the action of y on the action x on f(x,y) or the second partial derivative, $d^2f(x,y)/dxdy$. Alternatively, the term interaction may be used statistically to describe responses that have been shown to be interactions by proper use of Fisher's analysis of variance (5).

Most weed scientists view an interaction in plant physiology terms, specifically phytotoxicity. Interaction is defined as the total response to a combination of individual toxicants (8). This implies that each herbicide or compound in an interaction has a response of its own. Many papers have been published that report plant responses to mixtures of chemicals and describe the response without using the term interaction. In this review the term interaction shall be defined as a phytotoxic interaction.

Plant responses to herbicide mixtures are described as synergistic, antagonistic, additive, or enhanced effect. Scientists have defined a synergistic response as "if over a range of rates and ratios, the response is greater than that obtained when one chemical is substituted for the other at rates based on the activity of each chemical used singly" (1). There has been no agreement among researchers as to the definition of antagonism. Akobundo et al. (1) defined antagonism as the opposite of synergism or "if over a range of rates and ratios the response is less than that obtained when one chemical is substituted for the other at rates based on the activity of each chemical used singly". Nash (8) used a similar definition although he emphasized the comparison of the action of the mixture to the sum of the individual chemicals.

There is disagreement as to whether additive and enhancement effects can be considered interactions. An additive effect is what is normally expected from the mixture of two chemicals; the response is the same when one chemical is substituted for another. Nash (8) did not consider an additive effect an interaction. An enhancement is the "effect of a herbicide and a nontoxic adjuvant applied in combination on a plant...(if) the response is greater than that obtained when the herbicide is used at the same rate without the adjuvant (1). Enhancement is not usually included in discussions of interaction because the adjuvant alone is usually not active.

A great deal of the difficulty in defining an interaction comes from attempts to quantify the expected responses of a plant to a herbicide mixture. A formula devised by Colby (2) has been used frequently by weed scientists in describing the effect of a herbicide mixture on plants. The formula is:

$$E=XY/100$$

where E = the expected percent inhibition of growth by herbicides A and B at p and q rates; X = the percent inhibition of growth by herbicide A at p rate; and Y = the percent inhibition of growth by herbicide B at q rate. If the actual value of E is greater than the expected value the interaction is synergistic; if the actual value is less than the expected value the interaction is antagonistic. Statistical significance between the actual value and the expected value can be determined by using the modified Least Significance Difference equation developed by Hamill and Penner (4). Rummens (9) used the formula developed by Colby yet modified it to calculate inhibition (X and Y in Colby's formula). He gave this formula as:

$$X \text{ or } Y=A/(x/c)^{B}+1$$

where X or Y = measured response; x = concentration of herbicide used; A = response at x = 0; c = concentration of herbicide for which the response is reduced to 50% of A; and B = a dimensionless parameter defining the sharpness of the 'bend' in the response curve. Synergism was then defined as any significant deviation from E according to Colby's formula and could be either positive or negative. The negative response was defined as antagonism. These methods of dealing with herbicide mixtures are considered to be multiplicative survival models and are useful when studying a mixture containing herbicides that have dissimilar action (7).

Another method to describe chemical interactions was developed by Tammes (11). This method involves transforming the data through probit analysis and developing a new curve, or isobole, from constant inhibition values (usually ID_{50}). Drury (3) used calculus to calculate a multiple regression equation followed by differentiation with respect to each herbicide and then differentiating with respect to both herbicides combined. The second derivative values were then graphed to indicate the areas of interaction. Nash (8) noted that this method was no better than the multiple regression equation that is calculated and instead proposed a regression estimate method. In this method, best fit equations are obtained for separate herbicides and the response values were calculated as functions of pesticide dosage. This provided estimated value of reductions obtained from the product of the individual regression equations. This method also gave statistical significance to the deviation from the expected values, something that is lacking in some other methods of determining interactions.

Herbicide interactions remain an area that merits research. More time will need to be spent understanding and refining methods of calculating interactions and measuring or quantifying the interaction. With increased use of herbicide combinations to broaden weed control spectrums, there will continue to a demand for research on specific interactions.

LITERATURE CITED

1. Akobundo, I. O., R. D. Sweet, and W. B. Duke. 1975. A method of evaluating herbicide combinations and determining herbicide synergism. Weed Sci. 23:20-25.

2. Colby, S. R. 1967. Calculating synergistic and antagonistic responses of herbicide combinations. Weeds 15:20-22.

3. Drury, R. E. 1980. Physiological interaction, its mathematical expression. Weed Sci. 28:575-579.

4. Hamill, A. S. and D. Penner. 1973. Interaction of alachlor and carbofuran. Weed Sci. 21:330-335.

5. Lockhart, J. A. 1965. The analysis of interactions of physical and chemical factors on plant growth. An. Rev. Plant Phys. 16:37-52.

6. Montgomery, D. C. 1984. Design and Analysis of Experiments. John Wiley and Sons, New York. p. 190.

7. Morse, P. M. 1978. Some comments on the assessment of joint action in herbicide mixtures. Weed Sci. 26:58-71.

8. Nash, R. G. 1981. Phytotoxic interaction studies-techniques for evaluation and presentation of results. Weed Sci. 29:147-155.

9. Rummens, F. H. A. 1975. An improved definition of synergistic effects. Weed Sci. 23:4-6.

10. Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach. McGraw-Hill Book Co., New York. p. 340-342.

11. Tammes, P. M. L. 1964. Isoboles, a graphic representation of synergism in pesticides. Neth. J. Plant Path. 70:73-80.

HERBICIDAL EFFECTS ON PLANT PROCESSES

An understanding of the plant process affected by herbicides is essential in studying herbicide interactions. Traditionally, the nature of herbicidal uptake, translocation, and mode of action are usually not elucidated until herbicidal properties have been demonstrated, though this has changed in recent years. Therefore it has been possible for an interaction to be noted without an understanding of the basis for the interaction. An understanding of the chemical and/or physiological reasons for an interaction may allow for the prediction of interactions by similar chemicals, an opportunity to better understand the mode of action of a herbicide, and may offer a starting point in altering, overcoming, or preventing interactions.

Atrazine. Atrazine is in the triazine family of herbicides and is used for controlling annual broadleaf and grass weeds in corn (*Zea mays* L.). Atrazine has been noted for its carryover potential, especially in the Midwest and Great Plains, where a common rotation is corn followed by an atrazine-sensitive crop such as soybeans [*Glycine max* (L.) Merr.]. In Missouri, residual levels of atrazine of 0.19 kg ha^{-1} have been found one year after an application of 2.24 kg ai ha^{-1} (27).

Uptake of atrazine by the soybean seed is believed to be a physical process, such as diffusion, since there was no difference in uptake between living and dead seeds (23). The rate of absorption was rapid for the first few hours, but then decreased steadily until germination (24). Absorption by plant roots grown in an aqueous solution was also in two phases with initial rapid uptake followed by a slower continuous uptake. Rates of absorption and translocation were found to be proportional to the amount of water absorbed and/or the translocation rate (24). These results are indicative of apoplastic movement.

Once in the plant, atrazine inhibits photosynthesis with an associated decrease in transpiration. Atrazine blocks the electron transport chain in photosynthesis between the primary electron acceptor in photosystem II, a plastoquinone with special properties termed Q, and the plastoquinone (PQ) pool (1). This results in decreased photosynthesis and inhibition of carbon dioxide (CO_2) that has been noted by researchers (8,15). However, atrazine does not affect nonphotosynthetic CO_2 fixation (8). While the effects of atrazine on *Chlorella vulgaris* could be countered by the addition of glucose (1), it has been noted that the symptoms of atrazine injury are not consistent with a slow starvation of a plant and may be due to a secondary factor (1,5,25). However, the effects of the secondary factors have been found to be reversible if carbohydrate is not limiting (25).

Atrazine injury is indicated by chlorosis of plant tissue in susceptible species. The blocking of electron transport leads to the formation of excited chlorophyll that can only dissipate the excess energy by fluorescence or free radical formation. Free radical formation ultimately leads to formation of hydroxyl free radicals which attack cell membranes resulting in their peroxidation and eventual desiccation of the plant.

Metribuzin. Metribuzin, like atrazine, is also in the triazine family of herbicides. It is applied to control annual grass and broadleaf weeds in many crops including soybeans. Metribuzin has not been studied as extensively as atrazine and discussions of metribuzin activity have been dependent on findings of the action of other triazine herbicides. Movement in the plant is thought to be apoplastic. Metribuzin was more mobile in a sensitive species, hemp sesbania (*Sesbania exultata* L.), than in soybean (16). Metribuzin inhibits photosynthesis by blocking electron transport in photosystem II in the same manner as atrazine and was also found to inhibit plant respiration (4,10).

Soybean cultivars have displayed differential tolerance to metribuzin (13,26). Metribuzin has been found to be readily absorbed by the roots and translocated to the shoot in both tolerant and susceptible cultivars (14,26). Therefore, differences in tolerance are believed to be due to the metabolism of metribuzin (14,26), with tolerant cultivars detoxifying metribuzin more rapidly (18). Oswald and coworkers (21) have proposed that tolerance in soybeans is related to the presence of an unknown enzyme found in both tolerant and susceptible cultivars.

Linuron. Linuron is a substituted urea herbicide used for control of annual grass and broadleaf weeds in soybeans and other crops. Rapid initial uptake of linuron occurred in soybean roots followed by slower uptake, suggesting that the uptake process was passive (19). Absorption of linuron by soybean roots from nutrient solution appeared to be passive and governed by the entrance of water (20). Uptake by a susceptible species, giant foxtail (*Setaria faberii* L.), was greater by the plant shoot than by the root (17). However, Walker (30) reported that more linuron was taken up by the roots of turnip (*Brassica rapa* L.), lettuce (*Lactuca sativa* L.), and ryegrass (*Lolium perenne* L.) than by shoots. In tolerant species, linuron did not translocate out of the plant roots to the same degree observed in sensitive species (1).

The primary action of linuron, like most of the substituted urea herbicides, is thought to be inhibition of photosynthesis by blocking electron transfer between Q and PQ in photosystem II. Diuron, another substituted urea herbicide, is often used in research to block photosystem II to enable isolation of photosystem I.

Alachlor. Alachlor, a chloroacidanilide herbicide, controls grasses and some broadleaf weeds in many crops, including soybeans. Alachlor is absorbed by soybean roots and translocated to the shoots (7), although among other species there is considerable shoot uptake. In the plant, alachlor does not affect the Hill reaction or photosystems I or II (6). In barley (*Hordeum vulgare* L.), alachlor has been shown to inhibit gibberellic acid-induced alpha-amylase synthesis by repressing the genes that code for alpha amylase (9). Alachlor may also act as an alkylating agent.

Clomazone. Clomazone has been developed for grass and broadleaf weed control in soybeans. Information has only recently been published about the mode of action or uptake of clomazone. Susceptible plants in the field turn white, become chlorotic, and are shortened. Duke and coworkers (12), suggested that clomazone blocks both diterpene and tetraterpene synthesis in pitted morningglory (*Ipomoea lacunosa* L.). Growth of etiolated pitted morningglory plants treated with clomazone was inhibited, indicating a reduction in gibberellin levels. Cotyledons of treated plants had levels of protochlorophyllide equal to cotyledons of untreated plants; however, the Shibata shift, a shift in the absorbance of precursor of chlorophyll a because of the addition of phytol, was reduced. Carotenoid levels were also greatly reduced in treated plants. Both phytol and gibberillins are diterpenoids while carotenoids are tetraterpenoids. In similar research with cowpea (*Vigna unguicula* L.), a less susceptible species, only the reduction in growth of etiolated seedlings and elimination of the Shibata shift was observed (11). Uptake of clomazone by the susceptible species redroot pigweed (*Amaranthus retroflexus* L.) and livid amaranth (*Amaranthus lividus* L.) was greater then uptake by the tolerant species soybean and smooth pigweed (*Amaramthus hybridus* L.) (28)

LITERATURE CITED

1. Ashton, F. M., T. Bisalputra, and E. B. Risley. 1966. Effect of atrazine on *Chorella vulgaris*. Am. J. Bot. 53:217-219.

2. Ashton, F. M. and R. K. Glenn. 1979. Influence of chloro-, methoxy-, and methylthio-substitutions of bis(isopropylamino-*s*-triazine) on selected metabolic processes. Pestic. Biochem. Physiol. 11:201-207.

3. Ashton, F. M. and A. S. Crafts. 1981. Mode of Action of Herbicides. J. Wiley & Sons. New York. 525pp.

4. Boger, P. and U. Schlue. 1976. Long term effects of herbicides on the photosynthetic apparatus: I. Influence of diuron, triazines and pyridazinones. Weed Res. 16:149-154.

