

This is to certify that the
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Environmental and Edaphic Influences on the
Selectivity of Diphenamid and Alachlor
in Tomatoes and Snapbeans
presented by

Robert P. Rice Jr.

has been accepted towards fulfillment
of the requirements for

Ph.D degree in Horticulture

A handwritten signature in cursive script, reading "J. L. Gentry", written over a horizontal line.

Major professor

Date August 31, 1977



ENVIRONMENTAL AND EDAPHIC INFLUENCES ON THE SELECTIVITY OF
DIPHENAMID AND ALACILAR IN TOMATOES AND SNYPSBEANS

By

Robert P. Rice Jr.

A DISSERTATION

Submitted to

Michigan State University

in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

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ABSTRACT

ENVIRONMENTAL AND EDAPHIC INFLUENCES ON THE SELECTIVITY OF
DIPHENAMID AND ALACHLOR IN TOMATOES AND SNAPBEANS

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Environmental and edaphic factors were monitored to determine their effects on the tolerance of direct seeded tomatoes (*Lycopersicon esculentum* L.) to diphenamid (N,N-dimethyl-2,2-diphenylacetalanilide). Injury was enhanced by low temperatures within seven days of planting in both field and growth chamber tests and by increasing the soil pH from 5.5 to 8.0. The application of both soluble fertilizer and diphenamid at planting acted synergistically to increase tomato injury. Increasing the organic matter content of the soil inversely affected diphenamid injury while rainfall amounts up to 5 cm did not affect injury. Nine tomato cultivars exhibited differential tolerance to diphenamid. These factors alone or in combination may result in the sporadic injury which has been observed in the field.

affected. Volatilization of diphenamid from soil was

To determine the reason for reduced diphenamid selectivity at low temperatures and high pH's, tomatoes were grown under varying temperature and pH conditions, and uptake, translocation, and metabolism of ^{14}C -diphenamid were monitored. During the period prior to emergence of tomato seedlings there was little ^{14}C -diphenamid uptake, but this increased at high temperatures. Root growth was inhibited at both temperatures. Uptake and metabolism of ^{14}C -diphenamid by plants in the cotyledon stage was not greatly affected by temperature or pH, however translocation from the root to the shoot was significantly reduced with low temperatures and high pH's. These differences in translocation parallel reported differences between tolerant and susceptible species and may account for the increased diphenamid injury to tomatoes under stress conditions.

The influence of several environmental and edaphic factors on alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide] selectivity in snapbean (*Phaseolus vulgaris* L.) was also examined. Maximum injury occurred either when temperatures during germination were cool and rainfall was light or when temperatures approached or exceeded 27 C and the soil was moist. Ten cultivars of snapbeans varied in their early tolerance to alachlor, however yield was not affected. Volatilization of alachlor from moist soil

resulted in bean injury in growth chamber and greenhouse tests. Injury was less severe in soils high in organic matter. Alachlor leached readily in a loamy sand soil, but less leaching occurred in a loam soil. Applications of 10 cm of simulated rainfall after treatment reduced alachlor injury to beans growing in a loamy sand soil.

The uptake, translocation, and metabolism of ^{14}C -alachlor in germinating and emerged snapbeans were studied under 16 C night/21 C day and 27 C night/32 C day temperature regimes. Total uptake of ^{14}C -alachlor by germinating snapbeans was greater under the higher temperatures, however the label was located primarily in the roots where it was rapidly metabolized. Under lower temperature conditions less of the alachlor was metabolized and there were equal amounts of the label in all plant parts except the cotyledons. Root uptake of ^{14}C -alachlor by emerged snapbeans was significantly greater under high temperatures than under low temperatures and translocation of the label to the shoots was more rapid under high temperatures. Approximately 60% of ^{14}C -alachlor was shown to volatilize from a watchglass after 48 hours at 27 C. After volatilization, uptake of alachlor occurred in adjacent snapbean plants in a closed system.

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During the last few years as the ability of growers to detect herbicide injury has improved, reports of injury have become more common. Occasionally the injury cannot be linked to misapplication or equipment failure and the reason for crop injury associated with a normally selective herbicide is difficult to explain. The water herbicides, alachlor and diphenamid, have been mentioned

in crop injury under certain environmental conditions which have not been well defined.

The purpose of this study was to determine the weather and soil conditions that may enhance the activity or reduce the selectivity of alachlor and diphenamid on selected crops.

INTRODUCTION

With the increase in world population, demand for food is increasing rapidly and at the same time cities are encroaching on farmland resulting in a need for increasing crop production per hectare to economically supply needed food. In the United States, it has been estimated that weeds result in greater crop loss than insects and diseases. Agriculturists have found that herbicides are often the least expensive and most energy efficient means of weed control available. Although herbicides have been an important tool in maximizing yields, all the ramifications of their use are not understood.

During the last few years as the ability of growers to detect herbicide injury has improved, reports of injury have become more common. Occasionally the injury cannot be linked to misapplication or equipment failure and the reason for crop injury occurring with a normally selective herbicide is difficult to explain. The amide herbicides, alachlor and diphenamid have been implicated

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The purpose of this study was to determine the weather and soil conditions that may enhance the activity or reduce the selectivity of alachlor and diphenamid on selected crops.

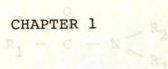
CHAPTER 1

General Literature Review

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Amides. Amide herbicides are a broad group of compounds with the basic structure shown below:



General Literature Review

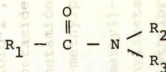
There is considerable confusion in nomenclature of amide herbicides which are also called acid amides, acetamides, or anilides. Generally speaking, however, all are derived from carboxylic acids in which the hydroxyl (OH) portion of carboxyl group (COOH) is replaced by an amine (NH₂) group. The hydrogens in the amino group are then displaced by other functional groups. In the case of acetanilides for example, one of the hydrogens is replaced by a phenyl group.

Most amide herbicides (Table 1) are used as selective preemergence herbicides with the exception of propaqui-

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Table 1. Common and chemical names of selected amide herbicides (5).

Common Name	Chemical Name
alachlor	2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide
butachlor	N-(butoxymethyl)-2-chloro-2',6'-diethylacetanilide
CDA	N,N-diallyl-2-chloroacetamide
CDEA	N,N-diethyl-2-chloroacetamide
metolachlor	2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methyl-ethyl)acetamide
delachlor	2-chloro-N-(isobutoxymethyl)-2',6'-acetoxylidide
diphenamid	N,N-diethyl-2,2-diphenylacetamide
naproamide	2-(α -naphthoxy)-N,N-diethylpropionamide
propamide	N-(1,1-dimethylpropynyl)-3,5-dichlorobenzamide
propachlor	2-chloro-N-isopropylacetanilide
propanil	3',4'-dichloropropionanilide
prynachlor	2-chloro-N(1-methyl-2-propynyl)acetanilide

which is applied postemergence and has little preemergence activity. Several amides including alachlor, CDAA, propachlor, and pronamide have some postemergence activity against very young weed seedlings of plant species into sensitive, moderately sensitive and tolerant categories.

The sensitive category: Diphenamid such common grasses as barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), downy brome (*Bromus tectorum* L.), quackgrass (*Alopecurus repens* L.), orchardgrass (*Dactylis glomerata* L.), and hairy woodcock (*Eleusine indica* L.). It is a white or off-white crystalline solid with no appreciable odor. The melting point is 134.5 - 135.5 °C with some decomposition occurring at 210 °C. Diphenamid is relatively resistant to decomposition by ultraviolet radiation. It is soluble in phenyl cellosolve, dimethylformamide and acetone. Solubility in water at 27 °C is 260 ppm.

Diphenamid is nonflammable and non-corrosive and is compatible with most other wettable powder herbicide formulations and with non-alkaline fertilizers. The acute oral LD₅₀ is 686-776 mg/kg (34).

Diphenamid is a selective herbicide used for controlling annual grass and broadleaf weeds in tomatoes, peppers, sweet potatoes, cotton, tobacco, peanuts, soybeans, strawberries, fruit trees and ornamental plants. It is used primarily as a preemergence or preplant incorporated

herbicide although it may be safely sprayed over the foliage of several crops. Diphenamid controls a wide variety of weeds but is most active on grasses. Deli and Warren (16) have grouped a number of plant species into sensitive, moderately sensitive and tolerant categories. The sensitive category includes such common grasses as barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], downy brome (*Bromus tectorum* L.), quackgrass [*Agropyron repens* (L.) Beauv.], orchardgrass (*Dactylis glomerata* L.). Included in the moderately sensitive category are redroot pigweed (*Amaranthus retroflexus* L.), common lambsquarters (*Chenopodium album* L.) and pale smartweed (*Polygonum lapathifolium* L.). Velvetleaf (*Abutilon theophrasti* Medic.), ivyleaf morninglory [*Ipomoea hederacea* (L.) Jacq.], and jimsonweed (*Datura stramonium* L.) are selected species which show considerable tolerance to diphenamid. LeBaron (43) reported that under a wide variety of conditions, diphenamid gave excellent preemergence weed control with some postemergence activity on annual grasses and common chickweed [*Stellaria media* (L.) Cyrilo] in the early seedling stage. There have been numerous other reports on the efficiency of diphenamid on annual weeds (1,2,3,4,11,25,33,48,49,50).

Though tomatoes are considered tolerant to diphenamid, Behal (24) reported that there have been reports of variable effects of diphenamid

on tomato growth, yield and quality. Noll (48,49,50) reported an increase in tomato yields where diphenamid was used in 1962, 1963 and 1964. Others have also reported increased yields (43). Taylorson (60) reported improved tomato stands following diphenamid application. There have also been reports of tomato injury (25) resulting from diphenamid use. Taylorson (61) reported that 10 kg/ha reduced the levels of total or reducing sugars in ripe tomato fruit. There was little effect at lower rates. Sistrunk, *et al.* (57) reported that fruit from diphenamid-treated plants had improved color after canning, but that the drained weight and relative viscosity of the canned product was lowered.

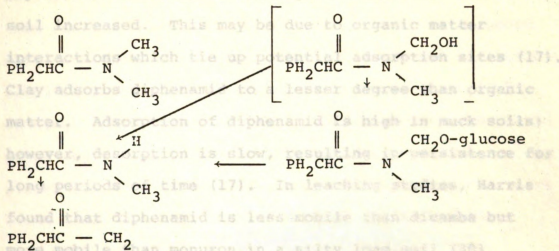
Uptake and Movement. Diphenamid uptake occurs both in the roots and shoots of both tolerant and susceptible plants; however, when applied to the shoots of tomatoes and oats, damage is less severe than root applications (15). In tomato and ivyleaf morninglory, both resistant plants, root absorbed diphenamid moves rapidly into the leaves while in susceptible oats (*Avena sativa* L.) movement into the shoots is slow (15). Bingham (10) reported that in tomato, labeled diphenamid moved rapidly in the xylem from the roots to the veins of the leaves and then to the mesophyll. Eshel (24) reported that in peppers, application of diphenamid to the root zone decreased growth slightly but

that when shoots grew through a diphenamid layer, growth was not decreased.

Mode of Action. According to Deli and Warren (15) diphenamid causes decreased root growth in susceptible plants but the effects are localized to treated zones. When diphenamid is removed after periods of up to seven days, root growth resumes in oat plants. Nashed and Illnicki (47) showed that in cabbage plants, diphenamid decreased the uptake of macronutrients, further indicating root effects. In this test, diphenamid decreased the uptake of Mg, Ca, P and K in that order. Leaf analysis supported uptake studies by showing reduced levels of all the above elements, especially Ca in the leaves. Deli and Warren (15) theorized that diphenamid is a reversible metabolic inhibitor which stops or slows mitosis in susceptible plants; however, Yaklich and Scott (64) stated that inhibition of mitosis does not occur and theorized that growth reduction is due to a reduction in the expansion of cell walls. Due to the differences in rate of movement of diphenamid out of the roots between tolerant and susceptible plants, Bingham postulated that the method of selectivity may be differential rates of translocation from roots to shoots (10).

however, several subsequent studies have shown this to be true (27,55).

Metabolism. Diphenamid is metabolized in both tolerant and susceptible plants. It is generally agreed that diphenamid is demethylated (27,50,55). Schultz and Tweedy (55) proposed the following scheme:



They further proposed that the reason for the resistance of certain plants to diphenamid was that neither diphenamid nor its metabolite, N-methyl-2,-diphenylacetamid (MDA) were toxic except at relatively high levels. In addition, a stable glucose conjugate is formed in the tolerant crop, tomatoes. In susceptible wheat, both diphenamid and its metabolite are more toxic than in tomato and in addition, only small amounts of the glucose conjugate are formed. Kesner (40) stated that MDA was more toxic than the parent compound; however, several subsequent studies reported the reverse to be true (27,55).

Edaphic Effects. Diphenamid leaching studies have shown that movement in the soil is greatest in sandy soils low in organic matter (17,31). Deli and Warren (17) showed that the efficiency of organic matter adsorption of diphenamid decreased as the organic matter content of the soil increased. This may be due to organic matter sorbed interactions which tie up potential adsorption sites (17). Clay adsorbs diphenamid to a lesser degree than organic matter. Adsorption of diphenamid is high in muck soils; however, desorption is slow, resulting in persistence for long periods of time (17). In leaching studies, Harris found that diphenamid is less mobile than dicamba but more mobile than monuron in a silty loam soil (30).

Since uptake of diphenamid is primarily via roots, diphenamid injury to both crop and weed species occurs when high concentrations are present in the root zones (24). In the case of pepper (*Capsicum frutescens* L.), which germinates slowly, injury can be decreased by delaying the application of diphenamid to just before pepper emergence. Earlier applications result in a longer time period during which rainfall may leach diphenamid into the root zone where uptake will occur (24).

