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To the Graduate Council:

I am submitting herewith a dissertation written by Kermit Bruce Kirksey entitled "Clomazone efficacy in snap beans and dissipation in soil." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant, Soil and Environmental Sciences.

Robert M. Hayes, Major Professor

We have read this dissertation and recommend its acceptance:

Charles Mullins, David Coffey, Fred Tompkins

Accepted for the Council:

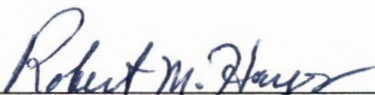
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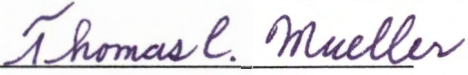
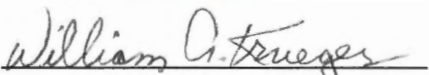
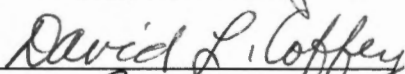
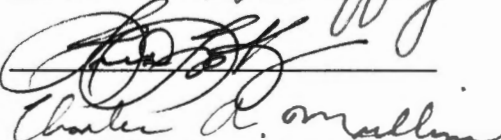
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To the Graduate Council:


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Robert M. Hayes, Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:


Associate Vice Chancellor
and Dean of The Graduate
School

CLOMAZONE EFFICACY IN SNAP BEANS AND DISSIPATION IN SOIL

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Kermit Bruce Kirksey

December, 1994

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Thesis
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DEDICATION

To my wife, Debra Brewer Kirksey, whose tremendous sacrifices made this effort a success; and to my daughter, Haley Marie Kirksey, I dedicate this dissertation.

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ABSTRACT

Studies were conducted from 1991 to 1993 to determine the effects of clomazone on snap bean (*Phaseolus vulgaris*) growth and yield. Field experiments were conducted on Etowah, Sequatchie, and Lily soils. Clomazone was applied preemergence at 0, 0.28, 0.56, 0.84, or 1.12 kg ai/ha in a randomized complete block with treatments replicated four times. Snap beans were evaluated for chlorosis, density and yield. Data were subjected to analysis of variance and means were separated utilizing Fisher's Least Significant Difference at the 0.05 probability level. Visual evaluations were made approximately 2, 4 and 6 wk after treatment in each experiment.

Preliminary studies in 1991 indicated an increase in snap bean yield as clomazone rates increased. In 1992 and 1993, snap beans were injured by clomazone at 1.12 kg ai/ha with chlorosis reaching 50%. However, injury was not observed in each planting. Yields were reduced 30% when significant injury occurred.

Clomazone dissipation under field conditions was also determined. Soil samples were obtained from field studies at approximately 0, 7, 14, 21, 28, 42 and 56 DAT. Clomazone was extracted and analyzed using high performance liquid chromatography.

Clomazone degradation in soil empirically fit pseudo-first order kinetics. Degradation was initially rapid and then degraded gradually resulting in a decrease in concentration. Half-life values ranged from 16 to 53 d. Clomazone adsorption was directly related to organic matter content. K_d values were 0.92, 0.85, and 1.12 for the Etowah, Sequatchie and Lily soil, respectively.

The effects of soil moisture (-1.5 and -0.033 mPa) and temperature (15 and 30 C) on clomazone degradation under controlled conditions were also evaluated. Clomazone degradation was slower under cool, dry conditions than warm conditions. Soil moisture had no influence on clomazone degradation except in the Sequatchie soil. After 84 d incubation, clomazone concentrations decreased 25% in the Sequatchie soil at 15 C compared with 75% in the Lily soil.

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PART I
INTRODUCTION

SNAP BEAN PRODUCTION IN TENNESSEE

Snap beans [*Phaseolus vulgaris* L.] are a warm season crop requiring a temperature range of 21 to 27 C. The majority of snap beans produced in Tennessee is located within a 80 km radius of Crossville. Temperatures at the higher elevations of the Cumberland Plateau are suitable for snap bean production.

There were approximately 4800 ha of snap beans produced in 1993 in Tennessee (3). Almost half was sold in the fresh market while the other half was sold as processing beans. Snap beans are very temperature sensitive, with temperatures above 32 C causing flower abortion and temperatures below 10 C drastically reducing plant growth. Higher temperatures can cause two maturity stages which would be undesirable for mechanical harvesting.

Snap beans require approximately 60 d from planting to harvest. Since snap beans are a short-season crop, it is possible for double cropping with another suitable crop. If this practice is performed, care must be taken to avoid pesticide carryover.

Clomazone (2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone) is a herbicide that received full registration in soybeans in February 1986. Clomazone is in the chemical family isoxazolidinone and bears the trade name Command™ (2). Clomazone is available as a 4EC (480 g/L emulsifiable concentrate) and the recommended application rate in other crops is 0.56 to 1.12 kg ai/ha. Clomazone is translocated via symplast or apoplast (2). Clomazone can be applied either preplant incorporated or preemergence.

Clomazone has been examined as a possible new herbicide in snap beans, and is actively being investigated through the IR-4 program. This program is responsible for pursuing pesticide labels on minor use crops. Clomazone would definitely have utility in snap bean weed management. Clomazone controls grasses and some broadleaf weeds such as prickly sida [*Sida spinosa* L.], velvetleaf [*Abutilon theophrasti* Medicus], and spurred anoda [*Anoda cristata* (L.) Schlecht.] (1). Clomazone also controls common purslane [*Portulaca oleracea* L.] which is one to the most troublesome weeds in snap beans because of the difficulty in separating the fleshy stems of common purslane from snap beans during mechanical harvest and grading. Clomazone can be tank-mixed with herbicides already labeled to provide a broader spectrum of control. Clomazone and pendimethalin (N-(1-ethylpropyl)-3,4-dimethyl -2,6-dinitrobenzenamine) are very compatible and this combination would control pigweeds (*Amaranthus spp.*) which is another major weed problem in snap bean production.

In some areas, two crops of snap beans are grown in the same year. It is common to plant a cover crop in the fall following snap bean harvest. Most farmers in Tennessee grow snap beans on the same site for 2 to 3 yr and then rotate to prevent soil-borne diseases. Problems can arise when herbicides persist longer than needed. A herbicide should be active for sufficient duration to prevent weed interference but not long enough to interfere with rotational and winter cover crops.

This research was conducted to determine the effects of clomazone on snap bean growth and yield and also to determine the dissipation and bioavailability of clomazone as affected by moisture and temperature.

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PART II

LITERATURE REVIEW

INTRODUCTION

There were approximately 4800 ha of snap beans grown in Tennessee in 1993. To date, there are relatively few herbicides labeled for use in snap beans. Clomazone was originally labeled only for soybeans [*Glycine max*] and would be expected to be an ideal herbicide for snap beans. Reports have indicated season long control of certain grass and broadleaf weed species with clomazone.

In most areas of Tennessee where snap beans are produced, two crops can be obtained in a single growing season. Where multiple plantings of the same crop are performed, a persistent herbicide could be used with little injury to the crop.

Clomazone. Clomazone is a relatively new herbicide that received full registration for soybeans in 1986. Clomazone (FMC-57020) is in the chemical family isoxazolidinone and bears the trade name Command™. The chemical structure of clomazone is in Figure 1. The first common name proposed for this herbicide was dimethazone. Clomazone is available as a 4EC (480 g/L emulsifiable concentrate) and the recommended application rate is 0.56 to 1.12 kg ai/ha. Clomazone is labeled to be applied either preplant incorporated (PPI) or preemergence (PRE) in Tennessee and is translocated via symplast or apoplast (2). Clomazone can be incorporated 2 to 5 cm deep to reduce the possibility of vapor drift. The use of an agriculturally approved drift additive is recommended when applying spray volumes of 94 to 140 L/ha and is required at higher spray volumes.

Clomazone has an acute oral LD₅₀ for male rats of 2077 mg/kg body weight. Clomazone has a vapor pressure of 19.2 mPa at 25° C and a water solubility of 1.1 g/L (2). The half-life of clomazone in soil has been reported at 15 to 45 d depending on soil series and environmental conditions (2).

Clomazone controls a variety of weeds and is currently labeled for soybeans, cotton [*Gossypium hirsutum*], peppers [*Capsicum annuum* L.], pumpkins [*Cucurbita maxima*], and succulent peas [*Pisum sativum*] (1). Clomazone is a non-ionic herbicide with an octanol-water partition coefficient (K_{ow}) of 350 (2).

Several soil factors can influence clomazone activity. High levels of soil organic matter and clay reduce herbicidal activity (27). Clomazone activity is highest in sandy soils when soil organic carbon levels and cation exchange capacities are low. No correlation between soil pH and clomazone activity exists (10). Soil/water partition coefficients ranged from 8 for a kaolin clay to 60 for a muck soil with 76% organic matter. Leaching studies indicate low mobility (Class 2) of clomazone in sandy loam, silt loam, and clay loam soils and intermediate mobility (Class 3) in fine sand. No crop injury has been noted following a 10 mon fallow period after the herbicide was applied at the labeled rate (6). Reduced organic matter and thus reduced binding would permit greater solubility and increased availability for degradation.

Mechanism of action. Clomazone is a carotenoid inhibitor that stops or reduces accumulation of plastid pigments that protect chlorophyll from photodegradation. It is commonly referred to as a bleaching herbicide, in that it makes susceptible plants white, yellow, or pale-green. The mechanism of clomazone's action is not precisely known.

Carotenoids play a part in light-harvesting and energy-transfer in plants, but probably the most important function of carotenoids is to prevent photodynamic damage to chlorophyll. Photodynamic damage is caused by singlet oxygen (O_1^{\cdot}). Carotenoids prevent photodynamic damage during photosynthesis (11). Clomazone drastically reduced plastid ultrastructure even though chlorophyll accumulation was only reduced 65 to 75% (8).

Phytylation of chlorophyllide is inhibited by clomazone either by inhibition of phytol or geranylgeraniol synthesis or through the inhibition of attachment of one of these terpenes to chlorophyllide (9). Duke et al. (9) examined the effect of clomazone on chloroplast development in 5-d old etiolated pitted morningglory [*Ipomoea lacunosa* L.] cotyledons. Protochlorophyllide content was not affected but clomazone exterminated carotenoid accumulation. The Shibata shift, which is the spectral shift that occurs when chlorophyllide is converted to chlorophyll, was reduced suggesting that phytol levels were also reduced. Duke concluded that clomazone blocks both diterpene and tetraterpene synthesis.

Clomazone's mechanism of action occurs in the carotenoid biosynthesis pathway (Figure 2.). Bleaching of treated tissue is observed, but phytoene and phytofluene do not accumulate (6). Carotenoids are not formed in the presence of clomazone and this leads to the development of etiolated, white seedlings. Unlike most carotenoid synthesis inhibitors, clomazone also inhibits seedling growth (7,8,9). More detailed analysis of the terpenoid synthesis pathway in the presence of clomazone has shown that inhibition by clomazone occurs after farnesyl-PP; the synthesis of sterols, which originate from the

triterpenoid squalene, is not affected (8). However, diterpene (C₂₀) and tetraterpene (C₄₀) syntheses are both inhibited in the presence of clomazone (7,8,23,24). The block in the *in vivo* synthesis of terpenoids by clomazone may therefore be localized between farnesyl-PP and geranylgeranyl-PP. The synthesis of the diterpenoid compound phytol is also not affected, resulting in decreased chlorophyll phytylation and membrane integration (7,9). Consequently, chlorophyll accumulation is reduced in the presence of clomazone. Another very important diterpene derivative is the plant growth hormone gibberellic acid (GA₃). Interestingly, 100 μM GA₃ reverses the growth inhibition induced by 100 μM clomazone in *Pisum sativum* (23).

Activity in soils. Most soil-applied herbicides require moisture for activation. However, due to the high water solubility of clomazone, only a small amount of moisture is needed for herbicide activation. Effective weed control can be obtained after 0.64 cm of rainfall (26). If sufficient rainfall does not occur within 14 d after clomazone application, but there is enough soil moisture to germinate weeds and/or crops, a light cultivation with a rotary hoe or similar implement will uproot these small weeds. The shallow mixing of clomazone with a rotary hoe will not interfere with its activity when sufficient soil moisture is available. Once clomazone is transported to the seed germination zone, it is tightly adsorbed to soil particles and organic matter and is thereby protected from leaching when rainfall occurs. Factors such as sunlight and soil pH that can influence the activity of some herbicides in the field, have little or no effect on clomazone. The seemingly incompatible properties of high water solubility, resistance to leaching,

insensitivity to sunlight and soil pH, help clomazone move into and remain in the germination zone for season-long control.

Other factors can influence clomazone activity in soils. High levels of organic matter and clay can reduce herbicidal activity (27). Soil organic matter appears to be the primary soil property influencing clomazone adsorption. Clomazone activity is highest when CEC and organic carbon levels are low in sandy soils. Research was conducted by Gallandt et al. (10) to evaluate clomazone dissipation in two Montana soils. Clomazone was applied at three rates and soil samples were taken at monthly intervals. The soil series used included a Bozeman silty clay loam and a Willow creek loam. Residual levels of clomazone were measured using an oat-shoot bioassay. Clomazone dissipated in the loam soil to levels below 0.1 mg/kg in 3 mon and 0.2 mg/kg after 6 mon in the silty clay loam. Half-lives were determined to be 33 and 37 d for the loam and the silty clay loam, respectively. Their results indicated that clomazone residues from labeled rates should not injure wheat in a wheat-fallow-wheat cropping system in Montana.

Loux et al. (19) conducted experiments to study the availability and persistence of clomazone in two soils (Cisne silt loam and Drummer silty clay loam). Bioassays were conducted to determine the range of concentrations that was required for corn [*Zea mays*] injury. Clomazone remaining in the soil was determined using HPLC. Clomazone availability was greater in the Cisne soil than in the Drummer soil. Half-lives were determined to be 22 d for the Cisne soil and 49 to 58 d for the Drummer soil.

Clomazone was not detected by extraction 1 yr following application in the Cisne soil but residues were detected in the Drummer soil 3 yr after initial application.

In a similar study conducted by Loux et al. (18), the adsorption of clomazone on soils, sediments and clays was evaluated. Clomazone adsorption on 19 different soils and sediments resulted in a positive correlation between adsorption constants and soil organic carbon content. Adsorption was not correlated with the clay content indicating that organic coatings on clays may block adsorption sites on clay surfaces. Organic matter content has the greatest effect on bioactivity of clomazone. Bioactivity has been negatively correlated with clay content and CEC (25).

Research was conducted by Salzman et al. (22) to determine if a synergistic interaction occurs with clomazone plus metribuzin (4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one) and clomazone plus linuron (*N*'-(3,4-dichlorophenyl)-*N*-methoxy-*N*-methylurea) due to the effects of one herbicide on root uptake, partitioning, or metabolism of the other. Their results indicate that binding of clomazone or its metabolites in an unextractable form may be a method of deactivating clomazone by soybean but not by common cocklebur (*Xanthium strumarium*). Results from their study also indicated that the metabolism of metribuzin or linuron is altered in both species when clomazone is applied, leading to increased phytotoxicity.

Selectivity mechanisms. The selectivity of clomazone appears to be from differential metabolism (2). Although very limited information is available concerning clomazone selectivity, several studies have been pursued.

Keifer (16) evaluated the tolerance of corn lines to clomazone. Corn hybrids differ in tolerance to various herbicides. The mechanism of selectivity among hybrids is the result of the operating level of a mechanism and not necessarily separate mechanisms. Corn hybrid injury was measured by levels of discoloration. Temperature effects on corn susceptibility to clomazone were also evaluated. Corn injury did not change with temperature and temperature did not affect relative phytotoxicity. Apparently, the tolerant corn hybrids transferred clomazone tolerance to the next generation. With a knowledge of the parents of a tolerant hybrid, it should be possible to breed corn that is tolerant to clomazone, or to determine a hybrid's tolerance.

Liebl et al. (17) conducted studies to evaluate mechanisms for clomazone selectivity in corn, soybean, smooth pigweed [*Amaranthus hybridus* L.], and velvetleaf. Plants were treated with clomazone 2 d after transplanting 6-d old seedlings. Soybean was the most tolerant followed by velvetleaf, corn, and smooth pigweed. Soybean did not translocate clomazone acropetally which may account in part for the tolerance of soybean to clomazone. Soybean tolerance to clomazone was not entirely due to uptake, translocation or metabolism. Liebl suggested that the mechanism of tolerance was differences at the enzymatic site of action.

The tolerance of tomato (*Lycopersicon esculentum*) and bell pepper to clomazone was evaluated by Weston et al. (30) to determine selectivity mechanisms. Clomazone injury was observed 10 d after soil application. Bell peppers were 40 times more tolerant than tomatoes. A higher percentage of ¹⁴C-clomazone was recovered in bell pepper roots than in tomato roots. Clomazone was metabolized into two methanol-

soluble metabolites in the roots 48 h after treatment. Differences between root and shoot metabolism of both plant species occurred and further studies indicated that these metabolites may have conjugated with sugar. Differential uptake, translocation, or metabolism did not account for observed selectivity.

The uptake of clomazone or its metabolism in higher plants is not well established. Vencill (28) et al. reported that clomazone absorption could be species dependent since uptake by sensitive species was greater than uptake by tolerant species. In another study, Vencill reported that, *in vitro*, a deactivating mechanism of clomazone could be conjugation with glutathione (29).

Off-site movement and injury situations. When herbicide vapors remain in the soil matrix, herbicide efficacy can be enhanced by volatilization (14). Once herbicide vapors escape the soil matrix and enter the environment, injury to non-target plants and reduced weed control can occur (21). Off-site movement is very common with clomazone and can cause chlorosis of non-target species. Due to high vapor pressure, clomazone can volatilize and move from the treatment site to non-target plants and cause chlorosis or whitening in the plant foliage. Clomazone can only be applied preplant incorporated in some northern states, but Tennessee has a label for preemergence application as well. In Tennessee, clomazone can not be applied within 457 m of towns and subdivisions, commercial fruit or vegetable production, and commercial greenhouses or nurseries (1). Off-site movement can be influenced by many factors including wind speed, spray pressure, particle size, nozzle type, boom height and drift retardants.

Soil must be in good tilth for adequate incorporation of clomazone. Poor weed control and off-site movement may occur through vapor drift if clomazone is not incorporated appropriately. Environmental factors that contribute to off-site movement or volatilization of clomazone include application to wet soil, air and soil temperature, wind speed, and precipitation (12). Other practices such as spray droplet size, herbicide incorporation and increased levels of surface residue characteristic of conservation tillage can also affect herbicide volatilization.

Application method and tillage practice affect volatilization of clomazone (26). Clomazone from volatilization was detected up to 2 wk after PRE or PPI application. Volatilization increased following rainfall but varied between years. Clomazone volatilization was in the order of no-tillage > minimum tillage > conventional tillage. Thelen's results agree with previous research indicating greater volatilization from a straw mulch compared to soil (13). Surface residue may increase surface area for volatilization of clomazone. Higher soil moisture associated with the soil cover may contribute to greater volatilization.

