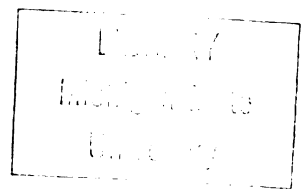

THE BASIS FOR THE DIFFERENTIAL RESPONSE OF SEVERAL WEED SPECIES TO QUINCLORAC

By

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ABSTRACT

THE BASIS FOR THE DIFFERENTIAL RESPONSE OF SEVERAL WEED SPECIES TO QUINCLORAC

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Quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) herbicide from BASF is currently registered in the United States for use in rice (*Oryza sativa* L.) and turfgrass. The spectrum of weed control with quinclorac includes annual grasses and several key broadleaf weeds. Quinclorac exhibits both preemergence and postemergence activity on susceptible weed species.

Past research has demonstrated that quinclorac requires the use of an effective adjuvant to maximize foliar activity. Greenhouse experiments were conducted to evaluate selected commercial and experimental adjuvants for their effectiveness and selectivity. Large crabgrass (*Digitaria sanguinalis* [L.] Scop.) and goosegrass (*Eleusine indica* [L.] Gaertn.) were used as indicator species for efficacy. In previous studies, large crabgrass was sensitive to quinclorac, while goosegrass was tolerant. Applications were made at the one to two tiller stage of the weeds. GR_{50}' (herbicide rate required to reduce plant growth 50%) values were calculated to quantify and compare the efficacy of the adjuvants. For large crabgrass, GR_{50} values ranged from 46 to 98 g ha^{-1} depending on adjuvant.

For goosegrass, no adjuvant provided sufficient activation to allow for a GR_{50} calculation within a commercial use rate range. Further studies were conducted with goosegrass to evaluate the effects of growth stage and the impact of both foliar and root uptake on resultant control. Across growth stages, quinclorac soil activity tended to increase goosegrass control; however, commercially acceptable performance could not be achieved within the proposed labeled use rates at any growth stage.

Absorption, translocation, and metabolism studies using ^{14}C-quinclorac were conducted with the two grass species. Results from the absorption studies showed that after an 80 hr exposure time, species had absorbed nearly equal amounts of applied ^{14}C- quinclorac (27% and 22%, respectively for large crabgrass and goosegrass). Translocation results showed that 95% of the absorbed ^{14}C-quinclorac remained in the treated leaf for large crabgrass after 80 hr. However, only 58% of the absorbed ^{14}C remained in the treated leaf of goosegrass. Nutrient vials did not contain any appreciable amounts of ^{14}C-quinclorac that may have been exudated by either species. Metabolism studies indicated that neither species metabolized the parent quinclorac herbicide. The data indicate that target site differences may contribute to selectivity.

DEDICATION

(DEDUKACJA)

In memory of my late father Edward Stanley Zawierucha, who was my mentor and friend.

(Ku pamięci mojego ojca, Edwarda Stanislawa Zawierucha, który był moim mentoriem i przyjacielem, dedykuję tą pracę.)

"Now to Him is able to do exceeding abundantly beyond all that we ask or think according to the power that works within us, to Him be the Glory forever and ever."

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First, my most grateful thanks goes to Dr. Hans von Amsberg for his encouragement and making it possible for me to complete my research project at BASF. I also want to convey my sincere gratitude to Dr. Donald Penner for his support and encouragement without which , I never would have made it. To my wife and children for their understanding and loyalty. Also, to Dr. David King for sharing his knowledge and expertise with me. To Reggie Sterling for his help and assistance. Also, a special thanks to the late Chad Cole for his help and who is deeply missed at BASF.

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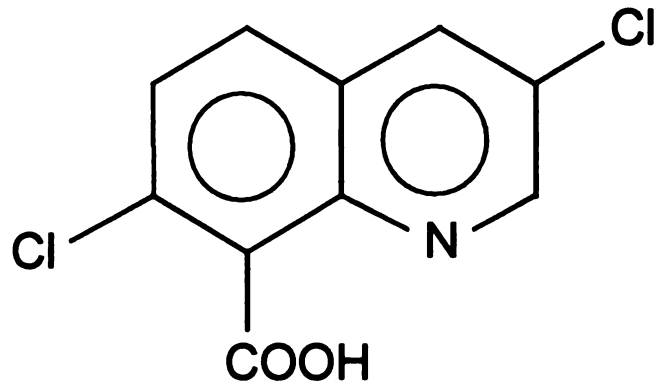
INTRODUCTION

Quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) herbicide was introduced in 1985 by BASF Atkiengesellschaft for the control of barnyardgrass (*Echinocola crus-galli* L. [Beauv.]) in rice. It has also been registered for control of foxtail species (*Setaria spp* L.) and specific key broadleaf weeds such as volunteer flax (*Linum usitatissimum* L.) in Canada in spring wheat (*Triticum aestivum* L.) production. It is under development in Canada for use in canola (*Brassica napus* L.) for control of cleavers (*Galium aparine* L.). Quinclorac also recently has received registration for use in turfgrass for the control of crabgrass (*Digitaria* spp L.) and certain broadleaf weeds such as white clover (*Trifolium repens* L.) and common dandelion (*Taraxacum officinale* L. [Weber]). Quinclorac has not been classified into a specific herbicide structure or mode of action group but it tends to cause auxin like symptoms in susceptible species (3).

Technical quinclorac is a colorless crystalline compound with a molecular formula of $C_{10}H_5Cl_2NO_2$. Its molecular weight is 242.06 grams. It has a fairly low vapor pressure of 1.1×10^{-7} mm Hg at 25^0C. The compound is considered stable to heat and light across a pH range of 3.0-9.0. Quinclorac is soluble in water at 0.62 mg/L, acetone at 0.25 mg/L and methylene chloride at 13.4 mg/L. The pKa value is 4.34 at 20^0C (21).



Quinclorac

Quinclorac demonstrates both preemergence and postemergence activity. Applied as a postemergence treatment, herbicidal effects are a result of both foliar and root uptake (2). Soil activity is primarily influenced by soil organic matter. Degradation is by soil microbes and the rate is primarily dependant on soil moisture and soil organic matter. Measured half life in soil is approximately 100 days (2).

One primary area of research with quinclorac has been the optimization of postemergence activity with adjuvants. One adjuvant, BAS 090 02S, has been identified as an effective adjuvant to use with quinclorac(2). However, due to specific properties of the adjuvant, it will not be registered for use in the U.S. Therefore, the screening of new potential adjuvants for use is of vital interest and was a major objective of this research.

In addition, factors contributing to the difference in susceptibility of two key grass weeds in turfgrass, namely, large crabgrass (*Digitaria sanguinalis* [L.] Scop.) and goosegrass (*Eleusine indica* [L.] Gaertn.) needed to be explored. Large crabgrass is very susceptible to quinclorac, whereas goosegrass is quite tolerant. This research focused on the dynamics of these two species and their response to quinclorac as quantified from an absorption, translocation metabolism, spray retention, and site of action perspective.

CHAPTER ONE

Literature Review

Quinclorac has demonstrated activity both as a preemergence and postemergence herbicide (3,10). It controls certain annual grasses such as barnyardgrass and foxtail species. Quinclorac also controls several annual and perennial broadleaf species including *Galium* spp. and field bindweed. Quinclorac is sold under the tradenames of Facet® in rice and Accord™ in Canada for use in spring and durum wheat. It has recently received federal registration for use in turfgrass under the tradename of Drive®. Pending registrations include the use in the United States in sorghum, spring wheat and chemical fallow under the tradename of Paramount™. Quinclorac is formulated as a 50% wettable powder (Facet) and 75% dry flowable.

Quinclorac has not been classified into a specific herbicide structure or mode of action group but it tends to cause auxin like symptoms in susceptible species (3). The first published research on the possible mode of action of quinclorac was presented by Berghaus and Wuerzer (3). Several experiments were conducted that suggested quinclorac exhibited auxin-like characteristics.

Root inhibition of cucumber seedlings was similar to that exhibited by 2,4-D [(2,4-dichlorophenoxy)acetic acid], picloram [4-amino-3,5,6-trichlor-2-pyridinecarboxylic acid] or IAA [indole-3-acetic acid]. In addition, the extension of wheat coleoptiles and ethylene biosynthesis by leaf discs was also induced.

Berghaus and Wuerzer (3) also showed that quinclorac was rapidly absorbed by leaves of both rice and barnyardgrass. Translocation of quinclorac occurred both basipetally and acropetally, as is observed with weak acid herbicides. Quinclorac tended to be more mobile in rice than in barnyardgrass and rice exhibited a greater extrusion of radiolabeled quinclorac through roots. The greater extrusion or exudation was suggested as a possible explanation for selectivity differences between these species. In root uptake studies, quinclorac was found to be readily absorbed and accumulated in the shoot and root tissue.

Work conducted by Koo *et al.* (15) further investigated the auxin-like characteristics of quinclorac vs. 2,4-D in grass species. In susceptible grass species, quinclorac caused a rapid onset of both chlorosis and necrosis. They also observed that in mesocotyl elongation assays, barnyardgrass did not exhibit auxin-like activity after treatment with quinclorac.

In addition, effects of quinclorac on respiration, protein, and RNA content in barnyardgrass shoot tissues were different from those of 2,4-D.

Further work by Koo *et al.* (16) showed that in susceptible grasses, quinclorac caused necrotic bands near the zones of elongation in both shoots and grasses. Quinclorac caused electrolyte leakage in smooth crabgrass and other susceptible species, but not in tolerant grasses (e.g. rice) or in susceptible broadleaf species. Koo proposed that this electrolyte leakage in susceptible grasses was a secondary effect to that of a primary metabolic effect of quinclorac on cell expansion.

Chism *et al.* (7) investigated the uptake, translocation and metabolism of quinclorac in southern crabgrass (*Digitaria ciliaris* [Retz.]) and Kentucky bluegrass (*Poa pratensis* L.). Their work showed that the uptake of quinclorac by both species was rapid. However, distribution of the herbicide between the species was different with more uniformity throughout Kentucky bluegrass plant tissues than southern crabgrass. Also, Kentucky bluegrass exuduated a significant amount of applied herbicide out of root tissue, similarly to rice (3). Metabolism studies suggested that very little of the parent quinclorac was metabolized by either species.

This finding suggested that the increased distribution and exudation of quinclorac by Kentucky bluegrass are responsible for selectivity.

Grossmann and Kwiatkowski (13) presented evidence for a causative role of cyanide, derived from ethylene biosynthesis, in the mode of action of quinclorac. Root applications of quinclorac to barnyardgrass caused shoot growth inhibition along with chlorosis and necrosis. After one day of herbicide exposure, measured cyanide levels in the barnyardgrass shoot tissue closely correlated with the increased rate of herbicide applied and reduction in shoot fresh weight. Four days after application of 10 and 100 μM quinclorac, the cyanide levels were approximately two to three times higher than the controls. Increases were noted in the β-cyanoalanine synthase activity (the major detoxifying HCN enzyme), ethylene production, and in the levels of 1-aminocyclopropane-1-carboxylic acid (ACC) prior to the accumulation of cyanide. When ACC was supplied exogenously to detached shoots of barnyardgrass, its accumulation coincided with increases in the formation of ethylene and cyanide. To test the hypothesis of the role of cyanide, they treated roots of intact barnyardgrass plants with a rate series of KCN. The levels of shoot cyanide levels correlated to the amount of KCN applied, as did reduction in shoot fresh weight.

The phytotoxic symptoms of applied KCN and quinclorac were similar. Phytotoxic response to exogenously supplied ethylene (from ethephon) was very low. On the basis of these findings, the authors suggested that cyanide, derived from the stimulation of ACC synthesis, is a causal factor in the herbicidal effects of quinclorac on the shoots of barnyardgrass.

Grossmann's and Kwiatkowski's work (13) suggested that quinclorac stimulates ACC synthase in the root tissue of sensitive species. The ACC is then transported via the vascular tissue to the leaf. In the leaf, the ACC undergoes oxidation by ACC oxidase to release ethylene and cyanide in stoichiometrically equivalent amounts. The ethylene itself did not cause phytotoxic effects (21). Cyanide has been demonstrated to cause growth inhibition and tissue chlorosis and subsequent necrosis that mimic the effects caused by quinclorac (11,12,14).

Miller and Conn (17) pointed out that several plants have the ability to produce HCN. More than 2000 species have been demonstrated to be cyanogenic. In most species, the mechanism for the production of HCN is the degradation of cyanogenic glycosides (9). Miller and Conn (17) investigated a variety of species that were known to be cyanogenic (e.g. sorghum) and noncyanogenic (e.g. soybeans). Each tested species was found to contain β-cyanoalanine synthase. Miller and Conn found that there was a trend between the enzyme activity and the HCN potential.

Miller and Conn defined HCN potential as the reflection of the concentration of cyanogenic glycosides in the plant which upon degradation, leads to the release of HCN. The higher the HCN potential, the higher the ß-cyanoalanine synthase activity. The activity of this enzyme was found to be lower in noncyanogenic plants.

The major precursor for the evolution of ethylene in plants was found to be the amino acid methionine (1). Work conducted by Adams and Yang (1) implicated SAM (*S*-adenosyl-l-methionine) as intermediate between methionine and ethylene. This lead to their discovery of a unique amino acid (1-aminocyclopropane-1-carboxylic acid) that was an immediate precursor to ethylene. Work by Yu *et al*. (26,27) determined that the enzyme involved in the conversion of SAM to ACC was ACC synthase. ACC undergoes an oxidation reaction that is catalyzed by ACC oxidase (13). Products of this reaction are ethylene and stoichimetrically equivalent amounts of cyanide (12,25). Free HCN is phytotoxic to plants, in that HCN has been found to block normal respiration in the mitochondria that is irreversible under physiological conditions (23).

The fate of the HCN formed as a result of this reaction has been studied by several researchers (4,5,6,9,20). Their work showed that plants have a specific mechanism for detoxifying cyanide.

Bluementahl-Goldschmidt *et al*. (4) were the first to describe the enzyme ß-cyanoalanine synthase which catalyzes the reaction of the amino acid cysteine and HCN to form ß-cyanalanine and H_2S. Wurtele *et al*. (22) suggested that this enzyme may be ubiquitous in plants since it has been detected in over 20 plant species. Plants have been found to covert ß-cyanalanine to asparagine by means of the enzyme ß-cyanalanine hydratase (6). This enzyme catalyzes the hydration of ß-cyanalanine. As a result of these two reactions, work with ^{14}C labeled cyanide was found to be incorporated into the amide carbon of asparagine (4,5).

Work conducted in barley by Wurtele *et al*. (22) showed that ß-cyanoalanine synthase was predominantly found in the mitochondria. This was not a surprising finding in that as previously discussed, free HCN actively blocks respiration in the mitochondria. Additionally, work has suggested that ethylene synthesis from ACC can occur in the mitochondria as well (21). Another fate of ACC in the plant is its conversion to 1-(malonylamino)cyclopropane-1-carboxylic acid (MACC) (18,24). Studies by Peiser *et al*. (18) investigating the fate of radiolabeled ACC in mungbean showed that the main metabolite of ACC was MACC. In fractionating tissue samples into cationic and noncationic by ion exchange resin, Peiser showed that the noncationic fraction contained 50-55% of the recovered radioactivity, and essentially all (98-99%) of the radioactivity in the fraction was identified as MACC.

The cationic portion contained unreacted ACC and a radiolabeled compound that was identified as asparagine. He also found that in the mungbean tissue approximately 16% of the administered ACC was converted into ethylene and 10% of the recovered radioactivity was accounted for as asparagine. MACC was found to be a poor precursor to the evolution of ethylene. This may be another mechanism within the plant to avoid the conversion of ACC to HCN.