Uses and Properties
Breakdown of herbicides in soils is due largely to the action of soil microorganisms (8). In the case of diphenamid, two saprophytic fungi (*Aspergillus candidus*

and *Trichoderma viride*) have been identified as demethylators of diphenamid (40).

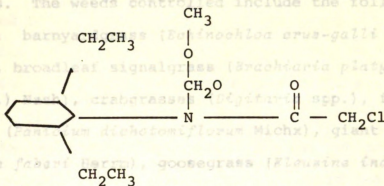
Various environmental parameters such as temperature, light intensity, humidity and rainfall may affect the activity of diphenamid. Schultz, *et al.* (55) showed that emerged tomato plants in nutrient culture absorbed more diphenamid when grown under low light and low humidity conditions than those grown similarly under high light and high humidity conditions. In addition, tomato plants grown under high light and high humidity had a higher percentage of a diphenamid-glucose complex than did those grown under low light, low humidity conditions (55). When diphenamid was sprayed on tomato leaves, high relative humidity for six hours prior to application enhanced its activity. Activity was further enhanced by growing the plants under low light prior to application. Emerged tomato plants also have shown increased diphenamid injury when grown under low light prior to soil application (28,55).

Alachlor

Uses and Properties. Alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide] like diphenamid, is an amide herbicide. Its structural formula is:

potatoes. The weeds controlled include the following

grasses: barnyard grass (*Echinochloa crus-galli* (L.) Beauv.), broadleaf signalgrass (*Brachiaria platyphylla* (Griseb.) Stapf), crabgrass (*Digitaria* spp.), fall panicum (*Panicum dichotomiflorum* Michx.), giant foxtail (*Setaria faberii* Pers.), goosegrass (*Eloisina indica* (L.) Gaertn.), green foxtail (*Setaria viridis* (L.) Beauv.),



yellow foxtail (*Setaria leucocoma* (Weigel) Hubb.), and It has a molecular weight of 269.8 and a specific gravity of 1.133 at 25/15.6 C. At room temperature, it is a cream-colored, odorless solid. Alachlor melts at between 39.5 and 41.5 C, boils at 100 C (0.02mm Hg) and decomposes at 105 C. The vapor pressure is 2.2×10^{-5} mm Hg at 25 C. Alachlor is relatively resistant to decomposition by ultraviolet light. It is moderately soluble in water (242 ppm at 25 C) and is also soluble in ether, acetone, benzene, chloroform, ethanol, ethyl acetate and is slightly soluble in heptane.

Alachlor is combustible so should not be stored near heat or open flames. Corrosion will occur when alachlor contacts steel or black iron, however, stainless steel and aluminum are resistant to corrosion. Alachlor is stable so can be stored indefinitely at moderate temperatures.

Alachlor is a selective herbicide used for controlling certain annual grass and broadleaf weeds in corn, cotton, dry beans, lima beans, red kidney beans, peanuts, peas and

through the shoot zone. Work by Enake and Wex has indicated that in giant foxtail the primary site of absorption was in the roots of the plant above the seed (41). Armstrong et al. have demonstrated that in yellow (Griseb.) Nash], crabgrasses (*Digitaria* spp.), fall panicum (*Panicum dichotomiflorum* Michx), giant foxtail above the tuber which includes both stem (rhizome) (*Setaria faberi* Herrm), goosegrass [*Eleusine indica* (L.) Gaertn], green foxtail [*Setaria viridis* (L.) Beauv.], measured by recording shoot emergence and height; it is yellow foxtail [*Setaria lutescens* (Weigel) Hubb], and therefore possible that significant uptake did occur witchgrass (*Panicum capillare* L.). In addition, alachlor in the root system below the tuber but was not translocated through the tuber to actively growing points in the carpetweed (*Mollugo verticillata* L.), Florida pusley (*Richardia scabra* L.), pigweed (*Amaranthus* spp.), and common purslane (*Portulaca oleracea* L.). Alachlor will also suppress the following weeds so that competition may be substantially reduced: red rice (*Oryza sativa* L.), sandbur (*Cenchrus* spp.), seedling johnsongrass [*Sorghum halepense* (L.) Pers.], yellow nutsedge (*Cyperus esculentus* L.), black nightshade (*Solanum nigrum* L.), Florida beggarweed [*Desmodium tortuosum* (SW.) D.C.], common lambsquarters (*Chenopodium album* L.) and smartweeds (*Polygonum* spp.) (32,52,53,54).

Uptake and Translocation. Reports on the site of uptake of alachlor are, at first glance, contradictory. In early work by Eshel (23) with cotton, uptake of alachlor occurred primarily via root tissue with only small amounts absorbed

through the shoot zone. Work by Knake and Wax has indicated that in giant foxtail the primary site of absorption was in that region of the plant above the seed coleoptiles and roots were exposed to alachlor, roots (41). Armstrong *et al.* have demonstrated that in yellow nutsedge, uptake of alachlor occurs largely in the area above the tuber which includes both stem (rhizome) tissue and root tissue (6). Uptake in this test was measured by recording shoot emergence and height; it is therefore, possible that significant uptake did occur in the root system below the tuber but was not translocated through the tuber to actively growing points in the shoot where it could affect shoot growth.

In studies with wheat (susceptible) and soybeans (tolerant), Chandler *et al.* (12) showed root uptake to be important in both plants. Using ^{14}C alachlor applied to the nutrient solution, uptake of alachlor occurred via the roots followed by translocation throughout the plant. The heaviest accumulation of alachlor was in the root system followed by the oldest leaves. Relatively little alachlor moved to the growing points. Alachlor was more mobile in wheat than in resistant soybeans. In this case, mobility seemed to be correlated with susceptibility to alachlor. When applied to the foliage, labeled alachlor moved apoplastically to the tip of the leaf with only a small amount moving to other plant parts. In soybean, 99 percent of the material applied remained in the leaf

with very slight accumulations in the cotyledons and the growing points. When excised sections of leaves, coleoptiles and roots were exposed to alachlor, roots obtained their maximum accumulation after four hours while coleoptiles and leaves continued to take up alachlor for up to 32 hours and eventually accumulated much higher levels of alachlor than did roots (11). It appears, then, that root uptake is important in some species but that shoot uptake is significant in yellow nutsedge and giant foxtail. It should be noted that the crops where shoot uptake has been shown to be important are all monocots which normally have some adventitious root production in the above seed ("shoot") portion of the plant.

Metabolism. Alachlor is rapidly metabolized in both resistant and susceptible plants as well as in soil and water ecosystems (29,58,62,65). In yellow nutsedge, Armstrong *et al.* found that within two days of application, alachlor is metabolized to a water soluble product (6). A similar product was obtained by Hammill and Penner in barley (29). In propachlor, a related amide herbicide, two water soluble metabolites were isolated from corn, sugarcane, sorghum and barley (21). These metabolites were identified as the glutathione and γ -glutamylcysteine conjugates of propachlor. Similar metabolites were also formed by 4-chloro-2-butynyl-m-chlorocarbonate (barban)

and N,N₁-diallyl-2-chloroacetamide (CDAA) also an α -chloroacetamide. The specific metabolites of alachlor have not been identified, however, it is possible that the metabolism is similar to the other α -chloroacetamides as outlined above. Devlin and Cunningham (18) have shown

that alachlor inhibits gibberellin acid induced α -amylase activity in embryonic barley (*Hordeum vulgare* L.). The mode of action of alachlor has not been precisely defined. Alachlor may stimulate or inhibit both nitrate ion uptake and nitrate reductase activity depending on the rate used and the relative timing of alachlor and nitrate uptake (12). Since nitrate is the primary ion utilized for protein synthesis and nitrate reductase is the enzyme involved in the rate limiting step, protein synthesis may be affected by alachlor. In wheat, nitrate reductase activity was inversely proportional to alachlor exposure time. High concentrations of alachlor were shown to decrease the amino acid and protein levels while low concentrations did not (12).

It has been postulated that alachlor may be a hill reaction inhibitor, however, Good (28) has shown that in all the herbicides tested the only common feature of Hill reaction inhibitors is that all contain a free imino hydrogen. In alachlor, this imino hydrogen has been replaced by a methoxymethyl group so one would not expect to find Hill reaction inhibition. In isolated wheat germinating seeds (23) by the depth the seed was placed

chloroplasts, alachlor was in fact shown not to inhibit the Hill reaction (28). With propachlor, a related compound, Duke *et al.* have shown that the primary mechanism of action is an effect on nascent protein biosynthesis (21). Devlin and Cunningham (18) have shown that alachlor inhibits gibberellic acid induced α -amylase activity in embryo-free barley (*Hordeum vulgare* L.). If this is the case, reduction in seedling growth could occur because of an inadequate supply of nutrients. Hickey and Kreuger suggested that in sorghum (*Sorghum bicolor*), alachlor may interfere with the action of gibberellic acid and indoleacetic acid, and further suggested that this interference may be due to inhibition of enzyme activity or synthesis (35). Narsaiah *et al.* reported that GA_3 induced growth in corn (*Zea mays* L.) can be counteracted by application of alachlor and conversely that applications of GA_3 to alachlor-treated plants resulted in growth comparable to non-treated plants (46).

In an earlier study by Eshel (15), it was reported that alachlor is most active on small seeded plants. Eshel related this to depth of planting and position of alachlor in the soil. In the light of more recent research cited above, it may be that selectivity is based on the relative timing of alachlor and nitrate uptake by germinating seeds (23) which, of course, would be affected by the depth the seed was planted and the time involved in

the movement of alachlor from the soil surface to the seed zone. inactivated in soils high in caly or organic matter (23). Timmons has reported that alachlor and Edaphic Effects. Alachlor is applied as either a preplant incorporated or a preemergence herbicide. There have been several reports of poorer weed control in preplant of alachlor do not occur until the soil contains 10-14% incorporated treatments (52) than non-incorporated applications. This difference in effectiveness may be due to such factors as leaching, adsorption by soil colloids, microbial decomposition and volatilization or codistillation of alachlor.

Beestman *et al.* reported that the principal avenue of acetanilide dissipation in the soil is by microbial decomposition and that volatilization is a significant factor only when the soil is wet and the weather is windy (9). Cox has, however, shown that alachlor vapor can cause plant damage in wheat even at relatively low concentrations so even though volatilization is minimal it could perhaps, under certain conditions, account for some crop injury (13). Smith and Phillips have shown that *Rhizoctonia solani* readily degrades alachlor in the presence of a carbon source. Although the fungi cannot utilize alachlor as a carbon source, they may use alachlor as a nitrogen source (58). Another fungus *Chaetomium globosum* is also capable of degrading alachlor and can use it as an energy source.

Alachlor is adsorbed by soil colloids and is thus partially inactivated in soils high in clay or organic matter (23). Timmons has reported that alachlor and propachlor are about equally effective in soils high in organic matter and that serious reductions in efficiency of alachlor do not occur until the soil contains 10-14% organic matter (63).

Eshel reported that alachlor is subject to leaching in both a sandy and a clay soil. When applied at the rates of 1 and 2 kg/ha alachlor leached two to three inches and one to two inches after a two-inch rainfall in sandy and in clay soil, respectively (23).

ENVIRONMENTAL AND EDAPHIC INFLUENCES ON THE ACTIVITY OF

DIPHENAMID ON DIRECT SEEDED TOMATOES

CHAPTER 2

ENVIRONMENTAL AND EDAPHIC INFLUENCES ON THE ACTIVITY OF DIPHENAMID ON DIRECT SEEDED TOMATOES

ABSTRACT

Environmental and edaphic factors were monitored to determine their effect on the tolerance of direct seeded tomatoes (*Lycopersicon esculentum* L.) to diphenamid. Environmental and edaphic influences on the tolerance of direct seeded tomatoes to diphenamid were determined by increasing the temperature within seven days of planting in both field and growth chamber tests. The application of base fertilizer and diphenamid at planting did not synergistically increase tomato injury. Increased soil water content of the soil inversely affected diphenamid injury but rainfall amounts up to 9 in did not affect injury. Nine commercial tomato cultivars were tested for tolerance to diphenamid. Some cultivars were more tolerant than others. A combination may result in the tolerance to diphenamid which have been observed in the field.

CHAPTER 2

ENVIRONMENTAL AND EDAPHIC INFLUENCES ON THE ACTIVITY OF
DIPHENAMID ON DIRECT SEEDED TOMATOES

ABSTRACT

In the last several years manufacturers have received occasional reports of injury to field grown tomatoes. Environmental and edaphic factors were monitored to determine their effects on the tolerance of direct seeded tomatoes (*Lycopersicon esculentum* L.) to diphenamid (N,N-dimethyl-2,2-diphenylacetanilide). Injury was increased by increasing the soil pH and by low temperatures within seven days of planting in both field and growth chamber tests. The application of both soluble fertilizer and diphenamid at planting acted synergistically to increase tomato injury. Increasing the organic matter content of the soil inversely affected diphenamid injury but rainfall amounts up to 5 cm did not affect injury. Nine commercial tomato cultivars exhibited differential tolerance to diphenamid. These factors alone or in combination may result in the sporadic injury problems which have been observed in the field.

INTRODUCTION

Diphenamid (N,N-dimethyl-2,2-diphenylacetanilide) has been used extensively for preemergence weed control in direct seeded tomatoes (*Lycopersicon esculentum* L.). In the last several years manufacturers have received occasional reports of injury to field grown tomatoes some of which have resulted in litigation. These sporadic cases of injury were not attributable to human error or other easily identifiable factors. For this reason, certain environmental or soil conditions are suspected to have contributed to the observed injury. Lynch and Sweet (3,4) reported that factors such as high humidity and low light intensity affect postemergence diphenamid activity but the effects of the environment on preemergence applications have not been elucidated. The purpose of this investigation was to determine how several environmental and edaphic factors affect the tolerance of direct seeded tomatoes to preemergence applications of diphenamid.

with zero being no damage to weeds or tomatoes and ten being complete kill. Yield data was obtained for all 1976 plots.

