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Quinclorac ester toxicity and metabolism in leafy spurge (*Euphorbia esula*) soil systems: Effects of foliar vs. soil applications[1]

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Abstract:

Quinclorac and 13 synthesized esters were monitored for toxicity in foliar- and soil-treated leafy spurge plants. Foliar treatment at 0.5 μmol/plant with quinclorac resulted with 100% mortality, whereas treatment with esters at 2 μmol/plant showed initial toxicity symptoms, but the plants recovered with time (24 weeks). Foliar toxicity symptoms and mortality increased throughout 24 weeks after soil treatment at 0.4 [μmol/ plant with the higher MW esters (C5-C16); however, toxicity symptoms decreased and plants recovered after quinclorac or low MW ester treatments. [^{14}C] Quinclorac esters also were monitored to ascertain if toxicity data could be related to ester mobility and/or metabolism. Foliar application showed volatile losses with the low MW esters; complete metabolism of esters occurred inside the treated leaves by 7 days, but little or no quinclorac was observed as a result; and little radiolabel migrated from the leaves. Soil application showed that the esters were metabolized by soil microorganisms through a series of ω- and β-oxidations and not by simple ester hydrolysis. These intermediate metabolites were identified by MS and NMR analyses. The C6 and C8 esters were metabolized to the quinclorac ester of 4-hydroxybutryic acid as a major intermediate in soil before conversion to quinclorac. The rate of quinclorac release from the various esters was dependent on the number of odd vs. even carbons in the ester side chain; the C7, C5, and C8 esters were metabolized to quinclorac at relative rates of 16, 3.2, and 1, respectively. Quinclorac acid was leached readily from soil with water, but the C8 ester was absorbed. As the higher MW esters

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released quinclorac in soil over time, concomitantly, the toxicity of leafy spurge after treatment with these esters also increased and was superior to quinclorac after 24 weeks. The slow release of quinclorac from the ω/β-oxidations of these esters appeared to increase efficacy in soil-treated leafy spurge.

Introduction

Quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) is a herbicide used for annual grass control in rice. The mechanism for quinclorac tolerance in rice and other grasses did not appear to be due to differences in herbicide absorption, translocation, or metabolism (1, 2). Its mode of action in sensitive grasses appeared to involve species selective induction of ACC synthase and elevation of cyanide levels (3-6) and/or inhibition of cell wall biosynthesis (7-9). Additionally, electrolyte leakage was induced by quinclorac in smooth crabgrass and other susceptible grasses but not in tolerant grass or susceptible broadleaf species; this quinclorac-induced electrolyte leakage appeared to be a secondary response in susceptible grasses (10). The cellular uptake and cytoplasmic accumulation of quinclorac could be predicted by an "ion trap mechanism" (9, 11). Quinclorac selectivity appeared to be associated with the differential sensitivity at the target site (cell wall biosynthesis) in susceptible grasses; the high degree of tolerance in rice and resistant smooth crabgrass also appeared to be associated with the water solubility and weak acid properties of quinclorac (9).

Quinclorac was one of the most effective herbicides tested for the control of leafy spurge (*Euphorbia esula* L.) in field studies (12, 13). The absorption, translocation, metabolism, and toxicity of quinclorac in foliar- and root-treated leafy spurge was evaluated previously (14). Upon foliar application, quinclorac was translocated basipetally and acropetally, and it was effluxed rapidly from the roots into the soil. About 30% of the applied radiolabel had effluxed into soil at 7 days. Upon foliar application of quinclorac to Kentucky bluegrass, 17% of applied radiolabel also had exuded from roots into nutrient solution at 5.3 days (2). Quinclorac also was taken up by the roots of leafy spurge and translocated acropetally (14). It was metabolized to a glucose ester and a subsequent pentosylglucose ester. However, the role of metabolism in quinclorac detoxification in leafy spurge was uncertain since these metabolites were labile and readily hydrolyzed. Quinclorac was toxic to leafy spurge when applied to the leaves, the soil, or to both the leaves and the soil (LD_{50} 2.0, 1.7, and 1.0 kg/ha, respectively).

It was hypothesized that conversion of quinclorac from the acid to ester(s) might alter the delivery properties of quinclorac into the leafy spurge and/or soil, and this change possibly could alter or improve the herbicidal activity. Therefore, various [^{12}C] and [^{14}C]quinclorac esters[2] were synthesized in order to evaluate the comparative toxicities of

[2] Abbreviations used:
C1, methanol; C2, ethanol; C3, *n*-propanol; C4, *n*-butanol; C5, *n*-pentanol; C6, *n*-hexanol; C7, *n*-heptanol; C8, *n*-

these esters relative to quinclorac in foliar- and root-treated leafy spurge. If increased toxicity was observed in one and/or the other treatment system, the additional objectives of this study were to ascertain the stability, movement, and metabolism of these esters in the plants and soil and how these factors might relate to toxicity. It was presumed that the esters ultimately would be hydrolyzed to the active form of the herbicide quinclorac in the plant and/or soil and the rate of hydrolysis would be dependent upon the esters chain length.

Materials and methods

General methods

Plant material, treatments, chemicals, instrumentation, and other general methods used in this study were as described previously (14).

HPLC analyses

HPLC analyses were conducted using a 3.9 × 300-mm. Bondapak C_{18} column (Millipore Corp.) with various linear gradients of acetonitrile:water:acetic acid (1.5 ml/minute). Both UV and radioisotope flow detectors were used. Metabolites were resolved from various esters and quinclorac by the following HPLC systems: (A) a linear 30-minute gradient from aqueous 25% acetonitrile/1% acetic acid to aqueous 55% acetonitrile/1 % acetic acid, followed by a 5-minute linear gradient from aqueous 55% acetonitrile/ 1% acetic acid to 99% acetonitrile/1% acetic acid; (B) a 7-minute isocratic elution with aqueous 35% acetonitrile/1% acetic acid, a 1-minute gradient to aqueous 70% acetonitrile/1% acetic acid, followed immediately by a 20-minute gradient to 99% acetonitrile/1% acetic acid with a 2-minute hold at the end; (C) a 7-minute isocratic elution with aqueous 35% acetonitrile/1% acetic acid, followed by a 10-minute gradient to aqueous 70% acetonitrile/1 % acetic acid and a 2-minute gradient to aqueous 99% acetonitrile/1% acetic acid with a 7-minute hold at the end. For analysis of the quinclorac propane 1,3-diol ester and metabolites, a 30-minute linear gradient from aqueous 10% acetonitrile/1% acetic acid to aqueous 40% acetonitrile/1 % acetic acid, followed by a 2-minute linear gradient to 99% acetonitrile/1% acetic acid, was used.

Mass spectrometry and NMR analyses

Positive ion fast atom bombardment (FAB) and ammonia-chemical ionization (CI) mass spectrometry (MS) was performed as previously described (14, 15). ^{1}H NMR spectra were acquired on a Brucker AM400 instrument equipped with a wide-bore, proton-observe probe. The quinclorac ester samples were prepared in deuterated solvent in

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octanol; C16, *n*-hexadecanol; G3, 2-propanol, iso-propanol; Cs4, 2-butanol, sec-butanol; Ci8, 3 octanol, iso-octariol; PD, 1,3-propanediol. These abbreviations also refer to the *n*-alkyl or branched alkyl side chain of the synthesized quinclorac esters.

standard 5-mm tubes, while metabolites were dissolved in 25 µl deuterated solvent, sealed in a capillary tube (1.8 mm i.d.) and placed in a 5 mm tube for analysis. To further increase sensitivity in the metabolite spectra, a 0.3-Hz linebroadening factor was applied to the data before Fourier transformation. Chemical shifts (δ) were referenced to tetramethylsilane at 0.00 ppm. Acquisition parameters were not optimized for peak integration; however, peak areas supported all proton assignments. One homonuclear decoupling experiment was performed with continuous low-level irradiation at the frequency of one of the methylene groups (H13) of metabolite II.

Synthesis of [^{12}C]quinclorac esters

Quinclorac (94%, 2.5 g) was dissolved in HC1-saturated alcohol (highest available commercial percentage of purity, 90-120 ml) and refluxed for 3 to 7 hours at 85 to 190º C dependent on increasing MW (C1 thru C8 esters) or for 13 hours (Ci3, Cs4, C16, and PD esters). Upon cooling, excess water was added and pH was adjusted to 7.0 with sodium bicarbonate. The mixtures were vacuum filtered, and esters were purified on a silica gel or aluminum oxide column which was eluted with ethyl ether. All esters were crystalized, melting points were determined, and retention times and purities were analyzed by HPLC system B and by UV analysis at 328 and 270 nm based on the assumption that the molar extinction coefficients were constant compared to quinclorac. Purities of the quinclorac esters were >90% except for the Ci8 (82%) and the C16 (47%). All ester structures were verified by FAB-MS. The C3, Ci3, C6, and C8 esters also were verified by NMR.