Chism *et al.* (8) evaluated the interaction between growth stage and rate of applied quinclorac to southern crabgrass. Several growth stages of crabgrass were evaluated as to susceptibility to an applied rate range of quinclorac. Growth stages included : pre (to crabgrass emergence), three to five-leaf stage, two to four tiller, and mature flowering plants. Each stage was treated with 70,140,280, 560, and 1120 g ai ha^{-1} of quinclorac. After 14 days, above ground tissue was harvested and fresh and dry weights were determined. Using non-linear regression techniques, equations were developed to model results. Results showed that quinclorac reduced both fresh and dry weights of southern crabgrass at all growth stages.

On a dry weight basis,plants treated at the preemergence and the true leaf stages had significantly lower GR_{50} values than when at either the tillering or the flowering stage. Dry weight data also suggested that crabgrass was most sensitive to quinclorac when applied at the tillering stage.

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CHAPTER TWO

ADJUVANT EFFECTS and GOOSEGRASS: STAGE OF GROWTH RESPONSE TO QUINCLORAC

ABSTRACT

Several commercial and experimental adjuvants were evaluated for selectivity and effectiveness in enhancing activity of quinclorac in canola (*Brassica napus* L.) and turfgrass. Weed species investigated included cleavers (*Galium aparine* L.), annual sowthistle (*Sonchus oleraceus* L.), large crabgrass (*Digitaria sanguinalis* L. [Scop.]), and goosegrass (*Eleusine indica* L. [Gaertn.]). Weed species were selected for their importance in canola (cleavers and annual sowthistle) and turfgrass (large crabgrass and goosegrass). Canola cultivars evaluated for selectivity included "Garrison" and "Goldrush". Turfgrass species evaluated included Kentucky bluegrass (*Poa pratensis* L.), perennial ryegrass (*Lolium perenne* L.), tall fescue (*Festuca arundinacea* L. [Schreb.]), and creeping bentgrass (*Agrostis palustris* L. [Huds.]. Adjuvants were selected to give a representative sample across adjuvant types such as methylated seed oil ("Sunit II"), petroleum based crop oil concentrate, silicone based ("Sylgard 309"), cationic surfactant ("Frigate" [fatty amine ethoxylate]), and modified crop oils ("Dash" and "Merge").

Effectiveness of adjuvants was evaluated for cleavers, annual sowthistle, and large crabgrass by calculating quinclorac GR_{50} (herbicide rate required to reduce plant growth 50%) values based on applied at rates of 0, 15.6, 31.2, 62.5, and 125 g ai ha^{-1}. For goosegrass, quinclorac rates evaluated were increased to 250, 500, 1000, and 2000 g ai ha^{-1}. Treatments also included quinclorac applied with no adjuvant at each rate.

Applications were made at the three to five-whorl stage for cleavers, four to six-leaf stage for annual sowthistle and the one to two-tiller stage for large crabgrass and goosegrass. Root uptake was minimized by the use of a vermiculite soil barrier. Shoot fresh weight data were recorded 14 days after treatment.

Adjuvant selectivity in canola and turfgrass was evaluated by applying the adjuvants alone with no added quinclorac. Applications were made at the six to eight-leaf stage for canola. Turfgrass species were maintained and treated at a clipped height of 6.25 cm. Crop selectivity was evaluated by rating visual injury 7 days after application.

All evaluated adjuvants provided similar enhancement of control for cleavers and annual sowthistle. Sylgard 309 was the only adjuvant that did not enhance control of large crabgrass. Goosegrass was tolerant to quinclorac across the evaluated rate range regardless of adjuvant, and therefore, GR_{50} values could not be determined. None of the adjuvants caused phytotoxicity to canola or any turfgrass species.

Goosegrass, at several stages of growth studies was treated with quinclorac at 0, 1, 2, 4, 8, and 16 kg ha^{-1} applied with 1% v/v of "Merge" spray adjuvant. The growth stages included preemergence, one to two true leaf, four to five true leaf and one to two-tiller. The effects of root uptake were also tested by evaluating treatments with and without a vermiculite soil barrier. Results showed differences in calculated GR_{50} values and improved control as a result of root absorption. The lowest GR_{50} value was 2.7 kg ha^{-1} for the one to two-leaf stage with no soil barrier. However, this value is approximately 3.5 times higher than the maximum labeled rate for turfgrass.

INTRODUCTION

An adjuvant can be defined as "any substance in a herbicide formulation or added to the spray tank to improve herbicidal activity or application characteristics" (12). The primary function of an adjuvant is to decrease the surface tension of the spray droplets, which results in more uniform spreading over the leaf surface (1,8). An effective adjuvant may also enhance the penetration of the herbicide through the major barriers to cell entry. An adjuvant must also be nonphytotoxic to the crop or desirable species. Efficacy of postemergence herbicides usually requires the addition of adjuvants (1,8,11). Work conducted with quinclorac has shown that a proper adjuvant is vital to enhance the postemergence activity (2,7).

Plant leaves are the main point of entry for foliar applied herbicides. However, entry can also occur via the stems and buds (1). Once the herbicide is delivered to the leaf surface, several factors can effect its fate. Environmental factors such as light, temperature, humidity, rainfall and wind can effect resultant absorption (1). The degree of pubescence or makeup of the cuticular waxes on the leaf surface can also affect absorption. For foliar applied herbicides to be effective, the herbicide molecule must be delivered to the site of action.

Foliar applied herbicides face three main barriers of entry into plant cells via the leaves. The barriers include the leaf cuticle, the cell wall and the plasmalemma. (1,11). The cuticle consists of waxes, pectin, cutin and cellulosic material (11). The structure has been likened to a sponge in which the framework is of spongy cutin and the holes are filled with waxes (1).

The movement of herbicides across the cuticle is by simple diffusion (9). There are three main pathways along which the herbicides may diffuse: 1). penetration via intermolecular spaces; 2). for water-soluble material, via water-filled and swollen pectin corridors between lipid platelets; and 3). for oil-soluble materials, directly through the waxy portions of the cuticle (1). The cell wall is composed of a dense network of cellulose and hemicellulose microfibrils with interfibrillar spaces that are commonly filled with water (3). The cell wall is known to offer little resistance to herbicide penetration (1,11). The main process for movement through this barrier is diffusion. The final barrier to herbicide movement is the plasmalemma ,which is a semipermeable, bimolecular membrane composed of tightly packed, globular lipoprotein molecules. (1). The penetration of herbicides through this barrier may require energy and a carrier (1,11).

The stage of growth of weeds at the time of application can affect resultant control. In general with postemergence herbicides, weeds tend to be more readily controlled in the early seedling stages than in advanced growth stages (1). Chism *et al.* (6) demonstrated differences in sensitivity of southern crabgrass (*Digitaria ciliaris* [Retz.]) to applied quinclorac when applied at different growth stages. He found that flowering crabgrass plants had a higher GR_{50} value than preemergence, three to five true leaf or two to four tiller stages.

The first objective of these studies was to investigate selected commercial and experimental adjuvants for their selectivity and effectiveness in enhancing quinclorac activity on important weed species in canola and turfgrass. A wide range of adjuvant types were evaluated including a series of experimental adjuvants from BASF. Adjuvants were selected to give a representative sample across adjuvant types such as methylated seed oil ("Sunit II"), petroleum based crop oil concentrate ("Herbimax"), silicone based ("Sylgard 309"), cationic surfactant ("Frigate" [fatty amine ethoxylate]), and modified crop oils ("Dash" and "Merge"). Cleavers and annual sowthistle represent important broadleaf weeds in canola production in Canada. Large crabgrass and goosegrass are major grassy weed problems in both cool and warm season turfgrass(4,5).

Adjuvant selectivity was evaluated in canola (*Brassica napus* L.) and several cool season turfgrass species including Kentucky bluegrass (*Poa pratensis* L.), perennial ryegrass (*Lolium perenne* L.), tall fescue (*Festuca arundinacea* L. [Schreb.]), and creeping bentgrass (*Agrostis palustris* L. [Huds.]. Canola was selected for evaluation based on the plans of BASF to pursue a future registration for the use of quinclorac. The second objective of these studies was to evaluate the effectiveness of quinclorac in controlling goosegrass at different stages of plant growth including preemergence, one to two true-leaf, four to five true-leaf and one to two-tiller. As part of this second objective, the role of root absorption of quinclorac was also investigated.

METHODS AND MATERIALS

Adjuvant Studies :

Cleavers (*Galium aparine* L.), annual sowthistle (*Sonchus oleraceus* L.), large crabgrass (*Digitaria sanguinalis* L. [Scop.]), and goosegrass (*Eleusine indica* L. [Gaertn.]) were seeded in Metro Mix 360[1] greenhouse potting soil in 946 ml plastic pots. The pots received an application of OSMOCOTE [2] fertilizer (10-10-10) at planting and were maintained with daily overhead irrigation. Greenhouse conditions were maintained at approximate day/night temperatures of 30 ^{o} /20 ^{o} C. Plants were grown in a 16 hour photoperiod and consisted of natural light supplemented with metal halide light at 600 uE m^{-2} s^{-1} photosynthetic photon flux density (PPFD). After emergence, plants were thinned to one per pot.

Quinclorac was applied at rates of 0, 15.6, 31.2, 62.5, and 125 g ai ha^{-1} to all species except goosegrass. For goosegrass, quinclorac rates used were 0, 250, 500, 1000, and 2000 g ai ha^{-1}. Adjuvants were applied at a rate of 1% (v/v) except for Sylgard 309 (0.125% (v/v)), and Frigate 0.5% (v/v). A description of the adjuvants used is presented in Table 1. Separate experiments were conducted with annual sowthistle and large crabgrass to determine GR_{50} values with quinclorac applied without an adjuvant.

A rate range of 0, 250, 500, 1000, and 2000 g ai ha^{-1} was used for annual sowthistle. For large crabgrass evaluated rates were 0, 1, 2, 4, 8, and 16 kg ha^{-1}.

Treatments were applied when the weed species were in the following growth stages : Cleavers (three to five-whorl), annual sowthistle (four to six leaf), large crabgrass and goosegrass (one to two-tiller). For cleavers and sowthistle, spray applications were made with an overhead track sprayer set to deliver 187 l ha^{-1} at an operating pressure of 275 kPa using an 8001 even flat fan nozzle [3]. For large crabgrass and goosegrass, applications were made at 748 l ha^{-1} at 275 kPa using an 8004 even flat fan nozzle. The spray volumes were selected to approximate those used under field conditions. Root uptake of quinclorac was prevented by covering the soil with a one cm layer of vermiculite before spraying. The vermiculite was removed after the spray had dried. Pots were watered by subsurface irrigation after treatments were applied. At 14 days after treatment, weeds were harvested at soil level and fresh weights recorded.

For the crop selectivity evaluations, canola cultivars "Garrison" and "Goldrush" were seeded in Metro Mix 360 greenhouse potting soil and maintained as discussed with the evaluated weed species. After emergence, pots were thinned to one plant per pot. Applications were timed when the plants reached the six to eight-leaf stage.

Spray parameters were the same as discussed for cleavers and annual sowthistle.

Evaluated turfgrass species, Kentucky bluegrass (*Poa pratensis* L.), perennial ryegrass (*Lolium perenne* L.), tall fescue (*Festuca arundinacea* L. [Schreb.]), and creeping bentgrass (*Agrostis palustris* L. [Huds.]. were broadcast seeded into pots containing Metro Mix 360 greenhouse potting soil and maintained at a clipped height of 6.25 cm. Applications were made after the grasses were well established and clipped several times. Turfgrass growth was supplemented with periodic applications of liquid fertilizer solution on an as needed basis. Adjuvants were applied alone (with no herbicide) at the rates discussed with the evaluated weed species. Spray parameters for the turfgrass species were the same as discussed for large crabgrass and goosegrass.

[1] Metro Mix 360, Scotts-Sierra Horticultural Products Company , Marysville, OH 43041.

[2] OSOMOCOTE Fertilizer, Scotts Company, Marysville, OH 43041.

[3] Flat fan Nozzle, Spraying Systems Co., Wheaton, IL 60188.

Table 1. Adjuvant description and source.

Name	Description	Address
Sylgard 309	Organosilicone mixture: the active ingredient 2 -(3-hydroxypropyl)-heptamethyl-trisiloxane,ethoxylated acetate	Dow Corning Corp. Midland,MI 48686
Herbimax	83% Petroleum hydrocarbons,17% surfactant (mono and diesters of omega hydroxypoly oxyethylene)	Loveland Indust. P.O. Box 1289 Greeley,CO 80632
Sunit II	Methylated seed oil	AGSCO, Inc., Fargo,ND,58105
Dash	45% petroleum hydrocarbons, 5% naphthalene, 1.5% phosphoric acid, and 48.5% mixture of alkyl esters and anionic surfactant	BASF Corp., RTP, NC 27709
Frigate	Mixture of ethoxlated long - chain fatty amines	ISK Biosciences Corp., Mentor,OH 44061
Merge	Proprietary Adjuvant	BASF Canada,Inc., Toronto, Ont M9W 6N9
Exp 1,2, 3,& 4	Proprietary Adjuvants	BASF Corp.,RTP,NC 27709

Goosegrass Stage of Growth Studies:

Goosegrass plants were grown and spray applications made as previously described in the adjuvant studies except that an actual field soil was used in place of a potting mix. The soil used for these studies was characterized as a silt loam with 3.8 % organic matter, a cation exchange capacity of 21.5 meq/100 grams and a pH of 6.6. Quinclorac was applied at rates of 0, 1, 2, 4, 8, and 16 kg ai ha^{-1} with "Merge" adjuvant at 1% v/v. Treatments were applied as preemergence (i.e. applied immediately after seeding), one to two-leaf, four to five-leaf and one to two-tiller stage of goosegrass. Each treatment was applied with and without a vermiculite soil barrier. The method used for the vermiculite barrier was the same as outlined in the adjuvant studies. Immediately after the spray solution had dried on the leaf surface, the "without vermiculite" treatments were surface irrigated with enough water to approximate a 1.25 cm depth applied per pot. Care was taken not to allow any water to come in contact with the treated leaves. The vermiculite barrier was removed for those specific treatments as well after the spray solution had dried on the leaf surface. Pots were subsequently subsurface irrigated daily. At 14 days after treatment, weeds were harvested at soil level and fresh weights recorded.

Data Analysis :

All experiments were conducted in completely randomized designs. For the adjuvant studies, treatments were arranged as a two factor (herbicide rate by adjuvant) factorial. For the goosegrass stage of growth studies, treatments were arranged as a three factor (growth stage by herbicide rate by soil barrier) factorial. Each treatment was replicated four times (one plant per replication) and each experiment was repeated once. Each weed species was evaluated as a separate experiment. Linear regression was conducted for the fresh weight data for each replication across the range of evaluated rates and GR_{50} values were calculated. Data were subjected to Analysis of Variance (ANOVA). No interactions were present between experiments; therefore, data were combined over time. Means were separated by Fisher's Protected LSD at $\alpha = 0.05$ (10).

RESULTS AND DISCUSSION

Adjuvant studies :

Results are presented in Fig. 1. The calculated GR_{50} values across both selected commercial and experimental adjuvants were equivalent in providing control on cleavers. All adjuvants combined with quinclorac provided significantly greater control than quinclorac without an adjuvant.

Results obtained from the annual sowthistle study followed a similar trend to that observed on cleavers (Fig. 2). All evaluated adjuvants provided greater control of annual sowthistle compared to the use of no adjuvant. However, there was no statistical difference observed among adjuvants. Quinclorac applied without adjuvant failed to provide adequate control of sowthistle within the evaluated rate range (i.e. 0 to 125 g ha^{-1}). The calculated GR_{50} value from the separate experiments for quinclorac on sowthistle without the use of an adjuvant was 0.98 kg ha^{-1}. These results suggested that there may be major differences in the cuticular makeup of the leaf surfaces of these two species that affects the absorption of formulated quinclorac (12); however, these differences are virtually overcome with the use of adjuvants.