Weed control and insecticide safening. There are several herbicides currently labeled for snap bean production in Tennessee. These include EPTC (*S*-ethyl dipropyl-carbamothioate), pendimethalin, trifluralin (2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl)benzenamine), metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide), bentazon (3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide), and sethoxydim (2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-

3-hydroxy-2-cyclohexen-1-one). Clomazone is not labeled for weed control in snap beans.

Clomazone controls grass weeds such as barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), broadleaf signalgrass (*Brachiaria platyphylla* (Griseb.) Nash), large crabgrass (*Digitaria sanguinalis* (L.) Scop.) and smooth crabgrass (*Digitaria ischaemum* (Schreb. ex Schweig.), foxtails (*Setaria spp.*), and other annual grasses, and broadleaf weeds such as velvetleaf, spurred anoda, and prickly sida (1).

Combinations of two or more pesticides are commonly used in crop production. Herbicide-insecticide combinations can interact to either reduce control or enhanced control. Mullins et al. (20) conducted studies in 1988, 1989, and 1990 to determine the effects on insect and weed control when sethoxydim was applied alone or in combinations with various insecticides currently labeled in Tennessee. Various combinations of sethoxydim and insecticides did not appear to cause interactive effects on weed control.

Hurst (15) studied cotton injury from clomazone. Clomazone was applied at 0.84 or 1.1 kg ai/ha. Very poor emergence of cotton was observed 21 DAP. However, after replanting, clomazone at 1.1 kg/ha reduced the replant stand at 21 d when aldicarb ([2-methyl-2-(methylthio)propionaldehyde-*O*-(methylcarbamoyl)oxamine]) was used in-furrow but no stand reductions occurred when phorate (*O,O*-diethyl *S*-[2-(ethylthio)methyl]phosporodithioate) or disulfoton (*O,O*-diethyl *S*-[2-(ethylthio)ethyl]phosporodithioate) was used in-furrow. His results indicated that cotton was protected from clomazone injury when phorate or disulfoton was used in-furrow. By using clomazone with phorate or

disulfoton applied in-furrow, cotton producers can take advantage of the weed control spectrum of clomazone without injury (5,33).

Clomazone is also labeled for weed control in cotton. The product can only be used if either phorate or disulfoton is applied at planting. York et al. (32) reported the effect of aldicarb, disulfoton, and phorate on the safening of cotton to clomazone. All insecticides were applied in-furrow and clomazone was applied at 0 to 1.12 kg ai/ha PPI or PRE. Disulfoton and phorate greatly reduced clomazone-induced chlorosis, stunting and death of cotton seedlings. Their results are in agreement with similar studies concluding that disulfoton and phorate effectively protect cotton seedlings from clomazone toxicity (3,4). Cotton injury ranged from 15 to 63% when clomazone was applied with aldicarb. However, when aldicarb was applied in combination with disulfoton and phorate, cotton was protected (31).

Due to the persistent nature of clomazone, rotational crop restrictions apply. Cotton and soybeans may be planted anytime after application at any labeled rate. No more than 1.4 kg ai/ha can be applied in one season. At the 0.56 kg ai/ha rate, cotton, soybeans, peppers, pumpkins and peas can be rotated. At the 0.84 to 1.12 kg ai/ha rate, all of these may be planted except peas. Corn, cucurbits (*Cucumis spp.*), dry beans (*Phaseolus spp.*), peanuts (*Arachis hypogaea*), rice (*Oryza sativa*), snap beans, sorghum (*Sorghum bicolor*), sugar beets (*Beta vulgaris*), sweet potatoes (*Ipomoea batatas*), tobacco (*Nicotiana tabacum*) and transplanted tomatoes may be planted as rotation crops following clomazone at 0.56 kg ai/ha. All of the above plus peas may be planted after 9 mon. Pumpkins can be planted following clomazone at 1.4 kg ai/ha.

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APPENDIX

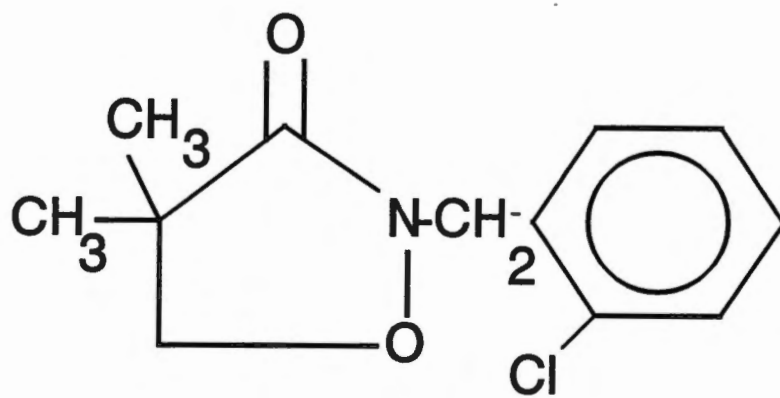


Figure 1. Chemical structure of clomazone.

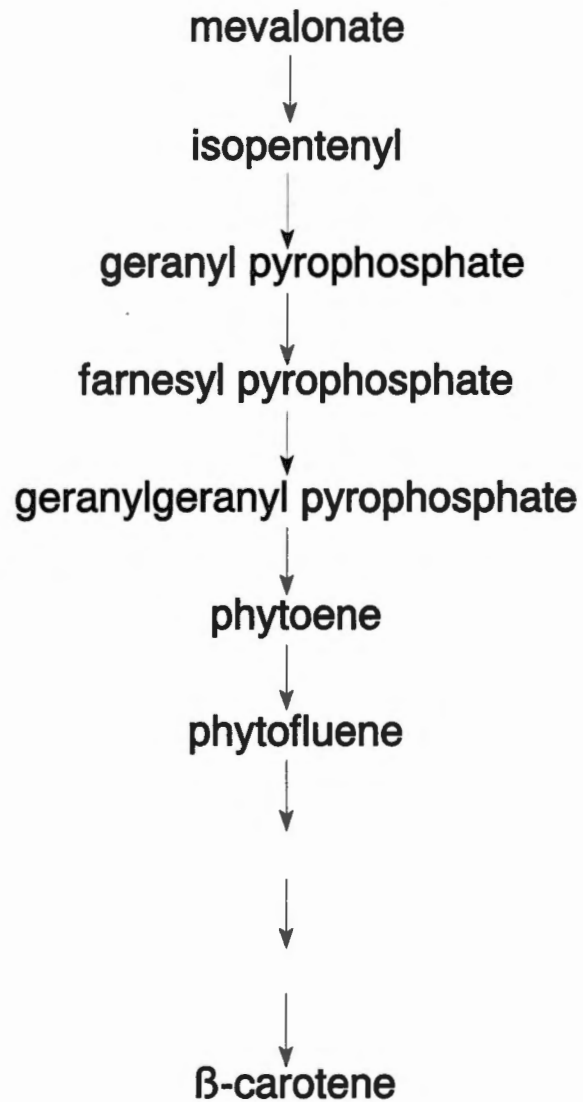


Figure 2. Pathway of carotenoid biosynthesis.

PART III

CLOMAZONE EFFICACY IN SNAP BEANS AND DISSIPATION IN SOIL.

1. FIELD EFFICACY AND CLOMAZONE BIOAVAILABILITY

ABSTRACT¹

Field and greenhouse studies were conducted to determine the effect of clomazone on growth and yield of snap beans. Field experiments were conducted at Knoxville in 1991 (one planting), 1992 and 1993 (two plantings each year) and at Crossville in 1992 and 1993 (three plantings each year). Clomazone injured snap beans (stand reduction, delayed emergence, bleaching) and reduced yield 1500 kg/ha on the Sequatchie soil at rates ≥ 0.84 kg ai/ha. No injury was observed at rates ≤ 0.56 kg/ha clomazone. Approximately 0.3 $\mu\text{g/g}$ clomazone remained in the soil 56 DAT in all experiments except for one planting at Crossville, suggesting a potential for injury to rotational crops such as wheat. Where a second crop of snap beans is grown in the same season, clomazone residues could be detrimental to the second crop or other crops in rotation if additional herbicides are applied. Nomenclature: Clomazone, (2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone); Snap beans, *Phaseolus vulgaris* L. cv. 'Blue Ridge'.

Additional index words. Persistence, rotation.

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INTRODUCTION

Clomazone (2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone) is a herbicide that is registered for use in soybeans [*Glycine max* (L.) Merr.], succulent peas (*Pisum sativum*), peppers (*Capsicum annuum* L.), pumpkins (*Cucurbita maxima*) and cotton (*Gossypium hirsutum*) (1). Weed control advantages of clomazone have been previously documented (4,6,9,19). Clomazone controls prickly sida (*Sida spinosa* L.), velvetleaf (*Abutilon theophrasti* Medicus), spurred anoda [*Anoda cristata* (L.) Schlecht.], several other broadleaf weeds and annual grasses. Clomazone is applied either PPI or PRE.

Clomazone is classified as a carotenoid inhibitor. Carotenoids help protect chlorophyll from photodynamic damage (3). This inhibition appears as a pale or whitish-green color in susceptible plants, often called 'bleaching'.

Weed control in vegetable production is often difficult due to the limited number of labeled herbicides. Clomazone has been evaluated for weed control in vegetable crops, including sweet potatoes (*Ipomoea batatas*) (13), spinach (*Spinacia oleracea*)(8), sugar beets (*Beta vulgaris*) (14,15), crambe (*Crambe abyssinica*)(18), pinto beans (*Phaseolus vulgaris*)(5), cabbage (*Brassica oleracea* L.)(7), navy beans (*Phaseolus vulgaris*)(16), and other cole crops (17). Clomazone is being considered as a new herbicide in snap beans through the IR-4 program (12).

The major weed problems in snap beans include common ragweed (*Ambrosia artemisiifolia* L.) and common purslane (*Portulaca oleracea* L.). Both are difficult to

separate during mechanical harvest operations. Clomazone controls these weeds (1). Pendimethalin (*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitro benzenamine) is labeled for snap beans and can be tank-mixed with clomazone to control pigweed (*Amaranthus spp*).

Previous research has indicated carryover potential with clomazone. Loux et al. (11) reported clomazone detection in soil 3 yr after the initial application. Kendig et al. (10) reported that clomazone at 0.28 kg/ha did not affect wheat yield but it was believed that wheat had increased seeds per head and increased tillers per plant to compensate for the injury.

The objectives of this study were to evaluate the effects of clomazone on the growth and yield of snap beans, to study dissipation of clomazone under field conditions, and to determine if clomazone could play a major role in snap bean production.

MATERIALS AND METHODS

Field studies. Preliminary studies were performed in 1991 to evaluate the effects of clomazone on snap bean growth and yield. Clomazone was applied both PPI and PRE at 0.28, 0.43, 0.56, 0.72, 0.86 and 1.12 kg/ha. Plots were maintained weedfree through the duration of the experiment. Stand density and yields were determined.

Snap beans were planted under conventional tillage system at three planting dates (June 23, July 14, July 25; and June 24, July 12, August 12) at the Plateau Experiment Station in Crossville and two dates (May 19, August 18; and June 8, August 26) at the Knoxville Experiment Station-Plant Science Unit in 1992 and 1993, respectively. Each

experiment was fertilized according to standard procedures for snap bean production. The experiments were conducted at different sites for every planting each year and a complete pesticide history was determined for each location to insure no contaminants or persistent herbicides had been used. The soil at Knoxville was of the Etowah series (fine-loamy, siliceous, thermic typic Paleudults) with a pH of 6.0 and organic matter of 1.7% in 1991 and 1992, and a Sequatchie series (fine-loamy, siliceous, thermic humic Hapludults) with a pH of 5.9 and organic matter of 1.7% in 1993. The soil at Crossville was a Lily loam (fine-loamy, siliceous, thermic typic Hapludults) with a pH of 6.0 and organic matter of 2.2%.

Snap beans var. 'Blue Ridge', were planted in 91 cm rows at Knoxville and 107 cm rows at Crossville in 1992 and 1993. Equipment and management practices were typical of snap bean production. Plant spacing was approximately one plant per 15 cm. Treatments included clomazone at 0, 0.28, 0.56, 0.84 and 1.12 kg/ha PRE. The entire experiment was maintained weed-free by mechanical cultivation and hand-hoeing. Data collected included plant density, visual chlorosis, and yield. Soil samples were taken at approximately 0, 7, 14, 21, 28 and 56 days after treatment (DAT) from plots containing 1.12 kg/ha to determine clomazone activity. Soil samples were taken with a core sampler to a depth of 15 cm and were immediately placed in a freezer. These samples were used in the greenhouse bioassay. Plot size consisted of four rows by 9.2 m and treatments were replicated four times.

Time of application study. Based on observed injury in 1992, additional field studies were performed in 1993. Snap beans were planted in 91 cm rows at Knoxville and 107

cm rows at Crossville on June 6, 1993, and June 16, 1993, respectively. Clomazone was applied at 1.12 kg/ha 0, 1, 2, 3 and 5 days after planting. By day five, snap bean plants had emerged. Visual evaluations were made at both locations and yield was taken at Crossville.

Greenhouse bioassay. The greenhouse bioassays were initiated on November 25, 1992, and on January 30, 1994. Soil samples were removed from the freezer and allowed to thaw overnight (16 h). Approximately 450 g soil was placed into a 0.5 L plastic cup. Twelve seeds of 'Madison' wheat were planted approximately 1.3 cm deep into each cup. All cups were filled with soil, planted, and watered for 1 hr with a mist irrigation system. Wheat injury ratings were taken 4 wk after planting (WAP²). Wheat stand, height and percent chlorosis were determined. Height was recorded in cm and percent injury was measured on a scale of 0 to 100 where 0 was green and 100 was completely white. Shoot fresh weights and dry weights were also determined. A standard curve was performed each year with clomazone at 0, 0.25, 0.5, 0.75 and 1.0 $\mu\text{g/g}$ in 1992 and 0, 0.125, 0.25, 0.38, 0.5, 0.625, 0.75, 0.85, and 1.0 $\mu\text{g/g}$ in 1993. Standard curves were used to predict clomazone concentration based on responses to wheat. Only the linear portion (the part of the line that is changing) was used to predict clomazone concentration.

Statistical analysis. Treatment means were calculated using least squares analysis and least squares means were separated using Fisher's protected LSD at $P = 0.05$. The means were fit to a simple linear model by least squares statistical methods.

²Abbreviations: WAP, weeks after planting.

RESULTS AND DISCUSSION

Field studies. In preliminary studies in 1991 at Knoxville, greater yields were obtained with clomazone PRE than with PPI treatments (Figure 1). Snap bean injury was more prevalent and yields were reduced when clomazone was applied PPI. Yields increased as clomazone (PRE) rates increased.

At 15 DAT in the early planting at Knoxville in 1992, clomazone at 1.12 kg/ha injured snap bean 15% (Figure 2). Injury due to clomazone has been observed in several crops, however, plants such as soybeans can metabolize clomazone to non-toxic compounds and no yield reduction occurs. At Knoxville in 1993, chlorosis occurred early but snap beans recovered except where clomazone was applied at 1.12 kg/ha. Snap bean density was reduced with clomazone at 1.12 kg/ha. Snap bean injury 15 DAT increased by 17% for every kg clomazone at Knoxville. Dry conditions in 1993 forced snap beans to abort flowers and yields were not taken from either planting.

At the late planting at Crossville in 1992, snap bean injury 15 DAT was 60% when treated with clomazone at 1.12 kg/ha (Figure 2). Snap bean injury decreased to 20% by 35 DAT (data not shown). Snap bean stands did not differ among treatments either year. Some injury (5%) was detected early at all plantings in Crossville in 1993, but by 28 DAT, no injury was observed. Clomazone did not influence snap bean yield in 1993.

Clomazone reduced plant height 3.4 cm/kg of clomazone at 15 DAT in 1992 at Knoxville (Figure 2). By 23 DAT, snap bean height ranged from 22 cm in the untreated

check to 7 cm with clomazone at 1.12 kg/ha. At 23 DAT, snap bean height was reduced 11.8 cm/kg of clomazone. Snap bean injury was reflected in reduced yields at Knoxville as clomazone rate increased (Figure 3).

Periods of cold weather followed by warm weather and excessive rainfall caused injury and snap beans developed root rot at Crossville in 1992. Yields were acceptable however, and no injury from clomazone was observed. Yield from snap beans treated with clomazone was not different from the untreated checks.

Time of application study. Snap beans were planted in 1992 at Knoxville but because of rainfall, clomazone was not applied until 3 d later. No injury was observed from applying clomazone to germinated snap beans. This led to experiments involving application timings to determine if snap beans were more tolerant to clomazone as the germination process progressed. Experiments conducted at Crossville and Knoxville in 1993 indicated <5% injury to snap bean plants when clomazone was applied within 5 d after planting, the last application being to emerged plants. After 14 DAT on the fifth day, some visual chlorosis was observed, but by 28 DAT, no injury was detected. Snap bean stands or yields were not affected among application timings (data not shown).

Greenhouse bioassay. Greenhouse bioassays with wheat indicated that $\geq 0.5 \mu\text{g/g}$ of clomazone was present at 0 DAT in 1992 and 1993 when compared to the standard curve. Chlorosis was observed in some untreated checks due to contamination by drift, soil splashing with mist irrigation, or factors unknown. Clomazone was usually detected through the end of the sampling period, with the exception of Crossville's first planting in 1992.

Visual evaluation of chlorosis was taken prior to wheat harvest. Once concentration levels reached $0.5 \mu\text{g/g}$ when compared to the standard curve, no further chlorosis could be detected from any trials, except the first planting at Crossville in 1992, where the plants were completely bleached (Figure 4). Chlorosis decreased with time from samples in both years of the bioassay.

Wheat height was reduced by clomazone (Figure 4). As clomazone concentration increased to $0.5 \mu\text{g/g}$, no difference in wheat height could be detected. No difference occurred with dry weights (Figure 5) probably due to the length of time wheat had to grow. As concentration increased to $0.5 \mu\text{g/g}$, fresh weights decreased.

Using data from the standard curve (Figure 6 and 7), clomazone concentrations were predicted based on observed measurements. Chlorosis was the most obvious symptom of clomazone, however the upper limit of detection in the bioassay was approximately $0.5 \mu\text{g/g}$. Predicted clomazone concentration decreased as the number of days after treatment increased (Figure 8).

Similar results from the greenhouse study were obtained in 1993. As clomazone concentration decreased, wheat height and fresh weight increased, and chlorosis decreased. Standard curve data was variable in 1993 and concentrations were not predicted. The greenhouse study was performed in January and cold temperatures may have affected wheat growth.

Snap bean injury from clomazone was observed in field studies. Most injury occurred in the Sequatchie soils when clomazone was applied at 1.12 kg/ha . Symptoms included chlorosis, reduction in snap bean height, stand, and yield. Clomazone was

applied up to 5 d after planting without significant injury to snap bean. Injury was observed 14 DAT to emerged snap bean, however, snap beans recovered and yields were not affected.