Synthesis of [^{14}C]quinclorac esters

In a typical reaction, 12×10^6 dpm (562 nmol) [^{14}C]quinclorac in acetone was added to a 1-ml microflex vial and dried under N_2. Alcohol (800 µI) was added and HCI was bubbled into the solution to saturation. The vial was sealed and heated at 100 to 140º C for 4 to 14 hours (higher temperatures and longer times with increasing MW). Upon cooling, the alcohol solvent was removed by rotary evaporation, and the sample was redissolved in 35% acetonitrile for purification in HPLC system B. The ^{14}C and nonradioactive ester retention times were verified by cochromatography in HPLC systems A and B. The [^{14}C]quinclorac esters (21,360 dpm/nmol) synthesized were C1, C2, C3, Ci3, C4, C5, C6, C7, C8, Ci8, C16, and the PD ester. In some cases where the boiling point of the alcohol solvent was very high (e.g., *n*-octanol at 196º C), the reaction mixture was diluted with 70% acetonitrile in H_2O until the alcohol was solubilized. The HPLC gradient was modified (70% CH_3CN linear gradient to 99% acetonitrile in 1% acetic acid in 20 minute) to separate the alcohol and quinclorac (at solvent front) from the octyl ester (at 10.5 minute).

Plant toxicity studies

Quinclorac or quinclorac esters were dissolved in acetone, and aliquots were diluted with H_2O (0.2% acetone in 20 ml). Solutions were applied to Sunshine mix in cones which contained single-stemmed greenhouse-grown leafy spurge plants (ca. 22 cm height). Quinclorac was applied (10 replicates/dose) at 0.01, 0.03, 0.1, 0.4, 0.5, and 1.0

μmol/plant (0.02-2.0 kg/ha) (14), and quinclorac esters (corrected for purity) were applied (7 replicates/dose) at 150 or 400 nmol/plant. Control plants were treated with H_2O or 0.2% acetone in 20 ml H_2O. Individual plant heights (cm from soil surface) were measured at time of treatment (0 weeks). Foliar toxicity symptoms were scored (14), and changes in heights were calculated at 4 and 8 weeks. The plant portions above the soil surface were cut back for continued incubation in the greenhouse. Plants were irrigated with 25 ml H_2O on a daily basis to minimize loss of solutions through the bottom of the cones. After foliar tissues were cut back, plants were watered every other day until new shoots had emerged. Shoot regrowth was monitored through 16 weeks post-treatment, before the new shoot regrowth was cut back to the soil surface a second time. Foliar regrowth was monitored for an additional 8 weeks. At 24 weeks post-treatment, plant heights and weights (stems + foliar tissue) were measured. The experiment was initiated in May and was repeated in December at 0.4 [μmol ester/plant (12 replicates/dose) with similar results.

At the same times as above soil treatments, soil-shielded foliage of intact leafy spurge plants were treated as a spray (Badger Air Brush, Franklin Park, IL) in 1.5 ml of an aqueous 0.3% lutensol emulsion (14) at the following levels: 0.01, 0.03, 0.1, 0.5, and 2.0 μmol/plant with seven replicates per dose. Quinclorac esters also were treated as a foliar spray (soil-shielded) in 1.5 ml acetone at 0.1 and 2 μmol/plant (seven replicates per dose). The esters did not penetrate the leaves when applied in 0.3% lutensol (refer to following section). Controls were untreated plants and plants treated with acetone or 0.3% aqueous lutensol (10 replicates each). Plants were ca. 22 cm tall at treatment. Growth was monitored, and plants were cut back at 8 and 16 weeks in the same manner as the above soil treatments. Changes in plant growth (height at 4 and 8 weeks, weight at 24 weeks) relative to controls were expressed as percentage of growth inhibition ± SE, and the percentage of dead plants for each treatment was noted at the 24-week termination.

Translocation and metabolism of quinclorac esters in foliar-treated leafy spurge

Single-stemmed leafy spurge plants (84NDl) were grown in cones, which contained Sunshine mix (Sun Gro Horticulture, Belevue, WA) (ca. 17.5 g dry wt per 130 ml, 4 cm diameter cone). Plant heights were ca. 22 cm after ca. 60 days in the greenhouse. In a previous study (14), [^{14}C]quinclorac was applied by syringe to the upper horizontal leaves immediately below the unfolded apical leaves by streaking 2.4 nmol in 8 μl 0.3% lutensol per ca. 2 cm^2 leaf surface (the equivalent of a subtoxic, ca. 0.03 kg/ha dose). Preliminary leaf application studies with the [^{14}C]quinclorac esters showed that uptake was not accomplished when applied in 0.03% lutensol, but uptake was relatively rapid when applied in acetone; i.e., 36% of a 2.4 nmol/leaf dose of the C6 ester was inside the leaf at 2 hours, 68% at 6 hours, and ca. 78% at 1 day. However, quinclorac did not enter the leaf when applied in acetone. Therefore, the quinclorac esters were applied to leaves of intact plants as described previously (14), except acetone was used as the solvent. Duplicate treated plants were incubated *in situ* for 7 days. The treated leaves were excised and immediately dip-rinsed in 5 ml acetonitrile for 5 s. The treated leaves, remaining plant portions, and Sunshine mix were extracted and analyzed (14). For controls, [^{14}C]quinclorac and [^{14}C]quinclorac esters were applied to leaves, extracted at zero time, and assayed as

above. Treated leaf extracts (25 ml) were concentrated, diluted to 3 ml with H_2O, acidified to pH 1 with HC1 at 4º C, and immediately partitioned with 2 vol CH_2Cl_2. Under these conditions, quinclorac and the esters were partitioned (> 98%) into CH_2C1_2 The CH_2C1_2-soluble samples were rotary-evaporated at ambient temperature and redissolved in 25% acetonitrile for analysis and quantitation in HPLC system A. HPLC analysis of the aqueous phase of these samples consistently resulted in chromatograms with no distinct ^{14}C peaks; broad unresolved chromatographic bands were observed. Direct HPLC analyses of control or zero time quinclorac- or quinclorac ester-treated leaf extracts consistently resulted in sharp quinclorac or ester peaks at the correct retentions; no unresolved chromatographic bands were observed. The experiment was repeated with similar results.

Metabolism of quinclorac esters in soil and nutrient solution

A fresh sample of medium textured sandy loam soil with a pH of 7.7 and an organic matter content of 3.5% that had been used to grow leafy spurge for several years was used to study soil metabolism of quinclorac and the quinclorac esters. The soil (1 g) and 2.4 nmol (21,360 dpm/nmol) of [^{14}C]quinclorac or an ester of [^{14}C]quinclorac dissolved in 120 µl of acetone were mixed with a vortex shaker in a 20-ml glass vial, air-dried for 40 minute, and mixed with an additional 1 g of soil and 400 µl of water. Duplicate samples of quinclorac and the various esters were incubated at 23º C in the dark. Samples were readjusted to the original weight by addition of water on alternate days. Samples were extracted three times for 30 minute each on a rotary shaker with 10 ml of aqueous 70% acetonitrile and centrifuged, and then the extract was decanted. The extracts from each sample were combined, concentrated under vacuum, dissolved in 1.2 ml of aqueous 20% acetonitrile, and analyzed by HPLC system A. The extracted soil was dried and analyzed for $^{14}CO_2$ by combustion analysis (14). Duplicate six-point, 28-day time course soil incubations also were assayed with the C7 and C8 esters.

Leafy spurge plants were grown under hydroponic conditions in Hoagland's nutrient solution (one-third strength) for 4 to 7 days. Plants were removed and the residual solution was filtered through Whatman No. 1 filter paper and added (3 ml/vial) to 24 sterile 7-ml screw cap vials. Another portion of this nutrient solution (100 ml) was sterilized by filtration through a 0.22 µm membrane before being added (3 ml/ vial) to an additional 24 sterile vials. Duplicate samples of each of the 12 esters of [^{14}C]quinclorac (3.0 nmol, 64,080 dpm in 33 µl acetone) were added to the 24 vials of nonsterile nutrient solution and to the identical series of 24 vials of sterile nutrient solution. The vials were lightly capped and incubated in the dark at 23º C. The vial contents were analyzed after 7 and 14 days for total ^{14}C by LSS and for radiochemical composition by HPLC systems A and B. The experiment was repeated with similar results. A detailed 45-day time course study also was assayed by HPLC system A for the C5, C7, and C8 ester nonsterile nutrient solution systems.

