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FIELD AND LABORATORY INVESTIGATIONS ON THE EFFICACY, SELECTIVITY, AND ACTION OF THE HERBICIDE CLOMAZONE

by

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(ABSTRACT)

Clomazone is a recently introduced herbicide for the selective control of grass and broadleaf weeds in soybeans. Field studies were conducted in full-season no-till soybeans to determine the efficacy of clomazone as a preplant and preemergence herbicide. Clomazone applied preemergence provided large crabgrass (Digitaria sanguinalis L.) control equivalent to that of oryzalin applied preplant or preemergence and provided better control of several broadleaf weeds. Control from preplant applications of clomazone was not adequate. Preemergence and preplant incorporated applications of clomazone were compared in conventionally-tilled soybeans. Clomazone efficacy at two depths of incorporation was also investigated. Clomazone applied preemergence generally provided control of large crabgrass and several broadleaf weed species equivalent to preplant incorporated applications. The addition of imazaquin or chlorimuron plus linuron improved smooth pigweed (Amaranthus hybridus L.)

control over that provided by clomazone alone. These combinations generally did not improve large crabgrass, jimsonweed (Datura stramonium L.), and common lambsquarters (Chenopodium album L.) control over that of clomazone alone. Shallow incorporation (4 cm) of clomazone provided better weed control than deep incorporations (8 cm). Studies were conducted to evaluate efficacy and to quantify volatilization of three clomazone formulations (emulsifiable concentrate, wettable powder, and a microencapsulated formulation) following soil application. Samples were collected at the first, second, and tenth day after clomazone application. The three clomazone formulations provided control of large crabgrass. Clomazone volatilization was greatest 24 h after application from the emulsifiable concentrate and wettable powder formulations and declined at the second and tenth day after application. Volatilization from the microencapsulated formulation was lower than the other two formulations at all sampling times. Clomazone volatilization was greater from preemergence than preplant incorporated applications. Differential selectivity studies were initiated to determine the absorption, translocation, and metabolism of clomazone in tolerant soybean and smooth pigweed and susceptible redroot pigweed and livid amaranth exposed to foliar and root applied clomazone. Redroot pigweed and

livid amaranth absorbed more clomazone through the roots than soybean and smooth pigweed. Absorption of foliar-applied clomazone was limited in all species. Of the clomazone absorbed in all species, most was translocated to the leaf tissue. Two metabolites of clomazone were found. One was determined to be a GS-clomazone conjugate. Differences in clomazone metabolism among species examined were not found. Growth and physiological responses of a normal hybrid ('DeKalb XL67'), a dwarf mutant, and an albino mutant of corn (Zea mays L.) to clomazone and interactions of gibberellin with clomazone on normal corn were examined. The dwarf mutant displayed greater tolerance to clomazone than normal corn. Growth measurements suggested that gibberellin was antagonistic with clomazone.

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I. LITERATURE REVIEW

Current Weed Control Systems in Soybeans

In Virginia, losses of soybean [Glycine max (L.) Merr.] yield due to weed competition are very significant. According to Hagood (34), soybean yield losses due to weed competition amounted to 4 million dollars in 1986. For example, one common cocklebur plant per 1.5 m of soybean row can reduce soybean yields by 26% (67). The most troublesome weeds competing with soybean growth in Virginia include morningglories (Ipomoea spp.), common cocklebur (Xanthium strumarium L. #[1] XANST), smooth pigweed (Amaranthus hybridus L. #AMACH), common lambsquarters (Chenopodium album L. #CHEAL), jimsonweed (Datura stramonium L. #DATST), and large crabgrass (Digitaria sanguinalis L.#DIGSA) (23). Many of the standard preemergence herbicides for soybeans such as oryzalin [4-(dipropylamino) -3,5-dinitro benzenesulfonamide], alachlor [2-chloro-N- (2,6-diethylphenyl)-N-(methoxymethyl) acetamide], metolachlor [2-chloro-N-(2-ethyl-6-

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[1] Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Weed Sci. 32, Suppl. 2. Available from WSSA. 309 W. Clark St., Champaign, IL 61820.

methylphenyl)-N-(2-methoxy-1-methylethyl)-acetamide], linuron [N-(3,4-dichlorophenyl)-N-methoxy-N-methylurea, and metribuzin [4-amino-6-(1,1-dimethyl-ethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one] provided inadequate control of several important weed species such as cocklebur, morningglories, and velvetleaf (Abutilon theophrasti Medik. #ABUTH) (80). Many new preemergence and postemergence herbicides have been introduced in soybeans for control of these problem broadleaf weeds. Clomazone (2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone), one of the new herbicides, is presently labeled for annual grass and broadleaf control in soybeans (5, 24, 42).

Efficacy and Potential Applications of Clomazone in Soybeans

Clomazone has been shown to control many broadleaf weeds in addition to several common annual grass species including barnyardgrass [Echinochloa crus-galli (L.) Beav. #ECHCG] and large crabgrass and small seeded broadleaf weeds including common lambsquarters, with preemergence and preplant incorporated applications in conventionally-tilled as well as minimum tillage soybeans (73, 83). Control of some small- seeded broadleaf weeds such as pigweed species (Amaranthus spp.) with clomazone is sporadic (79).

Because of a relatively long growing season in eastern Virginia, soybeans can be grown either as the primary crop (full-season) or as a second crop following harvest of wheat (Triticum aestivum L.) or barley (Hordeum vulgare L.). In recent years, no-till planting has become an increasingly important method of soybean production with approximately 50% of the soybeans being planted without tillage in Virginia (35). Successful no-till soybean production can only be achieved with adequate weed control (72). No-till production systems are dependent on the use of non-selective herbicides for control of existing vegetation at or prior to the time of planting and additional herbicides for preemergence and postemergence weed control during the season. In full-season no-till soybeans, herbicides have been applied 30 to 60 days prior to planting (early preplant) to prevent weed interference at planting. However, residual control by early preplant herbicides such as oryzalin have been dependent on soil moisture. According to Fawcett et al. (26), early preplant treatments provide better weed control under dry conditions than under conditions of frequent and heavy rainfall. Stougaard et al. (72) found that winter and spring early preplant applications of oryzalin reduced establishment of many annual broadleaf weeds and grasses and continued to provide control during the following summer months in

soybeans. However, oryzalin does not provide control of many broadleaf weeds such as common ragweed (Ambrosia artemisiifolia L. #AMBEL), common cocklebur, velvetleaf, and spurred anoda (Anoda cristata L. #ANCVR) that clomazone controls.

Currently, because of the potential off-site damage to desirable vegetation, the clomazone label is limited to preplant incorporated application. Herbicide incorporation prevents herbicide loss due to wind, volatilization, and photodegradation. In addition, incorporation allows the placement of the herbicide precisely into the zone of germinating weed seed (4). The disadvantages of incorporating herbicides include purchase of extra equipment for herbicide incorporation, extra trips across the field, and the loss of activity of some herbicides when incorporated (4).

Environmental Fate of the Herbicide Clomazone

There are many routes of loss for an applied pesticide such as loss due to runoff, leaching into the soil below zone of plant growth, photodecomposition, adsorption to soil particles, and volatilization or movement of the pesticide from the point of application to the atmosphere. Volatilization.Volatilization is defined as the loss of chemical vapor from soil and water surfaces (46). Once the

pesticide has left the soil or other surfaces, it is quickly dispersed in the air as a result of turbulence (36). Pesticide vapors may be removed from the atmosphere by decomposition, adsorption on aerosols followed by dry deposition or removal by rainfall. Oxidation and photolytic reactions are the most likely reactions to occur (36). The rate of movement away from an evaporating surface is a diffusion-controlled process such that diffusion away from the surface is related to vapor pressure of the pesticide and temperature (36).

Potential volatility is related to the vapor-pressure of the compound, but actual volatility rates are dependent on environmental conditions such as temperature, soil moisture, soil properties, and air movement. Vapor pressures for organics of intermediate molecular weight such as most pesticides increase 3 to 4 times for each 10 C increase in temperature (36). Gray and Weireich (32) reported EPTC (S-ethyl dipropyl carbamothioate) loss to volatility was related to soil temperature and depth of incorporation. EPTC volatility was greater from moist soils at 18 C than at 0 C. However, in dry soils, temperature had little effect on volatilization losses (32). Danielson and Gentner (16) reported that air movement had a major influence on soil-applied EPTC with persistence related inversely to air velocity between 1.1 and 6.5 km/h.

Soil moisture affects pesticide volatility by displacing the pesticide from soil adsorption sites (69). The actual amount of pesticide volatility during a given period is related to the time it takes dry soil to sufficiently reduce vapor density to an insignificantly low value (46, 69). Short-term studies indicated that pesticide volatilization was dependent on soil moisture but not on the rate of water loss from the soil (69).

Volatility is strongly influenced by adsorption to soils or other surfaces. Adsorption to soil reduces the biological activity below that of the pure compound. Most of the more volatile pesticides are either weakly polar or nonionic, thus adsorption is related to soil organic matter content and total cation exchange capacity (i.e. clay) (69). Several investigators report an inverse relationship between rate of pesticide volatilization and soil organic matter content (46). Parochetti and Warren (59) found that losses of propham (1-methylethyl phenylcarbamate) and chlorpropham (1-methylethyl 3-chlorophenyl carbamate) decreased as percent clay and organic matter as well as cation exchange capacity increased.

When a pesticide is mixed into the soil, its volatilization rate is dependent on the rate of movement away from the soil surface, effective vapor pressure within the soil, and rate of movement of chemical to the surface

(46). Initially, the rate of loss by volatilization will depend on soil concentration-vapor density relationship at the soil surface, but the volatilization rate decreases rapidly as concentration at soil surface is depleted and soon becomes dependent upon rate of movement of the pesticide to the soil surface (46). Water evaporating from the soil surface creates a gradient that results in an upward movement of water to replace that evaporating from the soil surface. Pesticides in the soil solution move toward the surface by mass flow with water. However, volatilization rates from soil incorporated pesticides decrease with time after application and level off at rates dependent upon rate of pesticide movement to the soil surface.

The degradation products of pesticides may also be volatile and in some instances this could account for greater pesticide dissipation than direct loss of the applied parent material (46).

The first indication of pesticide volatility losses in the field comes from pesticide effects on susceptible plant and insect species (69). Bioassays have been used by many researchers to evaluate the potential volatility of insecticides and herbicides under field conditions using crops as bioassay species (14, 15, 76). Direct measurements of volatilization rates are the best way to address the

importance of pesticide volatility in the field (47). Bardsley et al. (3) and White et al. (81) have quantitatively studied trifluralin [2,6-dinitro-N,N-dipropyl-4-(trifluormethyl)benzeneamine] losses under field conditions. Trifluralin losses were proportional to the concentration applied and the greatest concentrations of volatilized trifluralin were closest to the ground. White et al. (81) found seasonal losses of trifluralin due to volatility to be approximately 22% of that applied. Of this 22%, 13 to 15% were lost during application and through the first 24 hours. Approximately one-half was lost during the first 9 days and 90% in the first 35 days. Combined losses through other pathways such as chemical and microbial degradation and photodecomposition were 2.5 times that lost to volatilization.

Clomazone was first labeled for use in soybeans in 1986. Following the first applications of clomazone in the spring of 1986, there were numerous reports from several midwestern states of non-target vegetation damage caused by clomazone (8). Clomazone apparently volatilizes off the treated soil surface and moves downwind to areas where non-target plant foliage is bleached. For most non-target vegetation, the bleaching is a temporary phenomenon. Some of the desirable plant species affected are ornamentals such as roses (Rosa spp.) , trees such as flowering and

edible cherries (Prunus spp.), agronomic crops including alfalfa (Medicago sativa L.) and small grains, and some vegetable crops including lettuce (Lactuca sativa L.), cole crops (Brassica spp.), and radishes (Raphanus sativus L.) (8).

Halstead and Harvey (37, 38, 39) have used a field bioassay technique to qualitatively characterize some of the aspects of clomazone off-site movement with environmental factors such as soil moisture, rainfall after application, surface crop residues, and rates of application. It was found that the greatest off-site movement occurred from wet soil. Preplant incorporated treatments of clomazone were found to reduce off-site movement by 75%. The greater amount of off-site movement occurred with the current emulsifiable concentrate formulation; the granular formulation provided the least. Limited off-site movement of clomazone occurred for up to two weeks after treatment (37, 38). Carrier volume did not affect clomazone volatilization rates (39). Thelen et al. (75) found greater clomazone volatility after rainfall and from applications to no-till or minimum tillage soybeans. The increased clomazone volatility from no-till application could be due to less soil contact and the greater soil moisture present in no-till soils. To date, studies have not been conducted to quantitatively determine clomazone

volatilization.

Soil Persistence. The ideal soil-applied herbicide should persist long enough to give an acceptable period of weed control but not long as to injure rotational crops (43). The three principal processes by which herbicides dissipate from the zone of germinating weed seed in the soil are 1) downward leaching with precipitation or irrigation, 2) volatilization from soil surface into the atmosphere, and 3) chemical or biological degradation. The residual activity of a particular herbicide is a function of the herbicide's chemical properties, soil components such as organic matter and clay content, and environmental conditions including temperature and moisture.

Environmental factors affect pesticide residues in the soil (9, 13). Temperature affects pesticide decomposition by increasing abiotic reaction rates and increasing microbial activity (56). The half-life of atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine] on a clay loam can vary from one month in moist soil at 25 C to one year in dry soil at 25 C (57). Harris et al. (40) found that persistence of atrazine and fenac (2,3,6-trichloro-benzeneacetic acid) was more persistent in cooler, drier soils of the western states than in the warm, moist soils of the southeast. Soil moisture has an effect on soil-applied herbicide persistence by its effect on

leaching and binding to soil particles. Aerobic soil conditions can also affect pesticide dissipation rates. Under anaerobic conditions, compounds with benzene rings and carbon to carbon single bonds decompose faster than under aerobic conditions (56). In virtually all agronomic situations, net water movement in the soil during the growing season is upward because natural evaporation plus transpiration exceeds rainfall; therefore leaching of herbicides is insignificant during the summer months (78). The dependence of herbicide persistence on soil pH is a function of the chemical's stability over pH range (57, 78). For example, the sulfonylurea herbicide, chlorimuron (2-[[[[4-chloro-6-methoxy-2-pyrimidinyl]amino]carbonyl] amino]sulfonyl] benzoic acid) which has a pK_a around 4.0 exhibits a longer residual activity at higher soil pH (6, 78). The reduced rate of metribuzin loss at lower soil pH has been attributed to the increased soil adsorption of the protonated herbicide (50).

Pesticide adsorption by organic matter has been shown to be a key factor in the behavior of many pesticides in the soil (71). Most but not all pesticides have a greater affinity for organic rather than mineral surfaces (71). This has been shown to be true for linuron and simazine (6-chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine). Organic matter exists in many forms in the soil including

unmodified remains of plant and animal tissues (plant detritus, roots, bacterial, and fungal tissue) and secondary products of metabolism known as humic substances (71). Humic material which consists of high molecular weight polyelectrolytes high in nucleophilic functional groups including carboxylic, phenolic, aliphatic, and enolic-OH is the most reactive component of soil organic matter (71). Humic material can bind with pesticides via electrostatic, hydrogen bonding and ligand exchanges. The humic substances are strong reducing agents that are able to transform many pesticides. For example, the hydrolysis of chloro-s-triazines is a non-biological transformation dependent on soil humic matter (71). There is substantial evidence to indicate that pesticide residues form stable chemical linkages to soil matter increasing the persistence of the pesticide by preventing leaching and enzymatic degradation. However, these bound pesticide residues are not available for uptake by susceptible plant species (71).

Degradation of some pesticides is characterized by an initial lag period followed by rapid degradation which appears to be linear with time (43). This pattern is true for short-residual herbicides such as 2,4-D [(2,4-dichlorophenoxy)acetic acid], dalapon (2,2-dichloropropanoic acid), and propham (43). However, for other compounds, there is no lag period and the rate of

degradation is more or less proportional to concentration.

A study of sorption of clomazone on to soils and clay indicated that clomazone primarily binds to organic carbon rather than clay particles in the soil (48, 54). Tymonko et al. (77) found that high levels of organic matter negatively influenced clomazone activity. Under aerobic conditions, degradation of clomazone was by CO_2 evolution and formation of bound soil residues. After 9 months of the study, the parent clomazone was the predominant product detected (30). Trifluralin behaves in a similar fashion (58). These researchers found that although clomazone has a relatively high water solubility of 1100 ppm, it is strongly adsorbed to soil organic matter and thus has relatively long soil life. No correlation between soil pH and performance was found. In flooded soils where anaerobic conditions can exist, clomazone was degraded rapidly to the metabolite [N-[2'-chlorophenyl)methyl]-3-hydroxy-2,2-dimethylpropanamide].

Clomazone seems to have limited potential for injuring rotational crops such as corn (Zea mays L.) and sorghum (Sorghum bicolor (L.) Moench.) crops planted into soybean fields treated with the herbicide the preceding year. Curran et al. (11, 12) found that clomazone at rates of 0.8 to 2.2 kg/ha did not adversely affect the yields of corn planted the following year, although early season injury

was noted from the higher rates. No varietal differences in corn response to clomazone were observed (12). Loux (55) reported that sorghum injury increased with clomazone rate from 0.14 to 2.24 kg/ha, 12 months following clomazone application. However, small grains such as wheat and oats (Avena sativa L.) are very sensitive to clomazone residues (47, 48). Stougaard et al. (74) reported that clomazone applied 6 months prior to wheat planting caused a 75% decrease in dry weight and an 80% yield reduction. Gunsolus (33) found injury to oats and wheat planted 12 months after application from clomazone at rates of 1.4 to 2.8 kg/ha. Most of these studies were conducted in the midwest on fine soils with high organic matter content and no information has been reported on clomazone residual effect on coarse soils low in organic matter such as are present in eastern Virginia.