Results from the greenhouse bioassay indicated that 100% bleaching of wheat can occur when clomazone concentrations reach 0.5 $\mu\text{g/g}$. This is equivalent to applying 0.56 kg/ha in the field. Results indicate that 28 DAT, 50% of the applied clomazone may remain and injure susceptible crops such as wheat.

Clomazone may cause initial yellowing or chlorosis to snap beans with the majority of snap beans overcoming symptoms with no reduction in yield. Yield was not reduced with low rates (0.56 kg/ha) of clomazone. Clomazone at 1.12 kg/ha caused the greatest injury and yield was reduced on the Sequatchie soils. These data also demonstrate that during late plantings of snap bean, clomazone has the potential to persist into a cover crop such as wheat. These results are in agreement with those of Kendig et al. (10) where they observed visual injury to wheat following clomazone. These low concentrations are capable of causing detrimental damage to wheat. Even low concentrations (0.25 $\mu\text{g/g}$) of clomazone can cause 'bleaching' of wheat.

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APPENDIX

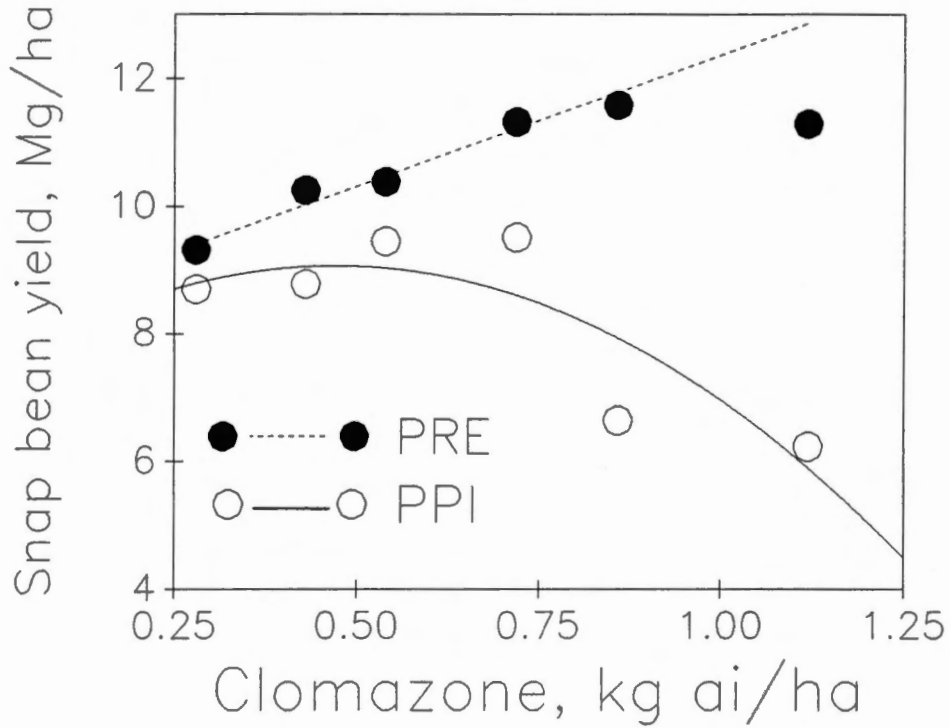


Figure 1. Effects of application method and rate of clomazone on snap bean yield in 1991 at Knoxville. Lines represent regression curves for each application method. Regression equations: PPI: $\hat{Y} = 7.4 + 7.07(\text{rate}) - 7.51(\text{rate})^2$, $r^2 = 0.72$, PRE: $\hat{Y} = 2.47(\text{rate}) + 9.06$, $r^2 = 0.72$.

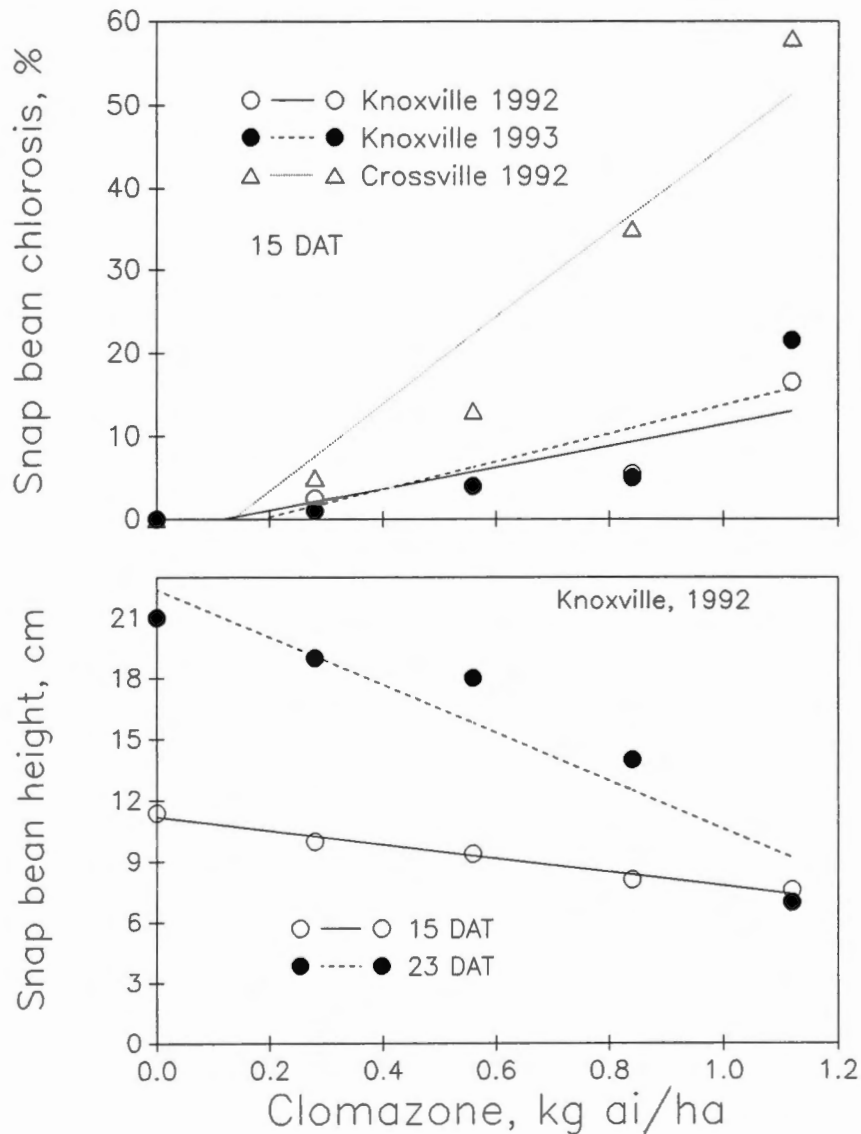


Figure 2. Effect of clomazone rate on snap bean injury and height. Injury data are pooled over plantings and height data for Knoxville are pooled over plantings. Regression equations for snap bean injury: Knoxville 1992, $\hat{Y} = 12.9(\text{rate}) - 1.5$, $r^2 = 0.80$; Knoxville 1993, $\hat{Y} = 16.8(\text{rate}) - 3.1$, $r^2 = 0.72$; Crossville 1992, $\hat{Y} = 52.1(\text{rate}) - 7.0$, $r^2 = 0.92$. Regression equations for snap bean height: 15 DAT, $\hat{Y} = -3.4(\text{rate}) + 11.2$, $r^2 = 0.98$; 23 DAT, $\hat{Y} = -11.8(\text{rate}) + 22.4$, $r^2 = 0.89$.

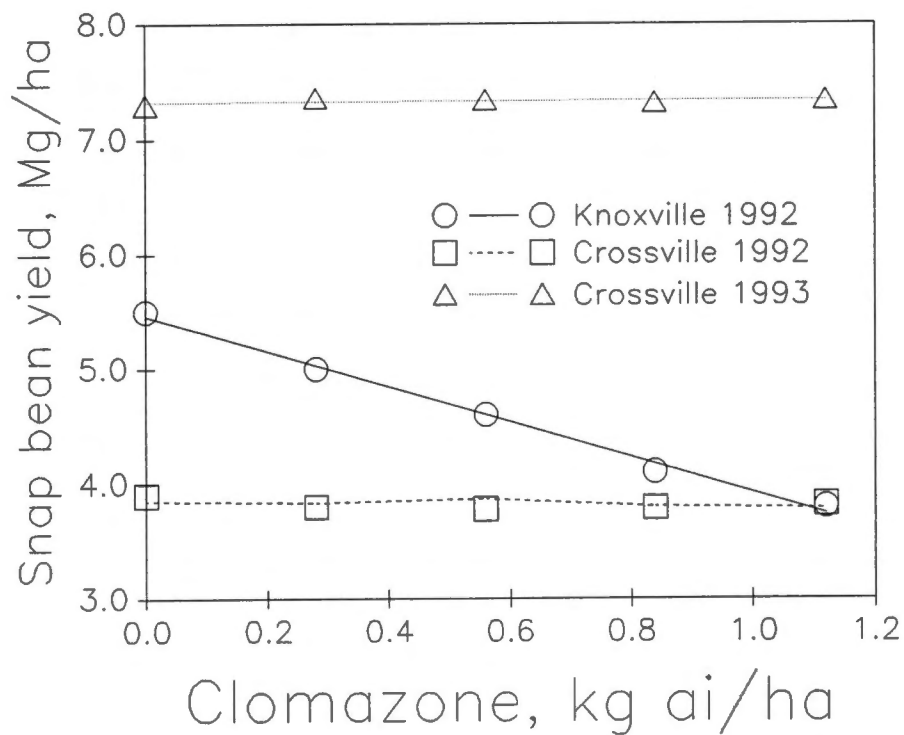


Figure 3. Effect of clomazone rate on snap bean yield. Data are pooled over plantings each year. Regression equation: Knoxville, 1992, $\hat{Y} = -1.53(\text{rate}) + 5.46$, $r^2 = 0.99$. Clomazone did not affect snap bean yield at Crossville in 1992 or 1993.

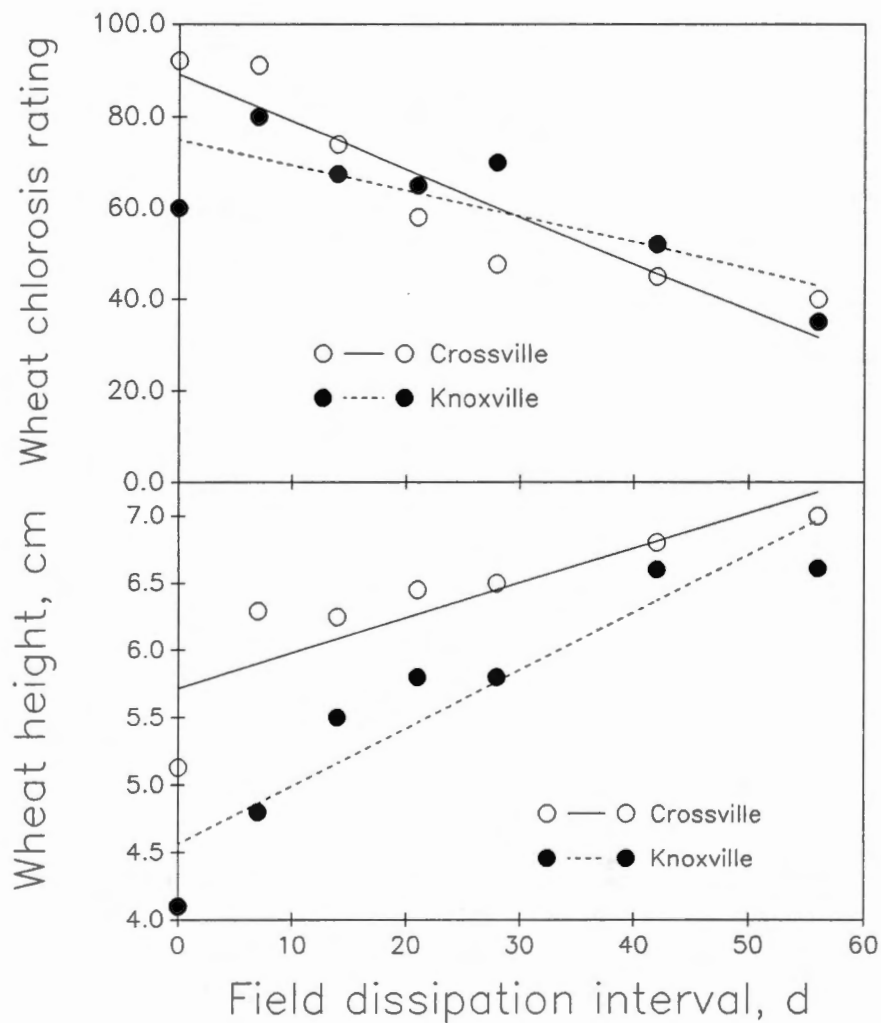


Figure 4. Chlorosis and height of wheat grown in the greenhouse in soil removed at various times from snap bean plots treated with clomazone at 1.12 kg/ha in 1992. Evaluations made 28 d after wheat was planted. Data pooled over plantings. Regression equations: Knoxville-chlorosis, $\hat{Y} = -0.56(\text{days}) + 75$, $r^2 = 0.60$; Crossville-chlorosis, $\hat{Y} = -1.04(\text{days}) + 89.2$, $r^2 = 0.86$; Knoxville-height, $\hat{Y} = 0.043(\text{days}) + 4.56$, $r^2 = 0.87$; Crossville-height, $\hat{Y} = 0.026(\text{days}) + 5.72$, $r^2 = 0.73$.

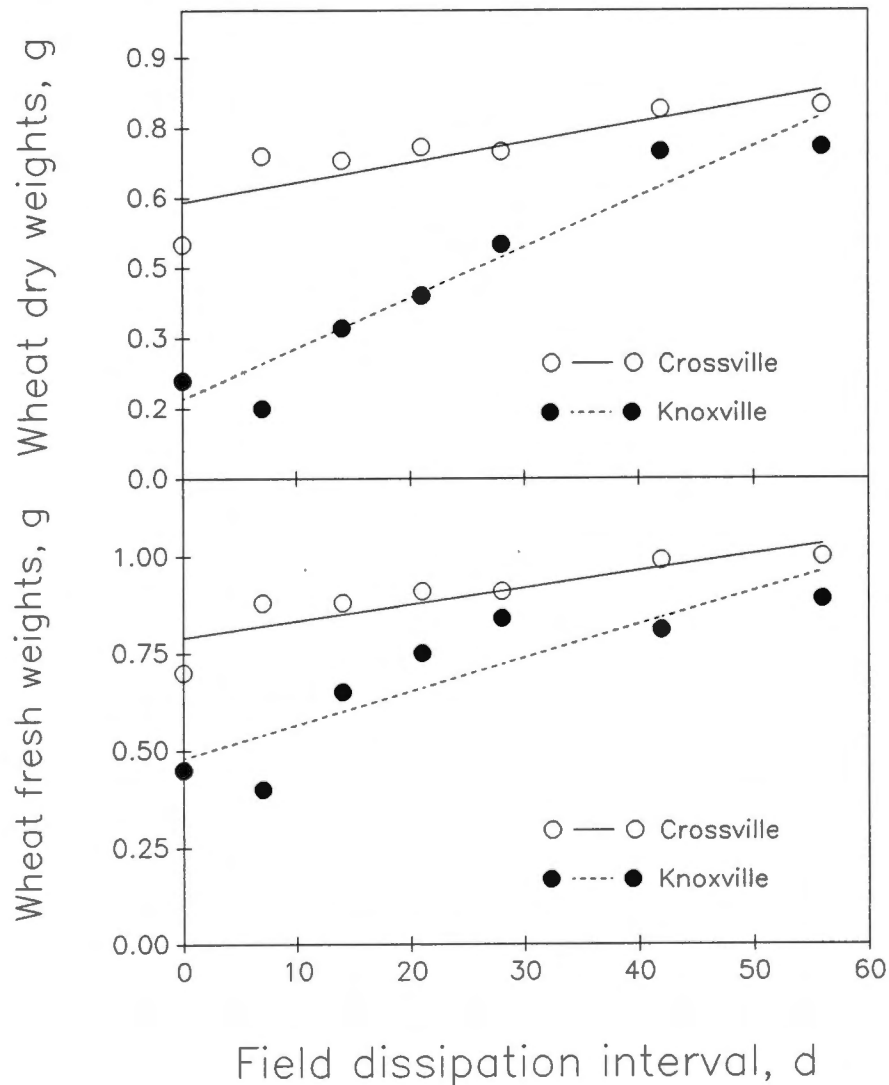


Figure 5. Fresh and dry weights of wheat grown in the greenhouse in soil removed at various times from snap bean plots treated with clomazone at 1.12 kg/ha. Evaluations made 28 d after wheat was planted. Data pooled over plantings and years. Regression equations: Knoxville-dry weight, $\hat{Y} = .0108(\text{days}) + 0.17$, $r^2 = 0.93$; Crossville-dry weight, $\hat{Y} = .0043(\text{days}) + 0.59$, $r^2 = 0.72$; Knoxville-fresh weight, $\hat{Y} = .0086(\text{days}) + 0.48$, $r^2 = 0.77$; Crossville-fresh weight, $\hat{Y} = .0043(\text{days}) + 0.79$, $r^2 = 0.75$.