Translocation and metabolism of quinclorac esters in root-treated leafy spurge

Plants were grown in cones for 45 days and were transferred and incubated in aerated one-third strength Hoagland's nutrient solution for an additional 20 days. The roots of these intact plants were immersed partially in 75-ml nutrient solutions which contained 406,000 dpm (19 nmol) of each [^{14}C]quinclorac ester in acetone (0.6% v/v) for treatment. Duplicate plants were incubated for 3 days without aeration at 50% humidity, 30º C day-light at 145 μmo/m$^{2\bullet}$s for 16 hours, and 23º C night. Plants were removed from the ^{14}C treatment solutions and were transferred to fresh, nonradioactive, aerated nutrient solution at 3 day for continued incubation until day 7. The 0- to 3-day treatment and 3- to 7-day incubation solutions were assayed for ^{14}C content and were concentrated directly for HPLC analyses. The rinsed roots and foliar tissues were extracted and assayed as described previously. The experiment was repeated with similar results.

Sunshine mix percolation studies

Sunshine mix was added to 4 ml (5.1 × 1.0 cm diameter) columns, and [^{14}C]quinclorac or [^{14}C]quinclorac-C8 ester (124,200 dpm, 5.8 nmol) was applied in acetone to the top of the column. The acetone was evaporated overnight, and H_2O was pumped through the columns at 0.2 ml/minute. Fractions (1.0 ml) were assayed directly by LSS. After 100 column vol, the Sunshine mix was dried with a N_2 stream, and the column contents were divided into four parts from top to bottom. Each portion was extracted with 25 ml 100% CH_3CN with shaking for 30 minutes followed by centrifugation. The extract supernatants were assayed directly by LSS.

Biosynthesis and purification of metabolites I, II, III, and IV

The C5 ester of [^{14}C]quinclorac (1.0 μmol, 1 × 10^6 dpm) was incubated in 1 liter nutrient solution (1% acetone v/v) which had been used to grow leafy spurge plants. The system was slowly stiffed for aeration at 23º C in the dark for 24 days. Time course studies showed this concentration and incubation time produced optimum concentrations of metabolites I and III (21 and 26 min Rt, respectively, in HPLC system A). The experiment was repeated and also was accomplished with the C6 and C8 esters of [^{14}C]quinclorac for the production of metabolite II (24 min Rt, HPLC system A). The incubation mixtures were chilled to 4º C, acidified to pH 1 with HC1, and immediately partitioned two times with 0.5 vol CH_2C1_2 where 93% of the ^{14}C was CH_2C1_2-soluble, 1% was associated with the lipoprotein interface, and the remainder was H_2O soluble. The organic phase was concentrated to dryness, and acetonitrile was added until the residue was solubilized. The solution was diluted with H_2O to 33% acetonitrile. The sample was centrifuged and the supernatant was used directly for HPLC separation of quinclorac, ester, and metabolites. Initial separation of components was accomplished with HPLC system C, and metabolites I, II, and III were purified in HPLC system A. Final purifications were accomplished in isocratic aqueous acetonitrile HPLC systems in the absence of acetic acid. Elution systems used were isocratic 35% acetonitrile for metabolites I and II at 14 and 17 min Rt, respectively, and isocratic 40% CH_3CN for metabolite III at 16 min Rt.

In one of the three separate C5 ester incubations, another component (metabolite IV at 31 min Rt in HPLC system A) was observed. Final HPLC purification of metabolite IV was in isocratic aqueous 45% acetonitrile at 19 min Rt.

Results and discussion

Foliar toxicity

When quinclorac or the quinclorac esters were applied as a foliar spray, the percentage of growth inhibition (height) data correlated with foliar toxicity symptom scores (r = 0.949) during the initial 8-week incubations. Upon foliar application of quinclorac, 100% mortality was observed after 4 weeks at 0.5 µmol/plant and above. Treatment with quinclorac at 0.1 µmol/ plant showed initial toxicity symptoms and growth inhibition; however, the plants recovered after 24 weeks. Foliar growth at 4 weeks post-treatment with the esters at 2 µmol/plant was inhibited 74 ± 7% for all esters except for the Cs4, C8, Ci8 and C16 esters (25 ± 14%).

However, no toxicity symptoms were evident and the plants had recovered at 24 weeks; growth inhibition was now only 10 ± 7% with all 13 esters (Fig. 1). The quinclorac esters also were less phytotoxic than quinclorac when applied to foliage at 0.1 µmol/plant. With the exception of the C1 ester, growth inhibition at 4 weeks was inversely proportional to ester chain length (62% for the C2 ester; 37%, C3; 24%, C4; 13%, C5; and 8%, C6). The plants had recovered after 8 weeks (data not shown). No differences in growth for the lutensol- or acetone-treated controls compared to untreated controls were observed throughout the 24-week total incubations, and no toxic symptoms were observed in these control treatments. The quinclorac esters were not effective for control of leafy spurge when applied as a foliar spray.

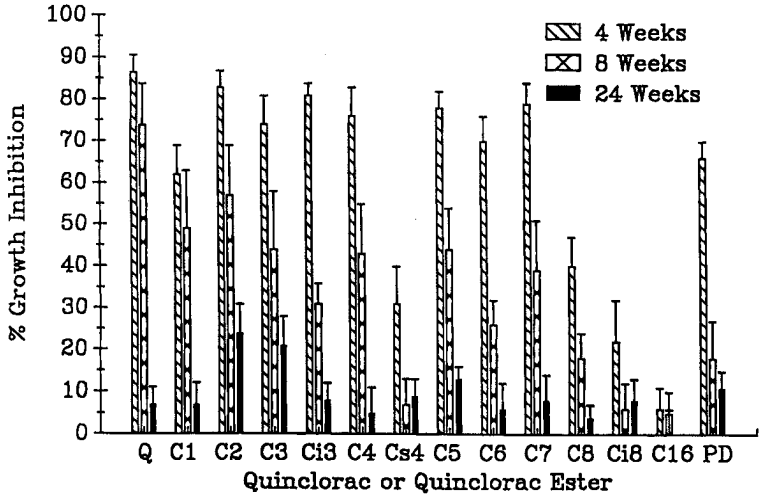


Fig. 1. Toxicity of quinclorac or quinclorac esters when applied as a foliar spray at 0.1 (quinclorac) or 2.0 (esters) µmol/plant. At 0.5 µmol/plant, 100% plant mortality was observed with quinclorac after 4 weeks. Abbreviations are indicated in Footnote 2 of the text.

Soil toxicity

When quinclorac or the quinclorac esters were applied to soil which contained intact plants, the percentage of growth inhibition (height) data correlated with the foliar toxicity symptom scores (r = 0.974) during the initial 8-week incubations. Foliar toxicity symptom scores correlated with the percentage of inhibition (weight) data at 24 weeks (r = 0.935) but did not correlate with percentage of inhibition (height) data. Reemerged shoots from some of the ester treatments (C4, C8, Ci8, C16) were approximately the same height as control plants; however, apical and lower leaves were injured and dwarfed, and lower portions of plants were defoliated in some cases. Foliar fresh vs dry weights also correlated (r = 0.993) in a linear manner. Growth inhibition data (% of controls) were based on plant height at 4 weeks and on weight at 24 weeks (Fig. 2). No significant difference in growth heights or weights of controls with 0.0, 0.2, or 0.3% acetone in water was observed.

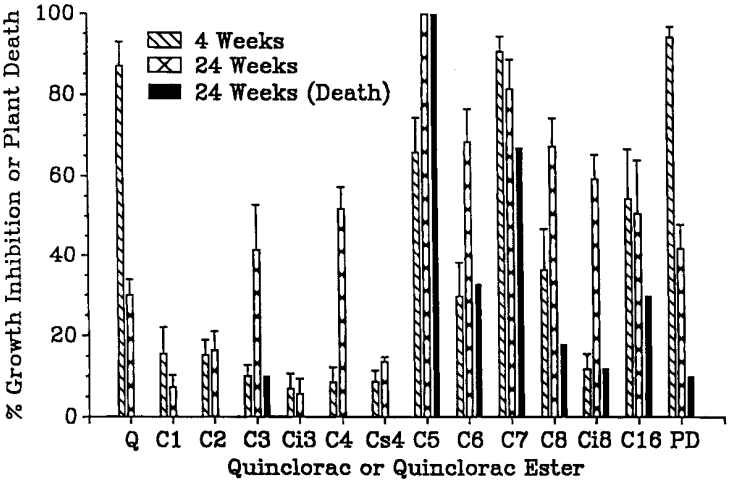


Fig. 2. Toxicity of quinclorac and quinclorac esters when applied to soil which contained intact plants (0.4 µmol/plant). Abbreviations are indicated in Footnote 2.

