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.

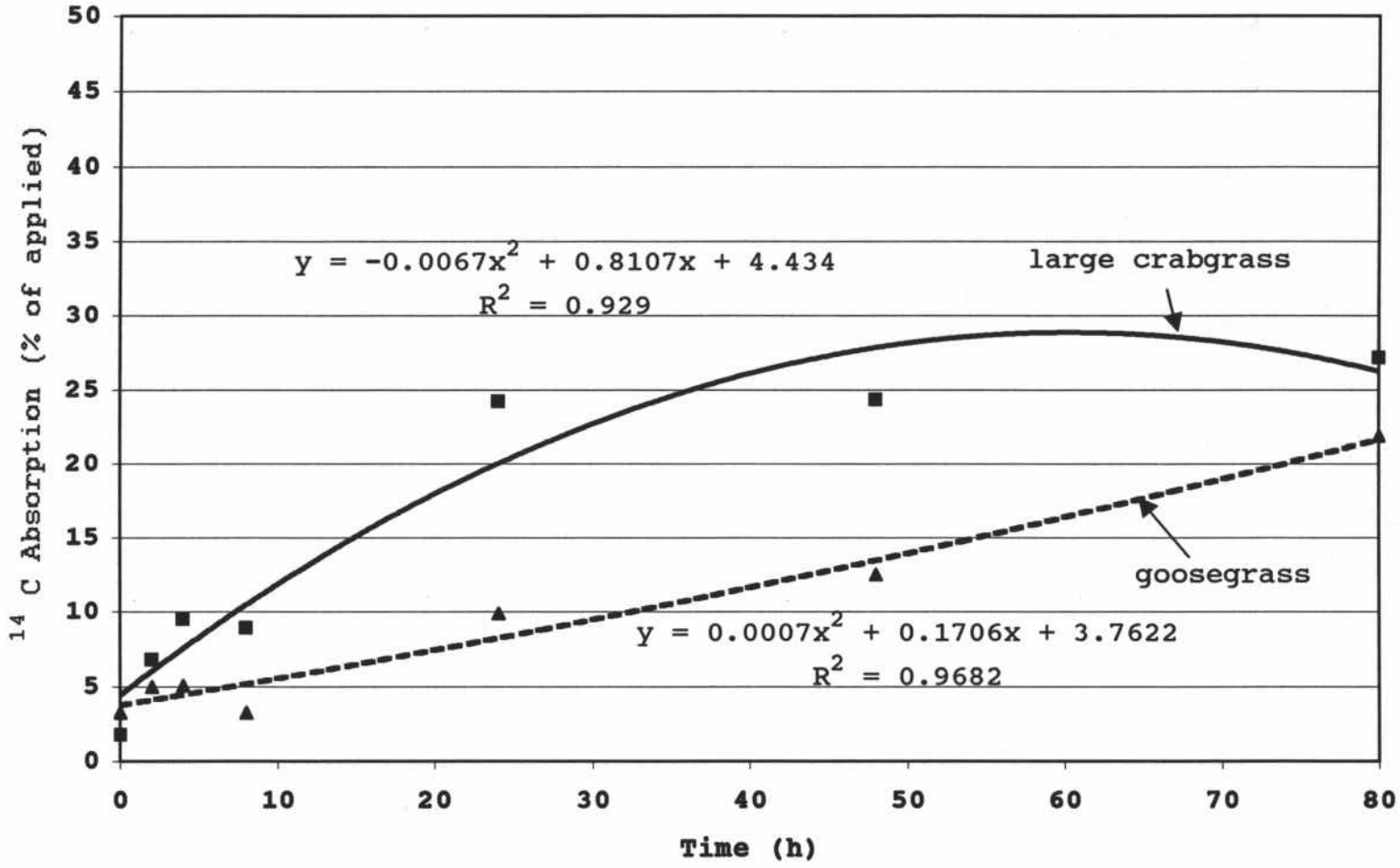

Figure 1. Comparative absorption of ^{14}C-quinclorac treated large crabgrass and goosegrass leaves as influenced by time.

