MCPA (4-Chloro-2-ethylphenoxyacetate) Resistance in Hemp-nettle (*Galeopsis tetrahit* L.)

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The physiological basis for MCPA resistance in a hemp-nettle (*Galeopsis tetrahit* L.) biotype, obtained from a MCPA-resistant field population, was investigated. Dose−response studies revealed that the resistance factor for MCPA, based on GR_{50} comparisons of total dry weight of resistant (R) and susceptible (S) plants, was 3.3. Resistance factors for fluroxypyr, dicamba, 2,4-D, glyphosate, and chlorsulfuron were 8.2, 1.7, 1.6, 0.7, and 0.6, respectively. MCPA resistance was not due to differences in absorption, because both R and S biotypes absorbed 54% of applied [^{14}C]MCPA 72 h after treatment. However, R plants exported less (45 vs 58% S) recovered ^{14}C out of treated leaves to the apical meristem (6 vs 13% S) and root (32 vs 38% S). In both biotypes, approximately 20% of the ^{14}C recovered in planta was detected as MCPA metabolites. However, less of the ^{14}C recovered in the roots of R plants was MCPA. Therefore, two different mechanisms protect R hemp-nettle from MCPA phytotoxicity: a lower rate of MCPA translocation and a higher rate of MCPA metabolism in the roots. In support of these results, genetic studies indicated that the inheritance of MCPA resistance is governed by at least two nuclear genes with additive effects.

KEYWORDS: *Galeopsis tetrahit*; MCPA; fluroxypyr; auxinic herbicide; herbicide resistance; dose−response; radiolabeled herbicide; uptake and translocation; metabolism

INTRODUCTION

The introduction of the auxinic herbicides during the 1940s revolutionized modern agriculture and weed control. Furthermore, the ability of auxinic herbicides to selectively control dicotyledonous weeds in cereal crops and pastures has made them the most widely used group of herbicides in the world (*1*).

In many susceptible species, auxinic herbicides activate the metabolic processes that lead to ethylene accumulation (within 1−2 h after application), resulting in abnormal symptoms, for example, stem curling, tissue swelling, and leaf cupping (within 3−4 h). In cleavers, abscisic acid (ABA) accumulates shortly after ethylene (within 5−8 h). Growth and photosynthesis are inhibited within 24 h. Later, foliar senescence along with the formation of reactive oxygen species (ROS) causes destruction of cellular compartments and vascular integrity, ultimately resulting in plant death (*2, 3*). Often, continuous stimulation of the metabolic system results in the disruption of growth integrity. Also, cell division, growth, and differentiation in meristematic and cambial tissues occur at inappropriate times. This abnormal growth acts as a strong sink that depletes carbohydrates and proteins by diverting them from essential tissues, causing lethal damage to the vascular system, that is, phloem crushing (*2*). However, despite the extensive knowledge on the mode of action

of auxinic herbicide, their primary biochemical site of action remains unknown.

Prolonged and repeated use of the same herbicide or herbicides sharing the same target site imposes intense selection pressures that can result in the selection of herbicide-resistant weed biotypes (*4*). Even so, after 60 years of widespread and repeated use of auxinic herbicides, only 35 resistant biotypes within 24 species have developed resistance. This is a relatively low number compared to the hundreds of biotypes that have developed resistance to acetolactate synthase (ALS) and photosystem II inhibitors within 92 and 65 species, respectively (*5*).

Resistances to picloram and dicamba in wild mustard (*6, 7*), dicamba in kochia (*8*), picloram and clopyralid in yellow starthistle (*9, 10*) and quinclorac in false cleavers (*11*) cannot be attributed to differences in absorption, translocation, root exudation, or metabolism. These reports have resulted in many researchers suggesting that resistance to these herbicides may be due to changes at the site of action. This hypothesis was supported by other observations, for example, the production of ethylene in wild mustard and false cleavers, and ABA production in false cleavers was enhanced in the susceptible biotypes only, indicating that the primary target sites of auxinic herbicides or differences along the signal transduction pathway may be the basis for the resistance (*11, 12*). In dicamba-resistant and -susceptible biotypes of kochia, the expression of the key enzyme in ethylene production, 1-aminocyclopropane-1-car-