Quinclorac applied to the soil at 0.4 µmol/intact plant caused 87% inhibition of leafy spurge shoot growth at 4 weeks post-treatment, but only 30% inhibition at 24 weeks post-treatment (Fig. 2). Very little inhibition of shoot growth was observed with the C1 through C4 esters; 65 to 90% growth inhibition was observed at 4 weeks post-treatment with C5, C7, and the PD esters; and the C5 through the C16 esters were all more effective than quinclorac in providing inhibition of shoot growth evaluated at 24 weeks post-treatment (Fig. 2). Based upon extrapolations from the dose-response curve of quinclorac, these esters were from 2.7 to 3.2 times more effective than quinclorac at 16 to 24 weeks post-treatment.

Translocation and metabolism of quinclorac esters in foliar-treated leafy spurge

Distribution of radiolabel after foliar application of [^{14}C]quinclorac at 2.4 nmol/leaf to soil grown intact plants resulted with 4% of dose on the treated leaves, 21% inside the leaves, 38% in remaining plant portions, and 31% in the soil after 7 days with 94% recovery (14). Seven days following application of the same concentration of [^{14}C]quinclorac esters to the leaves of intact plants, the highest percentage of the dose remained in the treated leaves (Table 1). Due to the volatility of the low MW esters, total recovery of ^{14}C ranged from 35 to 78% for the C1 through C6 esters, and recovery increased approximately 9% (r = 0.960) for each additional carbon in the *n*-alkyl ester side chain. At 7 days, 1.1% or less of the dose remained on the treated leaf surfaces of intact plants. The volatilization half-life of the C1, C2, C3, and C7 esters applied to glass plates and incubated under similar conditions was 1.9, 2.4, 2.5, and 46.0 hours, respectively. Movement of radiolabel from the treated leaves to other plant parts was ca. 5% or less of the dose in 7 days with the exception of the C2 and Ci3 esters (11% of dose), and virtually no ^{14}C was present in the Sunshine mix (Table 1).

Table 1. Distribution of radioactivity in leafy spurge plants 7 days following leaf treatment with [^{14}C]quinclorac esters.

	% of dose (location of ^{14}C)				
Ester	Treated leaves (out)	Treated leaves (in)	Apical leaves	Low leaves, stems, roots	Total recovery
C1	0.2 ± 0.1	30.9 ± 1.0	1.8 ± 0.1	2.2 ± 0.1	35.1 ± 1.1
C2	1.1 ± 0.0	23.1 ± 1.2	7.0 ± 0.2	4.7 ± 0.1	35.9 ± 1.4
C3	0.8 ± 0.0	56.3 ± 0.2	1.8 ± 0.1	1.6 ± 0.1	60.5 ± 0.3
Ci3	0.6 ± 0.1	34.7 ± 3.0	6.3 ± 0.8	4.1 ± 0.3	45.7 ± 2.7
C4	0.6 ± 0.1	60.4 ± 0.4	1.6 ± 0.1	1.4 ± 0.1	64.0 ± 0.4
C5	0.9 ± 0.2	68.0 ± 0.6	1.7 ± 0.1	1.6 ± 0.1	72.1 ± 0.9
C6	0.6 ± 0.1	72.2 ± 3.4	3.4 ± 1.3	1.8 ± 0.2	78.0 ± 3.6

Note: Less than 1% of the doses were present in the Sunshine mix. Abbreviations are indicated in Footnote 2 of the text; out, [^{14}C] found on leaf surfaces; in, [^{14}C] found inside leaves.

Metabolism of the esters in the treated leaves of intact plants was complete in 7 days for all esters with the exception of the C2 ester, and metabolism to quinclorac was seen only with the C3 and Ci3 esters (Table 2). The majority of the H_2O-soluble ^{14}C radiolabel from extracts of the treated leaves HPLC-chromatographed as broad, unresolved bands; however, sharp peaks corresponding to quinclorac or the appropriate ester were observed when these ^{14}C standards were added to the extracts prior to chromatography. Recovery of the ^{14}C standards from the HPLC columns was nearly quantitative. Therefore, the broad bands of ^{14}C observed during analysis of the extracts did not appear to be due to chromatographic artifacts. They may have been due to the formation of ester complexes or conjugates with endogenous plant products. Hydrolysis (2.5 N NaOH, 100º C, 3 hours) of the unresolved HPLC metabolite bands from the C4 and C5 metabolite preparations resulted in the liberation of 70 and 78%, respectively, of the ^{14}C as quinclorac (HPLC

system A), but the preparation derived from the Ci3 ester preparation did not produce quinclorac. The HPLC metabolite bands derived from the esters of quinclorac apparently contained the intact quinclorac structure as demonstrated by cochromatography of the ^{14}C hydrolysates with quinclorac, but it is uncertain whether they contained the original ester structure and were simply complexed or chelated to endogenous compounds or whether they had undergone oxidation, conjugation, transesterification, amination, and/or similar reaction(s). Nonextractable residues in the treated leaves of intact plants increased with increasing carbon number of the *n*-alkyl side chain and ranged from 2.0 to 9.8% of the dose (Table 2).

Table 2. Metabolism of quinclorac esters in the treated leaves of intact leafy spurge plants at 7 days after treatment.

	% of Dose (form of ^{14}C isolated)			
Ester	Ester	Quinclorac	Soluble metabolites	Insoluble metabolites
C1	—	—	28.9 ± 1.0	2.0 ± 0.1
C2	7.5 ± 0.4	—	13.3 ± 0.4	2.3 ± 0.0
C3	—	9.0 ± 0.9	42.0 ± 0.9	5.3 + 0.1
Ci3	—	2.2 ± 0.3	28.6 ± 0.3	3.9 ± 0.1
C4	—	—	50.6 ± 0.1	9.8 ± 0.4
C5	—	—	59.0 ± 0.0	9.0 ± 0.6
C6	—	—	63.0 ± 3.4	9.2 ± 0.8

Note. Metabolism data were obtained from the treated leaves used in Table 1. Abbreviations are indicated in Foot-note 2; –, not detected.

Metabolism of quinclorac esters in nutrient solution and soil

The esters of [^{14}C]quinclorac were metabolized in non-sterile nutrient solution and in a moist, aerobic soil at rates dependent upon ester chain length and chain branching (Fig. 3). The short-chain esters (C1-C3) were relatively resistant to metabolism while the intermediate-chain esters (C4-C8) were metabolized at rates that generally increased with chain length. The Ci3 and Ci8 esters were metabolized more slowly than the corresponding C3 and C8 esters. No appreciable metabolism of these esters occurred for up to 14 days in sterile nutrient solution (data not shown). Recovery of radiolabel in the soil extracts ranged from 87 ± 1% (C1 ester) to 100% (C16 ester). Nonextractable radiolabel in soil ranged from not detectable (C16 ester) to 13 ± 2% of dose (C1 ester). Recovery of radiolabel at 14 days in the nonsterile nutrient solution incubations ranged from 90% (PD ester) to 100% (C6 ester). Quinclorac was the primary product of metabolism of the odd carbon-numbered esters (C5, C7, and the PD esters) while metabolite II was the primary product of metabolism of the even carbon-numbered esters (C4, C6, C8, and C16) (Figs. 4A and 4B).

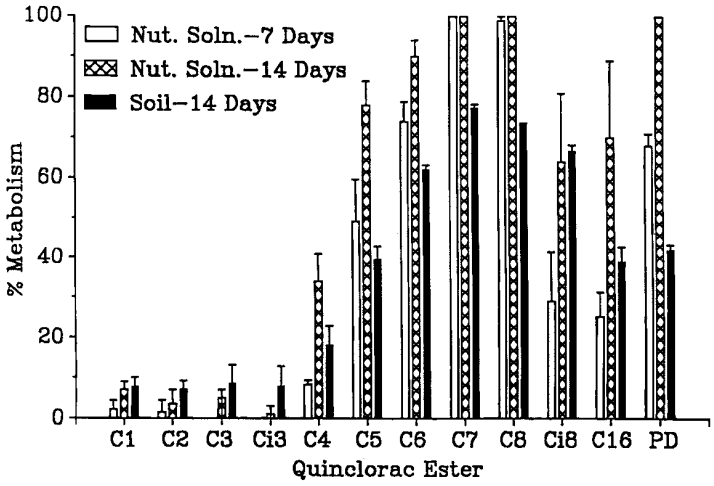


Fig. 3. Metabolism of quinclorac esters in nutrient solution and in moist aerobic soil which had been exposed to roots of leafy spurge plants. Abbreviations are indicated in Footnote 2.