Correlations were made utilizing crop injury as the dependent variable. Environmental factors such as average high, low, and mean temperature seven days after

MATERIALS AND METHODS

A wide range of environmental conditions in the field was obtained by planting 'Heinz 1350' tomatoes at approximately one week intervals in two growing seasons, at two different locations, and treating with diphenamid at various rates. In 1975, plantings were made on May 20, May 29 and June 10 in a Spinks loamy sand (Location II, Table 1) and a Miami clay loam (Location III), and treated with diphenamid (Enide 50% WP)* at 0, 6.75 and 9 kg/ha applied in a volume of 342 l/ha. In 1976, plantings were made on May 10, 17, 24 and June 14 in the loamy sand (Location II) and on May 10, 24, June 14 and 21 in the sandy loam (Location I). The experiment was arranged in a randomized complete block design with a plot size of 1.8 m by 7.5 m. Plots were treated with diphenamid at 0, 9, 13.5 and 18 kg/ha. Crop injury and weed control ratings were made on all plots 10 and 20 days after treatment (DAT). Plots were rated on a zero to ten basis

* Upjohn Co., Kalamazoo, Michigan.

with zero being no damage to weeds or tomatoes and ten being complete kill. Yield data was obtained for all 1976 plots. Five Correlations were made utilizing crop injury as the dependent variable and several environmental factors such as average high, low, and mean temperature seven days after planting (DAP), and rainfall as the independent variables. Test treatments were replicated a minimum of three

Table 1. Organic matter content, pH and mechanical analysis of soil from three test locations. applicable.

location	type	constituents (%)				pH
		organic matter	sand	silt	clay	
I	Cohover sandy loam	3.0	64	23	12.5	6.5
II	Spinks loamy sand	1.7	85	0.8	7.0	6.3
III	Miami clay loam	3.6	25	44	30.7	5.4

Cultivar tolerance

Since crop cultivars often differ in their tolerance to herbicides, nine commercial tomato cultivars were planted at location II in July 1975 and treated with diphenamid at 0, 9 and 13.5 kg/ha prior to emergence. Stand counts and crop injury ratings were obtained 20 DAT.

Greenhouse and growth chamber tests

Greenhouse and growth chamber tests were conducted utilizing 10 cm styrofoam pots and Spinks loamy sand soil. Five tomato seeds (Heinz 1350) were planted 0.6 cm deep in each pot. Greenhouse tests received only natural light while light intensities at plant level in the growth chambers were standardized at approximately $160 \text{ e/m}^2/\text{sec}$. In each test treatments were replicated a minimum of three times and the tests were repeated twice. Periodic visual injury ratings and fresh weight data were obtained where applicable.

Rainfall

The effects of various amounts of rainfall on diphenamid activity were tested by planting tomato seeds in pots, spraying with diphenamid at several rates and applying appropriate amounts of water to simulate 0, 1.3, 2.5 and 5 cm of rainfall. The water was applied with a chain driven boom which applied the water as fine droplets over a five to ten minute period. Plants were then grown in the greenhouse under conditions previously described.

Temperature

Tomato plants were seeded in styrofoam pots and treated with diphenamid prior to being placed in growth chambers at 13 C night/18 C day and 24 C night/29 C day temperature regimes with 16 hr photoperiods. After ten

days all pots were moved to a 24 ± 5 C night temperature greenhouse for further growth. Pots were sub-irrigated as needed.

Fertility

Tomatoes planted and seeded as above were placed in a 24 ± 5 C night temperature greenhouse and fertilized at ten day intervals. A 20-20-20 water soluble fertilizer was used at three rates: 0, 0.25 and 0.5 g/pot. Another test was performed as above except the fertilizer was applied only at the time of planting and the rate of application was extended to include 1.0 g/pot.

Organic matter

Organic matter in soil is known to adsorb herbicides and thereby decrease their activity. To test the effects of organic matter on diphenamid activity, a Spinks loamy sand soil with an organic matter content of 1.7% was amended with a Houghton muck soil (80% organic matter) to produce two additional soils with organic matter contents of 4.8 and 12.2%, respectively.

Tomatoes were seeded in pots and sprayed with diphenamid at 0, 9 and 18 kg/ha. Crop injury ratings were obtained 10 DAT.

Soil pH

Calcium hydroxide and sulfuric acid were utilized to adjust the pH of Spinks loamy sand soil to pH 5.5, 7.0 or 8.0. Tomatoes were seeded and sprayed with diphenamid at 9.5 kg/ha to determine the effects of soil pH on diphenamid activity.

Statistical procedures

All data was subjected to an analysis of variance and means were compared with Duncan's multiple range test at the 5% level of probability.

RESULTS AND DISCUSSION

In 1975, tomato injury was slight in all plots probably because of favorable conditions for tomato growth which existed during the time following seeding. No significant differences were observed between treatments at site II. There was not significant correlation between mean temperature after treatment and crop injury during 1975. The greatest injury occurred during the coolest periods (Table 2). In 1976, crop injury at both

Table 2. Mean air temperatures first 7 DAP and crop injury ratings for direct seeded tomatoes grown at site III in 1975.

Planting Date	Mean Temperature (C)	Crop Injury (20 DAP)
5/20	22	1.3 ab
5/29	17	2.7 b
6/10	20	0.3 a

sites differed between planting dates. The greatest amount of injury again occurred when germination took place

during periods of relatively low temperatures (Tables 3, 4).

Table 3. Mean air temperatures first 7 DAP and crop injury ratings for direct seeded tomatoes grown at site II in 1976.

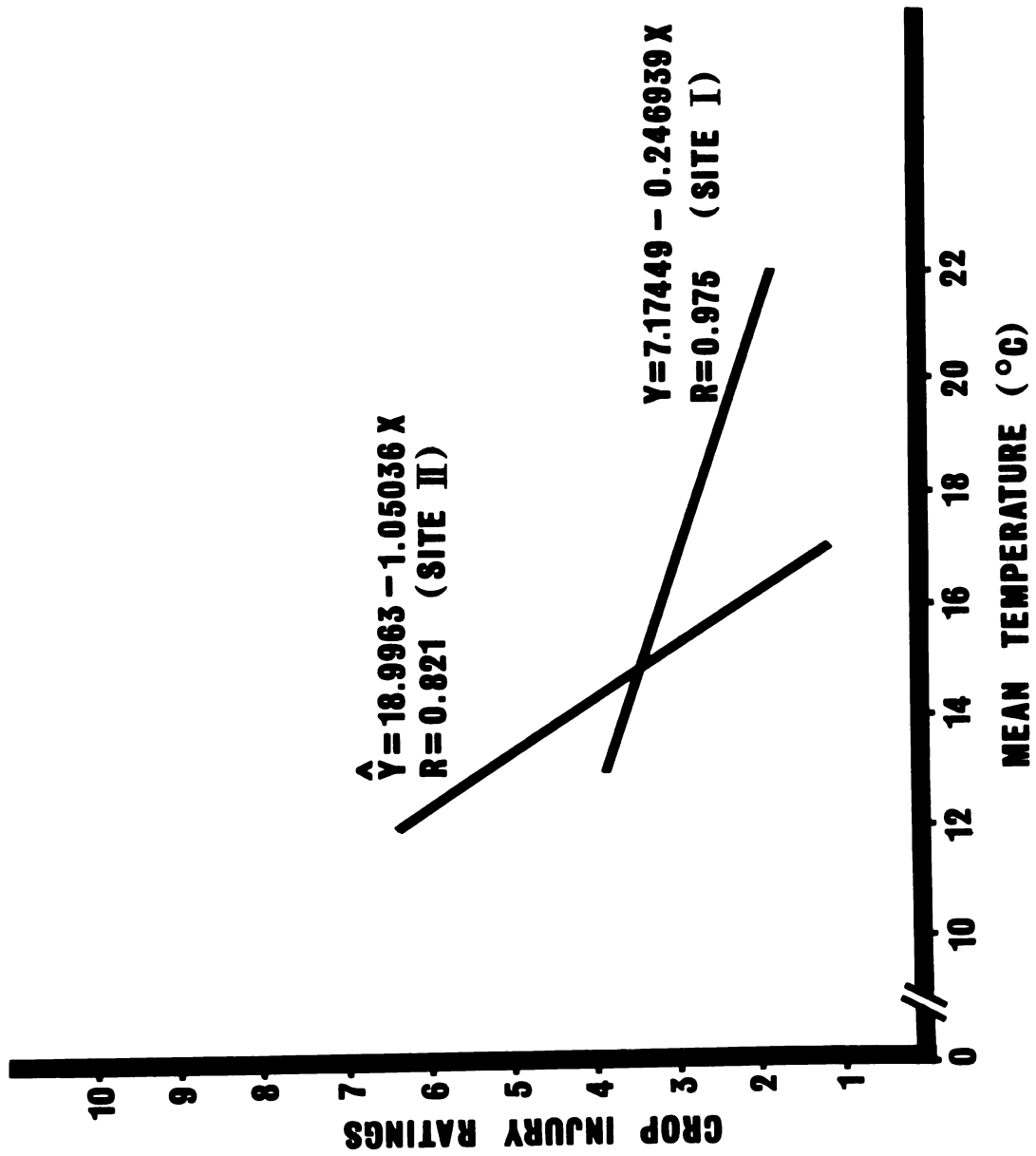
Planting Date	Mean Temperature (C)	Crop Injury 20 DAP		
		(kg/ha)		
		9	13.5	18
5/10	14	7.0 c	6.3 b	9.3 c
5/17	12	6.3 bc	5.0 b	4.3 b
5/24	13	4.3 b	4.7 b	5.7 b
6/14	17	0.3 a	0.7 a	1.7 a

Table 4. Mean air temperatures first 7 DAP and crop injury ratings for direct seeded tomatoes grown at site I in 1976.

Planting Date	Mean Temperature (C)	Crop Injury		
		Diphenamid (kg/ha)		
		9	13.5	18
5/10	14	3.7 b	4.0 b	5.7 bc
5/24	13	0.3 a	3.7 b	6.3 c
6/14	17	1.7 ab	3.0 ab	2.7 a
6/21	22	1.3 a	1.7 a	4.3 b

Severity of crop injury at location I and II correlated negatively ($R = .98$ and $.80$) with the mean air temperature the first seven days after planting (Fig. 1).

Figure 1. Relationship between crop injury ratings and mean air temperature the first 7 DAP at two sites.



Yield differences due to planting date did not occur at location III. The greatest yield reductions at location II occurred when temperatures after planting were relatively cool (Table 5). Yield differences correlated with

Table 5. Effects of planting date and diphenamid on tomato yields at location II in 1976.

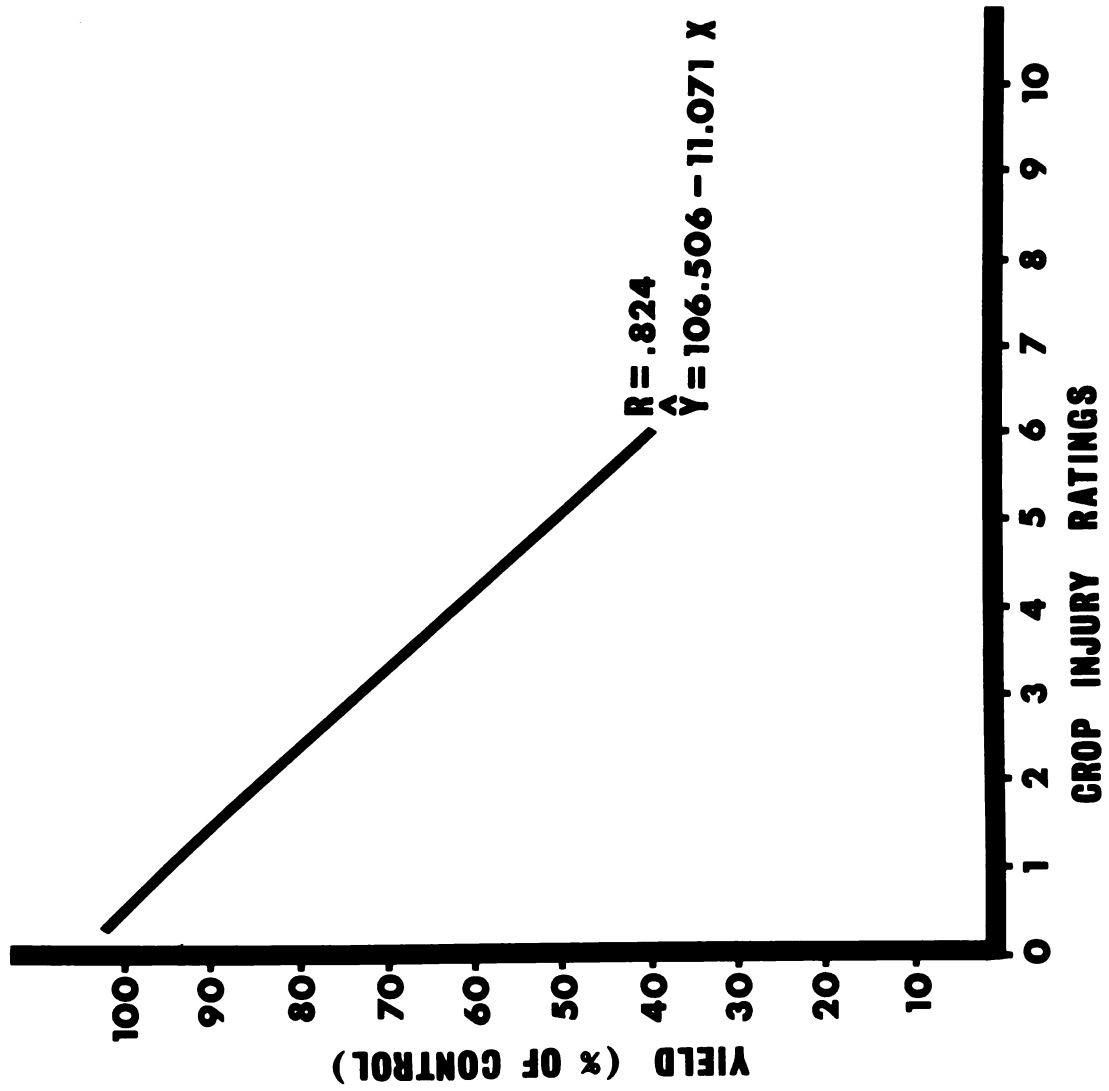
Planting Date	Yield (% of control)		
	rate (kg/ha)		
	9	13.5	18
5/10	73 b	25 a	17 a
5/17	39 a	47 a	52 b
5/24	77 b	84 b	65 b
6/14	87 b	86 b	79 c

earlier crop injury ratings ($R = .82$, Fig. 2). Yield expressed as a percent of the yield of the check for each planting date increased as mean temperatures following planting increased. None of the other environmental factors such as high or low temperatures, and rainfall after planting was significantly correlated with crop injury rating or yield.