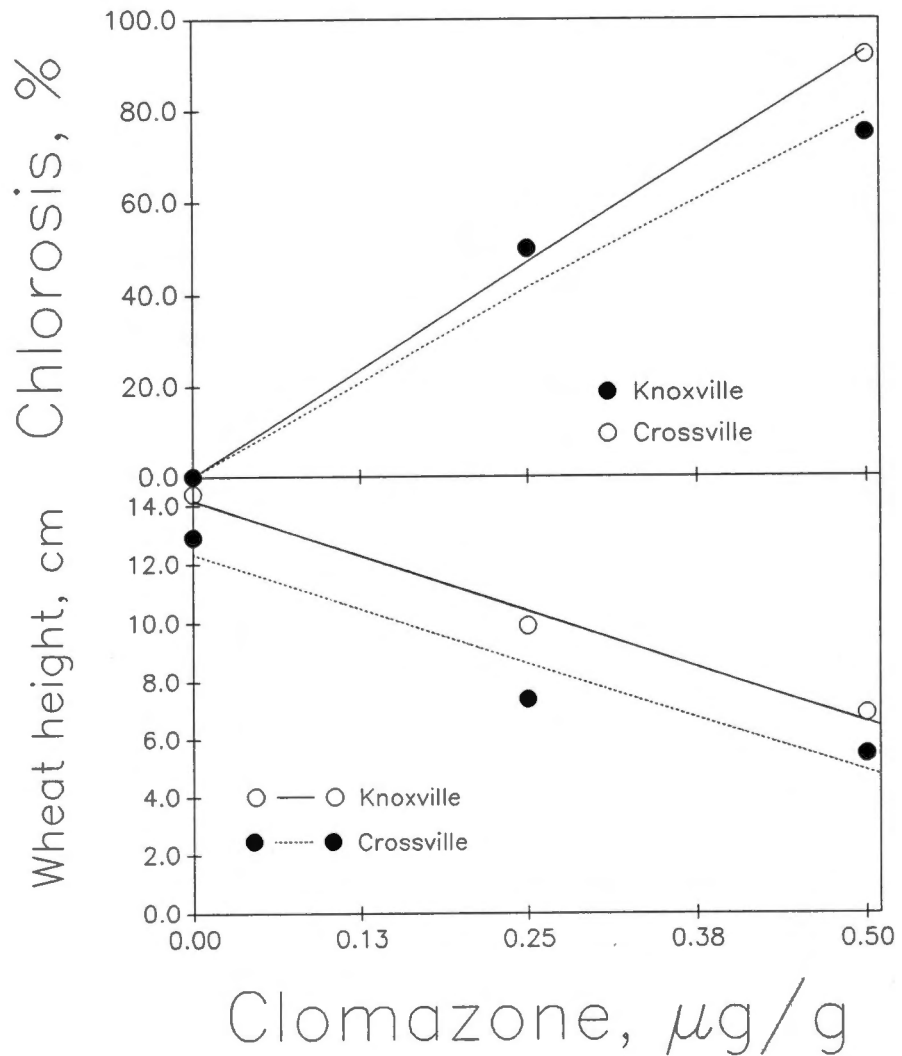


Figure 6. Standard curves for wheat chlorosis and height in soil containing various rates of clomazone in 1992. Evaluations made 28 d after wheat was planted. Regression equations: Knoxville-chlorosis, $\hat{Y} = 183.33x + 1.39$, $r^2 = 0.99$; Crossville-chlorosis, $\hat{Y} = 150x + 4.17$, $r^2 = 0.96$; Knoxville-height, $\hat{Y} = -15x + 14.16$, $r^2 = 0.99$; Crossville-height, $\hat{Y} = -14.84x + 12.3$, $r^2 = 0.93$.

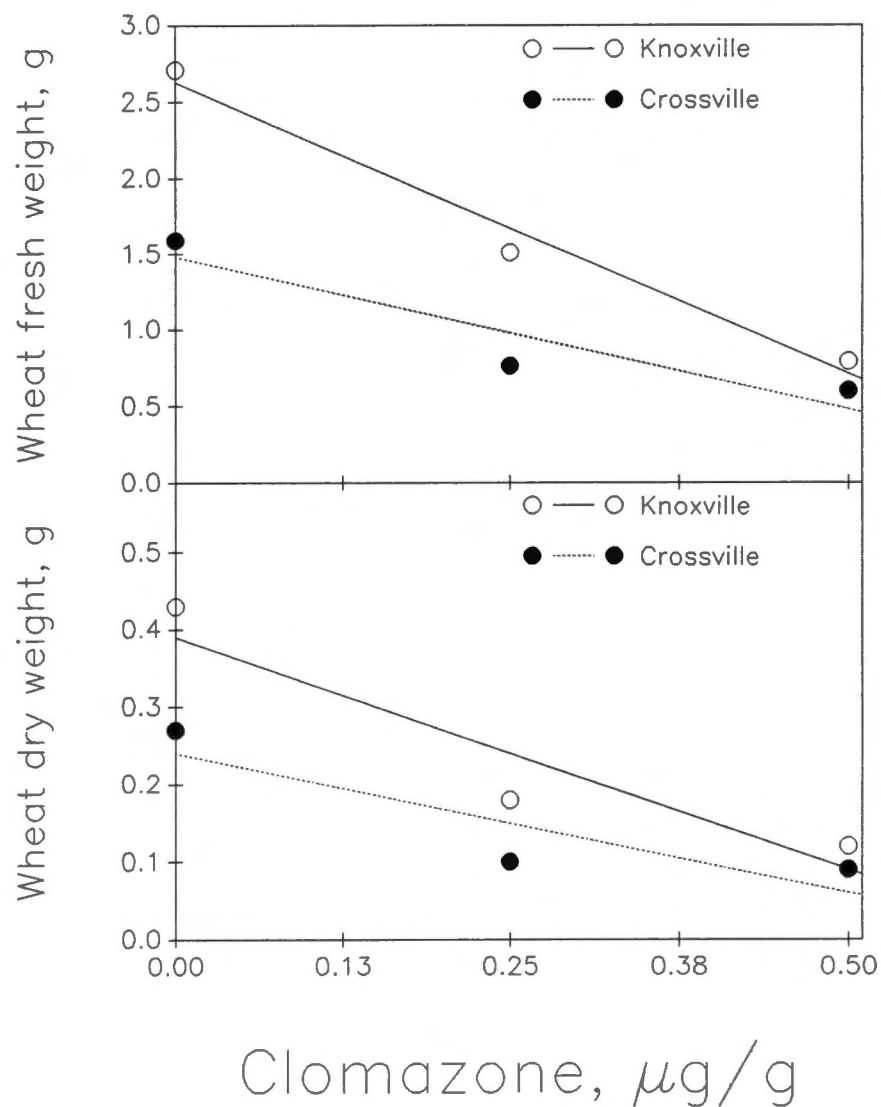


Figure 7. Standard curves for wheat fresh and dry weights in soil containing various rates of clomazone in 1992. Evaluations made 28 d after wheat was planted. Regression equations: Knoxville-fresh wt., $Y = -3.84x + 2.63$, $r^2 = 0.98$; Crossville-fresh wt., $Y = -1.98x + 1.48$, $r^2 = 0.87$; Knoxville-dry wt., $Y = -0.61x + 0.39$, $r^2 = 0.88$; Crossville-dry wt., $Y = -0.36x + 0.24$, $r^2 = 0.87$.

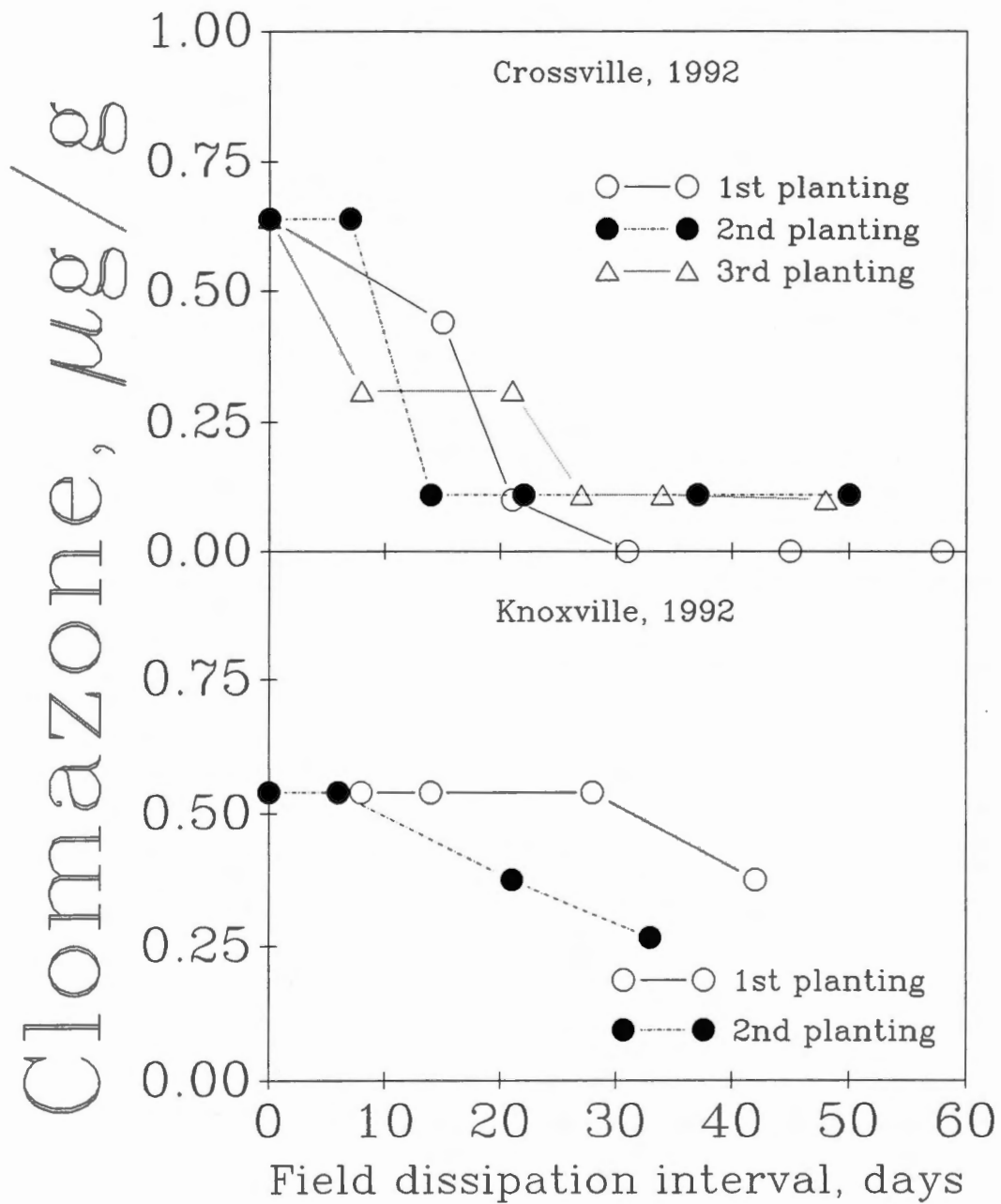


Figure 8. Predicted clomazone concentration according to wheat chlorosis bioassay in soil sampled at various times after application of 1.12 kg ai/ha at Knoxville and Crossville in 1992. All concentrations designated 0.5 are actually $\geq 0.5 \mu\text{g/g}$.

PART IV

CLOMAZONE EFFICACY IN SNAP BEANS AND DISSIPATION IN SOIL.

**2. EFFECT OF EXTRACTION SYSTEMS ON CLOMAZONE RECOVERY
FROM AGED SOIL SAMPLES.**

ABSTRACT¹

Seven extraction methods were examined to determine which method provided a simple, efficient clomazone extraction from aged soil. The method selected was when 80 ml acetonitrile was added to moist soil and samples were allowed to equilibrate 16 h. The extract was filtered and an additional 80 ml acetonitrile was added and the sample allowed to equilibrate for another hour, and filtered again. The filtrates were combined and concentrated using a rotary evaporator. Clomazone concentrations were then determined using UV detection coupled with reverse-phase liquid chromatography. Recovery using this method was equal to a 16 h + 1 h extraction with methanol, but acetonitrile was selected for the extraction solvent due to its ease of removal with a rotary evaporator.

INTRODUCTION

Clomazone (2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone) is a soil-applied herbicide registered for use in soybeans (*Glycine max*), cotton (*Gossypium hirsutum*), succulent peas (*Pisum sativum*), peppers (*Capsicum annum*) and pumpkins (*Cucurbita maxima*) (2). Clomazone inhibits carotenoid synthesis thus reducing accumulation of plastid pigments that protect chlorophyll from photodegradation. It is

¹To be submitted to J. Assoc. Off. Anal. Chem. Authors: Bruce Kirksey and Thomas Mueller. Research Associate and Asst. Prof., respectively, The University of Tennessee, Dept. of Plant and Soil Sci., Knoxville, TN 37901.

commonly referred to as a 'bleaching' herbicide, in that the new growth of susceptible plants are white, yellow, or pale-green in appearance (3). Most soil-applied herbicides need water for activation. However, due to its high water solubility (1.1 g/L), only a minimal amount of moisture is needed for activation. Research and field trials have demonstrated effective weed control following 0.64 cm of rainfall (1). Once clomazone is transported to the seed germination zone, it is tightly adsorbed to soil particles and organic matter, and is thereby protected from leaching.

Clomazone can be applied in the field at 0.5 to 1.1 kg/ha and normal soil concentrations at the time of application are $< 2.0 \mu\text{g/g}$. Clomazone extractions have been performed by Loux et al. (4). Clomazone residues were extracted by shaking 50 g of soil with 100 ml of acetonitrile. Samples were centrifuged for 10 min. This procedure was performed twice, samples were filtered, and the extracts concentrated with a rotary evaporator. The sample was redissolved in solvent prior to UV detection with liquid chromatography. Loux employed a C_{18} reversed-phase column with a mobile phase of acetonitrile and water at a flow rate of 1.5 ml/min for separation. Clomazone recovery using this technique was 82%.

The objective of this study was to determine a simple, efficient extraction method for clomazone from aged soil samples. Clomazone has been reported to be tightly adsorbed to soil constituents, especially organic matter (4). We were concerned that our extraction system be sufficiently exhaustive to remove clomazone, even that tightly adsorbed to soil. Due to the chemicals having a relatively weak chromophore, concentration of the extraction solvent to enhance method sensitivity was required (data

not shown).

MATERIALS AND METHODS

Apparatus and reagents

(a) *Liquid chromatograph.* - Waters liquid chromatography system, including model 680 control unit, model 717 auto-injector, model 510 solvent delivery system, model 486 UV detector at 220 nm, and a Waters model 740 integrator.

(b) *Analytical column.* - LC-C₁₈ 25 cm by 4.6 mm id, 5 μm, in-line 1 cm by 1.5 mm pellicular C₁₈ guard column (Alltech, Chicago, IL USA).

(c) *Solvents.* - LC-grade (J.T. Baker, Inc., Phillipsburg, NJ 08865; and Burdick and Jackson, Muskegon, MI).

(d) *Mobile phase.* - Isocratic acetonitrile-water (60 + 40 v/v) (0 to 9 min); followed by solvent flush (acetonitrile-water (80 + 20 v/v) for 9 min).

(e) *Analytical standards.* - Clomazone (FMC Corporation, Philadelphia, PA). Standards were >95% pure and each was used without purification.

Soil selection. The soil used in method development was a Lily silt loam (fine-loamy, siliceous, thermic typic Hapludults) with a pH of 6.0 and an organic matter content of 2.0%. The sample utilized for method development was a field sample taken 14 d after clomazone (1.12 kg ai/ha) application. The sample was taken from the center of a plot and three soil cores were taken from that plot, homogenized in the field, and placed in

freezer at -10 C for 30 d. The soil was allowed to thaw, passed through a 2 mm sieve and thoroughly homogenized again for use.

UV-detection. Clomazone absorbance spectrum was determined utilizing a Shimadzu UV-Visible Recording Spectrophotometer Model UV-260. Maximum absorbance occurred between 215 and 220 nm (spectrum not shown). Sherma reported previous clomazone methods measured absorbance at 254 nm (5). Various combinations of acetonitrile:water and methanol:water were evaluated to determine clomazone retention time and capacity factor (K') for the mobile phase (Table 1). Methanol was also used but did not improve results and had higher operating pressure. The mobile phase selected was 60:40 acetonitrile:water (v/v). Due to the interference from soil extracts, a solvent flush (80% acetonitrile) was added. Total run time per sample was 30 min.

Extraction. Seven extraction regimes were examined. The methods included:

- 1) acetonitrile for 1 h
- 2) acetonitrile for 16 h (overnight)
- 3) methanol for 16 h
- 4) acetonitrile for 1 h followed by (fb) acetonitrile for 1 h
- 5) acetonitrile for 16 h fb acetonitrile for 1 h
- 6) methanol for 16 h fb methanol for 1 h
- 7) acetonitrile for 1 h fb acetonitrile for 1 h fb acetonitrile for 1 h

Moist soil (40 g dry weight basis) was placed in 250 ml Nalgene™ bottles with screw-top caps (Nalgene, Rochester, NY) and either acetonitrile or methanol (80 ml) added. The bottles were then placed on a reciprocating shaker set to operate at 240 excursions per minute, then shaken for the indicated times. After equilibration, the bottles were allowed to settle for < 2 h, and then the extract was filtered through two Whatman #1 filter papers (Whatman, Clinton, NJ, USA) to remove particulates. The samples were filtered once and most of the liquid was removed. Once all extractions were made, the samples were then concentrated with a rotary evaporator in a 45° C water bath at 8 rad/s. The concentrated clomazone extract (<2 ml) and three acetonitrile rinses were brought up to 10 ml in a volumetric flask, and duplicate samples were placed in 4 ml autosampler vials.

RESULTS AND DISCUSSION

Injection of 50 μL of clomazone standard produced a peak with retention time of 4.9 to 13.7 min, depending on mobile phase composition (Table 1). Peak tailing was encountered under isocratic conditions, so a solvent flush was used to improve resolution. The system was flushed with acetonitrile-water (80:20) for 9 min before injection of the next sample. The clomazone standard curve (0 to 10 $\mu\text{g/g}$) was linear with an r^2 of 0.99 (data not shown). Figure 1 depicts liquid chromatograms of clomazone detection.

Extracting soil samples in 80 ml acetonitrile 16 h followed by an additional extraction with 80 ml acetonitrile for 1 h extracted 458 ng/g \pm 18 (Table 2). Extractions

for 16 h with methanol followed by an additional extraction with 80 ml methanol for 1 h also were efficient (448 ng/g \pm 14). Advantages to using acetonitrile rather than methanol include faster concentration on rotary evaporator and full compatibility between extraction solvents and mobile phase.

This method of clomazone extraction is simple but tedious. However, all solvents used are relatively safe for use and represent minimal health risk to researchers.

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APPENDIX

Table 1. Solvent systems and retention times for clomazone.

Mobile Phase	Solvent ratio	Retention time	K' ^a
	v/v	min	
acetonitrile:water	80 : 20	4.9	0.97
acetonitrile:water	70 : 30	6.1	1.45
acetonitrile:water	60 : 40	8.4	2.37
acetonitrile:water	50 : 50	13.7	4.46
methanol:water	80 : 20	7.7	2.08
methanol:water	75 : 25	9.7	2.88

^aDwell time for system = 2.5 min.

Table 2. Clomazone concentrations from soil with various extraction methods.