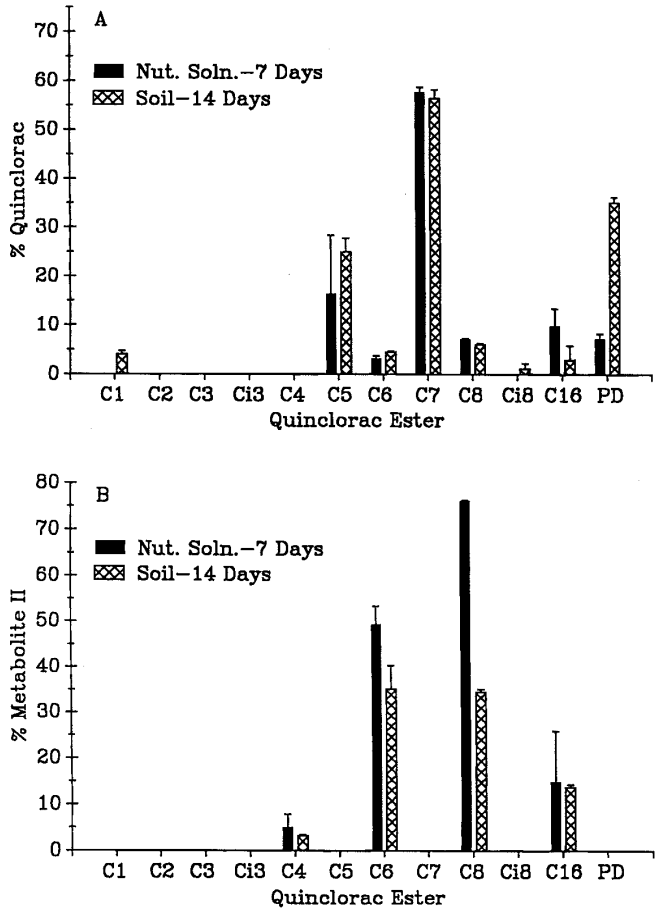


Fig. 4. Metabolism of quinclorac esters to quinclorac (A) or metabolite II (B) in nutrient solution and in moist aerobic soil which had been exposed to roots of leafy spurge plants. Abbreviations are indicated in Footnote 2.

Time-course study

A time-course study in non-sterile nutrient solution showed that the C7 ester was metabolized rapidly to quinclorac while the C8 ester was metabolized rapidly to metabolite II, which was then metabolized slowly to quinclorac (Figs. 5A and 5B). The C5 ester was metabolized at a slower initial rate than the C7 and C8 esters; during the metabolism of the C5 ester, two transitory metabolites (I and III) were produced that may have been intermediates in the formation of quinclorac (Fig. 5C). The same two intermediary metabolites as well as one additional metabolite were detected during the metabolism of the C7 ester, and two additional metabolites in addition to metabolite II and quinclorac were detected at low concentrations during the metabolism of the C8 ester (Figs. 5A and 5B).

A 28-day time-course study in soil with the C7 and C8 esters showed the same metabolism trends as seen in non-sterile nutrient solution (data not shown). Both esters were rapidly metabolized (70%) during the first week; quinclorac and metabolite II (40%, each) were observed from the C7 and C8 esters, respectively, and transitory intermediates (5-10%) occurred in both systems. At 4 weeks, both esters were metabolized (>90%), and quinclorac increased to 60% at 4 weeks (C7 ester system), whereas metabolite II decreased to 20% with the concomitant increase (15%, each) of a polar intermediate and quinclorac (C8 ester system). Nonextractable radiolabel slowly increased to 10 and 20% in 4 weeks in the C7 and C8 ester-treated soil systems. Essentially complete recovery of radiolabel was attained in both systems.

Translocation and metabolism of quinclorac esters in root-treated leafy spurge

After root treatment of duplicate intact plants with 12 [^{14}C]quinclorac esters in nutrient solutions, between 10.8 ± 1% (PD ester) and 27.5 ± 4.2% (C7 ester) of the radiolabel remained in the treatment solutions at 3 days. Upon transfer of the plants to fresh nonradioactive nutrient solutions and continued incubation from 3 to 7 days, the amount of ^{14}C present at 7 days in these incubation solutions was approximately equal to that found previously in the treatment solutions at 3 days except for the PD ester (41.3 ± 0.3%) and the C7 ester (47.8 ± 9.9%). It appeared that esters and/or metabolites were released from the roots or microorganisms associated with the roots into the fresh nutrient solutions. At 7 days the roots, stems, and leaves were analyzed, and recovery of ^{14}C from the plants and nutrient solutions was >90% from the 12 quinclorac ester treatments (96.0 ± 3.9% of dose). Therefore, no volatility problems were observed with root treatment of quinclorac esters, whereas foliar treatment resulted in volatile losses from the lower MW esters. Translocation of radiolabel from roots to foliar tissues decreased with increasing MW of the *n*-alkyl esters (16.6 ± 2.2% of dose in the leaves at 7 days for the C1 ester; 11.9 ± 3.0%, C2 ester; 7.0 ± 1.6%, C3; 4.4 ± 0.1%, C4; 3.7 ± 0.0%, C5; to 1.4 ± 0.3% and less for the C6 and larger esters).

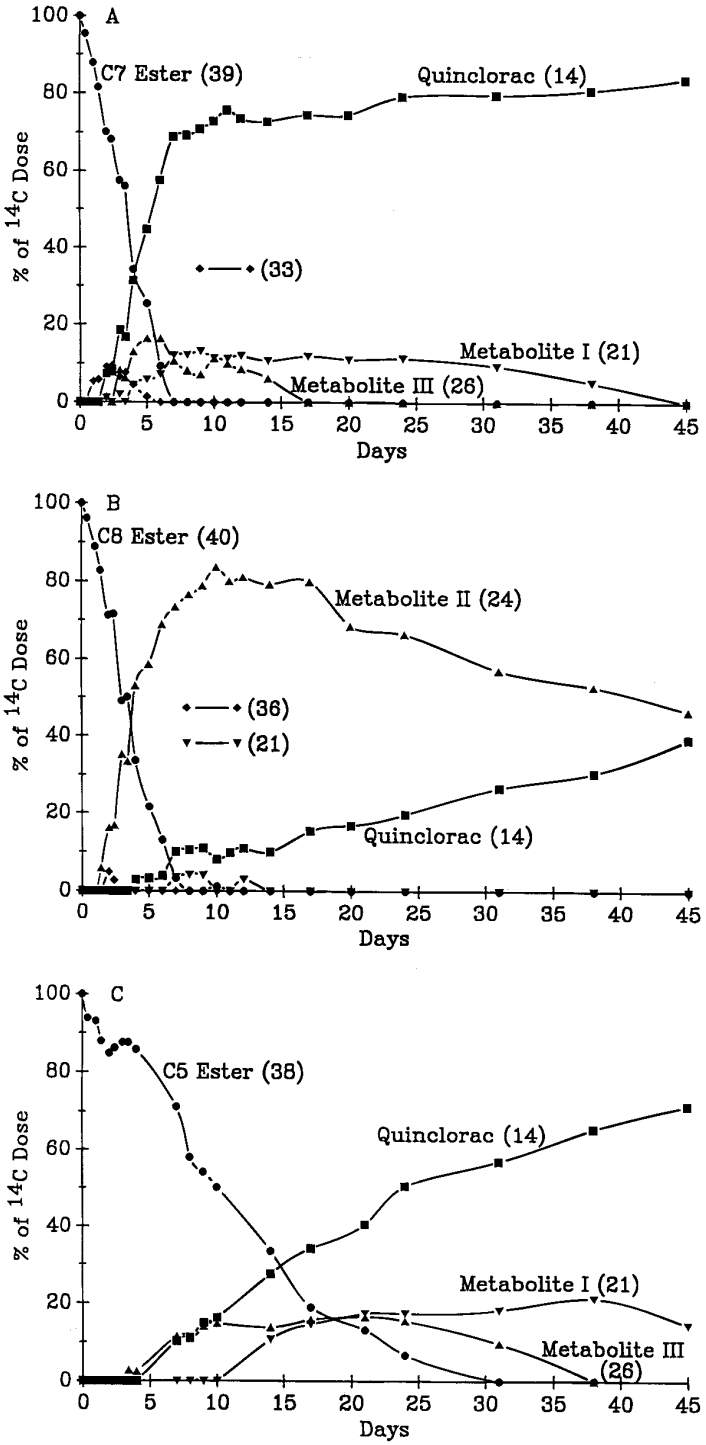


Fig. 5. Metabolism of quinclorac-C7 (A), -C8 (B), and -C5 (C) esters in nutrient solution which had been exposed to roots of leafy spurge plants. HPLC system A retentions (min) for quinclorac, quinclorac esters, and metabolites are indicated in parentheses. Unresolved ^{14}C radiolabel from the three treatments were in the range 4.9 ± 0.7, 8.2 ± 0.8, 11.7 ± 1.2, 14.1 ± 1.6, and 14.8 ± 0.7% of dose at 0.4, 7, 14, 31, and 45 days, respectively.