5. Bush, P. B. and S. K. Ries. 1974. Effect of atrazine on elongation of the embryonic axis of red kidney bean. Weed Sci. 22:227-229.

6. Chandler, J. M., L. I. Croy, and P. W. Santelmann. 1972. Alachlor effects on plant nitrogen metabolism and Hill reaction. J. Agric. Food Chem. 20:661-664.

7. Chandler, J. M., E. Basler, and P. W. Santlemann. 1974. Uptake and translocation of alachlor in soybean and wheat. Weed Sci. 22:253-258.

8. Couch, R. W. and D. E. Davis. 1966. Effect of atrazine, bromacil, and diquat on $^{14}CO_2$-fixation in corn, cotton, and soybeans. Weeds 14:251-255.

9. Devlin, R. M. and R. P. Cunningham. 1970. The inhibition of gibberellic acid induction of α-amylase activity in barley endosperm by certain herbicides. Weed Res. 19:316-320.

10. Draber, K., K. H. Buchel, K. Dickore, A. Trebst, and E. Pistorius. 1969. Structure-activity correlation of 1,2,4-triazinones, a new group of phototsynthetic inhibitors. pp. 1789-1795 *in* H. Metzner ed. Prog. Photosynthesis Research. Vol. III.

11. Duke, S. O. and W. H. Kenyon. 1986. Effects of dimethazone (FMC 57020) on chloroplast development: II. Pigment synthesis and photosynthetic function in cowpea (*Vigna unguiculata* L.) primary leaves. Pestic. Biochem. Physiol. 25:11-18.

12. Duke, S. O., W. H. Kenyon, and R. N. Paul. 1985. FMC 57020 effects on chloroplast development on pitted morningglory (*Ipomoea lacunosa*) cotyledons. Weed Sci. 33:786-794.

13. Eastin, E. F., J. W. Sij, and J. P. Craigmiles. 1980. Tolerance of soybean genotypes to metribuzin. Agron. J. 72:167-168.

14. Falb, L. N. and A. E. Smith, Jr. 1984. Metribuzin metabolism in soybeans: Characterization of the intraspecific differential tolerance. J. Agric. Food Chem. 32:1425-1428.

15. Funderburk, H. H. and M. C. Darter. 1965. The effect of amitrole, atrazine, dichlobenil, and paraquat on the fixation and distribution of $^{14}CO_2$ in beans. Proc. 18th Southern Weed Control Conf. P. 607.

16. Hargroder, T. G. and R. L. Rogers. 1974. Behavior and fate of metribuzin in soybean and hemp sesbania. Weed Sci. 22:238-245.

17. Knake, E. L. and L. M. Wax. 1968. The importance of the shoot of giant foxtail for uptake of preemergence herbicides. Weed Sci. 16:393-395.

18. Mangeot, B. L., F. E. Slife, and C. E. Rieck. 1979. Differential metabolism by two soybean (*Glycine max*) cultivars. Weed Sci. 27:267-269.

19. Moody, K., C. D. Kurst, and K. P. Buchholtz. 1970. Release of herbicides by soybean roots in culture solutions. Weed Sci. 18:214-218.

20. Nashed, R. B., and R. D. Ilnicki. 1970. Absorption, distribution, and metabolism of linuron in corn, soybean, and crabgrass. Weed Sci. 18:25-28.

21. Oswald, T. H., A. E. Smith, and D. V. Phillips. 1978. Phytotoxicity and detoxification of metribuzin in dark-grown suspension cultures of soybeans. Pestic. Biochem. Physiol. 8:73-83.

22. Rao, V. S. and W. B. Duke. 1976. Effect of alachlor, propachlor, and prynachlor of GA^3-induced production of protease and α-amylase. Weed Sci. 24:616-618.

23. Rieder, G., K. P. Buchholtz, and C. A. Kurst. 1970. Uptake of herbicides by soybean seed. Weed Sci. 18:101-105.

24. Scott, H. D. and R. Phillips. 1973. Absorption of herbicides by soybean seed. Weed Sci. 21:71-76.

25. Shimabukuro, R. H., V. J. Masteller, and W. C. Walsh. 1976. Atrazine injury: Relationship to metabolism, substrate level, and secondary factors. Weed Sci. 24:336-340.

26. Smith, A. E., and R. E. Wilkinson. 1974. Differential absorption, translocation, and metabolism of metribuzin (4-amino-6-tert-butyl-3-(methylthio)-as-triazine-5(4H)one) by soybean cultivars. Physiol. Plant. 32:253-257.

27. Talbert, R. E. and O. H. Fletchall. 1964. Inactivation of simazine and atrazine in the soil. Weeds 12:33-37.

28. Vencill, W. K., K. K. Hatzios, and H. P. Wilson. 1990. Absorption, translocation, and metabolism of ^{14}C-clomazone in soybean (*Glycine max*) and three *Amaranthus* weed species. J. Plant Growth Regul. 9:127-132.

29. Vostral, J. J., K. P. Buchholtz, and C. A. Kust. 1970. Effect of root temperature on absorption and translocation of atrazine in soybeans. Weed Sci. 18:115-117.

30. Walker, A. 1973. Vertical distribution of herbicides in soil and their availability to plants: Shoot compared with root uptake. Weed Res. 13:407-415.

CHAPTER 2

INTERACTION IN SOYBEAN OF CLOMAZONE, METRIBUZIN, LINURON, ALACHLOR, AND ATRAZINE

ABSTRACT

Field observations in 1986 indicated that a synergistic interaction in soybean could occur from combinations of clomazone plus metribuzin and clomazone plus linuron. Field experiments were conducted in 1988, 1989, and 1990 at two locations in Michigan. Atrazine treatment consisted of rates of 0, 1.1, 2.2, and 3.4 kg ai ha^{-1} to determine if residues in soil would influence the soybean herbicide interactions. Atrazine was applied the year previous to the plots. Herbicide treatments in soybean included clomazone, metribuzin, linuron, and alachlor alone, and in various combinations. A synergistic response in soybean from clomazone plus linuron and clomazone plus metribuzin occurred, however, soil characteristics and weather conditions impacted soybean response. Experiments were conducted in the growth chamber to measure these synergistic interactions and to determine if they were influenced by ambient air and/or soil temperature and placement of the herbicide in the soil. The temperature regime did not affect the response of soybean to these herbicides applied alone or in combination. Under both cool and warm temperature regimes there was a synergistic interaction from a combination of clomazone plus metribuzin. Leaf area and shoot dry weight were equally reduced from a combination of clomazone plus metribuzin when placed in any soil zone. There was a decrease in leaf area and shoot dry weight only when soil treated with clomazone plus linuron or atrazine plus metribuzin was placed in the same zone as the soybean seed and not when the herbicide-treated soil was either above or below the germinating seed.

Nomenclature: alachlor, 2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide; atrazine, 6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine; clomazone, 2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone; linuron, *N'*-(3,4-dichlorophenyl)-*N*-methoxy-*N*-methylurea; metribuzin, 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one; soybean, *Glycine max* (L.) Merr. 'Century'.

INTRODUCTION

Increased phytotoxicity and stand reduction to soybean have occurred following clomazone plus metribuzin and clomazone plus linuron applications when compared to alachlor or metolachlor combined with either metribuzin or linuron (personal observations). Increased injury to soybeans was noted in eastern Arkansas from combinations of clomazone plus metribuzin applied preemergence (PRE) compared to metribuzin applied PRE alone (17). Soybean yield was reduced by applications of clomazone plus metribuzin compared to untreated soybeans. Werling and Buhler (16) observed increased injury to no-till soybeans from applications of clomazone plus metribuzin compared to clomazone applied alone in Wisconsin. Yields were reduced when clomazone and metribuzin were applied together early preplant (EPP) or PRE compared to when clomazone was applied EPP followed by metribuzin applied PRE. In addition to the synergistic interactions of clomazone plus metribuzin and clomazone plus linuron there exists the potential for synergistic interactions among other soybean herbicides and residual atrazine. Atrazine applied for weed control in corn may persist in the soil and injure soybeans (5), and a synergistic injury response in soybean to residual atrazine and metribuzin in soil has been reported (8).

Rainfall patterns, soil moisture, and soil and/or air temperature during the initial stages of soybean development may influence soybean response to herbicides. Increased soybean injury from applications of either metribuzin alone or clomazone plus metribuzin on soils with organic matter contents of 1.3% was attributed to rainfall totals of 7.7 cm 1 week after herbicide application making the herbicide readily available and rapidly moving the herbicide to the soybean roots (17). Significant injury had not been noted the year previously, a year with normal rainfall patterns. In both years, however, applications of metribuzin at 560 g ha^{-1} alone or combined with clomazone resulted in soybean yield reduction. Conversely, in studies conducted on a soil with an organic matter content of 5.5%, decreased soybean injury occurred from metribuzin applied alone when 8.5 cm of rain fell 10 days after treatment, compared to soybean in plots that received 0.6 cm of rainfall during that same time period (15). Yields were not reported in the study. The affinity of metribuzin for organic matter (11, 13), and planting depth may have influenced these differences in soybean response to metribuzin following heavy rainfall within 10 days of application.

Early season injury from metribuzin is not always reflected in yield reductions. Wax observed yield reductions only from metribuzin applied preplant incorporated (PPI) at 1.1 kg ha^{-1}, while significant visual injury also occurred from metribuzin applied PPI or PRE at 0.6 and 0.8 kg ha^{-1}. Hagood et al. (6) studied the relationship between early-season soybean injury and yield response under weed-free conditions. Significant injury from metribuzin at the one to two trifoliolate-leaf growth stage was not an adequate indicator of yield response unless there was a concomitant reduction in soybean stand.

The objectives of these studies were to: (a) determine if combinations of clomazone plus metribuzin and clomazone plus linuron were synergistic, (b) determine if soybean injury increased and yield decreased from combinations of clomazone plus metribuzin and clomazone plus linuron compared to combinations of alachlor plus metribuzin and alachlor plus linuron, and (c) determine if either temperature or herbicide placement in the soil influenced soybean response to these herbicide combinations.

MATERIALS AND METHODS

Field studies. Field experiments were conducted in 1988, 1989, and 1990 at two locations in Michigan. Soil characteristics for each location and year are summarized in Table 1.

The experimental design was a split plot with atrazine levels as the main plot treatments and soybean herbicides as the subplot treatments. Treatments of atrazine consisted of rates of 0, 1.1, 2.2, and 3.4 kg ha^{-1}. The atrazine was applied to the plots 1 year before soybean planting. Corn was grown using standard crop production practices. The following spring the main plots were moldboard plowed and disked in one direction, perpendicular to the corn rows to evenly distribute the atrazine residues and prepare the soil for planting. Two soil samples consisting of five cores each to a depth of 15 cm were taken from each atrazine rate in each replication. The atrazine was extracted from the soil for quantification following the method developed by Smith (14). The procedure in brief is as follows. Twenty grams of wet soil were extracted for 23 hr in a Soxhlet tube containing 150 ml of methanol:water (9:1). The methanol was removed by rotoevaporation, the residue suspended in methylene chloride, and extracted in methylene chloride with distilled water. The methylene chloride was removed by rotoevaporation and the sample resuspended in

Table 1. Soil characteristics at East Lansing and Hickory Corners in 1988, 1989, and 1990[a].

	Location	
Year	East Lansing	Hickory Corners
1988	Capac loam Mixed, mesic Aeric Ochraqualf 46% sand, 40% silt, 14% clay pH=6.5, 2.5% OM	Oshtemo sandy loam Mixed, mesic Typic Hapludalf 71% sand, 15% silt, 14% clay pH=6.3, 1.6% OM
1989	Capac loam Mixed, mesic Aeric Ochraqualf 48% sand, 39% silt, 13% clay pH=6.0, 4.4% OM	Oshtemo sandy loam Mixed, mesic Typic Hapludalf 65% sand, 23% silt, 12% clay pH=6.3, 1.6% OM
1990	Celina loam Mixed, mesic Aquic Hapludalf 25% sand, 35% silt, 40% clay pH=6.1, 2.2% OM	Kalamazoo loam Mixed, mesic Typic Hapludalf 39% sand, 30% silt, 31% clay pH=5.9, 2.6% OM

[a]OM=organic matter

isooctane. The atrazine residues were quantitated by using a gas chromatograph[1] equipped with a nitrogen/phosphorous detector. The column temperature was set on a gradient program with an initial temperature of 100 C and a final temperature of 240 C. The temperature was increased 20 C min^{-1}. Quantification was accomplished by injecting samples containing known levels of atrazine to make a standard curve. Samples were then compared against the standard curve to determine the total atrazine in the isooctane. Total amount in the soil was based on the soil dry weight.