Treatment	Clomazone
	ng/g \pm sd ^a
1. acetonitrile 1 h	276 (\pm 29)
2. acetonitrile 16 h	421 (\pm 23)
3. methanol 16 h	313 (\pm 8)
4. acetonitrile 1 h fb acetonitrile 1 h	358 (\pm 35)
5. acetonitrile 16 h fb acetonitrile 1 h	458 (\pm 18)
6. methanol 16 h fb methanol 1 h	448 (\pm 14)
7. acetonitrile 1 h fb acetonitrile 1 h fb acetonitrile 1 h	375 (\pm 21)

^a Mean values from triplicate samples, \pm standard deviation

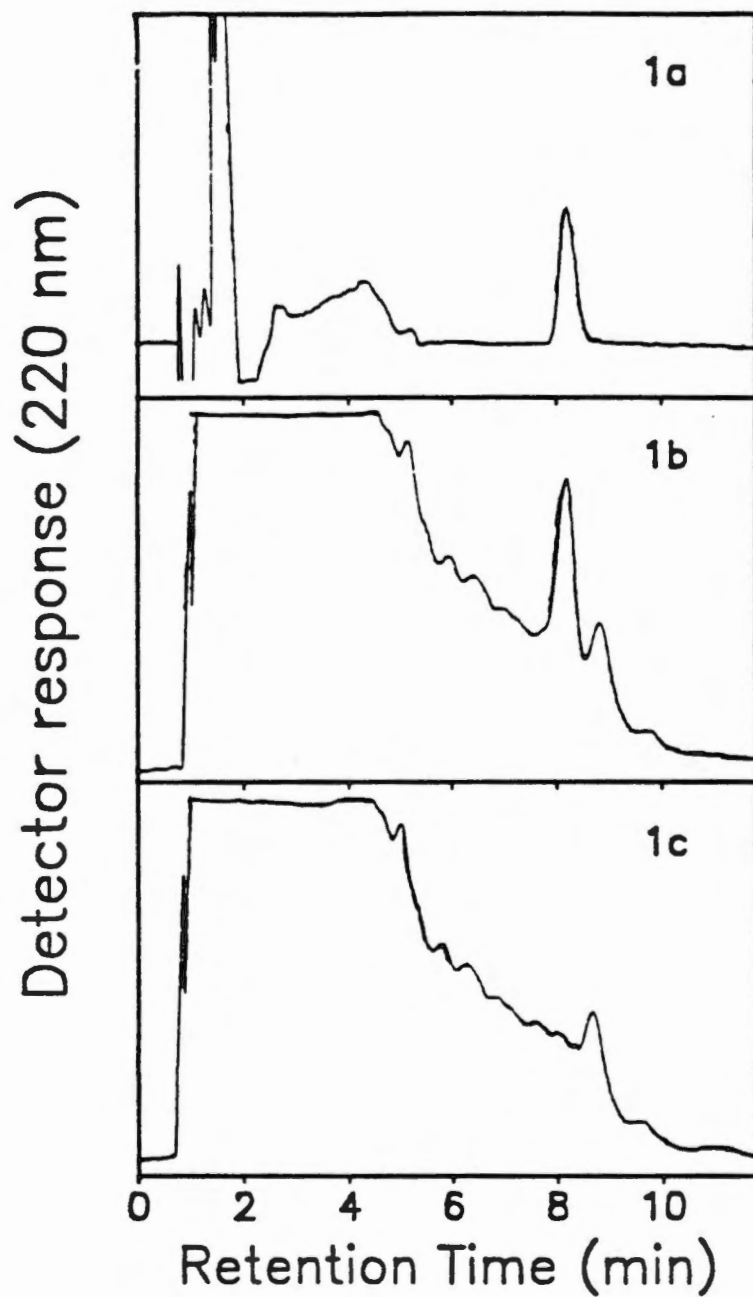


Figure 1. Liquid chromatograms of (1a) analytical standard containing 250 ng/g clomazone; (1b) extracts from soil containing 450 ng/g clomazone; and (1c) extracts from soil containing no clomazone using acetonitrile:water (60:40) mobile phase.

PART V

CLOMAZONE EFFICACY IN SNAP BEANS AND DISSIPATION IN SOIL.

**3. FIELD DISSIPATION AND DEGRADATION UNDER CONTROLLED
CONDITIONS**

ABSTRACT¹

Clomazone dissipation in Etowah, Sequatchie, and Lily soils was described in field and laboratory experiments. Soil samples were collected to a depth of 8 cm at 0, 7, 14, 21, 28, 42, and 56 DAT, immediately frozen, and later analyzed. Clomazone degradation was examined in incubator studies, with temperatures of 15 and 30 C and moisture contents of -1.5 and -0.033 mPa. Liquid chromatographic analysis indicated initial clomazone concentrations of 0.44 to 1.03 $\mu\text{g/g}$ soil where 1.12 kg/ha was applied PRE. Clomazone concentrations decreased with time, and by 56 DAT approximately 30% of initial clomazone was detected in all plantings except the early planting at Crossville in 1992 where none was detected at 42 DAT. Other than the latter exception, clomazone detected at 56 DAT could injure rotational crops. Half-lives (DT_{50}) in the field were 10 to 42 d for the Etowah and Lily soils and 15 to 64 d for the Sequatchie soil. Clomazone degradation was slower in Sequatchie soil at 15 than 30 C. Soil moisture had no effect on degradation rate except in the Sequatchie soil at 15 C where approximately one-half of the applied clomazone was detected 84 DAT. DT_{50} ranged from 42 to 66 d for the Lily loam and 60 to 90 d for the Sequatchie soil. Where a second crop of snap beans is grown in the same season, clomazone residues from 1.12 kg ai/ha could be detrimental to the second crop or other crops in rotation.

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Nomenclature, (2-[(2-chlorophenyl) methyl]-4,4-dimethyl -3-isoxazolidinone).

Additional index words. Persistence, soil temperature, soil moisture, rotational crops.

INTRODUCTION

Clomazone (2-[(2-chlorophenyl) methyl]-4,4- dimethyl-3 -isoxazolidinone) is a versatile herbicide that is labeled for soybean [*Glycine max*], cotton [*Gossypium hirsutum*], peas [*Pisum sativum*], peppers [*Capsicum spp.*], and pumpkins [*Cucurbita spp.*]. Residual velvetleaf [*Abutilon theophrasti* Medicus], spurred anoda [*Anoda cristata* (L.) Schlect.] and prickly sida (*Sida spinosa* L.) control is obtained with clomazone at the labeled rate.

Clomazone applied at labeled rates did not inhibit wheat in a wheat-fallow-wheat cropping system (4). Injury has been observed in corn [*Zea mays*], wheat, alfalfa [*Medicago sativa* L.], and oats [*Avena sativa* L.] the year following clomazone application (5). Severe damage can occur that may result in the death of wheat plants. Clomazone applied in the spring to conventional tobacco [*Nicotiana tabacum*] plant beds continued to have activity to fall-planted wheat (9).

Soil factors exist that influence clomazone activity. High soil organic matter, cation exchange capacity and clay content can reduce herbicidal activity (6,11,15). Clomazone activity is highest in sandy soils where organic carbon levels and cation exchange capacity are low. No correlation exists between soil pH and herbicide activity (4).

Clomazone is a non-ionic herbicide with a water solubility of 1100 $\mu\text{g/g}$ and an octanol-water partition coefficient of 350 (2). Clomazone has a vapor pressure of 19.2 mPa at 25° C (2). The half-life of clomazone in soil has been reported at 15 to 45 d depending on soil series and environmental conditions (2). In previous studies, activity and rate of dissipation of clomazone were reduced in fine-textured soils compared to medium-textured soils (10). Soil series also influences rate of degradation and mobility of clomazone in soil (3).

To date, studies involving the effects of temperature and moisture on the dissipation of clomazone are not well documented. The objectives of the following studies were to evaluate clomazone degradation under field conditions in two Tennessee soils and to evaluate the effects of ambient temperature and moisture content on clomazone degradation under controlled conditions.

MATERIALS AND METHODS

Field dissipation. Clomazone dissipation under field conditions was studied at Crossville and Knoxville, TN from 1991 through 1993. Treatments included clomazone at 1.12 kg ai/ha PRE and an untreated control. The soil at Knoxville was of the Etowah series (fine-loamy, siliceous, thermic typic Paleudults) with a pH of 6.0 and organic matter of 1.7% in 1991 and 1992, and a Sequatchie series (fine-loamy, siliceous, thermic humic Hapludults) with a pH of 5.9 and organic matter of 1.7% in 1993. The soil at Crossville was a Lily loam (fine-loamy, siliceous, thermic typic Hapludults) with a pH of 6.0 and

organic matter of 2.2%. Field plots were tilled and clomazone was applied with a CO₂-pressurized tractor-mounted sprayer. Plot size was 3.7 m by 9.2 m and treatments were replicated four times in a randomized complete block design. There was one experiment at Knoxville in 1991 and two in 1992 and 1993. There were three experiments at Crossville in 1992 and 1993. Soil samples were taken at approximately 0, 7, 14, 21, 28 and 56 DAT from each plot. Three representative soil samples were obtained from plots with a core-type sampler to a depth of 8 cm, homogenized in a container and subsampled. The subsamples were sealed and immediately frozen to await extraction (<90 d).

Chemical extractions. Moist soil (40 g) from each treatment was weighed and placed in 250 ml polyethylene bottles with screw-top caps with 80 ml of acetonitrile. The bottles were placed on a reciprocating shaker which was then operated at 240 excursions per min for 16 h. Each sample was extracted, filtered through two Whatman² #1 filter papers, and the filtrate collected. An additional 80 ml of acetonitrile was added and allowed to equilibrate on a shaker for 1 h, and filtered as previously described. The filtrates were combined, weighed, and concentrated to near dryness (< 2 ml) at 50 C with a rotary evaporator. The concentrate was transferred to a 10 ml volumetric and brought to volume. An aliquot was transferred to a 4 ml vial and stored until quantification by high performance liquid chromatography (HPLC)³ using an external

²Whatman, Clinton, NJ.

³Abbreviations: HPLC, high performance liquid chromatography; K_d, distribution coefficient.

standard technique.

Clomazone (95% purity) was dissolved in HPLC grade acetonitrile to obtain 0, 0.25, 0.50, and 1.0 $\mu\text{g/g}$ standards. The total run time was 30 min with a conservative lower limit of detection of 0.2 ng/g. The mobile phase was acetonitrile and water (60:40) with UV detection at 220 nm. Retention time on samples was approximately 8 min. The standard curve for clomazone had an $r^2 = 0.99$ (data not shown).

Clomazone adsorption study. Adsorption of clomazone onto Sequatchie and Lily soils was determined using a slurry technique similar to that of Talbert and Fletchall (14). Soil was passed through a 2 mm screen and 10 g moist soil containing no clomazone was added to 50 ml plastic screw-top centrifuge tubes. Twenty ml of 0.01 M CaCl_2 solution containing either 0 or 1 $\mu\text{g/ml}$ of analytical clomazone was added to each tube. Tubes were capped and placed on a reciprocating shaker then operated at 240 excursions per min for 16 h. After equilibrating, samples were centrifuged at 2600 g for 10 min. The samples were filtered through two Whatman No. 1 filter papers to remove particulates. Supernatants were then quantified by HPLC as previously described. Centrifuge tubes containing clomazone solution but no soil were included to account for any clomazone adsorption by the tube. Any reduction in clomazone concentration was assumed to be from adsorption by the soil. Distribution coefficients (K_d), the ratio of herbicide adsorbed to that remaining in solution, were calculated. Chemical and physical properties of the soils are in Table 1.

Degradation study. Degradation studies under controlled conditions were conducted to quantify clomazone degradation in two Tennessee soils. Bulk surface soil (50 kg)

samples were obtained from each location. The soils had no prior clomazone treatment. Soil was screened to pass a 2 mm sieve. Soil moistures were determined for each soil using pressure plate method (8) to obtain two soil moistures: permanent wilting point (-1.5 mPa) and field capacity (-0.033 mPa).

The study was established as a split-split plot design with a factorial arrangement of treatments, two moisture levels, two temperatures, three soils (Lily, Sequatchie and an autoclaved Sequatchie soil) with fortification level of 3 $\mu\text{g/g}$ clomazone. Sterile soil was autoclaved at 103.4 kPa and 121 C for 10 min. The soil samples were incubated in the dark for 84 d with triplicate samples of each treatment being removed from two incubators at 0, 14, 28, 42, 56, and 84 DAT. One incubator was maintained at 15 C and the other at 30 C.

Moist soil (40 g dry weight equivalent) with the respective moistures was added to a 250 ml polyethylene bottle. Soil moisture content for permanent wilting point and field capacity for the Lily soil was 17.5 and 33.9% and 14 and 28.2% for the Sequatchie soil, respectively. Each bottle was fortified with 1.0 ml of a 120 $\mu\text{g/ml}$ aqueous solution to give an initial clomazone concentration of 3 $\mu\text{g/g}$. Bottles were capped and soil thoroughly mixed to incorporate clomazone into the soil. Samples were incubated the respective number of days and then removed from the incubator and immediately frozen at -10 C until further analysis.

The study included untreated controls that were sampled at 0 and 84 DAT. These soils were not fortified with clomazone, but were otherwise analyzed in the same manner as treated soils. Samples were vented at 42 DAT for 30 min to prevent the formation

of anaerobic conditions. Extraction and chemical analyses were conducted as described previously.

Statistical analysis. Analysis of field and controlled degradation studies utilized General Linear Mixed Models⁴ to obtain treatment means. Regression analysis was performed on the generalized least squares means. Herbicide concentration from field dissipation studies was regressed against time for either linear or quadratic models. Clomazone concentration from degradation studies was regressed against time for first-order or linear models. Half-lives (DT_{50}) for first-order models were calculated by the equation $DT_{50} = 0.693/k$ where k is the first order dissipation rate constant (16).

RESULTS AND DISCUSSION

Field dissipation. Preliminary studies in 1991 at Knoxville indicated a decrease in clomazone concentration in soil over time. Initial concentrations at 0 DAT were 0.7 to 1.3 $\mu\text{g/g}$ (Figure 1). Linear regression analysis revealed a r^2 value of 0.92 and DT_{50} was 35 d.

Dissipation was empirically fit to first-order kinetics at the early planting at Knoxville in 1992 with a gradual decrease in clomazone concentration with time (Figure 2). The approximate clomazone DT_{50} for the first planting at Knoxville was 42 d. Soils containing 0.25 $\mu\text{g/g}$ clomazone will injure susceptible crops such as wheat.

Dissipation followed pseudo-first order kinetics for all other plantings at both

⁴GLMM. General Linear Mixed Models User's Guide. Baton Rouge, LA.

locations each year. The late planting at Knoxville in 1992 had an initial concentration of 0.95 $\mu\text{g/g}$ and DT_{50} was determined to be 11 d.

More clomazone was present 30 DAT in the early planting than in the late planting. This was probably due to lower temperatures and lower soil moisture early in the season.

The early planting at Crossville in 1992 was delayed by rainfall. The experiment was not planted until June 23, which is later than normal for early-season snap bean production. Soil moisture at the time of application was high and could have contributed to vapor loss of clomazone via co-distillation (Table 2). This may account for the low recovery of clomazone since clomazone has a high vapor pressure and volatilization makes 100% recovery unlikely. Initial clomazone concentration was 0.65 $\mu\text{g/g}$ (Figure 3), and concentrations decreased rapidly the first 15 d and to an undetectable level at 45 DAT. Rainfall from 0 to 15 DAT was 14 cm, and the DT_{50} was 10 d. The dissipation curve of the first planting at Crossville demonstrates the importance of soil moisture on clomazone availability.

DT_{50} values were 21 d for the second planting at Crossville (Figure 3). This may have been affected by the high moisture availability at the time of application. Initial concentration was 0.45 $\mu\text{g/g}$ and concentrations gradually decreased with time to 0.15 $\mu\text{g/g}$ by 56 DAT. Soil moisture at all plantings at Crossville in 1992 was unusually high and may have decreased clomazone availability.

The late planting at Crossville in 1992 had an initial concentration of 0.76 $\mu\text{g/g}$. DT_{50} value was determined to be 16 d (Figure 3). By 56 DAT, 0.2 $\mu\text{g/g}$ was detected.

According to results from greenhouse studies reported earlier, this is adequate clomazone in soil to damage wheat.

The data from the first planting at Knoxville in 1993 fit ($r^2=0.98$) pseudo first-order degradation (Figure 4), with a DT_{50} of 15 d. DT_{50} was 64 d at the second planting in Knoxville in 1993 probably due to dry conditions. Initial concentration was $0.73 \mu\text{g/g}$ and at 53 DAT, more than 50% remained in the soil.

Results at Crossville in 1993 indicated the same general trend as in 1992. Clomazone concentration decreased over time (Figure 5). Clomazone DT_{50} values were calculated to be 19 d at Crossville's first planting, 21 d at the second planting, and 17 d for the third planting.

Clomazone adsorption study. Clomazone adsorption was directly related to organic matter content. K_d values were 0.92, 0.85, and 1.12 for the Etowah, Sequatchie and Lily soil, respectively. Clomazone adsorption was greater in the Lily loam with an organic matter content of 2.2% than either of the other soils.

Degradation study. Variations among initial concentrations were greater than anticipated. While the exact source of error is unknown, it is postulated that clomazone, with its high vapor pressure, may have volatilized and was trapped in the bottle during incubation. As sample preparation occurred, clomazone vapors may have been lost to the atmosphere. Clomazone recovery was $>70\%$ in all treatments at 0 DAT except in the sterile soil where sterilization may have altered soil characteristics. Recovery could be influenced by clomazone not being in soil solution, because samples were treated, homogenized, and immediately frozen.

Soil moisture affected degradation in the Sequatchie soil at 15 C (Figure 6). Even though concentrations increased from 0 to 14 DAT, a gradual decrease in concentration was observed. Clomazone degradation was faster in dry soil. At permanent wilting point, clomazone was degrading at the rate of 20 ng/g/d, while at field capacity the rate was only 9 ng/g/d. DT_{50} values were 65 and 90 d for -1.5 and -0.033 mPa, respectively. Soil moisture did not affect degradation rate at 30 C in the Sequatchie soil (Figure 7), with DT_{50} , pooled over moistures, of 60 d.

Neither soil moisture nor temperature affected degradation in Lily loam, so data were pooled across moisture and temperature. It should be noted, however, that a greater initial concentration was detected in the cooler soil. Clomazone dissipated at the rate of 20 ng/g/d (Figure 8), and the corresponding DT_{50} was 42 d.

Initial clomazone concentration as determined by HPLC in autoclaved Sequatchie soil was one-fourth of the other treatments, although they had been identically fortified (Figure 9). Autoclaving may have created binding sites and recoveries do not correspond to that of field conditions. The DT_{50} for sterile soil was 84 and 63 d at 15 and 30 C, respectively. Clomazone at 15 C was degrading at 4.5 ng/g/d while at 30 C, the rate was 6 ng/g/d. Autoclaved soils were vented 42 DAT and this caused loss of sterile conditions. Clomazone degradation was slower in sterile soils compared to nonsterile soils, indicating microbial degradation to be a major mechanism of clomazone degradation.

There are still questions concerning the initial recovery and variability. Clomazone may have been adsorbed to soil colloids or lost as a vapor during sample

preparation. Further study is needed in this area to determine why recovery was lower in the autoclaved soil. Clomazone dissipation gradually decreased with time and under most situations, dissipation was described by first-order kinetics. Clomazone dissipation was slower in the Sequatchie soil that was cool and at field capacity. Approximately one-half of the applied clomazone was detected 84 DAT. These data indicates DT_{50} of 42 to 90 d. In these soils under the described conditions, longer DT_{50} 's were observed than in other field dissipation studies (10).

In field dissipation studies, soil temperature and moisture may play a vital role in clomazone activity. There seems to be a relationship between clomazone dissipation and availability. Clomazone dissipation was most rapid where availability was greatest. In degradation studies where moisture and temperatures were controlled, data indicates that under cool conditions, clomazone degradation is slower and there is a potential for injury to rotational crops. After 84 d incubation, greater clomazone concentrations were detected in cooler soils, regardless of soil moistures. Since snap beans are a short season crop, careful planning in rotational crops is vital to prevent injury from carryover.