Adjuvant effects on quinclorac activity on large crabgrass are presented in Fig. 3. All adjuvants increased quinclorac activity except for Sylgard 309. This suggested that a silicone based adjuvant that does exhibit excellent spreadibility and leaf coverage (8), may not be as effective in aiding quinclorac to penetration of the leaf cuticle in the case of large crabgrass.

As observed with annual sowthistle, the calculated GR_{50} for quinclorac used alone was greater than the scope of evaluated rates (i.e. > 125 grams ha $^{-1}$). The calculated GR_{50} value from the separate experiments for quinclorac on large crabgrass without the use of an adjuvant was 12.9 kg ha^{-1}.A separate experiment was conducted to determine the GR_{50} value for quinclorac without an adjuvant for large crabgrass and was determine to be 12.9 kg ha [redacted]^{1}.

An initial study was conducted with goosegrass across the same evaluated rate range and cultural conditions as used for the other weed species (i.e. 0 to 125 g ha $^{-1}$). However, no growth suppression was observed with quinclorac applied with any adjuvant at even 125 g ha $^{-1}$. An additional study was conducted to evaluate effects at 0, 250, 500, 1000, and 2,000 g ha $^{-1}$. The only noted growth suppression observed with quinclorac applied with adjuvants occurred at a rate of 2000 g ha $^{-1}$ as presented in Fig. 4. The 2000 g ha $^{-1}$ rate represents about a 2.5X rate over the projected maximum use rate in turfgrass for quinclorac (0.84 kg ha $^{-1}$).

Only three of the adjuvants, "Merge" and two experimentals coded #1 and #2, enhanced quinclorac activity to provide significant growth suppression of goosegrass in comparison to the untreated control.

A follow-up study was initiated to determine a GR_{50} value for the control of one to two-tiller goosegrass with quinclorac without an adjuvant. A rate titration of quinclorac up to 16 kg ha $^{-1}$ was evaluated. Results showed that the calculated GR_{50} was greater than the evaluated 16 kg ha $^{-1}$.

The GR_{50} results presented in Fig. 5 are an overview of the values determined for each species to quinclorac applied without the use of an adjuvant. Values ranged from 0.052 kg ha^{-1} for cleavers to greater than 16 kg ha^{-1} for goosegrass. These comparisons suggested that there may be major differences in the cuticular makeup of the leaf surfaces of these species that affects the absorption of formulated quinclorac (8,11). However, these studies showed that control with quinclorac can be markedly increased with the use of effective adjuvants. Since it was difficult to induce significant growth suppression in goosegrass with several adjuvant types, tolerance may involve other mechanisms.

In addition to effects on quinclorac efficacy, the adjuvants were evaluated for crop safety in canola and several turfgrass species. Canola varieties evaluated included "Garrison" and "Goldrush". Turfgrass included Kentucky bluegrass (*Poa pratensis* L.), tall fescue (*Festuca arundinacea* L. [Schreb.]), perennial ryegrass (*Lolium perenne* L.), and creeping bentgrass (*Agrostis palustris* L. [Huds.]). No injury was observed at 7 days after treatment with any adjuvant in either canola or turfgrass species.

Goosegrass studies :

Based on the results of the adjuvant studies, studies were conducted with goosegrass to investigate the effects of growth stage and the role of both foliage and roots on quinclorac uptake. Results presented in Fig. 6 summarize the sensitivity of goosegrass to quinclorac applied at several growth stages with and without a vermiculite soil barrier. Calculated GR_{50} values ranged from 2.7 kg ha $^{-1}$ to greater than 16 kg ha $^{-1}$. Quinclorac applied as a preemergence treatment had a measured GR_{50} value of 3.4 kg ha $^{-1}$. At the one to two-leaf stage, the GR_{50} value was similar for both the with and without vermiculite treatments 2.7 and 3.1 kg ha $^{-1}$, respectively. At the four to five-leaf stage, however, there was a large difference between the GR_{50} values for the with (4.3 kg ha $^{-1}$) and without (> 16 kg ha $^{-1}$) vermiculite treatments. Once the goosegrass reached the one to two-tiller stage, the calculated GR_{50} values for the presence or absence of vermiculite were above the scope of inference for the experiment (i.e., greater than 16 kg ha $^{-1}$). Mean separations were made among the GR_{50} values for the preemergence, the one to two-leaf and four to five-leaf stages without vermiculite treatments (3.4, 2.7 and 4.3 kg ha $^{-1}$, respectively). Among these three means, there was a significant difference between the one to two, and four to five-leaf stage.

These results suggested that quinclorac did exhibit preemergence activity on goosegrass although only at very high rates that were beyond the scope of the suggested labeled rates in turfgrass. Postemergence results demonstrated that, at the one to two and four to five-leaf stages, root uptake enhanced resultant control. The difference was markedly pronounced at the four to five-leaf stage where the no vermiculite treatment had a measured GR_{50} value of 4.3 kg ha $^{-1}$ vs. the vermiculite treatment of > 16.0 kg ha $^{-1}$. These data suggested that root uptake can be an important factor in resultant weed control with quinclorac. However, in the case of goosegrass (even at the more "sensitive" stage of one to two-leaf, it is not enough to increase control to within labeled rates.

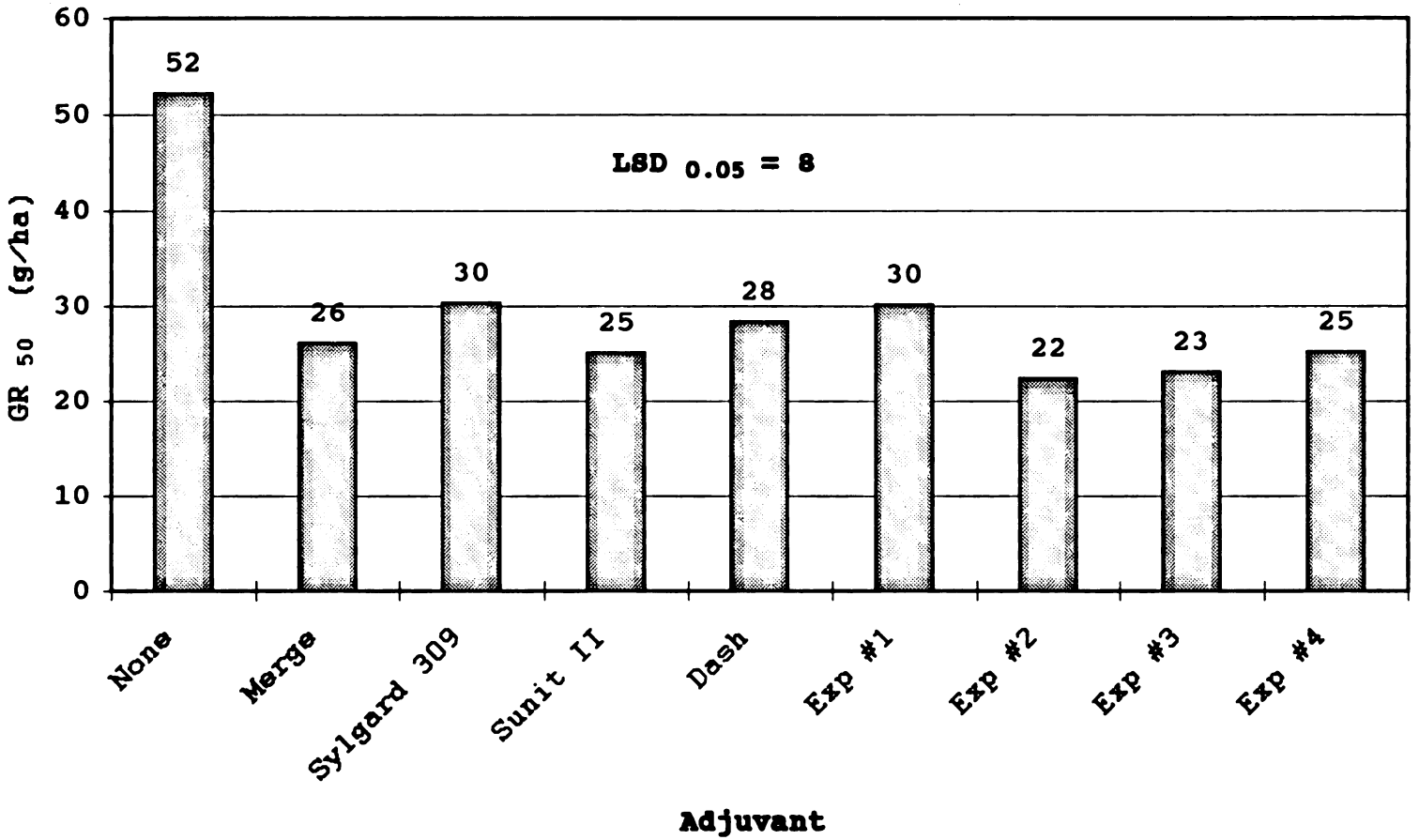

Figure 1. Calculated GR_{50} values for cleavers treated with quinclorac using several adjuvants.

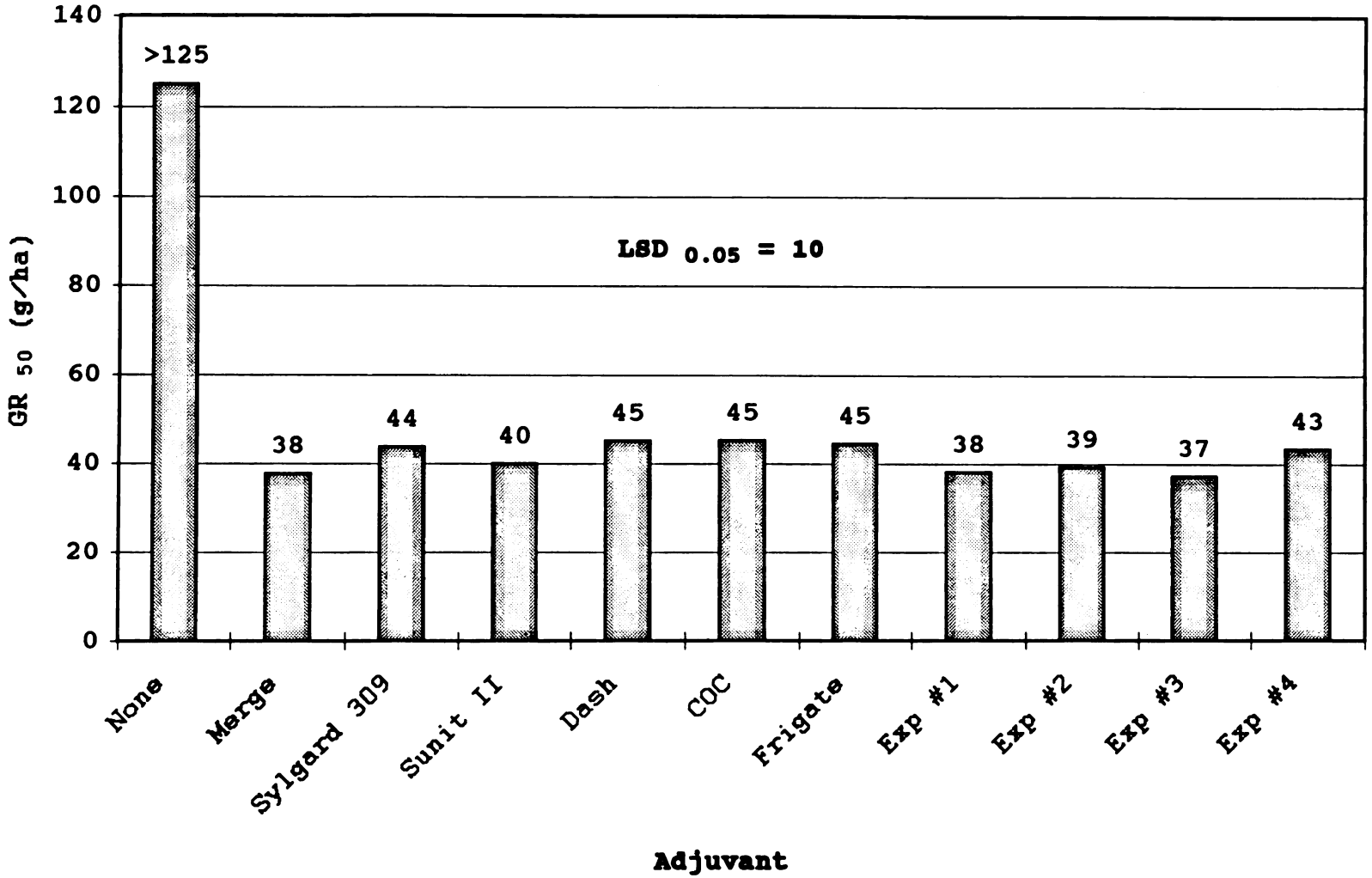

Figure 2. Calculated GR_{50} values for annual sowthistle treated with quinclorac using several adjuvants.

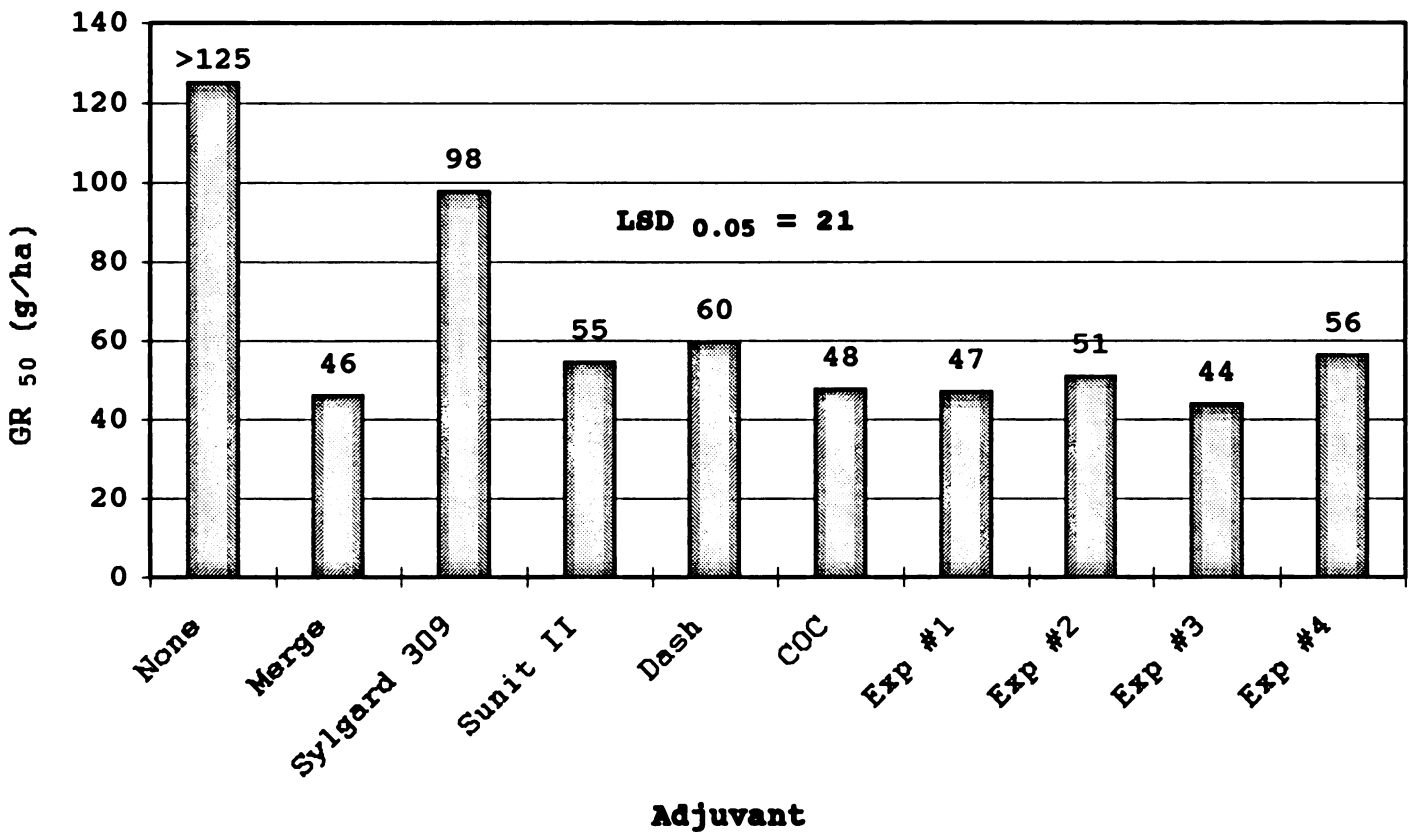

Figure 3. Calculated GR_{50} values for large crabgrass treated with quinclorac using several adjuvants.