HPLC analyses of the nutrient solutions showed that the esters were completely metabolized in the C5 ester through the C16 ester systems (Table 3). Quinclorac was present in all quinclorac ester nutrient solutions at 7 days; however, the level of quinclorac was significantly higher (56%) in the C7 system. Concomitantly, metabolite II was observed in the even numbered C4, C6, C8, and C16 ester systems; the maximum level (41%) occurred with the C8 ester. Metabolite II apparently was produced from even numbered quinclorac esters by microorganisms in soil and nutrient solutions which previously had been exposed to roots of intact leafy spurge plants (Fig. 4B) and in nutrient solutions in the presence of the plants (Table 3). This observation was confirmed from nutrient solution studies where [^{14}C]quinclorac esters incubated in sterile nutrient solution produced no metabolites.

Table 3. Metabolism of quinclorac esters in nutrient solutions which were exposed to the roots of intact leafy spurge plants at 7 days after treatment.

	% of dose (form of ^{14}C isolated).				
Ester	Ester	Quinclorac	Metabolite II	Soluble metabolites	Total in nutrient solutions
C1	9.9 ± 1.2	6.0 ± 0.6	—	13.4 ± 1.2	29.3 ± 2.3
C2	21.8 ± 1.2	3.8 ± 0.3	—	10.4 ± 1.2	36.0 ± 2.2
C3	14.9 ± 4.0	2.5 ± 0.4	—	11.0 ± 3.6	28.4 ± 3.6
Ci3	23.3 ± 5.1	0.3 ± 0.3	—	10.1 ± 5.1	33.7 ± 5.9
C4	4.5 ± 0.8	9.8 ± 3.5	3.7 ± 3.7	19.2 ± 3.5	37.2 ± 3.2
C5	0.6 ± 0.3	8.0 ± 5.8	—	29.1 ± 5.8	37.7 ± 5.4
C6	—	4.6 ± 0.5	23.5 ± 4.0	23.5 ± 4.0	51.6 ± 1.7
C7	—	56.1 ± 10.4	—	19.2 ± 10.4	75.3 ± 7.0
C8	—	6.5 ± 1.1	41.0 ± 2.0	7.4 ± 2.0	54.9 ± 2.5
Ci8	—	4.1 ± 2.8	—	19.3 ± 2.8	23.4 ± 3.2
C16	—	3.6 ± 0.0	8.4 ± 0.8	11.4 ± 0.8	23.4 ± 0.8
PD	29.5 ± 2.6	14.1 ± 1.0	—	8.5 ± 2.6	52.1 ± 2.3

Note: The 0- to 3-day treatment and 3- to 7-day incubation solutions were both analyzed by HPLC; ester and metabolite distributions were expressed as percentage of dose in the combined 0- to 7-day nutrient solutions. Abbreviations are indicated in Footnote 2; –, not detected.

HPLC analysis of the 7-day root extracts (Table 4) showed that some low MW esters (C1-C5) were present in the roots, and almost 100% metabolism occurred following treatment with the higher MW esters (C6 and greater). Quinclorac was not observed as a metabolite in the roots except for the C2, C3, and PD ester systems. The majority of soluble metabolites were chromatographically unresolved broad ^{14}C bands as observed previously in the leaf-treated plants. Metabolite II was not detected in root extracts.

Sunshine mix percolation studies

Upon application of [^{14}C]quinclorac or the C8 ester to Sunshine mix columns and percolation with 100 vol of water, 93% of the applied quinclorac was recovered in the first 4 column vol, and 99% was recovered by the 14th volume. Alternatively, radiolabel from the C8 ester did not elute with H_2O through 100 column vol. Extraction of the residual Sunshine mix with CH_3CN resulted with 97.4% of the applied dose in the top half of the column and 98% from extraction of the total column. Whereas quinclorac was leached readily from the Sunshine mix, the long-chained alkyl ester was adsorbed.

Table 4. Metabolism of quinclorac esters in the treated roots of intact leafy spurge plants at 7 days after treatment.

	% of dose (form of ^{14}C isolated)				
Ester	Ester	Quinclorac	Soluble metabolites	Insoluble metabolites	Total in roots
C1	4.2 ± 0.9		34.6 ± 1.2	10.8 ± 0.8	49.6 ± 2.0
C2	15.4 ± 0.8	3.7 ± 0.0	19.3 ± 0.8	7.0 ± 0.8	45.4 ± 2.8
C3	14.2 ± 1.0	7.6 ± 1.6	31.2 ± 1.6	6.0 ± 1.0	59.0 ± 4- 2.8
Ci3	na	na	na	4.7 ± 0.9	52.4 ± 9.2
C4	13.2 ± 8.0	—	32.8 ± 8.0	6.6 ± 1.2	52.6 ± 1.2
C5	11.9 ± 9.6	—	41.3 ± 9.6	8.0 ± 1.9	61.2 ± 9.7
C6	0.5 ± 0.5	—	35.6 ± 0.5	10.4 ± 2.0	46.5 ± 2.0
C7	—	—	19.0 ± 2.2	3.2 ± 0.2	22.2 ± 2.2
C8	—	—	32.5 ± 1.4	6.4 ± 1.9	38.9 ± 3.6
Ci8	na	na	na	7.4 ± 0.6	77.1 ± 7.0
C16	na	na	na	11.9 ± 1.0	64.7 ± 1.0
PD		10.4 ± 1.2	15.0 ±-1.2	9.2 ± 0.5	34.6 ± 1.7

Note. Abbreviations are indicated in Footnote 2; –, not detected; na, not assayed (radiolabel from the Ci3, Ci8, and C16 ester treatments adhered to glassware during concentration of root extracts for HPLC.

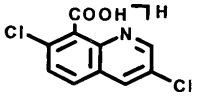
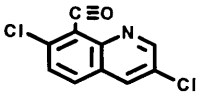
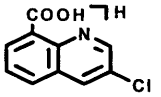
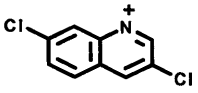
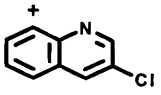
Identification of metabolites

Preparations of metabolite I (Met. I) produced by incubation of the C5 ester in nonsterile nutrient solution or soil were chromatographically indistinguishable; likewise, preparations of metabolite III produced from nutrient solution or soil were indistinguishable. However, in one of three separate nutrient solution incubations, an additional nonpolar metabolite (IV) was isolated. This metabolite had not been detected in detailed time-course studies with the C5 ester in soil. Metabolite IV appeared to arise from the C5 ester substrate, since the composition of the system at termination contained 26% quinclorac, 18% Met. I, 10% Met. III, 17% Met. IV, 24% C5 ester, and 5% unresolved material. Preparations of metabolite II produced by incubation of the C6 and C8 esters in nonsterile nutrient solution and soil also were indistinguishable from each other by HPLC (24 min Rt). The FAB and ammonia CI mass spectra of metabolites I, II, III, and IV and quinclorac C6 ester are summarized in Table 5. Each metabolite spectrum showed ion fragments at *m*/*z* 242/244, *m*/*z* 224/226, and/or *m*/*z* 197/199 consistent with a dichlorinated aromatic nucleus identical to that of quinclorac. The FAB mass spectra of metabo-

lites I, III, and IV were characterized by M + 1 quasi molecular ion clusters at *m*/*z* 314/316, 342/344, and 326/328, respectively. Additionally, an ion at *m*/*z* 85 consistent with a pentanone side-chain rearrangement was observed from metabolite IV. The FAB and ammonia CI mass spectra of metabolite II produced from the C6 and C8 esters were both characterized by an M + 1 quasi molecular ion cluster at *m*/*z* 328/330 that was consistent with two chlorines. Ions at *m*/*z* 102 (C6 ester) and at *m*/*z* 104 (C8 ester) in the CI mass spectra of Metabolite II can be attributed to an alkanoic acid side chain.