Cultivar tolerance

Injury in this study was slight as it was in all the 1975 tests due to the favorable growing conditions.

Figure 2. Relationship between crop injury ratings and yield of tomatoes treated with diphenamid at 13.5 kg/ha.



Significant differences in cultivar tolerance existed at only the 9.0 and 13.5 kg/ha rates, however diphenamid injured the tomatoes at both rates. Red Pak (891) and Heinz 1350 were the most sensitive varieties (Table 6).

Table 6. Effects of diphenamid on crop injury to nine different cultivars of tomatoes.

Cultivar	Crop Injury		
	rate (kg/ha)		
	0	9	13.5
Ace 55	0.3	1.3 a	2.3 a
Campbell 28	0.0	1.7 a	3.0 a
Chico III	1.0	0.3 a	3.0 a
Heinz 1350	1.0	1.7 a	4.0 b
Heinz 1439	1.3	0.3 a	2.3 a
6718 Hybrid	0.0	0.3 a	0.7 a
891 Red Pak Hybrid	0.0	3.7 b	2.0 a
Setmore	0.3	1.3 a	2.3 a
Veebrite	1.7	0.7 a	2.7 a
Mean	0.6	1.2	2.2

Greenhouse and Growth Chamber Tests

Rainfall

The amount of simulated rainfall which occurred after application of diphenamid did not affect the toxicity to tomato under the conditions of this study. Apparently

movement of diphenamid in the soil was not a major factor causing increased injury to tomato. Rainfall is, however, necessary to activate the herbicide (1), but as long as this minimal amount occurs, activity appears to be largely unaffected by rainfall amounts up to 5 cm under the conditions tested. Diphenamid has been reported to be more resistant to leaching than certain other herbicides including dicamba (2).

Temperature

Tomato injury was directly affected by temperatures in the critical period during and immediately following germination. In the growth chamber, injury to tomato seedlings was greatly increased when temperatures during and immediately following germination were cool (Table 7).

Table 7. Effects of diphenamid (9 kg/ha) and varying temperatures on visual injury and fresh weight of seedling tomatoes.

Temperature (C)		Injury Rating 15 DAP	Fresh Weight (% of control)
night	day		
13	18	9.0	45.3
24	29	1.7	99.7

F value for difference between treatments is significant at 1% level by F test.

This agrees with observations in the field. Injury appeared first as necrosis at the cotyledon tips on both the cool and warm temperature treatments, however, when temperatures were cool the area of injury increased and in some cases killed the seedling. When temperatures were warm, the injury was generally confined to the cotyledon tips and the tomatoes rapidly formed true leaves and grew out of the injury. Under the cool temperature regime, those seedlings which survived the initial injury eventually recovered in two to three weeks.

Fertility

The application of a soluble fertilizer alone increased the fresh weight of tomato seedlings at rates up to 0.5 g/pot when only one application was made (Table 8)

Table 8. Effects of fertilizer and diphenamid on fresh weight of seedling tomatoes when fertilizer was applied only at planting.

Diphenamid (kg/ha)	Seedling Fresh Weight (mg)				Mean
	Fertilizer		(g/pot)		
	0	.25	0.5	1.0	
0	108 b	134 b	143 b	78 b	116
9.0	88 a	94 a	55 a	57 ab	74
13.5	66 a	77 a	31 a	31 a	51
Mean	87	102	76	55	

Interaction of diphenamid rate with fertilizer rate is significant at the 5% level.

and up to 0.25 g/pot when three applications were made (Table 9). Diphenamid regardless of fertilizer rate

Table 9. Effect of fertilizer and diphenamid on fresh weight of seedling tomatoes when fertilizer was applied at ten day intervals

Diphenamid (kg/ha)	Seedling Fresh Weight (mg)			Mean
	Fertilizer		(g/pot)	
	0	0.25		
0	128 a	170 b	83 b	127
9.0	106 a	102 a	62 ab	90
13.5	114 a	82 a	30 a	75
Mean	116	118	58	

Interaction of diphenamid rate with fertilizer rate is significant at the 5% level.

applied at either 9.0 kg/ha or 13.5 kg/ha reduced the fresh weight of seedlings. When both soluble fertilizer and diphenamid were applied after planting, the effect was synergistic resulting in a decrease in fresh weight greater than that caused by the sum of the independent effects. For example, when 0.5 g fertilizer and 9.0 kg/ha diphenamid were applied (Table 8), growth was reduced 62% as compared to 19% with diphenamid alone.

It is now a common practice to place a soluble fertilizer in the row at the time of seeding tomatoes, followed by an application of diphenamid. At the 0.5 g/pot

rate, where the synergistic effect was most pronounced (Table 8) only 6.65 kg/ha (based on one application in a 2 cm band over the seed with a row spacing of 150 cm) of soluble fertilizer (20-20-20) would have to be applied to obtain similar synergistic effects. This is well within the range of fertilizer rates used by many growers.

Organic matter

The presence of organic matter decreased the injury from high rates of diphenamid (Table 10).

Table 10. The effect of organic matter content of the soil and diphenamid on crop injury to seeded tomatoes.

Diphenamid Rate (kg/ha)	Crop Injury Rating		
	% Organic Matter		
	1.7	4.8	12.2
0.0	0.0 a	0.0 a	0.0 a
9.0	1.0 b	0.0 a	0.0 a
13.5	4.0 c	0.7 a	0.3 a

The interaction of organic matter and crop injury is significant at the 1% level.

The apparent reduction in diphenamid activity may be due to adsorption or to increased metabolism by microbes in the organic matter. Regardless of the reasons for the

reduction in injury, it is possible that less injury could occur in fields with a relatively high organic matter content however it is doubtful that enough variation in organic matter would exist within a field to account for the reported cases of unexplained injury.

Soil pH

Visual injury symptoms were increased as the soil pH increased in greenhouse studies, however effects were noticeable in most plants for only about ten days after germination (Table 11) followed by rapid recovery. Within

Table 11. Interaction of soil pH and diphenamid on injury to direct seeded tomatoes

Diphenamid Rate (kg/ha)	Crop Injury		
	Soil pH		
	5.5	7.0	8.0
0.0	0.0	0.0	0.0
9.6	0.4	2.0	3.0

Interaction of soil pH with diphenamid rate is significant at the 1% level.

each pot of five tomato plants there appeared to be individual plants which were unharmed while the majority of plants exhibited typical injury symptoms. While some variation occurred in all experiments, presumably due to

variation in the seed, it was particularly noticeable in this test and occurred both times the study was repeated. Since the same lot of seed was used for all tests and conditions were similar to those of other greenhouse tests, the reason for this variation could not be explained.

From the above tests, it can be seen that a number of environmental and edaphic factors affect diphenamid safety on direct seeded tomatoes. At recommended rates injury is difficult to consistently induce, and in order to reliably produce injury during the course of this research it was necessary to use high rates of diphenamid. Apparently the use of diphenamid on direct seeded tomatoes places a temporary stress on the plant. Under optimum growing conditions, the tomato may be able to metabolize the herbicide rapidly enough so that symptoms of injury do not occur. When the plant is, however, placed under a situation where it is under additional stresses such as cool temperatures, high soil pH, high soluble salts in the soil, or other factors, it is not able to inactivate or metabolize the diphenamid and under this situation the crop is injured. It has been noted in both field and greenhouse tests that if the plant can recover from the injury caused by early stresses it will probably grow and yield as well as non-injured plants.

In the intensive agriculture of our time where crops are grown for maximum yield with high fertilizer rates, insecticides and fungicides, and where soil organic matter is often low due to continuous cropping, there are several factors which can interact with herbicides to enhance their action. Application errors or adverse weather after seeding may accentuate diphenamid injury. Therefore, it is important that all the factors affecting the early growth of the crop be evaluated for potential interaction with diphenamid.

LITERATURE CITED

1. Deli, J. and G. F. Warren. 1971. Relative sensitivity of several plants to diphenamid. Weed Sci. 19: 70-72.
2. Harris, C. I. 1964. Movement of dicamba and diphenamid in soils. Weeds 12: 112-115.
3. Lynch, R. and R. D. Sweet. 1969. The effect of environment on tomato response to diphenamid. Proc. WSSC.
4. _____. 1971. Effect of environment on the activity of diphenamid. Weed Sci. 19: 332-337.

CHAPTER 3

ENVIRONMENTAL INFLUENCES ON UPTAKE, TRANSLOCATION, AND METABOLISM OF DIPHENAMID IN TOMATO

CHAPTER 3

ENVIRONMENTAL INFLUENCES ON UPTAKE, TRANSLOCATION, AND METABOLISM OF DIPHENAMID IN TOMATO

ABSTRACT

Tomato (*Lycopersicon esculentum* cv. Heinz 1350) plants were grown under various temperature and pH conditions to determine the reasons for reduced diphenamid (N,N-dimethyl-2,2-diphenylacetanalide) selectivity at low temperatures and high pH's. During the period prior to emergence of the tomato seedling, ^{14}C -diphenamid uptake was slight but increased at high temperatures, although root growth was inhibited at both temperatures. Uptake and metabolism of ^{14}C -diphenamid by plants in the cotyledon stage was not greatly affected by temperature or pH, however translocation from the root to the shoot was reduced under low temperatures and high pH. These differences in translocation parallel reported differences between tolerant and susceptible species and may account for the increased diphenamid injury to tomatoes under stress conditions.

INTRODUCTION

Various environmental parameters have been shown to affect diphenamid (N,N-dimethyl-2,2-diphenylacetanilide) selectivity in tomato (*Lycopersicon esculentum* L.).

Cool weather during the critical period during and immediately after germination and a high soil pH greatly increased diphenamid injury to seedling tomatoes. Although Schultz (55) has shown that reduced light intensities and low humidity increased the absorption of diphenamid by emerged plants grown in nutrient culture, the reasons for increased injury under cool temperatures and high pH have not been elucidated. The purpose of this investigation was to determine how temperature and pH affect the uptake, translocation and metabolism of diphenamid.

MATERIALS AND METHODS

Determination of purity

^{14}C diphenamid (11.1 mg, 54.3 μCi) labeled in the carbonyl position was dissolved in 2 ml of acetone. A

10 μ l aliquot was streaked on a 250 micron silica gel thin layer plate and developed in a benzene:ethanol (85:15 V/V) solvent system to 15 cm. Strips (0.5 cm) were scraped off the plate after drying and added to 15 ml of scintillation fluid (4 g ppo*, 50 mg dimethylpopop**/l toluene) and counted. Activities at each R_f were plotted.

Temperature effects on uptake, translocation and metabolism

Five seeds of tomato were planted in each of six petri dishes containing silica sand and 20 ml of distilled water spiked with 0.3 μ Ci of 14 C-diphenamid. The petri dishes were sealed and half were placed in a growth chamber with a 16 to 21 C night/day temperature regime and the remainder were maintained with a 27 to 32 C night/day temperature regime. Plants were harvested for assay when roots reached 1.25 cm in length.

Additional seeds were pregerminated in vermiculite in the greenhouse until the first true leaf began to appear and then placed in cups containing 150 ml of half strength Hoagland's solution spiked with 0.3 μ Ci of labeled diphenamid. Seedlings were supported by sections of

*2,5-diphenyloxazole

**1,4-bis[2-(4-methyl-5-phenyloxazolyl)]-benzene

sponge which fit into the top of the plastic cups containing the Hoagland's solution. The seedlings were then placed in growth chambers as above and removed after 24 or 48 hours.

Plants were rinsed thoroughly with distilled water, separated into roots and shoots and quick frozen in a dry ice, acetone mixture. Samples were then lyophilized and weighed. Half the samples were placed in liter flasks which were then purged with oxygen and stoppered with septum caps prior to combustion in a Nuclear Chicago model 3151 oxidizer unit with magnetic stirrers. After combustion, 10 ml of ethanol:ethanolamine (2:1 V/V) solution was injected and stirred for 15 minutes to capture the $^{14}\text{CO}_2$. One ml of this solution was added to 15 ml of scintillation fluid and counted. This combustion and counting procedure was used for all tests.

DPM was calculated by multiplying the cpm minus the background by an efficiency factor for the procedure which was calculated by counting a standard containing similar ingredients.