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APPENDIX

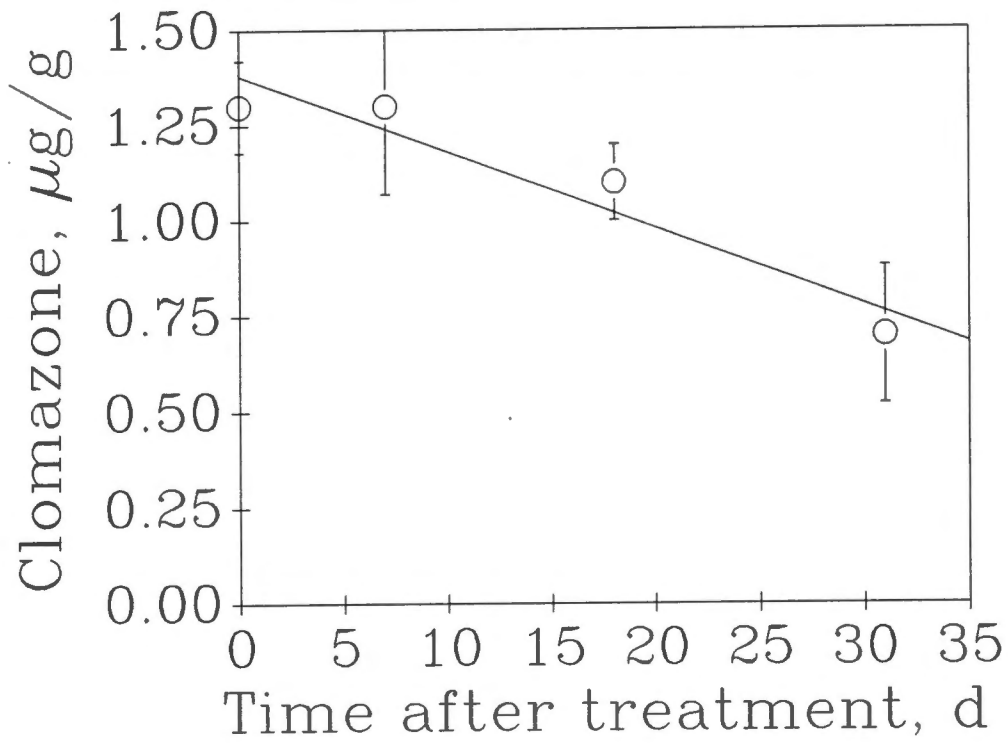


Figure 1. Field dissipation of clomazone with time after treatment in Etowah soil at Knoxville in 1991. Data fit to linear regression equation of $\hat{Y} = -.02(\text{days}) + 1.38$, $r^2 = 0.92$.

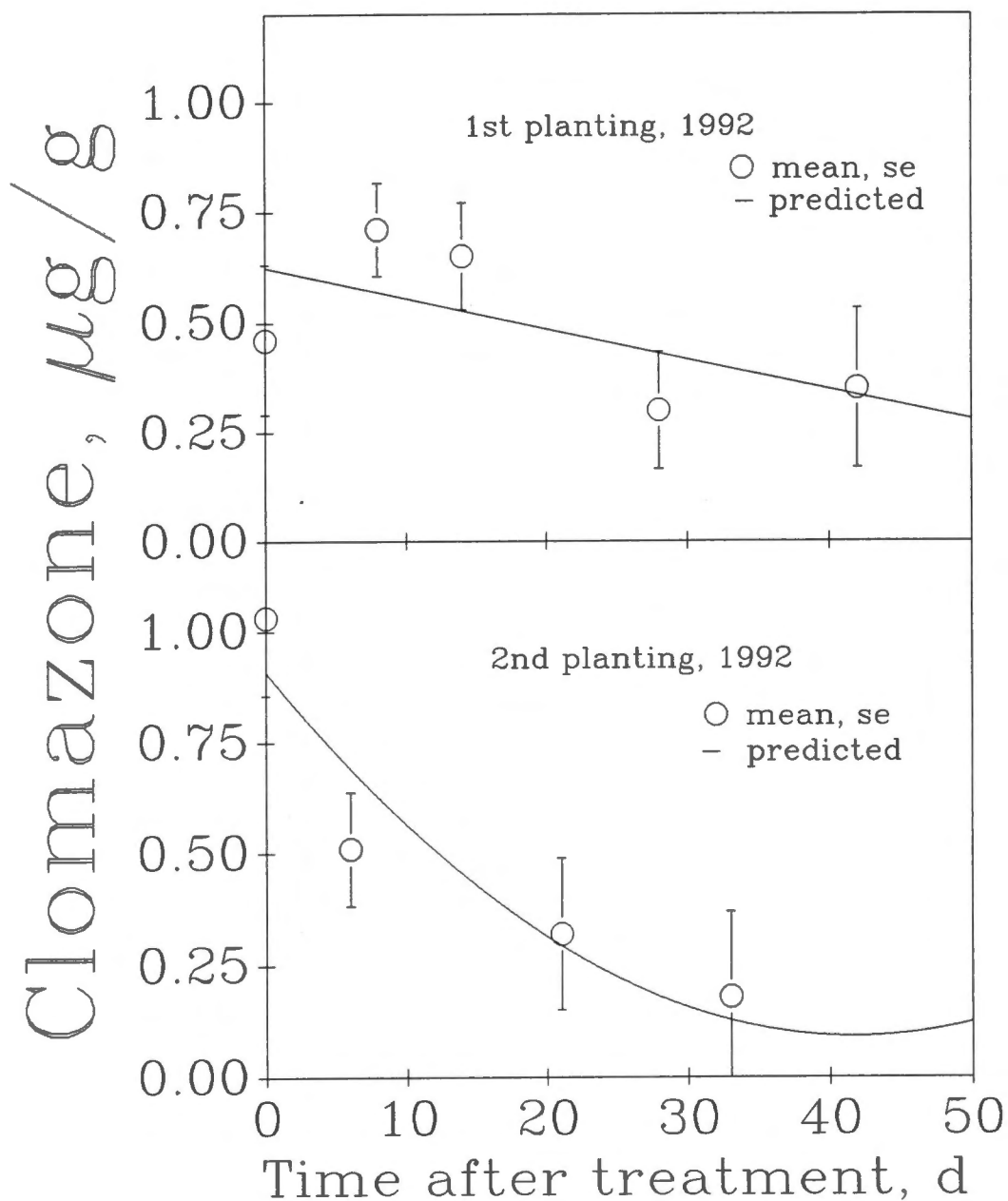


Figure 2. Field dissipation of clomazone with time after treatment in Sequatchie soil in two plantings at Knoxville in 1992. Regression equations: first planting: $\hat{Y} = -0.007(\text{days}) + 0.62$, $r^2 = 0.86$; second planting: $\hat{Y} = 0.95 - 0.05(\text{days}) + 0.001(\text{days})^2$, $r^2 = 0.92$.

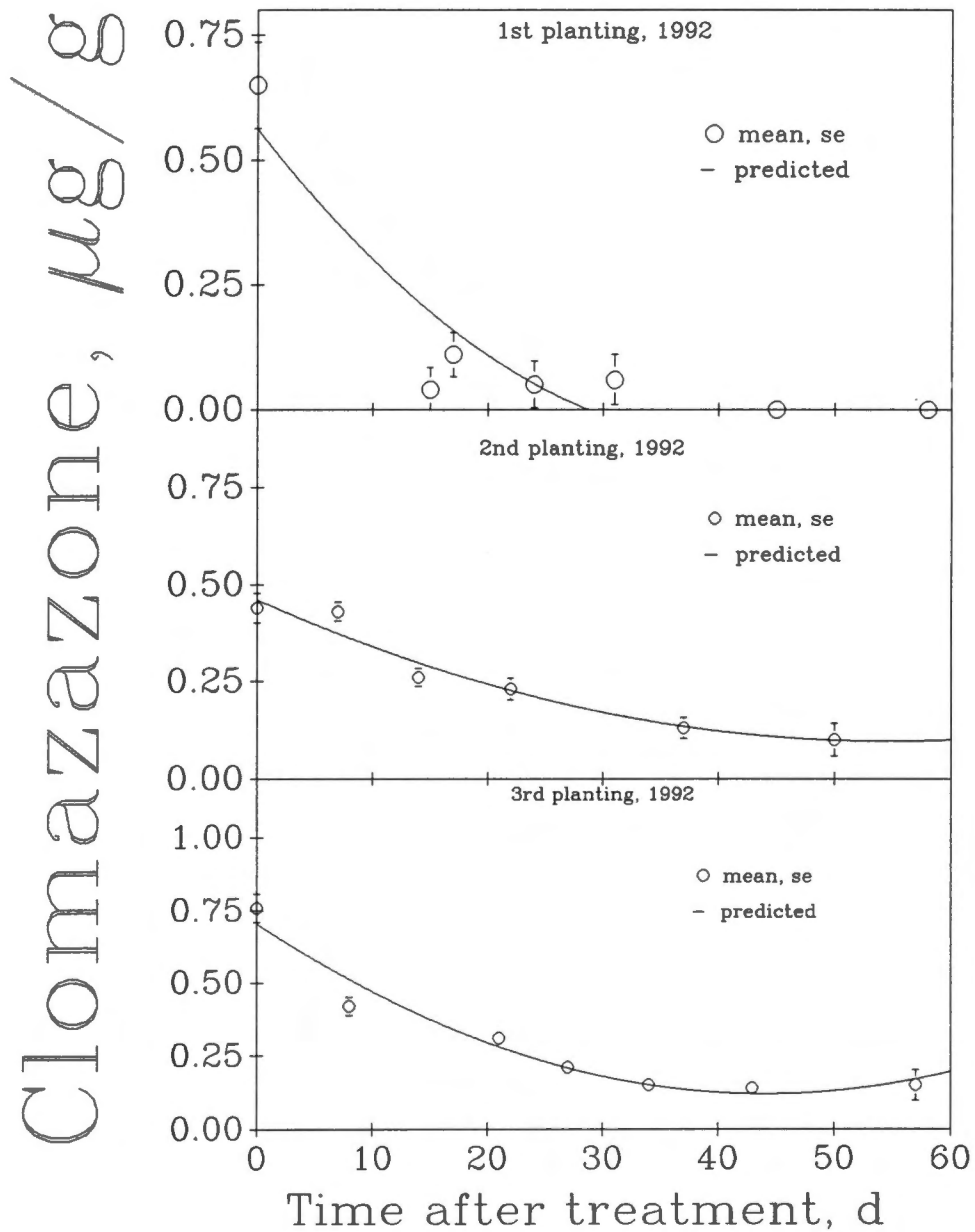


Figure 3. Field dissipation of clomazone with time after treatment in Lily loam in three plantings at Crossville in 1992. Regression equations: first planting: $\hat{Y} = 0.58 - 0.03(\text{days}) + 0.0004(\text{days})^2$, $r^2 = 0.89$; second planting: $\hat{Y} = 0.46 - 0.01(\text{days}) + 0.0001(\text{days})^2$, $r^2 = 0.94$; third planting: $\hat{Y} = 0.71 - 0.03(\text{days}) + 0.0003(\text{days})^2$, $r^2 = 0.96$.

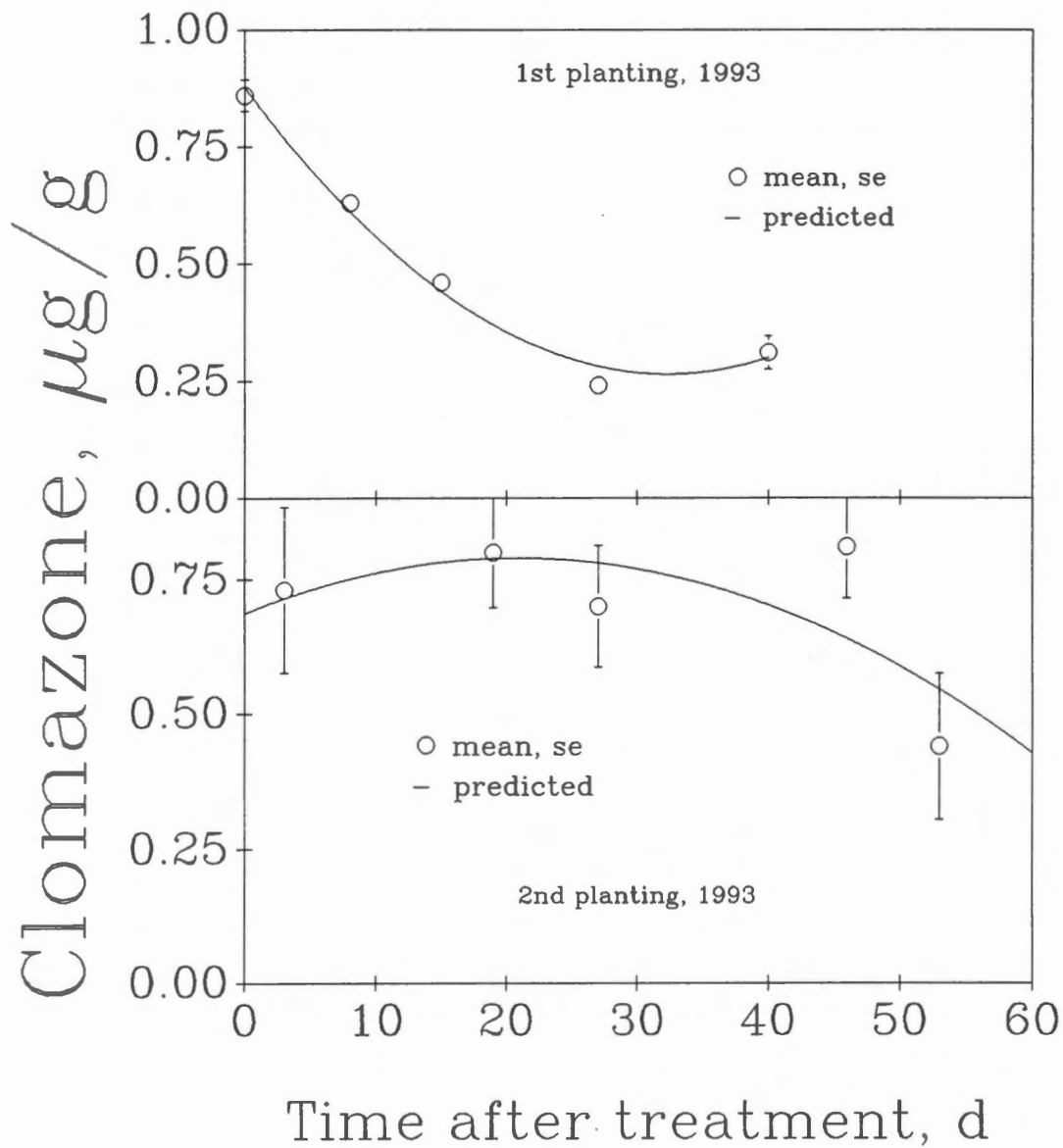


Figure 4. Field dissipation of clomazone with time after treatment in Sequatchie soil in two plantings at Knoxville in 1993. Regression equations: first planting: $\hat{Y} = 0.87 - 0.04(\text{days}) + 0.0006(\text{days})^2$, $r^2 = 0.98$; second planting: $\hat{Y} = 0.69 + 0.01(\text{days}) - 0.0002(\text{days})^2$, $r^2 = 0.46$.

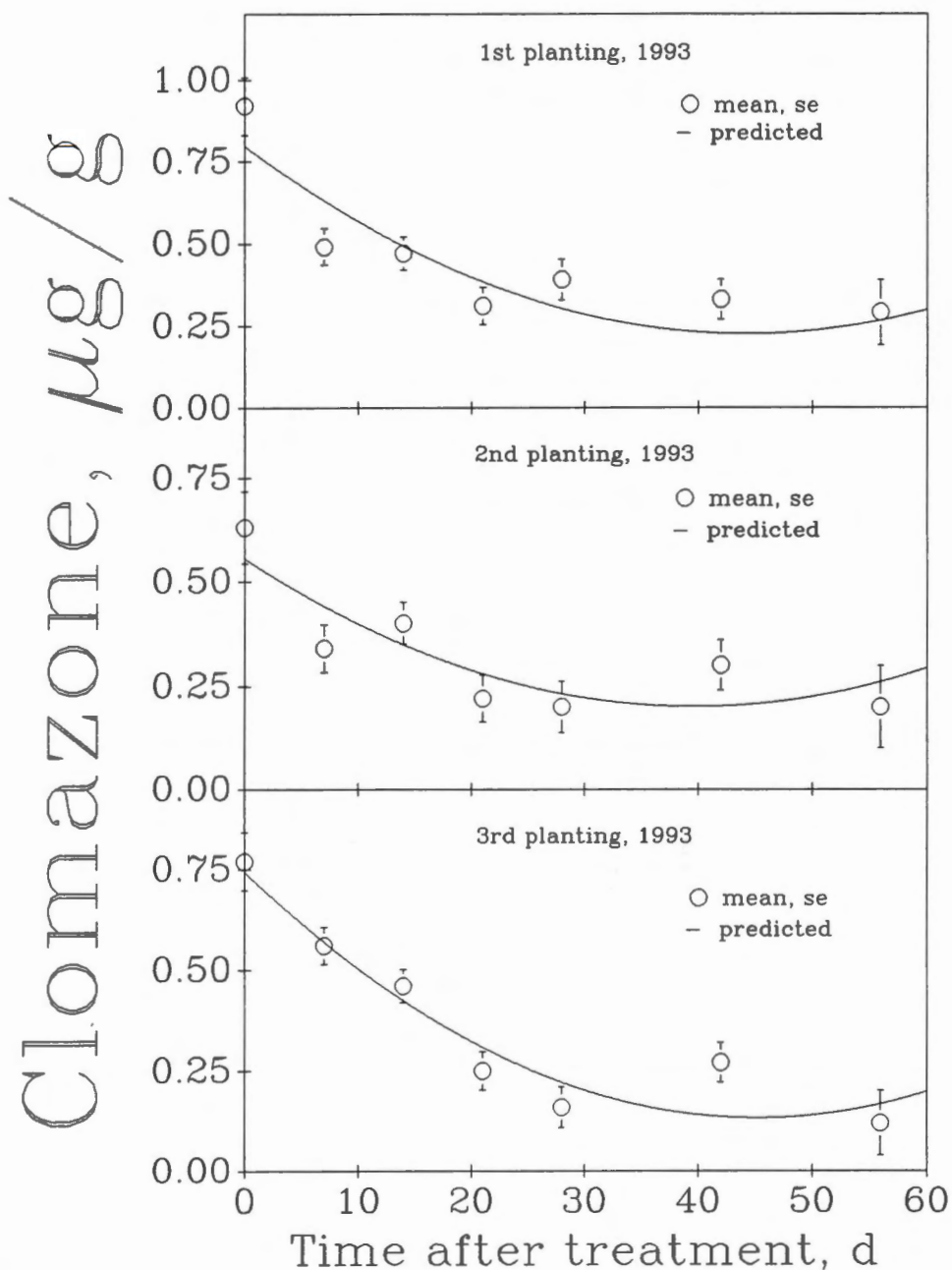


Figure 5. Field dissipation of clomazone with time after treatment in Lily loam in three plantings at Crossville in 1993. Regression equations: first planting: $\hat{Y} = 0.81 - 0.03(\text{days}) + 0.0004(\text{days})^2$, $r^2 = 0.84$; second planting: $\hat{Y} = 0.55 - 0.02(\text{days}) + 0.0002(\text{days})^2$, $r^2 = 0.68$; third planting: $\hat{Y} = 0.74 - 0.03(\text{days}) + 0.0003(\text{days})^2$, $r^2 = 0.91$.