Selectivity of the Herbicide Clomazone

Amaranthus species are among the ten most important weeds in Virginia soybean production (83). Smooth pigweed (Amaranthus hybridus L. #AMACH) is the predominant weed species in Virginia. Redroot pigweed (Amaranthus retroflexus L. #AMARE) and livid amaranth (Amaranthus lividus L. #AMALV) are also present in the state. Field studies have indicated that smooth pigweed control by

clomazone is sporadic (79). This sporadic weed control could be due to more than one species or biotype of a single species of pigweed being present which display differential absorption, translocation or metabolism of clomazone.

Selective herbicides control undesirable plants in a specific desirable crop. Selective herbicides are the basis of chemical weed control in agriculture today. The crop selectivity of herbicides is due to either differential penetration, absorption, translocation, rates of metabolism of the herbicide and intrinsic differences of sites of herbicide action (41).

With non-selective herbicides such as glyphosate [N-(phosphonomethyl)glycine] and paraquat (1,1´-dimethyl-4,4´-bipyridinium ion), selectivity can be achieved via placement or time selectivity such as application before emergence of the crop. An example of placement selectivity is the use of post-directed application of cyanazine (2-[[4-chloro-6-(ethylamino)-1,3,5-triazin-2yl]amino]-2-methylpropanenitrile) to cotton (Gossypium hirsutum L.).

Herbicides must enter the plant and move to their site of action to be effective (2). Absorption and translocation are dependent on the molecular configuration of the herbicide and characteristics of the target plant species. Variations in sensitivity of crops and weeds to any

particular herbicide can be attributed to differences in plant growth stage, genetic differences, herbicide chemical characteristics, and environmental conditions. Young plants are generally more susceptible to herbicides than older plants. For example, acifluorfen [5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid] must be applied to common lambsquarters at the 1 to 2 leaf stage to provide adequate control (82). Differences in herbicide selectivity due to genetic differences are particularly evident among crop species. For example, considerable varietal tolerance of soybeans to metribuzin exists which is based on differential metabolism rates (25). Some plant biotypical differences can be attributed to the site of action instead of uptake or herbicide metabolism differences. Triazine-resistant weed biotypes have a genetic change in the chloroplast such that triazine herbicides do not bind to the protein rendering the plant resistant to the herbicide (1). The chemical characteristics of a herbicide can affect selectivity. For example, 2,4-dichlorophenoxy alkanoic acids with an even number of carbon atoms in the side chain such as butyric, caproic, and caprylic acids are less active on legumes such as soybeans (41). The tolerance of legumes is due to their inability to convert even numbered 2,4-dichlorophenoxy alkanoic acids via ß-oxidation to the biochemically active

2,4-dichlorophenoxy acetic acids in susceptible weed species (41).

Differential metabolism and differential rate of metabolism have been considered as two important factors contributing to interspecific differential tolerance of plants to certain herbicides (41). Differential rate of metabolism is important in propanil [N-(3,4-dichlorophenyl)propanamide] selectivity of rice (Oryza sativa L.) and barnyardgrass (68). Environmental conditions can also adversely affect crop sensitivity and weed control by a particular herbicide. Quite often, cool, moist conditions such as are present in spring vegetable crops can reduce herbicide efficacy because of reduced herbicide uptake and translocation. For example, bentazon [3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4-(3H)-one-2,2-dioxide] provided reduced control of common lambsquarters in cool conditions as compared to warmer temperatures later in the season (61).

Plants metabolize herbicides through a series of intermediates leading ultimately to insoluble residues of the herbicide (41, 68). Herbicide metabolism in plants take place in three stages. In the first stage of metabolism known as phase I, the herbicide is modified and is predisposed for phase II metabolism. In phase II metabolism, the herbicide is further detoxified and

conjugated. In phase III metabolism, the herbicide is converted into insoluble or bound residues which are more common than in animals (68). Phase I metabolism is most important to herbicide selectivity (68).

Similar classes of compounds or functional groups are frequently metabolized by comparable mechanisms such as oxidation, reduction, hydrolysis, and conjugation reactions (52). Glycoside conjugation appears to be the most common xenobiotic conjugation reaction in both plants and animals (52). Glycoside reactions most frequently involve functional groups such as hydroxyl, carboxyl, and amines. Phenols and alcohols or xenobiotics metabolized to phenols and alcohols are metabolized to ß-O-glucosides. Most ß-O-glucosides are further metabolized to bound residues. For chlorsulfuron [2-chloro-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide]-treated wheat, 60% of applied chlorsulfuron was converted to 5-hydroxyglucoside within 24 h after application with no free chlorsulfuron present (6, 52). Most ß-O-glucosides are thought to be formed by glucosyl transferase enzymes that require UDPG as a glucosyl donor (52). N-glucosides are common in a variety of plant species. N-glucosides are formed most commonly on aromatic or heterocyclic xenobiotics containing a primary or secondary amino group. Chloramben (3-amino-2,5-dichlorobenzoic acid) is

exclusively metabolized in tolerant species to the N-glucoside (52). Xenobiotic and endogenous substances containing free or potential carboxyl groups are commonly metabolized to O-1 glucose ester conjugates. Glucose ester conjugates are more important than amino acid conjugates in plant systems (52). Glucose ester conjugates are formed by UDPG glucosyl transferases although an ATP/CoA dependent system has been reported for glucose esters of indole acetic acid (61). S-glucosides are rare in plant systems. Xenobiotic glucose conjugates frequently undergo additional metabolism by conjugation of the glucose moiety with other endogenous substances such as ß-gentiobiosides [ß-(1->6) glucosyl ß-(1->O)glucose] which were the first complex glucose conjugates identified (52).

The conjugation of xenobiotics with glutathione (GSH) in higher plants was first demonstrated to be a major detoxification mechanism for atrazine in sorghum, corn, and other atrazine-tolerant species (52). Glutathione conjugation can occur in plants and detoxify xenobiotics in the presence or absence of glutathione-S-transferase (GST) (41, 52, 67). Glutathione conjugation has been reported to be a very rapid reaction (52). Atrazine and propachlor [2-chloro-N-(1-methylethyl)-N-phenylacetamide] are metabolized to their glutathione-conjugate in corn within 6 h (52). There are several means by which glutathione conjugates are

formed such as by alkyl transfer, aralkyl transfer, aryl transfer, epoxide transfers, and the transfer of nitrogen heterocycles (44). For example, the α-chloroacetamide herbicides are metabolized by nucleophilic displacement of chloro group by glutathione. Most xenobiotic conjugates are catabolized at least to cysteine conjugates which are N-acylated with malonic acid. The lanthionine conjugate produced from N-cysteine conjugate of atrazine in sorghum, and the deamination of xenobiotic cysteine conjugate are converted to thio-acetic compounds (EPTC) (44). In leguminous species such as soybeans, glutathione (-glutamylcysteinylglycine) is not abundant; homoglutathione (-glutamylcysteinyl-ß-alanine) appears to be the most abundant tripeptide that conjugates xenobiotics (44). This has been shown to occur for acifluorfen, metribuzin, and chlorimuron in soybeans (52).

The current methodology for characterizing herbicide metabolism in plants depends on radioassays and the isolation of the parent herbicide and it's metabolites by various chromatographic techniques such as thin-layer chromatography, gas-liquid chromatography, and high-performance liquid chromatography (10, 22). From 1965 to 1975 plant pesticide metabolism studies focused primarily on metabolite purification and identification (51). Mass spectrometry has become a widely utilized tool

for metabolite identification, but most plant pesticide metabolites are polar conjugates which are not readily analyzed by conventional mass spectrometric techniques. In the early 1980's, a new soft ionization technique was developed for non-volatile compounds known as fast-atom-bombardment (FAB) which provides molecular weight and fragmentation data for structural assignments of unknown metabolites (62).

Mode of Action of Bleaching Herbicides

Carotenoids are diterpenoid compounds found in both non-photosynthetic and photosynthetic plant tissues (31). In higher plants, the most common carotenoids are the carotenes α-and ß-carotene and xanthophylls lutein, violaxanthin, and neoxanthin (31). Carotenoids absorb light in wavelengths of 400 to 500 nm whereas chlorophylls absorb light in the blue (450nm) and red (650-700 nm) regions of the spectrum. Chlorophyll *a* is the most important photosynthetic pigment for it is present in all photosynthetic plants (31). Chlorophylls *b*, c_1, c_2, *d*, and carotenoids are called accessory pigments.

The chloroplast carotenoids function as accessory light-harvesting pigments and as agents protecting chlorophylls against photooxidation (31). At high light

intensities, the chlorophyll of light-harvesting antennae is capable of absorbing more light energy than can be passed on through photosynthetic reaction centers and through photosynthetic electron flow such that a large proportion of chlorophyll molecules become excited. The excited chlorophyll molecules can deexcite by many ways including intersystem crossing in which triplet state chlorophyll molecules are formed. These triplet state chlorophyll molecules can return to ground state by passing excess energy to molecular oxygen which leads to the formation of singlet oxygen, $^1O_2$ (18, 31). The initiation of lipid peroxidation of unsaturated fatty acids in the cellular membranes may be accomplished by species such as singlet oxygen (18, 70). Carotenoids prevent oxidative damage by singlet oxygen. ß-Carotene is the carotenoid that protects against photooxidative action (31). Singlet oxygen is quenched very efficiently by ß-carotene so that even low ß-carotene levels can adequately serve as chlorophyll protectants. The carotenoid compounds can efficiently quench the excess energy of triplet chlorophyll molecules, thus preventing singlet oxygen production or they can quench the excess energy of the singlet oxygen species produced. Triplet carotenoids produced by deexcitation of singlet oxygen or triplet chlorophyll molecules can lose their excess energy by heat, thus preventing photooxidative

damage. When carotenoid biosynthesis is inhibited, the protection of the chlorophyll and chloroplast apparatus cannot be achieved. Thus, the plant bleaches and can no longer function normally (7, 18, 27, 28, 29, 49).

Mechanisms of Inhibition of Carotenoid Biosynthesis by Clomazone and Other Herbicides

At present, clomazone is considered to be an inhibitor of isoprenoid biosynthesis (17, 21, 65, 66). Isoprenoid biosynthesis is an important plant biosynthetic pathway which synthesizes essential plant products such as carotenoids and gibberellin. Carotenoids are formed by the condensation of eight isoprene units (45). Isopentenyl pyrophosphate is the five-carbon building unit for all isoprenoid compounds. It is derived from acetyl-CoA, ß-hydroxy-ß-methylglutaryl-CoA (HMGCoA), and mevalonate.

The synthesis of carotenoids may be divided into three stages: 1) the synthesis of geranylgeranyl pyrophosphate from isopentenyl pyrophosphate, 2) condensation of two molecules of geranylgeranyl pyrophosphate which gives rise to phytoene, and 3) desaturation and cyclization of phytoene which gives rise to carotenoids (45). The synthesis of geranylgeranyl pyrophosphate is the first committed step of carotenoid biosynthesis. The first biosynthetic step of geranylgeranyl pyrophosphate is the

isomerization of isopentenyl pyrophosphate to dimethylallyl pyrophosphate by isopentenyl pyrophosphate isomerase. This biosynthetic step is followed by a sequence of three prenyltransferase reactions from which geranyl pyrophosphate, farnesyl pyrophosphate, and geranylgeranyl pyrophosphate are formed (45). Two molecules of geranylgeranyl pyrophosphate are condensed in a head to head fashion to form phytoene. Phytoene undergoes a stepwise sequence reaction to form cis-phytofluene, trans-phytofluene, ß-carotene, neurosporene, and lycopene which have 5, 7, 9, and 11 conjugated double bonds, respectively. Cyclization of lycopene leads to the formation of the major carotenoids in plants including α-carotene and ß-carotene.

Herbicidal inhibitors of carotenoid biosynthesis include pyridazinone, aminotriazole, and pyridone derivatives (7, 27, 28, 63, 64). Aminotriazoles have been shown to inhibit carotenoid biosynthesis at the phytoene desaturation step and at the cyclization step that leads to carotene formation (45) . Bleaching herbicides such as the pyridazinones and difunone inhibit phytoene desaturase exclusively (64). The photodestruction of chlorophyll by treatments of carotenogenesis inhibitors requires the presence of oxygen (18). The breakdown of chlorophyll is of a photooxidative nature due to the absence of protecting

carotenoids and not enzymatic in nature. The constituents of the protein synthesizing machinery are sensitive to photodestruction so that increasing photodestruction gradually diminishes the cell's ability to synthesize chlorophyll (27, 53).

Several investigators have investigated the mode of action of clomazone (19, 20, 21, 65, 68). Duke and Paul (20) investigated the ultrastructural effects of clomazone on cowpea (Vigna unguiculata L.). The plastids were similar to those treated with aminotriazole (1H-1,2,4-triazol-3-amine) and fluometuron (N,N-dimethyl-N'-[3-(trifluoromethyl)phenyl]urea), but not indicative of the effects of a herbicide with a single mechanism of action. Duke et al. (19, 21) reported that clomazone, unlike other bleaching herbicides, did not cause the buildup of phytoene and phytofluene via inhibition of phytoene desaturase enzymes. Duke et al. (21) reported that clomazone prevented the Shibata shift of chlorophyll biosynthesis which indicated a possible inhibition of phytol biosynthesis. The Shibata shift is a spectral shift of chlorophyllide from 684 to 673 nm which occurs when chlorophyllide is phytylated to chlorophyll. Sandmann and Böger (65, 66) found that in a cell-free system of spinach (Spinacea oleracea L.), in vitro conversion of mevalonate to geranyl pyrophosphate, farnesyl pyrophosphate, and geranylgeranyl

pyrophosphate is inhibited with subsequent decrease in carotenes and phytol. Enzyme kinetic studies indicated that clomazone is a non-competitive inhibitor of isopentenyl pyrophosphate isomerase leading to geranyl, farnesyl, and geranylgeranyl pyrophosphate (65, 66). Other investigators have shown that clomazone inhibits longitudinal growth of higher plants (28). Sandmann and Böger (65, 66) found that exogenous applications of gibberellin to clomazone-treated peas (Pisum sativum L.) reversed longitudinal growth inhibition. It has been postulated that clomazone affects isoprenoid metabolism preventing the biosynthesis of several terpenoid biosynthetic products such as carotenoids, gibberellin, and phytol.

OBJECTIVES

The objectives of the studies reported in this dissertation were: 1) to evaluate the efficacy of clomazone as a preplant and preemergence herbicide alone and in combination with other herbicides in full-season no-till soybeans, 2) to determine the effects of incorporation depth on the efficacy of clomazone applications in conventionally-tilled soybeans, 3) to characterize the effect of formulation and application type on clomazone volatilization, 4) to determine the basis of differential selectivity of clomazone among clomazone-sensitive livid amaranth and redroot pigweed and clomazone-tolerant soybean and smooth pigweed, and 5) to examine the response of corn mutants to clomazone.

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Table 1. Clomazone technical information [2]

Chemical name: 2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone.

Molecular weight: 239.7

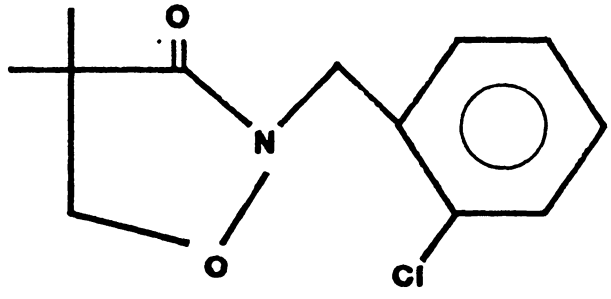
Specific gravity: 1.192 at 20 C

Vapor pressure: $1.92 \times 10_2$ Pa at 25 C

Water solubility: 1100 ppm

Completely soluble in: chloroform, acetone, hexane, methanol, acetonitrile,
xylene, and toluene.

Structure:



[2] Clomazone technical data sheet. FMC Corp., Princeton, NJ.

II. CLOMAZONE EFFICACY IN FULL-SEASON NO-TILL SOYBEANS (*Glycine max*)

INTRODUCTION

Because of a relatively long growing season in eastern Virginia, soybeans can be grown as the primary crop (full-season) or as a second crop following harvest of wheat or barley. In recent years, no-till planting has become an increasingly important method of soybean production with approximately 50% of the soybeans being planted without tillage in Virginia [1]. Successful no-till soybean production can only be achieved with adequate weed control (4, 11). No-till production systems are dependent on the use of non-selective herbicides for control of existing vegetation at or prior to the time of planting and additional herbicides for preemergence and postemergence weed control during the season.

In full-season no-till soybeans, herbicides have been applied 30 to 60 days prior to planting (preplant) to prevent weed interference at planting. However, residual control by preplant herbicides has been dependent on soil

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[1] Hagood, E.S. 1986. Personal communication, Virginia Polytechnic. Inst. & State Univ., Blacksburg, VA 24061.

moisture. According to Fawcett et al. (6), preplant treatments provide better weed control under dry conditions than under conditions of frequent and heavy rainfall. Stougaard et al. (11) found that winter and spring preplant applications of oryzalin reduced establishment of many annual broadleaf weeds and grasses and continued to provide control during the following summer months in soybeans. However, oryzalin does not control many large-seeded broadleaf weeds such as common ragweed, common cocklebur, velvetleaf, and spurred anoda. Recently, new soybean herbicides such as clomazone, imazaquin (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-quinolinecarboxylic acid) , and chlorimuron have shown promise for season-long broadleaf weed control in full-season no-till soybeans (3, 8, 14, 15, 16, 17).

In conventional tillage soybeans, clomazone has provided good to excellent control of annual grasses and several species of annual broadleaf weeds including large crabgrass, yellow foxtail (Setaria glauca L. #SETFA), common lambsquarters, common cocklebur, and velvetleaf (1, 2, 5, 9, 11, 16). Clomazone has also been found to provide weed control in full-season no-till soybeans (3, 12, 14, 15). In contrast, Krausz et al. (9) reported that preplant applications of clomazone provided control of many species including common lambsquarters but did not control others

including giant foxtail.