The remainder of the samples were extracted with acetone (10 ml) for 24 hours. Aliquots (200 μl) were then streaked on 250 micron silica gel thin layer plates and developed in a benzene-ethanol (85:15 V/V) solvent system. After drying, the plates were divided into 1 cm bands,

each band scraped, placed in scintillation vials, and counted.

Seedlings were also grown under the same system as above except that a 10^{-5} M technical diphenamid solution was substituted for the labeled diphenamid. The growth of roots and shoots was monitored to detect growth differences.

pH effects on uptake, translocation and metabolism

Tomato seedlings were pregerminated in vermiculite and five seedlings were placed in each plastic cup containing 150 ml of half strength Hoagland's solution which had been adjusted to pH 5.0, 6.0, 7.0, and 8.0 with H_2SO_4 . Each cup also contained a 0.3 μ Ci of labeled diphenamid. Plants were removed after 96 hours and rinsed with distilled water prior to dividing the plants into roots and shoots, and freeze drying. Half of each sample was then combusted while the other half was extracted and chromatographed as above.

RESULTS AND DISCUSSION

Determination of purity

Thin layer chromatography revealed that the labeled diphenamid was over 96% pure diphenamid ($R_f = .55$) with about 0.6% accounted for as the demethylated metabolite ($R_f = .43$). The nature of the studies to be conducted

did not require further purification of the ^{14}C -diphenamid.

Temperature effects on uptake, translocation and metabolism

Although uptake by germinating tomato seedlings was relatively small under both low and high temperature regimes, uptake was at least 15 fold greater under high temperatures (Table 1). The amount of labeled diphenamid

Table 1. Uptake of ^{14}C diphenamid by roots and shoots of germinating tomato seedlings

Location	Temperature (C)			
	27 - 32		16 - 21	
	Dry Wt. (mg)	DPM/mg ^{A/}	Dry Wt. (mg)	DPM/mg ^{A/}
root	2.5	47	4.0	3
shoot	7.1	23	8.2	0

^{A/} Interaction of plant location x temperature is significant at the 1% level.

was about two-fold higher in roots than in shoots. In this experiment, both the roots and shoots of the germinating plant were exposed to ^{14}C -diphenamid so no conclusions can be made regarding the relative importance of sites of uptake or movement after uptake.

Seeds exposed to a 10^{-5} M concentration of technical diphenamid in a manner similar to the above exhibited a 33% reduction in root growth within two days of

radical emergence (Table 2). Apparently, even the relatively small amounts of diphenamid taken up during the early stages of germination affected root growth.

Table 2. Effects of a 10^{-5} M diphenamid solution on root growth of germinating tomato two days after radical emergence.

Diphenamid	Mean Root Length (mm)
0	3.1
+	2.4

Differences in root growth significant by F test at the 1% level.

The temperature at which seedling tomato plants were grown did not significantly affect diphenamid uptake after 48 hours. However, diphenamid translocation from the roots into the shoots, although limited in the cool temperature regime, was nearly complete in the high temperature treatment (Table 3).

Table 3. Location of ^{14}C -diphenamid after root uptake by seedling tomato plants.

Location	DPM/mg	
	Temperature (C)	
	16 - 21	27 - 32
root	28.5	6.5
shoot	9.0	58.0

Interaction of location and temperature is significant at the 1% level.

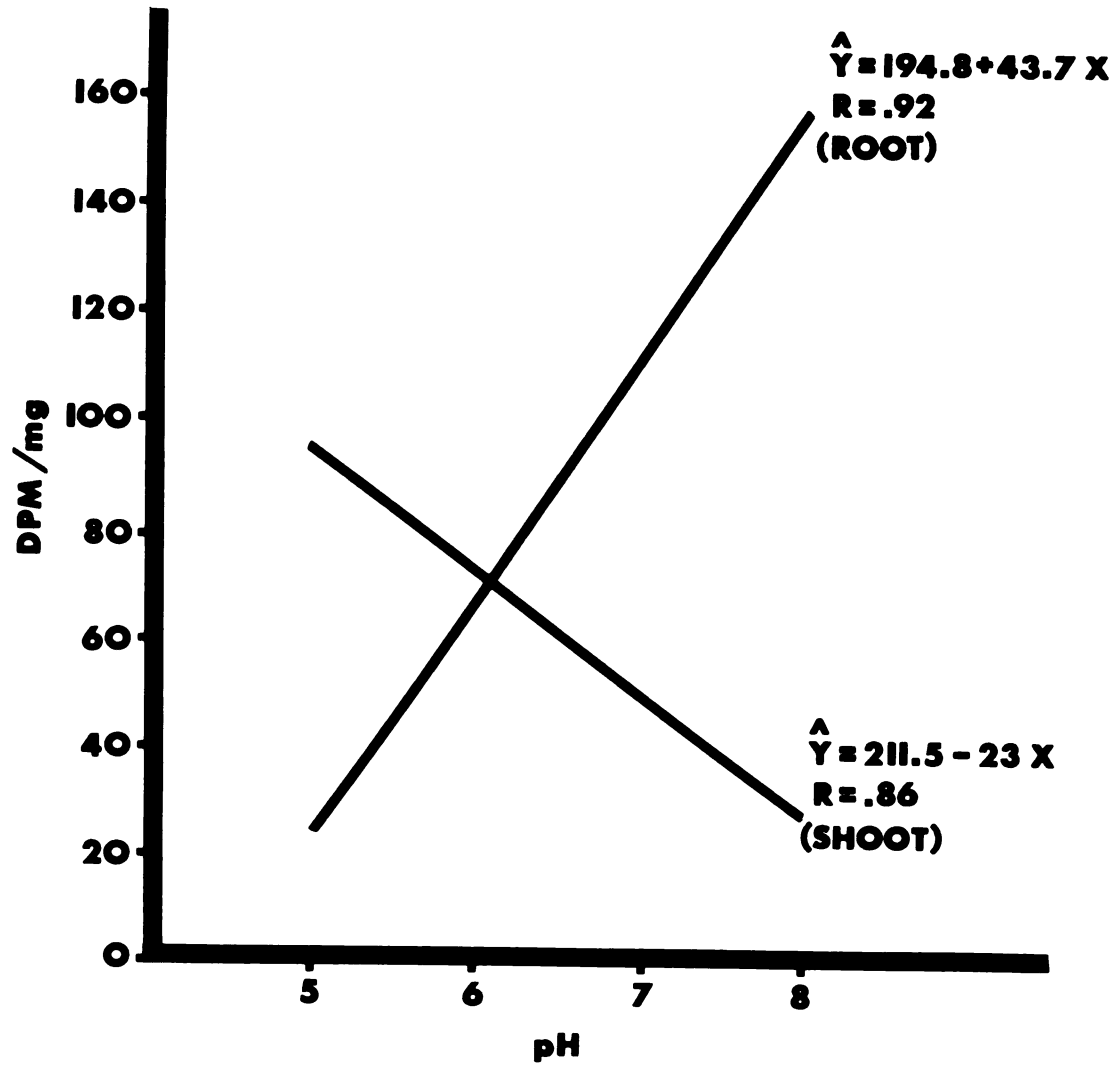
Thin layer chromatography revealed three major peaks at R_f values of 0.13, 0.50 and 0.58. The 0.50 and 0.58 peaks have been identified by co-chromatography with known samples as N-methyl-2,2-diphenylacetamid (MDA) and diphenamid, respectively. The peak at $R_f = 0.13$ has not been identified but may be a glucose conjugate as proposed by Schultz (2). There was no difference in metabolism between temperatures or between the root and shoot.

pH effects on uptake, translocation and metabolism

Varying the pH of nutrient solution in the range of pH 5.0 to 8.0 did not affect the uptake of ^{14}C -diphenamid. As the pH of the solution increased, less diphenamid was translocated out of the root into the shoot resulting in relatively small amounts of diphenamid in the roots at low pH's and large amounts at the upper pH range of this study (Fig. 1). The quantity of label in the roots increased in a linear manner with the pH ($R = .92$) and the quantity in the shoots decreased linearly ($R = .86$) with increasing pH. Again when samples were chromatographed, three major peaks could be identified. Their R_f values were 0.13, 0.50 and 0.58. There were no significant differences in metabolites attributable to pH.

It appears that the reason for increased tomato injury from diphenamid under either cool temperature

Figure 1. Relationship between ^{14}C -diphenamid content and pH in both roots and shoots of tomato seedlings.



conditions or high pH levels is similar. In both cases, the tomato plant is unable to translocate the diphenamid from the roots to the shoot fast enough to prevent injury. Since the site of action of diphenamid is in the roots (1), this may be the cause of the increased injury. Deli and Warren (1) found that in both tomato and ivyleaf mornin-glory [*Ipomoea hederacea* (L.) Jacq.], diphenamid tolerant species, movement from the roots to the shoot was rapid, however in the susceptible species, oats (*Avena sativa* L.), movement into the shoots was slow. It is likely that the same differences in translocation of diphenamid that differentiate tolerant and susceptible species also affect the tolerance of normally tolerant species such as tomato under certain environmental conditions.

LITERATURE CITED

1. Deli, J. and G. F. Warren. 1970. Uptake, translocation, and herbicidal effect of diphenamid. Weed Sci. 18: 692-696.
2. Schultz, Donald P. and B. G. Tweedy. 1972. The effect of light and humidity on absorption and degradation of diphenamid in tomatoes. J. Agr. Food Chem. 20: 10-13.

CHAPTER 4

ENVIRONMENTAL AND EDAPHIC INFLUENCES ON THE ACTIVITY OF ALACHLOR ON SNAPBEANS

CHAPTER 4

ENVIRONMENTAL AND EDAPHIC INFLUENCES ON THE ACTIVITY OF ALACHLOR ON SNAPBEANS

ABSTRACT

The influence of several environmental and edaphic factors on alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide] selectivity in snapbean (*Phaseolus vulgaris* L.) was examined. Maximum injury occurred either when temperatures during germination were cool and rainfall was light or when temperatures approached or exceeded 27 C and the soil was moist. Ten cultivars of snapbean varied in their early tolerance to alachlor, however yield was not affected. Volatilization of alachlor from moist soil resulted in bean injury in growth chamber and greenhouse tests. Injury was less severe in soils high in organic matter. Alachlor leached readily in a loamy sand soil, but less leaching occurred in a loam soil. Applications of 10 cm of simulated rainfall after treatment resulted in less alachlor injury to beans growing in a loamy sand soil.

INTRODUCTION

Increased awareness of incidents of marginal herbicide injury which occurs even when label directions have been followed, has promoted interest in determining the factor which may influence herbicide selectivity. Several environmental factors may alter the activity and selectivity of certain herbicides (3,4,5,6).

In spring of 1974, injury to crops treated with alachlor was widespread in Michigan. Prior to this time injury from alachlor had been uncommon. It was hypothesized that some environmental peculiarity which occurred during the spring of 1974 contributed to the observed alachlor injury.

The objective of this research was to examine the effects of various temperatures, rainfall quantities, fertility levels, and organic matter contents of the soil on the activity of alachlor on snapbeans (*Phaseolus vulgaris* L.). Snapbeans were selected for this study because they are only marginally tolerant to alachlor and differences in tolerance had been observed in field experiments for

several years (7,8,9). In particular, placement of the herbicide had been demonstrated to influence selectivity (7,8,9,10).

MATERIALS AND METHODS

A randomized complete block design with three replications was utilized for all the field experiments. Plots were 1.8 m by 7.5 m. Herbicides were applied with a small plot, CO₂-powered sprayer which delivered 342 l/ha of water. Bean seeds were planted 2.5 cm deep with a Planet Jr. planter. Crop injury was rated on a scale of zero to ten with zero representing no injury and ten representing complete kill of the crop. Analysis of variance was performed on all data and means were compared using Duncan's multiple range test at the 5% level.

Time of planting

In 1975 and 1976, snapbeans (cv. Spartan Arrow) were planted at seven to ten day intervals in three soils at Michigan State University Horticultural Research Center to observe the effects of environmental conditions accompanying the various plantings on snapbean tolerance

to alachlor. The soils analysis is shown in Table 1.

Table 1. Organic matter content, pH and mechanical analysis of soil from three test locations.

location	texture	Constituents (%)				pH
		organic matter	sand	silt	clay	
I	Conover sandy loam	3.0	64	23	12.5	6.5
II	Spinks loamy sand	1.7	85	08	7.0	6.3
III	Miami clay loam	3.6	24.9	44.4	30.7	5.4

In 1974, plantings were made at approximately ten day intervals from May 20 to June 23. In 1975, plantings were begun on May 10 and continued at seven day intervals until June 14. Alachlor was applied at 0 and 3.3 kg/ha in 1974 and 0, 3.3, 4.5 and 5.6 kg/ha in 1975. In 1974, crop injury and stand count data were obtained. In 1976 in addition to the above data, plots were harvested to obtain yield data. Daily high and low temperatures and rainfall were monitored during the entire period.

Cultivar tolerance

In order to ascertain whether commercially important cultivars of snapbeans differed in their tolerance to alachlor, ten different cultivars of snapbeans were

treated preemergence with alachlor at 0, 3.3 and 5.6 kg/ha in 1976. Stand count, crop injury and yield data were obtained.

Greenhouse and Growth Chamber Experiments

After observing various patterns of injury in the field and correlating injury with certain environmental parameters, greenhouse and growth chamber tests were conducted so that the effects of individual parameters could be studied.

All greenhouse and growth chamber tests were conducted utilizing 10 cm styrofoam pots and Spinks loamy sand soil. Five snapbean (cv. Spartan Arrow) seeds were planted 2.5 cm deep in each pot. Plants in the greenhouse received only natural light while light intensities in the growth chambers were standardized at approximately $106 \text{ e/m}^2/\text{sec}$. Visual injury ratings and fresh weight data were obtained at appropriate intervals after treatment. All tests were placed in a randomized complete block design with treatments replicated at least three times and experiments repeated at least twice.

