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CORN (*ZEA MAYS* L.) TOLERANCE TO CHLOROACETANILIDE HERBICIDES

By

Loston Rowe

A DISSERTATION

Submitted to

Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Crop and Soil Sciences

1989

604230

ABSTRACT

CORN (ZEA MAYS L.) TOLERANCE TO CHLOROACETANILIDE HERBICIDES

by

Loston Rowe

Greenhouse, laboratory, and field studies were conducted to evaluate factors influencing corn tolerance to chloroacetanilde herbicides. Studies were conducted to determine the effectiveness of CGA-154281 in protecting corn from metolachlor injury and to determine the mechanism for the protective action.

Applications of alachlor and metolachlor to ten Great Lakes corn hybrids at four application rates showed some of the hybrids were more tolerant of alachlor and others were more tolerant of metolachlor. There was a linear response of increasing injury with increasing application rate. In a soil moisture study, more injury was evident as the soil moisture content increased.

Evaluation of 200 commercial corn hybrids and 29 corn inbreds revealed a high degree of variability in metolachlor tolerance. The distribution of tolerance resembled a normal distribution curve, with most of the hybrids and inbreds having a midlevel of tolerance. Laboratory studies with metolachlor tolerant and sensitive hybrids indicated that the variability of tolerance was due to differences in

absorption, metabolism, as well as differences at the site of action of metolachlor.

Greenhouse and field studies showed that CGA-154281 was very effective in protecting corn seedlings from metolachlor injury. This protection was evident even with sensitive hybrids at high soil moisture levels and high herbicide application rates. Studies with ^{14}C-metolachlor indicated that the protectant CGA-154281 did not reduce metolachlor absorption or alter the pathway of metolachlor metabolism. However, the protectant did appear to enhance the metabolism of metolachlor to a non-phytotoxic glutathione conjugate. Nomenclature: Corn, (*Zea mays* L.); alachlor, 2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)-acetamide; metolachlor, 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide; CGA-154281, 4-(dichloro-acetyl)-3,4-dihydro-3-methyl-2H-1,4-benzoxazine.

Additional index words. Corn tolerance, distribution of tolerance, soil moisture content, protectant, glutathione conjugate.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Dr. Donald Penner for his guidance, and counsel throughout this project. I also offer appreciation to Drs. James J. Kells, Elmer Rossman, Alan Putnam, Bruce Branham, and Bernard H. Zandstra for serving as members of my guidance committee, for their assistance with the research, and for their suggestions in the preparation of this manuscript.

A special thanks is extended to Frank C. Roggenbuck for his valuable assistance with all aspects of my research and for his sincere friendship during my study.

I want to acknowledge the Ciba Geigy Corporation for their financial support. Also, appreciation is offered to Dr. James Jay for his kindness and assistance.

Finally, appreciation is extended and my wife, Rita, whose support and patience has made this goal attainable.

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CHAPTER 5

INTRODUCTION

The chloroacetanilides are a class of herbicides that are commonly used in corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) production. They control many annual grasses and certain small seeded broadleaf weeds and are generally selectively safe on corn. However, under certain conditions injury symptoms can occur. The factors that affect the extent of corn injury exhibited are not clearly understood.

Alachlor (2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide) and metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide) are the primary chloroacetanilide herbicides used in corn. These compounds have very similar chemical structures and control essentially the same weeds. However, differences in corn tolerance to the two herbicides have been reported. The accuracy and basis of these reported differences have been disputed.

Soil conditions, specifically soil moisture content, may also influence the observed corn injury. Genetic variability among corn hybrids and inbred lines has been linked to the differences in corn susceptibility to chloroacetanilides. Inherited traits like herbicide tolerance could result in observed differences in injury. However, the range of variability in chloroacetanilide tolerance among commercial hybrids is not well defined.

If the factors that cause variability in corn tolerance to the chloroacetanilides can be identified, then management practices and products such as chemical protectants can be developed to prevent the problem. Protectants are already used extensively in sorghum (Sorghum bicolor L.) production for protection from alachlor and metolachlor. A new protectant, CGA-154281 (4-(dichloroacetyl)-3,4-dihydro-3-methyl-2H-1,4-benzoxazine), is currently being evaluated for the protection of corn from metolachlor injury.

The objectives of this research were to: 1) identify the factors associated with chloroacetanilide injury to corn, including herbicidal differences, soil moisture conditions, and genetic variability; 2) determine the range of chloroacetanilide tolerance among inbreds and hybrids; 3) determine the basis for the observed difference in tolerance between tolerant and sensitive hybrids; 4) evaluate the efficacy of CGA-154281 in protecting corn from metolachlor injury; and 5) determine the mechanism of the protection associated with CGA-154281.

Chapter 1

REVIEW OF LITERATURE

CHLOROACETANILIDES

Mode of Action

Chloroacetanilides are preemergence herbicides which control many grass and several broadleaf weed species. Chloroacetanilides inhibit the early development of susceptible weed species. The treated seeds usually germinate, but the seedlings do not emerge from the soil. These compounds are generally selective and safe for use on corn. However, under certain conditions, chloroacetanilides may cause stunted or abnormal growth of corn seedlings (Figure 1).

Most of the research on the modes of action of chloroacetanilides indicates that these herbicides inhibit growth (21,28,56), inhibit protein synthesis (24,87), alter lipid synthesis (25,104), or interact with plant hormones (75,107).

Deal and Hess (21) concluded that the growth inhibition of plants caused by chloroacetanilides results from an inhibition of cell division and cell elongation. The degree of growth inhibition is mostly a function of concentration and duration of the treatment. They found that significant inhibition of etiolated oat (*Avena sativa* L.)

coleoptiles was caused by slightly lower concentrations of alachlor than metolachlor.

Chloroacetanilides are absorbed by both shoot and root. Grass species are generally more suseceptible when the herbicide is absorbed by the emerging shoot, especially when absorbed near the coleoptilar node (34,58,59,76,82,95). Translocation of these herbicides can occur both in the xylem and phloem. However, data indicating primarily xylem transport were obtained on emerged plants, which would have a much more active transpiration stream than unemerged plants (17,34).

Metabolism

Several studies have been conducted to determine the fate of chloroacetanilides in higher plants. Most researchers agree with Breaux et al. (14), that the basis of chloroacetanilide selectivity is related to the plant's success in metabolizing these compounds (23).

The metabolism of chloroacetanilides herbicides in higher plants is not fully understood. However, most researchers have concluded that glutathione plays a major role in the inactivation of these compounds (11,12,13,14,34, 63,64,67). When a chloroacetanilide herbicide enters a tolerant plant seedling, the glutathione conjugates to it, producing a non-phytotoxic metabolite, which is harmless to the plant. Glutathione conjugates chloroacetanilides nonenzymatically in vitro (38,67) and enzymatically in vitro with glutathione S-transferases isolated from etiolated corn (72) and sorghum (38). Isozymes of glutathione S-transferase isolated from etiolated corn seedlings varied in their reactivity with the chloroacetanilides (72). The chloroacetanilides have been reported to be alkylating agents (70). The conjugation of these herbicides with glutathione could be considered an alkylation reaction.

Alachlor and metolachlor have very similar structures (Figure 2) and serve the same applications in corn production. While some researchers have determined that there is no significant difference in the phytotoxicity of alachlor and metolachlor to corn (9,47,102), others maintain that there are differences, and that the differences are due to differences in glutathione conjugation (19,30,80).

Harvey et al. (47) stated that preliminary greenhouse studies indicated that metolachlor had greater potential for injury to corn than alachlor. However, field studies over a 12-year period indicated corn yields following metolachlor treatments were at levels not significantly different from those following alachlor treatments. Thus, there was no evidence that either herbicide caused more injury to corn than the other.

In growth chamber studies, Boldt and Barrett (9) found that alachlor applications generally caused more injury and yield loss to Pioneer 3780 corn than metolachlor, while the response of Pioneer 3320 to the two herbicides was not consistently different.

A research team at Monsanto Chemical Company found that both alachlor and metolachlor were absorbed by the seedlings at the same rate. However, plants converted the alachlor into harmless by-products twice as easily. The researchers concluded that, because of the difference in their chemical structures, glutathione conjugation occurred more readily with alachlor than with metolachlor, and therefore, alachlor was less phytotoxic to corn seedlings (19,30,80).

Attempts to determine differences in metabolism and phytotoxicity between alachlor and metolachlor show many discrepancies, especially between times, concentrations and hybrids used. Studies usually compare the two herbicides at equal rates. However, metolachlor is labeled for and usually used at lower rates than alachlor. This factor also complicates the comparison of the two herbicides for chemical effects.

Soil Activity

The primary factors affecting soil inactivation of chloroacetanilide herbicides are adsorption to soil components and microbial degradation. The herbicide degradation rate by soil microbes decreased and adsorption to the soil components increased with increasing organic matter and clay content (51). Ninety percent of all chloroacetanilide loss in soil is due to microbial degradation (51). Because chloroacetanilides are degraded quickly by microbes, their soil persistence is relatively short. Beestman (8) found half-life values of 4.0 and 7.3 days for alachlor in a silt and a silty clay soil, respectively. The half-life of metolachlor has been estimated at 30 to 50 days in the northern areas and 15 to 25 days in the southern areas of the United States (51). Studies have shown that degradation of alachlor and metolachlor was greater at 50 and 80% than at 20% field capacity at 20 C. Degradation rates of alachlor and metolachlor at 50% field capacity were greater at higher temperatures (113).

These results indicate that metolachlor persists longer in the soil than alachlor. Therefore, metolachlor has the potential to provide a longer period of weed control. The persistence of the herbicides and their injury seems to be amplified under cool, wet conditions. The rate of herbicide required to achieve a certain level of weed control on a particular soil has often been related to the capacity of the soil to sorb the herbicide. It has been shown that alachlor moves more readily through the soil than metolachlor. Although metolachlor is more soluble in water than alachlor, less movement occurs in the soil because metolachlor is adsorbed more tightly to the soil particles

(20,51,105).

Weber and Peter (105) found that adsorption of the herbicides was not related to molecular size (weight or volume), or molecular surface area. However, differences in adsorption were apparently due to slight, molecular structural differences in the two herbicides. In a study by Banks and Robinson (5), less than 10% of the original alachlor remained in the soil 10 days after treatment, compared to 26% of the original metolachlor. They concluded that straw and decaying organic matter provided for a greater retention of the metolachlor than of alachlor.

Peter and Weber (86) found that alachlor and metolachlor adsorption was postively correlated with soil organic matter content, clay content, and surface area as measured by ethylene glycol monoethyl ether (EGME) or benzyl ethyl ether (BEE) and inversely correlated with herbicidal activity. Alachlor was adsorbed in slightly greater amounts by soil than metolachlor. Metolachlor had slightly greater bioactivity than alachlor on grass weeds, but the herbicides had similar activity on broadleaf weeds. Slightly greater amounts of metolachlor than alachlor were leached through the soil and slightly greater amounts of alachlor were retained in the upper soil zones.

A study of adsorption and mobility of the chloroacetanilides by Jordan (57) indicated that adsorption of alachlor and metolachlor did not differ in 10 different soils.

He also reported that the mobility of the chloroacetanilides was inversely related to their adsorptivity.

Although previous studies of adsorption and mobility are conflicting, their results indicate a difference in corn tolerance to alachlor and metolachlor could be due to differences in their location and availability in the soil.

FACTORS AFFECTING CROP INJURY

Soil Moisture

Soil water content influences the phytoxicity of several herbicides (15,43,44,59,61,62,71,99,100,103,106). Rainfall or irrigation is accepted as being necessary for the activation of preemergence herbicides, such as chloroacetanilides. Surface applied water moves the herbicide into the soil thus preventing its loss from the soil surface via phototransformation and volatization. This movement of the herbicide into the soil by rainfall also brings it into contact with the germinating weed seedlings. Along with herbicidal activity, herbicidal injury to crops, which is associated with increased rainfall, could be due to movement of the herbicide into the soil.

Although rainfall usually has been associated with herbicide effectiveness and injury, perhaps soil moisture should be the primary consideration with rainfall of secondary importance as it affects soil moisture content.

Walker (103) stated that herbicidal response depends, in part, on the soil water content, which influences herbicide concentrations in the soil solution, the rates of mass flow, diffusion, and plant root extension. He further concluded that herbicidal phytotoxicity generally increases as soil water content increases. However, it may not be closely correlated with the amount or concentration of herbicide in the available soil solution (103).

Green and Obien (37) concluded that the principle effect of soil water content on herbicide phytotoxicity probably is associated with herbicide transport, which is more sensitive to changes in water content than is the concentration of herbicide in the soil solution (55).

Stickler et al. (99) showed that the effectiveness of atrazine (6-chloro-N-ethyl-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine) and EPTC (S-ethyl dipropylcarbamothioate) was increased when soil moisture was raised from 25% to 31%, but no further increase was obtained at 37% moisture. Response to chloramben (3-amino-2,5-dichlorobenzoic acid) increased linearly and response to trifluralin (2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzenamine) decreased linearly with increased moisture. They also concluded that three possible functions of surface-applied moisture were to: 1) move the herbicide into the soil and thus reduce loss of the herbicide from the soil surface, 2) move the herbicide into the soil for contact with germinating weeds or emerging weed seedlings, and 3) create sufficiently moist conditions in the soil for absorption of the herbicide by the seedlings.

There is some data that indicates certain herbicides have less activity with increasing soil water content. Grover (40) found that bioactivity of picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid) decreased as the soil moisture content increased. He stated that this was due to the effect of varying soil moisture levels on the concentration of the picloram in the soil-water phase. Therefore increasing soil moisture had a dilution effect on the herbicide concentration.

Genetic Variability

Differential tolerance to the same herbicides has been reported in several crops (2,3,4,10,36,45,53,78,89,93,96). Narsaiah and Harvey (74) found differential alachlor tolerances among both corn inbreds and hybrids. Alachlor severely injured inbreds W117, W182E, and A65, but inbreds W153R and W59M were not affected, even at 10.0 kg/ha. Penner et al. (84,92) evaluated the sensitivity of 108 inbred lines and several hybrids and found the tolerance followed a normal distribution curve. Francis and Hamill (31) found significant differences in corn shoot weight in a study with three alachlor rates and 21 inbred lines. They also stated that hybrids appeared to exhibit a smaller range of response to high rates of alachlor than inbred lines. They concluded that variation in inbred and hybrid tolerance to alachlor would suggest that screening is necessary before assessing the suitability of alachlor for foundation and seed production fields.

Studies conducted by Renner et al. (90,94) showed that corn hybrids showed differential responses to imazaquin (2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl)-3-quinolinecarboxylic acid) for all parameters that were measured. Great Lakes 422 and 5922, Cargill 921, and Pioneer 3901 were significantly more tolerant to imazaquin than the others tested. They concluded that the tolerance shown did not appear related to corn maturity groups. Wright and Rieck (111) found that Pioneer 3030 and Coker 71 were tolerant to butylate (S-ethyl cyclohexylethylcarbamothioate), whereas Pioneer 511A and PAG644 were observed as being sensitive. Laboratory studies showed that the tolerant hybrids absorbed less ^{14}C-butylate and metabolized more to $^{14}CO_2$ than the sensitive ones.

Other Factors

Susceptibility of a plant to a herbicide differs with variation in environmental factors. High temperatures and high humidities generally increase susceptibility (15,41, 60,101). However, Penner (85) found that alachlor was injurious to navy beans (*Phaseolus vulgaris* L.) at a high application rate at 20 and 25 C but not at 30 C. The alachlor injury to the navy beans was characterized by plant growth reduction and growth inhibition of the leaf apex. Muzik and Mauldin (73) found that 2,4-D ((2,4-dichlorophenoxy)acetic acid) absorption and translocation in both leaves and roots was less under low temperatures. Therefore, sensitivity of wheat (Triticum aestivum L.) to 2,4-D was greater at 26 C than at 10 C and 5 C, at all stages of growth.

Burt and Akinsorotan (15) noted that EPTC and butylate reduced corn growth more at 30 C than at 20 C. High temperatures before coleoptile emergence was more critical than high temperatures after coleoptile emergence. Penner (83) also reported that the phytotoxicity of linuron (N-(3,4-dichlorophenyl)-N-methylurea) to corn and soybean seedlings increased with increasing temperatures from 20 C to 30 C. He concluded that there was a relationship between increased herbicide transport to the shoot and higher temperatures, therefore causing increased toxicity. However, Thompson et al. (101) noted that cold, wet conditions produced more severe injury than warmer temperatures. They concluded that lower temperatures caused a decrease in the detoxication rate of atrazine in corn. Therefore, the accumulation of absorbed atrazine was responsible for the injury to corn under cooler temperatures. Other researchers agree that an increase in phytotoxicity can occur at high temperatures because of increased herbicide uptake. On the other hand, an increase in phytotoxicity at lower temperatures may be attributed to reduced detoxication of the herbicide (16,60,73).

Several reports indicate that soil pH may play a role in the amount of herbicide injury which occurs (46,108). Harris and Warren (46) showed that soil pH altered the adsorption and desorption properties of several herbicides, thereby, altering the amount available for plant uptake.

Soil texture is also known to influence the activity of soil applied herbicides. Generally, more injury was observed when the herbicides were applied to coarser textured soils (39,42,77,81,106). Soils with higher organic matter contents showed less herbicide activity than did soils of lower organic matter (39).