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Table 1. Herbicides and Dosage Ranges Used on Resistant and Susceptible Hemp-nettle Biotypes

herbicide	formulated product	IUPAC name	field rate (x), g of ai ha^{-1} (dose range)
MCPA- dimethylamine	MCPA-amine 500	dimethylamine salt of 4-chloro-2-methylphenoxyacetate	$X = 850$ (1/16x–64x)
2,4-D-dimethylamine	DMA 4 IVM	dimethylamine salt of 2,4-dichlorophenoxyacetic acid	$X = 850$ (1/4x–64x)
dicamba-dimethylamine	Banvel	dimethylamine salt of 3,6-dichloro-2-methoxybenzoic acid	$X = 1200$ (1/16x–64x)
fluroxypyr-methylheptyl	Vista	methylheptyl ester of 4-amino-3,5-dichloro-6-fluoro-2-pyrdiloxyacetic acid	$X = 200$ (1/64x–16x)
quinclorac	Accord	3,7-dichloroquinoline-8-carboxylic acid	$X = 125$ (1/16x–64x)
chlorsulfuron	GLEAN	2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazine-2-yl)amino]carbonyl]benzenesulonamide	30 (1/256x–4x)
glyphosate-isopropylamine	Roundup	isopropylamine salt of *N*-(phosphonmethyl)glycine	$X = 1250$ (1/256x–4x)

boxylic acid synthase, is increased 7- and 5-fold, respectively, 60 min after dicamba treatment (*13*). Thus, it is unlikely that reduced or altered ethylene production plays a role in dicamba resistance in kochia.

Inheritance mechanisms for resistance to auxinic herbicides vary with species and/or type of auxinic herbicide. For example, resistance results from one locus with a dominant allele in a dicamba-, picloram-, and 2,4-D-resistant wild mustard biotype (*14*, *15*). In contrast, a recessive allele is responsible for resistance to quinclorac in false cleavers (*16*) as well as to clopyralid and picloram in yellow starthistle (*17*). Furthermore, the inheritance of picloram and 2,4-D resistance in wild mustard is attributed to the same locus or closely linked loci, suggesting that cross resistance is operating (*1*, *15*).

Hemp-nettle (*Galeopsis tetrahit* L.), a member of the mint family (Labiatae) and a native of Eurasia, was introduced to North America in the 1800s. It is a self-fertile, annual species that overwinters only as seed (*18*). In North America, hemp-nettle (*G. tetrahit*) is a hetero-tetraploid with 32 chromosomes, that is, most likely a natural hybrid of *Galeopsis pubescens* and *Galeopsis speciosa*, with a haploid chromosome number of ($n + n'$) = 16 (*19*, *20*). Hemp-nettle is a noxious weed that commonly infests cereal crops in Europe and Canada (*18*, *21*, *22*). Chemical control of hemp-nettle depends on the use of the auxinic herbicides MCPA and fluroxypyr, the sulfonylurea herbicides chlorsulfuron and metsulfuron-methyl, or the photosystem II inhibitor metribuzin (*18*).

Hemp-nettle biotypes with resistance to sulfonylureas have been found both in Denmark (*23*) and in Manitoba, Canada (*5*). However, these cases of resistance have not yet been characterized. Recently, a MCPA-resistant biotype of hemp-nettle was found near Lacombe, AB, Canada, in a field that had been subjected to the following chemical treatments: 1994, metsulfuron and MCPA; 1995, linuron and MCPA; 1996, metribuzin and 2,4-D; 1997, metribuzin and MCPA; and 1998, fluroxypyr plus 2,4-D (as a mixture) and tralkoxydim (O'Donovan, personal communication).