Soybean herbicides were applied on May 11, 1988, May 18, 1989, and May 9, 1990 at East Lansing and May 6, 1988, May 16, 1989, and May 2, 1990 at Hickory Corners (Table 2). Soybean herbicide treatments were applied with a tractor-mounted, compressed-air sprayer in 206 L ha^{-1} of water at 207 kPa. Herbicides were incorporated with a Danish S-tine field cultivator[2] set to a depth of 7 cm. 'Century' soybean, intermediate in metribuzin tolerance[3], were planted in 76-cm rows. Plots were four rows wide and 9 m in length. Plots were kept weed free all season with a tractor-mounted cultivator and hand hoeing.

Injury ratings and stand counts were recorded 3 weeks after planting (WAP) from observations made on soybean plants in the middle 6 m of the two center rows in each plot. Injury ratings were based on soybean

[1]HP 5890A. Hewlett-Packard Co. Palo Alto, CA 94304.

[2]Kongskilde. Kongskilde Corp. Bowling Green, OH 43402.

[3]Field sheet, "Soybean tolerance to metribuzin-northern edition.", January, 1991. Mobay Corporation, Kansas City, MO 64120.

Table 2. Herbicide treatments, application methods, and rates in 1988, 1989, and 1990.

Herbicide	Application method[a]	Rate
		kg ha^{-1}
Clomazone[b]	PPI	0.8
Clomazone	PPI	1.1
Metribuzin[c]	PPI	0.3
Metribuzin	PPI	0.4
Linuron	PRE	0.6
Alachlor	PPI	2.2
Clomazone + metribuzin	PPI + PPI	1.1 + 0.4
Clomazone + metribuzin[c]	PPI + PPI	1.1 + 0.3
Clomazone + metribuzin[c]	PPI + PPI	0.8 + 0.4
Clomazone + linuron	PPI + PRE	1.1 + 0.6
Alachlor + linuron	PPI + PRE	2.2 + 0.6
Alachlor + metribuzin	PPI + PPI	2.2 + 0.4
Untreated	--	--

[a]PPI=preplant incorporated; PRE=preemergence.
[b]Treatment at MSU in 1989 and KBS in 1990 only.
[c]Treatments at KBS and MSU in 1989 and 1990 only.

stunting, leaf discoloration, chlorosis, and necrosis. An injury rating of 0 indicated no visible injury while a rating of 100 indicated all plants in the observation area were dead. At 7 WAP, six plants were harvested from each plot outside of the area to be harvested for yield. These six plants were evaluated for number of nodules, leaf area, dry root weight, and dry shoot weight. Nodulation counts were included because linuron has been implicated in reducing soybean nodulation (4). Plant response to the herbicide treatments was more pronounced 3 weeks after planting, but the later harvest date accounted for plant recovery from earlier injury, thus, it may be a better indicator of season-long response. At maturity the center 6 m of the middle two rows was harvested for yield with a small plot combine. Yields were adjusted to 13.5% moisture.

In 1988, all data collected was subjected to analysis of variance and means separated by Least Significant Differences (LSD) at the 5% level. The data collected in 1989 and 1990 was converted to the percent of untreated plants, with the exception of visual injury data, before analysis of variance and mean separation. Data were not combined over years because of significant year by location by treatment interactions. For the herbicide combinations the expected mean was also calculated following the method of Colby (2). The modified LSD test developed by Hamill and Penner (7) was used to determine if the response of soybean to two herbicides was additive or synergistic. Data is averaged over three replications at each location each year with the exception of Hickory Corners in 1989 which is averaged over four replications.

Temperature effects. Experiments were conducted in the growth chamber[4] to determine if the ambient air temperature at the time of soybean emergence would influence soybean response to herbicides applied alone and in combination. Air-dried Capac loam soil (fine-loamy, mixed, mesic Aeric Ochraqualf, 52% sand, 25% silt, 23% clay, pH=6.4, 2.5% organic matter (OM)) was placed into 473-ml plastic pots prior to herbicide application. After the herbicide was incorporated with a soil mixer, it was placed into 946-ml plastic cups, already containing 473 ml of untreated soil. Herbicide treatments included clomazone PPI at 1.1 kg ai ha^{-1}, metribuzin PPI or PRE at 0.4 kg ai ha^{-1}, linuron PRE at 0.6 kg ai ha^{-1}, alachlor PPI at 2.2 kg ai ha^{-1}, clomazone PPI at 1.1 kg ha^{-1} plus metribuzin PPI at 0.4 kg ha^{-1}, clomazone PPI at 1.1 kg ha^{-1} plus metribuzin PRE at 0.4 kg ha^{-1}, clomazone PPI at 1.1 kg ha^{-1} plus linuron PRE at 0.6 kg ha^{-1}, and clomazone plus alachlor PPI and PPI at 1.1 plus 2.2 g ha^{-1}. In addition, untreated plants were maintained for use as comparisons. All herbicides were applied with a stationary, air-pressurized, greenhouse pot sprayer in 206 L ha^{-1} of water at 207 kPa. Three Century soybean seeds were planted in each pot and thinned to one plant per pot following emergence. Pots did not have any drainage holes to prevent herbicides from leaching out the bottom from watering. To prevent overwatering, pots were weighed after planting and herbicide application, and soil moisture levels based on 18% by weight were calculated and recorded. When the soil surface was observed to be dry, soil was irrigated to 18% moisture.

[4]Conviron CMP 3244. Controlled Environments, Ltd. Winnipeg, Manitoba, Canada.

Pots were subjected to one of two temperature regimes. The warm regime had temperatures of 18 C for the daily low and 28 C for the daily high. The cool regime had temperature settings of 8 C for the daily low and 18 C for the daily high for 0 to 10 days after planting and 13 C and 23 C for daily high and low, respectively, for 10 to 21 days after planting. Lights in the growth chambers had an intensity of 320 μE $m^{-1}s^{-2}$ and were set for a 15 hour day. Plant shoots were harvested at soil level when the third trifoliolate leaf was fully expanded, cotyledons removed, air dried, and weighed.

The experimental design was a split plot with temperature regime as the main plot and herbicide treatments as the subplot. The experiment was repeated twice in time with four replications each time. Means were subjected to analysis of variance and significant treatment means separated by LSD at the 5% level. Expected response from herbicide combinations and the Hamill-Penner LSD were calculated as described in the field studies.

Herbicide placement. Experiments were conducted in the greenhouse to determine if the placement of herbicide-treated soil in relation to the soybean seed would influence soybean shoot weight or leaf area. Air-dried Capac loam soil (fine-loamy, mixed, mesic Aeric Ochraqualf, 52% sand, 25% silt, 23% clay, pH=6.4, 2.5% OM) received applications of clomazone PPI at 840 g ha^{-1} plus metribuzin PPI 280 g ha^{-1}, clomazone PPI at 840 g ha^{-1} plus linuron PRE at 560 g ha^{-1}, and atrazine PPI at 5 g ha^{-1} plus metribuzin PPI at 280 g ha^{-1}. Herbicide applications were made with a stationary, compressed air, pot sprayer in 206 L ha^{-1} of water at 207 kPa. All treatments were thoroughly mixed with a soil mixer. Pots with a volume of

946 ml were filled to the 473-ml level with untreated soil; this was measured to be 7 cm below the final soil surface. A 2.5 cm layer of treated soil was positioned either below, above, or in the same zone as the soybean seed. The treated soil was separated from the untreated soil by placing a 0.5 cm layer of activated charcoal above and below the treated soil layer. When the treated soil was placed above the seed, charcoal was only placed below the layer as the top was also the soil surface. In all placement regimes, three soybean seeds were planted 3.5 cm below the soil surface. The soybean seedlings were thinned to one per pot. Soil was maintained at 18% soil moisture by surface irrigation as described in the previous experiment. Natural sunlight was supplemented by sodium lamps with an intensity of 300 $\mu E\ m^{-1}s^{-2}$ set for a 15 hour day. Pots were arranged in a completely randomized design and the experiment was repeated twice in time with four replications each time. Plants were harvested at soil level at the V3 growth stage, cotyledons removed, leaf area measured, and shoots dried and weighed. All data was subjected to analysis of variance. Mean comparisons were made by LSD at the 5% level for soil placement within each herbicide treatment.

RESULTS AND DISCUSSION

Field studies. Atrazine. Extractable atrazine remaining in the soil one year after application was below 200 parts per billion, a level considered harmful to soybean[5] (Table 3). Atrazine levels varied depending on location and year. At five of six plot locations, atrazine was detected

[5]Field sheet, "General guidelines to crop sensitivity to popular herbicides", Grower Service, Lansing, MI 48906.

Table 3. Residual atrazine in soil 1 year after application.[a]

Year sampled	Application rate	Location: East Lansing	Location: Hickory Corners
	kg ha^{-1}	ppb[b]	
1988	0	6 ± 8	28 ± 27
	1.1	17 ± 72	33 ± 35
	2.2	24 ± 32	58 ± 50
	3.4	25 ± 43	70 ± 62
1989	0	0	8 ± 2
	1.1	7 ± 5	9 ± 17
	2.2	11 ± 9	13 ± 11
	3.4	28 ± 16	15 ± 10
1990	0	7 ± 1	6 ± 1
	1.1	18 ± 79[c]	33 ± 20
	2.2	34 ± 10	49 ± 18
	3.4	39 ± 7	88 ± 34

[a]ppb=parts per billion. Lower detection limit was 0.5 ppb in the soil.
[b]± standard deviation.
[c]Mean of three replications was 17 ppb. Mean of one replication was 221 ppb.

in all main plots, including those that had not received an atrazine application the year previously. Atrazine is a commonly used herbicide in corn as part of a rotation with soybean in Michigan and occasionally has been found to persist in the soil up to 9 years after application (1).

1988. Nodulation, leaf area, dry shoot weight, and dry root weight at Hickory Corners and East Lansing in 1988 were not influenced by atrazine residues or by soybean herbicide treatments (data not presented). Soybean injury and stand count were not affected by atrazine residues at Hickory Corners or East Lansing, but differences were noted among soybean herbicide treatments (Table 4). Visual injury from the combination of clomazone plus metribuzin was greater than that from each herbicide applied alone at East Lansing but not at Hickory Corners, while clomazone plus linuron reduced soybean stand compared to clomazone applied alone at Hickory Corners only. However, yield differences amongst treatments were not significant at East Lansing and Hickory Corners in 1988 (data not presented).

For 7 weeks after planting, rainfall was 6 and 29% of the following 2 years at Hickory Corners and East Lansing, respectively (Table 5). This lack of rainfall may have reduced herbicide availability to soybeans.

1989. Despite the low moisture levels of the 1988 growing season, atrazine residues were below 28 ppb at East Lansing and 15 ppb at Hickory Corners when measured at soybean planting in 1989 (Table 3). These low residual atrazine levels did not influence soybean response to herbicide treatments, and all results in 1989 are averaged over atrazine levels.

Table 4. Response of soybean to herbicides applied alone and in combination at Hickory Corners and East Lansing in 1988.

Treatment	Herbicide rate	Visual injury		Soybean stand
		Location		
		East Lansing	Hickory Corners	
		3 WAP[a]		
	kg ha^{-1}	%		Plants 12 m^{-1}
Clomazone	1.1	7	13	84
Metribuzin	0.4	3	26	82
Linuron	0.6	0	37	78
Alachlor	2.2	1	16	77
Clomazone + metribuzin	1.1 + 0.4	14	21	68
Clomazone + linuron	1.1 + 0.6	5	29	68
Alachlor + linuron	2.2 + 0.6	0	23	66
Alachlor + metribuzin	2.2 + 0.4	-	16	68
LSD(0.05)	-	6	12	11

[a]WAP=weeks after planting.

Table 5. Total rainfall recorded for 7 weeks after planting soybeans at East Lansing and Hickory Corners in 1988, 1989, and 1990.

	Location	
Year	East Lansing	Hickory Corners
	cm	
1988	1	6
1989	22	21
1990	19	18

Combinations of clomazone plus metribuzin and clomazone plus linuron resulted in more injury than did combinations of alachlor plus metribuzin and alachlor plus linuron, respectively at Hickory Corners (Table 6). Nodule number was reduced by metribuzin at 0.4 kg ha^{-1} and combinations of clomazone plus metribuzin and clomazone plus linuron. Reductions in leaf area, shoot weight, and root weight occurred from combinations of clomazone plus metribuzin. Reductions in leaf area, shoot weight, and root weight occurred from clomazone plus linuron compared to alachlor plus linuron or untreated plants; this response was also synergistic. Yield was reduced, compared to untreated plants, by applications of clomazone plus linuron at Hickory Corners and the response was synergistic. Rainfall of 13 cm within 16 days after planting may have moved herbicides in the soil to where they were readily taken up by the emerging soybeans. The sandy loam soil had 1.6% OM, which would be conducive to herbicide movement and increased availability for uptake.