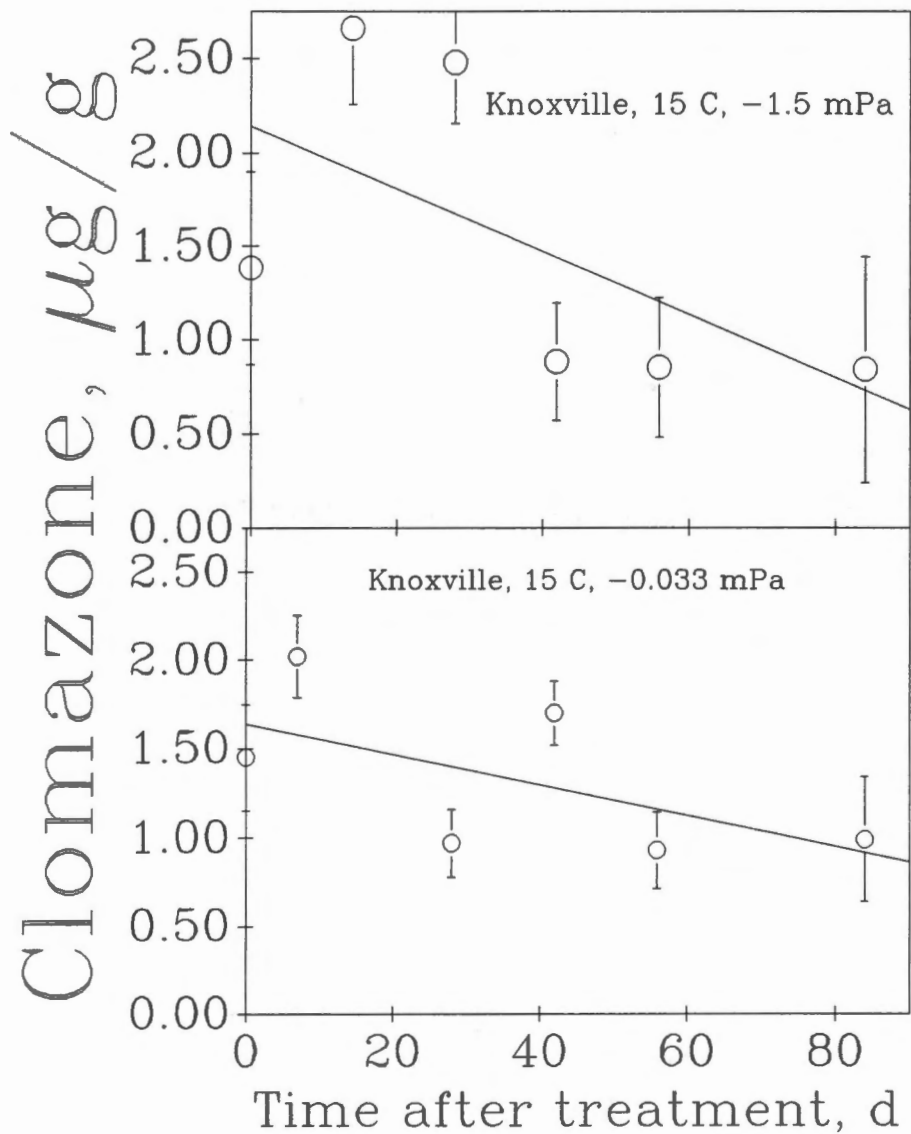


Figure 6. Clomazone degradation with time after treatment in Sequatchie soil in incubators. Regression equations: (-1.5 mPa): $\hat{Y} = 2.15 - 0.02(\text{days})$, $r^2 = 0.37$; (-0.033 mPa): $\hat{Y} = 1.66 - 0.009(\text{days})$, $r^2 = 0.33$.

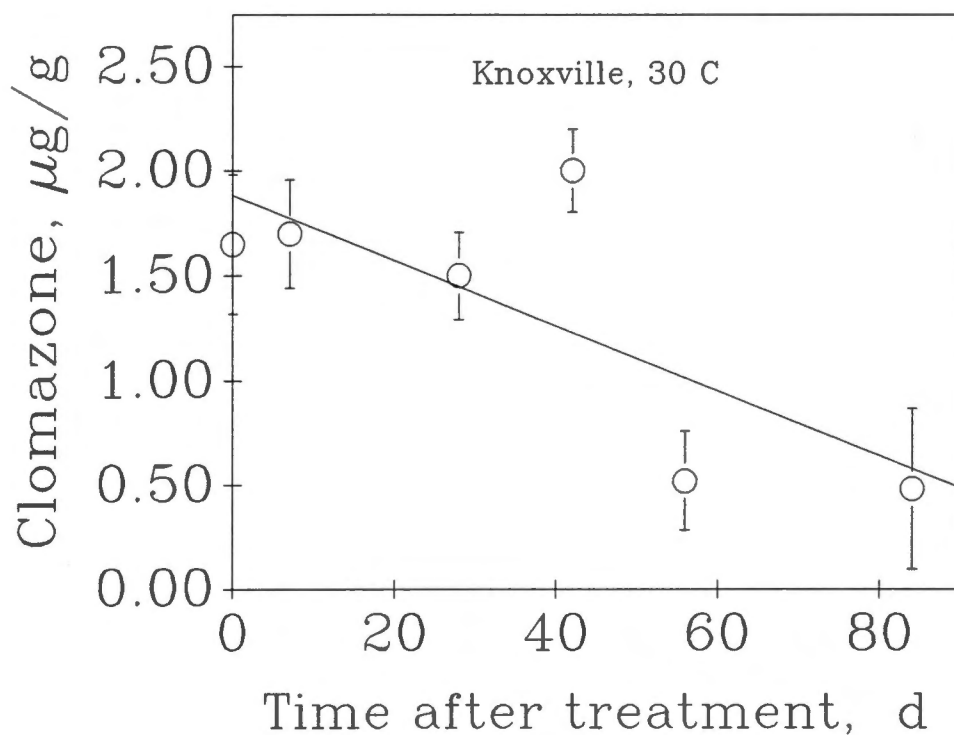


Figure 7. Clomazone degradation with time after treatment in Sequatchie soil in incubator at 30 C. Since moisture levels did not differ, data are pooled over moisture levels. Regression equations: $\hat{Y} = 1.93 - 0.02(\text{days})$, $r^2 = 0.57$.

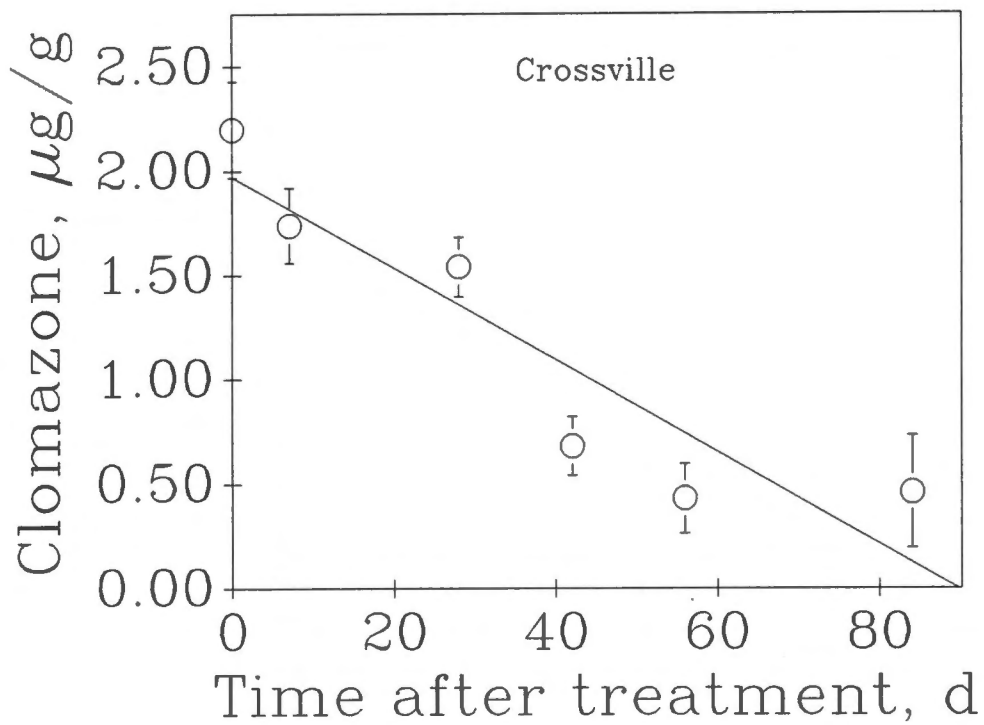


Figure 8. Clomazone degradation with time after treatment in Lily loam in incubator.

Data are pooled over moisture levels and temperature. Regression equation: $\hat{Y} = 2.04 - 0.02(\text{days})$, $r^2 = 0.84$.

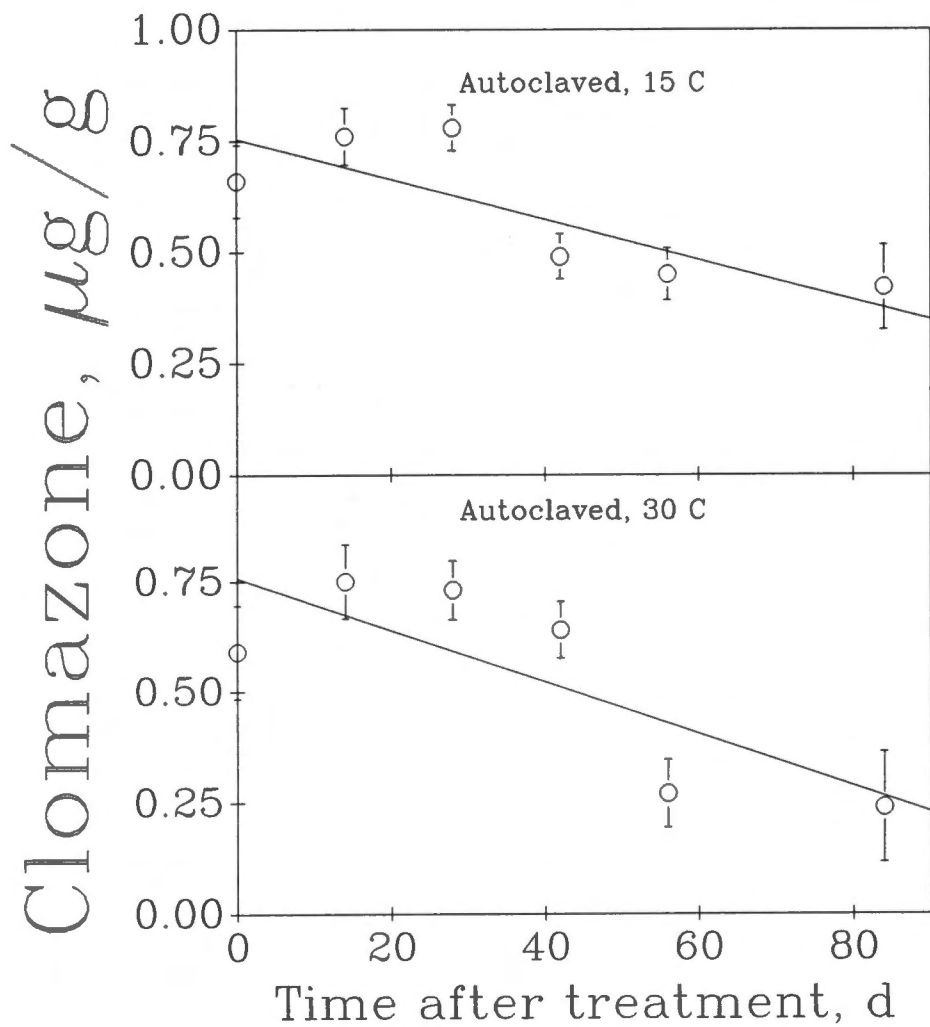


Figure 9. Clomazone degradation with time after treatment in autoclaved Sequatchie soil in incubators. Data are pooled over moisture levels. Regression equations: (15 C): $\hat{Y} = 0.75 - 0.0045(\text{days})$, $r^2 = 0.62$; (30 C): $\hat{Y} = 0.66 - 0.006(\text{days})$, $r^2 = 0.66$.

Table 1. Physical and chemical characteristics of three Tennessee soils.

Soil ^a	pH ^b	CEC ^c	Organic			
			Matter ^d	Sand ^e	Silt ^e	Clay ^e
		cmol/kg	%	-----	%	-----
Etowah	6.2	8.65	1.7	20	36	44
Sequatchie	6.1	10.02	1.7	19	37	44
Lily	6.0	8.23	2.2	45	34	21

^aEtowah (fine-loamy, siliceous, thermic typic Paleudults); Sequatchie (fine-loamy, siliceous, thermic humic Hapludults); Lily (fine-loamy, siliceous, thermic typic Hapludults).

^bSoil pH was determined using a 1:1 soil to water suspension (13).

^cCEC, cation exchange capacity was determined by extraction and subsequent calculation of exchangeable bases and acids (1).

^dSoil organic matter was determined by dry combustion (12).

^eParticle size analyses were performed using the hydrometer method (7).

Table 2. Summary of rainfall and temperatures occurring in May through September of 1992 and 1993 at Crossville and Knoxville, TN.

Month	Crossville				Knoxville			
	Mean monthly temperature		Monthly precipitation		Mean monthly temperature		Monthly precipitation	
	1992	1993	1992	1993	1992	1993	1992	1993
	-----C-----		-----cm-----		-----C-----		-----cm-----	
April	11.8	10.3	6.8	12.5	13.5	12.5	6.2	10.5
May	15.3	16.9	10.5	8.0	17.0	18.6	7.2	11.8
June	19.0	21.3	16.0	4.0	21.5	23.2	11.0	6.9
July	22.7	25.4	17.8	2.7	25.0	26.8	17.1	10.3
August	19.6	23.4	13.8	10.5	22.5	24.6	12.0	15.1
September	18.2	18.4	13.9	12.9	21.5	20.9	6.3	12.1

PART VI
GENERAL SUMMARY

The objectives of this research were to: (1) determine the effect of clomazone application to soil on snap bean growth and yield and (2) determine the behavior of clomazone in soil.

Injury from clomazone was observed in field studies. Most injury occurred when clomazone was applied at the 1.12 kg/ha rate. Symptoms included whitening or chlorosis, reduction in plant height and stand counts, and reduction in yield in 1992 at Knoxville. In some instances, snap beans outgrew injury and yields were not affected.

Results from the time of application study indicated that clomazone (1.12 kg/ha) could be applied up to 5 d after planting without reducing snap bean yield. Environmental conditions were optimum at both locations. Injury was observed 14 d after the application of clomazone to emerged snap beans, however, snap beans outgrew the injury symptoms and yields were not effected at Crossville in 1993.

Results from the greenhouse bioassay indicated that 100% bleaching of wheat can occur when clomazone concentrations are $\geq 0.5 \mu\text{g/g}$. At these concentrations, further visual or measurable differences could not be distinguished. Concentrations $< 0.5 \mu\text{g/g}$ could be predicted based on chlorosis and plant height.

Results from the extraction experiment indicated equilibrating soil samples in 80 ml acetonitrile or methanol 16 h followed by an additional extraction with 80 ml acetonitrile or methanol for 1 h extracted the most clomazone. Acetonitrile was selected for the extraction solvent due to its ease of removal with a rotary evaporator. This provided a good test to ascertain if our technique was sufficient to extract clomazone which is tightly adsorbed to soil.

Clomazone concentration in the field gradually decreased with time and under most situations, dissipation empirically fit pseudo-first order kinetics. Clomazone dissipation was slower in soils that were cool and dry. Approximately one-half of the applied clomazone had dissipated after 56 d.

In field dissipation studies, soil temperature and moisture may play a vital role in clomazone dissipation. In degradation studies where moisture and temperatures were controlled, data indicates that under cool, moist conditions, clomazone degradation is slow and there is a potential for injury to rotational crops. After 84 d incubation, greater clomazone concentrations were detected in cooler soils, regardless of soil moisture. Since snap beans are a short season crop, careful planning of rotational crops is prudent when clomazone is used.

APPENDIX

Table A-1. Maximum and minimum temperatures at the Knoxville Experiment Station in 1992.

Date	April		May		June		July		August		Sept	
	max	min	max	min	max	min	max	min	max	min	max	min
C												
1	12.8	0.0	24.0	7.8	22.9	7.8	29.6	19.6	29.1	15.6	29.6	14.5
2	12.8	-3.9	28.0	10.0	21.8	8.4	26.8	20.1	29.1	15.6	30.8	15.6
3	7.8	-5.6	28.5	16.2	26.8	10.0	26.8	19.6	30.8	18.4	29.6	17.9
4	11.2	-1.6	26.3	5.6	23.5	15.6	28.0	18.4	29.6	17.3	26.8	17.9
5	18.4	-2.8	21.8	5.0	24.0	15.6	30.2	17.3	29.6	16.2	25.7	18.4
6	15.6	0.5	16.8	3.9	25.7	14.0	29.6	19.0	29.6	17.9	28.0	16.8
7	16.8	2.2	13.4	6.1	30.2	15.6	28.5	16.8	23.5	18.4	29.6	17.9
8	12.3	5.6	8.9	6.7	30.2	18.4	30.2	18.4	28.0	19.6	30.8	18.4
9	22.9	6.1	11.7	6.7	30.2	19.6	32.4	19.6	29.1	20.1	31.9	16.8
10	27.4	7.8	19.0	7.2	26.3	18.4	32.4	19.6	30.2	19.0	31.9	16.8
11	28.5	9.5	26.8	8.9	29.1	16.8	33.0	19.6	29.1	17.9	30.8	16.2
12	26.8	11.7	28.0	10.6	29.1	16.8	32.4	19.0	30.8	19.0	27.4	11.7
13	26.3	7.2	28.5	12.8	23.5	16.2	33.6	20.1	28.0	19.0	28.5	15.1
14	25.7	7.8	24.6	11.7	22.9	17.9	33.6	21.8	26.8	15.6	28.5	16.2
15	26.3	11.7	27.4	14.0	26.8	16.8	31.9	21.2	29.1	15.6	28.5	14.5
16	29.1	14.0	29.6	13.4	29.6	17.3	28.0	19.6	25.2	12.8	28.5	12.8
17	27.4	12.8	31.3	12.8	31.9	17.9	30.2	21.2	27.4	13.4	29.1	12.8
18	28.0	12.3	29.1	14.0	30.8	16.8	29.6	19.6	30.8	16.8	30.2	12.8
19	29.1	11.7	29.6	16.2	26.8	18.4	29.6	16.8	29.1	16.2	26.8	19.0
20	26.8	13.4	29.1	15.1	30.2	13.4	30.8	16.8	28.0	16.8	28.0	15.6
21	28.5	15.1	25.7	15.1	29.6	16.8	31.9	17.9	29.1	16.8	28.5	17.9
22	23.5	7.8	27.4	11.2	24.0	8.4	32.4	19.0	26.3	17.3	26.8	21.8
23	24.6	6.1	28.5	8.4	25.2	8.9	30.8	20.1	24.0	17.9	26.8	17.3
24	27.4	8.9	30.2	12.3	27.4	12.8	29.1	20.7	26.8	16.8	20.7	11.2
25	28.5	10.0	26.3	13.4	30.2	17.9	30.2	19.6	31.9	17.9	22.9	9.52
26	18.4	5.6	20.7	11.7	31.3	17.3	31.9	21.2	31.9	19.0	25.7	10.0
27	10.6	6.1	20.1	6.7	28.0	15.6	31.3	22.4	31.9	17.9	22.9	13.4
28	14.0	5.0	21.2	10.0	28.5	14.0	29.6	15.1	29.6	18.4	25.2	16.2
29	15.6	1.6	13.4	11.2	30.8	17.3	29.6	16.2	25.7	10.6	25.2	9.52
30	19.0	3.9	19.0	11.7	31.3	17.9	30.8	16.8	25.2	10.6	21.2	5.04
31			21.2	9.5			30.2	19.0	28.5	10.6		

Table A-2. Rainfall information at the Knoxville Experiment Station in 1992.