The objectives of this study were to characterize the MCPA resistance of this hemp-nettle biotype and to examine potential cross-resistance to other herbicides. Studies were carried out to (1) quantify hemp-nettle's resistance to MCPA and six other herbicides, (2) examine the absorption, translocation, and metabolism of [^{14}C]MCPA in both resistant (R) and susceptible (S) biotypes as possible explanations for resistance, and (3) determine the inheritance of MCPA resistance in this biotype.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Resistant hemp-nettle seeds were collected during the fall of 1998 from a field near Lacombe, AB, that had been subjected to the following chemical treatments: 1994, metsulfuron and MCPA; 1995, linuron and MCPA; 1996, metribuzin

and 2,4-D; 1997, metribuzin and MCPA; and 1998, fluroxypyr plus 2,4-D (as a mixture) and tralkoxydim (O'Donovan, personal communication). Susceptible seeds were collected from a field outside Edmonton, AB. To confirm the resistance and susceptibility of the two respective biotypes, seeds were germinated and plants sprayed with 500 g of active ingredient (ai) ha^{-1} of MCPA (336 g L^{-1}) when the first and second pairs of opposite leaves were approximately equal in size. In these preliminary experiments, resistance and susceptibility were confirmed by visual assessment of injury.

Plants used for dose−response, uptake, translocation, and metabolism studies were germinated and grown in pots containing a commercial peat mixture, Promix PGX (Premier Brands, Brampton, Canada), whereas Promix BX was used to grow plants for seed production. All plants were grown in a controlled environment growth room maintained at 21/16 ± 1 °C day/night temperature, 16-h photoperiod, and an average relative humidity of 65%. Light intensity was constant at 350 μeinstein m^{-2} s^{-1}. Plants were grown as a single plant per pot (450 mL) and watered daily. Water-soluble fertilizer (20% N, 8% P$_2$O$_5$, 20% K$_2$O, and micronutrients, 20 g/l) was applied once a week to promote optimal growth.

Single plants from each biotype were self-pollinated by covering the whole plant with a perforated plastic bag to prevent cross-pollination. Self-pollinations were conducted for another five consecutive generations. Seeds from the third to the sixth generations of self-pollinated plants were used. Seeds were harvested when plants started wilting. The plants were manually threshed, and seeds were cleaned with a laboratory specific gravity separator and stored at 5 °C in a desiccator.

Dose−Response Experiments. When the first and second pairs of opposite leaves were approximately equal in size, plants were treated with commercial formulations of MCPA-dimethylamine, 2,4-D-dimethylamine, dicamba-dimethylamine, fluroxypyr-methylheptyl, quinclorac, chlorsulfuron, or glyphosate-isopropylamine (**Table 1**). Quinclorac treatments also contained the surfactant Merge (BASF, Limburgerhof, Germany), at 1% v/v, whereas chlorsulfuron and glyphosate were applied with the surfactant Agral 90 (DuPont, Mississauga, Canada) at 0.1 and 0.3% v/v, respectively. Plants were sprayed with a track sprayer (Mandel Scientific Corp., Guelph, Canada) equipped with a single 80015E flat-fan nozzle, mounted 55 cm above the top of the plant canopy, set to deliver 110 L ha^{-1} at 276 kPa. During the application, the soil was covered with vermiculite to prevent root uptake of the herbicides. The vermiculite was removed several hours after spraying. Plants were harvested 21 days after treatment (DAT). The soil was removed from the roots by gently washing them under flowing water. Plant material was dried for 72 h at 55 ± 5° C.

Radiolabeled MCPA. [^{14}C]MCPA, with a specific activity of 1.874 MBq μmol^{-1}, was dissolved in acetone (pesticide grade) and kept at −20 °C. Formulated MCPA-amine and [^{14}C]MCPA were combined to 7.86 mM in aqueous treatment solutions, which is equivalent to 212.5 g of ai ha^{-1} of MCPA-amine applied at 110 L ha^{-1}, a dose that generated the greatest difference between the responses of R and S plants (**Figure 2**).

Uptake and Translocation of [^{14}C]MCPA. Hemp-nettle plants were treated when they had two pairs of true leaves. Ten microliters of the treatment solution containing 2 kBq of [^{14}C]MCPA was applied to the adaxial side of the second pair of opposite leaves using a Wiretrol II 10 μL micropipet that delivered 0.5−1.0 μL droplets. Plants were

harvested 6, 12, 24, 48, and 72 HAT; TL were rinsed six times with 5 mL of 20% ethanol and 0.5% Tween 20 (enzyme grade) in water (v/v/v). Each plant was dissected into the TL, shoot above TL, shoot below TL, and root. Each plant part was wrapped with Kimwipe EX-L paper tissues (Kimberly Clark Inc., Roswell, GA) and then dried at 60 °C.