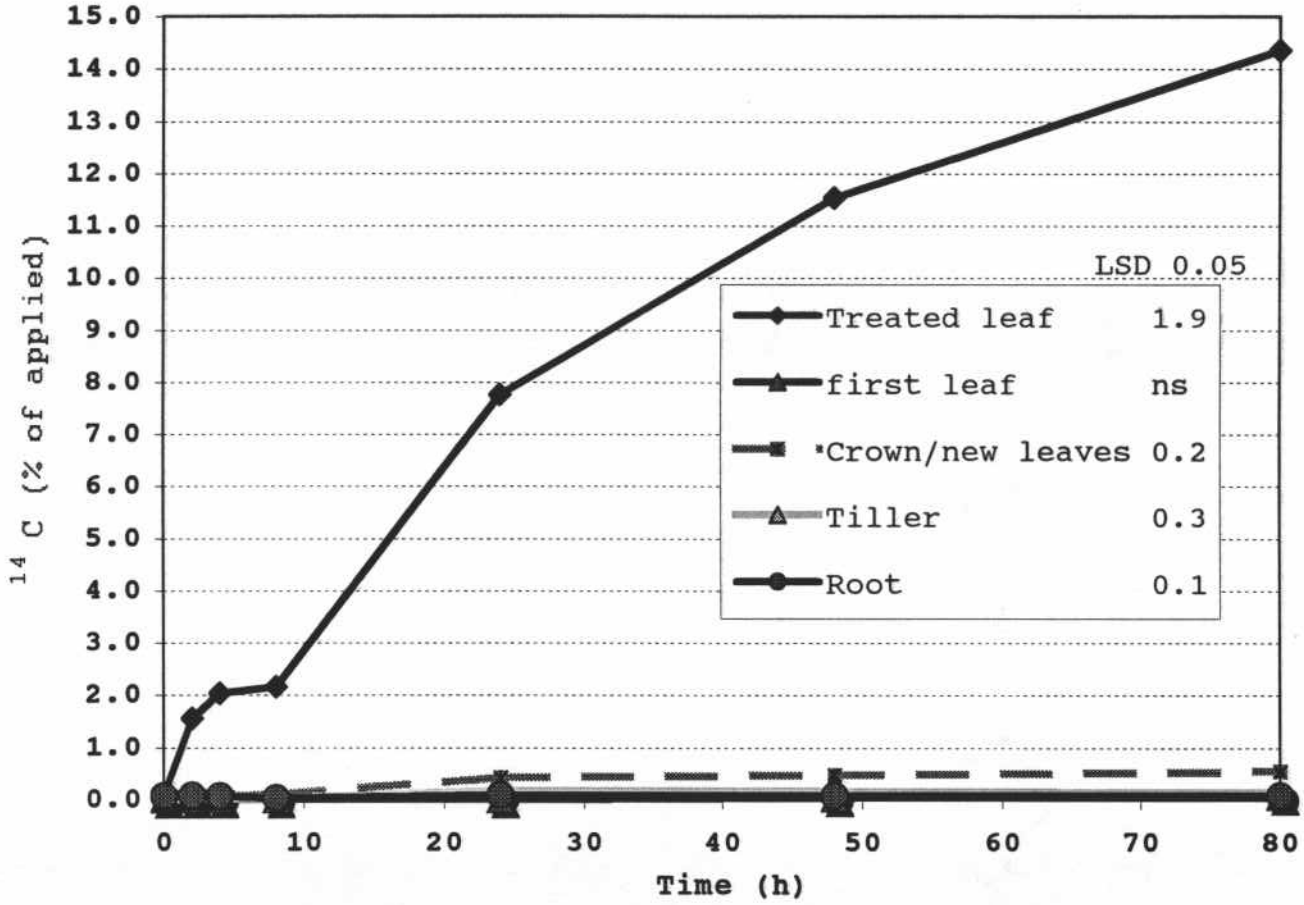

Figure 2. Distribution of ^{14}C-quinclorac in large crabgrass plant parts as influenced by time of absorption.