Date	April	May	June	July	August	September
	-----cm-----					
1					0.20	
2	0.15			2.62		
3		0.03		1.32		0.28
4			1.60	0.28		
5		0.43	0.33		0.05	0.23
6				1.65		0.10
7	0.08	0.33		0.08	4.72	
8		2.18			0.13	
9		0.61	0.86			
10			2.18			0.08
11	1.47				0.81	0.61
12	0.74		3.84			
13		1.27			0.20	
14		0.13	0.64		0.46	
15	0.05		0.51		0.66	
16	0.23			1.57		
17		0.10				
18				4.47	0.38	
19			0.56			1.27
20	0.51	1.32	0.08	0.05		0.41
21	1.32		0.36			
22	2.54			0.05	0.91	
23				0.64	0.43	1.52
24						
25	1.40			1.47		
26						
27	0.05			1.83		1.50
28	0.18	0.05		0.91	2.97	0.13
29		0.33			0.03	0.15
30	0.03	0.64		0.05		
31				0.10		
Total	8.75	7.42	10.96	17.09	11.95	6.28

Table A-3. Maximum and minimum temperatures at the Plateau Experiment Station in 1992.

Date	April		May		June		July		August		September	
	max	min	max	min	max	min	max	min	max	min	max	min
	-----C-----											
1	12.7	0.56	22.22	8.33	21.11	7.78	25.55	17.78	26.66	13.33	27.78	11.67
2	6.11	-3.89	26.66	8.89	21.11	7.22	25.55	17.22	26.66	11.67	27.22	12.78
3	4.44	-6.67	26.11	13.33	23.89	11.67	27.78	17.78	26.66	10.00	25.00	15.00
4	6.11	-2.22	22.22	5.00	17.22	13.89	27.78	15.55	27.22	11.67	23.89	15.00
5	15.00	-2.78	21.11	0.56	21.11	13.89	27.22	16.67	26.66	13.33	21.11	15.00
6	13.89	-1.11	12.78	1.11	24.44	12.22	26.66	17.22	27.78	13.89	25.00	16.11
7	14.44	3.89	12.22	3.33	27.78	15.00	27.78	15.00	22.78	15.00	26.66	13.89
8	12.78	2.22	13.33	4.44	27.22	14.44	29.44	16.67	24.44	17.78	28.33	13.89
9	22.22	6.11	10.55	2.78	26.66	17.22	30.55	18.89	27.78	17.78	29.44	12.78
10	24.44	8.33	21.11	6.67	23.89	16.67	31.11	17.78	30.00	16.11	27.78	15.55
11	25.00	10.00	25.00	8.89	25.55	13.89	30.55	18.33	30.55	13.89	28.33	8.33
12	23.89	10.55	27.22	9.44	25.00	15.00	31.11	16.67	29.44	15.00	22.22	7.22
13	23.89	3.33	25.00	12.78	19.44	15.00	31.66	17.78	29.44	16.67	25.55	9.44
14	23.89	3.89	21.66	10.55	19.44	15.55	32.22	17.22	23.89	10.55	25.00	13.33
15	25.55	12.22	26.66	12.78	25.00	13.33	29.44	18.33	26.11	11.67	26.66	11.67
16	27.22	12.78	27.78	14.44	27.78	15.00	27.22	16.67	21.66	10.55	25.55	10.55
17	26.11	13.33	26.66	14.44	30.55	17.78	28.33	17.78	24.44	12.78	26.11	10.55
18	25.55	11.67	26.66	12.78	26.66	18.89	27.78	16.67	26.66	11.11	27.22	11.67
19	26.11	13.33	28.33	13.89	25.55	14.44	28.33	13.89	26.66	10.00	27.22	15.55
20	22.22	14.44	25.55	14.44	28.33	13.33	29.44	13.89	25.55	11.11	25.00	10.55
21	22.22	13.33	26.11	14.44	27.22	13.33	29.44	15.00	26.66	13.89	26.11	13.33
22	21.66	5.56	22.78	11.67	20.55	5.56	30.00	15.55	24.44	13.89	27.22	16.11
23	21.11	6.67	26.66	10.55	23.33	7.78	27.22	15.00	20.55	16.67	22.78	9.44
24	26.11	9.44	26.66	11.67	25.55	12.22	29.44	17.22	23.33	16.11	15.55	8.33
25	26.66	5.56	23.89	9.44	27.78	15.00	29.44	17.78	28.33	15.00	22.22	8.33
26	13.89	1.67	15.55	6.11	29.44	16.67	31.11	18.33	28.89	15.55	23.33	9.44
27	6.11	2.22	17.22	5.56	23.33	13.89	30.00	18.33	29.44	16.67	20.55	12.78
28	9.44	0.56	19.44	8.33	24.44	10.55	25.55	13.89	26.11	14.44	20.55	11.11
29	12.78	-1.67	11.11	8.33	26.11	16.11	27.78	13.33	18.33	7.78	22.78	12.22
30	17.78	-0.56	14.44	10.55	28.89	16.67	28.89	13.33	22.22	7.78	17.78	1.11
31			16.11	5.00			29.44	16.11	26.66	10.55		

Table A-4. Rainfall information at the Plateau Experiment Station in 1992.

Date	April	May	June	July	August	September
	-----cm-----					
1				1.63	0.61	
2	0.08			4.50		
3		0.79	0.08	4.52		0.56
4			6.45	1.57		0.20
5		0.25	0.71	0.05		0.79
6		0.05	0.05	0.41	0.18	
7	0.43	0.08	0.03	0.38	0.38	
8	0.33	1.42			0.03	
9		1.35	3.45			
10		0.03	0.38		2.18	1.07
11	0.46					0.74
12	0.91		1.78		0.08	
13		0.28	0.08		1.60	
14		0.10	0.84		0.43	
15					0.25	
16		0.84		0.79		
17				0.03		
18				0.76		2.36
19		0.56	1.07			1.57
20	0.15	1.50				0.03
21	2.34	0.03	0.18			
22	0.51			0.46	0.86	2.59
23				1.14	0.13	2.90
24				0.64	0.38	0.05
25	0.81		0.28	0.03		
26		0.08	0.18			
27	0.10		0.48	0.03		1.09
28	0.48			0.74	6.65	
29		0.58			0.08	
30	0.23	2.51				
31		0.08		0.13		
Total	6.83	10.53	16.04	17.81	13.84	13.95

Table A-5. Maximum and minimum temperatures at the Knoxville Experiment Station in 1993.

Date	April		May		June		July		August		September	
	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min
C												
1	22.2	9.4	23.8	9.9	23.8	11.6	32.1	19.4	32.1	11.6	34.4	18.8
2	12.2	2.2	24.4	11.1	22.2	9.9	32.7	19.9	31.0	11.6	34.9	19.4
3	4.4	0.5	22.7	11.6	27.2	11.6	32.7	19.9	32.7	19.4	30.5	20.5
4	14.9	0.5	21.0	13.8	29.4	17.2	32.7	19.4	31.6	20.5	31.0	19.9
5	16.1	7.7	25.5	9.9	29.4	19.9	34.9	19.9	28.8	18.3	27.7	13.8
6	12.2	5.5	27.2	11.1	27.2	8.8	33.8	19.4	28.8	17.2	29.4	14.4
7	15.5	5.5	29.4	11.6	26.0	14.9	34.4	18.3	24.4	13.8	31.0	14.4
8	20.5	5.0	29.9	11.6	31.6	16.6	35.5	18.32	28.8	13.8	30.5	15.5
9	22.2	5.5	29.9	13.8	32.7	16.6	36.6	19.9	30.5	13.8	29.4	15.5
10	14.4	8.8	26.6	16.1	33.8	17.7	36.0	18.8	31.0	14.4	30.5	16.6
11	19.9	3.3	28.3	13.3	32.7	16.6	35.5	19.4	31.0	16.6	27.2	6.1
12	25.5	5.5	28.3	15.5	32.1	17.7	34.4	20.5	31.0	18.8	26.0	6.6
13	26.0	6.6	27.2	11.1	29.9	17.2	34.9	20.5	29.9	19.9	29.4	8.8
14	26.0	8.8	22.7	11.1	29.9	18.3	34.9	19.9	28.8	20.5	31.0	12.2
15	28.8	9.4	23.3	9.4	31.0	16.6	33.3	20.5	31.6	18.3	32.1	14.4
16	23.8	8.8	26.0	8.8	28.8	13.3	29.9	21.6	33.3	18.3	27.7	17.7
17	12.2	4.4	27.2	14.4	31.0	16.1	32.7	19.9	33.8	19.4	26.6	17.7
18	17.2	0.5	27.2	16.1	32.1	18.8	31.6	19.9	32.1	19.4	29.9	13.3
19	20.5	2.7	28.8	15.4	32.7	18.8	33.3	21.0	32.7	18.8	27.2	9.9
20	24.4	5.5	22.2	8.3	32.1	17.7	34.4	19.9	32.7	19.4	28.8	11.6
21	23.3	9.4	19.4	5.0	31.0	18.3	33.3	17.7	33.3	18.8	24.4	12.7
22	10.5	0.0	16.1	6.1	24.4	18.3	34.4	18.8	30.5	17.2	28.8	10.5
23	14.9	0.0	22.2	5.5	30.5	14.9	34.9	19.9	31.6	17.2	28.3	11.1
24	19.9	3.3	25.5	5.5	32.1	17.7	35.5	19.9	33.3	18.3	22.2	13.3
25	23.3	6.6	26.6	10.5	31.6	17.7	35.5	20.5	32.1	19.9	28.3	15.5
26	27.7	12.7	19.9	14.4	31.0	19.4	34.4	19.9	33.8	18.8	28.3	17.7
27	19.4	3.8	24.4	8.8	29.9	13.3	36.0	21.0	33.8	18.8	33.8	15.4
28	21.6	2.7	28.3	13.3	32.1	13.3	36.0	19.9	32.7	17.7	21.0	4.4
29	23.8	4.4	29.4	15.5	31.0	19.9	36.0	21.0	32.7	18.3	22.2	5.0
30	25.5	6.6	29.4	14.4	31.0	18.3	34.7	14.9	33.8	18.8	22.2	2.7
31			29.9	16.1			31.0	12.7	34.4	18.8		

Table A-6. Rainfall information at the Knoxville Experiment Station in 1993.

Date	April	May	June	July	August	September
	-----cm-----					
1	2.79		0.38	2.54		
2	0.51		0.74			
3	0.03				0.46	0.53
4		1.27				0.36
5	0.89	0.56			0.28	
6	0.25				3.33	
7	0.03				1.17	
8						
9		0.36				
10	2.01					0.05
11	0.05					
12					0.61	
13		0.08			1.40	
14		3.51		0.20	2.03	
15				3.28		
16	0.71		2.31	2.06		2.79
17	0.03	0.08				0.81
18				1.19	0.36	
19		4.29			0.03	
20		0.20	3.05	0.10		
21	1.19	0.03			5.44	0.03
22	0.25	0.86	0.13			
23	0.03		0.05			
24						1.70
25						0.36
26	1.73	0.28	0.20			1.32
27						4.11
28				0.94		
29						
30		0.25				
31					0.38	
Totals	10.50	11.77	6.86	10.31	15.49	12.06

Table A-7. Maximum and minimum temperatures at the Plateau Experiment Station in 1993.

Date	April		May		June		July		August		September	
	max	min	max	min	max	min	max	min	max	min	max	min
	C											
1	19.4	6.6	22.2	9.9	22.2	6.1	31.6	17.7	31.0	11.6	32.1	17.7
2	8.3	-2.7	21.6	13.3	19.9	7.2	31.0	18.8	28.3	16.6	32.5	18.3
3	2.2	-5.5	17.2	12.7	24.4	9.9	31.6	17.7	33.3	16.1	28.3	19.9
4	13.3	0.0	18.3	12.7	28.3	14.4	32.1	17.2	31.6	16.6	25.5	16.6
5	13.8	1.1	22.2	8.8	26.6	16.6	33.3	17.7	27.2	16.1	26.0	12.2
6	9.4	5.0	25.5	12.2	20.5	7.22	31.6	15.5	27.7	16.1	26.6	12.2
7	10.5	0.0	27.7	10.5	25.5	11.1	33.3	14.9	22.7	11.1	28.8	12.2
8	17.2	2.2	28.8	11.1	30.5	15.5	33.8	16.6	27.2	13.3	28.8	12.7
9	18.8	9.9	29.4	14.4	30.5	17.2	34.9	18.3	28.3	13.8	25.5	13.8
10	12.7	2.7	26.6	12.1	31.0	16.1	32.1	16.1	29.4	13.3	25.5	13.3
11	17.7	3.8	26.6	9.9	31.0	14.4	32.1	18.3	29.9	14.9	23.8	5.0
12	23.3	5.0	26.6	12.7	30.5	16.1	32.1	17.2	31.0	16.6	22.7	5.5
13	23.3	7.7	23.8	9.4	29.9	15.5	29.9	18.3	29.9	18.3	27.2	9.4
14	23.8	7.7	19.9	5.5	28.3	15.5	30.5	18.3	28.3	18.8	26.0	18.3
15	26.0	11.6	22.7	5.5	29.4	14.9	30.5	18.3	29.4	16.6	28.8	19.4
16	19.9	3.3	24.9	8.8	27.2	12.7	30.5	17.7	32.1	16.6	21.6	13.8
17	8.33	-1.1	26.0	9.4	28.3	15.5	33.8	18.8	32.1	16.6	24.4	14.4
18	14.4	-1.1	24.9	13.3	29.9	17.7	32.1	18.3	32.1	16.6	26.0	7.2
19	19.4	4.4	27.2	12.2	30.5	16.6	34.9	19.9	31.6	17.2	22.7	8.8
20	22.2	9.9	19.4	5.5	29.4	14.4	34.4	18.3	32.1	17.7	26.0	8.8
21	16.1	2.7	19.4	1.6	28.3	17.7	33.8	16.1	33.8	17.7	23.8	13.3
22	4.44	-2.2	16.6	2.2	25.5	16.6	34.4	17.7	29.9	13.8	26.6	9.4
23	12.7	-1.1	21.0	6.1	28.8	13.8	33.8	18.3	31.6	15.5	27.2	9.9
24	19.4	3.3	23.3	6.1	29.4	17.2	33.8	18.3	32.7	18.3	19.4	12.2
25	21.0	11.6	24.9	12.7	29.4	17.2	35.5	18.8	30.5	18.3	25.5	12.2
26	21.6	7.7	18.3	9.4	27.2	16.6	36.0	19.4	32.1	18.3	27.2	11.6
27	17.2	1.1	21.6	7.2	28.8	11.6	36.0	19.4	32.1	17.2	21.0	12.7
28	20.5	2.7	26.0	11.1	30.5	16.1	36.6	18.8	31.6	17.2	17.2	1.6
29	22.7	6.1	28.3	16.6	30.5	16.6	36.0	19.9	32.1	17.7	20.5	3.3
30	23.3	9.4	28.3	13.3	28.8	15.5	33.3	13.3	32.1	17.2	18.3	1.1
31			27.7	16.1			30.5	12.7	32.1	17.2		

Table A-8. Rainfall information for the Plateau Experiment Station in 1993.

Date	April	May	June	July	August	September
	-----cm-----					
1	0.58		0.94	0.86		
2	0.84					
3	0.13	0.08			0.25	0.30
4		0.81				0.89
5	1.02				2.01	
6	1.04				3.10	
7	0.05		0.18		0.10	
8						
9	0.18					2.34
10	1.30			1.24		0.05
11			0.23			
12						
13		0.43	0.08	0.18	1.04	
14		0.18	0.03		0.03	
15			1.52			1.70
16	1.63					1.37
17	0.18	1.07				0.05
18		0.13		0.18	0.66	
19		2.08	0.28			
20				0.05		
21	1.55	0.05	0.08			
22	0.23	0.03				
23				0.18		
24						2.87
25		0.18	0.51		2.79	0.48
26	3.78	1.73			0.10	1.88
27					0.38	0.89
28			0.05			0.03
29		0.89	0.13			
30						
31		0.33				
Total	12.51	7.99	4.03	2.69	10.46	12.85

VITA

Kermit Bruce Kirksey was born in Memphis, Tennessee on August 6, 1963. He graduated from Harding Academy in Memphis in May 1981. He entered The University of Tennessee at Martin in September 1981 and completed a Bachelor of Science degree in Plant and Soil Science in August 1987. In September 1987, he entered The University of Tennessee at Knoxville and became a graduate research assistant in March 1988. He received his Master of Science degree with a major in Weed Science in May 1990. In June 1990 he began to pursue the Doctor of Philosophy at The University of Tennessee at Knoxville in the Department of Plant and Soil Science. He received his Ph.D in December 1994 with an emphasis in Weed Science.

The author is a member of the Weed Science Society of America, the Southern Weed Science Society, and the Tennessee Agricultural Chemical Association. The author received the Outstanding Ph.D Graduate Student Award from the Tennessee Agricultural Chemical Association in 1992 and was a member of the University of Tennessee weed team from 1989 to 1992. Following graduation, he plans to work for an agricultural chemical company in research and development.

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