The objectives of this research were to determine the efficacy of clomazone as a preplant and preemergence herbicide alone and in combination with other herbicides in full-season no-till soybeans. Preplant and preemergence applications of clomazone were compared to similar applications of oryzalin.

MATERIALS AND METHODS

Field studies were conducted from 1984 through 1987 at the Eastern Shore Agricultural Experiment Station in Painter, VA on a State sandy loam soil (Typic Hapladults). The prior crop for each year of the study was soybeans except in 1986 when soybeans were planted into corn stalks remaining from the previous year. Soybean varieties planted, row spacing, planting and herbicide application dates for each year are shown in Table 1. Existing vegetation was controlled with non-selective herbicides that were applied over the entire study areas prior to or after planting. From 1984 through 1986, 0.4 kg a.i./ha paraquat was applied and in 1987, 1.7 kg a.i./ha glyphosate was applied. Paraquat applications included 0.25% (v/v)

Table 1. Soybean varieties, row spacing, planting dates, and herbicide application dates from 1984 through 1987.

Year	Variety	Row spacing	Planting date	Non-selective herbicide	Herbicide application dates		
					Non-selective	Residual	
						Preplant	Preemergence
		-(cm)-					
1984	'Pioneer 5482'	76	May 25	Paraquat	May 25	April 18	May 29
1985	'Asgrow 3966'	17	May 20	Paraquat	May 15	-------	May 21
1986	'Essex'	46	June 13	Paraquat	May 15 & June 16	April 25	May 17
1987	'Essex'	46	May 21	Glyphosate	May 22	March 22	May 25

non-ionic surfactant [2] in the spray mix.

Clomazone and other residual herbicides were applied to plots 2 m wide by 6 m long. The number of soybean rows per plot varied with row spacing but was a minimum of five. Treated plots were separated from each other by two untreated soybean rows which also served to indicate density of weed populations throughout the study sites. Flat fan tips[3] were used to apply all non-selective and residual herbicides in a spray volume of 200 L/ha at 275 kPa pressure.

The experimental design used in these studies varied with year. In 1984, 1986, and 1987, a randomized complete block design was used to evaluate the effects of application time (whole plots) and herbicides (sub-plots). In 1985, a single herbicide application timing (preemergence) permitted the use of a randomized complete block design. There were three replications of all treatments from 1984 through 1986 and four replications in

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[2] X-77. Chevron Chem. Co., San Francisco, CA 94119. Principal functioning agents are alkylarylpolyoxy -ethylene glycols, free fatty acids, and isopropanol.

[3] TeeJet 8003 tips. Spraying Systems Co., Wheaton, IL 60287.

1987.

Weed species and populations varied with year. Large crabgrass was present in all 4 yr at populations of 60 to 120 plants/m^2. Smooth pigweed, common lambsquarters, and common ragweed were each present in 2 yr of the study. Populations of smooth pigweed averaged 3 plants/m^2 in 1985 and 5 plants/m^2 in 1987, common lambsquarters populations varied and were present in 1985 and 1986 at 25 to 60 plants/m^2, and common ragweed populations were 80 to 100 plants/m^2 in 1984 and 1985.

Visual control ratings of individual species were collected twice during the season and subjected to analysis of variance following arcsine transformation. Treatment means were separated by Fisher's Protected Least Significance Difference Test. Data presented in the tables are non-transformed values. Soybeans were harvested for yield determinations in all years except 1985 when drought conditions were judged severe enough to mask any yield differences that might otherwise have been present.

RESULTS AND DISCUSSION

Control of large crabgrass and common ragweed in 1984 was influenced by herbicide treatment and application time at both rating dates (Table 2). Large crabgrass control from clomazone was higher when applied at planting than

when applied 40 days prior to planting. Preemergence clomazone applications were frequently comparable to those of oryzalin which generally provided nearly 80% or higher control at either application timing. Common ragweed control was low from both clomazone and oryzalin and although preemergence applications of linuron+chlorimuron or imazaquin improved control, remaining populations of common ragweed were high in most instances. Soybean yields in 1984 were low due to high weed populations and low rainfall in August but were highest when clomazone or oryzalin treatments were applied preemergence rather than preplant.

Since clomazone was more effective preemergence than preplant in 1984, only preemergence comparisons of clomazone with oryzalin were made in 1985. Large crabgrass control was high in 1985 with all clomazone and oryzalin treatments providing greater than 85% control through October (Table 3). Control of broadleaf weeds differed between clomazone and oryzalin. Although both clomazone and oryzalin provided early control of smooth pigweed, only oryzalin provided season-long control of this species. Common ragweed control was higher from clomazone than from oryzalin. Chlorimuron+linuron and imazaquin were more effective than linuron for enhancing control of broadleaf weeds from clomazone and oryzalin. Low rainfall in late

Table 2. Comparison of application time of clomazone and oryzalin for weed control in full-season no-till soybeans in 1984[a].

Herbicide[b]	Application timing[c]	Rate	Weed Control[d,e]				
			4 WAP		18 WAP		
			DIGSA	AMBEL	DIGSA	AMBEL	Soybean Yield
		---(kg/ha)---	----------(%)----------				(kg/ha)
Clomazone	PRPL	1.4	7	0	15	47	609
Clomazone	PRE	1.4	75	17	68	27	937
Clomazone+ Chlor. +Linuron	PRPL+ PRE+ PRE	1.4+ 0.06+ 0.5	28	10	20	65	682
Clomazone+ Chlor. +Linuron	PRE+ PRE+ PRE	1.4+ 0.06+ 0.5	33	92	68	85	1254
Clomazone+ Imazaquin	PRPL+ PRE	1.4+ 0.2	7	3	0	73	572
Clomazone+ Imazaquin	PRE+ PRE	1.4+ 0.2	52	79	35	70	804
Oryzalin	PRPL	1.1	94	0	80	0	511
Oryzalin	PRE	1.1	92	7	92	0	657
Oryzalin+ Chlor.+ Linuron	PRPL+ PRE+ PRE	1.1+ 0.06+ 0.5	88	10	77	63	767
Oryzalin+ Chlor.+ Linuron	PRPL+ PRE+ PRE	1.1+ 0.06+ 0.5	93	57	84	67	1181
Oryzalin+ Imazaquin	PRPL+ PRE	1.1+ 0.2	84	0	82	53	597
Oryzalin+ Imazaquin	PRE+ PRE	1.1+ 0.2	85	91	86	94	1230
Check			0	0	0	0	742
LSD (0.05)			9	9	12	9	267
Significance[f, g]:							
PRPL vs. PRE			**	**	**	**	**
Clomazone vs. Oryzalin			**	**	**	**	**

[a] Means within columns are the averages of three replications.

[b] Chlor. denotes chlorimuron.

[c] PRPL denotes preplant; PRE denotes preemergence.

[d] Percent control were transformed by arcsine prior to analysis with original rating percents reported. Untreated checks were not included in the analysis.

[e] WAP denotes weeks after planting.

[f] Indicated significance values at 1% (**), 5% (*), and nonsignificant (NS).

[g] Contrast of clomazone and oryzalin treatments alone.

Table 3. Comparisons of preemergence applications of clomazone and oryzalin for weed control in full-season no-till soybeasn in 1985[a].

Herbicide[b]	Rate	Weed Control[c,d]					
		3 WAP			16 WAP		
		DIGSA	AMACH	CHEAL	DIGSA	AMACH	AMBEL
	---(kg/ha)---	----------(%)----------					
Clomazone	1.1	96	67	88	94	0	81
Clomazone+ Chlor.+ Linuron	1.1+ 0.06+ 0.5	92	99	99	89	99	99
Clomazone+ Imazaquin	1.1+ 0.2	95	99	98	86	90	99
Clomazone+ Linuron	1.1+ 0.5	90	73	99	92	56	77
Oryzalin	1.1	91	82	55	97	85	57
Oryzalin+ Chlor.+ Linuron	1.1+ 0.06+ 0.5	91	98	96	90	98	99
Oryzalin+ Imazaquin	1.1+ 0.2	92	99	93	97	91	98
Oryzalin+ Linuron	1.1+ 0.5	91	98	96	97	82	65
Check		0	0	0	0	0	0
LSD (0.05)		10	11	23	11	20	27
Significance[e,f]:							
Clomazone vs. Oryzalin		NS	**	**	**	**	NS

[a] Means within columns are the average of three replications.

[b] Chlor. denotes chlorimuron.

[c] Percent control were transformed by arcsine prior to analysis with original percent control reported.

[d] WAP denotes weeks after planting.

[e] Indicated significance values at 1% (**), 5% (*), and nonsignificant (NS).

[f] Contrast of clomazone and oryzalin treatments alone.

July and August resulted in poor soybean pod development so soybeans were not harvested.

Application timing initially influenced large crabgrass response to herbicide treatments in 1986 (Table 4). Large crabgrass control by clomazone was higher than by oryzalin with control from preemergence clomazone applications being higher than from those made 21 days prior to planting. Imazaquin and linuron+chlorimuron occasionally enhanced early control of large crabgrass by clomazone and oryzalin. Twelve WAP, large crabgrass control was higher from clomazone applied preemergence than from oryzalin applied preplant or preemergence. In addition, combinations of clomazone or oryzalin with imazaquin more consistently controlled large crabgrass than combinations with linuron or linuron+chlorimuron.

Common lambsquarters control by clomazone was approximately 80% from preplant and preemergence applications but oryzalin was ineffective against this species in 1986 (Table 4). Linuron, linuron+chlorimuron, and imazaquin increased the control of common lambsquarters by both clomazone and oryzalin although the combination of oryzalin+linuron resulted in the lowest control. Soybean yields were generally high in 1986 with the exception of yields from soybeans treated with the combinations of oryzalin plus linuron+chlorimuron. These treatments

Table 4. Comparisons of application timing of clomazone and oryzalin for weed control in full-season no-till soybeans in 1986[a].

Herbicide[b]	Application timing[c]	Rate	Weed Control[d,e]			
			4 WAP		12 WAP	
			DIGSA	CHEAL	DIGSA	Soybean Yield
		---(kg/ha)---	----------(%)---------			(kg/ha)
Clomazone	PRPL	1.1	86	77	82	2265
Clomazone	PRE	1.1	94	82	93	1848
Clomazone+ Chlor.+ Linuron	PRPL+ PRE+ PRE	1.1+ 0.06+ 0.5	78	99	75	1906
Clomazone+ Chlor.+ Linuron	PRE+ PRE+ PRE	1.1+ 0.06+ 0.5	96	99	84	1853
Clomazone+ Imazaquin	PRPL+ PRE	1.1+ 0.2	95	99	94	2161
Clomazone+ Imazaquin	PRE+ PRE	1.1+ 0.2	95	99	91	2052
Clomazone+ Linuron	PRPL+ PRE	1.1+ 0.5	83	98	87	1884
Clomazone+ Linuron	PRE+ PRE	1.1+ 0.5	92	99	92	2343
Oryzalin	PRPL	1.1	59	0	72	1968
Oryzalin	PRE	1.1	68	7	72	1921
Oryzalin+ Chlor.+ Linuron	PRPL+ PRE+ PRE	1.1	68	99	52	1093
Oryzalin+ Chlor.+ Linuron	PRE+ PRE+ PRE	1.1	81	99	52	1265
Oryzalin+ Imazaquin	PRPL+ PRE	1.1+ 0.2	92	99	88	2296
Oryzalin+ Imazquin	PRE+ PRE	1.1+ 0.2	93	99	83	1702
Oryzalin+ Linuron	PRPL+ PRE	1.1+ 0.5	62	68	58	1410
Oryzalin+ Linuron	PRE+ PRE	1.1+ 0.5	69	68	72	1921
Check			0	0	0	232
LSD (0.05)			9	13	12	633
Significance[f,g]:						
PRPL vs. PRE			**	NS	NS	NS
Clomazone vs. Oryzalin			**	**	**	**

[a] Means within columns are the average of three replications.

[b] Chlor. denotes chlorimuron.

[c] PRPL denotes preplant; PRE denotes preemergence.

[d] Percent control were transformed by arcsine prior to analysis with original percent control reported.

[e] WAP denotes weeks after planting.

[f] Indicated significance at 1% (**), 5% (*), and nonsignificant (NS).

[g] Contrast of clomazone and oryzalin treatments alone.

initially caused up to 30% reduction in soybean growth (data not presented) from which the crop apparently did not recover. Injury was not observed from combinations of clomazone plus linuron+chlorimuron.

Application timing significantly affected weed responses to treatments in 1987. Clomazone applied at 1.65 kg/ha 64 days prior to planting failed to provide adequate large crabgrass and smooth pigweed control both early and late in the season (Table 5). Preemergence applications of clomazone alone provided good large crabgrass and smooth pigweed control early in the season. Linuron did not significantly increase the control from 1.1 kg/ha clomazone applied preplant or preemergence. Soybean yields were higher from preemergence clomazone applications and treatments containing linuron than from preplant clomazone applications.

In these studies, clomazone alone or in combination with other herbcides did not cause soybean injury (data not shown). Clomazone applied preemergence provided better overall weed control than when applied 22 to 64 days preplant in full-season no-till soybeans. Clomazone provided large crabgrass control equivalent to that of oryzalin and provided better control of common lambsquarters. However, control from clomazone was inconsistent for smooth pigweed and common ragweed.

Preemergence applications of imazaquin or linuron+chlorimuron with clomazone provided adequate control of smooth pigweed when clomazone alone was not adequate; preemergence applications of linuron were less effective. A possible explanation for significant differences in weed control with application timing for clomazone is moisture. Preplant applications in these studies were always exposed to more rainfall than preemergence applications. Studies conducted on clomazone volatility and soil residual aspects indicate that clomazone is strongly adsorbed to the soil, but is easily displaced by moisture leading to off-site movement (7,10). Thelen et al. (13) found that when clomazone is applied to no-till soybeans, residual activity was less than with applications in conventionally tilled soybeans. They further reported that clomazone volatility is greater from no-till applications which could reduce clomazone activity.

Table 5. Comparisons of application timing of clomazone and oryzalin for weed control in full-season no-till soybeans in 1987 [a].

Herbicide	Application timing [b]	Rate	Weed Control [c,d] 4 WAP		12 WAP		Soybean
			DIGSA	AMACH	DIGSA	AMACH	Yield
		(kg/ha)	----------(%)----------				(kg/ha)
Clomazone	PRPL	1.1	35	37	18	35	804
Clomazone	PRE	1.1	89	88	69	88	969
Clomazone	PRPL	1.7	50	30	40	52	495
Clomazone	PRE	1.7	95	90	92	89	1649
Clomazone+ Linuron	PRPL+ PRE	1.1+ 0.6	75	48	55	57	1320
Clomazone+ Linuron	PRE+ PRE	1.1+ 0.6	95	90	86	88	1155
Check			0	0	0	0	1072
LSD (0.05)			9	11	21	14	410
Significance [e] :							
PRPL vs. PRE			**	**	**	**	**

[a] Means within columns are the average of four replications.
[b] PRPL denotes preplant; PRE denotes preemergence.
[c] Percent control were transformed by arcsine prior to analysis with original percent control reported.
[d] WAP denotes weeks after planting.
[e] Indicated significance level at 1% (**), 5% (*), and nonsignificant (NS)

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III. INFLUENCE OF APPLICATION METHOD AND INCORPORATION DEPTH ON CLOMAZONE EFFICACY IN SOYBEANS (Glycine max)

INTRODUCTION

Clomazone is a recently introduced herbicide for selective grass and broadleaf weed control in soybeans which is used at rates ranging from 0.6 and 1.1 kg/ha (7, 12, 13). At present, data on the effect of application method on clomazone efficacy is limited.

Soil-applied herbicides must move into the zone of weed seed germination for adequate weed control to take place. This can be achieved by water from precipitation or irrigation, or by mechanical incorporation (4). Some herbicides such as the carbamothioates and dinitroanilines must be incorporated to prevent loss due to volatilization and photodecomposition (4, 9). Clomazone is a volatile herbicide and off-site movement from preemergence applications has been observed (2, 3, 9). Incorporation reduces off-site movement of clomazone (5, 11).

The optimal depth of herbicide incorporation for weed control also depends on the chemical and physical characteristics of the herbicide. EPTC provided optimal green foxtail (Setaria glauca L. #SETLU) and barnyardgrass control from incorporations of 2.5 cm (4). At incorporation

depths greater than 5 cm, a dilution effect occurred resulting in a loss of weed control (4).

The objectives of these studies were to compare efficacy of clomazone following preemergence and preplant incorporated application and to determine the optimal clomazone depth of incorporation.

MATERIALS AND METHODS

Field studies were conducted from 1985 to 1988 at the Eastern Shore Agricultural Experiment Station in Painter, VA on a State sandy loam soil (Typic Hapladults). Soybean variety, row spacing, planting and harvest dates, and herbicide application dates for each year are shown in Table 1.

Clomazone and other residual herbicides were applied to plots 2 m by 7.6 m long. Treated plots were separated from each other by two untreated crop rows that served to indicate density of weed population throughout the study sites. Large crabgrass populations varied from 75 to 113 plants per m^2 for all 4 years of the study, smooth pigweed populations varied from 40 to 72 plants per m^2 in 1987 and 1988, and jimsonweed populations were approximately 40 plants per m^2 in 1985 and 1987. Common lambsquarters

Table 1. Soybean variety, planting, herbicide application, and harvest dates for 1985 through 1986.

Year	Variety	Planting date	Herbicide application date	Harvest date
1985	Asgrow 3966	June 5	June 4	October 9
1986	Essex	June 20	June 19	October 15
1987	Essex	May 25	May 22	October 15
1988	Essex	June 9	June 9	October 25

populations ranged from 27 to 54 plants per m^2 in 1986 and 1987. All herbicides were applied with flat fan tips in a spray volume of 200 L/ha at 275 kPa pressure.