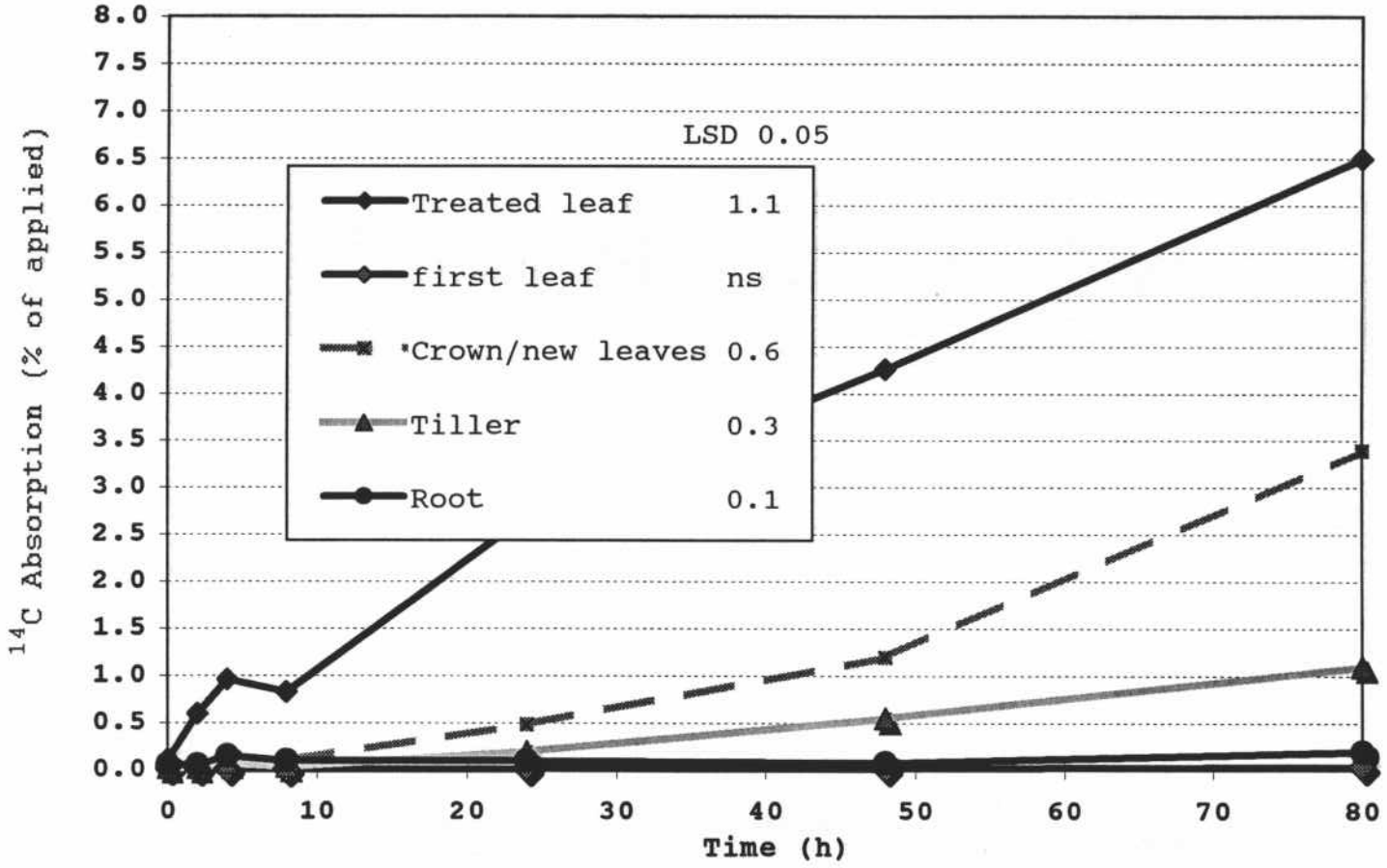

Figure 3. Distribution of ^{14}C -quinclorac in goosegrass plant parts as influenced by time of absorption.