Soil movement

Fifteen cm diameter tiles were filled with soil from locations I and II and the surface was sprayed with alachlor at 5.6 kg/ha. Water was then applied to the

surface in amounts equal to 2.5, 5 and 7.5 cm of rainfall. The tiles were then split lengthwise and bioassayed. In addition, one treatment was allowed to surface dry prior to the bioassay to see if alachlor would move back up to the surface with the capillary water. An additional treatment was allowed to surface dry then again treated with 2.5 cm of water to simulate sequential rains occurring several days apart. All treatments were bioassayed with barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] and the zone of alachlor activity indicated by the distance from the surface barnyardgrass manifested injury.

Rainfall

In order to test the effects of various amounts of rainfall on alachlor activity, snapbeans were planted in soil from location II, treated with alachlor at 5.6 kg/ha, and then 1.25, 2.5 and 5.0 cm of water was applied with a chain operated boom which applied the water as fine droplets over a five to ten minute period.

Volatility

The possibility of alachlor volatility causing injury under certain conditions was investigated by spraying beans with alachlor at 5.6 kg/ha and covering half the pots with plastic bags and leaving the other half uncovered. Additional pots were not sprayed with alachlor but

treated in the same manner. These were then grown in the greenhouse until the first trifoliate leaf appeared.

Beans were also planted in paper cups which were not treated with alachlor and placed on the surface of flats filled with loamy sand soil half of which had been treated with alachlor at 5.6 kg/ha. Flats were kept moist and covered with a polyethylene bag. These were then placed in the greenhouse with a $24\text{ C} \pm 5$ night temperature and the beans allowed to germinate.

Temperature

Five bean seeds were planted in 10 cm pots filled with soil from location II and treated with alachlor at 0 and 5.6 kg/ha. Pots were then placed in two growth chambers, with night and day temperatures of 16 C to 21 C and 27 C to 32 C, respectively. The light period was 16 hours per day. Pots were covered with polyethylene bags to prevent contamination of controls by gaseous alachlor.

Organic matter

The effects of organic matter on alachlor activity were tested by amending Spinks loamy sand soil (1.7% o.m.) with Houghton muck soil (80% o.m.) to provide soils with 4.8 and 12.2% organic matter. The beans were planted in the three soils and sprayed with alachlor at 5.6 and 11.3 kg/ha.

Soil fertility

Snapbeans grown in pots in the greenhouse were subjected to various fertilizer regimes to test the effects of various fertility levels on snapbean tolerance to alachlor. Beans were planted in a Spinks loamy sand soil, treated with alachlor at 0, 4.5 and 5.6 kg/ha and then fertilized weekly with 100 ml of water soluble 20-20-20 fertilizer at the following rates: 0.0, 0.75, 1.5 and 3.0 g/l.

RESULTS AND DISCUSSION

Time of planting

Significant differences in snapbean tolerance to alachlor occurred between six planting dates and between the two different locations (Tables 2 and 3).

Table 2. Effects of five different planting dates on alachlor injury to snapbeans (Location I, 1976).

Planting Date	Conditions for first 7 DAP		Injury Rating (18 DAP)			
	mean temp (C)	total rainfall (cm)	Rate (kg/ha)			
			0	3.4	4.5	5.6
5/10	14.4	0.7	1.3 abc	7.0 c	6.3 c	6.7 c
5/17	12.2	0.0	2.3 c	6.7 c	7.3 c	7.0 c
5/24	13.3	4.2	0.0 a	0.0 a	0.0 a	0.7 a
6/07	23.0	7.6	0.3 ab	4.0 b	4.3 b	5.7 bc
6/14	22.0	7.1	0.3 ab	1.0 a	3.0 b	4.3 b

Interaction between planting date and rate is significant at the 1% level.

Table 3. Effects of four different planting dates on alachlor injury to snapbeans (Location II, 1976).

Planting Date	Conditions for first 7 DAP		Injury Rating			
	mean temp (C)	total rainfall (cm)	Rate (kg/ha)			
			0	3.4	4.5	5.6
5/10	14.4	0.7	0.7 a	1.7 a	3.7 b	3.0 a
5/17	12.2	0.0	1.0 a	5.7 c	7.7 c	7.3 c
5/24	13.3	4.2	0.0 a	1.0 a	1.7 a	2.0 a
6/07	23.0	7.6	0.0 a	3.0 b	2.7 ab	5.3 b

Interaction between planting date and rate is significant at the 1% level.

In 1975 differences occurred only at location III (Table 4).

Table 4. Effects of three different planting dates on alachlor (3.4 kg/ha) injury to snapbeans (Location III, 1975).

Planting Date	Conditions for first 10 DAP		Crop Injury
	mean high temp (C)	total rainfall (cm)	
5/20	22	3.6	5.0 c
5/29	17	2.8	2.0 b
6/10	19	4.1	0.0 a

In 1976, the most severe injury occurred in the May 17, June 7 and June 14 plantings. This injury corresponded

with low rainfall and cool temperatures during germination (May 17) or moderate rainfall and warm temperatures (June 4 and 14) during and immediately after germination.

Temperature and rainfall conditions during the period immediately prior to emergence are believed to be critical because it is during this time period that the bean epicotyl could absorb alachlor from the soil, if the shoot is an important site of alachlor uptake. Presumably, the greater the amount of rainfall that occurred immediately following alachlor application, the more the alachlor will move down through the soil diluting it in the uppermost layer. Thus, less alachlor is in contact with the shoot portion of the germinating seedling. At the same time if the temperature is cool at the time germination is occurring, growth and emergence of the bean plant will be slow resulting in a longer period of time that the shoot is in contact with the alachlor in the upper layer of soil, providing an opportunity for increased uptake.

Conversely, when the soil is warm, the bean emerges rapidly and the shoot is in contact with the alachlor layer for a lesser period of time, providing time for less shoot uptake. If this is indeed the case, one would expect to find that when only a small amount of rainfall has occurred, the decrease in alachlor injury by dilution would be greater on a sandy soil than on a clay soil due to the greater movement of alachlor in sandy soils. This

could explain the responses in the first planting, where injury on the sandy soil was less than on the heavier soil. In the June 7 and 14 plantings, injury is approximately equal on the sandy soil and the loam soil which may indicate that rainfall was not as important a factor in these plantings which both received adequate amounts of rainfall during the germination period to dilute the alachlor in the shoot zone.

The relatively high amount of injury occurring in the June 7 and 14 plantings could have resulted from volatilization of the alachlor. Beestman showed that under wet, windy conditions volatilization of alachlor may occur (9). Cox has shown that alachlor vapor can cause plant damage even at relatively low concentrations (13). Weather data from the period immediately following snapbean germination in the June 7 and 14 plantings, indicates that more than 6.3 cm of rain fell during that period and that the average high temperature was near 27 C. These weather conditions would be conducive to volatilization of alachlor. These conditions could also be conducive to greater root uptake and movement via the transpiration stream. In 1975 where adequate rainfall also occurred, the greatest injury occurred where the temperature was the warmest while less injury occurred where air temperatures were lower. Apparently volatilization is not a problem until the air temperature

approaches 27 C. Where temperatures were adequate for rapid bean germination but below 27 C, bean injury was minimized.

The yield data reveal that early injury due to alachlor was largely outgrown as the plants reached maturity. Early yield was affected by planting dates (Tables 5, 6), however total yield was not. Both early and total yield were reduced by alachlor at the 4.5 and 5.6 kg/ha rates at both locations.

Table 5. Effect of alachlor at various planting dates on early yield (first picking) of snapbeans on a loamy sand (Location II) soil.

Planting Date	Early yield (% of weed control)		
	Alachlor (kg/ha)		
	3.4	4.5	5.6
5/10	90 c	93 c	97 d
5/17	35 a	13 a	22 a
5/24	56 b	57 b	44 b
6/07	101 c	85 c	72 c

Table 6. Effect of alachlor at various planting dates on early yield of snapbeans on a sandy loam (Location I) soil.

Planting Date	Early yield (% of weeded control)		
	Alachlor (kg/ha)		
	3.4	4.5	5.6
5/10	28 a	72 b	26 a
5/17	26 a	10 a	33 ab
5/24	73 b	69 b	56 abc
6/07	67 b	60 b	55 abc
6/14	84 b	75 b	74 c

Cultivar tolerance

Ten different cultivars exhibited differential tolerance to alachlor by visual observation 15 DAT. Of the cultivars tested, Contender and Bountiful were the most resistant while Topcrop was the most susceptible (Table 7). The cultivar differences in tolerance exhibited in the early crop injury ratings (Table 7) did not result in yield differences. Apparently differences in cultivar susceptibility which did exist soon after treatment were slight and are overcome so that yield is not affected.

Table 7. Tolerance of ten different cultivars of snapbeans to alachlor at two rates (Crop Injury Ratings),

Cultivar	Crop Injury Ratings	
	Rate (kg/ha)	
	3.4	4.5
Bountiful	0.7 a	2.0 a
Burpee's Stringless	1.7 abcd	3.3 bc
Commodore Bush Kentucky Wonder	2.7 de	3.7 cd
Contender	0.7 a	2.3 ab
Greencrop	1.3 abc	2.3 ab
Harvester	1.0 ab	3.7 cd
Provider	3.0 e	3.7 cd
Spartan Arrow	2.3 cde	3.7 cd
Tendergreen	2.0 bcde	4.0 cd
Topcrop	4.7 f	4.7 e

Greenhouse and Growth Chamber Experiments

Soil movement

Alachlor moved readily in soil in response to rainfall but showed little tendency to move upwards with capillary water as the soil surface dried (Table 8).

Leaching would result in a dilution of alachlor in the upper soil layers so that a lower concentration is in contact with the shoot of the germinating bean. If shoot uptake is a major site of alachlor uptake, less injury

would then be expected. It should be noted, however, that the alachlor did not completely leach out of the surface layers of the soil but remained in sufficient concentrations to inhibit the barnyardgrass at the soil surface.

Table 8. Movement of alachlor in two soil types in response to various amounts of rainfall.

Rainfall (cm)	Movement (cm)	
	sandy loam ^{A/}	loamy sand ^{B/}
0.0	0.0	0.0
2.5	5.7	7.2
5.1	10.7	11.0
7.6	9.0	15.7

^{A/}Quadratic influence of rainfall is significant at the 1% level.

^{B/}Linear influence of rainfall is significant at the 1% level.

Rainfall

Alachlor growth inhibition was substantially decreased by 5 cm of rainfall as compared to 2.5 cm or

1.25 cm (Table 9). Since alachlor leaches readily in a sandy soil, it is probable that the alachlor in the shoot zone of the germinating bean was diluted so that less shoot uptake occurred.

Table 9. Effects of simulated rainfall on alachlor (5.6 kg/ha) injury to snapbeans in a loamy sand soil.

<u>Rainfall (cm)</u>	<u>Fresh Weight (g)</u>
1.25	8.8 a
2.50	11.2 ab
5.00	16.0 b

The linear influence of rainfall is significant at the 1% level.

Volatility

Alachlor volatility has been shown to occur under wet windy conditions (1) and under laboratory conditions has been shown to cause crop injury (2). Volatility was first suspected of being a factor in snapbean injury in a growth chamber test (27-32 C) where alachlor injury symptoms appeared in the controls and the degree of injury increased over time (Table 10). After 11 days there were no differences in weights between the treated and the untreated beans. Since temperatures were high and the

soil moist, volatility was suspected. The symptoms which appeared in the growth chamber were similar to symptoms found in the field the previous summer.

Table 10. Injury to alachlor treated and untreated snapbeans in a growth chamber.

Alachlor Rate (kg/ha)	Crop Injury Ratings		
	Days after treatment		
	5	7	9
0	1.7	4.7	4.8
5.6	6.0	6.0	6.3

Interaction of rate with time of rating is significant at the 1% level.

When pots containing treated and untreated beans were covered with plastic bags, the most severe injury occurred in the sprayed pots which were covered (Table 11).

Table 11. Effects of alachlor volatility on crop injury and fresh weight of snapbeans.

Alachlor Rate (kg/ha)	Pot Maintenance	Crop Injury		Fresh Weight (g)
		Days	after treatment	
		5	15	
5.6	covered	4.6 a	8.3 a	2.7 a
5.6	uncovered	1.5 b	3.0 b	4.9 b
0	covered	1.5 b	1.8 c	5.1 b

Since plants were confined within a plastic bag any alachlor which volatilized would have remained in the bag and hence in contact with the bean plant. It is highly probable that alachlor in a gaseous form increased the snapbean injury.

When untreated pots were placed on top of treated soil and enclosed in plastic bags alachlor injury symptoms developed although the bean roots were not in contact with the treated soil (Table 12).

Table 12. Injury to snapbeans in pots placed on alachlor treated soils.

Alachlor Rate (kg/ha)	Visual Injury	Fresh Weight (g)
5.6	6.0	2.1
0	3.4	2.3

F value for comparison of treatments significant at 1% level.

In all cases where alachlor volatility caused plant damage, the soils were moist and the air temperature was above 27 C. Injury first appeared as a mottling of the foliage and cupping of the leaves and then progressed to necrosis of the leaf margins (Fig. 1).

The crinkling of the leaf commonly associated with alachlor injury occurred only when the soil in which the

Figure 1. Symptoms of alachlor injury to snapbean.

Top: Crinkling of leaves associated with cool temperatures following germination.

Bottom: Marginal necrosis of leaves associated with high temperatures or alachlor volatility.



beans were planted had been treated. Apparently this type of injury occurs as a result of root or shoot uptake in soil and not by foliar contact with gaseous alachlor.