For the comparison of application method studies, clomazone and other residual herbicides were incorporated twice with a field cultivator[1] to a depth of 4 cm. For depth of incorporation studies, clomazone was incorporated twice with a field cultivator to a depth of approximately 4 cm (shallow) and 8 cm (deep).

Control ratings of large crabgrass were made in all years of the study, smooth pigweed in 1985, 1987, and 1988, jimsonweed in 1985 and 1987, common lambsquarters in 1986 and 1987.

The experimental design in all studies was a randomized complete block design. There were three replications in 1985 and 1986 and four replications in 1987 and 1988. Percent weed control data were subjected to analysis of variance following arcsine transformation. Means presented in the tables are non-transformed values. Percent weed control and yield means were separated by Fisher's Protected Least Significance Difference test.

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[1] McKee Field Cultivator, McKee Bros, Lincoln, NE.

RESULTS AND DISCUSSION

Comparison of Application Method. Generally, preemergence applications of clomazone provided weed control equivalent to preplant incorporated applications. The effect of the timing of clomazone application was not significant for large crabgrass control in 1985 and 1988 (Table 2 and 5). In 1986, clomazone applied preemergence provided significantly better large crabgrass control than preplant incorporated applications 4 and 12 weeks after treatment (WAT) (Table 3). The addition of imazaquin to clomazone did not improve large crabgrass control in these studies (Tables). The addition of chlorimuron plus linuron to clomazone did not improve large crabgrass control in 1987 or 1988 at 3 and 4 WAT, respectively (Tables 4 and 5). However, the addition of chlorimuron plus linuron did significantly improve large crabgrass control at 14 and 8 WAT in 1987 and 1988, respectively. Generally, clomazone applied preplant incorporated provided large crabgrass control equivalent to or better than trifluralin in these studies. Clomazone applied preemergence provided better large crabgrass control than metolachlor applied preemergence in 1985 (Table 2). In 1986, clomazone applied preemergence provided control equivalent to that of alachlor (Table 3).

The response of smooth pigweed to clomazone applied preemergence and preplant incorporated varied throughout the study. Clomazone applied preemergence provided significantly better smooth pigweed control than preplant incorporated in 1985 (Table 2). However, in 1988, an application method effect was not observed 4 WAT and preplant incorporated clomazone applications provided better smooth pigweed control 8 WAT (Table 5). The addition of imazaquin to clomazone improved smooth pigweed control over that of clomazone alone when applied preemergence or preplant incorporated in 1985. However, the addition of imazaquin to clomazone did not improve smooth pigweed control in 1987, 3 or 14 WAT (Table 4). The addition of chlorimuron plus linuron to clomazone did not improve smooth pigweed control from clomazone alone in 1987, 3 or 14 WAT (Table 4). However, the addition of chlorimuron plus linuron to clomazone applied preemergence or preplant incorporated improved smooth pigweed control over that of clomazone alone at 4 and 8 WAT (Table 5). Clomazone applied preplant incorporated did not provide smooth pigweed control equivalent to trifluralin in 1985. However, clomazone provided better smooth pigweed control than trifluralin in 1987 (Table 4).

A significant application method effect was not observed for either jimsonweed or morningglory species in

Table 2. Comparisons of application method for clomazone and imazaquin in conventionally-tilled soybeans in 1985[a].

Herbicide	Rate	Application type[c]	Weed Control[b] DIGSA 8 WAT	AMACH 8 WAT	DATST 8 WAT	Soybean Yield
	-(kg/ha)-		----------(%)----------			-(kg/ha)-
Clomazone	0.8	PE	98	71	98	675
Clomazone	0.8	PPI	88	26	94	1111
Clomazone + Imazaquin	0.8+0.1	PE +PE	96	99	99	873
Clomazone + Imazaquin	0.8+0.1	PPI+PPI	91	96	99	1251
Metolachlor	1.6	PE	66	97	0	448
Trifluralin	0.6	PPI	91	65	0	1140
Check			0	0	0	576
LSD (0.05)			14	19	8	NS
Significance[d] :						
PE vs. PPI			NS	*	NS	**
Clomazone (PPI) vs. Trifluralin			NS	NS	**	NS
Clomazone (PE) vs. Metolachlor			*	NS	**	NS

[a] Means within columns are average of three replications.

[b] Means were transformed by arcsine prior to analysis, non-transformed data are presented. Checks were not included in analysis.

[c] PE denotes preemergence ; PPI denotes preplant incorporated.

[d] Indicated significance values at 1% (**), 5% (*), and nonsignificant (NS).

Table 3. Comparisons of application method for clomazone and imazaquin in conventionally-tilled soybeans 1986[a].

Herbicide	Rate	Application type[c]	Weed Control[b] DIGSA 4 WAT	DIGSA 12 WAT	CHEAL 4 WAT	CHEAL 12 WAT	Soybean Yield
	-(kg/ha)-		----------(%)----------				-(kg/ha)-
Clomazone	0.6	PE	97	94	98	96	3136
Clomazone	0.6	PPI	92	78	91	88	1304
Clomazone	0.8	PE	97	98	98	96	2624
Clomazone	0.8	PPI	93	82	97	93	1207
Clomazone + Imazaquin	0.6+0.1	PE+PE	99	95	99	99	3136
Clomazone + Imazaquin	0.6+0.1	PPI+PPI	91	77	99	99	941
Alachlor	2.24	PE	98	95	96	96	2168
Trifluralin	0.6	PPI	95	93	98	98	843
Check			0	0	0	0	1146
LSD (0.05) Treatment			NS	11	NS	8	536
Significance[d]:							
PE vs. PPI			NS	**	NS	*	**
Clomazone (PPI) vs. Trifluralin			NS	**	NS	*	NS
Clomazone (PE) vs. Alachlor			NS	NS	NS	NS	*

[a] Means within columns are average of three replications.

[b] Means were transformed by arcsine prior to analysis, non-transformed data are presented. Checks were not included in analysis.

[c] PE denotes preemergence; PPI denotes preplant incorporated.

[d] Indicated significance values at 1% (**), 5% (*), and nonsignificant (NS).

Table 4. Comparison of preplant incorporated herbicides in conventionally-tilled soybeans in 1987 [a].

Herbicide	Rate	Weed Control [b] DIOSA 3 WAT	DIOSA 14 WAT	AMACH 3 WAT	AMACH 14 WAT	DATST 3 WAT	DATST 14 WAT	CHEAL 3 WAT	Soybean Yield
	-(kg/ha)-	-(%)-							-(kg/ha)-
Clomazone	0.6	71	30	80	45	86	51	85	593
Clomazone	0.8	84	65	81	60	89	56	81	470
Clomazone	1.1	80	68	85	49	90	62	83	688
Clomazone + Imazaquin	0.6 + 0.1	88	64	84	65	88	65	93	607
Clomazone + Imazaquin	0.8 + 0.1	86	69	89	72	92	72	96	756
Clomazone + Chlor.+Linuron	0.6 + 0.3	81	62	85	55	93	55	96	668
Clomazone + Chlor.+Linuron	0.8 + 0.3	91	71	88	60	93	60	96	716
Trifluralin	0.6	84	75	22	10	6	12	86	378
Check		0	0	0	0	0	0	0	475
LSD (0.05)		14	20	11	29	7	7	14	241
Significance [c]:									
Clomazone vs. Trifluralin		**	**	NS	**	**	**	NS	NS

[a] Means within columns are the average of four replications.

[b] Means within columns were transformed by arcsine prior to analysis; non-transformed means are presented. Checks were not included in analysis.

[c] Indicated significance values at 1% (**), 5% (*), and nonsignificant (NS).

Table 5. Comparisons of application method for clomazone and chlorimuron plus linuron in conventionally-tilled soybeans 1988[a].

Herbicide	Rate	Application[c]	Weed Control[b]				Soybean Yield
			DIGSA		AMACH		
			4 WAT	8 WAT	4 WAT	8 WAT	
	-(kg/ha)-		----------(%)----------				-(kg/ha)-
Clomazone	0.3	PE	85	49	81	35	3286
Clomazone	0.3	PPI	80	28	72	27	3530
Clomazone	0.6	PE	93	79	89	52	3396
Clomazone	0.6	PPI	92	85	89	84	4068
Clomazone	0.8	PE	94	80	90	62	4056
CLomazone	0.8	PPI	96	92	90	90	4080
Clomazone + Chlor.+Linuron	0.3+0.06+0.5	PE +PE	99	99	98	99	2537
Clomazone + Chlor.+Linuron	0.3+0.06+0.5	PPI+PPI	98	93	99	99	2777
Clomazone + Chlor.+Linuron	0.6+0.06+0.5	PE+PE	99	99	98	99	2598
Clomazone + Chlor.+Linuron	0.6+0.06+0.5	PPI+PPI	99	98	99	94	2613
Check			0	0	0	0	2364
LSD (0.05) Treatment			4	20	8	13	525
Significance[d]:							
PE vs. PPI			NS	NS	NS	**	*

[a] Means within columns are the average of four replications.

[b] Means within columns were transformed by arcsine prior to analysis; non-transformed means are shown. Checks were not included in analysis.

[c] PE denotes preemergence; PPI denotes preplant incorporated.

[d] Indicated significance values at 1% (**), 5% (*), and nonsignificant (NS).

1985. The addition of imazaquin to clomazone did not improve jimsonweed or morningglory species control in 1985 8 WAT (Table 2) from either application type or from preplant incorporated applications in 1987 (Table 4). Clomazone applied preplant incorporated provided better jimsonweed and morningglory species control than trifluralin in both 1985 and 1987 (Table 2 and 4).

An application method effect was not observed for common lambsquarters 4 WAT in 1986. However, at 12 WAT, clomazone applied preemergence provided better common lambsquarters control than clomazone applied preplant incorporated. The addition of imazaquin to clomazone did not improve common lambsquarters control over that provided by preemergence applications of clomazone alone in 1986 (Table 3). However, the addition of imazaquin to clomazone applied preplant incorporated did improve common lambsquarters control. The combination of clomazone plus imazaquin applied preplant incorporated did not improve common lambsquarters control over clomazone applied alone in 1987 (Table 4). Clomazone and trifluralin provided equivalent common lambsquarters control in 1986 and 1987 (Tables 3 and 4).

Soybean yield response to application method varied throughout the study. In 1985, a significant application method effect was not observed (Table 2). Soybean yields

were greater from preemergence clomazone applied in 1986 (Table 3). However, soybean yields were significantly greater from clomazone preplant incorporated in 1988 (Table 5). The addition of imazaquin to clomazone applied preemergence or preplant incorporated did not improve soybean yield over that provided by clomazone applied alone in 1985 and 1987 (Table 2 and 4). In 1986, the combination of clomazone and imazaquin applied preemergence did not improve soybean yields significantly over that provided by clomazone applied alone preemergence (Table 3). However, soybean yields from clomazone applied alone preplant incorporated were better than the combination of clomazone and imazaquin applied preplant incorporated. The addition of chlorimuron plus linuron to clomazone applied preplant incorporated did not improve soybean yields in 1987 (Table 4). In 1988, soybean yields were greater from clomazone applied alone than in combination with chlorimuron plus linuron (Table 5).

Comparison of Incorporation Depth. The response of weed species to clomazone incorporation depth was similar in both 1987 and 1988. Large crabgrass control was 92 % from the shallow (4 cm) clomazone incorporation in 1987 and 1988, 3 and 4 WAT, respectively (Table 6 and 7). In 1987, clomazone incorporated deep (8 cm) provided significantly lower large crabgrass control than the shallow

incorporation at all but the highest rate 3 WAT. In 1988, the effect of incorporation depth was not as great as in 1987. Shallow clomazone incorporation provided better large crabgrass control 14 WAT in 1987 and 8 WAT in 1988 at 0.6 and 0.8 kg/ha. As clomazone rate increased from 0.6 to 1.1 kg/ha, differences in large crabgrass control between shallow and deep incorporation narrowed in 1987 and 1988.

Although both shallow and deep incorporated applications of clomazone provided less than adequate smooth pigweed control in 1987, shallow incorporated clomazone applications provided better smooth pigweed at all rates 3 WAT (Table 6). Fourteen WAT, smooth pigweed control was weak enough so that significant differences between shallow and deep clomazone incorporated applications were not present. In 1988, clomazone incorporated shallow provided better smooth pigweed control than deep incorporated clomazone at all but the highest rates 4 WAT (Table 7). At 8 WAT, shallow incorporated clomazone provided better smooth pigweed control at all clomazone rates but as in previous instances, the largest differences were at the lower rates.

The response of jimsonweed to clomazone incorporation depth was similar to that of large crabgrass and smooth pigweed. At 3 WAT, differences in incorporation depth were only apparent at the 0.6 kg/ha rate where shallow

Table 6. Effect of incorporation depth on clomazone efficacy in conventionally-tilled soybeans in 1987[a].

Clomazone rate	Weed Control[b]											
	DIOSA				AMACH				DATST			
	3 WAT		14 WAT		3 WAT		14 WAT		3 WAT		14 WAT	
	4 cm	8 cm	4 cm	8 cm	4 cm	8 cm	4 cm	8 cm	4 cm	8 cm	4 cm	8 cm
(kg/ha)-	(%)											
0.6	92	48	76	46	66	46	18	29	92	48	62	14
0.8	94	80	85	70	83	47	20	15	95	88	72	52
1.1	96	88	88	80	81	65	35	38	96	88	74	55
Weedy Check	0		0		0		0		0		0	
Significance[c]:												
Rate	**		**		**		**		**		**	
Depth	**		**		**		NS		**		**	
Rate x Depth	**		NS		NS		NS		**		NS	

[a] Means within columns are the average of four replications.

[b] Means within columns were transformed by arcine prior to analysis; non-transformed means are shown. Checks were not included in analysis.

[c] Indicated significance level at 1% (**), 5% (*), and nonsignificant (NS).

Table 7. Effect of incorporation depth on clomazone efficacy in conventionally-tilled soybeans in 1988[a].

Clomazone rate	Weed Control[b]								Soybean Yield	
	DIOSA				AMACH					
	4 WAT		8 WAT		4 WAT		8 WAT			
	4 cm	8 cm	4 cm	8 cm	4 cm	8 cm	4 cm	8 cm	4 cm	8 cm
-(kg/ha)-	----------(%)----------								---(kg/ha)---	
0.6	92	91	96	47	92	67	92	9	3897	2980
0.8	98	90	93	56	93	88	86	50	3628	3628
1.1	99	98	89	72	95	94	89	71	3494	3359
Weedy Check	0		0		0		0		3530	
Significance[c]:										
Rate	**		**		**		**		NS	
Depth (shallow vs deep)	**		**		**		**		*	
Rate x Depth	NS		NS		NS		NS		*	

[a] Means within columns are the average of four replications.

[b] Means within columns were transformed by arcsine prior to analysis; non-transformed means are shown. Checks were not included in analysis.

[c] Indicated level of significance at 1% (**), 5% (*), and nonsignificant (NS).

incorporated clomazone provided better jimsonweed control than clomazone incorporated deep (Table 7). Fourteen WAT, clomazone provided better jimsonweed control from the shallow incorporation at all rates examined.

Due to a dry growing season and weed pressure, soybean yields were not made from clomazone incorporation studies in 1987. In 1988, differences in soybean yield were observed at the lowest rate of clomazone.

In conclusion, shallow incorporated applications of clomazone provided better weed control than deep incorporation, especially at the lower rates of clomazone. In addition, clomazone incorporated at greater depths may be below weed germination zone (upper 1 cm of soil) where most weed seeds germinate such that its effectiveness is reduced (1).

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IV. VOLATILIZATION AND EFFICACY OF CLOMAZONE AS AFFECTED BY FORMULATION

INTRODUCTION

Following its application in the field a herbicide may be lost by means of runoff, leaching into the soil below the activity zone, photodecomposition, adsorption to soil particles, and volatilization or movement of the pesticide from point of application to the atmosphere (7, 8). Volatilization is defined as the loss of chemical vapor from soil and water surfaces (7). The rate of movement away form an evaporating surface is a diffusion-controlled process related to the vapor pressure of pesticide, molecular weight, and temperature (3, 8).

Direct measurements of volatilization rates are the best way to address the importance of pesticide volatility in the field (8). Bardsley et al. (1) and White et al. (10) have quantitatively studied trifluralin losses under field conditions. They found trifluralin losses to be proportional to concentration applied and the greatest concentrations of volatilizing trifluralin were closest to the ground. White et al. (10) found seasonal losses of trifluralin due to volatility to be approximately 22% of

that applied. Of this 22%, 13 to 15% was lost during application and through the first 24 hours. Approximately one-half was lost during the first 9 days and 90% in the first 35 days. Combined losses through other pathways such as chemical and microbial degradation and photodecomposition were 2.5 times that lost to volatilization.

Clomazone was labeled for use in soybeans in 1986. When the first applications of clomazone were made in the spring of 1986, numerous observations of non-target vegetation damage by clomazone in several midwestern states were reported (2). Clomazone apparently volatilizes off the treated soil surface and moves downwind to areas where non-target plant foliage is bleached. For most non-target vegetation, the bleaching is a temporary phenomenon. Some desirable plant species that are affected are ornamentals such as roses, trees such as flowering and edible cherries, agronomic crops such as alfalfa and small grains, and vegetable crops such as lettuce, cole crops, and radishes (2).

Halstead and Harvey (4, 5, 6) have used a field bioassay technique to qualitatively characterize some of the aspects of clomazone off-site movement with certain environmental factors such as soil moisture, rainfall after application, surface crop residues, and rates of

formulation. It was found that the greatest off-site movement occurred from wet soil. Preplant incorporated treatments of clomazone were found to reduce off-site movement by 75%. The greater amount of off-site movement occurred with the current emulsifiable concentrate formulation than with a granular formulation. Limited off-site movement of clomazone occurred for up to two weeks after treatment (7, 8). Carrier volume did not affect clomazone volatilization rates (6). Thelen et al. (9) found greater clomazone volatility after rainfall and from applications to no-till or minimum tillage soybeans. The increased clomazone volatility from no-till application could be due to increased surface area to volatilize from and the greater soil moisture present in no-till soils.