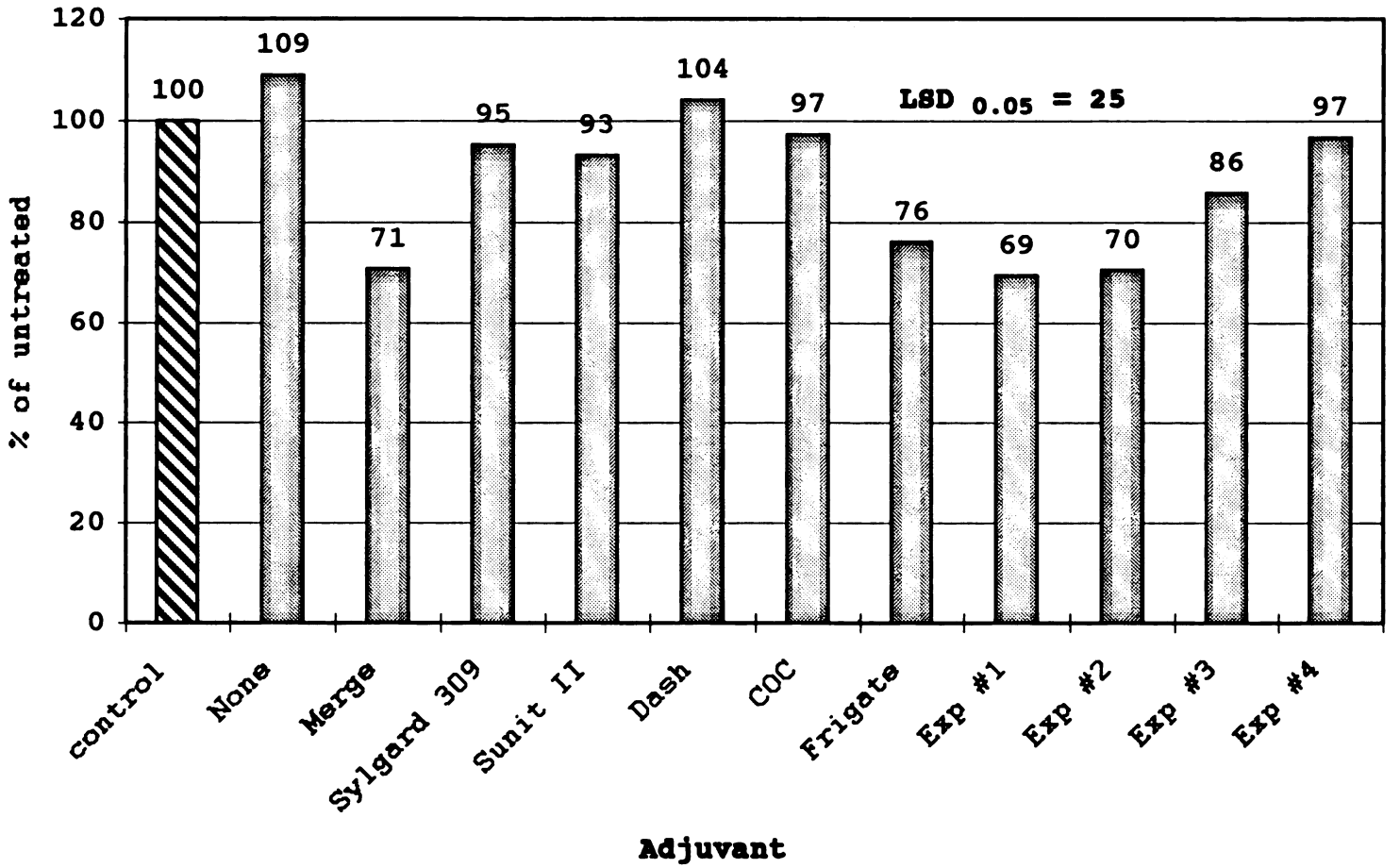

Figure 4. Influence of 2.0 kg ha^{-1} quinclorac applied at the 1 to 2 tiller stage on fresh weight values of treated goosegrass using several adjuvants.

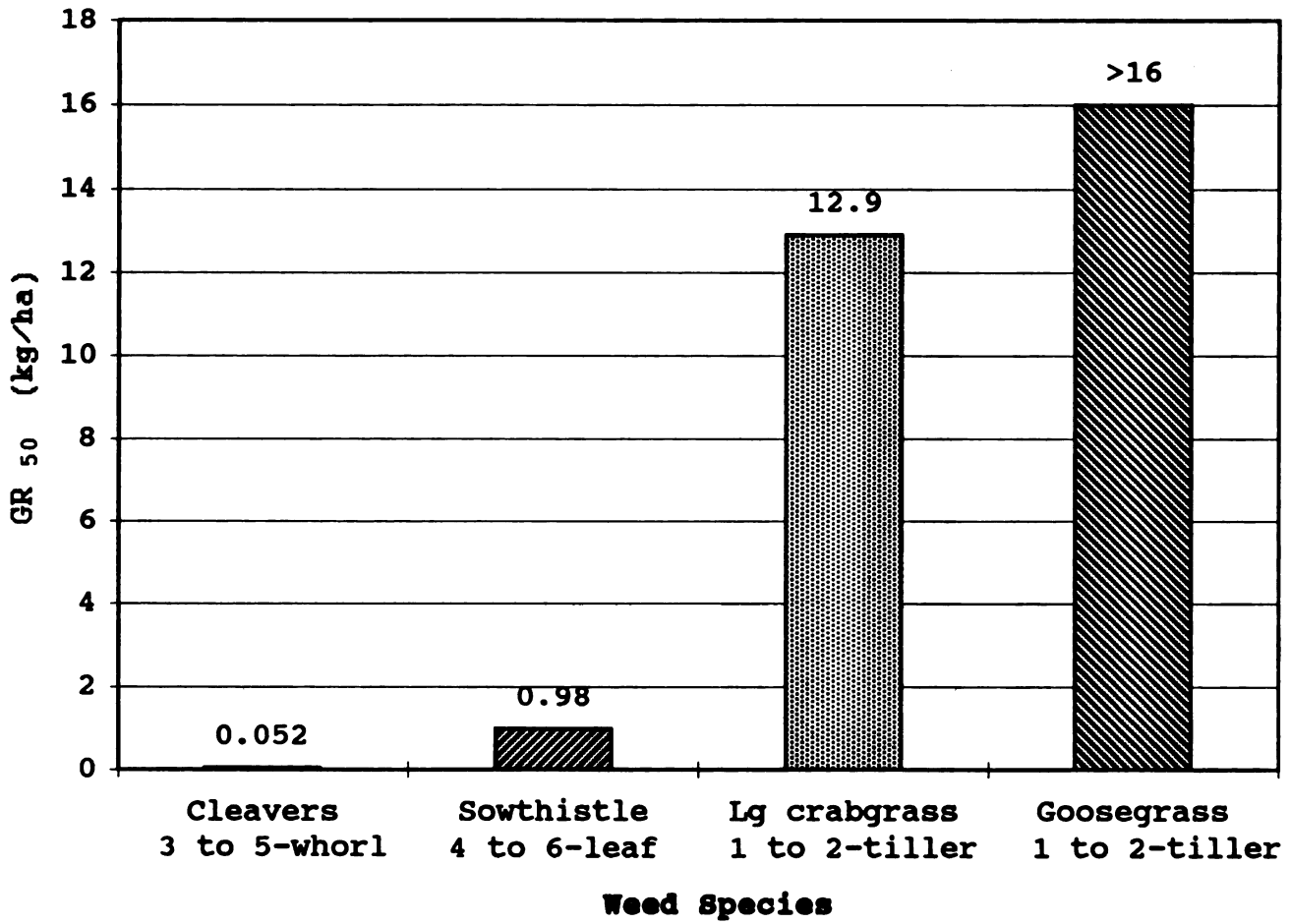

Figure 5. Comparative GR_{50} values for cleavers, annual sowthistle ,large crabgrass and goosegrass treated with quinclorac without adjuvants.

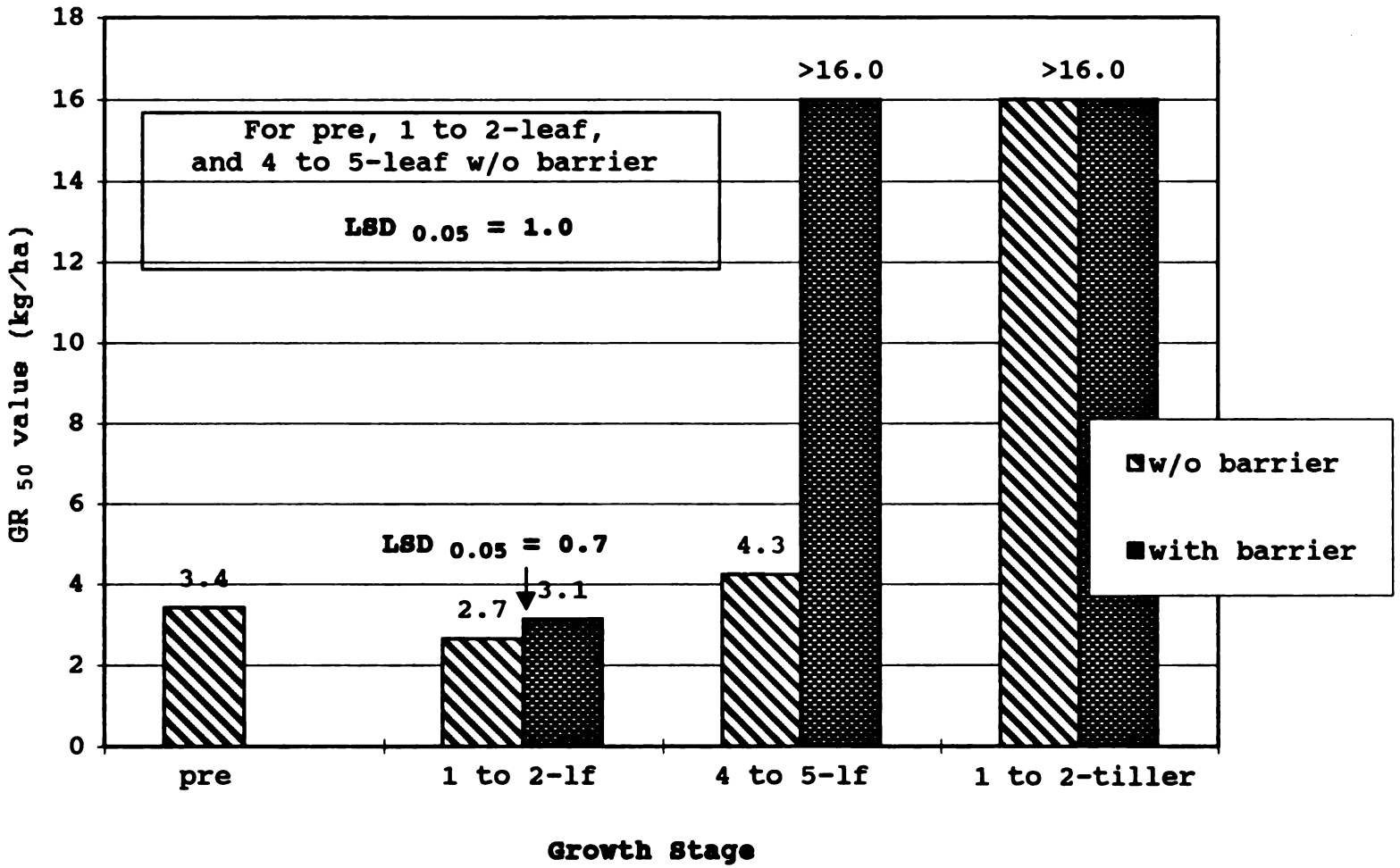

Figure 6. Calculated GR_{50} values for quinclorac as affected by goosegrass growth stage and presence of vermiculite soil barrier.

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CHAPTER THREE

ABSORPTION ,TRANSLOCATION, METABOLISM AND SPRAY RETENTION

ABSTRACT

Absorption, translocation, and metabolism studies using ^{14}C-quinclorac were conducted with large crabgrass and goosegrass at the one to two-tiller growth stage cultured under hydroponic conditions. After an 80 hr exposure time, both species had absorbed nearly equal amounts of ^{14}C-quinclorac (27% and 22% ,respectively) for large crabgrass and goosegrass. Over the exposure period, the absorption curve for large crabgrass tended to be curvilinear with the maximum absorption occurring approximately 48 hr after exposure. The response curve for goosegrass tended to be linear across the exposure period. Results from the translocation studies showed that 95% of the absorbed ^{14}C-quinclorac remained in the treated leaf for large crabgrass after 80 hr. However, only 58% of the absorbed ^{14}C remained in the treated leaf of goosegrass. Most of the ^{14}C translocated out of the leaves moved to the tiller and the crown and new leaf tissue. Sampling of nutrient vials did not reveal any appreciable amounts of ^{14}C-quinclorac that may have been exudated by either species during the absorption period.

Results of the metabolism studies showed that neither the susceptible species (large crabgrass) nor the tolerant species (goosegrass) was able to metabolize the parent quinclorac herbicide.

Spray retention studies showed that goosegrass (tolerant) retained more applied quinclorac than large crabgrass (sensitive). Overall results suggested that difference in tolerance of the two species to quinclorac involves mechanisms other than absorption, metabolism or spray retention. Translocation differences may play some role but since the site of translocation was to active meristematic tissue;however, it is somewhat difficult to explain how this may contribute to tolerance.

INTRODUCTION

Several factors can be involved in the differential tolerance of weed species to a particular herbicide. These factors include differences in herbicide uptake, translocation, metabolism and spray retention (1,11,15). Several parameters can affect differences in herbicide uptake. Species can differ in morphology, leaf angle, leaf structure, makeup of leaf cuticle, etc. The main focus of postemergence herbicide application is to maximize the amount of herbicide delivered to the site of action within the plant. This is where the use of effective adjuvants come into play. However, differences in absorption between species may not necessary be correlated with resultant control. Ma *et al.* (12) found a poor correlation between ^{14}C absorption of prosulfuron and the tolerance of specific weed species. ^{14}C absorption was found to be highest in common lambsquarters (*Chenopodium album* L.) followed by sicklepod (*Senna obtusifolia* L.)and common cocklebur (*Xanthium strumarium* L.). Tolerance rankings showed sickepod > common lambquarters > common cocklebur. The fate of the herbicide once delivered inside the plant cell also can be a factor in differential tolerance (1,7). A particular species maybe able to more readily translocate the herbicide to other areas of the plant as a dilution or as storage avoidance mechanism (1,7).

Metabolism can be an important factor in the differential tolerance of plant species (1,7). For example, Carey *et al.*(4) in their work on selectivity of nicosulfuron, and primisulfuron showed that weed species tolerant to the herbicides metabolized the compounds more rapidly and extensively than sensitive species.