CHEMICAL PROTECTANTS

The use of chemical antidotes or protectants has been widely studied. Because of the extensive commercial use of protectants for protection of sorghum (*Sorghum bicolor* L.) from chloroacetanilide injury, these studies have primarily dealt with chloroacetanilide protectants for sorghum (18,22,27,68,91,97). However, recent reports indicate that chemical protectants may be useful for other crops and other herbicides (6,7,49,52,65,69,79,109,110).

When used at higher rates, alachlor and metolachlor are often injurious to corn seedlings. Thus, the use of crop protectants or antidotes to minimize corn injury becomes important (69). Leavitt and Penner (66) reported that of the six potential protectants they evaluated dichlormid (N,N, diallyl-2-2-dichloroacetamide) provided the most protection to corn from alachlor, metolachlor and other chloroacetanilide herbicides. Spotanski and Burnside (97) said that 1,8 naphthalic anhydride was the most effective protectant they tested in reducing alachlor injury to sorghum. They concluded that the seed treatment was more effective than tank mixes. Rains and Fletchall (88) found that dichlormid was more effective than CDAA (2-chloro-N,N-di-2-propenylacetamide) in preventing yield reductions to corn from alachlor or metolachlor in the greenhouse.

The mechanism of the protective action of chloroacetanilide protectants is not fully understood. Herbicide protectants do indeed selectively reduce the biological activity of herbicides, which would otherwise result in crop injury. This reduction in crop injury must be the result of the protectant reducing or eliminating the herbicide from its site of action, or by an induction of an alternate pathway that will compensate for or override the effects of the herbicide. The biochemical antagonism can be a result of one or more factors, including reduced herbicide uptake, reduced herbicide translocation, enhanced herbicide metabolism, herbicide compartmentalization, or the induction of alternate plant metabolic pathways. There are conflicting reports in the literature as to which actually occurs.

Hatzios (50) proposed that biochemical, competitive, and physiological antagonisms of the activity of herbicides by the protectants are potential mechanisms of protective action. He said that biochemical antagonism occurs when a protectant prevents the penetration and/or translocation of a given herbicide into the protected plant, or when the protectant enhances the metabolic detoxication of the herbicide in the protected plant. Competitive or physiological antagonisms occur when a protectant competes with a given herbicide for the same site of action in the cells of the protected plant. Chemical antagonism involves a chemical reaction of the protectant with the herbicide to form an inactive herbicide-protectant complex.

Fuerst (34) proposed that two hypotheses for protectant mode of action seem plausible. Protectants induce rapid herbicide metabolism or they protect the biochemical site of action of the herbicide.

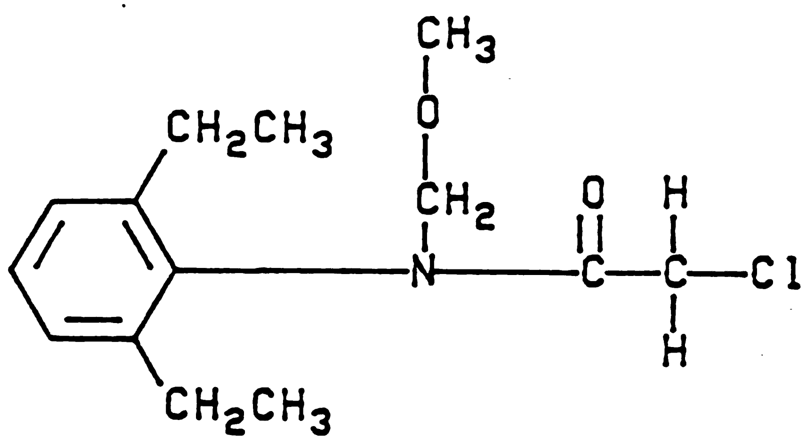
Protectants may induce rapid herbicide metabolism by increasing levels of glutathione and/or glutathione transferase which enhances the conversion of the herbicide to inactive metabolites. While looking at the effect of protectants on glutathione content and glutathione _S_-transferase (GST) activity in sorghum, Gronwald et al. (38) found a significant increase in GST activity when metolachlor was used as a substrate. The degree of protection from metolachlor injury conferred by a particular antidote was strongly correlated with its ability to enhance GST activity. This theory of protectant interaction with glutathione S-transferase enzymes to enhance chloroacetanilide metabolism has been reported from other studies as well (1,29,32,33,35,54,65,112).

Ebert (26) reported that cyometrinil (((cyano-methoxy) imino)benzeneacetonitrile) prevented the loss of cuticular integrity and therefore greatly reduced the amount of metolachlor taken up by sorghum seedlings.

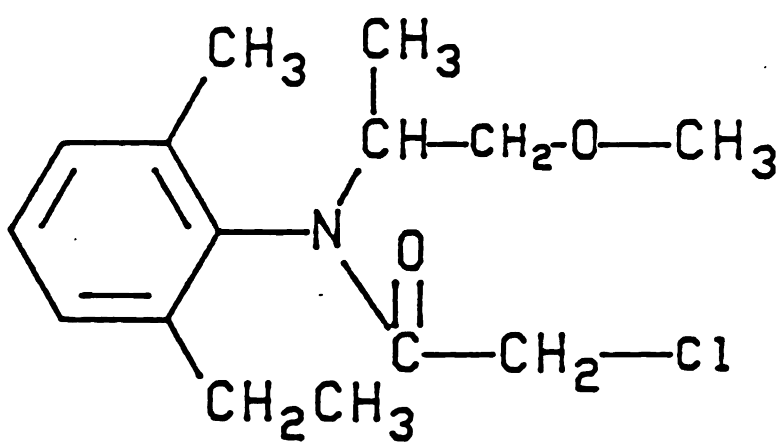
The other plausible hypothesis is that protectants protect the biochemical site of herbicide action. Compounds with similar structures to thiocarbamate herbicides are often effective protectants (50,58,98). For example, dichlormid is structurally very similar to EPTC.

There is evidence that there is an intermediate step in the metabolism of chloroacetanilide herbicides. As with the thiocarbamate herbicides, this intermediate step involves the oxidation of the herbicide to a sulfoxide. The oxidation occurs either by a mixed function oxidase or a peroxidase. The sulfoxide could then be conjugated enzymatically or nonenzymatically to the glutathione conjugate that has been widely observed (34). Recent studies showed that CGA-43089 fails to counteract metolachlor injury to sorghum grown in nutrient-solution culture or under conditions of excessive soil moisture (58). These reports indicate that the presence of molecular oxygen might be related to the protective effect offered by some herbicide protectants. The theory of an intermediate sulfoxidation step in the metabolism would explain the importance of molecular oxygen (48).

Figure 1. Corn injury symptom.



alachlor



metolachlor

Figure 2. Chemical structures of alachlor and metolachlor.

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Chapter 2

FACTORS AFFECTING CHLOROACETANILIDE INJURY TO CORN

ABSTRACT

A computer survey was conducted to evaluate public research reports in which alachlor and metolachlor were compared in the same trial. A summary of the data indicated that there was no significant difference in corn yield between alachlor and metolachlor treatments when used at labelled application rates. Greenhouse studies were conducted to determine the effects of herbicide, herbicide rate, genetic variability and soil moisture content on the tolerance of corn seedlings to two chloroacetanilide herbicides. Alachlor and metolachlor were applied preemergence at 2.2, 3.4, 4.5, and 6.7 kg/ha to ten Great Lakes corn hybrids. As was expected, there was a linear response of increasing herbicide injury with increasing application rate. When comparison was made between the two herbicides, metolachlor appeared to be less injurious at the low rate and more injurious at the high rate. There was a significant degree of variability in injury among the ten hybrids tested. This variability was more evident at higher herbicide application rates. Some of the hybrids appeared to be

more tolerant of alachlor, while others were more tolerant of metolachlor. Six soil moisture levels ranging from 8% to 22% soil moisture were evaluated for their effect on alachlor and metolachlor injury to corn seedlings. Increased herbicide injury occurred as the soil moisture level increased for both herbicides. The injury ranged from no injury at the lowest soil moisture level to about 70% at the highest soil moisture level. Nomenclature: Corn, *Zea mays* L.; alachlor, 2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide; metolachlor, 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methyl-ethyl)acetamide. *Additional index words*. moisture level, genetic variability, tolerance.

INTRODUCTION

Chloroacetanilide herbicides are commonly used in corn production to control a wide range of weed problems. The two most commonly used chloroacetanilides in corn are alachlor and metolachlor. These two compounds comprise a major segment of the United States corn herbicide market. Alachlor and metolachlor are generally safe for use on corn, however, under certain conditions injury symptoms do occur. These symptoms range from a twisting and curling of the leaves early in development to a more severe stunting and malformation of the plant, which may result in decreased corn yield.

The factors influencing the degree of chloroacetanilide injury to corn are not very well documented. Research and marketing claims by Monsanto Company imply that structural differences between alachlor and metolachlor afford a significantly reduced amount of corn phytotoxicity from alachlor than from metolachlor (1,2,3,8). However, independent research has found that there is really no significant difference in corn injury between the two compounds (5,9).

Inherited differences between inbreds and hybrids has been shown to provide differential tolerance to chloroacetanilide herbicides (7). Francis and Hamill (4) found significant differences in tolerance of 21 inbred lines when treated with alachlor. Narsaiah and Harvey (6) found that alachlor severely injured inbreds W117, W182E, and A65, but inbreds W153R and W59M were not affected at 10.0 kg/ha. Soil moisture content is an important factor in the amount of herbicide injury which occurs. With most soil applied herbicides there is the response of increasing crop injury and weed control effectiveness with increasing soil moisture. However, Grover (5) found that the bioactivity of picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid) decreased as the soil moisture content increased.

The objective of these studies was to evaluate the factors which influence the degree of corn injury from chloroacetanilide herbicides. The factors evaluated included herbicide, herbicide application rate, genetic variability, and soil moisture conditions.

MATERIALS AND METHODS

Computer survey

With the aid of Ball Research Services, East Lansing, MI., a computer survey was conducted to evaluate the public research reports in which alachlor and metolachlor were used in head to head comparisons. The data included corn grain yield for hybrids which received treatments of alachlor and metolachlor in 1984, 1985, or 1986. Comparisons included those that occurred at one location, done by one researcher, with tank mixed additional herbicide applied at the same rates. Alachlor and metolachlor were applied at the same rate or at the recommended differential application rate considered as an appropriate comparison by the researcher (i.e. alachlor 2.8 kg/ha, metolachlor 2.2 kg/ha).

General greenhouse procedure

Corn seed were planted in 946-ml plastic pots, which contained an air-dried Spinks sandy loam (mixed, mesic Psammentic Hapludalfs) soil consisting of 71.3% sand, 19.4% silt, and 9.4% clay with a pH of 6.2. The herbicides were applied preemergence with a chain-link belt, compressed air sprayer, which delivered a volume of 280 L/ha at 240 kPa. A known amount of water was then added to the soil surface for incorporation of the herbicide. The pots were then placed in the greenhouse which maintained 16 hr days at 25 +/- 2 C. The plants were grown with supplemental lighting from high-pressure sodium lamps. The light intensity was 500 $uE.m^{-2}.s^{-1}$ with only supplemental lights to 1200 $uE.m^{-2}.s^{-1}$ with both supplemental and natural sunlight. The greenhouse was maintained at 40 to 75% relative humidity. Plant height and injury ratings were evaluated 10 days after planting. Plant height as percent of the control was calculated. Each hybrid was compared to its own control, thus correcting for differences in shoot height among the hybrids. Plant injury rating was on a scale of 0 (no effect) to 100 (complete kill). The mean of three plants in each pot was considered one observation. Each treatment was replicated four times and the data are the means of two experiments. Following analysis of variance, means were separated at the 5% level of significance according to Duncan's multiple range test.

Influence of hybrid, herbicide, and rate

Ten Great Lakes hybrids were evaluated under the conditions described above. This experiment tested the effects of alachlor and metolachlor on the ten hybrids at four application rates. The ten Great Lakes hybrids used were 579, 313, 381, 422, 437, 466, 498, 516, 547, and 599. Alachlor and metolachlor were applied preemergence at rates of 2.2, 3.4, 4.5, and 6.7 kg/ha. After the herbicide application, 125 ml of water was applied to the soil surface for herbicide incorporation and activation. This gave a moisture content of 12% for each pot. Equal amounts of water were added to each pot thereafter. After 10 days, plant height and visual injury ratings were taken.

Influence of soil moisture content

Anderson 103 corn seed was evaluated as previously described in the general greenhouse procedure. Herbicide applications of alachlor and metolachlor at 4.5 kg/ha were used. Applications of surface applied water were made to obtain six moisture levels of 8, 10, 12, 14, 18, and 22 % soil moisture. Preliminary studies verified that adequate corn seedling growth could be obtained at these levels of soil moisture. The moisture levels were maintained daily by weighing and adding the appropriate amounts of water as needed. After 10 days plant height and injury ratings were taken as previously described.

RESULTS AND DISCUSSION

Computer survey

The computer data base contained a total of 158 comparisons of alachlor and metolachlor. These comparison were made with 31 different corn hybrids. The response of the hybrids showed that some were more tolerant to metolachlor while others are more tolerant to alachlor (Table 1). For example, in twelve comparisons with Pioneer 3906, the alachlor treatments averaged 625 kg/ha (10.8 bu/A) more yield than the metolachlor treatments. However, in six comparisons with Pioneer 3603, the metolachlor treatments averaged 387 kg/ha (6.2 bu/A) more than alachlor treatments. Of the 31 hybrids included in the trials, those receiving the alachlor treatments yielded more with 17 of the hybrids, while the corn receiving metolachlor treaments yielded more with 15 hybrids (Figure 1). The mean difference in corn yield between alachlor and metolachlor treatments for all 158 comprisons was only 25 kg/ha in favor of alachlor. This difference is negligible and insignificant.

Influence of hybrid, herbicide, and rate

In this experiment both parameters used for measuring injury showed a significant interaction between herbicide, hybrid, and application rate (Figure 2).

The interaction of hybrid, herbicide, and rate indicates that at the low herbicide application rate of 2.2 kg/ha there was generally no significant difference in injury between hybrids or herbicides. With seven of the 10 hybrids, the application rate of 2.2 kg/ha metolachlor was less injurious than alachlor, although this difference was rarely significant (Table 2).

As the herbicide appplication rate increased, the differences among the hybrids and between the herbicides also increased. At the 4.5 kg/ha appplication rate, Great Lakes hybrid 313 was more tolerant to metolachlor, whereas, hybrid 516 was more tolerant to alachlor.

At the highest application rate of 6.7 kg/ha, metolachlor was generally more injurious than alachlor for all hybrids. Based on visual observations over a period of time (data not included), corn seedlings treated with metolachlor at the highest appplication rates were unable to overcome the injury as well as those seedlings treated with alachlor.

Influence of soil moisture content

In this experiment there was an interaction between herbicide and soil moisture level. The interaction indicated a linear response of increasing injury with increasing moisture for both alachlor and metolachlor (Figure 3). However, at 12% soil moisture, which was about field capacity, there was no significant difference between alachlor and metolachlor treatments. There was significantly greater injury from metolachlor compared to alachlor at the higher moisture levels. At the 22% soil moisture level the alachlor treated seedlings were 40% of the control height compared to 25% for the metolachlor treated plants (Table 3).

From these studies it was conluded that the corn hybrid, the herbicide, the herbicide application rate, and the soil moisture content at the time of plant emergence all play a role in the degree of chloroacetanilide injury which occurred.

Table 1. Comparison[a] of corn grain yield for hybrids receiving alachlor versus metolachlor in public sector trails from 1984-1986.

Corn hybrid	Number of Comparisons	Sum of yield difference[b]
		(bu/A)
Bojac	2	-3
Carhart 793	2	-1
Coker 22	2	+2
Dekalb T-12-30	7	+10
Dekalb 1100	2	0
Dekalb 636	1	-3
Funk's 4733	2	+28
Funk's 4740	3	-15
Nebraska 611	3	-16
Olds 95	2	+9
Pioneer 3147	2	+4
Pioneer 3352	2	+7
Pioneer 3377	2	-7
Pioneer 3378	5	-18
Pioneer 3413	1	+17
Pioneer 3475	1	+21
Pioneer 3535	2	-22
Pioneer 3603	6	-37
Pioneer 3732	13	+45
Pioneer 3747	48	-10
Pioneer 3780	1	-5
Pioneer 3901	2	-18
Pioneer 3906	12	-130
Pioneer 3732	25	+59
Pioneer 3347	1	+6
Pioneer 3382	1	+7
Sokota 270	2	-2
Stuaffer 57751	1	-5
Sunbelt 1876	1	-25
Terra 3203	1	-10
Wilson 1700	2	+10
Unknown	4	-73
Totals	158	-69

[a]Mean difference between herbicide treatments (-69/158) was -0.44 bu/A.

[b]Negative numbers indicate the alachlor treatment had a yield advantage, positive numbers indicate the metolachlor treatment had a yield advantage.

Table 2. The effects of alachlor and metolachlor at four application rates on ten Great Lakes hybrids.