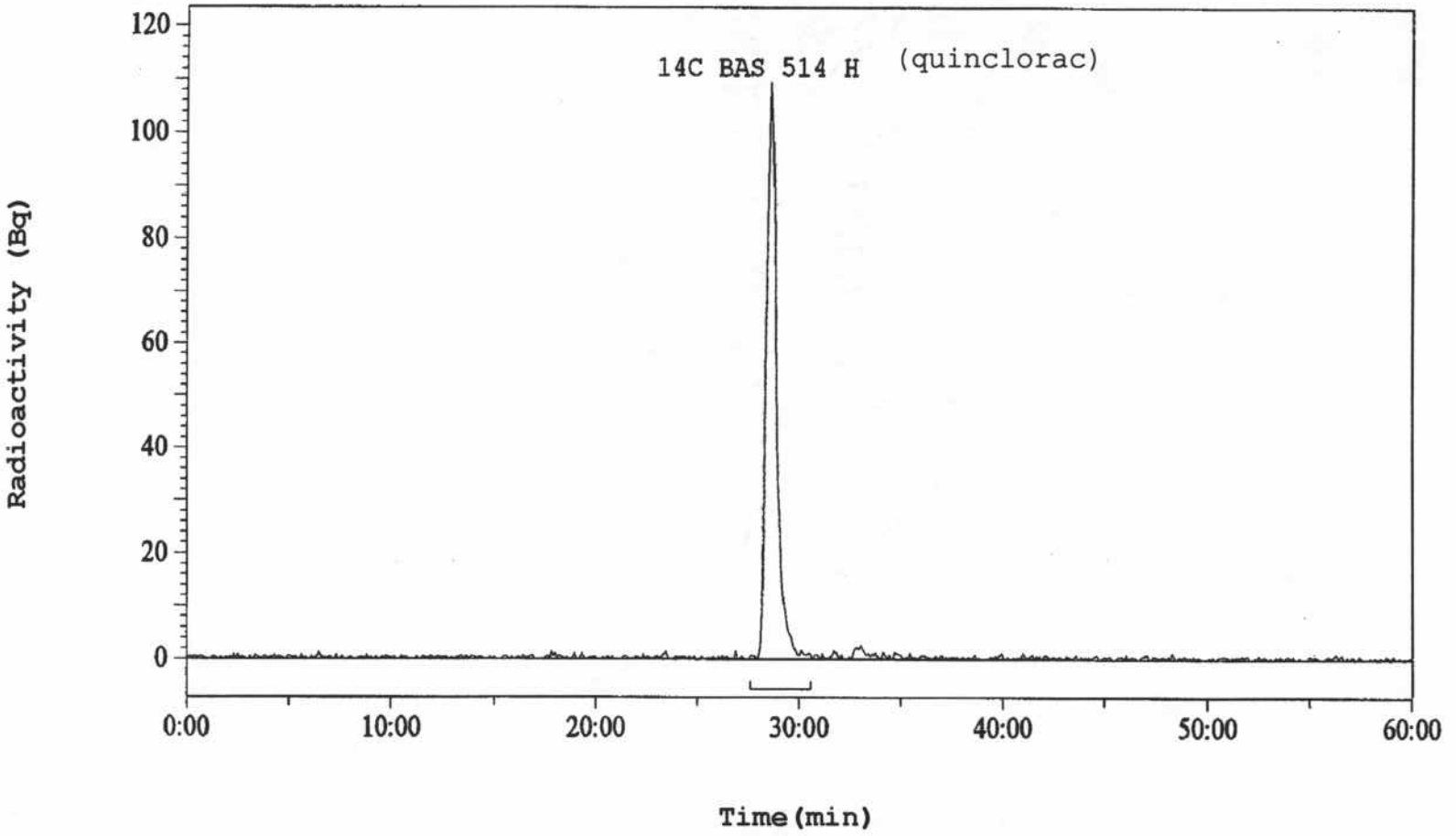

Figure 4. Reverse phase HPLC Chromatogram of ^{14}C-quinclorac standard