Spray retention differences between species can influence selectivity differences. Work by Sharma *et al.*(13) showed that susceptible wild oat (*Avena fatua* L.) retained four times more applied asulam [methyl[4-aminophenyl)sulfonyl carbamate] than flax. The researchers suggested that differences in retention partially explained the observed selectivity differences. However, it has also been demonstrated that increased spray retention in itself may not explain selectivity differences. Work by Boldt and Putnam (3) showed that tolerant soybeans (*Glycine max* L.) and cucumber (*Cucumis sativus* L.) retained the same amount of applied diclofop-methyl [±-2-[4-(2,4,- dicloropheno xy) phenoxy]propanoic acid] as sensitive barnyardgrass. The objectives of these studies were to investigate the role of spray retention, absorption, translocation and metabolism on the differential tolerance of large crabgrass and goosegrass to quinclorac.

METHODS AND MATERIALS

Absorption and Translocation Studies:

Large crabgrass (*Digitaria sanguinalis* (L.) Scop.), and goosegrass (*Eleusine indica* (L.) Gaertn.) were seeded into pure sand and covered with a one cm layer of Metro Mix 360 greenhouse potting soil in 946 ml plastic pots. The pots received an application of OSMOCOTE fertilizer (10-10-10) at planting and were maintained with daily overhead irrigation. Greenhouse conditions were maintained at approximate day/night temperatures of $30^0/20^0$ C. Plants were grown in a 16 hour photoperiod and consisted of natural light supplemented with metal halide light at 600 uE m^{-2} s^{-1} PPFD. After emergence, plants were thinned to 5 plants per pot.

At the 1^{st} tiller stage, intact plants were removed from the soil media pots and placed in a water bath maintained at room temperature. After all the excess sand was removed in the water bath, plants were transferred into amber vials (100 ml) that contained 70 ml of a 0.2X Hoagland nutrient solution.

Plants were supported in the vials by means of a foam sleeve. Each vial contained one plant. Plants were maintained under the same aforementioned greenhouse conditions. Vials were aerated throughout the experiment by means of attached tubing which supplied a constant air flow from an air compressor.

Plants were allowed to equilibrate to the nutrient solution culture for 48 hr prior to herbicide application. Plants at the one to two-tiller stage were oversprayed with nonlabeled, formulated, quinclorac at a rate of 0.56 kg ai ha^{-1} with "Merge" spray adjuvant @ 1% v/v. Overspraying with nonlabeled material was to ensure that the pattern of translocation and absorption would be similar to that under normal field conditions. The targeted leaf for ^{14}C application was the most fully expanded leaf above the tillers. This leaf was covered with a cellophane wrap during overspraying with nonlabeled quinclorac. The cellophane wrap was removed immediately after the spray solution dried.

Spray applications were made with an overhead track sprayer set to deliver 748 l ha^{-1} at an operating pressure of 275 kPa using an 8004 even flat fan nozzle. The radiolabeled spotting solution contained [$3^{14}C$] labeled quinclorac (with a specific activity of 1.5 x 10^3 kBq mg^{-1}), formulated, nonlabeled quinclorac and "Merge" spray adjuvant at 1% v/v. Nonlabeled quinclorac was added to the solution to approximate a rate of 0.56 kg ai ha^{-1} based on a spray volume of 748 l ha^{-1} . Each plant was spotted on the adaxial leaf surface with two, 1 μL droplets containing 500 Bq each of radioactivity (1000 Bq total per leaf).

Plants were harvested at 0, 2, 4, 8, 24, 48, and 80 hr after treatment. At harvest, each plant was divided into treated leaf, first leaf, tillers, crown and new leaf tissue, and roots.

The treated leaf was the first part to be dissected and was immediately placed into a vial containing 10 ml of a 0.5 % solution of ammonium hydroxide to remove unabsorbed herbicide. The vial was vortexed for 15 seconds. The treated leaf was removed and placed into a second vial and the rinse procedure repeated. One ml aliquots of the rinse and nutrient solutions were taken and radioassayed by liquid scintillation spectrometry (LSS). Plant parts were frozen and stored at -20^0 C until further analysis. Plant parts were oven dried at 80^o C. Samples were oxidized using a biological sample oxidizer (Packard, Model 387) and evolved CO_2 was trapped in 10 ml of CO_2 absorber plus 10 ml scintillation fluid. Samples were radioassayed by LSS.

Data Analysis :

All experiments were conducted in completely randomized designs. Each treatment was replicated four times (one plant per replication) and each experiment was repeated once. Each weed species was evaluated as a separate experiment. Data were subjected to ANOVA. No interactions were present between experiments; therefore, data were combined over time. Non-linear regression analysis was conducted to determine the best fit line equation to describe herbicide absorption over time. Means were separated by Fisher's Protected LSD at $\alpha = 0.05$.

^{14}C-Quinclorac Metabolism Studies :

Both large crabgrass and goosegrass plants were cultured as described in the translocation and absorption studies. For the metabolism studies, plants were not oversprayed with nonlabeled quinclorac. Overspraying was not deemed necessary since the main focus of these studies was strictly metabolism. Application of the ^{14}C - labeled quinclorac was at the same stage as described in the translocation and absorption studies. The radiolabeled spotting solution contained [3^{14}C] - labeled quinclorac (with a specific activity of 1.5 x 10^3 kBq mg^{-1}) and "Merge" spray adjuvant at 1% v/v. Each plant was spotted on the adaxial leaf surface with five, 1μL droplets containing 3333 Bq each of radioactivity (16,667 Bq total per leaf). The experiment consisted of 4 replications of each species (one plant per pot). The experiment was repeated once over time.

Plants were harvested at 80 hr after treatment. At harvest, each plant was divided into treated leaf, first leaf, tillers, crown and new leaf tissue, and roots. Leaf wash techniques were the same as previously described. One ml aliquots of the rinse and nutrient solutions were radioassayed by liquid scintillation spectrometry (LSS). Plant parts were frozen and stored at -20 ^{0} C until further analysis.

The treated leaf was homogenized in a tissue homogenizer using 10 ml of acetone:water (80:20,v/v). The homogenate was centrifuged at 3750 *g* for 10 min. The supernatant was decanted into a new tube and the acetone evaporated under a stream of nitrogen gas. A 0.5 ml aliquot of the concentrated supernatant was transferred into a mini-centrifuge tube fitted with a 0.45 μm filter and centrifuged at 16000 *g* for 2 minutes. The clarified supernatant was then transferred into a 1 ml vial in preparation for HPLC analysis.

A reverse phase HPLC system (Hewlett Packard, Model 1050) fitted with a 254-nm UV detector and an in-line radioactivity monitor was used for ^{14}C metabolite separation. Samples were injected individually onto a reverse phase C_{18} column (4.1 x 250 mm) and chromatographed. The mobile phase used was water plus 0.1% formic acid applied isocractically at a flow rate of 1.0 ml min^{-1}. A ^{14}C-quinclorac standard was chromatographed separately to make comparisons of retention times.

Spray Retention Studies

Both large crabgrass and goosegrass plants were cultured as previously described. Quinclorac was applied at 0.56 kg ai ha^{-1} along with Chicago Sky Blue dye[3] (2.5 g L^{-1}) when plants reached the one to two-tiller stage. "Merge" spray adjuvant was also added at a 1% (v/v) of the spray volume. The method used was modified from the technique described by Boldt and Putnam (10). Spray applications were made with an overhead track sprayer set to deliver 748 l ha^{-1} at an operating pressure of 275 kPa using an 8004 even flat fan nozzle.

Immediately after the spray application was made, plants were excised at the soil surface and the retained dye was collected by rinsing the plants in 5.0 ml of a water, non-ionic surfactant solution (0.25% v/v). A one ml aliquot of the rinse solution was arrayed spectrophotometrically (Beckman, Model DU 65) and absorbance read at 625nm. Absorbance values were compared to those of a standard curve prepared for the Chicago Sky Blue dye.

Plant leaves were dissected from the plants and leaf area determined (cm^2) using a belt driven leaf area meter (LI-Cor Leaf Area Meter, Model LI-3000). Plant parts were then transferred to an oven and dried at 80 ^0C for 24 hours and subsequent weights recorded.

The quantity of active quinclorac was estimated based on the concentration ratio with the Chicago Sky Blue dye.

In the spray solution, each ml contained 1.3 mg of active quinclorac and 2.5 mg of the dye. Dividing these two numbers yielded a conversion value of 0.51.

Data Analysis :

All experiments were conducted in completely randomized designs. Each treatment was replicated four times (one plant per replication) and the experiment was repeated once. Data were subjected to ANOVA. No interactions were present between experiments; therefore, data were combined over time. Means were separated by Fisher's Protected LSD at $\alpha = 0.05$.

[3] Chicago sky blue dye, Sigma Chemical Co., St.Louis,MO 63187.

RESULTS AND DISCUSSION

Absorption and Translocation :

Recovery of applied ^{14}C was over 90% at each harvest interval and grass species. The results of the ^{14}C absorption studies for large crabgrass and goosegrass are presented in (Fig. 1). The rate of leaf absorption tended to be higher with large crabgrass vs. goosegrass over the initial 24 hours. This difference in initial rate of absorption suggested that there may be physical, chemical, or morphological differences in the leaf tissue of the two species (6,7,11). By visual observation, the leaves of large crabgrass tend to be quite pubescent, while the leaves of goosegrass are quite smooth and have a glossy appearance (14). Also, the effectiveness of the adjuvant may be somewhat different for the breakdown rate of the cuticular waxes (8,15).

By the 80 hr harvest interval, the large crabgrass had absorbed 27% of applied ^{14}C vs. 21% for the goosegrass. The overall rate curve tended to be more linear for goosegrass but the final amount of absorbed ^{14}C was somewhat similar to large crabgrass. These data suggested that the 6% difference in final absorption is probably not enough to explain the great difference observed in the tolerance of the two species to quinclorac.

The measured absorption of quinclorac by large crabgrass was much less than reported by Chism (5). Chism noted a very rapid absorption of ^{14}C in smooth crabgrass that reached 85% by 0.5 hr. This difference may in part be explained by the application methodology used. In his studies, Chism applied ^{14}C - quinclorac in a pure solvent base of methanol and adjuvant and also used only a single 10 μL droplet to apply the labeled compound. Also, the treated plants were not oversprayed with nonlabeled quinclorac. Using pure methanol as a carrier along with the adjuvant may have acted as a very effective carrier across the lipophilic cuticle. Additionally, not having oversprayed the rest of the plant with formulated quinclorac may have affected the absorption obtained from the treated leaf. The application technique we utilized was an attempt to mimic as closely as possible what one may observe with a plant that had received a commercial spray application. Other factors contributing to the observed differences may include the morphological differences in the composition of the cuticle and leaf morphology differences between southern and large crabgrass. Also, one must note that Chism used the youngest expanded leaf to treat, while we targeted the most fully expanded leaf above the tillers for ^{14}C application.

The distribution of the ^{14}C-quinclorac in large crabgrass is summarized in (Fig. 2). As exposure time to the applied ^{14}C - quinclorac increased, the amount of measured ^{14}C - quinclorac in the leaf tissue increased.

The amount detected in the first initial harvest intervals of 2, 4 and 8 hr were similar. However, a significant increase in ^{14}C - quinclorac was measured in the treated leaf by the 24 hr harvest period.

For each subsequent harvest interval, a significant increase in detected ^{14}C - quinclorac was observed in the treated leaf with the maximum of 14.4% of applied absorbed by 80 hr. These data suggest that initial absorption into the leaf was at a somewhat slow, steady rate from the 2 to 8 hr period.

The marked increase at 24 hr and subsequent intervals, may be explained as the required time period for the ^{14}C /adjuvant solution to at least penetrate into the leaf cuticle and avoid wash off. Visual symptomology of the plants across the exposure period of leaf reddening, necrosis and dieback suggested that the ^{14}C - quinclorac was transported with the adjuvant system across the cuticle, the cell wall and through the plasmalemma to the site of action (1,11,15).

The data also suggested that very little of the ^{14}C - quinclorac was translocated either acropetally or basipetally. The crown and new leaf tissue did not show a significant increase in detectable ^{14}C- quinclorac until the 24 hr harvest period. The level remained steady through the rest of the harvest periods. The 24 hr harvest period coincided with the marked increase detected in the treated leaf tissue.

The tillers did not show a significant increase in detectable ^{14} C- quinclorac until the 24 hr harvest period and remained steady thereafter.

The percent of applied ^{14}C - quinclorac measured in the first leaf or the root tissue were very low. Additionally, only a very small trace of ^{14}C - quinclorac was measured in the nutrient solution (data not shown). By the 80 hr harvest period, only 5.6% was translocated out of the treated leaf (0.9 % of the 15.2 % of applied total) with most being translocated to the active meristematic regions of the tillers, crown and new leaf tissue. The results of this plant distribution study supported the work by Chism *et al.* (5) that showed that most of the applied ^{14}C - quinclorac remained in the treated leaf of smooth crabgrass.

The plant distribution of ^{14}C - quinclorac for goosegrass is presented in Fig. 3. Unlike large crabgrass, no visual quinclorac symptomology was noted. The observed retention in the treated leaf was very similar to that observed for large crabgrass. Initial absorption did not change over the 2, 4, and 8 hr sampling periods. However, as observed with large crabgrass, there was a significant increase in the ^{14}C - quinclorac in the treated leaf at the 24 hr harvest timing and each subsequent time thereafter. This similar pattern suggested that the dynamics concerning the leaf cuticle, morphology, etc. that affected absorption discussed with large crabgrass may apply to goosegrass.

The maximum retention in the leaf measured at 80 hr was 6.50 % of applied.

Very little translocation was found out of the treated leaf until the 24 hr sampling period. At 24 hr, there was an increase in the amount of detected ^{14}C in both the crown and new leaf tissue and tillers, or the site of active meristematic activity. For each subsequent harvest interval, there was a significant increase in detectable ^{14}C - quinclorac for the tillers and crown and new leaf tissue. A steady increase in detectable ^{14}C - quinclorac was observed for the tiller tissue across the 24 to 80 hr period. However, there was a marked increase noted with the crown and new leaf tissue from the 48 to 80 hr time period (183%). No difference was noted across harvest intervals for levels detected in the first leaf. This may in part be explained by the function of this leaf as an exporter of carbohydrate rather than a site that functions as a sink.

The amount of detectable ^{14}C - quinclorac in the root tissue remained at a low level throughout the experiment. However, a significant increase was observed between the 4 and 8 hr harvest interval. The level detected at 80 hr was significantly higher than all other harvest periods except the 8 hr timing. The increase at the 80 hr harvest coincided with increases noted for both the crown and new leaf and tillers.

The distribution pattern in goosegrass showed that by the 80 hr harvest interval, 42 % of the total absorbed herbicide was translocated out of the treated leaf (4.7 % of the 11.2 % of applied total). Most was translocated to the active meristematic regions of the tillers and crown and new leaf. The translocation of a higher percentage of quinclorac by goosegrass vs. large crabgrass may have some dilution effect and have a role in tolerance as observed in other species (2,5).

It was hypothesized by Berghaus and Wuerzer (2), Chism *et al.*(5) and Grossmann (9) that one of the possible modes of tolerance would be exudation of the parent quinclorac out of the root tissue as observed with tolerant species such as rice and Kentucky bluegrass. However, as observed with large crabgrass, only very small trace amounts ^{14}C - quinclorac were measured in the nutrient solution in the goosegrass study (data not shown).

The differences in absorption and translocation may be minor factors at best in explaining the magnitude of difference in sensitivity between the species that was determined in the previous GR_{50} studies (Chapter 2).