Great Lakes Hybrid	Herbicide	Rate	Shoot height	Injury
		(kg/ha)	(% of untreated)	(%)
579	Alachlor	2.2	94.5	28
		3.4	75.6	36
		4.5	62.0	39
		6.7	50.0	55
	Metolachlor	2.2	96.6	11
		3.4	95.8	11
		4.5	56.8	51
		6.7	39.4	69
313	Alachlor	2.2	85.8	34
		3.4	53.4	59
		4.5	44.6	64
		6.7	24.8	71
	Metolachlor	2.2	94.6	13
		3.4	77.6	34
		4.5	68.1	48
		6.7	25.7	81
381	Alachlor	2.2	93.9	8
		3.4	79.9	24
		4.5	68.6	17
		6.7	55.8	27
	Metolachlor	2.2	99.2	4
		3.4	73.6	21
		4.5	56.1	25
		6.7	35.8	56
422	Alachlor	2.2	78.0	34
		3.4	67.7	44
		4.5	49.8	41
		6.7	36.7	63
	Metolachor	2.2	88.6	16
		3.4	85.1	31
		4.5	52.3	59
		6.7	34.6	68
437	Alachlor	2.2	91.5	29
		3.4	68.8	38
		4.5	53.6	53
		6.7	36.4	54
	Metolachlor	2.2	83.6	28
		3.4	80.5	33
		4.5	59.2	52
		6.7	35.9	67

Table 2. Continued.

Great Lakes Hybrid	Herbicide	Rate	Shoot height	Injury rating
		(kg/ha)	(% of untreated)	(%)
466	Alachlor	2.2	83.3	19
		3.4	85.9	17
		4.5	57.2	23
		6.7	44.4	37
	Metolachlor	2.2	91.2	9
		3.4	69.5	26
		4.5	57.3	38
		6.7	30.4	57
498	Alachlor	2.2	83.2	17
		3.4	88.0	24
		4.5	53.4	45
		6.7	38.9	40
	Metolachlor	2.2	97.1	21
		3.4	81.6	30
		4.5	59.9	42
		6.7	35.4	65
516	Alachlor	2.2	84.8	14
		3.4	70.8	16
		4.5	56.9	36
		6.7	30.0	37
	Metolachlor	2.2	71.5	21
		3.4	56.9	36
		4.5	44.0	52
		6.7	27.9	70
547	Alachlor	2.2	82.4	18
		3.4	55.9	35
		4.5	52.9	26
		6.7	41.8	35
	Metolachlor	2.2	108.2	10
		3.4	68.4	22
		4.5	50.4	37
		6.7	32.2	58
599	Alachlor	2.2	87.6	21
		3.4	71.4	17
		4.5	44.0	20
		6.7	34.3	29
	Metolachlor	2.2	75.9	47
		3.4	64.3	42
		4.5	34.5	67
		6.7	35.5	73
LSD (0.05)			12.0	12

Table 3. Effect of soil moisture on response of Andersons 103 corn hybrid to alachlor and metolachlor.

	Shoot height		Injury rating	
Soil moisture	Alachlor	Metolachlor	Alachlor	Metolachlor
(%)	--(% of untreated)--		------(%)------	
8	95.5	98.3	8	11
10	80.5	75.7	13	10
12	74.8	73.9	15	16
14	56.7	58.4	34	35
18	51.8	28.2	49	59
22	40.2	25.2	69	70
LSD (0.05)	------ 10.4 ------		------ 12 ------	

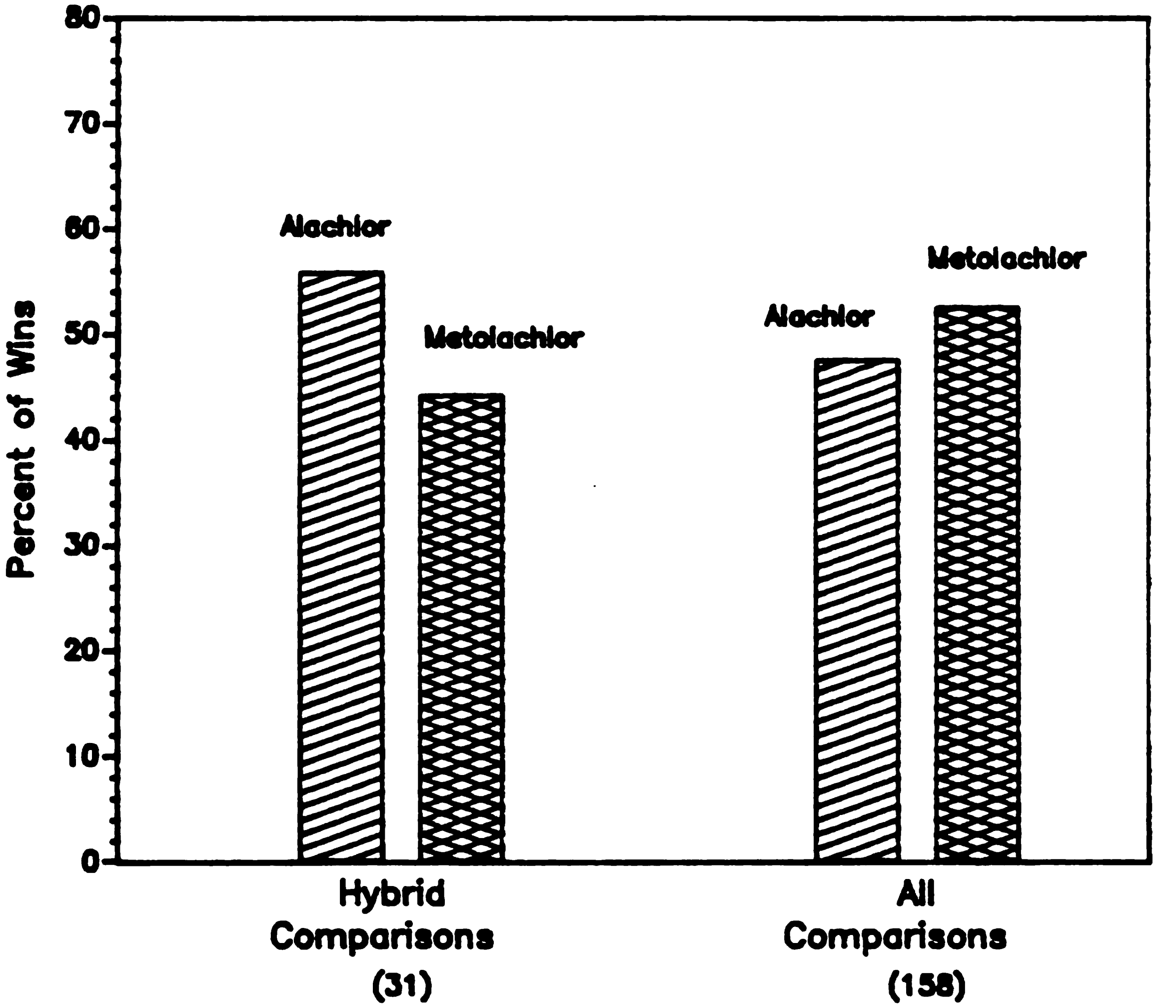
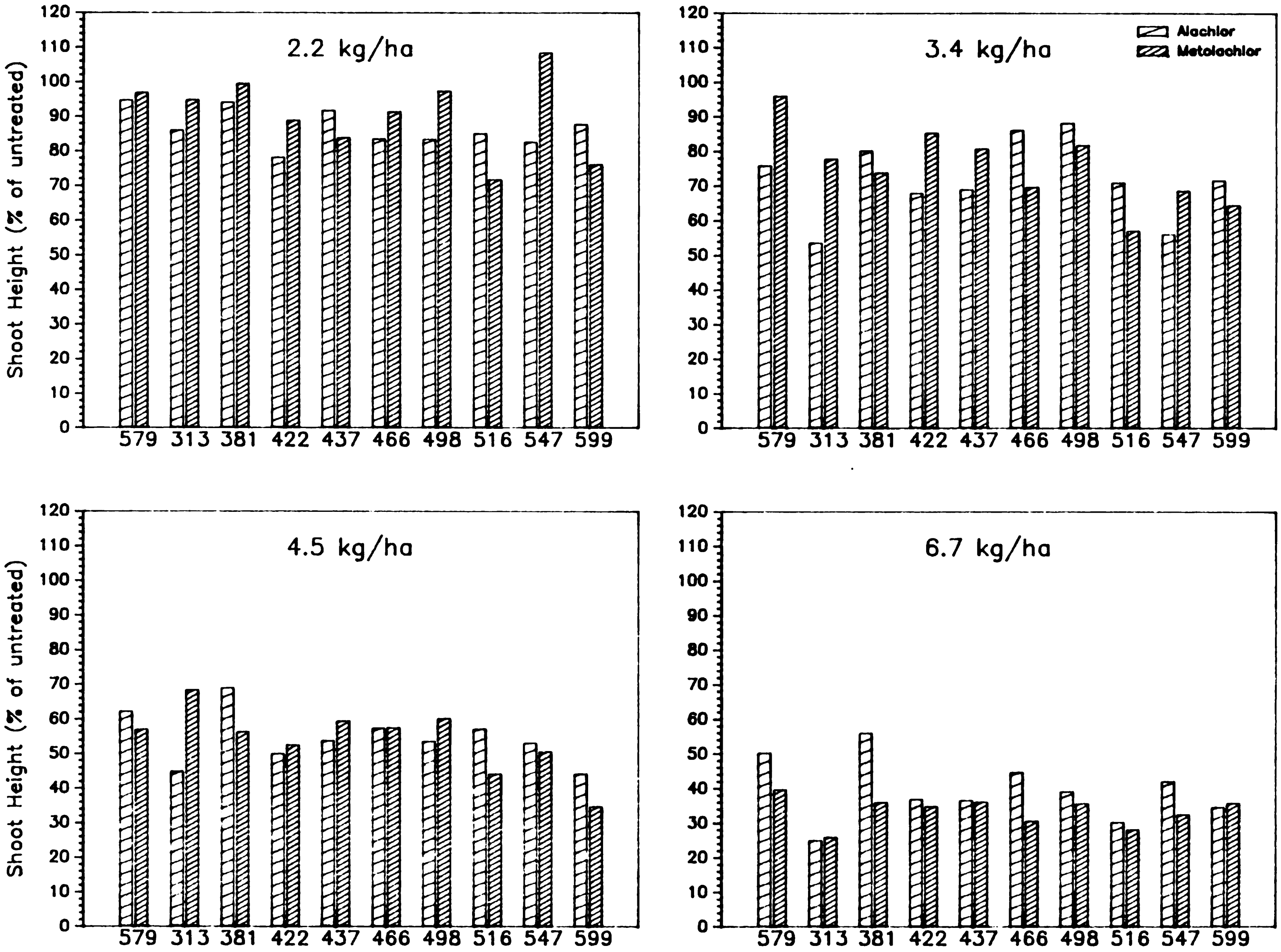


Figure 1. Comparisons of corn yield between alachlor and metolachlor treatments in 1984-1986.

Figure 2. Effects of alachlor and metolachlor at four application rates on shoot heights of 10 Great Lakes corn hybrids (LSD (0.05) = 12.0).



2.2 kg/ha
3.4 kg/ha
4.5 kg/ha
6.7 kg/ha
Alachlor
Metolachlor
Shoot Height (% of untreated)
579 313 381 422 437 466 498 516 547 599

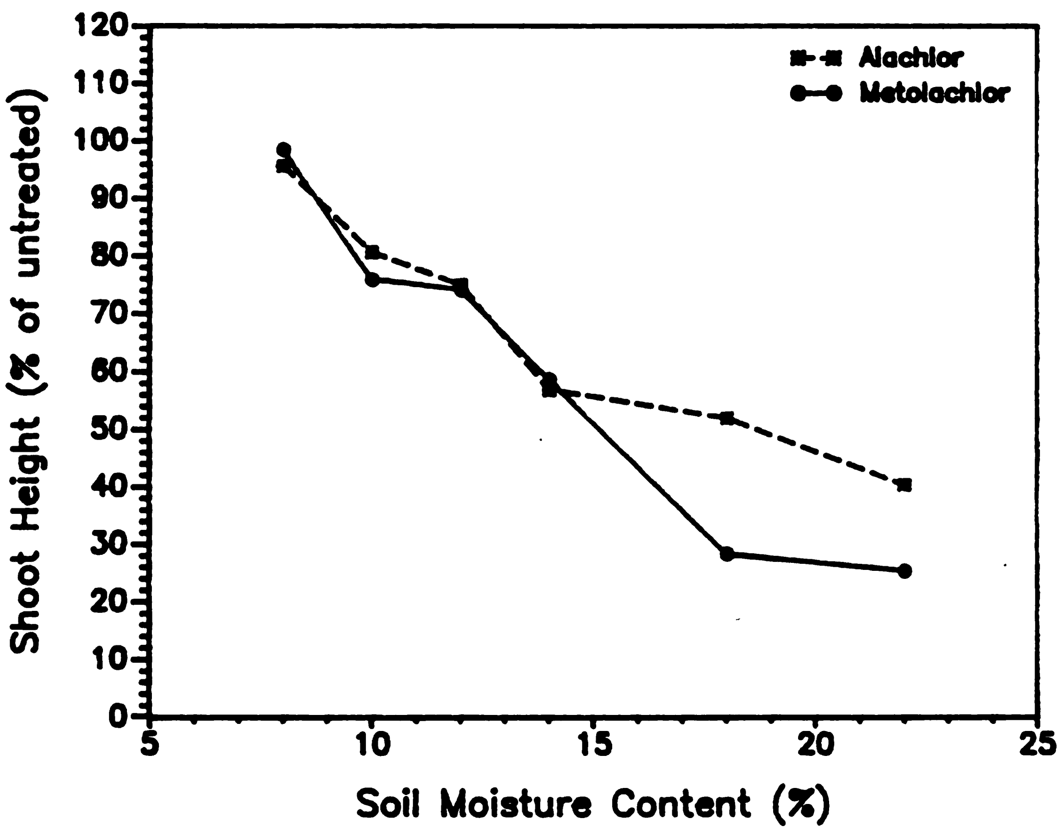


Figure 3. Effects of soil moisture content on the response Andersons 103 hybrid corn to alachlor and metolachlor applied at 4.5 kg/ha (LSD (0.05) = 10.4).

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Chapter 3

RESPONSE OF CORN HYBRIDS AND INBREDS TO METOLACHLOR

ABSTRACT

Greenhouse studies were conducted to determine the response of 200 corn hybrids and 29 inbreds to metolachlor applied at 4.5 kg/ha. Both hybrids and inbreds varied in their response to the herbicide. The distribution of injury resembled a normal distribution curve with most of the hybrids having a midlevel of tolerance. However, some of the hybrids were very tolerant, while others were quite sensitive. Laboratory studies were conducted to evaluate absorption and metabolism of ^{14}C-metolachlor for a subset of tolerant and sensitive hybrids. These studies showed that their was no difference in the pathway of metabolism for metolachlor in the tolerant and sensitive hybrids. The studies revealed that the basis for observed variability in metolachlor tolerance among hybrids was due to differences in rates of absorption and metabolism of metolachlor, and differences at the site of action of metolachlor. The tolerant Great Lakes 584 hybrid absorbed significantly less ^{14}C-metolachlor than did the sensitive Pioneer 3744, while the tolerant Cargill 7567 metabolized

^{14}C-metolachlor significantly faster than the other hybrids. The internal concentrations of available ^{14}C-metolachlor were the same for the tolerant Cargill 7567 and the sensitive Northrup King 9283, indicating differences at the site of action of metolachlor for these two hybrids. Nomenclature: Corn, *Zea mays* L.; metolachlor, 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide. *Additional index words*. Tolerance, normal distribution curve, midlevel tolerance, internal concentration.

INTRODUCTION

Metolachlor is a herbicide that is commonly used in corn production without significant injury to corn for the control of many grasses and several broadleaf weeds. However, under certain circumstances injury symptoms have been reported. The factors contributing to this increased crop injury from metolachlor have not been fully studied. In addition to several enviromental factors, genetic differences among hybrids is believed to play a role in the amount of visual injury observed (2,6,7).

Several other crop species are known to exhibit differential tolerance among cultivars to specific herbicides. The most documented of these is the response of soybean (*Glycine max* (L.) Merr.) cultivars to metribuzin (4-amino-6-(1,1-dimethylethyl)-3-(methylthio)1,2,4-triazin-5(4H)-one) (4). However, through extensive research and selective breeding, soybean tolerance to metribuzin can be identified and utilized at will. Differential tolerance of corn cultivars to several other herbicides has been reported. These include atrazine (6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine), trifluralin (2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)benzenamine), EPTC (*S*-ethyl dipropylcarbamothioate), and imazaquin (2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl)-3-quino-linecarboxylic acid) (1,8,9,10).

The objectives of these studies were to determine the distribution and degree of metolachlor injury among corn hybrids by testing a representative number of hybrids, and also to select and evaluate tolerant and sensitive hybrids to determine the physiological basis for the observed differences in tolerance.