At East Lansing, applications of alachlor plus metribuzin and alachlor plus linuron reduced root weight compared to applications of clomazone plus metribuzin and clomazone plus linuron (Table 7). Reductions in leaf area, shoot weight, and root weight occurred from combinations of clomazone plus metribuzin. At East Lansing, yield was reduced only by metribuzin plus clomazone applied at the highest rate, compared to untreated plants. No interactions at East Lansing were synergistic. High OM (4.4%) in the loam soil may have made herbicide

Table 6. Response of soybean to herbicides applied alone and in combination at Hickory Corners in 1989[a].

Treatment	Herbicide rate	Visual injury (3 WAP)	Nodules six $plants^{-1}$ (7 WAP)	Leaf area (7 WAP)	Shoot weight (7 WAP)	Root weight (7 WAP)	Yield
	kg ha^{-1}	%	% of untreated[b]				
Clomazone	1.1	16	96	86	93	92	105
Metribuzin	0.3	1	85	70	73	67	106
Metribuzin	0.4	3	76	56	70	67	98
Linuron	0.6	0	90	83	93	83	106
Alachlor	2.2	0	100	89	89	92	107
Clomazone + metribuzin	0.8 + 0.4	18	68	61	68	75	92
Clomazone + metribuzin	1.1 + 0.4	21	71	44	54	58	93
Clomazone + metribuzin	1.1 + 0.3	26	71	45	59	58	94
Clomazone + linuron	1.1 + 0.6	25	75	51*	59*	58*	81*
Alachlor + linuron	2.2 + 0.6	3	80	74	82	75	94
Alachlor + metribuzin	2.2 + 0.4	1	76	60	66	67	97
LSD(0.05)		6	20	17	14	14	15

[a]WAP=weeks after planting. Synergistic interactions are denoted with an asterisk.
[b]Average values for untreated plants were: number of nodules six $plants^{-1}$=93; leaf area=754 cm^2; shoot weight=4.4 g; root weight=1.2 g; yield=3520 g ha^{-1}.

Table 7. Response of soybean to herbicides applied alone and in combination at East Lansing in 1989[a].

		--3 WAP--	-----7 WAP-----				
Treatment	Herbicide rate	Visual injury	Nodules six plants^{-1}	Leaf area	Shoot weight	Root weight	Yield
	kg ha^{-1}	%	-----% of untreated[b]-----				
Clomazone	0.8	0	72	90	94	78	95
Clomazone	1.1	2	84	102	108	93	100
Metribuzin	0.3	12	76	95	98	86	93
Metribuzin	0.4	15	68	90	92	78	97
Linuron	0.6	0	84	107	112	93	103
Alachlor	2.2	2	89	114	117	93	101
Clomazone + metribuzin	0.8 + 0.4	38	66	77	82	64	93
Clomazone + metribuzin	1.1 + 0.4	31	55	80	85	64	89
Clomazone + metribuzin	1.1 + 0.3	25	71	82	88	71	95
Clomazone + linuron	1.1 + 0.6	12	76	93	95	71	94
Alachlor + linuron	2.2 + 0.6	0	88	101	111	86	97
Alachlor + metribuzin	2.2 + 0.4	16	78	91	94	86	105
LSD(0.05)		8	17	18	17	11	10

[a]WAP=weeks after planting.
[b]Average values for untreated plants were: number of nodules six plants^{-1}=128; leaf area=761 cm^2; shoot weight=6.5 g; root weight=1.4 g; yield=3490 g ha^{-1}.

unavailable to plants, even though 15 cm of rain fell within 14 days of planting. This may explain that reductions in leaf area, shoot weight, and root weight were not as great as at Hickory Corners, despite similar rainfall totals after planting.

1990. Clomazone at 1.1 kg ha^{-1} injured soybean at Hickory Corners in 1990 and addition of metribuzin did not elevate injury levels (Table 8). Leaf area and shoot weight measured 7 WAP were reduced by clomazone plus metribuzin at 1.1 plus 0.4 kg ha^{-1}, clomazone plus linuron, alachlor plus linuron, and alachlor plus metribuzin. In addition, shoot weight was reduced by clomazone alone at 1.1 kg ha^{-1}. Root weight was reduced by clomazone alone at 1.1 kg ha^{-1}, clomazone plus metribuzin at 1.1 plus 0.4 kg ha^{-1} and at 1.1 plus 0.3 kg ha^{-1}, clomazone plus linuron, and alachlor plus metribuzin. There was no decrease in leaf area, shoot weight, and root weight from combinations of clomazone plus metribuzin or clomazone plus linuron compared to alachlor plus metribuzin or alachlor plus linuron. Yield, as compared to untreated plants, was not affected by any of the herbicide treatments.

Neither visual injury, soybean stand, or yield were reduced from any of the soybean herbicide treatments at any atrazine level at East Lansing in 1990 (data not presented). There was an interaction between atrazine levels and soybean herbicide treatments for leaf area, dry shoot weight, and root weight. In plots that had atrazine treatments of 0 or 2.2 kg ha^{-1} in 1989, leaf area, shoot weight, and root weight were not significantly different (data not presented); however, when atrazine was applied at 1.1 kg ha^{-1} in 1989, the combination of clomazone plus metribuzin at 1.1 plus 0.4 kg ha^{-1} interacted synergistically for leaf area, shoot weight, and

Table 8. Response of soybean to herbicides applied alone and in combination at Hickory Corners in 1990[a].

Treatment	Herbicide rate	--3 WAP-- Visual injury	----7 WAP---- Nodules six plants^{-1}	Leaf area	Shoot weight	Root weight
	kg ha^{-1}	%	----% of untreated----			
Clomazone	0.8	12	104	95	93	95
Clomazone	1.1	23	96	90	89	89
Metribuzin	0.3	6	96	91	91	90
Metribuzin	0.4	0	104	101	96	97
Linuron	0.6	1	112	97	92	93
Alachlor	2.2	1	119	107	98	104
Clomazone + metribuzin	0.8 + 0.4	11	112	91	87	91
Clomazone + metribuzin	1.1 + 0.4	29	97	82	82	83
Clomazone + metribuzin	1.1 + 0.3	20	101	89	90	86
Clomazone + linuron	1.1 + 0.6	27	93	85	84	78
Alachlor + linuron	2.2 + 0.6	3	101	88	86	91
Alachlor + metribuzin	2.2 + 0.4	3	103	86	83	88
LSD(0.05)		8	17	12	11	11

[a]WAP=weeks after planting.
[b]Average values for untreated plants were: number nodules six plants^{-1}=100; leaf area=618 cm^2; shoot weight=3.7 g; root weight=1.7 g.

root weight (Table 9). Combinations of clomazone plus metribuzin at 1.1 plus 0.3 kg ha^{-1} and clomazone plus linuron also interacted synergistically for leaf area. When atrazine was applied at the highest rate of 3.4 kg ha^{-1} in 1989, applications in 1990 of clomazone plus metribuzin at 1.1 plus 0.3 kg ha^{-1} interacted synergistically and reduced leaf area, shoot weight, and root weight.

The soybean injury observed in this research may be partially explained by soil and herbicide sorption characteristics. Metribuzin is adsorbed by both clay and soil organic matter (12), while clomazone (9) and linuron (3) availability are primarily influenced by organic matter content. Injury from combinations of clomazone plus metribuzin and clomazone plus linuron occurred at Hickory Corners in 1988 and 1989 on soils of high sand content, and low organic matter and clay content. Injury was greater in 1989 than in 1988 perhaps because adequate rainfall allowed for herbicide uptake by germinating soybean seedlings. Soil organic matter alone did not appear to decrease soybean injury in 1989 because the organic matter content of the soil at East Lansing was 4.4%, yet soybean injury was observed. Injury was less severe in 1990, despite adequate rainfall, at both locations because soils had higher clay contents. Thus soil type and weather conditions appear to impact soybean response to clomazone, metribuzin, and linuron

Temperature effects Temperature during soybean germination and emergence did not influence soybean response to herbicide treatment (data not shown) and data was combined over temperature regime. Metribuzin applied PPI or PRE reduced shoot dry weight compared to untreated plants (Table 10). In both cool and warm temperature regimes, clomazone applied with metribuzin

Table 9. Response of soybean 7 WAP to herbicides applied alone and in combination at atrazine rates of 1.1 kg ha^{-1} and 3.4 kg ha^{-1} at East Lansing in 1990[a].

		Atrazine Rate					
		1.1 kg ha^{-1}			3.4 kg ha^{-1}		
Treatment	Rate	Leaf area	Shoot weight	Root weight	Leaf area	Shoot weight	Root weight
	kg ha^{-1}	--% of untreated[b]--			--% of untreated[c]--		
Clomazone	1.1	143	142	111	125	121	112
Metribuzin	0.3	109	111	92	111	123	114
Metribuzin	0.4	138	124	96	74	79	92
Linuron	0.6	97	102	83	73	78	81
Alachlor	2.2	114	102	95	60	61	75
Clomazone + metribuzin	0.8 + 0.4	90	110	90	95	112	98
Clomazone + metribuzin	1.1 + 0.4	58*	63*	66*	90	91	96
Clomazone + metribuzin	1.1 + 0.3	112*	107	84	54*	63*	78*
Clomazone + linuron	1.1 + 0.6	77*	101	72	80	86	90
Alachlor + linuron	2.2 + 0.6	94	85	76	113	97	111
Alachlor + metribuzin	2.2 + 0.4	164	157	112	91	95	90
LSD(0.05)		47	34	NS	35	29	22

[a]WAP=weeks after planting. Synergistic interactions are denoted with an asterisk.
[b]Average values of untreated plants were: leaf area=1169 cm^2; shoot weight=8.6 g; root weight=2.4 g.
[c]Average values of untreated plants were: leaf area=926 cm^2; shoot weight=7.2 g; root weight=1.8 g.

Table 10. Response of soybean shoot weight to applications of herbicides applied alone and in combination averaged over two temperature regimes[a,b].

Treatment	Application method[c]	Herbicide rate	Shoot weight
		kg ha^{-1}	% of untreated[d]
Clomazone	PPI	1.1	84
Metribuzin	PPI	0.4	60
Metribuzin	PRE	0.4	72
Linuron	PRE	0.6	103
Alachlor	PPI	2.2	91
Clomazone + metribuzin	PPI + PPI	1.1 + 0.4	14*
Clomazone + metribuzin	PPI + PRE	1.1 + 0.4	26*
Clomazone + linuron	PPI + PRE	1.1 + 0.6	89
Clomazone + alachlor	PPI + PPI	1.1 + 2.2	86
Untreated	--	--	100
LSD(0.05)			13

[a]Plants were harvested when the third trifolialate leaf was fully expanded.
[b]Temperature regime was not significant in the analysis of variance. Means are combined over temperature regime. Synergistic interactions are denoted with an asterisk.
[c]PPI-preplant incorporated; PRE-preemergence.
[d]Average shoot weight of untreated plants was 0.7 g.

either PPI or PRE reduced shoot weight further to levels of 14 and 27% of untreated plants, respectively and these interactions were determined to be synergistic.

Temperature has been found to alter herbicide uptake in soybean. Penner (10) found that soybean translocated more linuron and atrazine from the root to the shoot at 30 C than at 20 C resulting in decreased plant height and dry weight. The results in this study did not reflect any effect from the different temperature regimes.

Herbicide placement Response of soybean to clomazone plus metribuzin was similar regardless of the placement of the herbicide-treated soil (Table 11). Conversely, for combinations of clomazone plus linuron and atrazine plus metribuzin, placement of herbicide-treated soil in the same zone as the seed was necessary for a reduction in leaf area and dry shoot weight to occur.

These results suggest that the severity of injury from clomazone plus metribuzin is not dependent upon movement of the these herbicides in the soil to the area of the germinating soybean seed. Clomazone plus linuron, however, is similar to a combination of atrazine plus metribuzin in that movement of these herbicides in the soil to where the germinating seed is present may significantly increase the level of injury from these herbicides. As movement of herbicides downward in the soil profile is primarily dependent on rainfall, heavy rainfall shortly after planting and preemergence herbicide application may move the herbicides into the seed zone, resulting in increased injury to soybean.

Table 11. Response of soybean to placement of soil treated with clomazone plus metribuzin, clomazone plus linuron, or atrazine plus metribuzin in relation to the seed.

Herbicides	Placement	Leaf area	Shoot weight
		------------% of untreated[a]-------------	
Clomazone + metribuzin			
	Above	61	58
	With	52	52
	Below	66	66
Clomazone + linuron			
	Above	70	73
	With	48	45
	Below	69	69
Atrazine + metribuzin			
	Above	69	71
	With	37	32
	Below	65	65
LSD(0.05)		22	17

[a]Average values of untreated plants: leaf area=291 cm^2; shoot weight=1.4 g.