^{14} C - Metabolism :

Results of the comparative metabolism study are presented in Figures 4, 5, and 6. The results of the reverse phase HPLC showed that for the 80 hr exposure time, there was no apparent metabolism of the parent quinclorac in the leaf tissue for the sensitive species, large crabgrass and the tolerant species, goosegrass. The scale for the HPLC chromatogram was lower for goosegrass than the large crabgrass due to the higher % of ^{14} C- quinclorac that was translocated out of the treated leaf. For each species, only one peak with a retention time of approximately 28 minutes was detected. The retention time for this peak matched that of the standard ^{14}C- quinclorac (Fig. 4).

Metabolism work conducted by Chism (5) using southern crabgrass detected a water soluble metabolite using Thin Layer Chromatography (TLC) techniques. However, the amount of this metabolite was only 2.8% of the total. Berghaus and Wuerzer (2) and Grossmann (9) reported that quinclorac was metabolized at a moderate rate. At 24 hr, 5 to 10% of the absorbed quinclorac was transformed into a polar metabolite. No qualitative or quantitative differences between metabolism in the root and shoot tissues were observed (7,9). Since there was no apparent metabolism of the ^{14} C - quinclorac by goosegrass, this suggested that there must be another factor or group of factors that convey tolerance to quinclorac.

Spray Retention Studies :

Results presented in Table 1 describe the comparison of the amount of quinclorac retained both on a dry weight and leaf area basis. Expressed either way, the results showed that goosegrass retained significantly more quinclorac than large crabgrass. This may be in part due to differences in leaf morphology and cuticular makeup of the two species. The leaf blade and sheaths of large crabgrass tend to have a considerable amount of pubescence vs. goosegrass (14). Pubescence has been shown to affect spray retention (1,11,15). Spray droplets may be repelled off the leaf surface by these leaf hairs or they may impede the spreadibility of the spray solution on the surface of the leaf. The very smooth leaf blade of goosegrass also suggests that the cuticular layer may be different in its composition of waxes, etc (15).

The results of this retention study along with the findings of the GR_{50} studies (Chapter 2) suggested that the more sensitive species (large crabgrass) retained less applied quinclorac than the tolerant goosegrass. Based on these data, one must reject the hypothesis that a tolerance mechanism exhibited by goosegrass was the ability to retain less quinclorac than a sensitive species such as large crabgrass. These data also suggested that just measuring spray retention may not necessarily correlate with herbicidal efficacy.

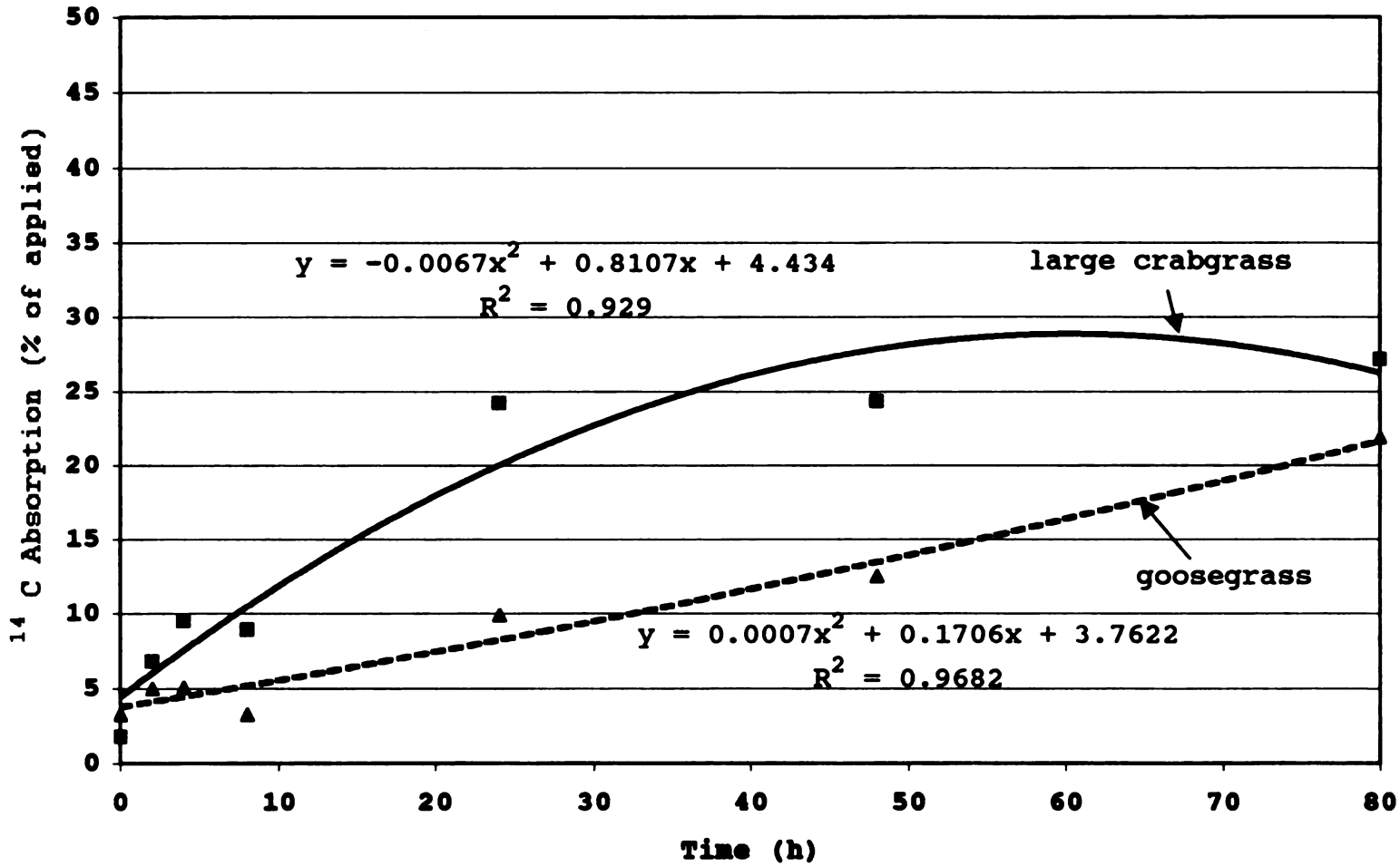

Figure 1. Comparative absorption of ^{14}C-quinclorac treated large crabgrass and goosegrass leaves as influenced by time.

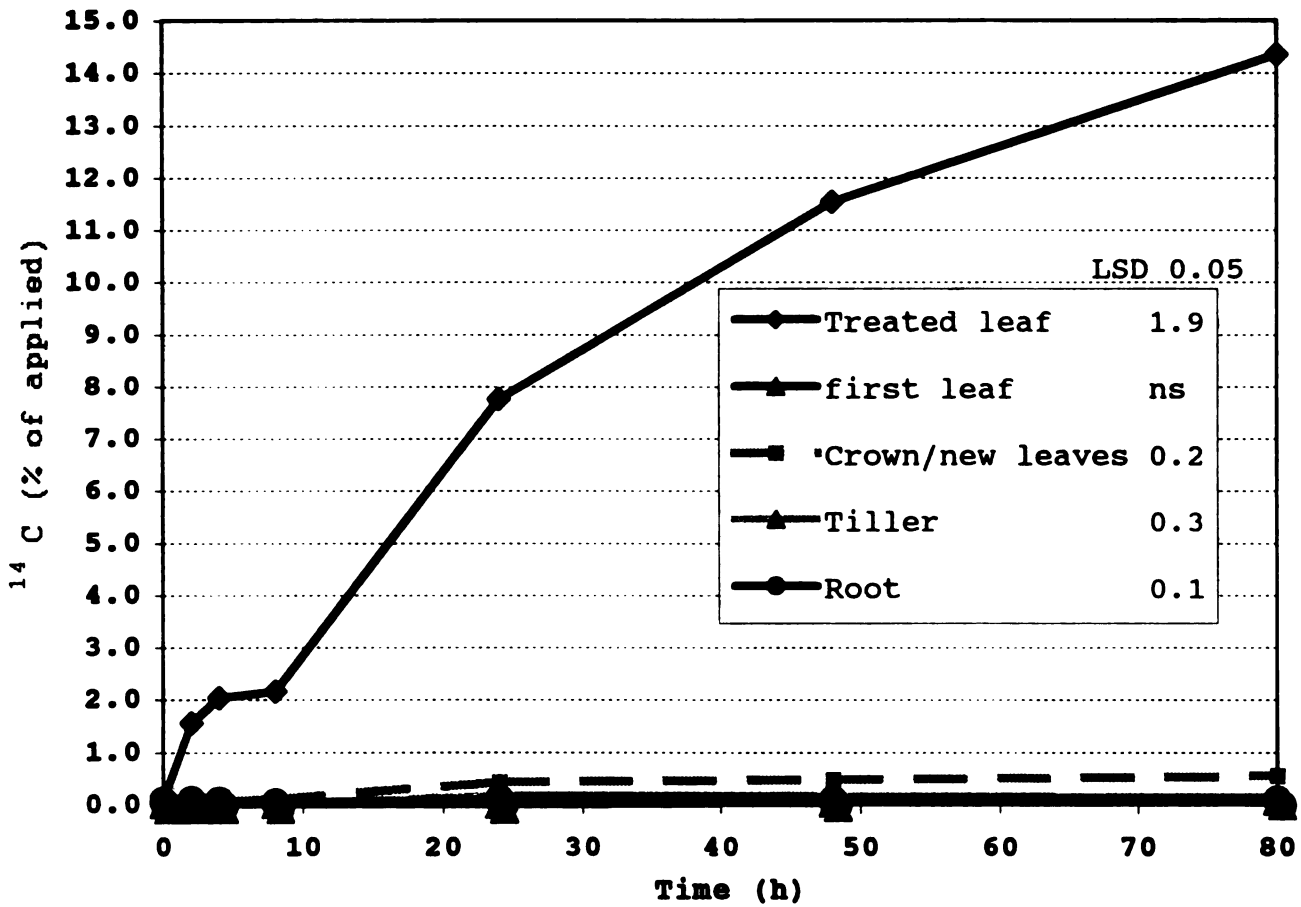

Figure 2. Distribution of ^{14}C-quinclorac in large crabgrass plant parts as influenced by time of absorption.

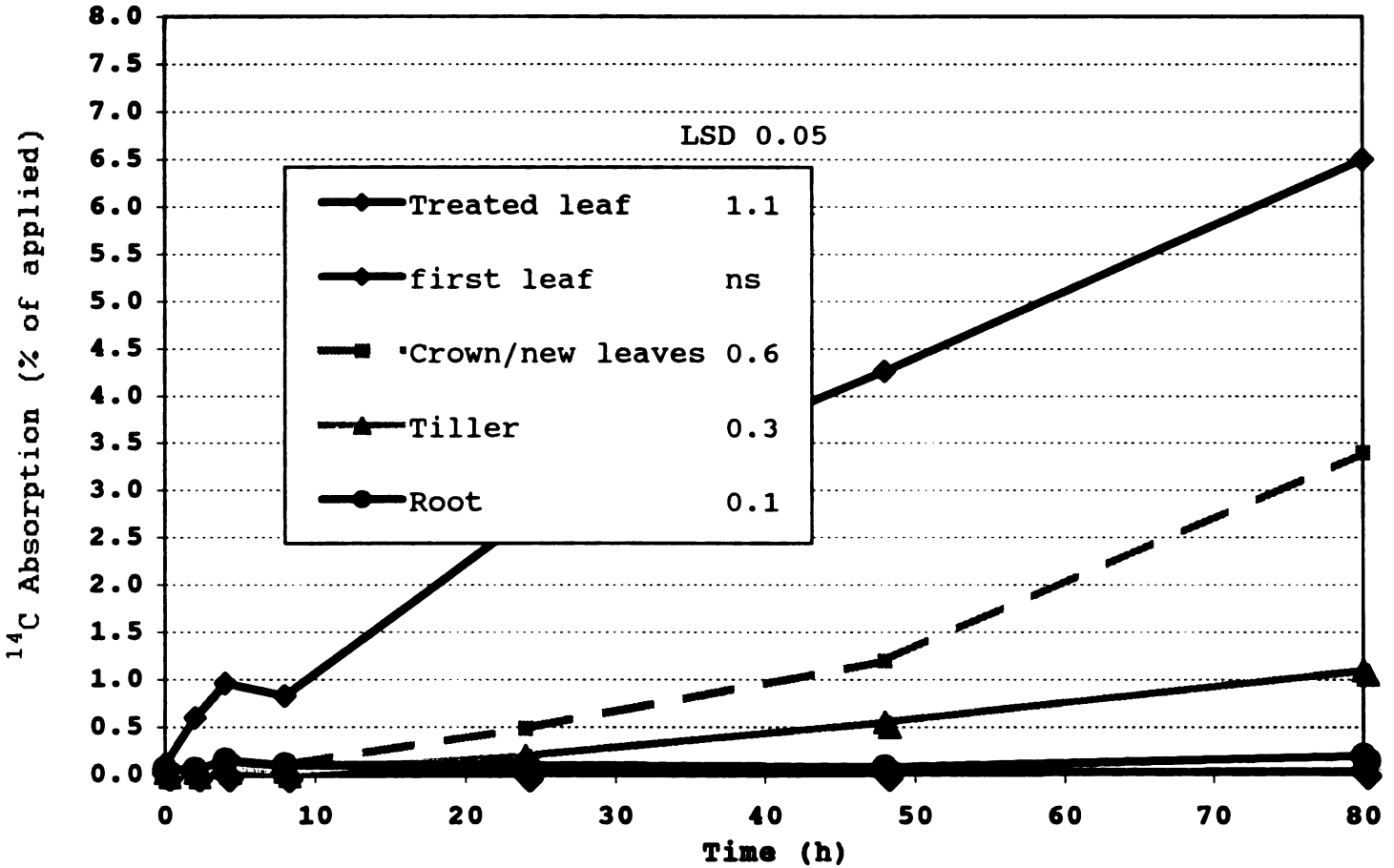

Figure 3. Distribution of ^{14}C -quinclorac in goosegrass plant parts as influenced by time of absorption.