MATERIALS AND METHODS

Inbred study

Twenty-nine public corn inbreds were obtained from the corn breeding program of Michigan State University, East Lansing, Michigan. The corn seed were planted in 50 by 30 cm pans with 8 inbreds in rows per pan. The soil was a Spinks sandy loam (mixed, mesic Psammentic Hapludalfs) consisting of 71.3% sand, 19.4% silt, and 9.4% clay with a pH of 6.2. The seeds were planted 4.0 cm deep and 4.5 kg/ha of metolachlor was then applied preemergence with a chain-link belt compressed air sprayer, which delivered 280 L/ha at 240 kPa. A total of 1200 ml of water was added per pot to the soil surface for incorporation and activation of the herbicide. This amount gave approximately 12% moisture content (w/w) to the previously air-dried soil. Greenhouse conditions were 16 h days at 24 C +/-2. The plants were grown with supplemental lighting from high-pressure sodium lamps. The light intensity was 500 $uE.m^{-2}\cdot s^{-1}$ with only supplemental lights to 1200 $uE\cdot m^{-2}\cdot s^{-1}$ with both supplemental and natural sunlight. The greenhouse was maintained at 40 to 70 % relative humidity. After 10 days, plant height was measured and visual injury was evaluated. The mean of four plants was considered one obsevation. Data is expressed as percent of control for that particular inbred. The experiment included four replications and was repeated.

Hybrid study and selection

Corn hybrids were evaluated in the greenhouse by obtaining 200 corn hybrids from 17 major seed companies across the Midwest. Twenty-four tolerant and sensitive hybrids were selected for further study based on the results from the 200 hybrids. The 24 hybrids were treated with 6.7 kg/ha of metolachlor for further evaluation and from these two tolerant and two sensitive hybrids were selected for ^{14}C-metolachlor absorption and metabolism studies. Cargill 7567 and Great Lakes 584 were tolerant, while Pioneer 3744 and Northrup King 9283 were sensitive to metolachlor (Figure 1).

Metolachlor absorption and metabolism

^{14}C-Metolachlor (specific activity 7.26 uCi/umole, uniformly ring labelled) was obtained from the CIBA-Geigy Corporation. The corn seed from the four hybrids mentioned above were placed on germination blotters and covered with paper towels. They were then placed in a dark growth chamber at 25 C. After 3 days the etiolated seedlings were removed from the growth chamber and the herbicide treatment was applied (Figure 2). A 2 ul drop, which contained 67.3 ug of metolachlor was applied just above the coleoptilar node of the corn seedling. Of the metolachlor applied only 2% was ^{14}C-metolachlor. The seedlings were placed back into the growth chamber for an 8-h absorption period. Based on preliminary studies this period of time allowed for maximum measurability of absorption and metabolism activity. After the 8-h absorption period the seedlings were rinsed with 3 ml of 100% methanol. Preliminary work verified that all surface radioactivity could be removed with 3 ml of 100% methanol. The seedlings were then placed in dry ice at -30 C until extraction. Two plants for each treatment were weighed, combined, and extracted with a Virtis grinder in 50 ml of 90% methanol for 5 min. The extract was filtered with Whatman No. 1 paper. The residue was oxidized with a Harvey Biological Oxidizer and counted with a Tri-Carb Liquid Scintillation Spectrometer. The volume of the extract was reduced under vacuum at 35 C and

1 ml of 90% methanol was added. A 100 ul aliquot of the concentrated extract was radioassayed with the spectrometer and a 50 ul aliquot was spotted on a Silica Gel GF TLC plate. The plate was then eluted with butanol:acetic acid: water (12:3:5) and radioactivity distribution was determined with an AMBIS Radioactivity Scanner. The results for absorption are presented as the percent ^{14}C absorbed. The results for metabolism are presented as the percent of ^{14}C absorbed that was converted to metabolite as determined by the scanner. Rf values were calculated for each area of radioactivity. The results represent the means of ten replications. By calculation based on absorption, metabolism, and weight of the corn seedling, internal concentration of metolachlor for each hybrid was determined.

RESULTS AND DISCUSSION

Inbred study

The results of this study showed a wide range in response of 29 corn inbreds to metolachlor applied at 4.5 kg/ha. The amount of injury ranged from a low of 16.3% for a tolerant inbred to 80.0% for a sensitive one. The shoot height of corn inbred LH91 was 82.4% of the height of the control compared to LH85 which was only 23.6% of its control height (Table 1). The majority of the hybrids had a midlevel of tolerance with their injury ranging from 35 to 55% of their controls (Figure 3).

Hybrid study and selection

This study also indicated a high degree of variability in metolachlor injury among the 200 commercial hybrids tested (Table 2). The range of distribution resembled that of a normal distribution curve with some of the hybrids being very tolerant, while others were quite sensitive (Figure 4). As in the inbred study the majority of the hybrids had a midlevel of tolerance.

The subset of 24 hybrids included the hybrids on the ends of the injury response spectrum. Further evaluation of these revealed distinct differences in injury when metolachlor was applied at a higher rate (Table 3). These differences led to the selection of two tolerant and two sensitive hybrids which exhibited dramatic visual differences in metolachlor injury (Figure 1). Great Lakes 584 and Cargill 7567 were metolachlor tolerant, while Northrup King 9283 and Pioneer 3744 were metolachlor sensitive.

Metolachlor absorption and metabolism

The four selected hybrids were evaluated to identify differences in the absorption and metabolism of ^{14}C-metolachlor. From the qualitative analysis of the TLC plates we concluded that there was no difference in the path of metabolism for tolerant and sensitive hybrids. The radioactivity in all hybrids was divided into two components (Figure 5). One was the parent compound metolachlor, which had a Rf value of about 0.82. The other component was a more polar metabolite which had a Rf value of 0.49. Based on previous reports (3,5,11) this metabolite is probably the inactive or non-phytotoxic conjugate of glutathione and metolachlor.

The laboratory study indicated significant differences in the absorption and rate of metabolism of ^{14}C-metolachlor for the four hybrids tested (Table 4). Great Lakes 584 absorbed only 23.0% of the ^{14}C-metolachlor, which was significantly less than the other three hybrids. Cargill 7567 metabolized greater amounts of metolachlor than the three other hybrids, while Great Lakes 584 metabolized more than the two sensitive ones. The calculations of the internal concentrations of metolachlor remaining as parent compound revealed, as expected, a significantly higher amount in the sensitive Pioneer 3744 and a lower amount in the tolerant Great Lakes 584 (Table 5). However, the tolerant Cargill 7567 and the sensitive Northrup King 9283 contained the same internal concentrations of available parent metolachlor. Based on the highly significant difference in visual injury symptoms exhibited by these two hybrids, there may be differences in the sensitivity at the site of action for metolachlor. Perhaps there is a difference in the number of sites or the nature of site of action.

Table 1. Response of 29 corn inbred lines to metolachlor applied at 4.5 kg/ha.

Inbred Line	Shoot height	Injury rating
	(% of untreated)	(%)
MBS838	82.4	16
LH91	71.5	30
LH108	70.0	43
LH82	69.7	40
LH146	63.3	46
FR31	62.5	43
LH109	61.5	48
MS71	60.6	46
MSB847	55.4	44
A632	55.1	49
LH119	53.5	45
LH132	51.6	49
LH74	48.9	51
FR19	46.0	61
LH59	45.4	60
FR1141	44.7	60
DF14	43.9	61
LH136	43.8	55
LH38	42.9	59
59G	41.5	56
MBS501	41.3	61
LH57	40.0	61
FR23	39.6	58
LH145	36.1	60
LH54	34.6	70
MS76	32.7	63
OF9	28.9	65
LH51	26.4	80
LH85	23.6	73
LSD (0.05)	14.9	12

Table 2. Response of 200 hybrids to metolachlor at 4.5 kg/ha.

Hybrid	Shoot height	Injury rating
	(% of untreated)	(%)
Asgrow 2545	88.1	18
Cargill 130411	86.7	43
Great Lakes 584	81.5	33
Northrup King 9470	79.2	32
Great Lakes 516	78.2	40
Northrup King 9251	76.0	32
Andersons 85	75.6	33
Terra 1125	75.6	48
Renk 68	75.4	23
Great Lakes 381	74.4	40
Asgrow 2330	74.0	35
Renk 76	73.9	35
Great Lakes 365	73.5	50
Terra 975	71.8	37
Callahan 19097x	71.5	37
Great Lakes 547	71.3	32
Callahan 19101X	71.3	42
Golden Harvest 2343	70.5	47
Callahan 19102x	70.3	43
Pioneer 3352	70.1	37
Crows 181	69.4	32
Cargill 7567	69.0	42
Asgrow 6882	69.0	35
Northrup King 9385	68.9	47
Great Lakes 487	68.9	42
Golden Harvest 2572	68.6	43
Great Lakes 482	68.2	48
Great Lakes 85553	68.1	33
Pioneer 3540	68.0	37
Dekalb 547	67.9	58
Great Lakes 437	67.6	48
Pioneer 3704	67.5	45
Asgrow RX788	67.1	43
Great Lakes 420	67.0	55
Dekalb 572	66.6	42
Great Lakes 5922	65.7	38
Dekalb 484	65.6	33
Great Lakes 498	65.2	38
Terra 3203	65.2	47
Andersons 107	65.1	35
Andersons 110	64.8	35
Callahan 19925X	64.5	30
Renk 73	64.4	33
Renk 1060	64.2	52

Table 2. Continued.

Hybrid	Shoot height	Injury rating
	(% of untreated)	(%)
Northrup King 9540	63.8	43
Stauffer 7751	63.8	43
Cargill HT115	63.7	45
Asgrow XP4506	63.4	50
Golden Harvest 2492	62.9	32
Great Lakes 599	62.6	45
Great Lakes 466	62.4	38
Crows 199	62.4	32
King 1184	62.3	40
Glenn-Garno 1005	62.0	42
Northrup King 9161	61.9	50
Payco 800	61.8	40
Golden Harvest EX536	61.5	50
Voris 2491	61.4	40
Glenn-Garno 1012	61.1	50
Glenn-Garno 988	61.0	38
Stauffer 4474	61.0	55
Stauffer 4590	60.9	37
Dekalb 524	60.7	50
Andersons 95	60.3	50
Asgrow RX498	60.1	48
Terra 1040	59.9	67
King 596	59.7	52
Renk 21	59.7	60
Crows 212	59.4	37
Voris 2515	59.2	40
Dekalb 464	59.1	58
Great Lakes 86647	59.1	45
Stauffer 4454WX	58.7	43
Asgrow 180	58.7	47
Cargill 7877	58.6	43
Andersons 103	58.6	52
Stauffer 5750	58.6	50
Pioneer 3902	58.5	55
Cargill HT110	58.3	52
Great Lakes 86601	58.3	50
Callahan 19908X	58.0	43
King 237	58.0	48
Great Lakes 87680	58.0	58
Pioneer 3790	57.8	53
Great Lakes 87671	57.8	57
Great Lakes 579	57.6	51
Callahan 766	57.2	42
Glenn-Garno 900X	57.1	42
Stauffer 4402	57.1	48

Table 2. Continued.

Hybrid	Shoot height	Injury rating
	(% of untreated)	(%)
Stauffer 2184	56.8	38
Cargill 893	56.6	48
Northrup King 9527	56.6	42
Pioneer 3475	56.5	50
Callahan 747	56.4	50
Renk 64	56.2	53
Glenn-Garno 1003	56.2	57
Cargill 809	55.6	58
Terra 32	55.4	52
Glenn-Garno 8885	55.1	57
Stauffer 5722WX	55.1	48
Northrup King 9060	55.0	40
Terra 162E	54.9	53
Terra 3100	54.9	48
Cargill SX239	54.8	60
Callahan 754	54.8	53
Cargill B53	54.7	45
Voris 2465	54.7	47
King 5574	54.5	58
Andersons 93	54.4	40
Glenn-Garno 944	54.3	42
Andersons 99	54.3	58
Cargill HT120	54.2	60
Stauffer 2206	53.8	50
Crows 444	53.5	40
Asgrow RX578	53.5	48
Crows 442	53.1	50
Payco 611	53.1	52
Renk 138	52.6	50
Payco 847	52.5	45
Golden Harvest 2465	51.6	62
Callahan 738	51.5	55
Andersons 100	51.5	55
Payco 786	51.3	47
Dekalb 397	51.3	43
Terra 3102	51.3	52
Voris 2365	51.2	60
Stauffer 6707WX	50.9	33
Asgrow 2230	50.8	53
Cargill HT95	50.8	62
Crows 488	50.6	50
King 4422	50.5	58
Northrup King 9353	50.5	52
Northrup King 4325	50.3	62
Payco 342	50.3	57

Table 2. Continued.

Hybrid	Shoot height	Injury rating
	(% of untreated)	(%)
Northrup King 9292	49.9	67
Pioneer 3803	49.5	57
Great Lakes 414	48.9	62
Crows 482	48.6	60
Pioneer 3901	48.3	57
Terra 29	48.1	63
King 416	47.9	50
Great Lakes 422	47.8	65
Renk 148	47.6	63
Golden Harvest EX615	47.4	70
Cargill 937	47.1	57
Cargill SX123	47.0	52
Crows 344	46.7	58
Terra 262E	46.7	45
Golden Harvest 2250	46.6	68
Renk 60	46.5	47
Renk 19	46.1	58
Voris 2331	46.1	62
Cargill 6127	45.8	68
Renk 27	45.5	60
Dekalb 435	45.3	63
Andersons 90	45.3	50
Stauffer 5340	45.3	60
Callahan 726	45.2	57
Great Lakes 82351	45.0	53
Stauffer 5650	44.5	62
Pioneer 3744	44.4	57
Golden Harvest 2344	44.3	72
King 647	44.2	63
Payco 686	43.7	50
Pioneer 3949	43.6	78
Cargill 3477	43.6	48
Asgrow RX626	43.6	57
Renk 7A	43.2	58
Payco 872	43.1	58
Cargill 3987	42.5	58
Northrup King 39	42.2	70
Dekalb 461	42.2	48
Cargill HT105	40.8	55
Great Lakes 313	40.4	77
Glenn-Garno 1007	40.0	63
Andersons 105	39.8	63
Crows 210	39.6	60
King 2203	39.5	45
Cargill 5157	39.1	68

Table 2. Continued.

Hybrid	Shoot height	Injury rating
	(% of untreated)	(%)
Pioneer 3737	38.5	78
King 2204	37.5	70
Stauffer 3306	36.9	57
Northrup King 9283	36.8	72
King 4484	36.5	65
Cargill 859	36.3	67
King 4464	36.0	60
Cargill 2787	35.7	68
Stauffer 3303	35.6	62
Cargill SX310	35.0	66
Voris 2471	34.1	63
Stauffer 2101WX	33.4	65
Great Lakes SX112	33.1	65
Terra 3200	32.6	72
Callahan 728	31.3	68
Pioneer 3779	31.0	73
Cargill 819	30.6	60
Glenn-Garno 900	29.6	72
Dekalb 415	28.2	77
Renk 24	26.3	60
Payco 500	24.7	75
LSD (0.05)	16.0	11

Table 3. Response of 24 hybrids to metolachlor applied at 6.7 kg/ha.

Hybrid	Shoot height	Injury rating
	(% of untreated)	(%)
Cargill 7567	74.2	30
Crows 181	65.0	40
Great Lakes 516	62.0	39
Great Lakes 584	61.2	42
Crows 212	60.3	41
Cargill Sx239	59.7	41
Terra 3203	58.3	44
Renk 68	54.4	42
Asgrow 2545	54.3	44
Great Lakes 547	51.2	51
Golden Harvest 2343	51.1	46
Stauffer 3303	48.6	58
Renk 64	46.9	59
Northrup King 39	43.7	55
Terra 29	43.7	62
Pioneer 3949	43.2	54
Payco 500	42.9	58
Dekalb 415	41.7	61
Andersons 93	37.8	56
Pioneer 3779	37.5	61
Glenn-Garno 1007	33.6	63
Great Lakes 313	31.7	69
Pioneer 3744	30.3	72
Northrup King 9283	27.2	68
LSD (0.05)	9.8	9

Table 4. Absorption and metabolism of ^{14}C-metolachlor by tolerant and sensitive corn hybrids.