The field studies support observations in producers fields in 1986, our own field studies, and studies in eastern Arkansas (17). Rainfall increased injury from linuron supporting the observation that it is necessary for this herbicide to be in the seed zone. Metribuzin placement was not critical and with PPI applications the herbicide was available to the soybean seed.

The field studies further indicate that a potential for a synergistic interaction from combinations of clomazone plus metribuzin and clomazone plus linuron exists, even though it may not appear every year. Other factors such as soil characteristics, primarily clay content, and rainfall play a role in determining if the interactions of clomazone plus metribuzin and clomazone plus linuron will be additive or synergistic. Temperature at the time of soybean germination and emergence, however, does not appear to be a factor in the interactions. Atrazine residues may enhance the interactions of clomazone plus metribuzin and clomazone plus linuron but this only occurred one location in one year and was not consistent with increasing rates of atrazine or the levels of atrazine extracted or detected. In the field studies we were able to detect the synergistic interactions by visual examination and quantify it by measuring leaf area, shoot weight, and root weight. Early season stand count was a poor predictor of the interaction as the herbicides did not usually prevent soybean emergence, but instead killed plants before the expansion of the first trifoliolate leaf. Visual injury, leaf area, shoot weight, and root weight did not predict yield loss, probably because of the ability of soybean to compensate for early season injury (6).

While these experiments did not directly examine the interactions of clomazone plus metribuzin and clomazone plus linuron on any weed species, the response of soybean to these herbicide combinations suggest that a similar response could occur in weed species. Weeds such as common cocklebur (*Xanthium strumarium* L.) or redroot pigweed (*Amaranthus retroflexus* L.), which are marginally affected by clomazone, metribuzin, and linuron, could exhibit increased injury from combinations of these herbicides. Research will need to be conducted to determine if these herbicides are synergistic in weed species. If synergism does exist, reduced rates of these herbicides could result in acceptable weed control without injury to soybeans. The behavior of these herbicides in both soybean and weed species will also provide insight as to how the interaction is occurring.

LITERATURE CITED

1. Caprial, P., A. Haisch, and S. U. Khan. 1985. Distribution and nature of bound (nonextractable) residues of atrazine in a mineral soil nine years after the herbicide application. J. Agric. Food Chem. 33:567-569.

2. Colby, S. R. 1967. Calculating synergistic and antagonistic responses of herbicide combinations. Weeds 15:20-22.

3. Dubrey, H. D. and J. F. Freeman. 1964. Influence of soil properties and microbial activity on the phytotoxicity of linuron and diphenamid. Soil Sci. 97:334-340.

4. Dunigan, E. P., J. P. Frey, L. D. Allen, Jr., and A. McMahon. 1972. Herbicide effects on the nodulation of *Glycine max* (L.) Merrill. Agron. J. 64:806-808.

5. Fink, R. J. and O. H. Fletchall. 1969. Soybean injury from triazine residues in soil. Weed Sci. 17:35-36.

6. Hagood, E. S., Jr., J. L. Williams, Jr., and T. T. Bauman. 1980. Influence of herbicide injury on the yield potential of soybeans (*Glycine max*). Weed Sci. 28:40-45.

7. Hamill, A. S. and D. Penner. 1973. Interaction of alachlor and carbofuran. Weed Sci. 21:330-335.

8. Ladlie, J. S., W. F. Meggitt, and D. Penner. 1977. Effect of atrazine on soybean tolerance to metribuzin. Weed Sci. 25:115-121.

9. Loux, M. M., R. A. Liebl, and F. W. Slife. 1989. Adsorption of clomazone on soils, sediments, and clays. Weed Sci. 37:440-444.

10. Penner, D. 1971. Effect of temperature on phytotoxicity and root uptake of several herbicides. Weed Sci. 19:571-576.

11. Peter, C. J. and J. B. Weber. 1985. Adsorption, mobility, and efficacy of metribuzin as influenced by soil properties. Weed Sci. 33:868-873.

12. Savage, K. E. 1977. Metribuzin persistence in soil. Weed Sci. 25:55-59.

13. Sharom, M. S. and G. R. Stephenson. Behavior and fate of metribuzin in eight Ontario soils. Weed Sci. 24:153-160.

14. Smith, A. E. 1981. Comparison of solvent systems for the extraction of atrazine, benzoylprop, flamprop, and trifluralin from weathered field soils. J. Agric. Food Chem. 29:111-115.

15. Wax, L. M. 1977. Incorporation depth and rainfall on weed control in soybeans with metribuzin. Agron J. 69:107-110.

16. Werling, V. L. and D. D. Buhler. 1988. Influence of application time on clomazone activity in no-till soybeans, *Glycine max*. Weed Sci. 36:629-635.

17. Westberg, D. E., L. R. Oliver, and R. E. Frans. 1989. Weed control with clomazone alone and with other herbicides. Weed Tech. 3:678-685.

CHAPTER 3

INTERACTION OF CLOMAZONE AND METRIBUZIN IN SOYBEAN, COMMON COCKLEBUR, AND REDROOT PIGWEED

ABSTRACT

Experiments were conducted in the greenhouse to determine if a synergistic interaction occurred in soybean, common cocklebur, and redroot pigweed to combinations of clomazone plus metribuzin. The soybean experiment was conducted on two silt loam soils, one with 2.5% organic matter (OM) and the other with 4.4% OM. Reductions in soybean shoot dry weight occurred in the soil with 2.5% OM soil metribuzin at 280 and 560 g ai ha^{-1} plus clomazone at 1680 g ai ha^{-1}, and from metribuzin at 560 g ha^{-1} plus clomazone at 420 and 840 g ha^{-1}. Soybean shoot dry weight was reduced by metribuzin at 560 g ha^{-1} plus clomazone at 420, 840, or 1680 g ha^{-1} in the 4.4% OM soil. The clomazone plus metribuzin interactions in soybean were synergistic. Metribuzin applied at 70 g ha^{-1} plus clomazone at 420 or 840 g ha^{-1} synergistically decreased common cocklebur dry shoot weight. Redroot pigweed dry weight was severely reduced from clomazone alone and an interaction between clomazone plus metribuzin was not evident. **Nomenclature:** clomazone, 2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone); metribuzin, 4-amino-6-(1,1 dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one; common cocklebur, *Xanthium strumarium* L. # XANST; redroot pigweed, *Amaranthus retroflexus* L. # AMARE; soybean, *Glycine max* (L.) Merr. 'Century'.

INTRODUCTION

Researchers have observed a synergistic response in soybean to applications of clomazone plus metribuzin (9, 12, 14). Clomazone is adsorbed to organic matter (OM) whereas metribuzin is adsorbed to clay and OM (7, 8, 11), thus an interaction at a given application rate may be dependent on soil type.

Herbicide interactions, definitions of synergism, and methods of measuring synergism have been reviewed by Hatzios and Penner (6). Difficulty has come in developing methods of measuring synergism. Care must be taken in examining the assumptions implicit in the method chosen. A test developed by Colby (2) has been used frequently because of its simplicity. It is based on a multiplicative survival model which assumes that the two compounds under study have different modes of action.

Clomazone and metribuzin have different sites of action in a plant. Metribuzin inhibits photosynthesis by blocking electron transport in photosystem II (1, 3). The site of action of clomazone has not been conclusively determined, but Duke et al. (4) suggest that clomazone blocks diterpene and tetraterpene synthesis. Specifically, clomazone inhibits the conversion of geranylgeranyl pyrophosphate from isopentenyl pyrophosphate (10). Symptomology of sensitive plant species, including bleaching of the leaves, indicate that clomazone is not a photosynthetic inhibitor. Therefore a multiplicative model for evaluating the interaction is appropriate.

The purpose of this study was to: (a) determine the range of application rates at which soybean demonstrated the synergistic interaction of clomazone and metribuzin on two soils with differing OM levels, and (b) determine if this synergistic interaction of clomazone and metribuzin could be exploited in providing common cocklebur and redroot pigweed control, two weeds that are not usually controlled when either of these herbicides are applied singly.

MATERIALS AND METHODS

The study was conducted with three soils. For the soybean study, two Capac loam soils (mixed, mesic Aeric Ochraqualf) that varied in organic matter (OM) content were used. Both soils contained 46 to 48% sand, 39 to 40% silt, and 13 to 14% clay. Soil pH was 6.5 and OM 2.5% for one soil while pH was 6.0 and OM 4.4% for the other soil. A Spinks sandy loam soil (mixed, mesic Psammentic Hapludalf, 71% sand, 19% silt, 10% clay, pH=6.2, 0.8% OM) was used for the study with common cocklebur and redroot pigweed. Plastic pots 24-cm tall and 11-cm in diameter with a total volume of 946 ml were filled with untreated soil to a level 10 cm below the top of the pot. Soil to be treated was put into smaller 473 ml plastic pots and herbicides were applied with a stationary, air pressurized greenhouse pot sprayer in 206 L ha^{-1} of water at 207 kPa. A factorial design was utilized with treatments consisting of clomazone at 0, 420, 840, and 1680 g ha^{-1} for all species and metribuzin at 0, 140, 280, and 560 g ha^{-1} for soybeans,; 0, 70, 140, 280 g ha^{-1} for common cocklebur; and 0, 35, 70, and 140 g ha^{-1} for redroot pigweed. Clomazone rates were selected based on the standard field recommendation of 840 g ha^{-1}. Metribuzin rates were based on the standard field recommendation of 280 g ha^{-1} for soybeans, and on preliminary common cocklebur and redroot pigweed experiments. Treated soil was mixed for 30 sec with a soil mixer and then used to fill the larger pots within 4 cm of the pot top. Three 'Century' soybean seeds or three common cocklebur burs were planted 2 cm deep in the treated soil in each pot and thinned to one plant per pot following emergence. Redroot pigweed seed was stirred into the soil. Emergence of redroot pigweed was erratic and plants were not thinned.

Pots were weighed after planting and herbicide application. Soil moisture levels based on 18% by soil weight were calculated and recorded. When the soil surface was observed to be dry, soil was irrigated to 18% moisture. This procedure precluded varying soil moisture levels from influencing herbicide availability.

Pots were placed in the greenhouse where natural sunlight was supplemented by sodium halide lights with an intensity of 300 $\mu E\ m^{-1} sec^{-2}$ set for a 15 hour day. Pots were arranged in a completely randomized design with four replications and the experiment was repeated twice in time. Soybean plants were harvested at the soil level at the when the second trifoliolate leaf was fully expanded; common cocklebur at the fourth leaf stage; and redroot pigweed 4 weeks after planting. The cotyledons were removed and shoots dried and weighed. Data was subjected to analysis of variance and Fishers Protected Least Significant Differences (LSD) calculated at the 5% probability level. For the herbicide combinations the expected mean was calculated following the method of Colby (2) and the modified LSD test developed by Hamill and Penner (5) used to determine if the response to the herbicide combinations was synergistic.

RESULTS AND DISCUSSION

Soybean. Response of soybean to clomazone plus metribuzin was dependent on soil OM content (Table 1). Clomazone alone did not reduce soybean shoot dry weight on either soil, regardless of application rate. Metribuzin alone at 560 g ha^{-1} reduced soybean shoot dry weight only when the soil OM was 2.5%, indicating a use rate too high for this soil and masking any herbicide interaction. Clomazone applied at 420, 840, and 1680 g ha^{-1} when combined with metribuzin application of 280 and 560 g ha^{-1} severely injured soybean grown in the 2.5% OM soil. When the soil OM was 4.4%, severe injury to soybean occurred only when metribuzin was applied at 560 g ha^{-1} plus any rate of clomazone. Several rates of metribuzin and clomazone were calculated to be acting synergistically.

In examining the analysis of variance for both organic matter levels, the F-value for metribuzin was much greater than the F-value for clomazone. This indicates that the rate of metribuzin had a greater influence in reducing shoot weight than the rate of clomazone.

Some of the synergistic interactions that were calculated for soybean dry shoot weights were for values equal or greater than the dry shoot weight of untreated plants. This is because the formula developed by Colby is based on data from plants treated with one of the herbicides in the combination. In this study soybean dry shoot weight from plants treated with clomazone alone at 420 and 840 g ha^{-1} and metribuzin at 140 and 280 g ha^{-1} on both soils were greater than plants treated with no herbicide. Using these observed values in Colby's formula results in expected values for the herbicide combinations being much larger than the observed values. When the observed value for a herbicide combination is

Table 1. Dry shoot weight of soybean treated with clomazone and/or metribuzin grown in Capac silt loam soil with 2.5% or 4.4% organic matter contents[a].