Temperature

Alachlor injured snapbeans at both temperatures however, the injury was more severe and the symptoms were exhibited earlier under lower temperatures (16-21 C) than at the higher temperatures (27-32 C) (Table 13).

Table 13. Effects of two temperatures on alachlor injury to snapbean.

Alachlor Rate (kg/ha)	Crop Injury (two days after emergence)	
	Temperature (C)	
	16 - 21	27 - 32
0	0.0	1.7
5.6	8.7	3.3

Interaction of temperature with rate is significant at the 5% level.

Injury in the high temperature treatments generally equaled the low temperature injury after 10 to 12 days, however the injury generally appeared as a cupping and marginal chlorosis rather than the leaf puckering seen in the low temperature treatments. Some of the early high temperature injury appeared to be related to leaves touching the

plastic bag which covered all pots in order to eliminate contamination by volatilization of alachlor.

Organic matter

Beans treated with alachlor were injured less in soils with a high organic matter content (Table 14). This may be due to adsorption or to microbial degradation of alachlor which would presumably be more active after the addition of a muck soil.

Table 14. Effects of the organic matter content of the soil on snapbean injury by alachlor.

Alachlor Rate (kg/ha)	Crop Injury Rating		
	organic matter (%)		
	1.7	4.8	12.2
0.0	1.0 a	1.7 a	1.0 a
5.6	3.3 b	1.7 a	0.7 a
11.3	6.7 c	3.7 b	1.7 a

Soil fertility

The soluble fertilizer applications did not influence the magnitude of alachlor injury on snapbeans.

A number of factors may interact to affect the severity of alachlor injury to snapbeans. Among the factors tested, temperature and rainfall appeared to be

the most important in the field. Though volatility was an important factor under greenhouse and growth chamber conditions, its importance in the field has not been demonstrated. It is probable that while volatility, cool temperatures, and other factors may singly contribute to alachlor injury, severe injury such as occurred during the spring of 1974 in Michigan is the result of the combination of adverse environmental factors which in themselves may have stressed crops. When these occurred in combination with factors enhancing the susceptibility of snapbeans to alachlor, injury occurred. Alachlor is not the only herbicide dependent on environmental conditions for efficiency and crop safety. When unexplained herbicide failure or crop injury occurs, the edaphic and environmental factors which alter herbicide activity and selectivity should be examined.

LITERATURE CITED

1. Beestman, G. B. and J. M. Deming. 1974. Dissipation of acetanilide herbicides from soils. *Agronomy Journal* 66: 308-311.
2. Cox, T. I. 1974. Experiments on vapor activity of alachlor and related herbicides. *Weed Research* 14: 379-383.
3. Davis, D. W., J. C. Cialone and R. D. Sweet. 1974. Some factors affecting the residual activity of diphenamid. *Proc. N,E,W,C,C*, 18: 100-108.
4. Hodgson, Richard, Kendall E. Dusabek and Barry Hoffer. 1974. Diphenamid metabolism in tomato: time course of an ozone fumigation effect. *Weed Sci.* 22: 205-210.
5. Lynch, R. and R. D. Sweet. 1969. The effect of environment on tomato response to diphenamid. *Proc. WSSC*.
6. ———. 1971. Effect of environment on the activity of diphenamid. *Weed Sci.* 19: 332-337.
7. Putnam, Alan R., A. Paul Love, Paul F. Boldt, Robert P. Rice Jr., Ronald H. Lockerman and Nancy E. Adams. 1976. Herbicide evaluations in vegetable and fruit crops. *Horticultural Report* 26. Department of Horticulture, Michigan State University, East Lansing.
8. Putnam, Alan R., A. Paul Love, Gregory Pagano and Robert P. Rice Jr. 1973. Weed control research. *Horticultural Report* 22. Department of Horticulture, Michigan State University, East Lansing.

9. Putnam, Alan R., A. Paul Love, Robert P. Rice Jr., Ronald H. Lockerman and Nancy E. Adams. 1975. Herbicide evaluations in vegetable and fruit crops. Horticultural Report 25. Department of Horticulture, Michigan State University.
10. Rice, Robert P. and Alan R. Putnam. 1977. Environmental effects on alachlor activity in snapbeans. Abstracts WSSA.

CHAPTER 5

ENVIRONMENTAL INFLUENCES ON UPTAKE, TRANSLOCATION, AND METABOLISM OF ALACHLOR IN SNAPBEANS

CHAPTER 5

ENVIRONMENTAL INFLUENCES ON UPTAKE, TRANSLOCATION, AND METABOLISM OF ALACHLOR IN SNAPBEANS

ABSTRACT

The uptake, translocation, and metabolism of ^{14}C -alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanalide] in germinating and emerged snapbeans (*Phaseolus vulgaris* L.) were studied under 16 C night/21 C day and 27 C night/32 C day temperature regimes. Total uptake of ^{14}C -alachlor by germinating snapbeans was greater under the higher temperature, however, the label was located primarily in the roots where it was rapidly metabolized. At the lower temperature, the label was located in approximately equal amounts in all plant parts except cotyledons and significantly less of the alachlor was metabolized. Root uptake of ^{14}C -alachlor by emerged snapbeans was significantly greater under the high temperature than under the low temperature and translocation of the label to the shoots was more rapid

under high temperatures. Approximately 60% of ^{14}C -alachlor was shown to volatilize from a watchglass after 48 hours at 27 C. After volatilization, uptake of alachlor occurred in adjacent snapbean plants in a closed system.

INTRODUCTION

Snapbeans are only marginally tolerant to alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanalide] applied at rates sufficiently high to adequately control weeds. In field tests, over a number of years, tolerance has ranged from excellent to poor with similar rates and application methods. Previous work has shown that differences in tolerance are due at least partly to environmental conditions. The most severe injury has been shown to occur either when the temperature during the germination period is below the optimum for bean growth and rainfall has been light, or when temperatures immediately following germination are above 27 C and the soil is moist. This paper relates the environmental conditions conducive to injury to differences in uptake, translocation, or metabolism of ^{14}C -alachlor by snapbeans.

MATERIALS AND METHODS

Determination of purity

Uniformly ring labeled ^{14}C -alachlor (15.46 mg, 100 μCi) was dissolved in 2 ml of acetone. A 10 μl aliquot was streaked on a 250 micron silica gel thin layer plate and developed in benzene:ethanol (85:15 V/V) to 15 cm. Strips of silica gel 0.5 cm wide were scraped from the plate after drying and added to 15 ml of scintillation fluid (4 g ppo*, 50 mg dimethyl-popop**/1 toluene) and counted in a liquid scintillation spectrometer.

Volatility

The amount of gaseous alachlor absorbed by the foliage of snapbeans under high temperature conditions was studied by placing 30 μl (0.9 μCi) of ^{14}C -alachlor in 1 ml of H_2O on a watchglass which was placed on top of the soil of pots containing a snapbean plant in the second trifoliate leaf stage. Plants were covered with a plastic bag and placed in a growth chamber with a 27 to 32 C night/day temperature regime. After 36 hours, the

*2,5-diphenyloxazole

**1,4-bis[2-(4-methyl-5-phenyloxazolyl)]-benzene

watchglass was washed with acetone into a scintillation vial and the amount of labeled alachlor remaining was determined. Shoots were washed, freeze dried, combusted, and counted to determine the amount of ^{14}C -alachlor in the shoots.

Ten μl ^{14}C -alachlor (0.3 μCi) were also placed in 1 ml of distilled water on watchglasses which were subsequently placed in growth chambers at 21 C, 27 C and 32 C. After 48 hours the residue was washed into scintillation vials with acetone and the amount of labeled alachlor remaining was determined.

Uptake and metabolism by germinating seedlings

Spartan Arrow bean seeds were planted in 150 cc of moist quartz sand to which 0.3 μCi of labeled alachlor had been added. Pots were placed in growth chambers at 16 C night/21 C day and 17 C night/32 C day temperature regimes. When seeds germinated and the first true leaves had expanded seedlings were removed (7 and 10 days), washed with distilled water, and sectioned into roots, hypocotyl, cotyledons, epicotyl, leaves and terminal bud. The samples were lyophilized, combusted and aliquots counted to reveal the location and quantity of ^{14}C -alachlor.

Combustion in this and in subsequent tests was performed by placing the sample (45 mg - 70 mg) into a 1000 ml combustion flask which had previously been purged

with oxygen. Samples were ignited in a Nuclear Chicago combustion apparatus. After combustion was complete the CO_2 was absorbed by 10 ml of ethanol-ethanolamine solution (85:15 V/V) which was injected through a rubber septum stopper and stirred for 15 minutes. One ml aliquots were then placed in scintillation vials and counted.

DPM was calculated by multiplying the cpm minus the background by an efficiency factor for the procedure which was calculated by counting a standard containing similar ingredients.

Site of uptake

Snapbean seeds were pregerminated between sheets of moist paper towelling prior to being transplanted into 15 cm pots (5 plants/pot) containing Spinks loamy sand soil. Pots were divided into root and shoot treatment zones utilizing a 2 mm layer of activated charcoal as a barrier. Treatments were made with alachlor at 4.5 kg/ha in (a) the shoot zone only, (b) the root zone only, (c) both root and shoot zones, and (d) neither root nor shoot zones (control). Plants were placed in growth chambers with a high temperature regime (32 C days and 27 C nights) and a low temperature regime (21 C days and 16 C nights). Crop injury ratings were taken at the time of primary leaf and first trifoliate leaf emergence.

Temperature effects on root uptake

Snapbeans were pregerminated in vermiculite and allowed to grow until the first unifoliate leaves had fully expanded. At this time they were washed and placed in a half-strength Hoagland's solution spiked with 0.3 μCi of ^{14}C -alachlor. Plants were then placed in growth chambers at 16 C nights/21 C days and 27 C nights/32 C days temperature regimes. Samples were removed after 48, 72 and 96 hours, washed with distilled water, quick frozen in dry ice and acetone and lyophilized. Each plant was divided into roots, hypocotyl, cotyledons, epicotyl, leaves, and terminal bud prior to combustion. After combustion, the quantity of labeled alachlor in each sample was determined.

RESULTS AND DISCUSSION

Determination of purity

Thin layer chromatography revealed that the ^{14}C -alachlor ($R_f = 0.46$) was 99.4% pure.

Volatility

When ^{14}C -alachlor was placed on a watchglass under the canopy of a snapbean plant activity was detected in the shoot of the bean 36 hours later. There were 191,573 DPM in the watchglass and 450 DPM in the 50 mg shoot sample.

When volatilization was measured from watchglasses, 65% of the ^{14}C -alachlor volatilized after 48 hours regardless of temperature.

Volatilization of ^{14}C -alachlor occurred in significant amounts under the conditions tested and uptake by snapbeans of the gaseous ^{14}C -alachlor did occur. Whether sufficient uptake of gaseous alachlor under field conditions would occur to cause serious injury is doubtful. The quantity of alachlor lost through volatilization is, however significant, and may account for the loss of efficacy

under certain conditions. In addition to temperature, soil moisture and wind may be important parameters affecting volatilization (9).

Uptake and metabolism by germinating seedlings

In the high temperature treatments, more than twice as much alachlor was taken up by the plants as was taken up in the low temperature treatments (Table 1). The bulk of the label was found in the roots in the high temperature treatments, however, in the low temperature treatments the alachlor was distributed throughout the plants with no one plant part accumulating a significantly greater amount of alachlor than any other.

Table 1. Location of ^{14}C -alachlor in snapbean plants germinated in moist sand spiked with ^{14}C -alachlor.

Location	DMP/MG Dry Weight	
	Temperature (C)	
	16-21	27-32
roots	1.6	7.1 c
hypocotyl	2.0	3.3 b
cotyledons	0.0	1.7 ab
epicotyl	1.2	2.6 b
leaves	0.4	1.4 ab
terminal bud	1.3	0.0 a
total uptake	6.5	16.1

Interaction of temperature and location is significant at the 5% level.

Thin layer chromatography revealed the presence of alachlor and two metabolites in the roots, and alachlor plus one metabolite in the shoots (Table 2). R_f values

Table 2. Percentage of alachlor and metabolites in extracts of the roots and shoots of snapbean plants grown at two temperatures

R_f	% of ^{14}C label			
	Roots		Shoots	
	temperature (C)		temperature (C)	
	16-21	27-32	16-21	27-32
0.06	37	41	0	0
0.21	17	18	18	23
0.46	46	41	82	76

for peaks present in the root extracts were 0.06, 0.21 and 0.46. In the shoot, peaks were present at R_f 0.21 and 0.46. In this solvent system, the R_f value for alachlor was 0.46, however no attempt was made to identify the metabolites. There are no reports in the literature on the identification of these metabolites. No quantitative differences existed between metabolites attributable to the temperatures at which the plants were grown. However, quantitative and qualitative differences in metabolism did exist between roots and shoots (Tables 2, 3).

Table 3. Quantity of unmetabolized ^{14}C -alachlor in roots and shoots of snapbeans.

<u>Location</u>	<u>% Unmetabolized ^{14}C-alachlor</u>
roots	19.5
shoots	36.5

F value for difference between shoots and roots is significant at the 1% level.

Translocation data indicated that although less uptake occurred at low temperatures, there were equal quantities of label in all plant parts except the cotyledons. At high temperatures the preponderance of label was located in the roots. Nearly 80% of the metabolites were located in the roots compared to only 45% in the shoot. If it is assumed that the metabolites are less toxic than the parent compound this may explain the increased crop injury at low temperatures.