The objectives of these studies were to evaluate and compare the potential for volatilization and field efficacy of three clomazone formulations following soil application. The formulations of clomazone examined included an emulsifiable concentrate, a wettable powder, and a microencapsulated formulation. In a separate study, volatilization of clomazone applied to soybean foliage was examined.

MATERIALS AND METHODS

General field studies. Field studies were conducted in 1986 through 1988 at the Eastern Shore Agricultural Experiment Station in Painter, VA on a State sandy loam soil (Typic Hapladults). Soybean variety, row spacing, planting and harvest date, herbicide application date for each year are shown in Table 1.

Clomazone was applied preemergence to plots 2 m by 7.6 m. Treated plots were separated from each other by two untreated crop rows that served to indicate density of weed population throughout the study sites. In 1986, an emulsifiable concentrate and a wettable powder formulation of clomazone were applied. In 1987 and 1988, a microencapsulated formulation (ME) was also applied. Soybeans were not planted into clomazone treated plots in 1987. All herbicides were applied with flat fan tips in a spray volume of 200 L/ha at 275 kPa pressure. Preplant incorporated applications of clomazone were incorporated twice with a field cultivator to a depth of 4 cm.

Control ratings of large crabgrass were made visually in 1986 and 1988. Weed control ratings were not made in 1987.

Table 1. Planting date, row spacing, application date, and harvest date for studies in 1986 to 1988.

Year	Variety	Row spacing	Application date	Harvest date
1986	Essex	76	June 27	October 12
1987	-	-	July 1	-
1988	Essex	76	June 8	October 25

Collection bottle apparatus. To trap clomazone volatilizing off the soil, a 2 L open ended bottle connected via tubing to a 125 ml flask containing 10 g of activated charcoal was inserted into clomazone-treated soil immediately after application. The collection bottle was not moved during the study period. Clomazone volatilization samples were taken separately at the first, second and tenth day after clomazone application by adding new charcoal to the flask. Samples collected at each of these days represent clomazone volatilization for a 24 h period and not cumulative volatilization of the herbicide 2 or 10 d after application. In 1987, the maximum and minimum daily temperature during the study was 28 $\pm$ 4 C and 22 $\pm$ 3 C, respectively and 2.9 cm of rainfall was received. In 1988, the maximum and minimum daily temperature during the study was 28 $\pm$ 5 C and 16 $\pm$ 4 C, respectively and 1.9 cm of rainfall was received. The temperature inside the collection bottle exceeded that of the ambient temperature by an average of 3 C.

Extraction of clomazone. An extraction procedure for clomazone from activated charcoal was developed. Ten g samples of activated charcoal were fortified with radiolabeled clomazone (28.0 mCi/mmole, aromatic ring labelled) for 5 d. Several organic solvents and solvent combinations for clomazone extraction were examined and

compared. The final procedure chosen for clomazone extraction was based on the addition of 75 ml of chloroform plus methanol (2:1 v/v) plus 1 ml of water with 4 h shaking and subsequent addition of 50 ml of chloroform plus methanol (2:1 v/v) plus 1 ml of water with 2 h shaking. The 75 ml and 50 ml washings were combined and concentrated to dryness under air. The samples were resuspended in 1 ml of hexane. This procedure yielded an average of 73 $\pm$ 9% of applied clomazone. A linear response between ^{14}C-clomazone applied to charcoal and clomazone extracted was observed (Figure 1).

Gas chromatographic analysis. The extracted clomazone was analyzed by capillary gas chromatography[1]. A splitless adapter was used with a 30 m, 0.32 mM fused silica glass capillary column[2] and ^{63}Ni electron capture detector. Nitrogen was used as the carrier gas at 275 kPa, the detector was set at 350 C , inlet temperature was 100 C, and the oven temperature was 50 C. A sample of 1 ul was injected for analysis. Clomazone volatilization data are presented as a percent of clomazone applied in the field. The retention time of clomazone was 5.97 min.

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[1] Tracor 540, Tracor Instruments, Austin, TX. 78721.

[2] SPB-35 fused silica capillary column, Supelco, Inc., Bellefonte, PA 16823.

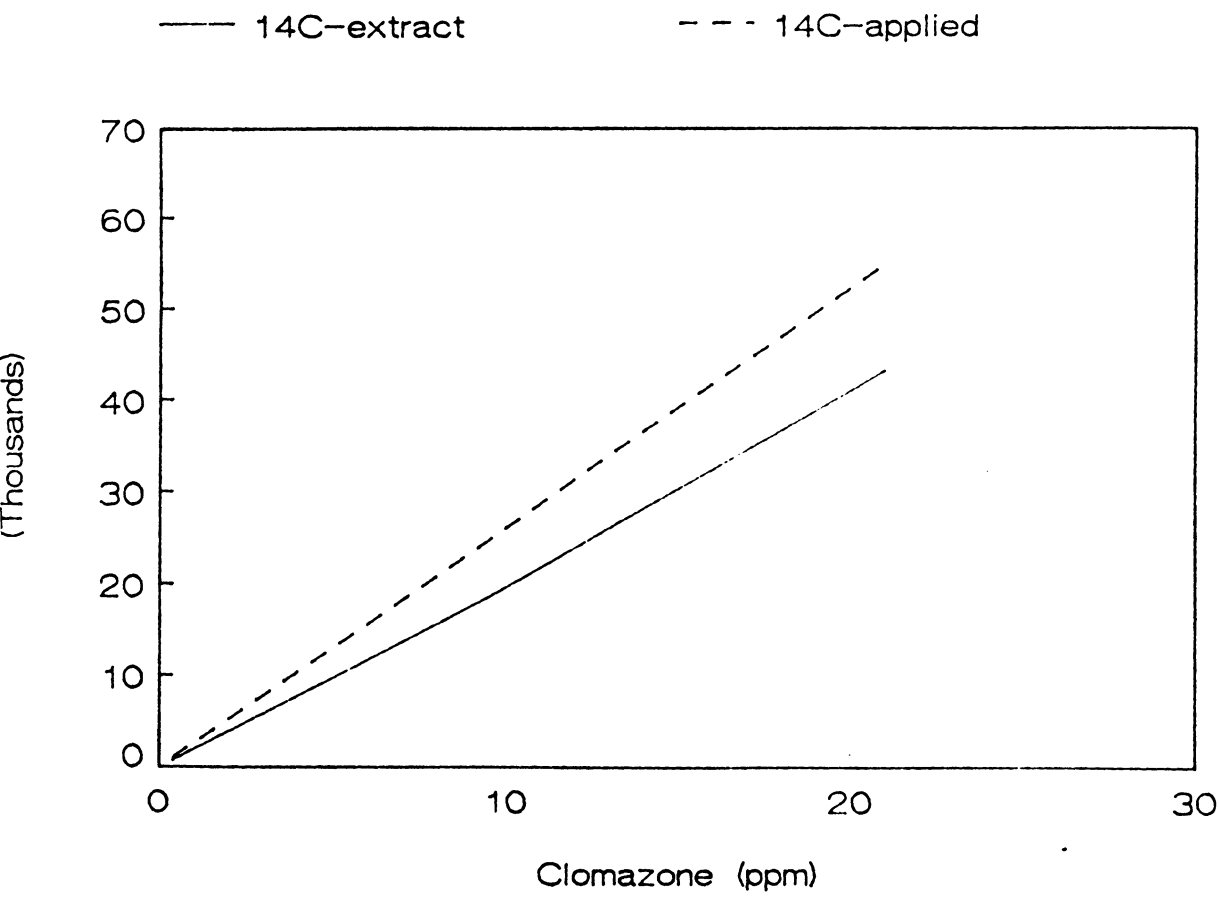

Figure 1. The recovery of ^{14}C-clomazone from activated charcoal over a concentration range.

Volatilization of clomazone from soybean foliage. To determine clomazone volatility applied to leaf surfaces, radiolabeled clomazone was applied to detached wet and dry soybean leaves. The leaves were placed in a chamber with an air flow of 1 cm/min for 12 h. The temperature inside the chamber was 25 C. The leaves were removed and oxidized using a biological sample oxidizer[3]. Following combustion, the released $^{14}CO_2$ was trapped in an appropriate liquid scintillation fluid and counted by liquid scintillation spectrometry[4]. This experiment was repeated three times.

Experimental design. The experimental design was a randomized complete block design. There were three replications in 1986 and four replications in 1987 and 1988. Percent weed control data were transformed by arcsine with non-transformed data presented. Percent weed control in 1986 and 1988 and volatilization means in 1987 and 1988 were separated by Fisher's Protected Least Significance Difference Test.

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[3] Tricarb Sample Oxidizer, Packard Instrument Co. Downers Grove, IL 60515.

[4] Beckmann LS 8100, Beckman Instruments. Fullerton, CA 92634.

RESULTS AND DISCUSSION

Field Efficacy. In general, differences in clomazone efficacy due to formulation were minor. In 1986, both the emulsifiable concentrate and wettable powder formulations applied preemergence provided good to excellent (>95%) season-long large crabgrass. No differences due to formulation were found (Table 1).

In 1988, early-season large crabgrass control was good at rates above 0.6 kg/ha from all clomazone formulations examined (Table 2). Differences between formulation or between preemergence and preplant incorporated applications were found. Large crabgrass control declined during the season for all treatments. Preemergence applications of the emulsifiable concentrate and the microencapsualted formulation provided better large crabgrass control than the wettable powder formulation. For preplant incorporated clomazone applications, no formulation differences were found.

Thus it appears that the efficacy of clomazone for weed control is independent of formulation and was more dependent on rate than on differences in formulation or timing of application.

Clomazone Volatilization. In 1987, clomazone volatilization was greater from the emulsifiable concentrate and wettable powder formulations than the microencapsulated formulation

Table 2. Effect of clomazone formulation applied preemergence on large crabgrass control in 1986.

Formulation[a]	Clomazone rate	Large crabgrass[b]	
		4 WAT	10 WAT
	(kg/ha)	---------(%)----------	
Weedy Check		0	0
4 EC	0.3	95	87
	0.6	97	98
	0.8	97	94
25WP	0.3	97	93
	0.6	94	86
	0.8	99	95
Significance[c] :			
Formulation		NS	NS
Rate		NS	NS
Formulation x Rate		NS	NS

[a] 4EC denotes 4 lb/gal emulsifiable concentrate; 25WP denotes 25% wettable powder.

[b] Weedy checks were not include in the analysis.

[c] Indicated significance values at 1% (**), 5% (*), and nonsignificant (NS).

Table 3. Effect of clomazone formulation and application method on large crabgrass control in 1988.

Formulation[a]	Clomazone rate	Large crabgrass[b]			
		4 WAT		8 WAT	
		PE	PPI	PE	PPI
	(kg/ha)	----------(%)----------			
4 EC	0.3	85	80	49	28
	0.6	93	92	79	85
	0.8	94	96	80	92
25 WP	0.3	86	81	30	35
	0.6	93	95	61	69
	0.8	94	90	64	82
1.8 ME	0.3	76	86	22	30
	0.6	94	87	50	64
	0.8	96	96	55	83
Untreated Control		0		0	
Significance[c]:					
Formulation		NS		**	
Application (PE vs PPI)		NS		NS	
Rate		**		**	
Formulation x Application		NS		NS	
Formulation x Rate		NS		NS	
Rate x Application		NS		NS	
Formulation x Application x Rate		NS		NS	

[a] 4EC denotes 4 lb/gallon emulsifiable concentrate; 25WP denotes 25% wettable powder; 1.8ME denotes microencapsulated formulation.

[b] Weedy checks were not included in analysis.

[c] Indicated significance values at 1% (**), 5% (*), and nonsignificant (NS).

at the first, second, and tenth day after application (Table 3). Clomazone volatilization was greatest 24 h after application and clomazone was not detectable from the microencapsulated formulation after 24 h.

In 1988, the study was expanded to examine clomazone volatilization from preemergence and preplant incorporated applications of clomazone (Table 4). As in 1987, the greatest volatilization occurred 24 h after application. One day after preemergence clomazone application the 23 % of the applied emulsifiable concentrate volatilized off of the soil. The emulsifiable concentrate exhibited the greatest volatilization followed by the wettable powder and the microencapsualted formulation. Clomazone volatilization was significantly greater from clomazone applied preemergence than preplant incorporated 24 h after application except for the microencapsulated formulation. Samples collected at the second or tenth day after clomazone application showed that volatilization had declined significantly and differences among formulations were not as apparent. Except for samples collected from the wettable powder formulation at the second day after application, no differences between formulation or application method were observed.

In conclusion, approximately 20 % of the emulsifiable concentrate formulation of applied clomazone volatilized

within 24 h after application in both years of the study. Volatilization was slightly lower for the wettable powder formulation. Volatilization was greater from preemergence than preplant incorporated clomazone 24 h after application. Volatilization from the microencapsulated formulation was less in 1987 and 1988 than the other two formulations 24 h after application. Differences in application method became less apparent later in the study. These results are in agreement with other researchers who found trifluralin volatilization to be greatest 24 h after application (11). These results are also in agreement with other researchers who used bioassay procedures and found that clomazone volatilization was greater from preemergence than preplant incorporated applications and volatilization was greater shortly after application (5, 6, 7, 11).

Clomazone volatilization from foliage. Although clomazone volatilization was greater than 60% from both wet and dry soybean foliage, clomazone volatilization from wet soybean leaves was 28% greater than from dry soybean leaves (Table 6). This could be attributed to differences in adsorption of clomazone to the leaf surface. Water present on the leaf surface may occupy sites for clomazone to bind.

In conclusion, these studies indicate that a significant portion of clomazone applied preemergence to the soil is lost to the atmosphere through volatilization.

Table 4. Effect of formulation on clomazone volatilization sampled at the first, second, and tenth day after preemergence application of 1.1 kg/ha in 1987.

Sampling time [a]	Clomazone volatilization [b]			
	4 EC	25 WP	1.8 ME	LSD (form)
d	-(% of applied clomazone volatilized)-			
1	23	19	<1	4
2	<1	<1	<1	NS
10	<1	<1	<1	NS

a Sampling within each day represents a 24-h period and not cumulative volatilization of clomazone.

b EC denotes emulsifiable concentrate; WP denotes wettable powder; ME denotes microencapsulated formulation.

Table 5. Effect of formulation and application type on clomazone volatilization sampled at the first, second, and tenth day after application of 0.8 kg/ha in 1988.

Sampling time[a]	Application type	Clomazone volatilization[b]			
		4 EC	25 WP	1.8 ME	LSD (formulation)
(d)		-(% of applied clomazone volatilized)-			
1	PE	23	15	8	6
	PPI	14	8	4	8
LSD (Application within day)		4	NS	NS	
2	PE	5	11	5	NS
	PPI	3	2	6	NS
LSD (Application within day)		NS	NS	NS	
10	PE	6	7	5	NS
	PPI	3	4	4	NS
LSD (Application within day)		NS	NS	NS	NS

a Sampling within each day represents a 24-h period and not cumulative volatilization of clomazone.

b EC denotes emulsifiable concentrate; WP denotes wettable powder; ME denotes microencapsulated formulation.

Table 6. Influence of moisture on volatilization of radiolabeled clomazone from soybean leaf surface[a].

Foliage	Clomazone volatilization
	-------(% volatilized)-----
Dry	61
Wet	72
LSD (0.05)	7

[a] Means are the average of three replicates..

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VI. ABSORPTION, TRANSLOCATION, AND METABOLISM OF ^{14}C-CLOMAZONE IN SOYBEANS (*Glycine max*) AND THREE *Amaranthus* SPECIES

INTRODUCTION

Clomazone is a herbicide introduced for the selective control of many grass and broadleaf weeds in soybeans. Species controlled include large crabgrass , foxtails (*Setaria spp.*), velvetleaf, and common lambsquarters (1, 13). Selective control is obtained at rates ranging from 0.6 to 1.1 kg a.i./ha.

Clomazone is an isoxazolidinone herbicide which causes reduction in carotenoid and chlorophyll levels resulting in a bleached appearance of susceptible plant species (4, 5). Sandmann and Böger (10, 11) reported that clomazone inhibits a prenyltransferase and disrupts the formation of isoprenoid pathway components needed for the synthesis of plant pigments such as carotenoids and chlorophylls.

Amaranthus species are among the ten most important weeds in Virginia soybean production (6). Smooth pigweed is the predominant *Amaranthus* species in Virginia, while redroot pigweed is also present in some areas (7). Field studies with clomazone have indicated that clomazone only sporadically controls pigweed species. It has been reported

that redroot pigweed is more susceptible to clomazone than smooth pigweed (13). Field studies have shown livid amaranth to be clomazone-susceptible. The basis of this observed differential plant selectivity of clomazone is unknown. Weston and Barrett (14) hypothesized that metabolism may not account for differences in selectivity between pepper (Capiscum annuum L.) and tomato (Lycopersicon esculentum L.) since sensitive tomato plants had greater amounts of two unidentified polar clomazone metabolites than tolerant peppers.

Differential absorption, translocation, and metabolism are recognized as important factors involved in herbicide selectivity (8, 12). The objectives of this research were to determine the absorption, translocation, and metabolism of clomazone in tolerant soybean and smooth pigweed and susceptible redroot pigweed and livid amaranth exposed to foliar and root applied herbicide.

MATERIALS AND METHODS

Chemicals. Technical (95%) and radiolabeled samples of clomazone were provided by FMC Chemical Corp., Princeton, NJ. Radiolabeled clomazone was uniformly labeled with ^{14}C on the aromatic ring (sp. act. 28.0 mCi/mmole). Herbicides were dissolved in 100% acetone.