Clomazone	Metribuzin	Shoot dry weight[b]	
		------Organic Matter------	
		2.5%	4.4%
-----g ai ha^{-1}-----	-----g ai ha^{-1}-----	-----% of untreated[c]-----	
420	0	121	112
840	0	135	123
1680	0	97	118
0	140	135	113
0	280	112	114
0	560	32	90
420	140	100*	104*
420	280	40*	112
420	560	17	53*
840	140	104*	112*
840	280	55*	101*
840	560	32	50*
1680	140	67*	108*
1680	280	38*	87*
1680	560	15	6*
LSD(0.05)		28	23

[a]Soybean plants were harvested when the second trifolialate leaf was fully expanded.
[b]Combinations determined to be synergistic are denoted with an asterisk.
[c]Average weight of untreated plants in 2.5% OM soil was 0.9 g. Average weight of untreated plants in 4.4% OM soil was 0.7 g.

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greater or similar to the observed value for untreated plants, any calculated synergism may not be of great consequence.

The data from applications of clomazone alone and metribuzin alone at low rates would suggest that these herbicide have a stimulatory effect on soybean shoot weight. The consistency of this response indicates that the phenomena was indicative of a true stimulatory effect. Sublethal doses of herbicides such as 2,4-D [(2,4-dichlorophenoxy)acetic acid] have been shown to stimulate plant development (12).

Common cocklebur. Increasing rates of clomazone or metribuzin applied alone decreased shoot dry weight (Table 2). Plants were stunted and extensive whitening (clomazone injury) or chlorosis (metribuzin injury) of the leaves were observed. Metribuzin alone at 280 kg ha^{-1} killed common cocklebur seedlings. A synergistic response occurred when metribuzin at 70 g ha^{-1} was combined with clomazone at 420 or 840 g ha^{-1}.

Redroot pigweed. Clomazone at 420 and 840 g ha^{-1} decreased redroot pigweed dry shoot weight 89 and 100%, respectively (data not presented). Metribuzin at 140 g ha^{-1} decreased redroot pigweed shoot dry weight 74% and all herbicide combinations resulted in complete kill of the seedlings. No synergism in redroot pigweed response was detected from any of the herbicide interactions in this study, because of the high degree of response of redroot pigweed to clomazone.

These experiments indicate that clomazone and metribuzin can interact synergistically in both soybean and common cocklebur. The metribuzin application rate should be reduced when combined with clomazone to reduce the potential for soybean injury. Synergism noted on common cocklebur would be beneficial for a broader spectrum weed control than

Table 2. Dry shoot weight of common cocklebur grown in soil treated with clomazone and metribuzin[a].

Clomazone	Metribuzin	Shoot dry weight[b]
-------g ai ha^{-1}-----	-------g ai ha^{-1}-----	---% of untreated[c]---
420	0	68
480	0	41
1680	0	30
0	70	86
0	140	20
0	280	0
420	70	21*
420	140	0
420	280	0
840	70	11*
840	140	0
840	280	0
1680	70	0
1680	140	0
1680	280	0
LSD(0.05)		22

[a]Common cocklebur plants were harvested with four fully expanded leaves.
[b]Combinations determined to be synergistic are denoted with an asterisk.
[c]Average dry shoot weight of untreated plants was 0.4 g.

from either herbicide alone. Other weeds such as eastern black nightshade (*Solanum ptycanthum* Dun.), that are poorly controlled by either herbicide alone, may be adequately controlled by the herbicides combined. Synergism could be exploited for weed control, without causing injury to the soybean crop.

LITERATURE CITED

1. Boger, P. and U. Schlue. 1976. Long-term effects of herbicides on the photosynthetic apparatus I. Influence of diuron, triazines and pyridazinones. Weed Res. 16:149-154.

2. Colby, S. R. Calculating synergistic and antagonistic responses of herbicide combinations. Weeds 15:20-22.

3. Draber, W., K. H. Buchel, K. Dickore, A. Trebst, and E. Pistorius. 1969. Structure-activity correlation of 1,2,4-triazinones, a new group of photosynthetic inhibitors. Prog. Photosyn. Res. 2:1789-1795.

4. Duke, S. O., W. H. Kenyon, and R. N. Paul. 1985. FMC 57020 effects on chloroplast development on pitted morningglory (*Ipomoea lacunosa*) cotyledons. Weed Sci. 33:786-794.

5. Hamill, A. S. and D. Penner. 1973. Interaction of alachlor and carbofuran. Weed Sci. 21:330-335.

6. Hatzios, K. K. and D. Penner. 1985. Interaction of herbicides with other agrochemicals in higher plants. *in* Reviews of Weed Science. Vol. 1 p. 1-63.

7. Loux, M. M., R. A. Liebl, and F. W. Slife. 1989. Adsorption of clomazone on soils, sediments, and clays. Weed Sci. 37:440-444.

8. Peter, C. J. and J. B Weber. 1985. Adsorption, mobility, and efficacy of metribuzin as influenced by soil properties. Weed Sci. 33:868-873.

9. Salzman, F. P. and K. A. Renner. 1991. The synergistic interactions of clomazone plus metribuzin and clomazone plus linuron. WSSA Abstr. 31:6.

10. Sandmann, G. and P. Boger. 1987. Interconversion of prenyl pyroposphates and subsequent reactions in the presence of FMC 57020. Z. Naturforsch. 42c:803-807.

11. Savage, K. E. 1977. Metribuzin persistence in soil. Weed Sci. 25:55-57.

12. Wax, L. M., L. A. Knuth, and F. W. Slife. 1969. Response of soybeans to 2,4-D, dicamba, and picloram. Weed Sci. 17:388-393.

13. Werling, V. L. and D. D. Buhler. 1988. Influence of application time on clomazone activity in no-till soybeans, *Glycine max*. Weed Sci. 36:629-635.

14. Westberg, D. E., L. R. Oliver, and R. E. Frans. 1989. Weed control with clomazone alone and with other herbicides. Weed Tech. 3:678-685.

CHAPTER 4

ABSORPTION, TRANSLOCATION, AND METABOLISM OF CLOMAZONE, METRIBUZIN, AND LINURON IN SOYBEAN AND COMMON COCKLEBUR

ABSTRACT

Research was conducted, using soybean and common cocklebur as test species, to determine if the synergistic interactions of clomazone plus metribuzin and clomazone plus linuron were due to the effect of one herbicide on the uptake, partitioning, or metabolism of the other. Treatments consisted of ^{14}C-clomazone alone and combined with metribuzin or linuron, ^{14}C-metribuzin alone and combined with clomazone, and ^{14}C-linuron alone and combined with clomazone. There were only slight differences in uptake and partitioning of clomazone applied alone in soybean and common cocklebur compared to clomazone plus metribuzin or linuron in soybean and common cocklebur. No differences were noted in uptake and partitioning in both species when either metribuzin or linuron were applied alone or combined with clomazone. Levels of parent clomazone were higher in common cocklebur roots when clomazone was combined with metribuzin and linuron compared to clomazone alone. The percent of parent metribuzin was higher in soybean roots, and common cocklebur roots and shoots when clomazone was combined with metribuzin compared to metribuzin alone. Levels of parent linuron were greater in soybean shoots when linuron was applied with clomazone compared to linuron alone. These results indicate that metribuzin and linuron metabolism are altered when clomazone is also applied, leading to increased phytotoxicity. **Nomenclature:** clomazone, 2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone; linuron, *N'*-(3,4-dichlorophenyl)-*N*-methoxy-*N*-methylurea;

metribuzin, 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one; common cocklebur, *Xanthium strumarium* L. # XANST; soybean, *Glycine max* (L.) 'Century'.

INTRODUCTION

Clomazone plus metribuzin and clomazone plus linuron provide broad spectrum weed control in soybeans. However, research in the field and greenhouse has indicated a synergistic interaction in soybean and common cocklebur to these herbicide combinations (11, 12, 16, 17). The response of soybean to clomazone plus metribuzin was neither temperature dependent, occurring under both cool and warm temperature regimes, nor was the response dependent on placement of the herbicides in the soil. The response of soybean to combinations of clomazone plus linuron was dependent on placement of the herbicides in the soil (11). Symptomology of soybean and common cocklebur growing in soil treated with metribuzin plus clomazone or linuron plus clomazone was typical of metribuzin and linuron injury, respectively.

Linuron is initially demethylated in soybean, and eventually metabolized to 3,4-dichloroaniline. This reaction is catalyzed by a mixed-function oxidase enzyme (9). Despite early reports indicating that metribuzin is metabolized to a deaminated diketo form and eventually conjugated to glucose (8, 10, 13), it is now proposed that the major metabolic pathways for metribuzin in soybean involves either the formation of a homoglutathione conjugate or degradation into the diketo form that is incorporated into insoluble residues (4) It was further suggested that the capacity of soybean to form a *N*-glucose conjugate is limited (4).

The pathway of clomazone metabolism in higher plants is not well established. Explanations include conjugation with glucose in tomato (*Lycopersicon esculentum*) (18). Conjugation with glutathione *in vitro* has been accomplished leading to the conjecture that this could be a deactivation mechanism in plants (15). Metabolism of these herbicide in common cocklebur, a weed species only suppressed by these herbicides individually, has not been determined.

A potential explanation for the synergistic interactions between clomazone plus linuron and clomazone plus metribuzin in soybean and common cocklebur may include one herbicide altering the uptake and/or partitioning of another. Alternatively, plant metabolism may be altered by competition between herbicides for a particular conjugate or enzyme that is specific for the deactivation of each.

The purpose of this study was to: (a) determine if the basis for the synergism in soybean and common cocklebur to clomazone plus metribuzin and clomazone plus linuron was due to differences in uptake, partitioning, and/or metabolism; and (b) determine if there were differences in uptake and partitioning of clomazone, linuron, and metribuzin between soybean and common cocklebur.

MATERIALS AND METHODS

Soybean and common cocklebur were germinated in silicone sand in the greenhouse under natural light supplemented with sodium vapor lights with an intensity of 300 $\mu E\ m^{-2} sec^{-1}$ and set for a 15 hr day. Sand was kept moist with water until emergence of the seedlings, after which the plants were irrigated with modified Hoaglands solution (1). When the first trifoliolate leaf of the soybean was fully expanded and common cocklebur had four fully expanded leaves, each plant was transferred to a jar containing 50 ml of modified Hoaglands solution that was continuously aerated. Preliminary experiments indicated that this system allowed plants to grow and develop normally for 10 days.

The plants were allowed to acclimate for 24 hr in the Hoaglands solution. The solution was then changed and as a preconditioning treatment herbicides were added to the solution at concentrations of 6.5 μM for clomazone, 1.0 μM for linuron, 1.0 μM for metribuzin, 6.5 plus 1.0 μM for clomazone plus linuron, or 6.5 plus 1.0 μM for clomazone plus metribuzin. The plants were allowed to remain in the preconditioning solutions for 24 hr.

The nutrient solution was again changed and the herbicide treatment added to the solutions. Treatments consisted of ^{14}C-uniformly phenyl ring-labelled clomazone (specific activity was 1,036,000 Bq mmol^{-1}, radiochemical purity was 94%), ^{14}C-uniformly ring-labelled linuron (specific activity was 2,257 Bq mg^{-1}, radiochemical purity was 95%), or ^{14}C-uniformly ring-labelled metribuzin (specific activity was 769,600 Bq mmol^{-1}, radiochemical purity was 98%) alone; ^{14}C-clomazone plus metribuzin; ^{14}C-metribuzin plus clomazone; ^{14}C-clomazone plus linuron; and ^{14}C-linuron plus clomazone. The total amount of activity in each jar was 3.7 MBq. Final concentrations of 6.5 μM for clomazone and 1.0 μM for metribuzin and linuron were achieved by the addition of technical grade herbicide. Plants were left in the treatment solutions for 18 hr after which the plants were removed and immediately placed on dry ice. Plants were kept frozen at -20 C until sampled for partitioning or metabolism or used for autoradiography. There were 15 plants of each species for each treatment and the experiments were repeated twice.

Six plants were divided into shoot meristem, leaves, stem, and roots and combusted in a biological oxidizer[1] to $^{14}CO_2$. The CO_2 was trapped in a solution of scintillator[2]:CO_2 absorber[3] (2:1 v/v). Total radioactivity in as sample was determined by liquid scintillation counting[4] (LSC).

Three plants were exposed for 5 weeks to X-ray film[5]. After the film was developed, visual comparisons were made between treatments for evidence of differences in uptake or partitioning.

Metabolites were extracted from six plants of each treatment. Two plants from each treatment were extracted simultaneously. The extraction procedures used were described by Weston and Barrett for clomazone (18), by Kuratle et al. for linuron (7), and by Falb and Smith for metribuzin (2). These methods are summarized as follows. The extraction solvents were methanol for clomazone, acetone for linuron, and ethanol:water (4:1 v/v) for metribuzin. Plants were separated into roots and shoots and the plant portions extracted separately. Plants were homogenized in solvent for 4 min in a tissue homogenizer[6]. The homogenate was filtered through filter paper[7] to remove the plant residue from the filtrate. Radioactivity in the residue was determined by oxidizing a preweighed

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[1]OX-300. R. J. Harvey Instrument Corp. Patterson, NJ 07642.