Hybrid	^{14}C Absorbed	Metabolite
	(% of applied)	(% of absorbed)
Cargil 7567	33.2	32.3
Great Lakes 584	23.0	26.6
Northrup King 9283	30.5	21.6
Pioneer 3744	36.6	18.7
LSD (0.05)	6.3	3.9

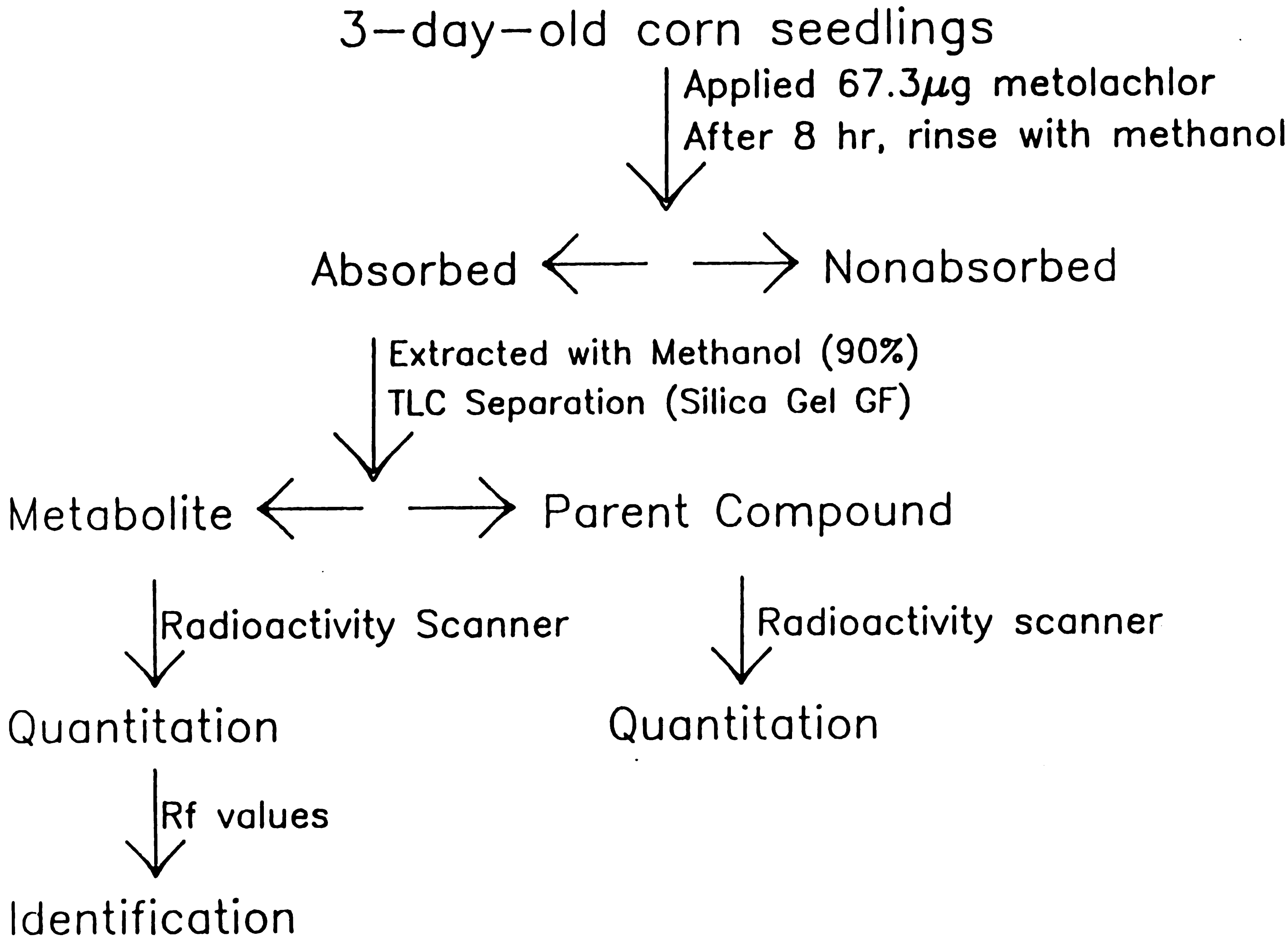
Table 5. Internal concentration of ^{14}C-metolachlor in tolerant and sensitive corn hybrids.

Hybrid	Concentration
	(ug/g)
Cargill 7567	1.13
Great Lakes 584	0.74
Northrup King 9283	1.04
Pioneer 3744	1.49
LSD (0.05)	0.20

__Figure 1__. Four selected corn hybrids, from left to right, Cargill 7567, Northrup King 9283, Great Lakes 584, Pioneer 3744.

right,
akes

Figure 2. Flow diagram for absorption and metabolism study.


3-day-old corn seedlings
Applied 67.3μg metolachlor
After 8 hr, rinse with methanol
Absorbed
Nonabsorbed
Extracted with Methanol (90%)
TLC Separation (Silica Gel GF)
Metabolite
Parent Compound
Radioactivity Scanner
Radioactivity scanner
Quantitation
Quantitation
Rf values
Identification

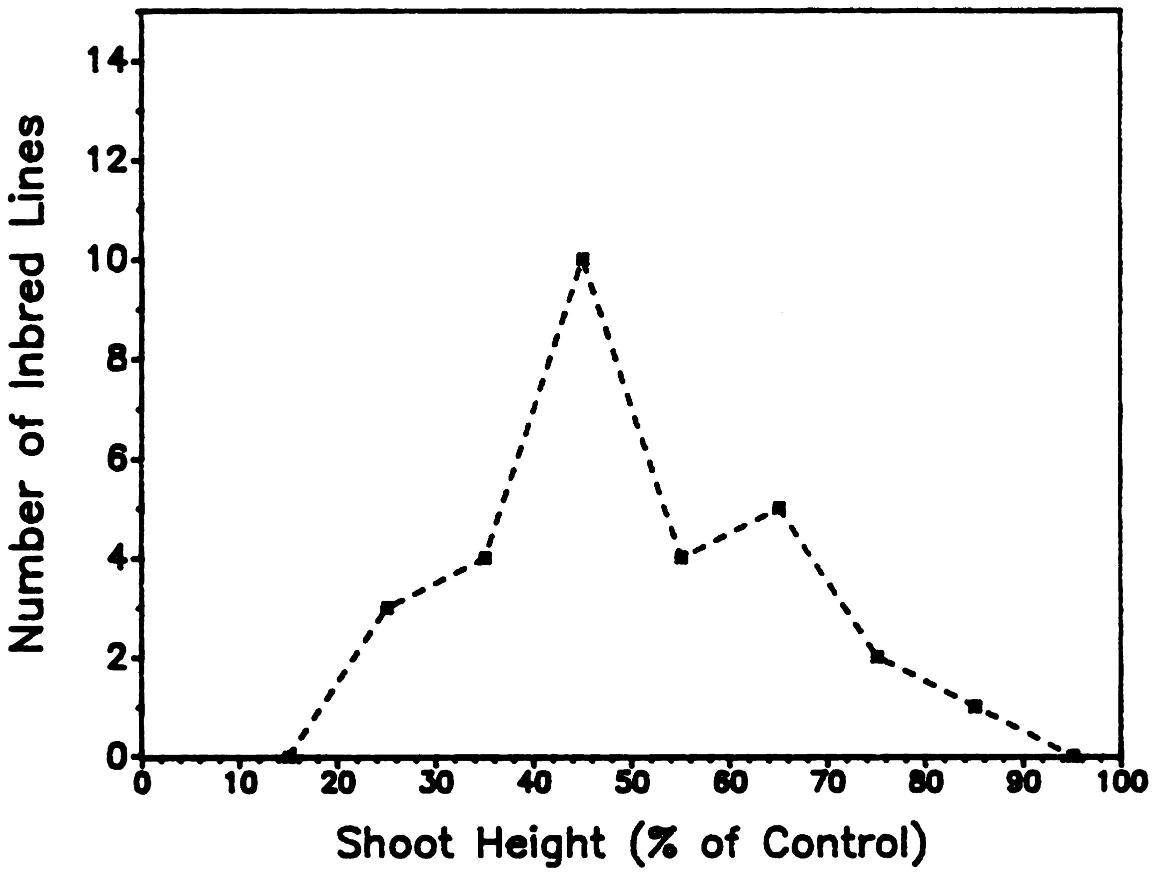


Figure 3. Distribution of injury for 29 corn inbred lines treated with 4.5 kg/ha of metolachlor.

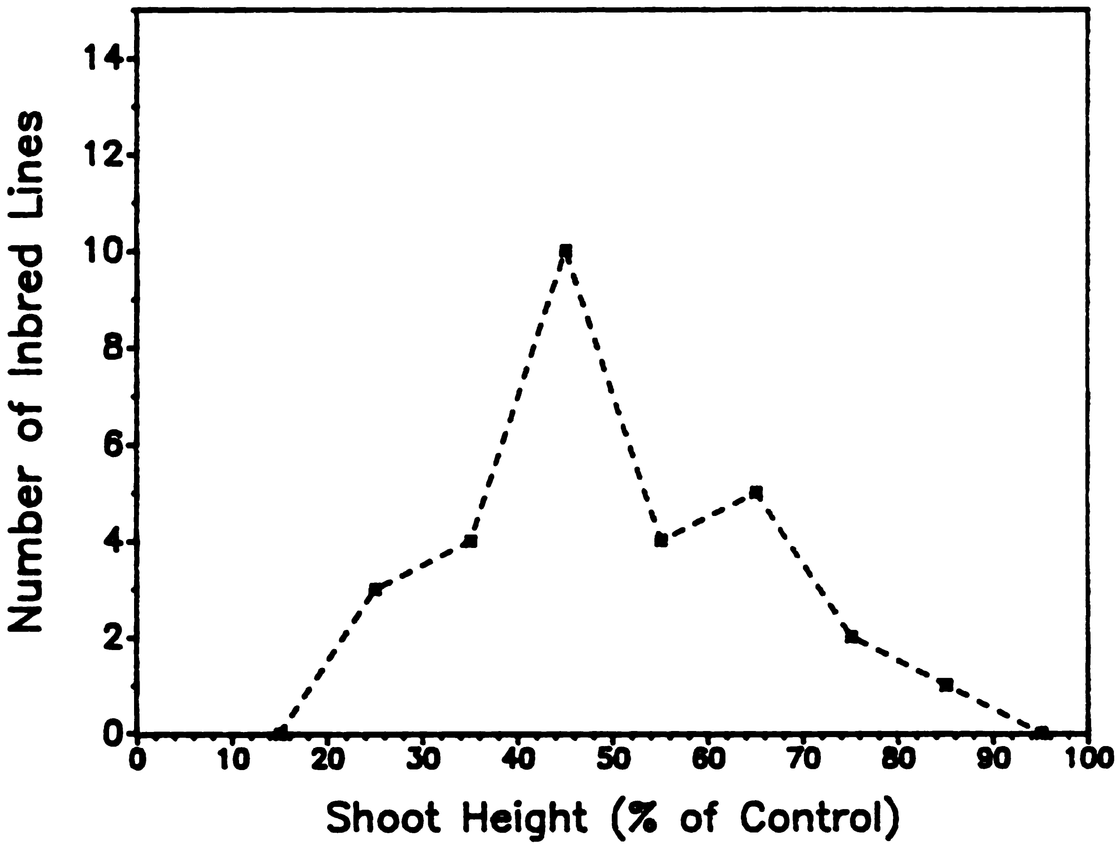


Figure 3. Distribution of injury for 29 corn inbred lines treated with 4.5 kg/ha of metolachlor.

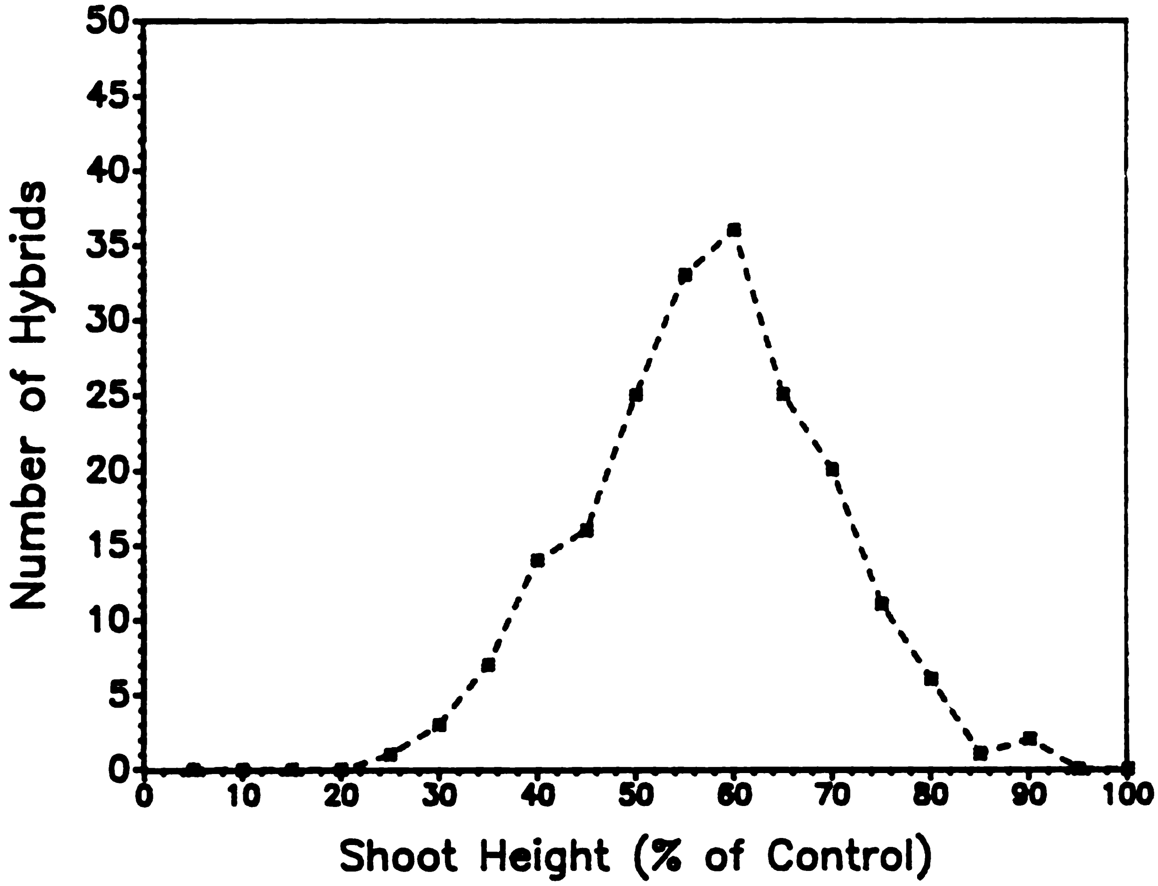


Figure 4. Distribution of injury for 200 corn hybrids treated with 4.5 kg/ha of metolachlor.

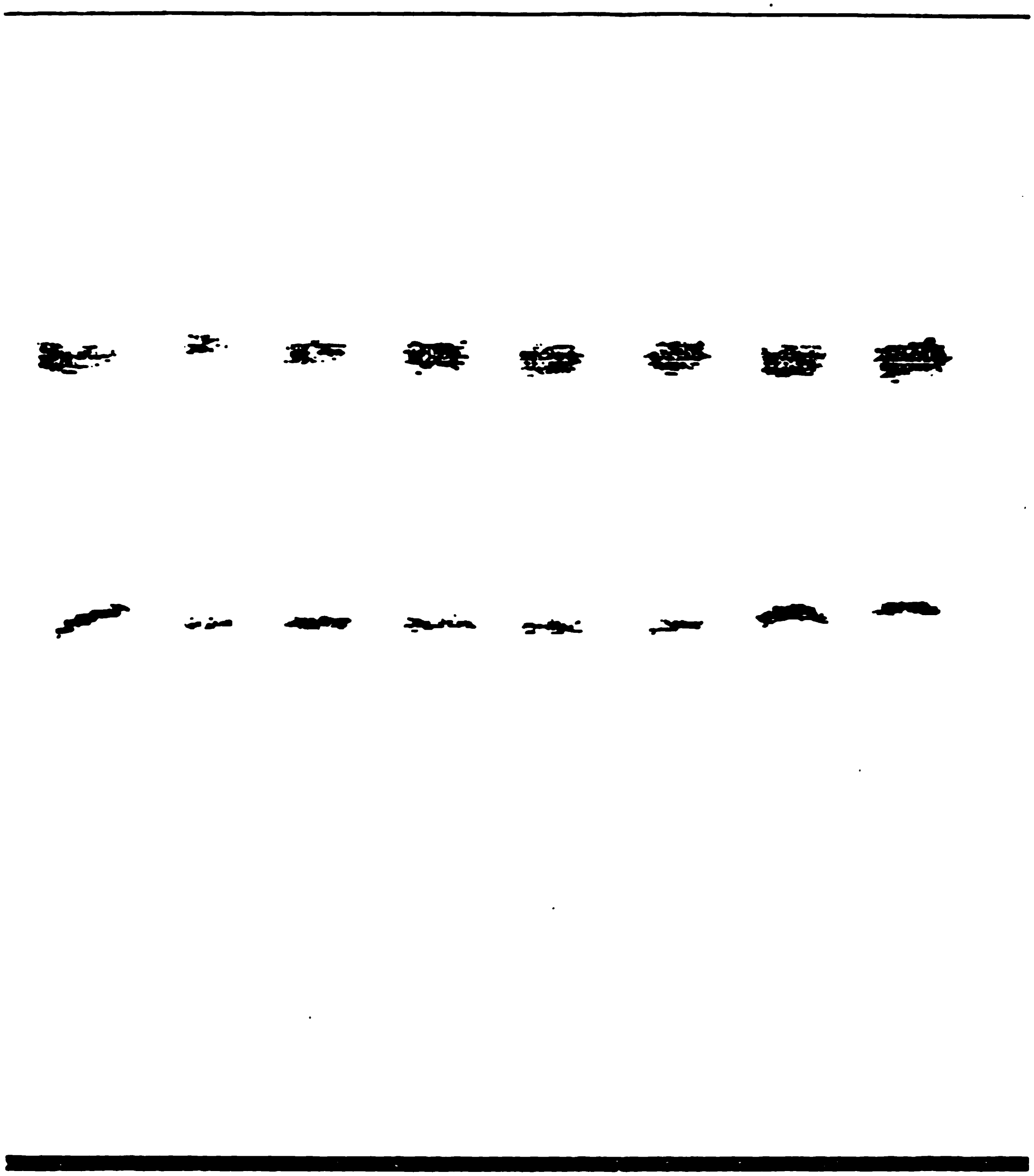


Figure 5. Output from AMBIS Radioactivity Scanner.