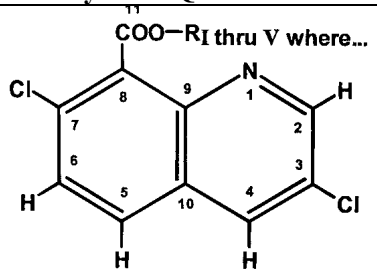
The NMR spectra of metabolites I-IV and the C6 ester of quinclorac are summarized in Table 6. Four aromatic protons (H2, H4, H5, and H6) detected in each metabolite spectrum were essentially identical to the aromatic protons in the spectrum of quinclorac C6 ester consistent with the proposed structures in which the aromatic nucleus was unchanged from that of quinclorac ester precursors. The side chain of metabolite II appeared to consist of three methylene groups, one that was attached to the oxygen of the quinclorac carboxyl group (H12, t, δ 4.60), one that was attached to another polar group (H14, t, δ 2.63), and one that appeared as a multiplet at δ 2.16 (H13). Selective irradiation of the methylene group at δ 2.16 decoupled the methylene protons at δ 4.60 and 2.63 which confirmed the connectivity of the three methylene groups.

Table 5. Mass spectral analyses of quinclorac nC6 ester and metabolites I through IV.

Metabolite ion structure (derived from Q ester substrate)		*m*/*z* (% of base peak) number of chlorines					
	MS	C6 ester	Met. I (C5 ester)	Met. II (C6 ester)	Met. II (C8 ester)	Met. III (C5 ester)	Met. IV (C5 ester)
Molecular ion M + H	FAB	326(100)2	314(71)2	328(100)2	328(100)2	342(90)2	326(37)2
	CI	326(100)2	na	328(10)2	328(100)2	na	na
	FAB	242(40)2	—	242(40)2	242(16)2	242(22)2	242(25)2
	CI	242(47)2	na	242(50)2	—	na	na
	FAB	224(45)2	224(47)2	224(65)2	224(73)2	224(100)2	224(100)2
	CI	224(78)2	na	224(8)2	—	na	na
	FAB	—	—	—	—	—	—
	CI	—	na	208(100)1	—	na	na
	FAB	—	—	—	—	—	—
	CI	197(70)2	na	198(60)2	198(8)2	na	na
	FAB	—	—	—	—	—	—
	CI	161(20)1	na	163(4)1	163(4)1	na	na
	CI	—	na	—	104(37)0	na	na
HO-CH_2-CH_2-CH_2-COOH O=CH-CH_2-CH_2-COOH	CI	—	na	102(30)0	—	na	na
CH_2-CH_2-CH_2-CO-CH_3	FAB	—	—	—	—	—	85(72)0

Note: —, not detected; na, not assayed.

Table 6. NMR Analyses of Quinclorac Ester Metabolites.



I = $CH_2(12)—CH_2(13)—COOH(14)$

II = $CH_2(12)—CH_2(13)—CH_2(14)—COOH(15)$

III = $CH_2(12)—CH_2(13)—CH_2(14)—CH_2(15)—COOH(16)$

IV = $CH_2(12)—CH_2(13)—CH_2(14)—CO(15)—CH_3(16)$

V = $CH_2(12)—CH_2(13)—CH_2(14)—CH_2(15)—CH_2(16)—CH_3(17)$

	V, hexyl ester of quinclorac								
Proton position:	H2	H4	H5/6	H5/6	H12	H13	H14/15	H16	H17
	Spectrum run in deutero chloroform								
δ in ppm	8.84	8.13	7.74	7.57	4.53	1.82	1.34	1.48	0.90
Peak multiplicity	d	d	d	d	t	pent.	m	m	t
J(hz)	2.4	2.4	8.8	9.0	6.7	7.0	NR	NR	7.1
	Spectrum run in deutero acetone[a]								
δ in ppm	8.90	8.56	8.10	7.76	4.47	1.80	1.36	1.49	0.90

	Metabolite I derived from the pentyl ester of quinclorac						
Proton position:	H2	H4	H5/6	H5/6	H12	H13	H14/15
	Spectrum run in deutero acetone[b]						
δ in ppm	8.90	8.56	8.11	7.76	4.73	3.59	NA
Peak multiplicity	d	d	d	d	t	m	NA
J (hz)	2.3	2.3	8.9	8.9	6.2	NR	NA

	Metabolite II derived from the hexyl and octyl esters of quinclorac							
Proton position:	H2	H4	H5/6	H5/6	H12	H13[c]	H14	H15
	Spectrum run in deutero chloroform							
δ in ppm	8.85	8.16	7.77	7.59	4.60	2.16	2.63	NA
Peak multiplicity	d	d	d	d	t	pent.	t	NA
J (hz)	2.1	2.1	8.9	8.9	6.1	6.7	7.4	NA

	Metabolite III derived from the pentyl ester of quinclorac							
Proton position:	H2	H4	H5/6	H5/6	H12	H13/14	H15	H16
	Spectrum run in deutero acetone							
δ in ppm	8.91	8.56	8.10	7.76	4.50	1.85	2.40	NA
Peak multiplicity	d	d	d	d	t	M	t	NA
J (hz)	1.8	1.8	8.9	8.9	5.9	NR	7.0	NA

	Metabolite IV derived from the pentyl ester of quinclorac							
Proton position:	H2	H4	H5/6	H5/6	H12	H13[d]	H14	H16
	Spectrum run in deutero acetone							
δ in ppm	8.92	8.57	8.11	7.77	4.48	NR	2.73	2.13
Peak multiplicity	d	d	d	d	t	ND	t	s
J (bz)	2.3	2.3	8.9	8.9	6.4	ND	7.2	NA
	Spectrum run in demero chloroform							
δ in ppm	8.86	8.16	7.77	7.59	4.56	2.10	2.69	2.18
Peak multiplicity	NR	NR	d	d	t	pent.	t	s
J (hz)	ND	ND	8.7	8.7	6.1	6.8	7.2	NA

Note: NA, not applicable; ND, not determined; NR, not resolved; pent., pentuplet; J, proton coupling constant.

[a]Multiplicity and J values were the same as in $CDCl_3$.

[b]Peaks due to the presence of water at 2.84 ppm, acetone at 2.07 ppm and an unexplained peak at 1.29 ppm were also observed.

[c]Assignment of the aliphatic methylene protons was facilitated by irradiation of H13 which resulted in decoupling at H12 and H14.

[d]In deutero acetone, H13 was obscured by the acetone peak at 2.06 ppm; a water peak at 2.82 ppm also was seen. In deutero chloroform, H13 was resolved; peaks due to water at 3.53 and 2.15 ppm and chloroform at 7.26 ppm also were observed.

In consideration of the MW (Table 5) and the NMR spectra (Table 6) of the four metabolites, it was concluded that metabolite I was the quinclorac ester of 3-hydroxypropionic acid; II, the quinclorac ester of 4-hydroxybutyric acid; III, the quinclorac ester of 5-hydroxypentanoic acid; and IV, the quinclorac ester of 5-hydroxy-2-oxopentane with the structures shown in Table 6.

ω-Oxidation and β-elimination

The metabolism of both the C6 and the C8 esters to metabolite II and the metabolism of both the C5 and the C7 esters to quinclorac by processes that appeared to produce several transitory intermediary metabolites (I, III, etc.) can be most reasonably explained by a sequence of reactions involving ω-oxidation followed by a series of β-elimination reactions (Fig. 6). ω-Hydroxylation and oxidation pathways have been reviewed for animals and bacteria (16, 17) and in plants (18-20). In bacteria, an ω-hydroxylation system isolated from *Pseudomonas oleovorans* hydroxylates the terminal carbon of long-chain fatty acids and hydrocarbons. In plants, the initial ω-hydroxylation of an *n*-alkyl fatty acid is a microsomal cytochrome P450-mediated reaction with NADPH and molecular oxygen. Subsequent oxidations of the ω-hydroxylated fatty acid product to an ω-oxo acid followed by an ω-dicarboxylic acid are catalyzed by soluble NADP-linked dehydrogenases. The K_m's for the oxidation of ω-oxo acid intermediates from the ω-hydroxylated carboxylic acids range from 7000 to 90 μM for the C3 to the C8 oxo acid; the K_m's above C8 remain at ca. 90 μM, but the enzymatic oxidation process stops with chain lengths above C20 (18). The failure of the short-chain quinclorac esters to undergo metabolism at an appreciable rate in soil and nutrient solution can be explained by a requirement for a chain length of at least four carbons for effective initiation of metabolism by ω-oxidation. This would be consistent with the observation that the PD ester was metabolized to quinclorac while the C3 ester was not. Branching of the alkyl side chain might inhibit both ω-oxidation and β-elimination and would be consistent with the slower rate of metabolism of the Ci8 ester compared to the C8 ester. Except for the requirement of the initial co-oxidation, metabolism of these esters appear to be somewhat similar to the metabolism of 2,4-dichlorophenoxyalkanoic acids with even and odd numbered carbons in the side chain (21). The ω-hydroxylase system also catalyzes epoxidations in plants and soil bacteria (17-19). The formation of the quinclorac ester of 5-hydroxy-2-oxo-pentane (metabolite IV) in nonsterile nutrient solution appeared to result from the initial oxidation of the quinclorac C5 ester. ω-Hydroxylation is the first step involved in the biosynthesis of most of the components of cutin and suberin in plants. Ferulic acid also is involved in suberization, and ferulic acid conjugate cross-linkages in the cell wall increase during stress and pathogen attack. A microsomal feruloyl-CoA transferase was coinduced transiently with the cytochrome P450 responsible for fatty acid ω-hydroxylation by elicitor treatments of French bean cells (20). The unresolved ^{14}C material observed in chromatograms of leafy spurge extracts of [^{14}C]quinclorac ester-treated leaves and roots might be attributed to ω-hydroxylation of the ester terminal methyl group with subsequent formation of many intermediates involved in the process of suberization. Since nonextractable ^{14}C residues from treated leaves and roots were detected at 2 to 12% of dose at 7 days after treatment, incorporation of radiolabel from the quinclorac ester substrates into cell wall material would be consistent with a suberization hypothesis. However, alternate

explanations for the formation of unresolved ^{14}C material in leafy spurge tissues are certainly possible.