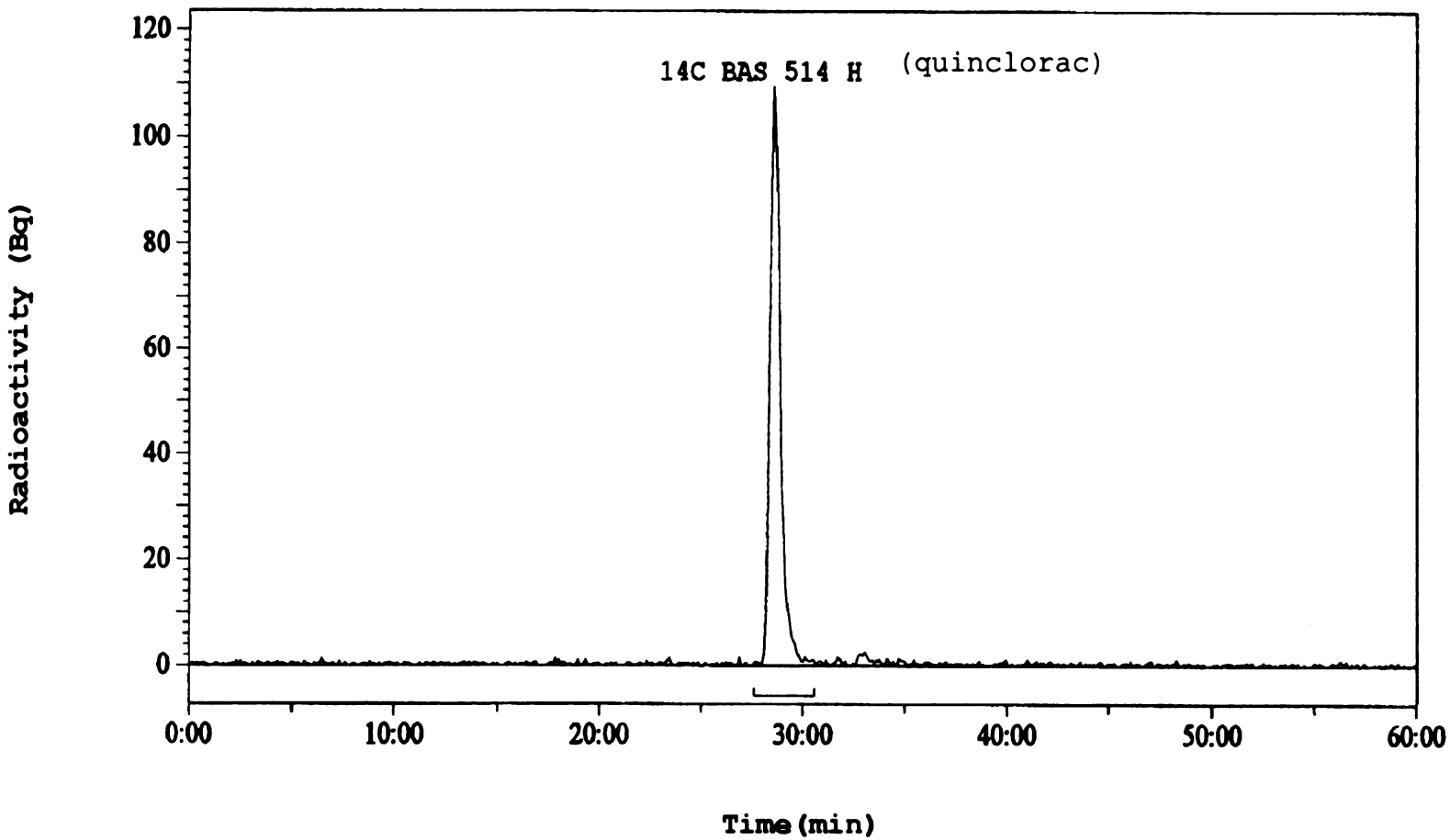

Figure 4. Reverse phase HPLC Chromatogram of ^{14}C-quinclorac standard

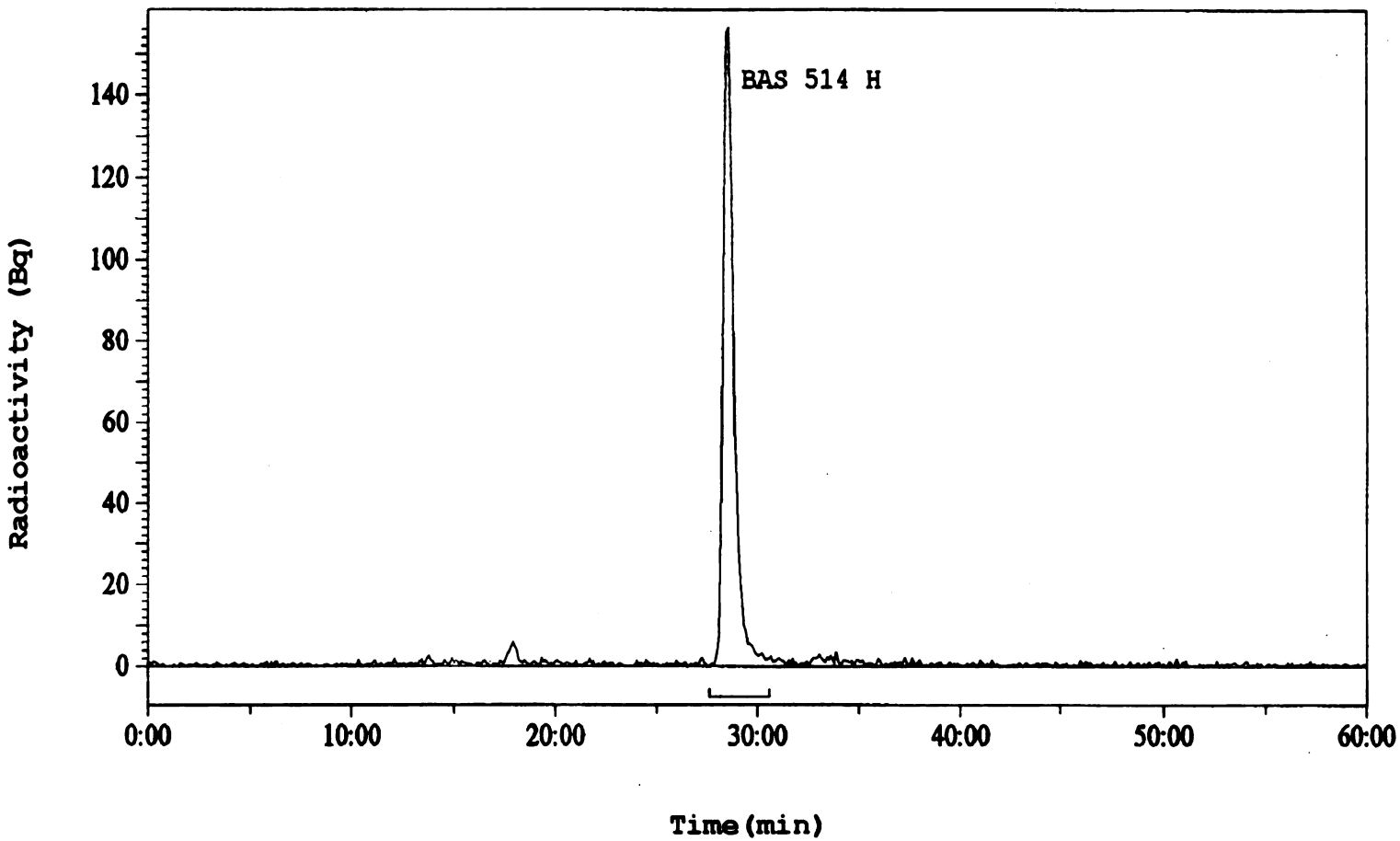

Figure 5. Reverse phase HPLC Chromatogram of ^{14}C-quinclorac treated large crabgrass 80 hr after treatment.

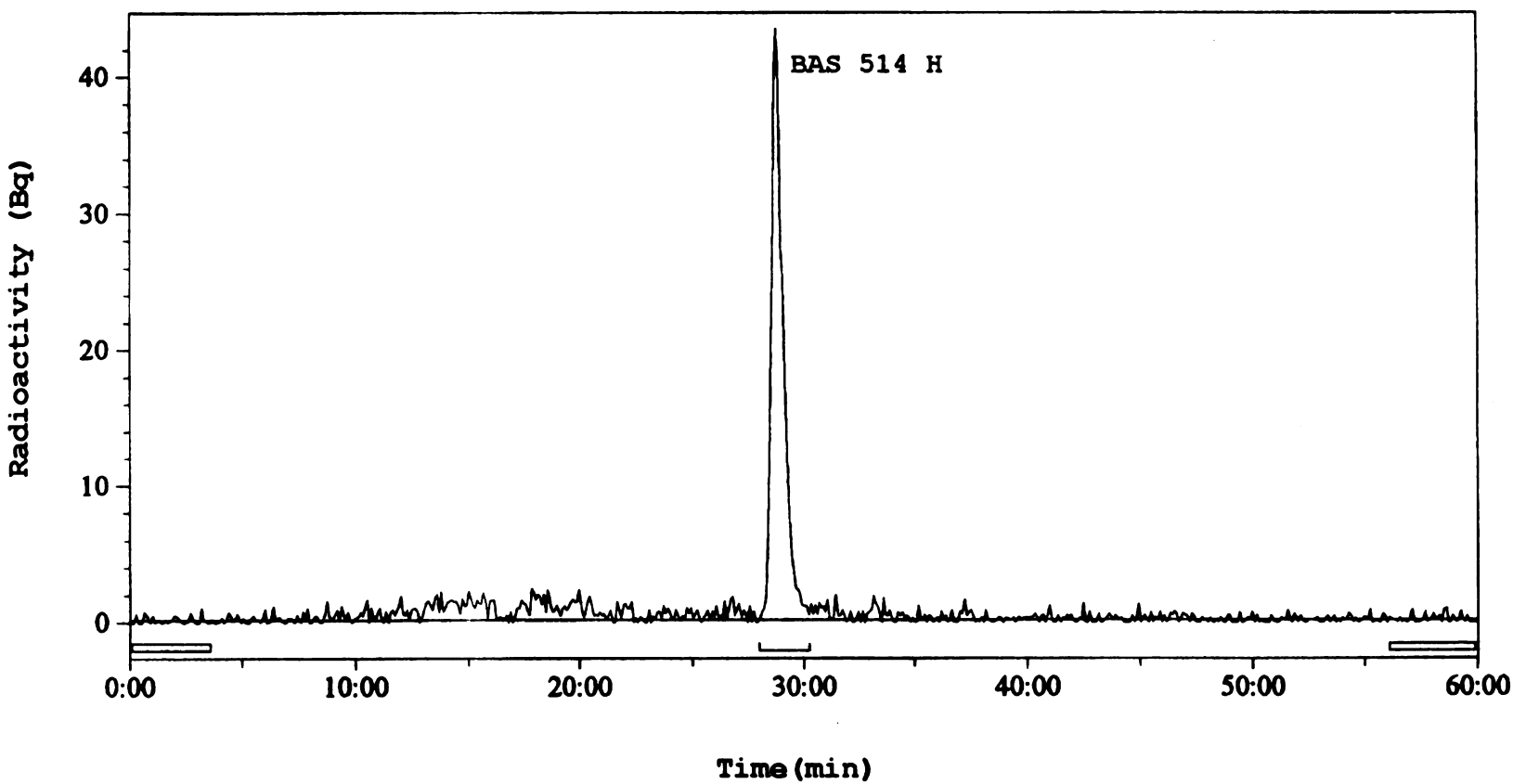

Figure 6. Reverse phase HPLC Chromatogram of ^{14}C-quinclorac treated goosegrass 80 hr after treatment.

Table 1.Retention of foliarly applied quinclorac[1] by large crabgrass and goosegrass measured immediately after application.

Species	quinclorac retained	
	dry wt. (μg/mg)	leaf area (μg/cm$^{2)}$
large crabgrass	0.27	0.87
goosegrass	0.45	1.21
LSD (0.05)	0.13	0.26

[1] quinclorac plus "Merge" spray adjuvant included at 0.56 kg ha^{-1} and 1% v/v, respectively

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CHAPTER FOUR

DETACHED SHOOT AND SITE OF ACTION STUDIES

ABSTRACT

Intact plants of large crabgrass and goosegrass were treated with 0.56 kg ha $^{-1}$ of quinclorac and 1% v/v of "Merge" spray adjuvant. Immediately after the spray had dried, plant shoots were excised at the soil surface and placed in vials containing nutrient solution. Plants were maintained under greenhouse conditions. Six days after treatment, shoot fresh weights and visual injury ratings were recorded. Injury response of large crabgrass was similar to that observed with treatment to intact plants (in previous studies) with 94% visual injury and a fresh weight reduction of 65%. Response of goosegrass was different than that observed with intact plants. Visual injury was 75% with a fresh weight reduction of 25%. Very little effects were noted at this evaluated rate (and higher) in previous work conducted with intact plants.

Additional work was conducted evaluating the response of both species to applied 1-aminocyclopropane -1-carboxylic acid (ACC). The stimulation of ACC synthase has been a proposed main mechanism of action of quinclorac.

The subsequent oxidation of ACC leads to the production of ethylene and hydrogen cyanide (HCN) in stoichimetrically equivalent amounts. The formation of HCN has been proposed to be the lethal agent resulting from applications of quinclorac in sensitive species. Exposure over a six day period to root applied ACC at 10mM to intact large crabgrass plants showed similar visual response to that of foliar applied quinclorac. No visual effects were noted to goosegrass. Results support the proposed model for the mode of action with quinclorac in the case of large crabgrass. Results of the detached shoot studies with goosegrass suggested that translocation may play a vital role in the detoxification of quinclorac. The lack of goosegrass response to applied ACC suggested that goosegrass may have a higher tolerance level for HCN, or may possess more efficient detoxifying mechanisms.

INTRODUCTION

Large crabgrass and goosegrass differ in their tolerance to quinclorac herbicide. Large crabgrass has been found to be sensitive, while goosegrass has been found to be quite tolerant. Investigations into differences in spray retention, absorption, and metabolism failed to reveal the actual mode of differential tolerance (Chapter 3). ^{14}C translocation studies did show that goosegrass translocated more ^{14}C quinclorac out of the treated leaf than large crabgrass. The main deposition sites of transported ^{14}C - quinclorac were the tillers and the crown and new leaf tissue. Differences in translocation are possible mechanisms for observed differences in response of weed species to herbicides (3,8,10). Additionally, results from the previous studies of this project (Chapter3) suggested that the differential tolerance between large crabgrass and goosegrass may involve physiological differences at the site of action.

Several papers have dealt with the investigation of the mode of action of quinclorac (5,13,14,15,16,17,18,19,22). The leading theory today has been proposed by Grossmann *et al.*(13,14,15,16) which strongly suggested that the synthesis of ACC (1-aminocyclopropane-1-carboxylic acid) and its subsequent oxidation into ethylene and cyanide is the key mechanism for the response observed with applied quinclorac.

Based on this theory, one could postulate that along this chain of reactions, the effect of quinclorac in large crabgrass is different than in goosegrass. Fig. 1 outlines the entire range of the major reactions that involve ACC and its subsequent oxidation and fate of co-products. One could speculate that, in goosegrass, quinclorac does not induce ACC synthase, thereby not allowing for the accumulation of ACC and subsequent oxidation to HCN. Alternatively, the induction may occur and ACC is formed and oxidized to ethylene and HCN as in large crabgrass. However, it may be that the activity and/or endogenous concentration of *ß*-cyanoalanine synthase (the major detoxifying enzyme for HCN) is higher in goosegrass than large crabgrass.

Another possible explanation of the tolerance exhibited by goosegrass may entail the alternate pathway of the metabolism of ACC to MACC as describe by Peiser *et al.* (26). If in goosegrass this mechanism is favored over the oxidation to ethylene and HCN, the accumulation of free HCN would be avoided along with its subsequent toxic effects. One also has to speculate on the role of the endogenous levels and synthesis formation of cysteine within the plant. Since this amino acid is the key substrate that is needed to trap the free HCN, its concentration within the plant would affect the efficacy of *ß*-cyanoalanine synthase and the capacity to trap the free cyanide.

The objectives of these studies were to investigate the response of detached shoots of large crabgrass and goosegrass to applied quinclorac and to evaluate the response of both species to applied ACC. A major assumption in this experiment was that some of the ACC would be converted to free HCN in the plant causing the phytotoxic effects.

METHODS AND MATERIALS

Detached Shoot Studies :

Both large crabgrass and goosegrass plants were cultured as previously described (Chapter 2). Quinclorac was applied at 0.56 kg ai ha^{-1} when plants reached the one to two-tiller stage. "Merge" spray adjuvant was also added at a 1% (v/v) of the spray volume. Spray applications were made with an overhead track sprayer set to deliver 748 l ha^{-1} at an operating pressure of 275 kPa using an 8004 even flat fan nozzle. Immediately after the spray dried, plants were excised at the soil surface and transferred into amber vials (100 ml) that contained 70 ml of a 0.1X Hoagland nutrient solution. Plants were supported in the vials by loosely fastening to plastic support stakes. Plants were maintained under incandescent lighting and temperature was maintained at 24 ^{0}C for the duration of the experiment. Visual injury ratings were taken at 2, 3 and 6 days after treatment. Plant fresh weights were also measured at 6 days after treatment.

Data Analysis.

The experiment was conducted twice and consisted of 4 replications (one plant per replication) and was arranged as a Completely Randomized Design.