Plant Material. Seeds of each species were planted in a potting soil mixture of vermiculite: weblite:sphagnum peat moss (2:2:1, v/v/v) in 400 ml cups. Plants were grown in a growth chamber at 25 C and 14 h light (400 uE/m^2/s^2) provided by both incandescent and fluorescent lamps. At the 1 to 2 leaf stage of pigweed species and unifoliate stage of soybeans, seedlings were transferred to half-strength Hoagland's solution (pH=6.0) in foil-wrapped 150 ml glass containers, one plant per container (9). After 2 d, plants were grown in full strength Hoagland's nutrient solution.

Uptake and distribution of [^{14}C]-clomazone. At the 3 to 4 leaf stage of pigweed species and first trifoliate stage of soybeans, plants were exposed to radiolabeled clomazone following root or foliar applications. For root uptake studies, the seedlings of all species were placed in nutrient solution containing 5 uCi/L of radiolabeled clomazone adjusted to 100 uM concentration with technical clomazone. Foliar treated plants were given a 10 ul drop on the midvein of the treated leaf containing 0.1 uCi of clomazone (adjusted to 100 uM concentration with unlabeled clomazone) and 0.1% surfactant[1].

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[1] X-77. Chevron Chem. Co., San Francisco, CA 94119. Principal functioning agents are alkylarylpolyoxyethylene glycols, free fatty acids, and isopropanol.

Plants were harvested at 12, 48, and 96 h after treatment. The roots of root-treated plants were washed with distilled water to remove any radioactivity from their surface. The treated leaves of foliar treated plants were washed twice in 10% ethanol to remove radiolabeled herbicide on the surface as described by Devine et al. (3). The plants were separated into roots, shoots, and leaves for root uptake studies and the treated leaf was separated from the other leaves for the foliar studies. Plant parts were weighed and ground to a fine powder with liquid nitrogen. Radiolabeled herbicide was extracted with two aliquots of 50 ml of 80% acetone. The acetone aliquots were combined and concentrated to 1 ml. A 100 ul sample of each extract was taken and a 1 ml sample of the remaining nutrient solution and counted by liquid scintillation spectrometry[2] to quantitatively determine absorption and translocation of radiolabeled clomazone. Unextracted clomazone in plant tissue was combusted in a biological

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[2] Beckmann LS-8100. Beckmann Instruments. Fullerton, CA 92634.

sample oxidizer[3] and counted by liquid scintillation spectrometry.

Autoradiography. A set of seedlings of all species treated with radiolabeled clomazone was used for analysis by autoradiography following the procedures of Crafts and Yamaguchi (3). The plant samples were dried after harvest and placed on X-ray film (Kodak X-Omat AR). Three weeks later, the film was developed for qualitative analysis of clomazone translocation.

Separation and Identification of Clomazone Metabolites. To analyze clomazone metabolites from plant extracts, 50 ul aliquots were spotted on silica-gel thin-layer chromatography plates[4] developed in butanol:acetic acid: water (12:3:5, v/v/v). Developed thin-layer plates were separated into 1 cm segments from the origin to the solvent front and scraped and radioactivity was quantitatively determined by liquid scintillation spectrometry. Clomazone and a synthetic clomazone-glutathione (GS-clomazone)

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[3] Tricarb Sample Oxidizer. Packard Instruments. Downers Grove, IL 60515.

[4] Silica gel TLC plates. LK6F. Whatman Inc., Clifton, NJ 07014.

conjugate were co-chromtographed with sample extracts.

Chemical Synthesis of GS-clomazone Conjugate. To determine if any of the plant extracts contained a GS-clomazone conjugate, a synthetic GS-glutathione conjugate was prepared as described by Brown and Neighbors (2). To 1 ml of phosphate buffer (pH=8.8), ^{14}C-clomazone (0.1 uCi) and unlabeled clomazone were added to make a final concentration of 1.3 mM. ^{3}H-glutathione (0.1 uCi) and reduced unlabeled glutathione were added to give a final glutathione concentration of 100 mM. The reaction ran for 3 d at 25 C without stirring. The mixture was concentrated to 100 ul. A 50 ul aliquot was spotted on silica-gel thin-layer chromatography plate and developed in butanol:acetic acid: water (12:3:5, v/v/v). Thin-layer plates were scraped and analyzed by high performance liquid chromatography (HPLC).

HPLC Chromatography. Twenty ul portions of clomazone and the synthetic GS-clomazone conjugate were injected into a Hewlett-Packard Model 1090 HPLC liquid chromatograph equipped with a Hewlett-Packard 200 mm by 46 mm Hypersil ODS 5 um C-18 pre column. The chemicals were analyzed by gradient elution chromatography. The procedure consisted of a 1 min elution in a solution of water and methanol (85:15) followed by a gradient change to a solution of water and methanol (90:10) over a 25 min period. The flow rate was

1.0 ml/min and the column temperature was 40 C. The chemicals were detected by a diode array detector monitoring at 254 nm. Clomazone and the synthetic GS-clomazone conjugate were identified by their absorption spectra and retention times (Figures 1 and 2).

Mass Spectrometry. Chemical ionization mass spectrometry was performed on a VG Analytical 7070E mass spectrometer[5] in isobutane. One ul samples of clomazone and the synthetic GS-clomazone conjugate were analyzed. Chromatography and mass spectrum analysis confirmed that the synthetic compound was a GS-clomazone conjugate (Figure 3 and 4).

Statistical design. Three replications of all treatments were made and each experiment was repeated twice in time. Mean were separated by Fisher's Protected Least Significance Difference Test.

RESULTS AND DISCUSSION

Clomazone Absorption and Translocation. In general, absorption of clomazone via root application increased over the course of the study in all four species (Table 1). Clomazone absorption by the sensitive plants livid amaranth and redroot pigweed was greater than that observed with

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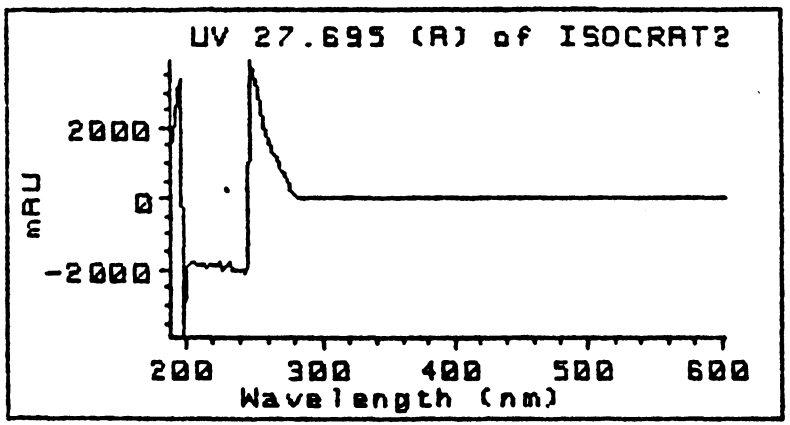
[5] Performed in Dept. of Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA.

a)



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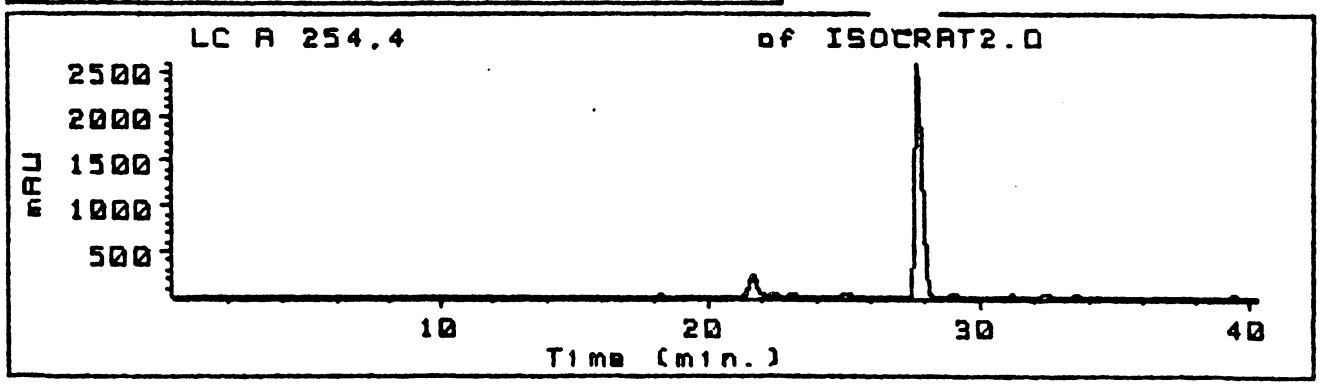
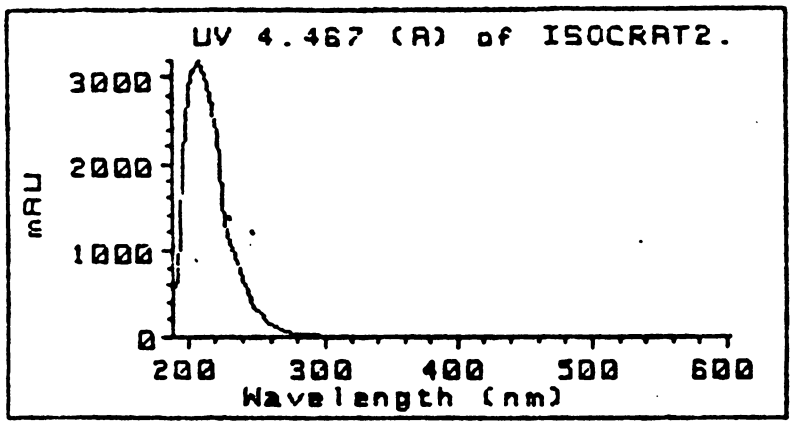


Figure 1. a) Absorption spectrum of clomazone measured in water:methanol

b) HPLC chromatogram of clomazone monitored at 254 nm.

a)



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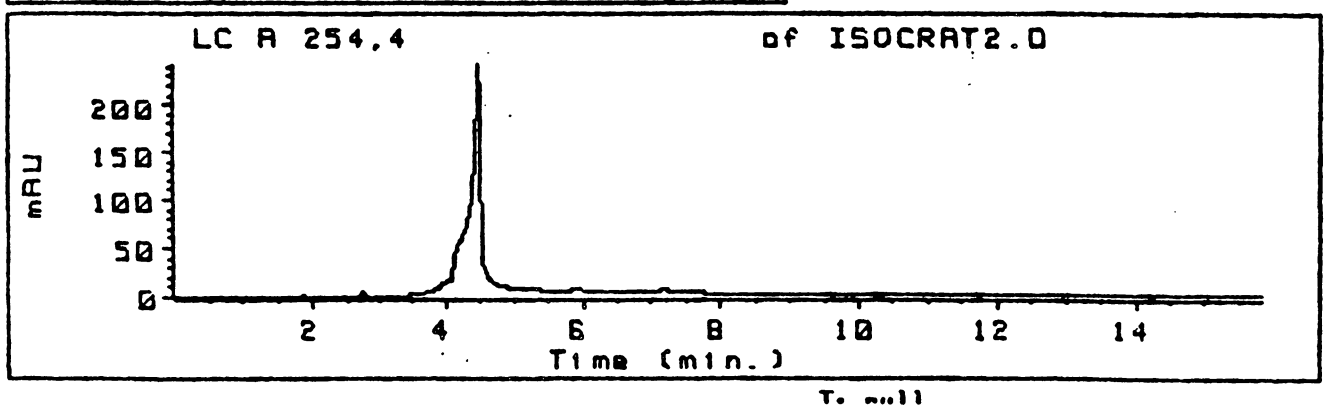

Figure 2. a) Absorption spectrum of synthetic GS-clomazone conjugate measured in water:methanol b) HPLC chromatogram of synthetic GS-clomazone conjugate monitored at 254 nm.

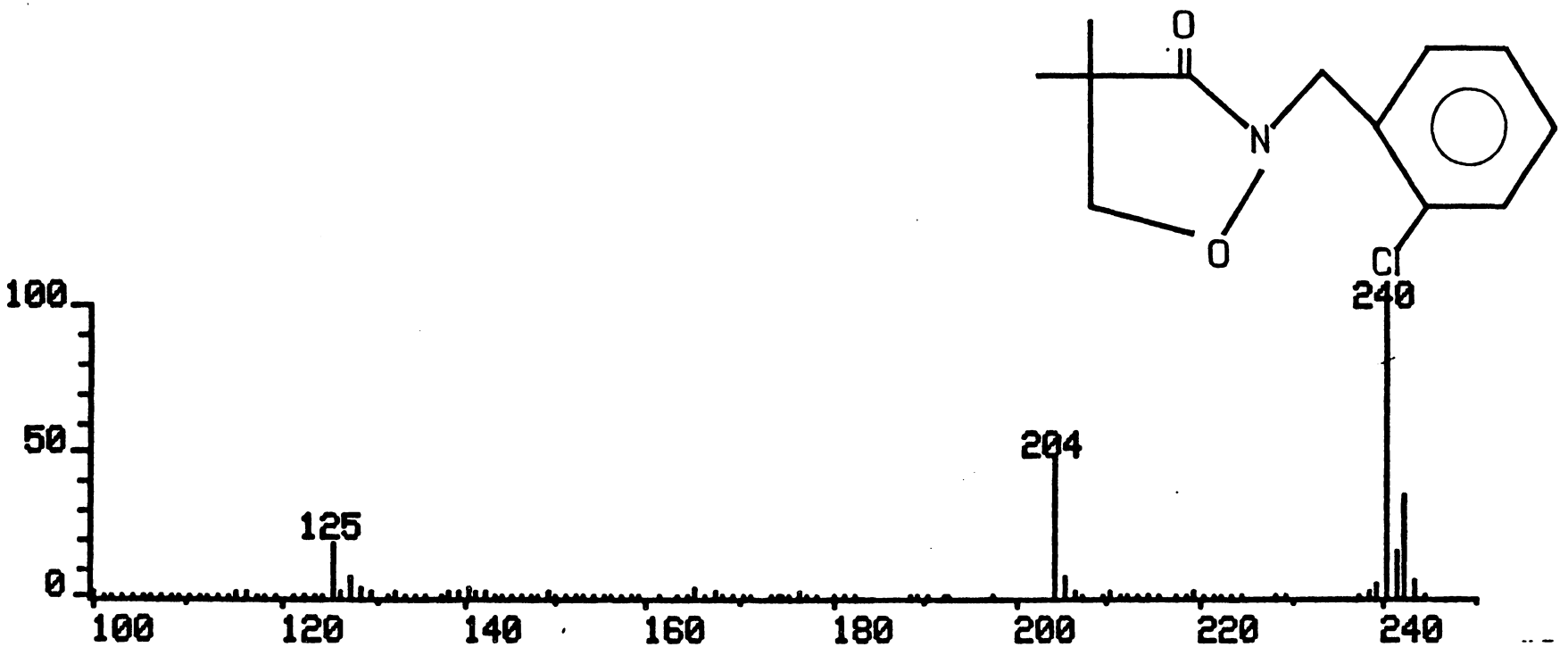

Figure 3. Mass spectrum of clomazone and corresponding structure.

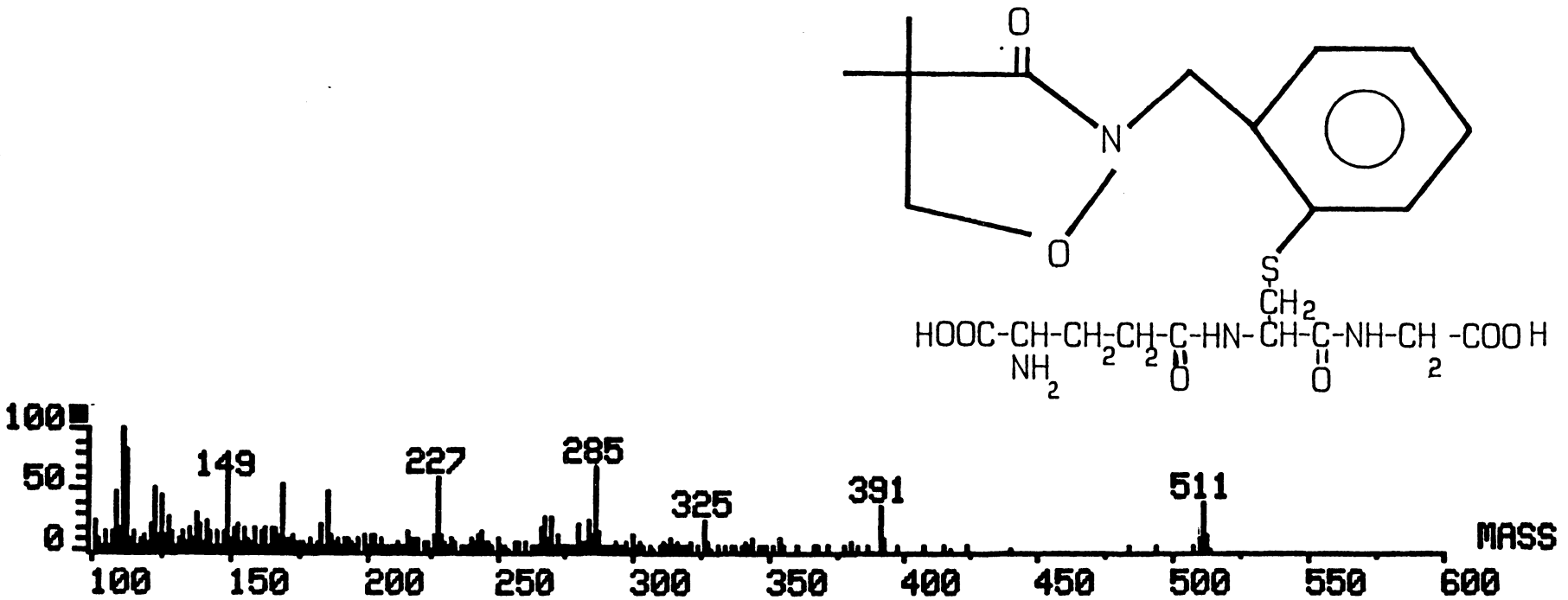

Figure 4. Mass spectrum of synthetic GS-clomazone and corresponding structure.