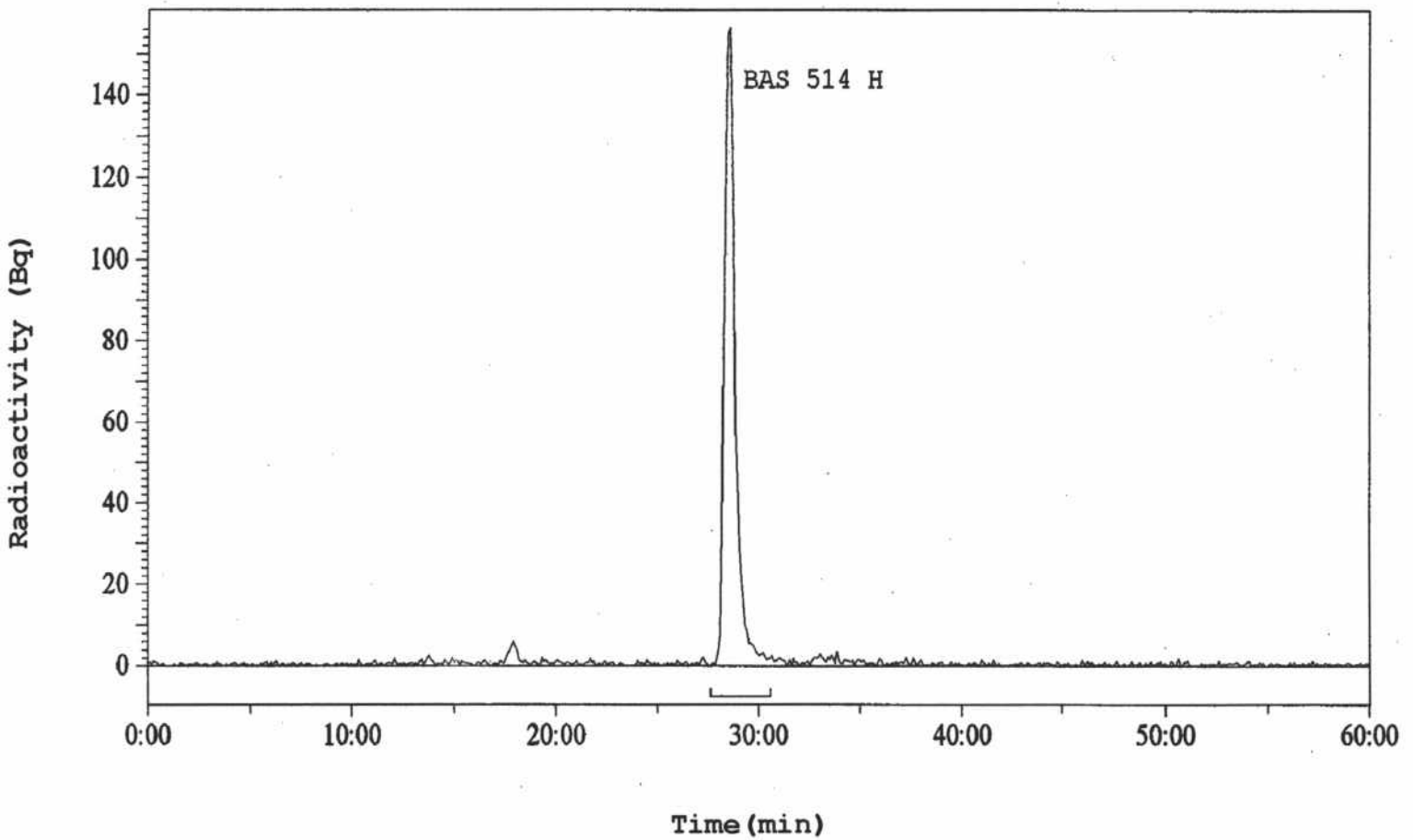

Figure 5. Reverse phase HPLC Chromatogram of ^{14}C-quinclorac treated large crabgrass 80 hr after treatment.