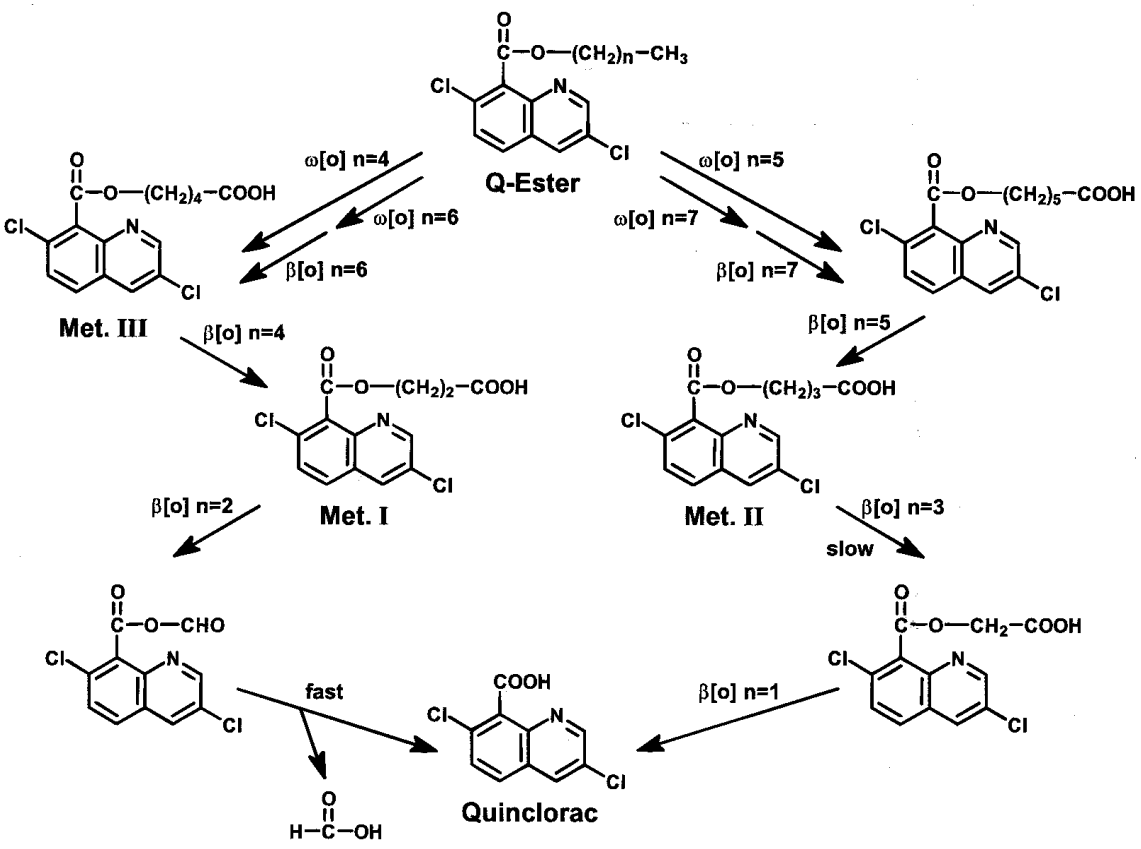


Fig. 6. Probable involvement of ω- and β-oxidation in the metabolism of quinclorac esters in nutrient solution and soil. ω[o], ω-oxidation pathway which includes hydroxylation of the ω-terminal alkyl group, oxidation of the ω-hydroxyalkyl group, and subsequent oxidation of the ω-aldehyde to the ω-carboxylic acid; β[o], β-oxidation of the ω-carbozylic acid with elimination of the acetyl-CoA; n, number of methylene groups in the side chain.

Summary and conclusions

Toxicity of quinclorac esters applied to the foliage

Most of the quinclorac esters showed initial toxicity symptoms and growth inhibition when applied to the foliage at 2 μmol/plant. However, the plants recovered with time; no toxicity symptoms were evident, and little growth inhibition occurred after 24 weeks. The lower toxicity of the short-chain-length esters may have been due to both volatility and incomplete metabolism to quinclorac. The longer-chain-length esters were metabolized at a more rapid rate than the short-chain esters, but metabolism led to products other than quinclorac. Little translocation of these products was observed and the low level of toxic-

ity of these compounds was probably due to the inability of the plant to convert these esters to quinclorac. Although 100% mortality was observed upon foliar treatment with quinclorac at 0.5 μmol/plant, treatment at 0.1 μmol/plant resulted with recovery from injury at 24 weeks. This recovery could be explained by movement of quinclorac to the roots and subsequent exudation into the soil (14).

Because of limitations in the greenhouse and how the plants were grown, it was necessary to cut the plants to ground level at 8 and 16 weeks. Therefore, the results of these studies cannot be readily translated to what effects might be observed under field conditions.

Toxicity of quinclorac esters applied to the soil

Most of the esters of quinclorac, except the C5, C7, and PD esters, were not very effective in reducing the growth of leafy spurge during the first 4 weeks following soil treatment of intact plants. Metabolism studies showed that most of these esters were not readily metabolized to quinclorac in the roots; however, the C7 and PD esters were metabolized rapidly to quinclorac by microorganisms present in the soil and nutrient solution. The C7 ester was metabolized to quinclorac at a rate 16 times greater than was the C8 ester (13%/day vs 0.8%/day in nutrient solution) and the C5 ester was metabolized to quinclorac at a rate of 2.6%/day or 3.2× faster than the octyl ester. The toxicity results were rather different after longer time periods. At 24 weeks post-treatment, the C5, C6, C7, C8, Ci8, C16, and PD esters all inhibited shoot growth. In fact, these esters, when assayed after the longer time period, were more phytotoxic than quinclorac in the soil-treated intact plant systems. This was probably due to their slow conversion to quinclorac in the soil. It is also possible that less quinclorac from high MW ester applications was leached from the pots during the 24 weeks study.

Relationship between leafy spurge toxicity and soil metabolism of quinclorac esters

The major points demonstrated in this work were: (a) toxicity occurred in leafy spurge only when quinclorac esters were applied by soil treatment and not by foliar application; (b) foliar application showed volatile losses with the low MW esters (C1-C6), and subsequent complete metabolism of the esters occurred in the leaves, but little or no quinclorac was observed as a result; (c) metabolism of quinclorac esters in soil occurred through a series of ω- and β-oxidations of the esters' side chain and not by simple hydrolysis of the quinclorac ester bond; (d) the rate of quinclorac release from the various esters was dependent on the number of odd vs even carbons in the ester side chain; (e) quinclorac acid was leached readily from soil with water, but the C8 ester was adsorbed; and (f) the C5-C8 esters released quinclorac in the soil over time and, concomitantly, the toxicity of leafy spurge after treatment with these esters also increased with time and was superior to quinclorac after 24 weeks.

Thus, both metabolism and mobility in the soil could be factors involved in the long-term toxicity of soil applied quinclorac esters. It appeared that these esters might be superior to quinclorac for control of leafy spurge in the field if applied to the soil. It is also

possible that a combination of quinclorac and the appropriate odd/even ester applied to the soil might increase the efficacy by acting as a mechanism for the slow release of quinclorac from the ω/β-oxidation of the esters in soil. The efficacy of other carboxylic acid herbicides in varied weed species also might be enhanced by application as their *n*-alkyl esters to soil, dependent upon the ability of these esters to be metabolized via the ω/β-oxidation pathways.

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