Data were subjected to analysis of variance and means separated using Fisher's Protected LSD at α =0.05. Data were combined across experiments since no experimental interactions were detected.

Site of Action Studies :

Large crabgrass and goosegrass plants were seeded and cultured as described in the translocation and absorption studies. A 10 mM stock solution of ACC was prepared using millipore water. The ACC rate selected was based on work conducted by Yip and Yang (35) with mungbean.
After removal of soil in the water bath, plants of both species were transferred into 15 ml centrifuge tubes containing either 10 mM ACC solution or millipore water. Plants were supported by means of a foam sleeve. No nutrient solution was introduced as to the unknown nature of possible interaction/ degradation that may occur with ACC. The experiment consisted of three treatments : untreated (millipore water), ACC, and foliar applied quinclorac at 0.56 kg ha^{-1}.

The quinclorac was only applied to plants immersed in millipore water. Quinclorac was applied with "Merge" adjuvant at 1% v/v using the spray chamber setup that was previously described in other sections (Chapter 2). Tubes were kept under greenhouse conditions as previously described (Chapter 2). Since no aeration was available, plants were supported with a portion of the root tissue above the solution level in the tubes. Tubes were checked each day and maintained at a constant volume with either millipore water or ACC.

To aid in aeration, as new solution was added, the entire volume of each tube was carefully removed momentarily by syringe. As the solution was reentered, air bubbles were introduced into the tubes via the syringe. At five days after treatment photographs of each treatment were taken.

RESULTS AND DISCUSSION

Detached Shoot Studies :

Results of the detached shoot studies are presented in Table 1 and Figures 1 & 2. The data showed that phytotoxicity was evident in both species at the 0.56 kg ha^{-1} rate. Phytotoxicity was higher for large crabgrass than goosegrass evaluated either on a visual or fresh weight basis. These results concur with previous studies that showed that large crabgrass was more sensitive to quinclorac than goosegrass. However, the difference in these detached shoot studies was the degree of injury to goosegrass. Very little injury was ever observed in studies on intact goosegrass plants treated with quinclorac within prospective labeled rates (22). The detached shoots were considerably more sensitive than effects observed on intact plants. These results suggested that confinement of quinclorac to the treated goosegrass shoots may have an impact on the tolerance mechanism to quinclorac. The response observed with goosegrass may somehow be related to stress-induced ethylene production as described by Yang and Hoffman (32). Yang and Hoffman suggested that stress induced ethylene can be caused by factors such as wounding, cutting, chilling, etc.

The other observation was that the effect on large crabgrass we observed was in contrast with work conducted by Grossmann *et al.* (14) on another sensitive grass species, barnyardgrass. Detached shoots of barnyardgrass were found to be very tolerant to applied quinclorac. These observed differences may just be species specific or may have something to do with application techniques. In Grossmann's studies, the detached shoots were not treated with a conventional foliar spray, but rather exposed to solution concentrations of quinclorac in reagent tubes (14).

Site of action studies :

Results of applied ACC are presented in Figures 2 and 3. Exposure to ACC caused similar phytotoxic effects to large crabgrass as observed with quinclorac (Fig. 2). However, results showed that there was little observable effect of either ACC or the applied quinclorac to the goosegrass plants (Fig. 3). In the case of large crabgrass, these observations supported Grossmann's proposed model on the role of ACC and the mode of action of quinclorac (13). In the case of resistant grasses, Grossmann proposed that ACC synthase is not stimulated in the root and therefore, the subsequent effects of resultant HCN are not produced (13). One may speculate that since we observed little effect of the applied ACC to goosegrass, and one assumes that absorption occurred, there may be other mechanisms that resistant plants employ to avoid toxicity to quinclorac.

Table 1. Herbicidal effects of foliar applied quinclorac [1] to detached shoots of large crabgrass and goosegrass.

Species	Rate	Visual Injury	Fresh Weight
	kg ha^{-1}	% control	% of untreated
Lg. Crabgrass	0	0	100
	0.56	94	35
Goosegrass	0	0	100
	0.56	75	70
LSD (0.05)		6	13

[1] quinclorac applied @ 0.56 kg ha^{-1} plus "Merge" @ 1% v/v

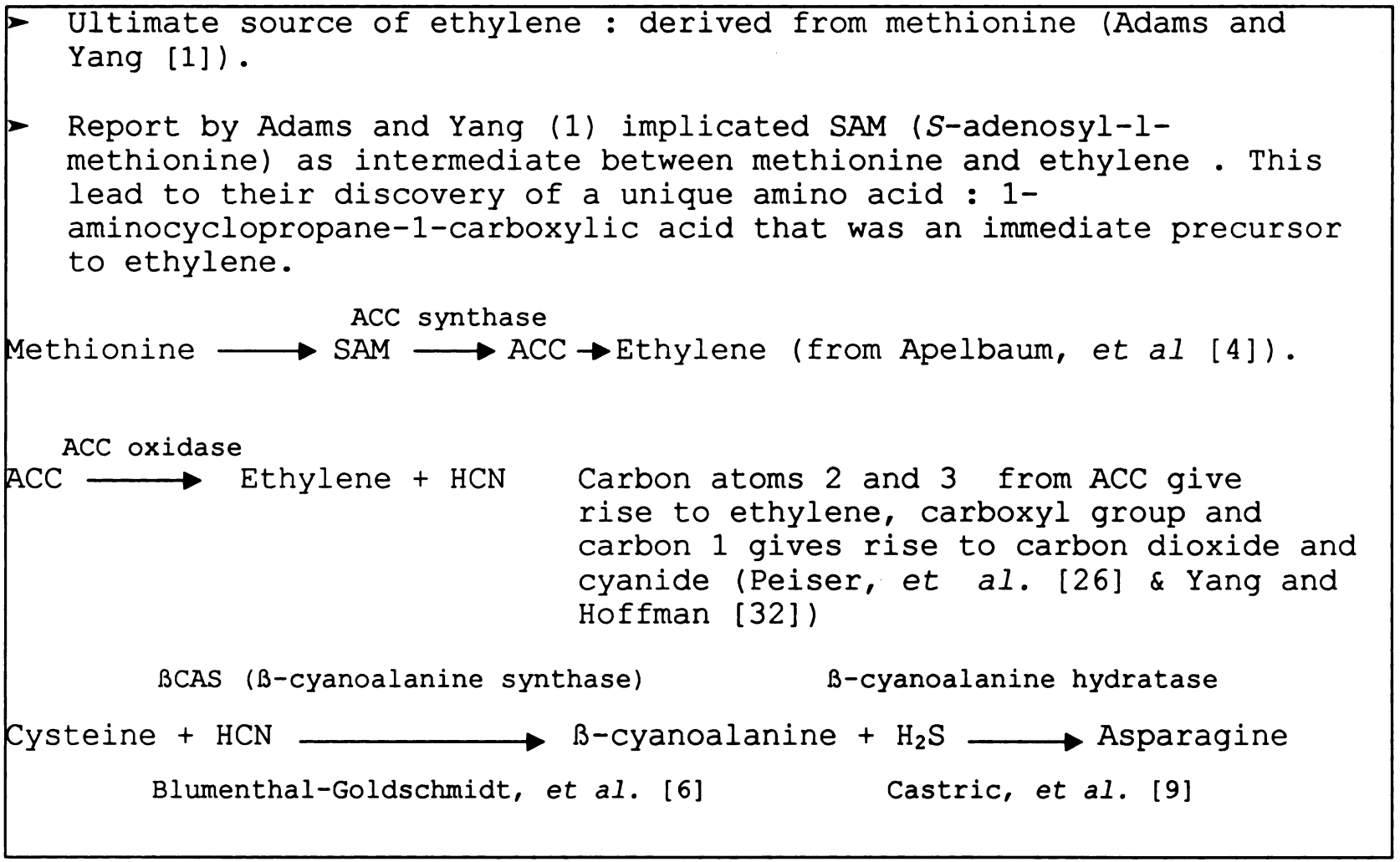

Figure 1. Schematic overview of the fate of cyanide derived from 1-aminocyccloпropane -1-carboxylic acid (ACC).

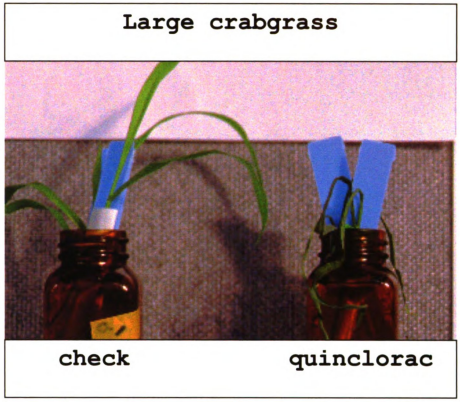

Figure 2. Influence of applied quinclorac at 0.56 kg ha^{-1} to detached shoot tissue of large crabgrass.

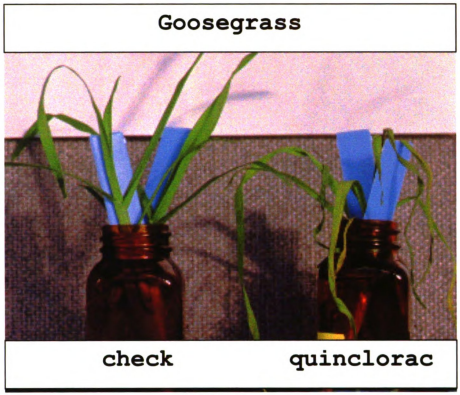

Figure 3. Influence of applied quinclorac at 0.56 kg ha^{-1} to detached shoot tissue of goosegrass.

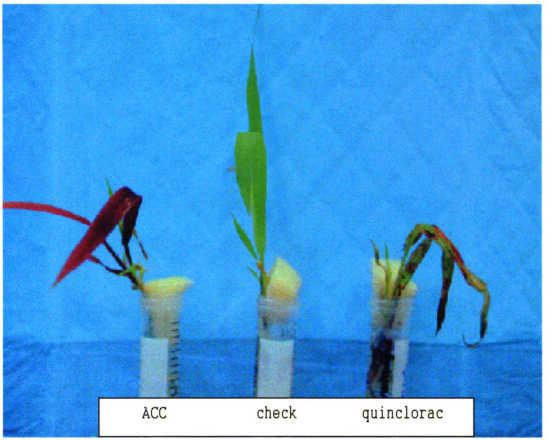

Figure 4. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) at 10mM and quinclorac at 0.56 kg ha^{-1} to large crabgrass.

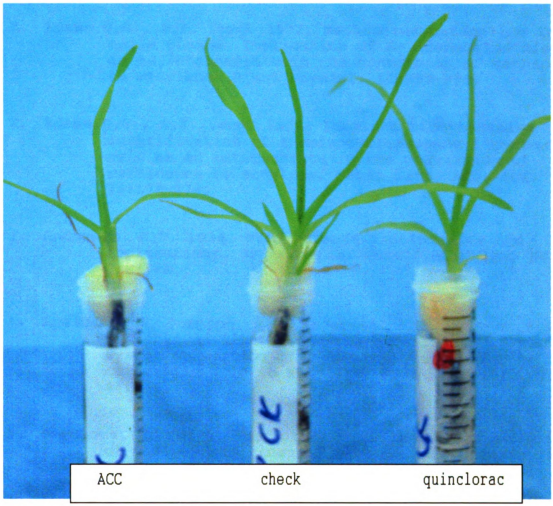

Figure 5. Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) at 10mM and quinclorac at 0.56 kg ha^{-1} to goosegrass.

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CHAPTER FIVE

SUMMARY AND CONCLUSIONS

The results of these studies showed that quinclorac herbicide has some very unique properties. It has not as yet been classified into a current, particular herbicide action or structural group. It is considered to belong to a new class of highly specific auxin-like herbicides (4). Quinclorac causes auxin-like symptomology in susceptible broadleaf species, but also causes chlorosis and necrosis in sensitive grass species (4). The findings of this research project reconfirmed these effects on sensitive broadleaf species such as cleavers and sensitive grasses such as large crabgrass.

Results of the adjuvant studies showed that species differed in their sensitivity to the herbicide and the use of quinclorac required the use of an effective adjuvant. As far as ranking of sensitivity to quinclorac the findings suggested that cleavers > annual sowthistle > large crabgrass > goosegrass. Across evaluated species, little difference was observed on the effectiveness of the evaluated adjuvants. The only noted exception where an adjuvant failed to provide comparable control with other materials tested, was in the case of Sylgard 309 applied to large crabgrass.

Goosegrass was found to be very tolerant to quinclorac regardless of adjuvant or stage of growth treated. The stage of growth studies did show that the one to two-leaf stage was the most sensitive stage to quinclorac. However, even at the one to two-leaf stage, the GR_{50} value was well above the labeled rates. The goosegrass studies also suggested that root uptake was a key component in the performance of quinclorac.

The ^{14}C absorption studies showed that after 80 hr of exposure, both large crabgrass and goosegrass absorbed over 20% of the applied herbicide. These data suggested that the quantity of quinclorac absorbed was not a causal factor in the difference observed in sensitivity. The translocation results showed that in both weed species, most of the applied ^{14}C-quinclorac remained in the treated leaf; however, the amount translocated out of the leaf was greater with goosegrass than large crabgrass. The translocation of ^{14}C-quinclorac was primarily into the meristematic regions of tillers and the crown and new leaf tissue. Dilution of herbicides by plants has been suggested as a mechanism used by plants to avoid or reduce phytotoxic effects from herbicides (3). Results also showed that goosegrass did not exudate any appreciable amount of ^{14} C-quinclorac which had been found to be a key tolerance mechanism in species such as rice (1) and Kentucky bluegrass (3).

Spray retention results showed that goosegrass actually retained more herbicide than large crabgrass.

These results ruled out the role of retention as a possible mechanism of differential tolerance of the two species.

The detached shoot study results suggested that some modifications in the leading proposed models of the mode of action of quinclorac may be necessary. In these tests, we were able to induce the phytotoxic effects of quinclorac to both large crabgrass and goosegrass. Grossmann's model suggested that quinclorac stimulates ACC synthase in root tissue and the subsequent ACC is then transported to the shoot (4,5,6,7). Since the roots were excised immediately after application, how then were the phytotoxic effects induced? Another question posed was why were we able to induce an effect to the shoots of a sensitive species (large crabgrass) and Grossmann was unable in his evaluations with sensitive barnyardgrass? Our results with detached large crabgrass plants suggested that ACC synthase may also be stimulated in leaf tissue as well.

The results observed with applied ACC suggested that one could mimic the effects of quinclorac to large crabgrass. This observation supported the proposed model put forth by Grossmann on the mode of action (4). However, the lack of observable response in goosegrass suggested that tolerant species may employ other physiological pathways to avoid the phytotoxic effects of quinclorac. Also, the endogenous concentration of ACC may have to be much higher in goosegrass for the reaction sequence to ethylene and HCN to be triggered.

In goosegrass, one could also speculate that if ACC sythase is stimulated and subsequent ACC produced, the plant employs other metabolic pathways for ACC, other than to ethylene and HCN. Conversion to MACC as described by Peiser *et al.* (8) may be a main alternate pathway.

The results of this research suggested that further work needs to be conducted to ascertain the specific mechanisms that goosegrass employs to avoid the phytotoxic effects of quinclorac. Suggested areas of investigation include: 1). Evaluate the effects of applied KCN to see as with Grossmann's work on large crabgrass (4), if one can induce symptomology? It is possible that goosegrass can tolerate higher free levels of HCN or has a very efficient ß-cyanalanine synthase to detoxify HCN, 2). Evaluate the effects of inhibitors of ß-cyanalanine synthase that are discussed in the literature and observe the effects of applied quinclorac to goosegrass (8,9) and 3). As an indirect effect, one should evaluate the endogenous concentrations and species capacity to synthesize the amino acid cysteine, which is the main substrate to which ß-cyanalanine synthase acts to capture free HCN (2).

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