Site of uptake

Early uptake occurred primarily via the shoot portion of the emerging seedling as evidenced by symptoms of leaf curling and later by marginal chlorosis. In the low temperature treatments, similar symptoms appeared in the root treatments about the time the first true leaf emerged (Table 4). In the high temperature treatments, the most severe injury occurred in the shoot treatments including

Table 4. Effects of zone treatments with alachlor and temperature on snapbean injury.

Treatment	Crop Injury Ratings			
	Temperature (C)			
	16-21		27-32	
Zone	Primary Leaf	First Trifoliolate	Primary Leaf	First Trifoliolate
Control	0.3 a	1.7 a	1.7 a	4.7 a
Root	2.7 b	7.3 c	2.7 a	6.0 a
Shoot	8.3 c	6.0 b	6.0 b	6.3 a
Root & Shoot	5.3 d	7.3 c	6.7 b	5.3 a

the checks and within a few days no differences in injury between treatments were apparent. This injury has been attributed to volatility within the confined atmosphere of the growth chamber.

Temperature effects on root uptake

Root uptake of ^{14}C -alachlor was significantly greater when plants were grown under 27 to 32 C as compared to the 16 to 21 C temperature regime (Table 5). There were no differences in the quantity or location of the label after 48 hours, however, after 72 hours, significantly higher quantities had accumulated in the epicotyl and terminal bud of plants in the high temperature treatment. After 96 hours the label had been translocated out of the

Table 5. Interaction of air temperature and exposure time on root uptake and translocation of alachlor.

Location	DPM/MG					
	48			96		
	Exposure Time (hrs)		Temperature (C)	Exposure Time (hrs)		Temperature (C)
	16-21	27-32		16-21	27-32	
roots	1.2	2.6	1.3	1.6	5.9	
hypocotyl	1.6	1.0	1.7	1.6	5.2	
cotyledons	0.7	0.2	0.0	0.3	0.1	
epicotyl	0.0	3.8	1.9	7.6	7.6	
leaves	0.8	5.4	1.6	2.1	14.4	
terminal bud	3.3	0.0	0.0	7.3	11.5	

Interaction of location x exposure time x temperature is significant at the 5% level.

epicotyl in the high temperature treatments and the highest concentrations were found in the leaves and the terminal bud. No significant accumulation of ^{14}C -alachlor occurred in the low temperature treatments at 48 or 72 hours, however after 96 hours the ^{14}C -alachlor began to accumulate in the epicotyl and terminal bud.

Alachlor uptake by snapbean roots apparently is much slower at cool temperatures than at warm temperatures. Under warm weather conditions root uptake and translocation to the shoot occurs within 72 hours while under cool conditions significant translocation of alachlor to the shoot does not occur until after 96 hours. This may account for alachlor injury which has been observed to occur in the field under warm weather conditions where sufficient rainfall has occurred to leach the herbicide into the root zone.

Alachlor injury to snapbeans has also been associated with cool temperatures during the germination period. Although alachlor uptake was greater when seeds germinated under warm temperature conditions, the majority of the alachlor was metabolized in the roots whereas under cool conditions the label was dispersed throughout the plant and a higher percentage of parent compound was present than under high temperatures.

Volatility of alachlor may result in loss of efficacy and limited crop injury under confined conditions, but is probably not responsible for crop injury with good air movement under field conditions.

BIBLIOGRAPHY

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1. Ahrens, J. F. 1963. Chemical control of weeds in deciduous nursery plantings. Northeastern Weed Control Conference Proc. 17: 208-212.
2. _____. 1963. Chemical control of weeds in Connecticut shade-grown and stalk-cut tobacco. Northeastern Weed Control Conference Proc. 17: 240-247.
3. Alder, E. F., W. L. Wright and Q. F. Soper. 1960. Control of weeds in vegetative crops with a substituted diphenylacetamide. North Central Weed Control Conference. Dec. 1960.
4. _____. 1962. Diphenamid for pre-emergent weed control in horticultural crops. Proc. N.E.W.C.C. 16: 54-59.
5. Anderson, Wood Powell. 1977. Weed Science: Principles. West Publishing Company. St. Paul. pp. 268-277.
6. Armstrong, Thomas F., William F. Meggitt and Donald Penner. 1973. Absorption, translocation, and metabolism of alachlor by yellow nutsedge. Weed Sci. 21: 357-360.
7. _____. 1973. Yellow nutsedge control with alachlor. Weed Sci. 21: 354-357.
8. Audus, L. J. 1964. The Physiology and Biochemistry of Herbicides. Academic Press. London and New York. pp. 163-203.
9. Beestman, G. B. and J. M. Deming. 1974. Dissipation of acetamitide herbicides from soils. Agronomy Journal 66: 308-311.

10. Bingham, S. W. and Richard Shaver. 1971. Uptake, translocation, and degradation of diphenamid in plants. *Weed Sci.* 19: 636-643.
11. Chandler, James M., Eddie Basler and P. W. Santelmann. 1974. Uptake and translocation of alachlor in soybean and wheat. *Weed Sci.* 22: 254-258.
12. Chandler, James M., Lavoy I. Croy and Paul W. Santelmann. 1972. Alachlor effects on plant nitrogen metabolism and the hill reaction. *J. Agr. Food Chem.* 20: 661-664.
13. Cox, T. I. 1974. Experiments on vapor activity of alachlor and related herbicides. *Weed Research* 14: 379-383.
14. Davis, D. W., J. C. Cialone and R. D. Sweet. 1974. Some factors affecting the residual activity of diphenamid. *Proc. N.E.W.C.C.* 18: 100-108.
15. Deli, J. and G. F. Warren. 1970. Uptake, translocation, and herbicidal effect of diphenamid. *Weed Sci.* 18: 692-696.
16. ———. 1971. Relative sensitivity of several plants to diphenamid. *Weed Sci.* 19: 70-72.
17. ———. 1971. Adsorption, desorption, and leaching of diphenamid in soils. *Weed Sci.* 19: 67-69.
18. 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 Research* 10: 316-320.
19. Dhillon, Neimal S. and S. Lamar Anderson. 1972. Morphological, anatomical, and biochemical effects of propachlor on seedling growth. *Weed Research* 12: 182-189.
20. Dubey, H. D., Roy E. Sigafut and J. F. Freeman. 1966. Effect of soil properties on the persistence of linuron and diphenamid in soils. *Agronomy Journal* 58: 208-211.
21. Duke, W. B., F. W. Slife, J. B. Hanson and H. S. Butter. 1975. An investigation on the mechanism of action of propachlor. *Weed Sci.* 23: 142-146.

22. Edmondson, J. B. 1969. Effect of 2-chloro-2',6'-diethyl-N-methoxymethyl acetanalide on cucumber seedling growth. Ph.D. Thesis. Univ. of Illinois. Urbana. 46 pp.
23. Eshel, Y. 1968. Phytotoxicity, leachability, and site of uptake of 2-chloro-2',6'-diethyl-N-methoxymethyl acetanalide. *Weed Sci.* 17: 441-444.
24. ——— and J. Katon. 1972. Effect of time of application of diphenamid on pepper, weeds, and disease. *Weed Sci.* 20: 468-471.
25. ——— and D. Palevetch. 1973. Selective action of diphenamid and napropamide in pepper (*Capsicum annum* L.) and weeds. *Weed Research* 13: 379-384.
26. Frank, R. and C. G. Waywell. 1970. Tomato tolerance to two preemergence herbicides. *Jour. Am. Soc. Hort. Sci.* 95: 370-377.
27. Genter, W. A. 1969. Phytotoxicity of demethylated analogs of diphenamid. *Weed Sci.* 17: 284-285.
28. Good, N. E. 1961. Inhibitors of the Hill reaction. *Plant Physiology* 36: 788.
29. Hamill, Allan S. and Donald Penner. 1972. Interaction of alachlor and carbofuran. *Weed Sci.* 21: 330-334.
30. Harris, C. I. 1964. Movement of dicamba and diphenamid in soils. *Weeds* 12: 112-115.
31. ———. 1967. Movement of herbicides in soil. *Weeds* 15: 214-216.
32. Heilman, Eugene A. 1976. *Herbicide Handbook*. Monsanto Ag. Prod. Co.
33. Hemphill, D. D. and R. L. Baumgartner. 1974. Weed control in transplanted tomatoes. *Proc. NCWCC* p. 71.
34. *Herbicide Handbook of the Weed Science Society of America*. 1974. Third edition.
35. Hickey, Stephen J. and W. A. Krueger. 1974. Alachlor and 1,8 naphthalic anhydride effects on sorghum seedling development. *Weed Sci.* 22: 86-90.

36. Hodgson, Richard, Kendall E. Dusabek and Barry Hoffer. 1974. Diphenamid metabolism in tomato: time course of an ozone fumigation effect. *Weed Sci.* 22: 205-210.
37. Horowitz, M. and Nira Hulin. 1971. Effects of gamma radiation of soil and diphenamid. *Weed Sci.* 19: 294-296.
38. Jaworski, Ernest G. 1969. Analysis of the mode of action of herbicidal α -chloroacetamides. *J. Ag. Food Chem.* 17: 165-170.
39. Katan, J. and Y. Eshel. 1974. Effect of the herbicide diphenamid on damping off disease of pepper and tomato. *Phytopathology* 64: 1186-1192.
40. Kesner, C. D. 1966. The Modes of Action of Diphenamid (N,N-dimethyl-2,2-diphenylacetamide) in Plants. Ph.D. Thesis. Michigan State Univ. East Lansing.
41. Knake, E. L. and L. M. Wax. 1968. The importance of shoot of giant foxtail for uptake of preemergence herbicides. *Weed Sci.* 16: 393-395.
42. Lamoureux, Gerald L., Lester E. Stafford and Fred S. Tanaka. 1971. Metabolism of 2-chloro-N-isopropyl acetanilide (propachlor) in the leaves of corn, sorghum, sugar cane, and barley. *J. Agr. Food Chem.* 19: 346-350.
43. LeBaron, H. M. 1963. Potential use of diphenamid for weed control in horticultural crops of eastern Virginia. *Proc. NEWCC* 17: 44-50.
44. Lynch, R. and R. D. Sweet. 1969. The effect of environment on tomato response to diphenamid. *Proc. WSSC*.
45. _____. 1971. Effect of environment on the activity of diphenamid. *Weed Sci.* 19: 332-337.
46. Narsaiah, D. Bala and R. G. Harvey. 1977. Alachlor and gibberellic acid interaction on corn tissues. *Weed Sci.* 25: 197-199.
47. Nashed, R. B. and R. D. Illnicki. 1968. The effect of diphenamid on the uptake and distribution of macronutrient elements in cabbage. *Proc. NEWCC* 22: 500.
48. Noll, C. J. 1962. An evaluation of chemicals used for the weeding of tomatoes. *Proc. NWCC* 16: 144-146.

49. _____. 1963. Chemical weed control in direct seeded and transplanted tomatoes. Proc. NEWCC 17: 121-124.
50. _____. 1964. Chemical weeding of tomatoes. Proc. NEWCC 18: 83-86.
51. Parochetti, J. V. 1973. Soil organic matter effect on activity of acetanilides, CDAA, and Atrazine. Weed Sci. 21: 157-160.
52. Putnam, Alan R., A. Paul Love, Paul F. Boldt, Robert P. Rice Jr., Ronald H. Lockerman, and Nancy E. Adams. 1976. Herbicide evaluations in vegetable and fruit crops. Horticultural Report 26. Department of Horticulture, Michigan State University. East Lansing.
53. Putnam, Alan R., A. Paul Love, Gregory Pagano and Robert P. Rice Jr. 1973. Weed Control Research. Horticultural Report 22. Department of Horticulture, Michigan State University. East Lansing.
54. Putnam, Alan R., A. Paul Love, Robert P. Rice Jr., Ronald H. Lockerman and Nancy E. Adams. 1975. Herbicide evaluations in vegetable and fruit crops. Horticultural Report 25. Department of Horticulture, Michigan State University. East Lansing.
55. Schultz, Donald P. and B. G. Tweedy. 1972. The effect of light and humidity on absorption and degradation of diphenamid in tomatoes. J. Agr. Food Chem. 20: 10-13.
56. _____. 1971. Uptake and metabolism of N,N,dimethyl-2,2-diphenylacetamide in resistant and susceptible plants. J. Agr. Food Chem. 19: 36-40.
57. Sistrunk, William A. and Ronald Talbert. 1968. The effect of herbicides on quality of tomato and snapbeans. J. Am. Soc. Hort. Sci. 93: 854-859.
58. Smith, A. E. and D. V. Phillips. 1975. Degradation of alachlor by *Rhizoctonia solani*. Agronomy Journal 67: 347-349.
59. Struckmeyer, B. Ester, L. K. Binning and R. G. Harvey. 1976. Effect of dinitroaniline herbicides in a soil medium on snapbean and soybean. Weed Sci. 24: 366-369.

60. Taylorson, R. B. 1965. Delayed preemergence weed control in seeded tomatoes and peppers. Weeds 13: 306-308.
61. ———. 1966. Some effects of herbicides on biochemical constituents of tomato plants. J. Am. Soc. Hort. Sci. 89: 539-543.
62. Tiedje, James M. and Myrna L. Hagedorn. 1975. Degradation of alachlor by soil fungus. J. Agr. Food Chem. 23: 77-80.
63. Timmons, F. D. and H. D. Coble. 1972. Performance of two acetanilide herbicides in organic soils of North Carolina. Proc. S. Weed Science Society 25: 446-451.
64. Yaklich, R. W. and E. G. Scott. 1973. The intracellular localization of the herbicide diphenamid in corn root tip. Physiol. Plant. 28: 447-451.
65. Yu, Ching-Chieh, Gary M. Booth, Dale J. Hansen and Joseph R. Larsen. 1975. Fate of alachlor and propachlor in a model ecosystem. J. Agr. Food Chem. 23: 877-879,