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Chapter 4

EFFICACY OF CGA-154281 AS A PROTECTANT FOR CORN FROM METOLACHLOR INJURY

ABSTRACT

Greenhouse and field studies were conducted to determine the influence of herbicide rate, hybrid variability, and soil moisture content on the effectiveness of CGA-154281 in protecting corn seedlings from metolachlor injury. In greenhouse studies, metolachlor and CGA-180937 (metolachlor + CGA-154281) were applied preemergence at seven rates ranging from 1.1 kg/ha to 7.8 kg/ha. Four corn hybrids, which were previously identified as being tolerant or sensitive to metolachlor, were used. As expected, high rates of metolachlor caused significant injury to the corn seedlings, especially the sensitive hybrids. However, with CGA-180937, very few injury symtoms were observed, even at the highest herbicide rate and with the most sensitive hybrid. Four watering regimes were used to evaluate protection by CGA-154281 at various soil moisture contents. Corn seedlings treated with CGA-180937 showed no significant injury, whereas, those treated with metolachlor alone showed 70% injury at the highest moisture level. Metolachlor injury increased as soil moisture content increased.

In field studies in 1987 and 1988, metolachlor and CGA-180937 were applied at rates up to 6.7 kg/ha to hybrids ranging in sensitivity to metolachlor. These studies also indicated that CGA-154281 was effective in protecting corn seedlings under conditions conducive to metolachlor injury. Nomenclature: Corn, *Zea mays* L.; metolachlor, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide; CGA-154281, 4-(dichloro-acetyl)-3,4-dihydro-3-methyl-2H-1,4-benzoxazine; CGA-180937, metolachlor + CGA-154281 (30:1). *Additional index words.* Protectant, hybrid variability, soil moisture contents.

INTRODUCTION

Metolachlor is generally safe for use on corn, however, it may injure corn seedlings under certain conditions. Factors which enhance metolachlor injury to corn are high application rates, inherent sensitivity of hybrids or inbred lines, and high soil moisture content (1,4,5).

Chemical antidotes or protectants are known to protect grain sorghum (*Sorghum bicolor* L.) from metolachlor. These antidotes have also been shown to protect corn seedlings as well (2,3,6). Since a seed treatment with some expense is required and the metolachlor injury to corn is infrequent and limited, these compounds have not been utilized in corn production.

The new experimental protectant CGA-154281 (Figure 1) is being evaluated specifically for the protection of corn from metolachlor. Very little is known about this protectant, thus the objectives of our research were to evaluate the effectiveness of this new protectant for corn seedlings growing under conditions that are known to be conducive to metolachlor injury. Also, we wanted to determine whether the CGA-154281 provided protection to several weed species.

MATERIALS AND METHODS

General greenhouse procedure

Corn seed of selected hybrids were planted in 946 ml pots, which contained an air-dried Spinks sandy loam (mixed, mesic Psammentic Hapludalfs) soil consisting of 71.3% sand, 19.4% silt, and 9.4% clay with a pH of 6.2. The herbicides were applied preemergence with a chain-link belt, compressed air sprayer which delivered a volume of 280 L/ha at 240 kPa. Varying amounts of water were added to the soil surface for incorporation of the herbicide. The pots were placed in the greenhouse which was maintained at 16 h days at 25 +/- 2 C. The plants were grown with supplemental lighting from high-pressure sodium lamps. The light intensity ranged from 500 $uE.m^{-2}.s^{-1}$ with only supplemental lights to 1200 $uE.m^{-2}.s^{-1}$ with both supplemental and natural sunlight. The greenhouse was maintained at 40 to 75% relative humidity. Shoot height and injury ratings were taken after 10 days. Shoot height is expressed as percent of the untreated plant's height. Plant injury rating was on a scale of 0 (no effect) to 100 (completely dead). The mean of three plants in each pot was considered one observation. Each treatment was replicated four times and the data are the means of two experiments. The data was analyzed, and means were separated with LSD values at the 5% level of significance.

Hybrid and rate response

Four corn hybrids, which were previously identified as being tolerant or sensitive to metolachlor, were evaluated under the above conditions at application rates ranging from 1.1 to 7.8 kg/ha of metolachlor in the Dual formulation or the CGA-180937 formulation (metolachlor + CGA-154281). The tolerant hybrids were Cargill 7567 and Great Lakes 584. The sensitive ones were Pioneer 3744 and Northrup King 9283. Metolachlor was applied preemergence at rates of 1.1, 2.2, 3.4, 4.5, 5.6, 6.7, and 7.8 kg/ha. After the herbicide application, 125 ml of water was applied to the soil surface for incorporation. This gave a moisture content of 12% for each pot, which was equilavent to field capacity for the soil. Equal amounts of water were added to each pot thereafter until the data was collected.

Soil moisture response

Pioneer 3744 corn hybrid was grown as previously described. Metolachlor was applied alone or in the presence of CGA-154281 at 4.5 kg/ha. Applications of water were made to obtain soil moisture contents of 8, 12, 18, and 22% moisture. Preliminary studies were conducted to verify that adequate corn seedling growth could be obtained at these soil moisture levels. The soil moisture contents were maintained daily by weighing and adding the appropriate amounts of water as needed. After 10 days plant heights and injury ratings were taken as previously reported.

Weed response

This study was conducted to determine if CGA-154281 provided protection from metolachlor to certain weed species. The study was conducted under the previously reported greenhouse conditions. Eight weed species were planted in 30 by 50 cm pans and metolachlor or CGA-180937 was applied preemergence at 1.1 and 2.2 kg/ha. After 14 days injury ratings were taken. The weed species planted were giant foxtail (Seteria faberii Herrm.), barnyardgrass (Echinochloa crus-gali (L). Beauv.), fall panicum, (Panicum dichotomiflorum Michx.), johnsongrass (Sorghum halapense (L.) Pers.), common ragweed (Ambrosia artemisiifolia L.), common lambsquarters (Chenopodium album L.), redroot pigweed (Amaranthus retroflexus L.), and shattercane (Sorghum bicolor L. Moench.).

Field studies

Field studies were conducted at two locations in 1987 and 1988 to determine if CGA-154281 was effective in protecting sensitive corn hybrids from high rates of metolachlor under field conditions. In these experiments, 6.7 kg/ha of metolachlor in the Dual formulation or in the CGA-

180937 formulation (metolachlor + CGA-154281) was applied on hybrids which were previously selected in greenhouse studies as being metolachlor tolerant or sensitive. In all experiments the herbicides were applied premergence with a tractor mounted compressed air sprayer which delivered 205 L/ha at 206 kPa. The experiments were arranged as split-plot design with herbicide treatment being the whole plot and hybrids being subplots. Plant height and injury ratings were taken after 21 days, and the height data was converted to percent of control.

In 1987, both locations were on the campus of Michigan State University in East Lansing. One location was on the Crops farm, which has a Riddles sandy loam (mixed, mesic Typic Hapludalfs, 67.6% sand, 10.4% silt, 21.1% clay) soil with a pH of 6.9, while the second location in 1987 was on the Soils farm. The soil type there is a Capac sandy clay loam (mixed, mesic Aric Ochraqualfs, 61.6% sand, 24.4% silt, 14.1% clay) with a pH of 6.8. Six hybrids marketed by three seed companies were used in 1987. In preliminary studies a relatively tolerant and sensitive hybrid from each company was selected. The tolerant hybrids were Pioneer 3352, Andersons 85, and Dekalb 584. The sensitive hybrids were Pioneer 3475, Andersons 103, and Dekalb 415. Metolachlor was applied at 2.2 and 6.7 kg/ha while CGA-180937 was applied at 6.7 kg/ha.

In 1988, one location was on campus in East Lansing, and the other location was near Battle Creek, Michigan at the Kellogg Biological Field Station (KBS). The campus soil is a Capac sandy loam (mixed, mesic Aeric Ochraqualfs) consisting of 71.6% sand, 10.4% silt, and 18.1% clay with a pH of 6.5. The KBS soil is a Oshtemo sandy loam (mixed, mesic Typic Hapludalfs) consisting of 71.3% sand, 19.4% silt, and 9.4% clay with a pH of 5.9. Four hybrids which were selected as being tolerant or sensitive were used. The tolerant hybrids were Cargill 7567 and Great Lakes 584, while the sensitive ones were Pioneer 3744 and Northrup King 9283. In 1988, both metolachlor and CGA-180937 were applied at 2.2 and 6.7 kg/ha. Plant height and injury ratings were taken after 21 days, and percent of control was calculated.

RESULTS AND DISCUSSION

Rate and hybrid response

In this study the CGA-154281 protected even the sensitive corn seedlings at the high metolachlor application rates (Table 1). The metolachlor alone treatment caused significant injury to Cargill 7567 at 4.5 kg/ha. However, for that same hybrid, the protectant protected the seedlings even at the highest rate of 7.8 kg/ha. With the other tolerant hybrid, Great Lakes 584, the protectant protected up to 5.5 kg/ha (Figure 2).

For the sensitive hybrid, Northrup king 9283, significant injury occurred with the metolachlor alone treatment at 2.2 kg/ha, while the protectant protected this hybrid up to 6.6 kg/ha. Significant injury was also observed with Pioneer 3744 at 2.2 kg/ha with the metolachlor alone treatment, however with the protected treatment no injury occurred up to 5.5 kg/ha.

Moisture response

CGA-154281 significantly protected Pioneer hybrid 3744 corn seedlings form the 4.5 kg/ha metolachlor application even at the highest soil moisture level (Figure 3). At 22% moisture the injury from metolachor was 78.8% compared to the 10% injury for the metolachlor plus protectant treatment (Table 2).

Weed response

When metolachlor plus protectant was used at the labelled rate of 2.2 kg/ha, there was no significant protection of any of the eight weed species tested. However, at one-half the labelled use rate or 1.1 kg/ha a significant degree of protection occurred for johnsongrass and shattercane (Table 3).

Field studies

In the field studies in 1987 there was a significant location effect so the study could not be combined over the two locations. In 1988 the study was combined over location and the data is presented as such.

In 1987 on the Crops Farm, there was no significant injury to any of the hybrids even at the high rate of metolachlor, as no significant rainfall occurred within 24 days after the preemergence application of the herbicides. Therefore, the protectant potential of CGA-154281 could not be accurately determined. However, that year at the Soils Farm 3.5 cm of rain fell within 5 days after appplication, resulting in significant injury to the sensitive hybrids at the high rate of metolachlor. This injury was not evident in the CGA-180937 treatment, indicating adequate protection with the antidote (Table 4).

In 1988 in the combined experiments, protection was again evident at the high rate and on the more sensitive hybrids (Table 5). The metolachlor alone treatment at 6.7 kg/ha resulted in a reduction in plant height to 72.4 and 72.9 percent of control for Northrup King 9283 and Pioneer 3744, respectively. However, the addition of the protectant prevented significant injury (Figure 4). In all the field studies there was generally no significant injury when metolachlor was applied alone at the labelled use rate of 2.2 kg/ha.

Table 1. Response of four hybrids to metolachlor at 8 application rates with and without CGA-154281.

Metolachlor rate	Hybrid	CGA-154281	Shoot height
(kg/ha)			(% of untreated)
1.1	Cargill 7567	-	99.2
		+	103.7
	NK 9283	-	98.5
		+	103.1
	GL 584	-	98.5
		+	102.7
	Pioneer	-	96.9
		+	101.8
2.2	Cargill 7567	-	96.7
		+	102.2
	NK 9283	-	82.8
		+	97.8
	GL 584	-	91.3
		+	97.8
	Pioneer 3744	-	83.8
		+	91.5
3.4	Cargill 7567	-	90.2
		+	100.8
	NK 9283	-	72.0
		+	92.9
	GL 584	-	86.5
		+	92.2
	Pioneer 3744	-	71.1
		+	91.9
4.5	Cargill 7567	-	81.1
		+	95.5
	NK 9283	-	54.8
		+	92.3
	GL 584	-	70.9
		+	95.9
	Pioneer 3744	-	61.8
		+	90.2
5.6	Cargill 7567	-	75.9
		+	98.3
	NK 9283	-	53.1
		+	93.8
	GL 584	-	70.8
		+	90.1
	Pioneer 3744	-	44.4
		+	86.4
6.7	Cargill 7567	-	69.3
		+	91.5
	NK 9283	-	40.5

Table 1. Continued.

Metolachlor rate	Hybrid	CGA-154281	Shoot height
(kg/ha)			(% of untreated)
		+	86.4
	GL 584	-	64.6
		+	83.9
	Pioneer 3744	-	28.8
		+	80.7
7.7	Cargill 7567	-	55.7
		+	88.5
	NK 9283	-	31.5
		+	80.8
	GL 584	-	47.6
		+	85.8
	Pioneer 3744	-	27.0
		+	76.3
LSD (0.05)			13.4

Table 2. Response of Pioneer 3744 to metolachlor at four soil moisture regimes with and without CGA-154281.

Soil moisture content	CGA-154281	Shoot height	Injury rating
(%)		(% of untreated)	(%)
8	-	89.8	15
	+	98.9	0
12	-	45.6	45
	+	89.1	1
18	-	34.1	64
	+	87.3	11
22	-	19.9	79
	+	70.1	10
LSD (0.05)		8.7	8

Table 3. Response of eight weed species to metolachlor at two application rates with and without CGA-154281.

Weed	Metolachlor rate	CGA-154281	Injury rating
	(kg/ha)		(%)
Giant foxtail	1.1	-	100
		+	98
	2.2	-	100
		+	100
Barnyardgrass	1.1	-	99
		+	98
	2.2	-	100
		+	100
Fall panicum	1.1	-	100
		+	100
	2.2	-	100
		+	100
Johnsongrass	1.1	-	97
		+	78
	2.2	-	98
		+	93
Common ragweed	1.1	-	72
		+	87
	2.2	-	98
		+	99
Common lambsquarters	1.1	-	92
		+	88
	2.2	-	99
		+	99
Redroot pigweed	1.1	-	98
		+	98
	2.2	-	100
		+	100
Shattercane	1.1	-	97
		+	72
	2.2	-	98
		+	97
LSD (0.05)			10

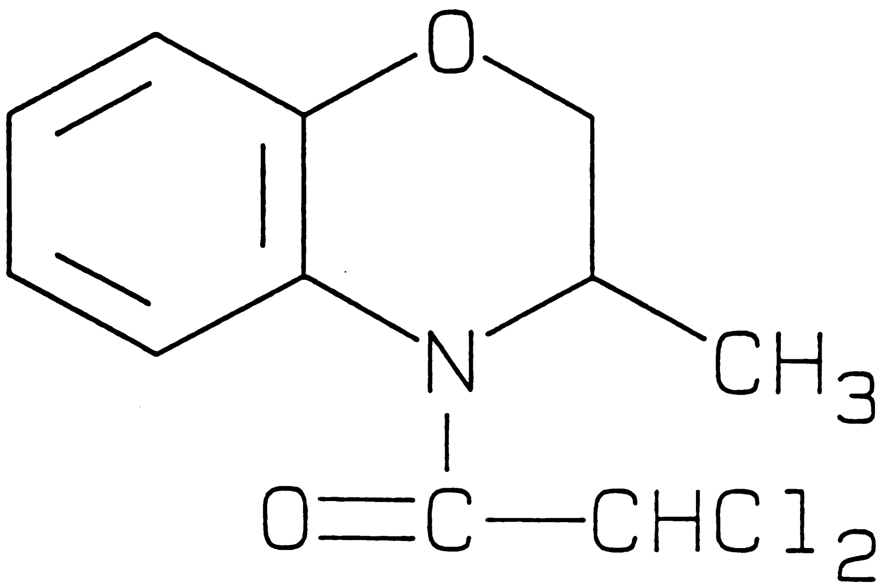
Table 4. Field response of six hybrids to metolachlor with and without CGA-154281 in 1987.

Hybrid	Metolachlor rate	CGA-154281	Shoot height: Crops Farm	Shoot height: Soils Farm
	(kg/ha)		--(% of untreated)--	
Pioneer 3475	2.2	-	98.7	93.8
	6.7	-	96.5	70.1
		+	103.5	95.7
Pioneer 3352	2.2	-	101.5	98.2
	6.7	-	99.1	82.0
		+	97.8	96.2
Andersons 103	2.2	-	93.5	101.7
	6.7	-	95.8	84.2
		+	99.7	101.6
Andersons 85	2.2	-	102.3	92.3
	6.7	-	92.0	86.1
		+	106.7	99.8
Dekalb 415	2.2	-	99.4	91.3
	6.7	-	100.3	73.4
		+	105.6	98.1
Dekalb 584	2.2	-	97.1	100.9
	6.7	-	96.4	89.1
		+	105.4	99.5
LSD (0.05)			ns	11.5

Table 5. Field response of four hybrids to metolachlor with and without CGA-154281 in 1988.