[2]Safety-Solve. Research Products International Corp. Mount Prospect, IL 60056.

[3]Carbo-Sorb II. Packard Instrument Co. Meriden, CT 06450.

[4]Model 1500. Packard Instrument Corp. Downers Grove, IL 60515.

[5]X-OMAT. Eastman Kodak Co. Rochester, NY 14650.

[6]VirTis 45. The VirTis Co., Inc. Gardiner, NY 12525.

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

portion of the sample and back calculating. The filtrate was placed in a flask and evaporated to about 5 ml by rotoevaporation[8]. Samples were transferred to a small test tube, and the flask was rinsed three times with 1 ml of solvent each time. Metribuzin samples were evaporated to the water phase. The filtrate was transferred to separatory funnels and extracted twice with an amount of benzene equivalent to the water in the funnel each time. The amount of water was recorded and the radioactivity in a 1 ml aliquot of the water was determined by LSC to quantify the polar metabolites. The water phase of the extraction was not used further. After drying samples in a stream of nitrogen, samples were resuspended in 1 ml of solvent. Efficiencies of extraction were 74% for clomazone, 75% for metribuzin, and 78% for linuron. Solvents used were methanol for clomazone samples, acetone for linuron samples, and benzene:acetonitrile (9:1 v/v) for metribuzin samples. Two 100 μl aliquot of each sample was spotted on a prechanelled, 15 nm silica gel thin layer chromatography (TLC) plate[9] and developed to 15 cm in solvent solution. Developing solutions were chloroform:methanol (7:1 v/v) for clomazone samples, chloroform:methanol:pyridine (100:5:1 v/v/v) for linuron samples, and benzene:chloroform:*p*-dioxane (4:3:3 v/v/v) for metribuzin samples. Parent compounds were also spotted on TLC plates and the plates developed to identify the parent compound. After developing, plate channels were scraped in 1 cm increments and the activity measured by LSC.

All data were subjected to analysis of variance. The data for total activity was analyzed on a Bq mg^{-1} basis. The partitioning data was

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[8]Büchi R110. Brinkmann Instruments, Inc. Westbury, NY 11590.

[9]LK5DF. Whatman, Inc. Clifton, NJ 07014.

converted to the percent of the total in all plant organs. The metabolite data was converted to the percent of the total in the gel scraped from the TLC plate. All data converted to percent was analyzed after an arc sin transformation had been performed. Means were separated by Fishers protected least significant differences test at the 5% level of probability to compare differences between species and between a single herbicide and herbicide combinations.

RESULTS AND DISCUSSION

Common cocklebur took up more clomazone than soybean, but retained a greater percentage of clomazone and metabolites in the roots than did soybean (Table 1). In the field and greenhouse common cocklebur will show symptoms of leaf whitening from clomazone while soybean will not. This data indicates that differential partitioning of clomazone and its metabolites within the plants did not account for the expression of clomazone injury in common cocklebur leaves.

In soybean, a greater percentage of clomazone was retained in the roots when metribuzin was in the nutrient solution compared to clomazone alone. Accordingly, a greater percentage of clomazone was translocated to the leaves when clomazone alone was in nutrient solution then when metribuzin was added. No differences occurred in partitioning of clomazone alone compared to clomazone plus linuron. Therefore, the increased injury observed from combinations of clomazone plus linuron and clomazone plus metribuzin cannot be explained by increased levels of clomazone in the leaves and meristems.

Table 1. Uptake and partitioning of clomazone, metribuzin, and linuron alone and as influenced by another herbicide in soybean and common cocklebur.[a]

Species	Treatment	Whole plant activity	Roots	Stem	Leaves	Shoot meristem
		dpm mg^{-1}	---------------% of activity---------------			
Soybean	^{14}C-clomazone	13.4 b	59.8 c	10.6 a	28.7 a	0.9 a
	^{14}C-clomazone + metribuzin	11.4 c	66.2 b	10.5 a	22.6 b	0.6 a
	^{14}C-clomazone + linuron	12.9 bc	63.1 bc	9.7 a	26.6 ab	0.6 a
Common cocklebur	^{14}C-clomazone	15.9 a	84.6 a	7.6 b	7.5 c	0.3 b
	^{14}C-clomazone + metribuzin	15.5 a	87.4 a	7.7 b	4.5 c	0.4 b
	^{14}C-clomazone + linuron	15.6 a	84.3 a	10.8 a	4.7 c	0.2 b
Soybean	^{14}C-metribuzin	10.3 a	54.0 a	16.4 b	28.8 c	0.8 a
	^{14}C-metribuzin + clomazone	9.4 a	50.9 ab	17.2 b	30.8 bc	1.1 a
Common cocklebur	^{14}C-metribuzin	9.6 a	45.7 bc	19.1 ab	33.8 ab	1.3 a
	^{14}C-metribuzin + clomazone	9.1 a	40.6 c	22.2 a	36.1 a	1.0 a
Soybean	^{14}C-linuron	22.5 a	85.5 b	7.9 a	6.4 a	0.2 a
	^{14}C-linuron + clomazone	20.9 ab	87.2 b	8.6 a	3.9 a	0.3 a
Common cocklebur	^{14}C-linuron	19.2 b	93.5 a	4.3 b	2.0 b	0.2 a
	^{14}C-linuron + clomazone	18.2 b	90.4 ab	6.6 ab	2.8 b	0.2 a

[a]Means followed by the same letter wtihin the same ^{14}C-herbicide grouping are not significantly different by Fishers Protected Least Signifcant Differences Test at 5%. Comparisons between herbicide treatment groupings are not valid.

There was no difference in the percentage of activity in soybean and common cocklebur tissues from linuron alone compared to linuron plus clomazone. The percentage of metribuzin in soybean and common cocklebur tissues was similar from treatments of metribuzin alone compared to metribuzin plus clomazone. Partitioning of metribuzin and linuron and their metabolites did not account for the increased injury observed from combinations of clomazone plus linuron and clomazone plus metribuzin.

There were no detectable visual differences in the autoradiographs of plants treated with ^{14}C-herbicide alone or in combination with another herbicide in either soybean or common cocklebur. More clomazone appeared in the roots than in the shoots of common cocklebur and in the shoots, clomazone was concentrated in the veins and surrounding areas. In soybean, clomazone was evenly distributed between roots and shoots and was evenly distributed throughout the leaf. Metribuzin appeared to be more evenly distributed between root and shoots in both species and in the shoots was found primarily in the veins and surrounding leaf portions. In both species, more linuron appeared to be in the roots then in the shoots. In the shoots linuron was concentrated in the veins in soybean and to a lesser extent in common cocklebur.

Differences between treatments in radioactivity in the plant residues were slight (Table 2). More radioactivity was detected in common cocklebur roots from ^{14}C-clomazone alone than from ^{14}C-clomazone plus linuron. No other differences among treatments in plant roots and shoots were significant. There was more radioactivity in the residue of soybean shoots from ^{14}C-linuron alone compared to ^{14}C-linuron plus clomazone.

Table 2. Unextracted radioactivity in plant residues[a].

Species	Organ	Treatment	% of total ^{14}C
Soybean	root	^{14}C-clomazone	15.9 a
		^{14}C-clomazone + metribuzin	16.7 a
		^{14}C-clomazone + linuron	16.0 a
	shoot	^{14}C-clomazone	7.1 bc
		^{14}C-clomazone + metribuzin	7.2 bc
		^{14}C-clomazone + linuron	5.3 cd
Common cocklebur	root	^{14}C-clomazone	8.5 b
		^{14}C-clomazone + metribuzin	6.7 bcd
		^{14}C-clomazone + linuron	5.1 cd
	shoot	^{14}C-clomazone	5.6 bcd
		^{14}C-clomazone + metribuzin	6.4 bcd
		^{14}C-clomazone + linuron	4.8 d
Soybean	root	^{14}C-metribuzin	19.4 bcd
		^{14}C-metribuzin + clomazone	25.0 abc
	shoot	^{14}C-metribuzin	12.1 d
		^{14}C-metribuzin + clomazone	18.6 cd
Common cocklebur	root	^{14}C-metribuzin	27.8 ab
		^{14}C-metribuzin + clomazone	32.1 a
	shoot	^{14}C-metribuzin	14.0 d
		^{14}C-metribuzin + clomazone	14.5 d
Soybean	root	^{14}C-linuron	4.9 ab
		^{14}C-linuron + clomazone	4.1 b
	shoot	^{14}C-linuron	7.3 a
		^{14}C-linuron + clomazone	3.7 b
Common cocklebur	root	^{14}C-linuron	4.2 b
		^{14}C-linuron + clomazone	4.6 ab
	shoot	^{14}C-linuron	4.8 ab
		^{14}C-linuron + clomazone	7.1 a

[a]Means followed by the same letter within the same ^{14}C-herbicide grouping are not significantly different by Fishers Protected Least Significant Differences at 5%. Comparisons between herbicide treatment groupings are not valid.

Differences in the level of radioactivity between roots and shoots did exist (Table 2). Activity of ^{14}C-clomazone averaged 16% of the total recovered in soybean roots, and 6% in soybean shoots. Activity of ^{14}C-clomazone was less than 9% of the total in common cocklebur roots and shoots. Significantly more radioactivity was detected in common cocklebur roots from treatments of ^{14}C-metribuzin then in shoots. Differences in soybean from ^{14}C-metribuzin treatments between roots and shoots were not significant. Radioactivity in plant residues accounted for less than 8% of the total recovered from ^{14}C-linuron treatments in organs of both species.

Clomazone (R_f 0.93) and two metabolites (R_f 0.13 and 0.33) were detected in soybean (Table 3). Three metabolites (R_f 0.13, 0.33, and 0.47) were detected in common cocklebur. Vencill and coworkers (14) found two major metabolites of clomazone in soybean and the three *Amaranthus* species they examined. They also found that levels of clomazone and the metabolites did not change significantly over a period of 12 to 96 hr.

A higher percentage of the parent clomazone extracted was present in soybean shoots than in roots. However, a greater percentage of metabolite 1 (R_f 0.13) was present in the roots than in the shoots. Metabolism of clomazone occurred in soybean roots 18 hr after addition of ^{14}C-clomazone to the nutrient solution. Despite the high level (83%) of parent compound present in soybean shoots, injury symptoms are not usually present in plants treated with clomazone in the field or greenhouse. In common cocklebur, similar distribution of metabolites was found in roots and shoots. More parent clomazone than metabolites were present in root and shoot of both species.

Table 3. Distribution of clomazone and major clomazone metabolites in soybean and common cocklebur.[a]

			R_f values			
Species	Organ	Treatment	0.13	0.33	0.47	0.93
			% of extractable ^{14}C			
Soybean	root	^{14}C-clomazone	18.8 a	4.7 b	-	68.9 d
		^{14}C-clomazone + metribuzin	17.2 b	5.9 a	-	65.6 d
		^{14}C-clomazone + linuron	17.6 ab	3.9 bc	-	70.7 d
	shoot	^{14}C-clomazone	5.7 d	2.6 de	-	85.0 b
		^{14}C-clomazone + metribuzin	5.7 d	3.2 cd	-	82.3 bc
		^{14}C-clomazone + linuron	6.0 d	2.4 de	-	82.3 bc
Cocklebur	root	^{14}C-clomazone	10.2 c	2.0 e	1.9 a	77.5 c
		^{14}C-clomazone + metribuzin	4.3 e	0.6 f	0.7 c	89.8 a
		^{14}C-clomazone + linuron	5.6 d	1.1 f	1.3 b	86.3 ab
	shoot	^{14}C-clomazone	2.6 f	2.8 de	0.4 cd	82.1 bc
		^{14}C-clomazone + metribuzin	2.8 f	3.1 d	0.1 e	84.5 b
		^{14}C-clomazone + linuron	2.6 f	2.4 de	0.5 cd	84.9 b

[a] Means followed by the same letter are not significantly different at the 5% level by Fishers Protected Least Significant Differences test. Comparisons are only valid within columns.

No differences in levels of metabolites were observed when metribuzin or linuron were added to clomazone in soybean or common cocklebur, indicating that the synergistic interactions between the two combinations is not based on altered clomazone metabolism.