Unabsorbed radioactivity was determined by adding 15 mL of EcoLite (MP Biomedicals Inc., Irvine, CA) to each of the 5-mL rinsates. Radioactivity was assayed by liquid scintillation spectrometry (LSS) using a Beckman LS6K-SC scintillation counter (Beckman Instruments Inc., Fullerton, CA). Absorbed [14C]MCPA was determined by subtracting the unabsorbed [14C]MCPA from the total amount of [14C]MCPA applied. Results are expressed as a percentage of [14C]MCPA applied. Translocation of 14C was determined by combusting the various plant parts to 14CO2 using an OX300, R. J. Harvey oxidizer (R. J. Harvey Instrument Co., Hillsdale, NJ). Samples were combusted for 4 min, at 890 and 665 °C for combustion and catalyst zone, respectively, with a flow rate of 350 cm^3 min^{-1} for O2 and N2; 14CO2 was trapped in 15 mL of carbon-14 cocktail (R. J. Harvey Instrument Co.) and quantified by LSS. The recovery efficiency of 14CO2 by the biological oxidizer was >91%, as determined by combusting a known amount of d-mannitol-1-14C. Data for translocation are expressed as a percentage of 14C recovered *in planta*, and total recovery is expressed as a percentage of total 14C applied.

Metabolism of [14C]MCPA. Hemp-nettle plants at the first leaf stage were carefully removed from the soil. The roots were rinsed with tap water, and the plants were transferred to 50-mL plastic centrifuge tubes containing 40 mL of hydroponic solution. Each plant was held in place by a punched rubber sleeve stopper placed into the 12-mm hole in the tube cap. A fine air stream was bubbled continuously through the hydroponic solution. The hydroponic solution contained 1.15 g L^{-1} Plant-Prod 7:11:27 (N/P/K) fertilizer (Plant Product Co. Ltd., Brampton, Canada) and 1.072 g L^{-1} Ca(NO3)·4H2O, with the pH adjusted to 5.8. Plants were acclimated in the hydroponic system for at least 24 h before [14C]MCPA was applied.

Eight microliters of the herbicidal solution containing 12.5 kBq of [14C]MCPA was applied equally to the adaxial surface of the first leaf pair with a 50-μL syringe equipped with a repeating dispenser (Hamilton Co., Reno, NV), which delivered 1-μL droplets. Treated plants were harvested 0, 12, 24, 48, and 72 HAT and dissected into TL, shoot, and root. The treated leaves were rinsed with 30 mL of wash solution as previously described; two aliquots of 1 mL were taken from both the TL rinsates and the hydroponic solution and dissolved in 5 mL of EcoLite, and 14C was measured by LSS so the foliar uptake of [14C]MCPA and root exudation of 14C could be calculated.

Acetone extraction of 14C was immediately executed upon harvest. First, up to 0.6 g of plant material was placed in a 2-mL microcentrifuge tube, frozen in liquid nitrogen, and then pulverized to fine powder by shaking for 30 s at 30 Hz in a MM301 mixer mill (Retsch GmbH, Haan, Germany) using two or three conical stainless tissue-grinding beads (3 mm in diameter) per tube. Acetone (750 μL) was added, and samples were shaken again and centrifuged for 30 s at 15000 rpm. The dark green supernatant was separated from the pellet and designated extract 1. The pellets were resuspended in 1000 μL of acetone and centrifuged, and the supernatant was removed (extract 2). The content of 14C in extracts 1 and 2 was determined by analyzing 50-μL aliquots by LSS. To avoid chlorophyll quenching during LSS analyses, 50-μL aliquots of extract 1 from the TL and the shoot were combusted and 14C determined was as described previously. Data from extracts 1 and 2 were pooled, and the distribution of 14C in plant parts was determined.