Hybrid	Metolachlor rate	CGA-154281	Shoot height[a]
	(kg/ha)		(% of untreated)
Cargill 7567	2.2	-	100.2
		+	97.7
	6.7	-	96.8
		+	102.9
Great Lakes 584	2.2	-	91.9
		+	98.4
	6.7	-	86.4
		+	98.2
Northrup King 9283	2.2	-	96.7
		+	102.5
	6.7	-	72.4
		+	99.7
Pioneer 3744	2.2	-	98.3
		+	95.8
	6.7	-	72.9
		+	94.5
LSD (0.05)			12.5

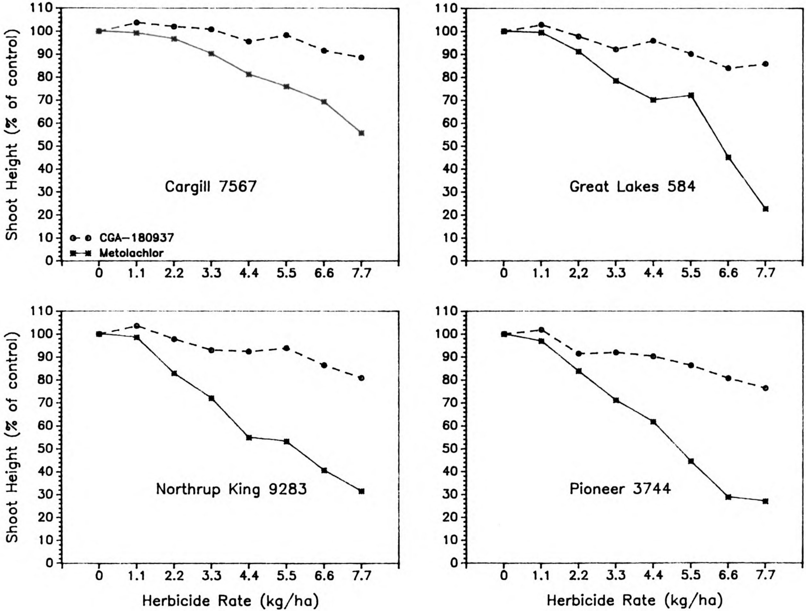
[a]Data combined over two locations in Michigan.



CGA-154281

Figure 1. Chemical structure of CGA-154281.

Figure 2. Response of 4 hybrids to metolachlor and CGA-180937 at 8 application rates (LSD (0.05) = 13.4).


Cargill 7567
Great Lakes 584
Northrup King 9283
Pioneer 3744
CGA-180937
Metolachlor
Shoot Height (% of control)
Herbicide Rate (kg/ha)
0
1.1
2.2
3.3
4.4
5.5
6.6
7.7
0
10
20
30
40
50
60
70
80
90
100
110

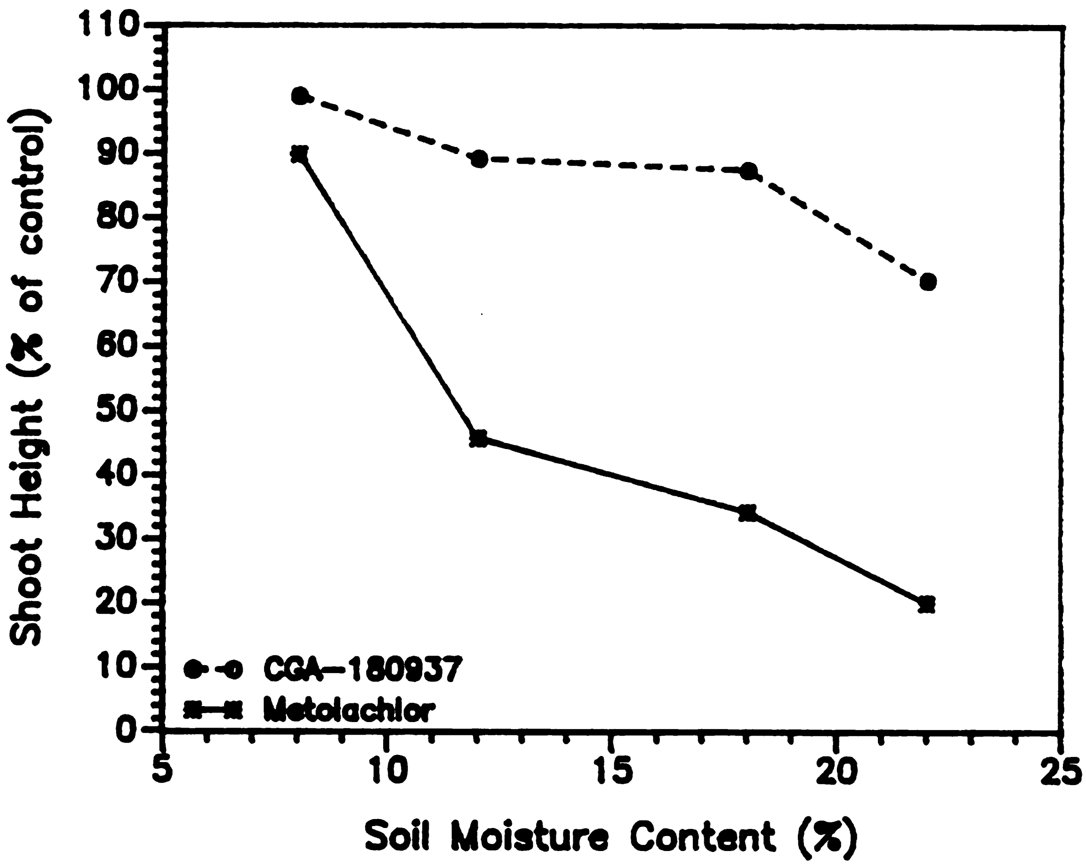
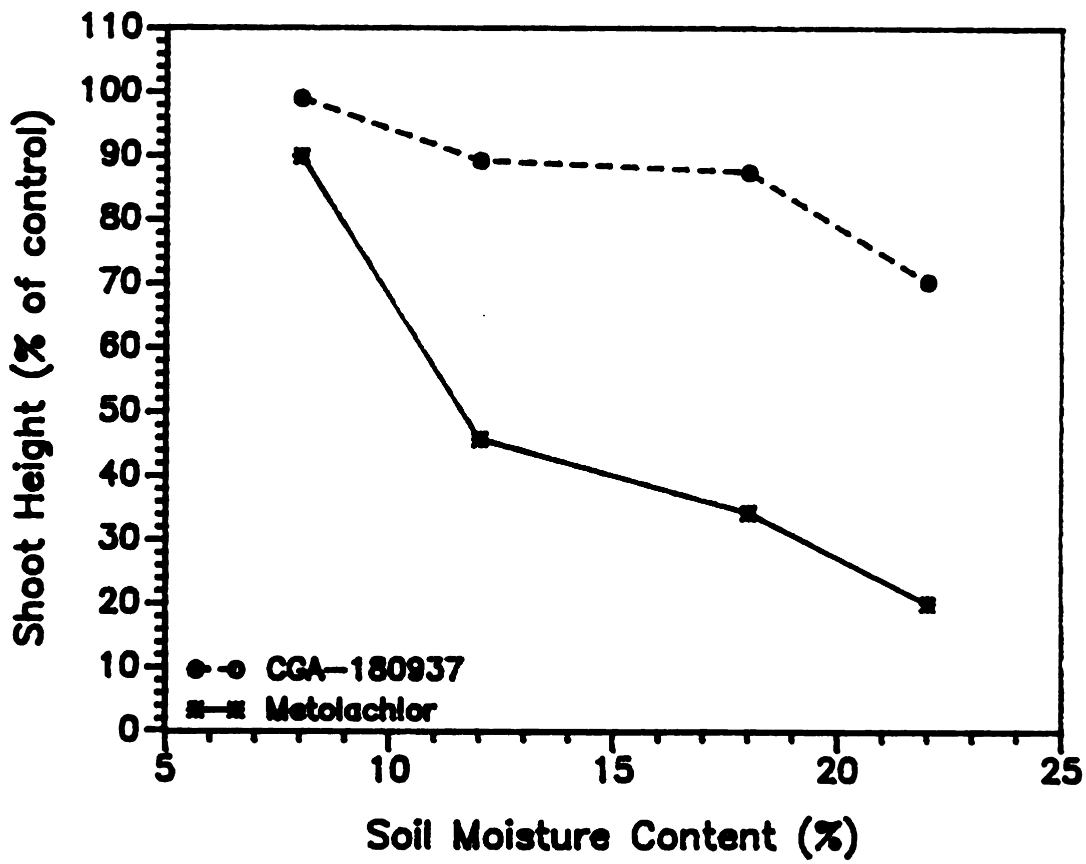
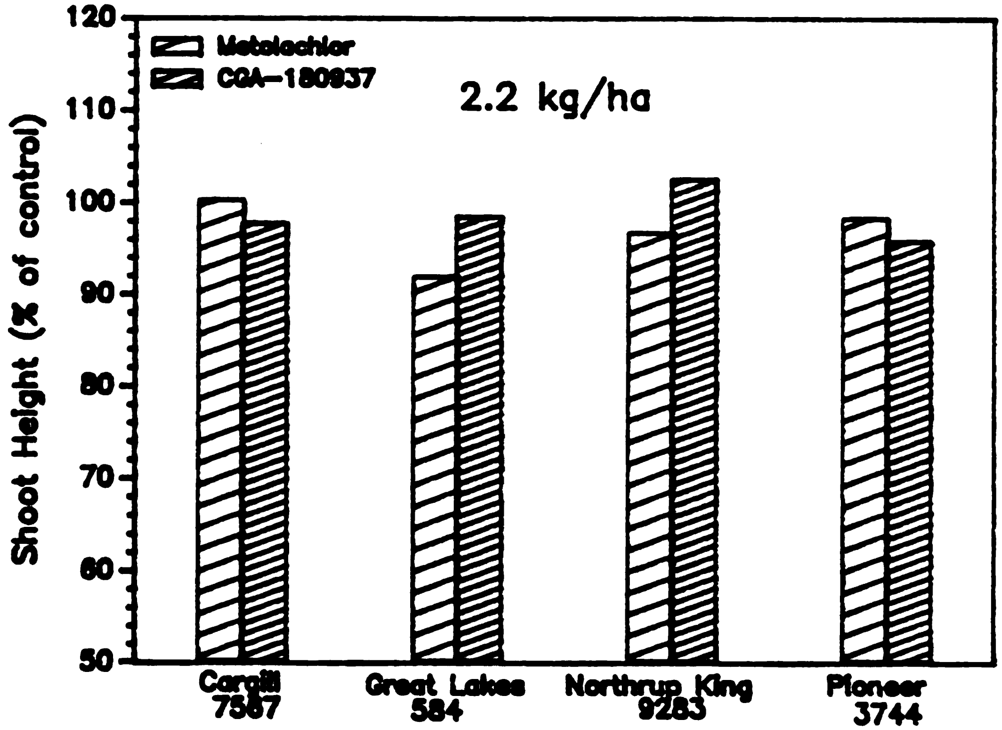


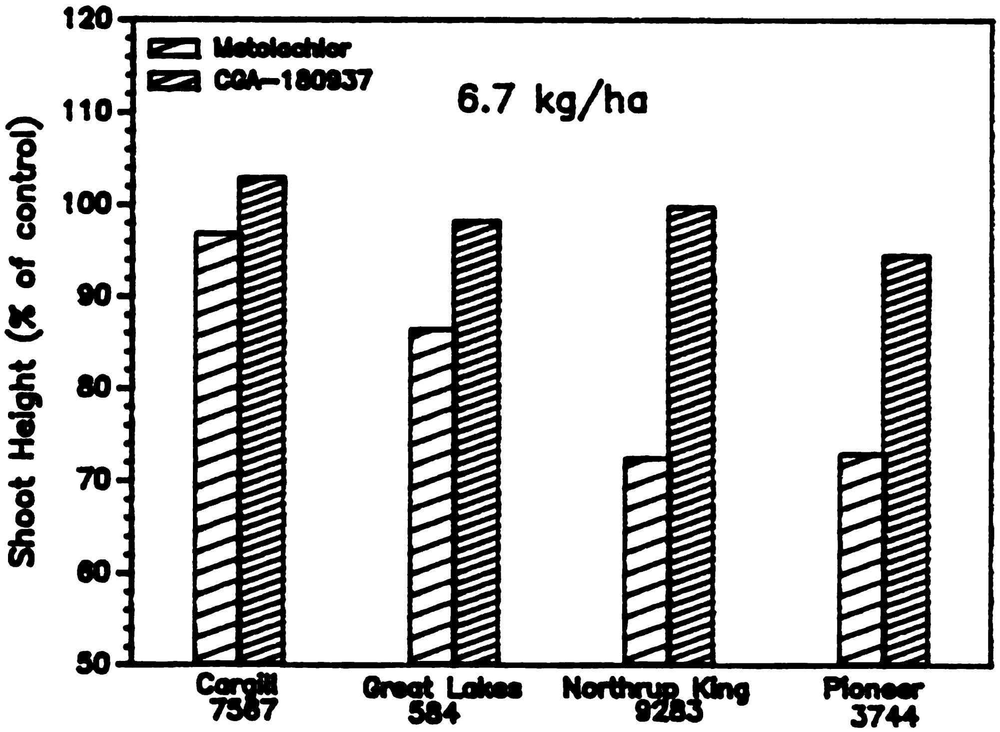
Figure 3. Response of Pioneer 3744 to metolachlor and CGA-180937 at four soil moisture contents (LSD (0.05) = 8.7).



<u>Figure 3</u>. Response of Pioneer 3744 to metolachlor and CGA-180937 at four soil moisture contents (LSD (0.05) = 8.7).

Figure 4. Field response of four corn hybrids to metolachlor and CGA-180937 in 1988 (LSD (0.05) = 12.5).


Metolachlor
CGA-180937
2.2 kg/ha
Shoot Height (% of control)
120
110
100
90
80
70
60
50
Cargill 7587
Great Lakes 584
Northrup King 9283
Pioneer 3744


Metolachlor
CGA-180937
6.7 kg/ha
Shoot Height (% of control)
120
110
100
90
80
70
60
50
Cargill 7587
Great Lakes 584
Northrup King 9283
Pioneer 3744

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Chapter 5

INFLUENCE OF THE PROTECTANT CGA-154281 ON THE ABSORPTION AND METABOLISM OF METOLACHLOR

ABSTRACT

Laboratory studies were conducted to determine the effect of the protectant CGA-154281 on the absorption and metabolism of metolachlor in two metolachlor sensitive and two metolachlor tolerant corn hybrids. During an 8 h period, the CGA-154281 did not alter the absorption of ^{14}C-metolachlor. Qualitative comparison of the metabolism of metolachlor in the presence or in the absence of the protectant revealed that CGA-154281 did not alter the pathway of metolachlor metabolism. In both instances the metolachlor was metabolized to a more polar metabolite, believed to be a glutathione conjugate. However, CGA-154281 significantly enhanced the rate of metabolism of metolachlor in three of the four hybrids tested. It appears from the data that the mechanism by which the antidote enhanced metolachlor metabolism activity was already at a maximum in the unaffected hybrid, Cargill 7567. Nomenclature: Corn, *Zea mays* L.; metolachlor, 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl) acetamide; CGA-154281, 4-(dichloroacetyl)-3,4-dihydro-3-methyl-2H-1,4-benzoxazine.

INTRODUCTION

Metolachlor is commonly used for weed control in corn. However, injury symtoms to the corn are known to occur under certain conditions. It has been reported that the new protectant CGA-154281 is effective in alleviating the injury of corn from metolachlor (9). Since this is a new protectant, very little is known about its mechanisms of protective action. Previous hypotheses, which have been proposed for other chemical protectants suggest that these compounds may act in several ways, depending on the protectant, the herbicide, and the crop which is protected. One popular hypothesis is that the protectant simply enhances the rate of degradation or metabolism of particular herbicide in the crop (1,6). Others believe that the protectant may reduce the absorption of the herbicide, thereby maintaining a sub-toxic dose in the crop plant (2). Another hypothesis is that the protectant may somehow alter or compete for the site of action for a particular herbicide, and by doing so, causes the herbicide to be inactive or non-phytotoxic in the crop (3,5,7).

The objective of this research was to study the absorption and metabolism of metolachlor in the presence or in the absence of CGA-154281 to determine the mechanism of its protective action.

MATERIALS AND METHODS

This study was conducted to determine the effects of CGA-154281 on the absorption and metabolism of metolachlor in the corn hybrids Cargill 7567, Northrup King 9283, Great Lakes 584, and Pioneer 3744. The corn seed were placed in plastic containers on germination blotters and covered with moist paper towels. The containers were then placed in a dark growth chamber which was maintained at 25 C. After 3 days the etiolated seedlings were removed and the herbicide treatment was applied (Figure 1). A 2 ul drop, which contained 67.3 ug of metolachlor in the Dual formulation or in the CGA-180937 formulation (metolachlor + CGA-154281), was applied just above the coleoptilar node of the corn seedling. The metolachlor treatment contained 2% ^{14}C-metolachlor (specific activity 7.26 uCi/umole, uniformly ring labelled). The seedlings were returned to the growth chamber for a 8-h period of herbicide absorption. Based on preliminary studies this period of time allowed for maximum measurability of absorption and metabolism activity. After the 8-h absorption period, the seedlings were rinsed with 3 ml of 100% methanol. Preliminary work verified that all surface radioactivity could be removed with 3 ml of methanol. The seedlings were then placed in dry ice at -30 C until extraction. Two plants for each treatment were weighed, combined, and extracted with 50 ml of 90% methanol for 5 min. The extract was filtered with Whatman No. 1 paper. The residue was oxidized with a Harvey Biological Oxidizer and counted with a TRI-CARB Liquid Scintillation Spectrometer. The extract was dried down completely under vacuum, and 1 ml of 90% methanol was added. A 100 ul aliquot of the concentrated extract was radioassayed and 50 ul was spotted on a Silica Gel GF60 TLC plate. The plate was eluted in butanol:acetic acid:water (12:3:5) and radioassayed with an AMBIS Ratioactivity Scanner. The results are shown as calculated Rf values. The results for absorption were calculated by dividing the amount of ^{14}C-metolachlor absorbed by the amount which was applied to get the percent of metolachlor absorbed. The results for metabolism were calulated by dividing the amount of ^{14}C-metolachlor that was metabolite by the total amount that was metobilite plus parent compound as quantified by the radioactivity scanner. The mass balance of ^{14}C was also calcuated (Figure 2). The results are from two experiments which included five replications each.