Three metabolites (R_f 0.13, 0.43, and 0.67) of metribuzin (R_f 0.90) were detected in soybean and four metabolites (R_f 0.13, 0.27, 0.43, and 0.67) were detected in common cocklebur (Table 4). Falb and Smith (2) identified four metabolites in soybean, in order of decreasing R_f value: 6-tert-butyl-3-(methylthio)-*as*-triazin-5(4H)-one, 6-tert-butyl-*as*-triazin-3,5-(2H,4H)-dione, 4-amino-6-tert-butyl-1,2,4-triazin-3,5(2H,4H)-dione, and an unidentified metabolite, using this extraction and developing procedure. In soybean, a greater percentage of activity was present as parent compound in shoots than in the roots. A greater percentage of metabolite 1 (R_f 0.13) and metabolite 4 (R_f 0.67) were found in soybean roots than in shoots. This indicates that metabolism of metribuzin occurs in soybean roots after uptake. A greater percentage of parent metribuzin and a lesser percentage of metabolite 1 (R_f 0.13) were found in soybean roots treated with ^{14}C-metribuzin plus clomazone than in roots treated with only ^{14}C-metribuzin. Differences in the levels of parent compound and metabolite 1 were not apparent between treatments in soybean shoots. In preliminary experiments, it took up to 5 days for injury symptoms to appear, therefore differences in metabolism in the shoot may take longer than 18 hr to be detectable or it may take longer for parent metribuzin to accumulate in the shoots.

In common cocklebur, there was a greater percentage of total activity present as parent metribuzin and a lesser percentage of

Table 4. Distribution of metribuzin and major metribuzin metabolites in soybean and common cocklebur.[a]

Species	Organ	Treatment	R_f values: 0.13		0.27		0.43		0.67		0.90	
			% of extractable ^{14}C									
Soybean	root	^{14}C-metribuzin	20.3	a	-		2.3	b	4.8	a	43.6	f
		^{14}C-metribuzin + clomazone	14.2	b	-		2.3	b	5.0	a	49.7	e
	shoot	^{14}C-metribuzin	5.7	cd	-		3.6	a	3.6	bc	74.8	b
		^{14}C-metribuzin + clomazone	5.9	cd	-		2.4	b	2.9	c	78.2	d
Cocklebur	root	^{14}C-metribuzin	16.1	b	1.3	c	2.1	b	5.2	a	56.6	d
		^{14}C-metribuzin + clomazone	6.9	c	1.7	b	4.2	a	1.9	d	68.1	c
	shoot	^{14}C-metribuzin	5.6	d	0.6	b	2.4	b	3.6	b	79.2	b
		^{14}C-metribuzin + clomazone	1.4	e	0	e	0.9	c	0.7	e	89.8	a

[a] Means followed by the same letter are not significantly different at the 5% level by Fishers Protected Least Significant Differences test. Comparisons are only valid within columns.

metabolite 1 (R_f 0.13) in roots and shoots of plants treated with ^{14}C-metribuzin plus clomazone than in plants treated with only ^{14}C-metribuzin. In preliminary experiments, common cocklebur exhibited injury symptoms from combinations of clomazone plus metribuzin before soybean did, indicating the movement of parent compound to the shoots of cocklebur is more rapid than in soybean. Total radioactivity in the water-soluble portion of the extract was greater in soybean roots treated with ^{14}C-metribuzin plus clomazone than from treatments of ^{14}C-metribuzin alone (data not presented). No differences were noted in soybean shoots or in common cocklebur. This water-soluble portion has been found to include metribuzin conjugates containing carbohydrates and often amino acids and lipids, and small amounts of the deaminated, diketo metabolites and deaminated metabolites (3).

The differences in metribuzin metabolism in soybean root and common cocklebur root and shoot could explain the synergistic interaction from clomazone plus metribuzin. The higher percentage of parent metribuzin, the active form, is consistent with the injury symptoms of metribuzin observed in treated plants in preliminary experiments. Metabolite 1 (R_f 0.13) is likely a large molecule, possibly the homoglutathione conjugate of metribuzin, the end product of metribuzin metabolism in soybean. The decrease in the percentage of this metabolite when clomazone is present indicates that clomazone is somehow interfering with metribuzin metabolism. The same explanation may be true for common cocklebur. Injury appears more quickly in common cocklebur because the parent compound is more rapidly transported to the shoot.

In both soybean and common cocklebur the majority of activity from the linuron treatments was parent compound (R_f 0.87) (Table 5). Four other metabolites were also detected (R_f 0.13, 0.33, 0.53, and 0.67). Identification of metabolites and R_f values for linuron and metabolites in soybean have been published (6, 7). Metabolites identified, in order of highest R_f value to lowest include linuron, 3,4-dichloraniline, 3-(3,4-dichlorophenyl)-1-methoxy urea, 3-(3,4-dichlorophenyl)-1-ethyl urea, and 3-(3,4-dichlorophenyl) urea. In soybean shoots, a greater percentage of parent linuron was present in plants treated with ^{14}C-linuron plus clomazone than in plants treated with ^{14}C-linuron alone. Higher percentages of metabolite 1 (R_f 0.13) and metabolite 3 (R_f 0.53) were detected in soybean shoots treated with ^{14}C-linuron alone than those treated with ^{14}C-linuron plus clomazone. This suggests that in soybean shoots the clomazone may be interfering with linuron metabolism. In common cocklebur, a greater percentage of parent linuron was present in the root than in the shoot, however there was no difference in the percentages of metabolites between treatments in either plant organ indicating that a synergistic interaction between the two herbicides would not be expected it this species.

These experiments demonstrate that the synergism observed from the combinations of clomazone plus metribuzin and clomazone plus linuron may be due to differences in metabolism of metribuzin and linuron when clomazone is also present in the plant, and not due to differences in the uptake or partitioning of metribuzin or linuron although there were some differences between species. Evidence suggests that the initial detoxication of both metribuzin and linuron are catalyzed by mixed-

Table 5. Distribution of linuron and major linuron metabolites in soybean and common cocklebur.[a]

Species	Organ	Treatment	R_f values				
			0.13	0.33	0.53	0.67	0.87
			% of extractable ^{14}C				
Soybean	root	^{14}C-linuron	1.3 b	1.2 bc	2.4 de	1.2 cde	86.0 bc
		^{14}C-linuron + clomazone	1.2 b	0.9 cd	1.8 ef	0.6 f	89.0 ab
	shoot	^{14}C-linuron	2.4 a	1.2 bcd	4.5 c	7.9 a	68.6 f
		^{14}C-linuron + clomazone	1.2 b	0.7 d	2.6 d	3.9 b	84.2 cd
Cocklebur	root	^{14}C-linuron	1.7 ab	1.4 b	2.3 def	0.7 def	90.0 a
		^{14}C-linuron + clomazone	1.6 ab	0.7 cd	1.7 f	0.4 f	90.3 a
	shoot	^{14}C-linuron	1.8 ab	3.0 a	6.0 b	1.9 c	77.8 e
		^{14}C-linuron + clomazone	2.2 a	2.9 a	7.5 a	1.5 cd	81.5 de

[a] Means followed by the same letter are not significantly different at the 5% level by Fishers Protected Least Significant Differences test. Comparisons are only valid within columns.

function oxidases (5, 9). If clomazone detoxication is also catalyzed by a mixed-function oxidase, there could be competition for the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) and/or activated O_2 that is required for mixed function oxidase activity, or for the active sites on the mixed-function oxidase. With the reduction in quantity of NADPH from the action of linuron and metribuzin in the light reaction, competition for NADPH could become acute. This research suggests that a mixed-function oxidase is involved in the detoxication of clomazone and that clomazone is preferentially metabolized by that enzyme over metribuzin and linuron in soybean and common cocklebur. Future research will need to elucidate the detoxication pathway(s) of clomazone and the enzymes involved.

LITERATURE CITED

1. Blankendaal, M., R. H. Hodgson, D. G. Davis, R. A. Hoerauf, and R. H. Shimabukuro. 1972. Growing plants without soil for experimental use. USDA Misc. Pub. 1251. 17 pp.

2. Falb, L. N. and A. E. Smith, Jr. 1984. Metribuzin metabolism in soybeans: Characteristics of the intraspecific differential tolerance. J. Agric. Food Chem. 32:1425-1428.

3. Falb, L. N. and A. E. Smith. 1987. Metribuzin metabolism in soybeans: Partial characterization of the polar metabolites. Pestic. Biochem. Physiol. 27:165-172.

4. Frear, D. S., H. R. Swanson, and E. R. Mansager. 1985. Alternate pathways of metribuzin metabolism in soybean: Formation of *N*-glucoside and monoglutathione conjugates. Pestic. Biochem. Physiol. 23:56-65.

5. Hatzios, K. K. and D. Penner. 1988. Metribuzin. Pages 191-243. *in* P. C. Kearney and D. D. Kaufman, eds. Herbicides: Chemistry, Degradation, and Mode of Action. Marcel Dekker, Inc. New York.

6. Katz, S. E. 1967. Determination of linuron and its known and/or suspected metabolites in crop materials. J. Assoc. Off. Anal. Chem. 50:911-917.

7. Kuratle, H., E. M. Rahn, and C. W. Woodmansee. 1969. Basis for selectivity of linuron on carrot and common ragweed. Weed Sci. 17:216-219.

8. Mangeot, B. L., F. E. Slife, and C. E. Rieck. 1979. Differential metabolism of metribuzin by two soybean (*Glycine max*) cultivars. Weed Sci. 3:267-269.

9. Nashed, R. B. and R. D. Ilnicki. 1970. Absorption, distribution, and metabolism of linuron in corn, soybean, and crabgrass. Weed Sci. 18:25-28.

10. Oswald, T. H., A. E. Smith, and D. V. Phillips. 1978. Phytotoxicity and detoxification of metribuzin in dark-grown suspension cultures of soybeans. Pestic. Biochem. Physiol. 8:73-83.

11. Salzman, F. P. and K. A. Renner. 1989. Interaction of clomazone and metribuzin in soybean. Proc. NCWSS 44:86.

12. Salzman, F. P. and K. A. Renner. 1991. The synergistic interactions of clomazone plus metribuzin and clomazone plus linuron. Proc. WSSA 31:18.

13. Smith, A. E. and R. E. Wilkinson. 1974. Differential absorption, translocation, and metabolism of metribuzin [4-amino-6-tert-3-

(methylthio)-as-triazine-5(4H)one] by soybean cultivars. Physiol. Plant 32:253-257.

14. Vencill, W. K., K. K. Hatzios, and H. P. Wilson. 1990. Absorption, translocation, and metabolism of ^{14}C-clomazone in soybean (*Glycine max*) and three *Amaranthus* weed species. J. Plant Growth. Regul. 9:127-132.

15. Vencill, W. K., K. K. Hatzios, and H. P. Wilson. 1990. Interactions of the bleaching herbicide clomazone with reduced glutathione and other thiols. Z. Naturforsch. 45c:489-502.

16. Werling, V. L. and D. D. Buhler. 1988. Influence of application time on clomazone activity in no-till soybeans, *Glycine max*. Weed Sci. 36:629-635.

17. Westburg, D. E., L. R. Oliver, and R. E. Frans. 1989. Weed control with clomazone alone and with other herbicides. Weed Tech. 3:678-685.

18. Weston, L. A. and M. Barrett. 1989. Tolerance of tomato (*Lycopersicon esculentum*) and bell pepper (*Capsicum annum*) to clomazone. Weed Sci. 37:285-289.

SUMMARY

These studies indicate that environmental factors, including soil characteristics, influence the synergistic interactions of clomazone plus metribuzin and clomazone plus linuron. The most severe reductions in soybean leaf area, shoot weight, root weight, and yield were noted in field soils of low organic matter and clay contents. These soils may allow more herbicide to remain available for plant uptake. The combination of clomazone plus linuron is most injurious to soybean when the herbicides are in the same soil zone as the seed, compared to herbicides in the soil above or below the seed. Combinations of clomazone plus metribuzin are equally injurious despite placement of these herbicide in the soil. Thus, a heavy rainfall after herbicide application could move clomazone and linuron in the soil to a zone where increased uptake by soybean results in increased injury. Ambient air temperature does not appear to influence these interactions. By examining the various rates of clomazone and metribuzin that interact synergistically, it appears reduced rates of clomazone and metribuzin could be used to control common cocklebur yet not harm soybeans. Studies conducted on the uptake, translocation, and metabolism of clomazone, metribuzin, and linuron alone and in combination in soybean and common cocklebur indicate that uptake and translocation of clomazone, metribuzin, and linuron are similar when applied alone compared to when clomazone was combined with metribuzin or linuron, linuron was combined with clomazone, or metribuzin was combined with clomazone, respectively. Metabolism of metribuzin was altered in soybean and common cocklebur when clomazone was present while metabolism of linuron was altered by the presence of clomazone only in soybean.

Clomazone detoxification was not significantly altered by the addition of metribuzin or linuron. This indicates that clomazone, metribuzin, and linuron are all detoxified in the plant similarly. Evidence suggests that metribuzin and linuron are deactivated in the plant by a mixed-function oxidase enzyme. Clomazone may also be deactivated in soybean and common cocklebur by a mixed-function oxidase enzyme.