Each remaining hydroponic solution was lyophilized to dryness for 72 h using Freeze Dryer 4.5 (Labconco Corp., Kansas City, MO). The dry powder was resuspended in 750 μL of acetone and filtered with a 0.2-μm nylon syringe filter (13-mm diameter). Radioactivity that remained in the solid material (unextractable) was estimated by combustion of air-dried pellets with a Harvey oxidizer. Samples were combusted for 3 min, under 800 and 625 °C temperatures of combustion and catalyst zone, respectively. Combustion efficiency was >98%. Radiolabeled MCPA was added to extracts from nontreated plants as a check for postextraction metabolism. All samples were kept at −20 °C until required for analysis. MCPA did not break down during storage.

The 14C-labeled metabolites and [14C]MCPA were separated by normal-phase thin-layer chromatography (TLC), using silica gel plates (20 × 20 cm aluminum backed; Whatman International Ltd., Kent, U.K.) as the stationary phase. The mobile phase was a mixture of methylene chloride, methanol, acetone, and glacial acetic acid (8:1:0.5:1 v/v/v/v, respectively). Portions (50−100 μL) of each extract solution were loaded with a Wiretrol II 50-μL micropipet, 2 cm above the bottom edge of the plate, in 14-mm-wide lanes. The loaded plates were air-dried (room temperature) and immediately placed, to a 5-mm depth, in mobile phase contained in a TLC developing tank. The development of the plates was continued until the mobile phase traveled 155 mm above the origin. Each lane was divided into 10-mm sections (16 per lane) starting 5 mm below the origin (i.e., the loading line). Each section was trimmed with scissors and placed in a 7-mL scintillation vial filled with EcoLite, and radioactivity was determined by LSS as previously described.

Recovered radioactivity in each of the TLC plate lanes was totaled, and the relative mobility (R_f) of each metabolite and MCPA was computed as the mean distance from the origin divided by the distance of the mobile phase front from the origin in that lane. The MCPA parent compound in each extract was identified by cochromatography with authentic [14C]MCPA dissolved in acetone as well as [14C]MCPA dissolved in untreated hemp-nettle extract.

Inheritance of MCPA Resistance in Hemp-nettle. R and S hemp-nettle plants from the third generation of self-pollination were reciprocally crossed to produce F1 progeny. The recipient flowers were emasculated after petal lobes were carefully separated using fine-point tweezers and scissors, and the premature anthers were clipped at the filament, before cleistogamy. The inner flower space, the pistil, the style, and especially the stigma were scanned visually for pollen. Pollen-contaminated flowers were excluded and detached. Pollen-free flowers were washed with 50 μL of 0.1 M phosphate buffer (pH 7.4) to minimize the possibility of fertilization by unseen pollen. Pollination was conducted 8−24 h after anthers were removed from the recipient flowers by fondling the stigma with pollen-lavished anthers from the donor plant. The whole process was conducted under a dissecting microscope. Unemasculated flower buds were removed on a daily basis, and recipient plants were covered with a perforated transparent bag. Pollen recipient, or female, plants included R 1, 3, 5, 6, and 7 and S 2 and 4, whereas R 13 and S 12 were male, or pollen donor, plants. Seeds were harvested at maturity.

A MCPA dose−response experiment was conducted with F1 S and R seedlings as described previously. In addition, F1 plants were self-pollinated and six F2 lines, 21, 22, 23, 41, 42, and 45, were established. Segregation of the resistance trait was then examined among F2 plants. F2 R and S plants were grown and treated at the second leaf stage with 425 g of ai ha^{-1} of MCPA-amine as described previously. Plants were not harvested; instead, 21 DAT the roots and shoots were each visually graded for herbicide injury on a scale of 0−10, where 0 indicated a dead plant or one that failed to flower, and 10 indicated a plant that had symptoms similar to those of a treated, vigorous R plant. The roots were scored by removing the root ball (soil and roots) from the pot, scoring them, and then placing the root ball in a new pot. The shoot and root scores were summed to generate a visual rating for each plant. Subsequently, for the sake of presentation, F2 survivors were sorted into five categories (i.e., 1−5) according to their visual ratings, whereas dead plants were categorized as 0.