RESULTS AND DISCUSSION

No significant difference was observed in the amount of metolachlor absorbed by the corn seedlings in the presence or absence of CGA-154281 (Table 1). This indicates that the protectant did not protect corn by reducing the amount of herbicide absorbed.

Two distinct areas of radioactivity were found on the TLC plate both in the presence and the absence of CGA-154281. The Rf values for these areas were 0.82 and 0.49 (Figure 3). Previous studies (4,8,10), along with a standard included in this study, suggest that the high Rf value of 0.82 is the parent compound metolachlor. Any metolachlor at this Rf is still in the active form and is considered to be available for herbicide activity. Previous studies (4,8,10) also indicate that the radioactivity at the lower Rf of 0.49 is the product of radioactive metolachlor conjugation with glutathione to form the inactive metabolite. These results show that the protectant did not protect corn seedlings by changing the pathway of metolachlor metabolism. In both cases the metoachlor was converted to a non-phytotoxic metabolite via the conjugation with glutathione.

Quantitation of the metabolite of metolachlor metabolism showed that in the presence of CGA-154281, there was significantly greater amounts of the metabolite present. The increase in the metolachlor metabolism rate occurred in three of the four hybrids tested (Table 2). The metabolism was not enhanced in Cargill 7567. The rate of metolachlor metabolism in this hybrid in the absence of the antidote was already significantly greater than in the other three hybrids. Apparently, in the Cargill 7567 the metolachlor metabolism was already functioning at a maximum level. Several hypothesis are available as to what mechanism is affected and therefore in turn speeds up metabolism. These include the increase of an enzyme which catalyzes the conjugation of metolachlor, or the increase in the glutathione concentration in the plant. Both of these possibilities deserve further study and review before final assesments can be made.

Table 1. Effect of CGA-154281 on the absorption of ^{14}C-metolachlor by four corn hybrids.

	CGA-154281	
Hybrid	-	+
	------(% absorbed)-----	
Cargill 7567	33.2 ab	28.6 bc
Great Lakes 584	23.0 cd	21.2 d
Northrup King 9283	30.5 ab	26.5 bcd
Pioneer 3744	36.6 a	31.7 ab

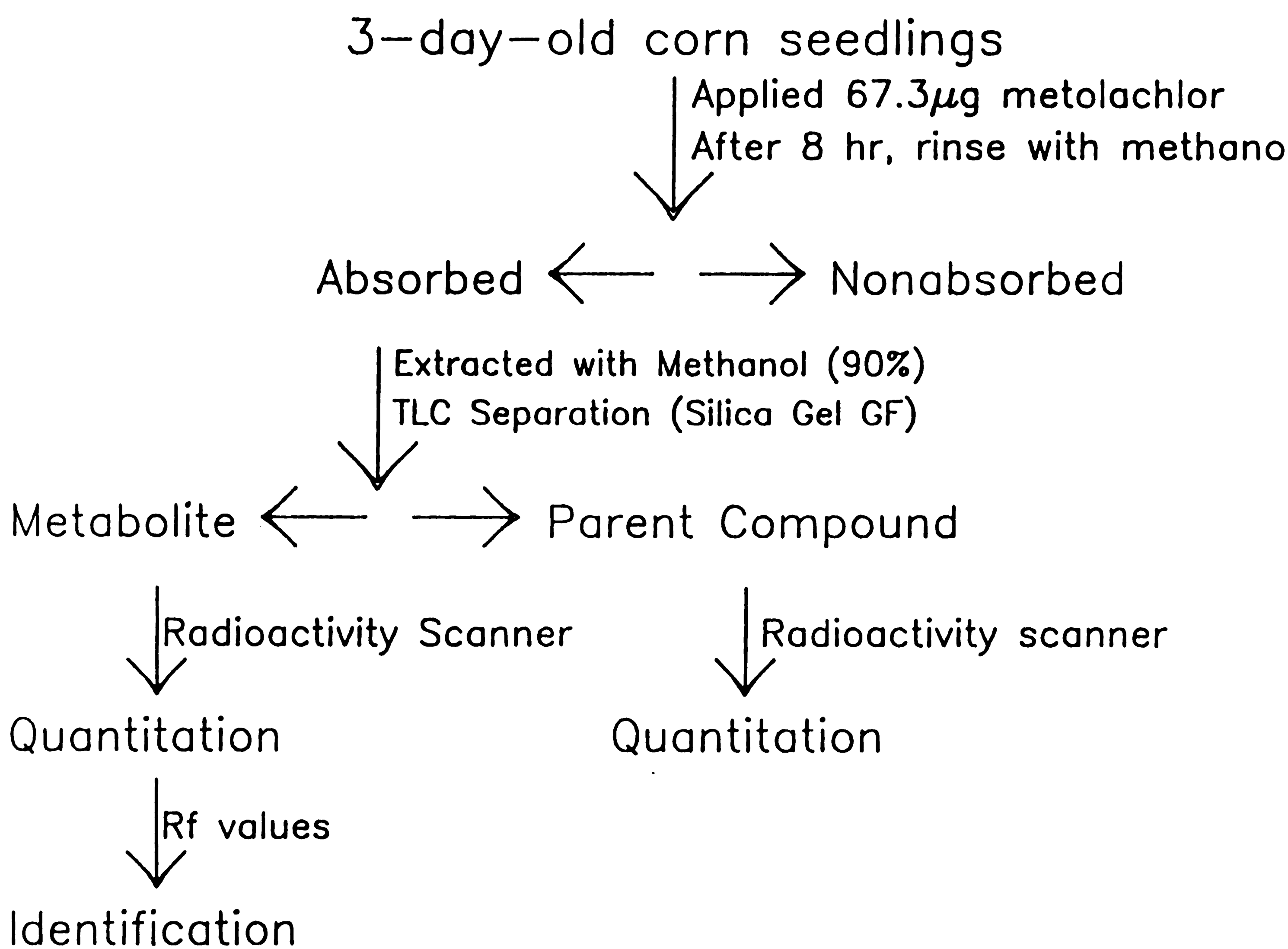
Means followed by a common letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

Table 2. Effect of CGA-154281 on the metabolism of ^{14}C-metolachlor by four corn hybrids.

Hybrid	CGA-154281 -	CGA-154281 +
	-----(% metabolite)----	
Cargill 7567	32.3 a	30.4 ab
Great Lakes 584	25.6 c	32.9 a
Northrup King 9283	21.6 d	29.0 abc
Pioneer 3744	18.6 d	27.3 bc

Means followed by a common letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

Figure 1. Flow diagram for absorption and metabolism study with ^{14}C-metolachlor.


3-day-old corn seedlings
Applied 67.3μg metolachlor
After 8 hr, rinse with methanol
Absorbed
Nonabsorbed
Extracted with Methanol (90%)
TLC Separation (Silica Gel GF)
Metabolite
Parent Compound
Radioactivity Scanner
Radioactivity scanner
Quantitation
Quantitation
Rf values
Identification

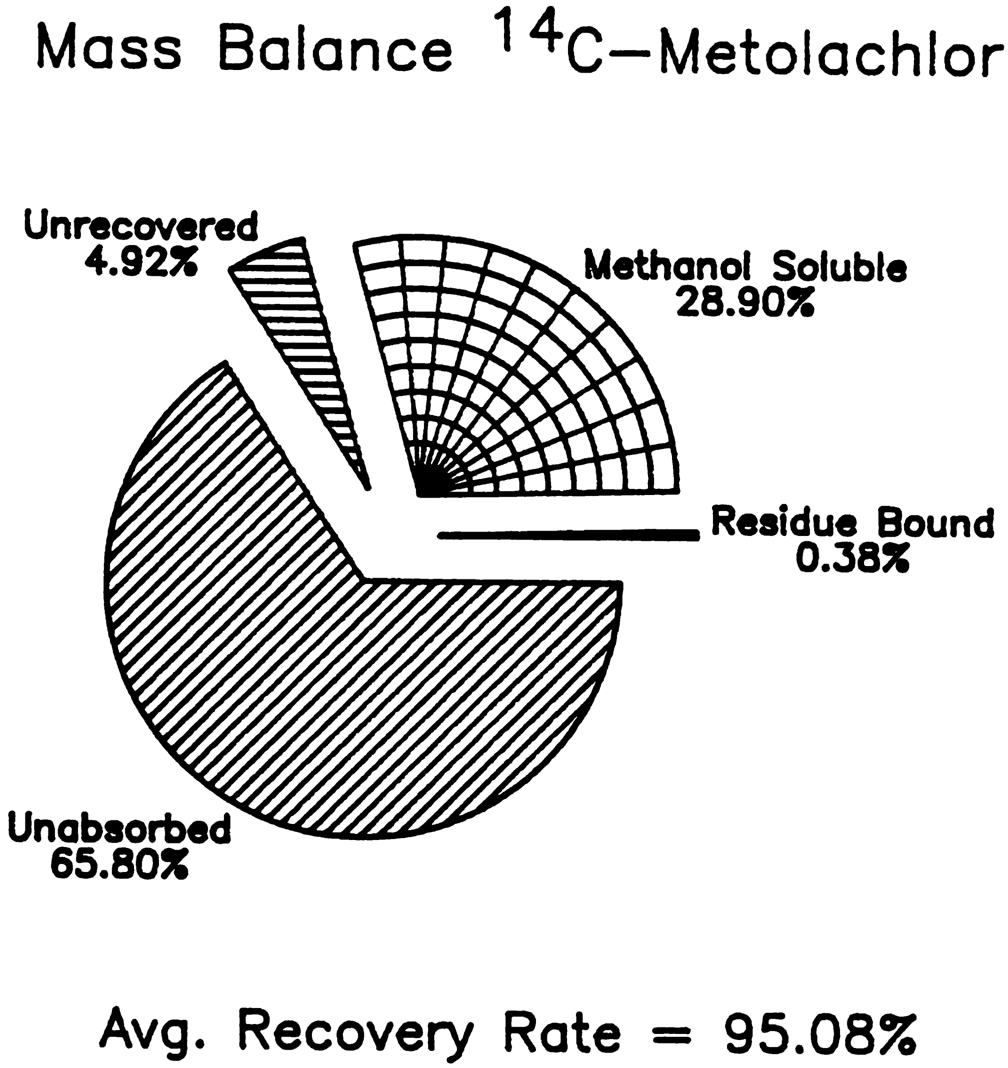


__Figure 2__. Mass balance for ^{14}C applied.

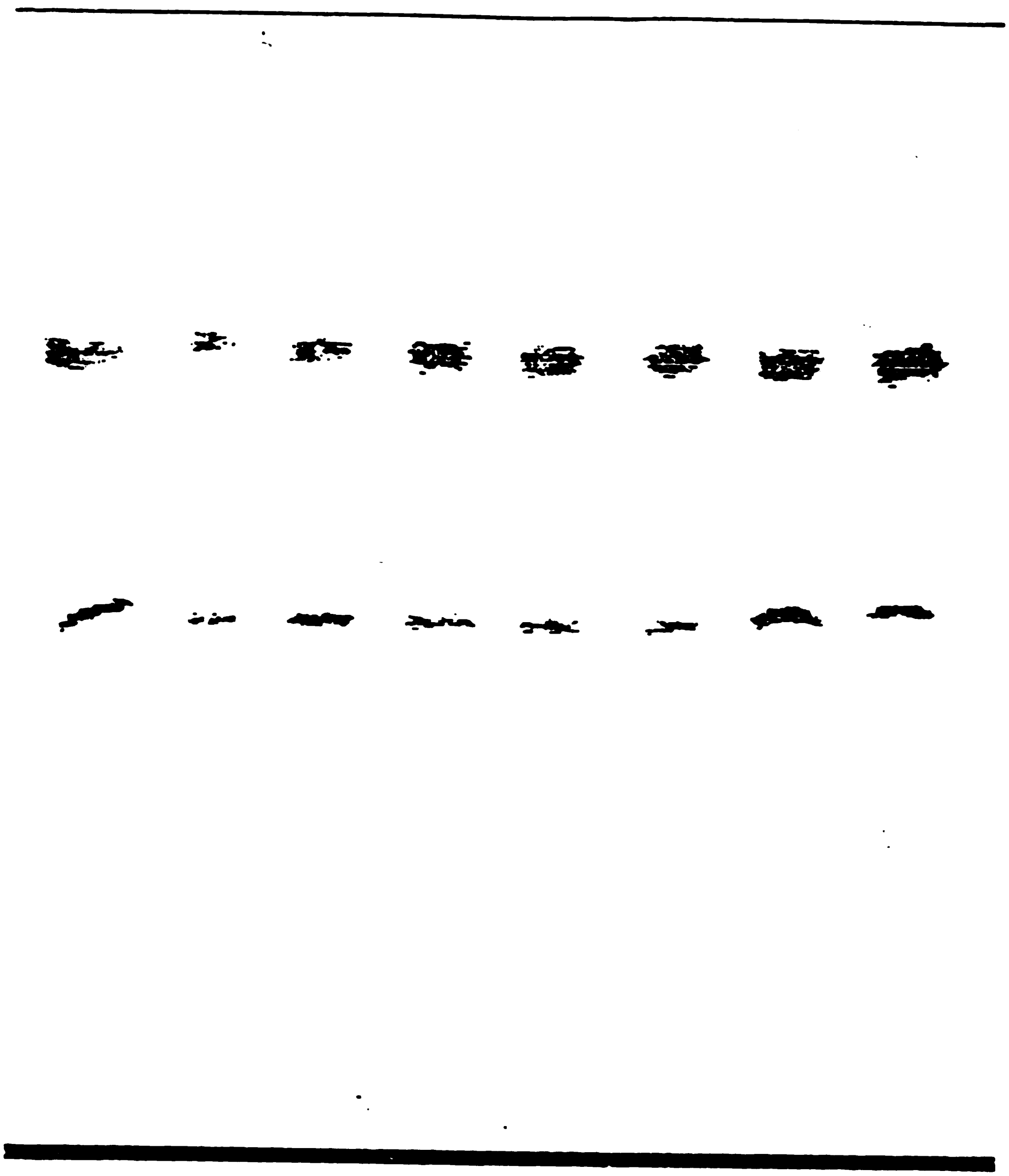


Figure 3. Output from AMBIS Radioactivity Scanner.

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SUMMARY AND CONCLUSIONS

A review of research literature indicated that corn tolerance to the chloroacetanilides involves a complicated interaction of many factors associated with the production system. Distinct differences between herbicides, enviromental factors, genetic variability, and use of protectants all seem to play roles in determining if and to what extent corn will be injured. Our research was an effort to evaluate these factors and increase our knowledge in this area of weed science.

From our first series of studies we concluded that the corn hybrid planted, the herbicide, the herbicide application rate, and the soil moisture content at the time of early corn emergence all play a significant role in the amount of chloroacetanilide injury which occurred. Some of the hybrids were relatively tolerant to both alachlor and metolachlor, while others appeared to be more tolerant to one or the other of the two herbicides. There was generally a linear response of increasing herbicide injury with increasing herbicide application rate and with increasing soil moisture content. However, we concluded that under normal conditions of using labelled herbicide application rates and soils at field capacity, there is generally not a significant difference in injury between hybrids and herbicides since under these conditions no herbicide injury is likely to occur.

We found that there was a high degree of variability in chloroacetanilide tolerance among corn inbred lines and commercial corn hybrids available to growers. The variability in tolerance resembled that of a normal distribution curve with some hybrids having a very high level of tolerance, while others had a low level of tolerance. However, the vast majority of the hybrids had a midlevel of tolerance. From the laboratory studies with ^{14}C-metolachlor, we concluded that the variability of tolerance appeared due to differences in absorption, metabolism, and perhaps differences at the site of action of metolachlor.

From our research with the new protectant, CGA-154281, we found that this compound was very effective in protecting corn seedlings from metolachlor injury. This protection occurred even under the conditions that are known to enhance metolachlor injury. In field studies, we showed that metolachlor injury generally did not occur when applied at the labelled use rates. However, CGA-154281 did give added assurance for safe use of metolachlor on corn under the most extreme conditions of high application rate on sensitive hybrids.

Finally, we concluded that CGA-154281 did not protect corn from metolachlor injury by reducing the amount of metolachlor absorbed by the seedling. Also, the protectant did not protect corn by altering the pathway of metolachlor metabolism. The protective action of CGA-154281 appeared due to the enhanced metabolism of metolachlor to a glutathione conjugate.

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