Table 1. Absorption and translocation of root-applied clomazone in soybeans and Amaranthus species over time[a].

Species	Time of Application	Absorption	Distribution of radioactivity		
			Root	Shoots	Leaves
	-(h)-	-(%)- (of applied)	---------(% absorbed)---------		
Soybean	12	38	20	17	62
	48	27	15	16	68
	96	53	17	17	65
LSD (time within species)		18	NS	NS	NS
Smooth pigweed	12	24	14	17	70
	48	17	13	15	72
	96	34	15	16	69
LSD (time within species)		NS	NS	NS	NS
Redroot pigweed	12	23	22	17	61
	48	43	16	22	62
	96	80	9	24	67
LSD (time within species)		14	NS	NS	NS
Livid Amaranth	12	46	8	19	73
	48	64	10	9	80
	96	83	13	15	72
LSD (time within species)		22	NS	NS	NS
LSD (between species)		23	NS	NS	NS

[a] Data are the means of three replications repeated in time.

tolerant soybeans and smooth pigweed. At 96 h after treatment, 80% or greater of the applied ^{14}C-clomazone was absorbed by redroot pigweed and livid amaranth. During the same time period, absorption of ^{14}C-clomazone by soybean and smooth pigweed was 53% and 34%, respectively (Table 1). Thus, in terms of their efficiency in absorbing root-applied clomazone, the four species can be ranked as follows: livid amaranth> redroot pigweed> soybean> smooth pigweed.

Of the clomazone absorbed through the roots, most was translocated to the leaves of all species examined (Table 1). Autoradiographs of dried plants indicated the same pattern (Figure 5). Clomazone distribution was similar in the stems and roots and reveal that acropetal movement of clomazone occurs.

Foliar absorption of clomazone was limited in all species examined (Table 2). Low amounts of radioactivity were recovered from leaf washings of treated leaves. Additional studies indicated that a majority of foliar applied clomazone volatilized from the leaf surface (data not shown). Foliar-applied clomazone remained in either the treated leaf or other leaves of all species examined (Table 2). In addition, autoradiographs indicated that clomazone moved acropetally within the plant (Figure 6).

These data combined with the root absorption data indicate that clomazone is a xylem-mobile herbicide. Clomazone absorption appears to be species-dependent, especially among the amaranths. Redoot pigweed and livid amaranth, which are clomazone-sensitive species, absorbed more clomazone through the roots than soybean and smooth pigweed, which are clomazone-tolerant species. Of the clomazone absorbed in all species, most was translocated to the leaf tissue which is in agreement with data of other researchers (1). Foliar absorption was limited in all species, perhaps due to volatilization from the leaf surface. Of the foliar-applied clomazone, most was translocated only within the treated leaf or leaves above the treated leaf. The fast recovery of vegetation damaged by clomazone off-site movement could be attributed to the limited foliar absorption and translocation.

Clomazone Metabolism. Approximately 50% of the applied clomazone was metabolized at 96 h after treatment in all species examined (Table 3). Two major metabolites other than the clomazone standard were found from thin-layer chromatography. These metabolites had R_f values of 0.4 (unknown #1) and 0.8 (unknown #2), respectively (Table 3). The R_f of the metabolite with a R_f of 0.4 was similar to the R_f the synthetic GS-clomazone conjugate. Based on previous reports (2), the GS-clomazone conjugate in

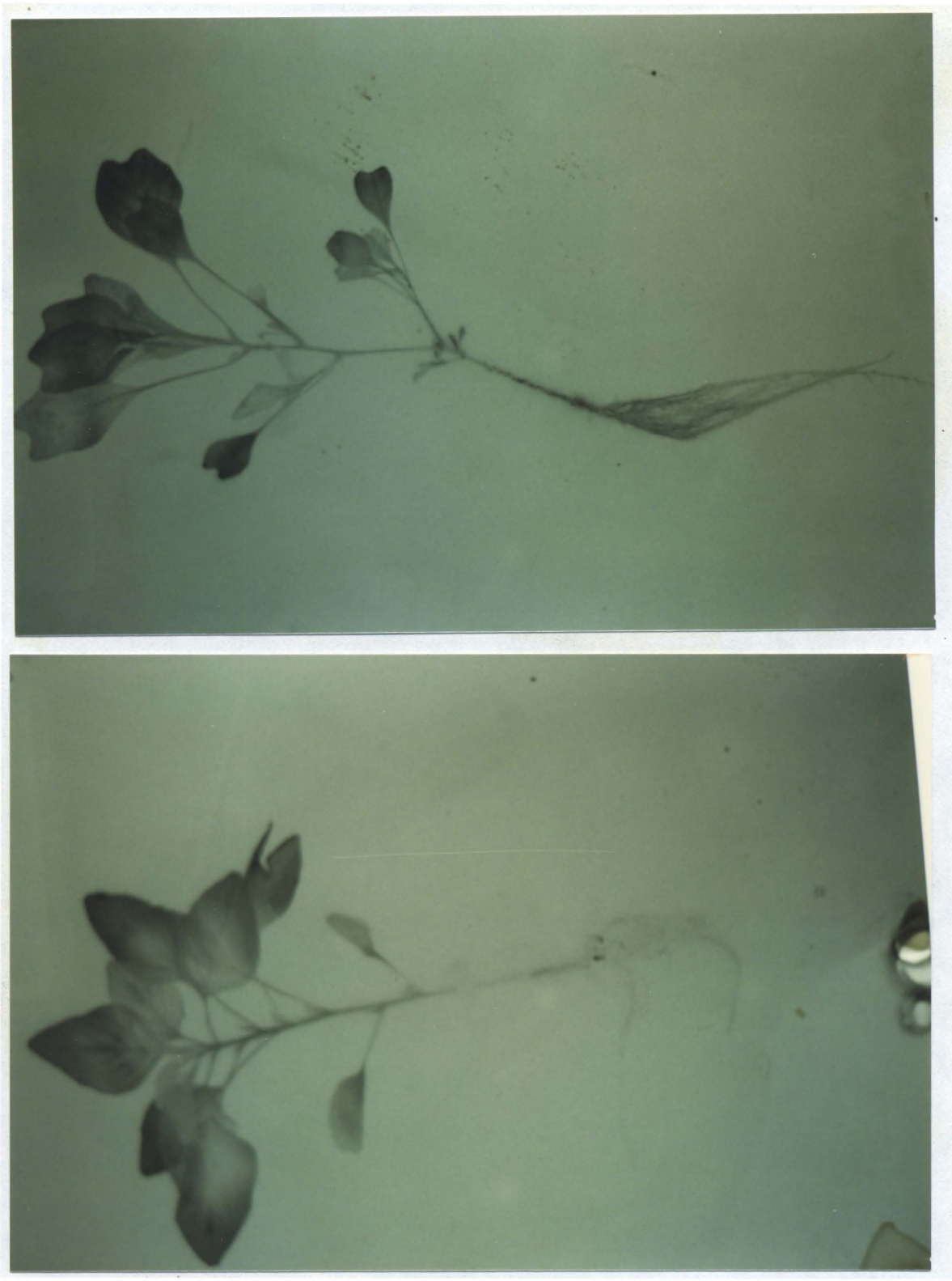

Figure 5a . Autoradiograph of root-applied clomazone in livid amaranth (top) and redroot pigweed (bottom).

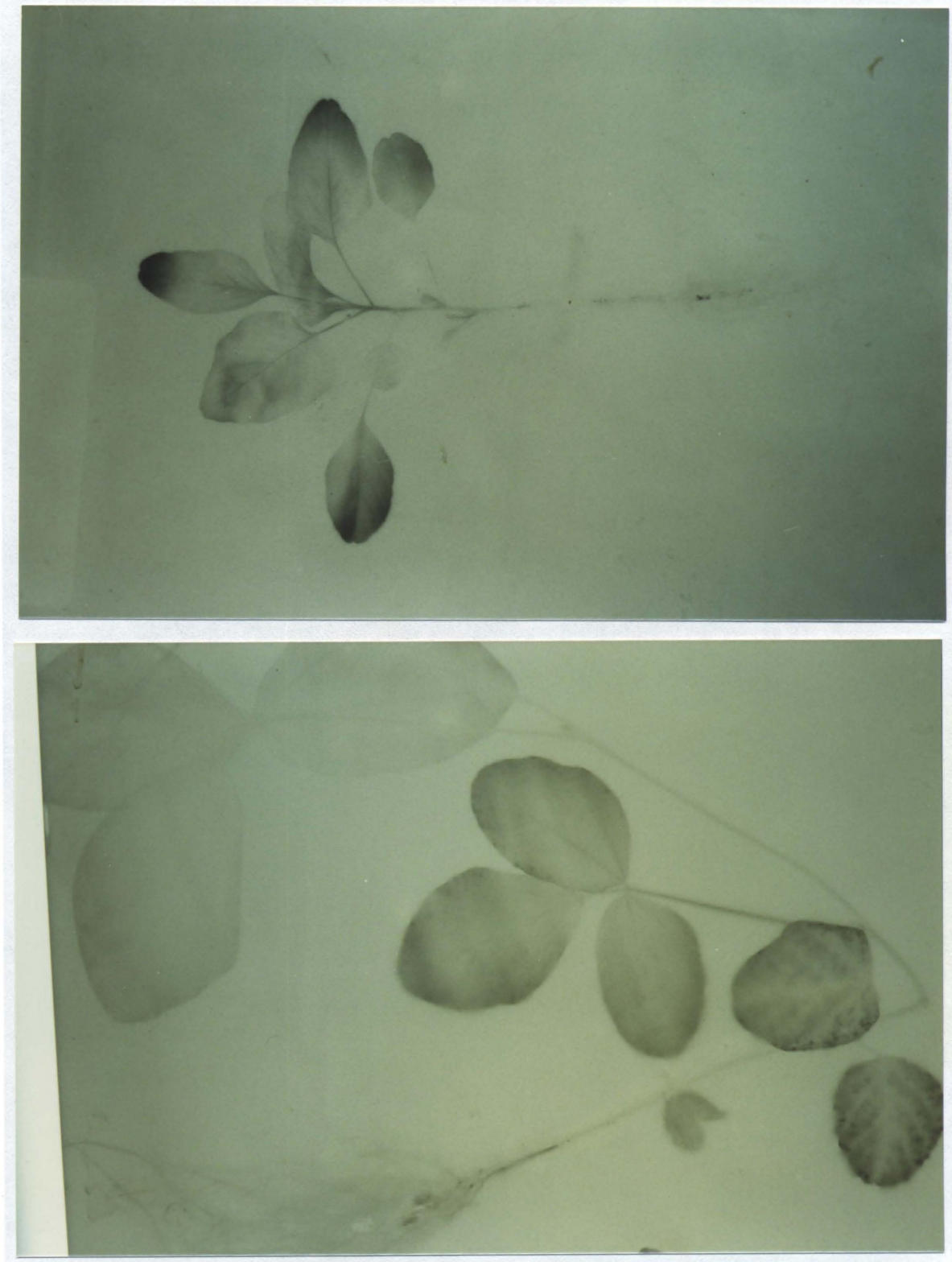

Figure 5b. Autoradiograph of root-applied clomazone in smooth pigweed (top) and soybean (bottom).

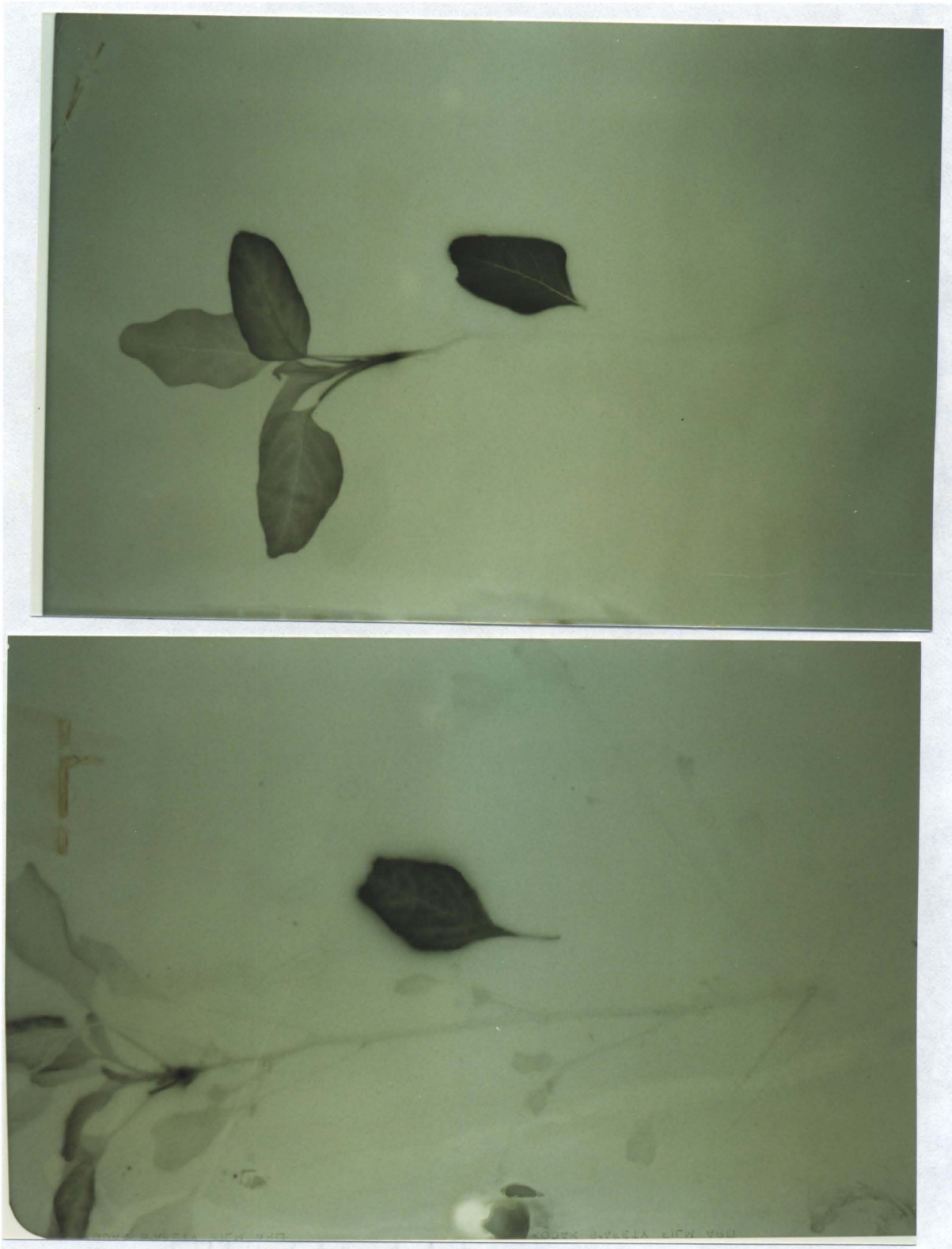

Figure 6. Autoradiograph of foliar -applied clomazone in redroot pigweed (top) and livid amaranth (bottom).

Table 2. Absorption and translocation of foliar-applied clomazone in soybeans and Amaranthus species over time.[a]

Species	Time of Application	Absorption	Distribution of radioactivity			
			Root	Shoots	Leaves	Trtd. Leaves
	-(h)-	-(%)- (of applied)	----------(% absorbed)----------			
Soybean	12	28	3	4	6	87
	48	24	2	3	4	90
	96	23	2	3	3	93
LSD (time within species)		NS	NS	NS	NS	NS
Smooth pigweed	12	20	6	17	47	30
	48	27	5	6	44	44
	96	48	<1	5	50	44
LSD (time within species)		14	NS	NS	NS	NS
Redroot pigweed	12	27	3	9	25	62
	48	25	3	6	7	83
	96	25	2	13	24	61
LSD (time within species)		NS	NS	NS	NS	NS
Livid Amaranth	12	17	6	6	9	78
	48	24	3	12	13	71
	96	38	1	11	40	47
LSD (time within species)		16	NS	NS	17	NS
LSD (between species)		NS	2	NS	23	26

[a] Data are the means of three replications repeated in time.

Table 3. Metabolism of root-applied clomazone in leaf tissue of soybeans and Amaranthus species, 96 h after treatment[a, b].

Metabolites	R_f Value	Species				
		Soybean	Smooth Pigweed	Redroot Pigweed	Livid Amaranth	LSD (among) (species)
		--------(% of radioactivity spotted)--------				
Unknown #1[c]	0.4	23	21	22	25	NS
Unknown #2	0.8	24	29	38	30	NS
Parent clomazone	0.95	52	50	40	45	NS
LSD (within species)		15	14	NS	13	

[a] Metabolites were separated by thin-layer chromatography using a solvent system of butanol:acetic acid:water (12:3:5, v/v/v).

[b] Data are the means of three replications repeated in time.

[c] Unknown #1 was identified as GS-clomazone.

soybeans may actually be homo-GS-clomazone since homoglutathione is far more abundant than glutathione in this plant. Another metabolite with a R_f of 0.8 was present. The levels of unknown #1 and #2 in soybean, smooth pigweed, and livid amaranth were similar. Similar levels of the parent clomazone and the two metabolites were present. A species response to clomazone metabolism was not observed.

In conclusion, these data would indicate that differential tolerance to clomazone may be due to differential absorption of clomazone. Differential absorption seems to be the important factor influencing differential smooth and redroot pigweed tolerance to clomazone. This finding is in agreement with those of other researchers who found differential absorption to be the major factor influencing clomazone tolerance between pepper and tomato (14). Additional factors such as intracellular compartmentalization may play a role in differential tolerance to clomazone.