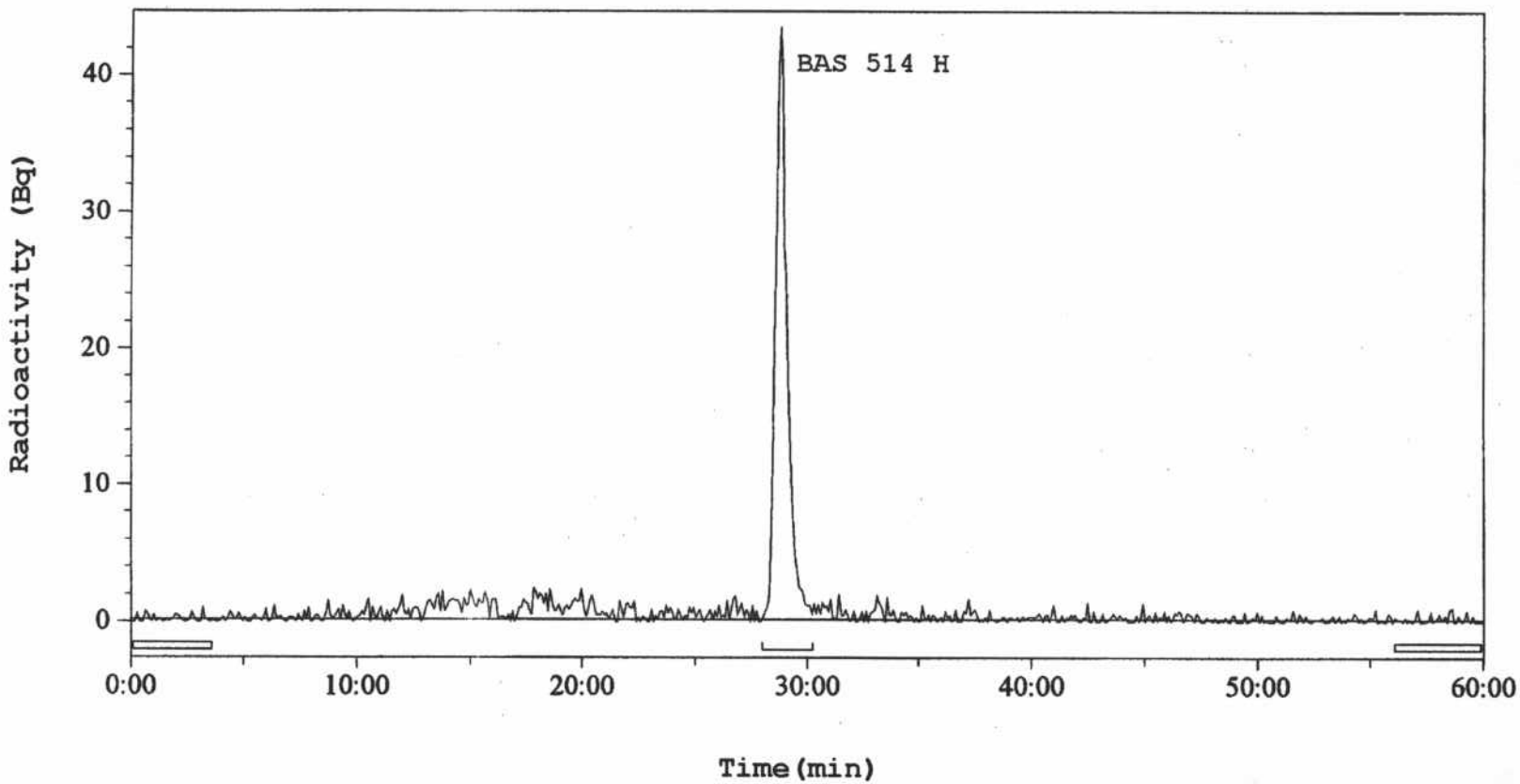

Figure 6. Reverse phase HPLC Chromatogram of ^{14}C-quinclorac treated goosegrass 80 hr after treatment.

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

Species	quinclorac retained	
	dry wt. ($\mu g/mg$)	leaf area ($\mu g/cm^2$)
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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