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VI. GROWTH AND PHYSIOLOGICAL RESPONSES OF NORMAL, DWARF, AND ALBINO CORN (*Zea mays*) TO CLOMAZONE TREATMENTS

INTRODUCTION

Clomazone is an isoxazolidinone herbicide used for selective weed control in soybeans. Clomazone inhibits pigment formation in sensitive plants causing a bleaching effect (2, 3, 4, 5, 10, 11). Unlike other bleaching herbicides such as norflurazon [4-chloro-5-(methylamino)-2-(3-(trifluoromethyl)phenyl)-3(2H)-pyradazinone], clomazone does not inhibit phytoene desaturase, but inhibits prenyltransferase which mediates the synthesis of many products of the isoprenoid pathway such as gibberellin, phytols, and carotenoids (3, 6).

Sandmann and Böger (10, 11) have reported that exogenous gibberellin (GA_3) applications reverse the clomazone-mediated reduction of shoot height in pea. This finding suggests a possible competitive interaction between the two compounds for a receptor site.

Genetic mutants provide useful tools for elucidating biochemical pathways (9). For example, single gene dwarf mutants in corn shoots have lead to conclusion that GA_3 is the main endogenous gibberellin active in the control of corn shoot growth (9).

There are several mutants of corn available for study such as albino mutants which lack the ability to produce photosynthetic pigments and dwarf mutants which lack ability to synthesize gibberellin. The major dwarf corn mutants are dwarf-1, dwarf-2, dwarf-3, and dwarf-5. The dwarf-5 mutant lacks the ability to synthesize ent-kaurene ß-synthetase and thus ent-kaurene from mevalonic acid and geranylgeranyl phosphate (9).

Since clomazone inhibits the formation of isoprenoid pathway components, it has been postulated that gibberellin-deficient mutants might have differential tolerance to clomazone from that of normal corn.

The objectives of the present study were to determine the growth and physiological responses of a normal hybrid, a dwarf mutant, and an albino mutant of corn to clomazone treatments and the interactions of exogenously applied gibberellin (GA_3) or CCC [(2-chloroethyl) trimethylammonium chloride] with clomazone on the growth of the normal hybrid of corn. The growth responses of the normal hybrid and the dwarf mutant of corn to norflurazon, another bleaching herbicide, were examined for comparative purposes.

MATERIALS AND METHODS

Plant material. A normal corn hybrid ('DeKalb XL67'), a dwarf corn mutant (d_5 mutant, gibberellin deficient[1]), and a lethal albino mutant (carotenoid and chlorophyll deficient[1]) were used in the study (8). Plants were grown from seed in 300 ml pots in a mixture of sand:weblite:sphagnum peat moss (2:2:1, v/v/v) in the greenhouse with 14 h light period at 22 to 25 C at 300 $uE/m^2/s$.

Chemical treatments. Clomazone was applied preemergence at rates of 0.3, 0.6, and 1.1 kg/ha at 191 L/ha and 275 kPa with a backpack sprayer. Gibberellin[2] (GA_3) was applied to untreated and clomazone-treated (0.6 kg/ha) XL67 corn hybrid at 1.6 kg/ha with an atomizer at the 1-leaf stage. CCC[3] was applied to untreated and clomazone-treated (0.6 kg/ha) XL67 corn hybrid plants at 0.6 kg/ha with an atomizer at the 1-leaf stage. Shoot height and dry weight determinations were made 10 d after germination.

Chlorophyll determination. Total chlorophyll content was determined for the normal corn hybrid and the dwarf corn

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[1] Carolina Biological Supply, Burlington, NC 27215.

[2] Pro-Gibb, Abbott Laboratories, Chicago, IL. 60004.

[3] Cyclocel, American Cyanamid Co., Princeton, NJ .

mutant. One cm pieces of fresh leaf tissue were cut and homogenized in 100% N,N-dimethylformamide (1). Pigment determinations were made spectrophotometrically[4] by measuring absorbance at 470 and 645 nm (1).

Statistical design. Each treatment was replicated three times and each experiment was repeated three times. Means were compared to untreated controls by a single degree of freedom analysis at the 5% level (8). Colby's method was used to analyze interactions between both gibberellin and CCC, respectively, and clomazone (7).

RESULTS AND DISCUSSION

Significant shoot height and weight reductions of XL67 corn hybrid were observed even with the lowest rate (0.3 kg/ha) of clomazone compared to the untreated control (Table 1). For the dwarf corn mutant, shoot height reductions occurred only at the 1.1 kg/ha rate of clomazone (Table 1). However, significant reductions of shoot weight were evident in dwarf corn treated with all rates of clomazone (Table 1). No significant shoot height or weight reductions were observed for the albino mutant. Visual observations revealed that seedlings of normal corn treated

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[4] Beckman DU-6 spectrophotometer, Beckman Instruments. Fullerton, CA 92634.

with clomazone were completely bleached at all rates of the herbicide (Figure 1a). In contrast, seedlings of dwarf corn treated with clomazone were only slightly bleached even at the higher rates of clomazone (Figure 1b).

These observations were supported by chlorophyll determinations of clomazone-treated XL67 and dwarf corn shown in Table 2. Total chlorophyll content of normal corn hybrid was significantly reduced at the lowest rate of clomazone (Table 2). Clomazone did not significantly reduce total chlorophyll content of dwarf corn mutant even at the 1.1 kg/ha rate.

Shoot height of XL67 corn treated with gibberellin was similar to that of the untreated control. The addition of gibberellin to the 0.6 kg/ha treatment of clomazone alleviated the bleaching symptoms and height reductions induced by clomazone on XL67 corn (Table 3). However, the addition of gibberellin did not fully alleviate the shoot weight reduction caused by clomazone on this normal hybrid (Table 3). Colby's analysis indicated that clomazone and gibberellin were anatagonistic with respect to corn shoot height and weight (Table 3). The addition of CCC to clomazone-treated XL67 corn did not cause any change in shoot height or weight. Colby's analysis indicated that the effects of the growth retardant CCC and clomazone on XL67 corn shoot height and weight were additive for shoot height

Table 1. Effect of clomazone on shoot height of normal, dwarf, and albino corn[a].

Clomazone rate	Corn Mutants					
	XL67		Dwarf		Albino	
	Height	Weight	Height	Weight	Height	Weight
(kg/ha)	-(cm)-	-(mg)-	-(cm)-	-(mg)-	-(cm)-	-(mg)-
0.0	39.1	890	10.1	506	8.8	65
0.3	21.7 *	124 *	9.0	197 *	8.3	65
0.6	19.1 *	50 *	7.8	113 *	8.4	63
1.1	16.3 *	40 *	5.9 *	68 *	7.7	68

[a] Means within columns followed by an (*) are significantly different from the control based on a single degree of freedom of analysis at the 5% level.

Table 2. Effect of clomazone on total chlorophyll content of normal and dwarf corn[a].

Clomazone rate	Total Chlorophyll	
	XL67	Dwarf
kg/ha	-------(mg/g fresh wt)--------	
0.0	24.9	18
0.6	5.1 *	23
1.1	0.4 *	13

[a] Means within columns followed by an (*) are significantly different from the control based on a single degree of freedom analysis at the 5% level.

Table 3. Growth responses of 'XL67' corn to combined treatments of clomazone and gibberellin (GA_3) or CCC [a].

Treatment	Rate	Height		Weight	
		Observed	Expected[b]	Observed	Expected[b]
	-(kg/ha)-	-(cm)-		-(mg)-	
Control	0.0	39	-	890	-
Clomazone	0.6	19 *	-	50 *	-
CCC	0.6	31	-	570 *	-
GA_3	1.6	39	-	400 *	-
CCC + Clomazone	0.6 + 0.6	15 *	(15)	50 *	(32)
GA_3 + Clomazone	1.6 + 0.6	25	(19)	140 *	(22)

[a] Means within columns followed by an (*) are significantly different from the control based on a single degree of freedom analysis at the 5% level.

[b] Expected values in parenthesis were calculated assuming no interactions (see Materials and Methods).

Figure 1. Comparison of XL67 and dwarf corn response to clomazone.
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and weight (Table 3). These results appear to confirm the growth retardant activity of clomazone and its possible interference with gibberellin biosynthesis.

Normal and dwarf corn exhibited a different response to norflurazon, another bleaching herbicide with a different mode of action (6). Both dwarf and XL67 corn were bleached significantly by norflurazon at 11 d after treatment (Figure 2a and 2b). However, shoot heights and weights of both normal and dwarf corn were not reduced significantly by any rate of norflurazon (Table 4).

In conclusion, normal corn appears to be very sensitive to clomazone. However, the dwarf corn mutant seems to display greater tolerance to clomazone. Since this dwarf mutant lacks the ability to convert ent-kaurene to gibberellin (5), a connection between the herbicidal activity of clomazone and gibberellin biosynthesis is evident (5). In addition, the ability of exogenous gibberellin to alleviate clomazone injury to normal corn hybrids indicates an interaction either between clomazone and gibberellin or a secondary effect of clomazone which affects gibberellin biosynthesis. Alternatively, the dwarf mutant could contain isozymes of prenyltransferases that render the isoprenoid pathway less sensitive to clomazone. Apparently, further results would be needed to clarify the primary or secondary nature of these actions of clomazone.

Table 4. Effect of norflurazon on shoot height and weight of normal and dwarf corn[a].

Norflurazon rate	Corn mutants			
	XL67		Dwarf	
	Height	Weight	Height	Weight
kg/ha	-(cm)-	-(mg)-	-(cm)-	-(mg)-
0.0	39	380	8	220
0.6*	33	340	10	220
1.1	34	390	14	230
1.6	28	300	11	190

[a] Means within columns followed by an (*) are significantly different from the control based on a single degree of freedom analysis at the 5% level.

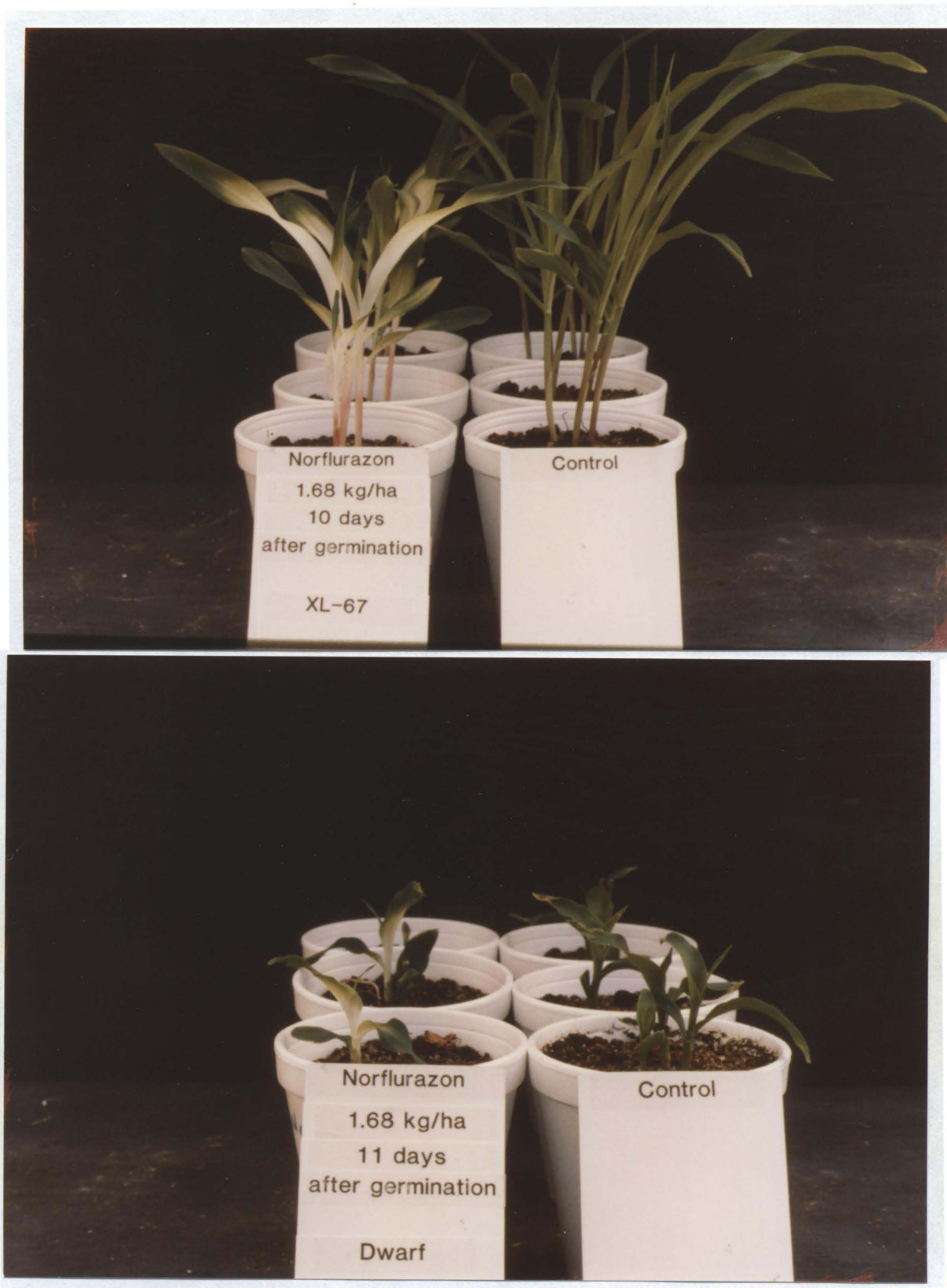

Figure 2. Comparison of XL67 and dwarf corn response to norflurazon.

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VIII. SUMMARY AND CONCLUSIONS

This research examined various aspects of the efficacy and physiological action clomazone, a recently introduced herbicide for selective grass and broadleaf weed control in soybeans. Field studies were conducted for 4 yr to evaluate preplant and preemergence applications of clomazone to full-season no-till soybeans. Weed control from preplant applications of clomazone was not adequate. However, clomazone applied preemergence provided large crabgrass (Digitaria sanguinlis L.) control equivalent to that from oryzalin applied preplant or preemergence and provided better control of common ragweed (Ambrosia artemisiifolia L.) and common lambsquarters (Chenopodium album L.) than oryzalin. Preemergence clomazone combinations with imazaquin or linuron+chlorimuron provided adequate control of smooth pigweed (Amaranthus hybridus L.); but control with clomazone alone was not adequate; preemergence combinations of linuron with clomazone were less effective than other combinations. Clomazone did not cause any soybean injury in these studies.

Field studies were conducted to compare efficacy of

clomazone applied preemergence and preplant incorporated in conventionally-tilled soybeans. In addition, the efficacy of clomazone incorporated at two depth was examined. Clomazone applied preemergence generally provided control of large crabgrass and several broadleaf weed species equivalent to than preplant applications. The addition of imazaquin or chlorimuron plus linuron improved smooth pigweed control over that provided by clomazone alone, especially later in the season. Shallow (4 cm) incorporated applications of clomazone provided better large crabgrass and smooth pigweed control than deep (8 cm) incorporation, especially at the 0.6 kg ai/ha rate. Differences in weed control between incorporation depth diminished with increasing rate of clomazone. Clomazone did not provide any soybean injury in any of these studies.

Field and laboratory studies were conducted to evaluate efficacy and volatilization of three clomazone formulations (emulsifiable concentrate, wettable powder, and a microencapsulated formulation) following soil application. Volatilized clomazone was collected in a closed bottle apparatus and trapped on activated charcoal. Samples were collected separately at the first, second, and tenth day after clomazone application. A method was

developed for extracting clomazone from activated charcoal and clomazone extracted was analyzed by capillary gas chromatography. Weed control was independent of formulation and was more dependent on clomazone rate. Clomazone volatilization was greatest 24 h after application from the emulsifiable concentrate and wettable powder formulation and declined considerably at the second and tenth day after application. Volatilization from the microencapsulated formulatiion was lower than the other two formulations 24 h after application. Clomazone volatilization was greater from preemergence than preplant incorporated applications. In a separate study, clomazone volatilization from wet and dry soybean foliage was examined. Volatilization was greater than 60% of applied clomazone from both wet and dry foliage. Clomazone volatilization was greater from wet than dry foliage.

Studies were initiated to determine if differences in clomazone absorption, translocation, and metabolism exist among smooth pigweed (Amaranthus hybridus L.), redroot pigweed (Amaranthus retroflexus L.), livid amaranth (Amaranthus lividus L.), and soybean (Glycine max L.). Differences in clomazone absorption were found among the four species. Redroot pigweed and livid amaranth, which are

clomazone-sensitive species, absorbed more clomazone through the roots than soybean and smooth pigweed, which are clomazone-tolerant species. Of the clomazone absorbed in all species, most was translocated to leaf tissue. Foliar absorption of clomazone was limited in all species. Of the foliar clomazone absorbed, most was translocated only within the treated leaf or to leaves above the treated leaf. Two major metabolites other than the clomazone standard were found. These metabolites had a R_f values of 0.4 and 0.8, respectively. The metabolite with a R_f of 0.4 was similar to the R_f of a synthetic clomazone-glutathione conjugate. Differences in clomazone metabolism were not found among the four species examined.

Studies were conducted to compare growth and physiological responses of a normal hybrid, a dwarf mutant, and an albino mutant of corn (Zea mays L.) to clomazone and interactions of clomazone with gibberellin and CCC on normal corn hybrid. The normal corn hybrid was sensitive to clomazone at rates as low as 0.3 kg/ha. The dwarf corn mutant exhibited greater tolerance to clomazone. Growth measurements suggested that clomazone and CCC effects were additive and that clomazone and gibberellin were antagonistic.

These studies suggest that clomazone has potential in both no-till and conventionally-tilled soybean production in Virginia. However, volatilization of clomazone and subsequent off-site damage to desirable vegetation is of considerable concern in Virginia where a variety of crops are grown in close proximity to one another.

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