Statistical Analyses. In all experiments, the experimental unit was a single hemp-nettle plant and the location in the growth room of each individual plant was chosen randomly. Each treatment, in any given experiment (except for F2 segregation test), was repeated five times (i.e., $n = 5$). Data were analyzed with SAS, version 8.2 (SAS Institute Inc., Cary, NC). The type I error rate was set to 0.05.

Initially, data from all experiments were subjected to analysis of variance (ANOVA), and residuals were analyzed using PROC MIXED, PROC GLM, and PROC UNIVARIATE to reveal the source of variance, determine if the residual status met the criteria for ANOVA, and identify outliers (*24*).

Dose−Response Experiments. Dose−response experiments were organized in a randomized complete block design (RCBD) and conducted twice (two blocks) except for the glyphosate experiment,
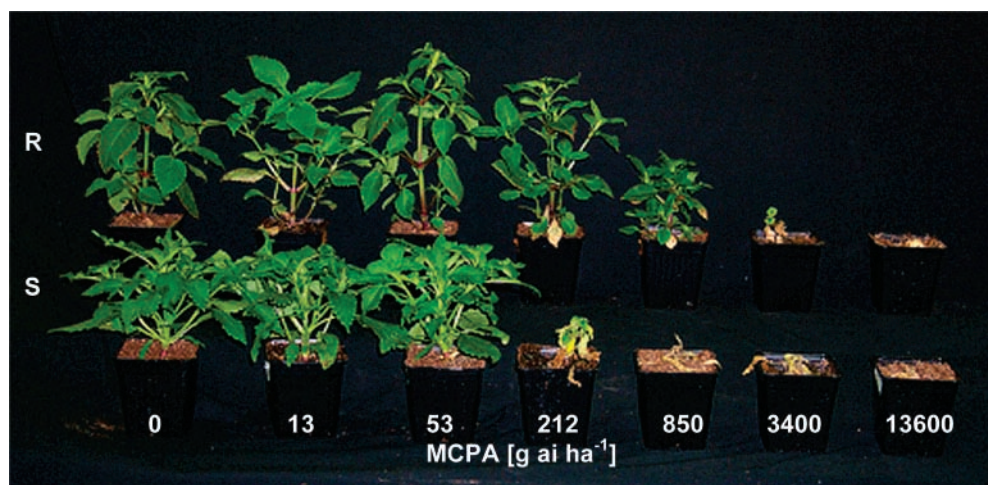
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Figure 1. Hemp-nettle plants 21 DAT with various doses of MCPA. Resistant (R) and susceptible (S) plants are in the top and bottom rows, respectively.

which was conducted once. Total DW per plant (shoot plus the roots) was expressed as a percentage of the mean of R or S plants treated with H_2O (dose = 0). The root parameter was the DW of the root expressed as percentage of total plant DW. Blocks were pooled upon lack of block by biotype interactions as indicated by ANOVA, and outliers were excluded from further analysis. Nonlinear regression (using PROC NLIN) analyses were employed to test the correlation of the data with the log-logistic model (eq 1), which related the response y to dose x with the following sigmoidal curve variables: C = lower limit, D = upper limit, b = slope, and GR_{50} = dose that results in 50% growth reduction (*25*).

$$y = C + \frac{D - C}{1 + \exp[b(\log{(x)} - \log{(I_{50})})]} \qquad (1)$$

The 95% confidence ranges of the GR_{50} values were used to determine if the R-GR_{50} was significantly different from the S-GR_{50} value. The level of resistance was determined by dividing the R-GR_{50} value by the S-GR_{50}, which generated the resistance ratio for each tested herbicide.

Uptake and Translocation of [^{14}C]MCPA. Uptake and translocation studies were organized as a three-factor factorial: (1) biotypes, (2) HAT, and (3) plant parts, arranged in a RCBD. Each treatment had five replicates, and experiments were conducted twice (two blocks).

Total ^{14}C recovered and uptake data were expressed as percentage of [^{14}C]MCPA applied, whereas translocation data were expressed as a percentage of the radioactivity recovered in planta. Blocks were pooled when block-related interactions were not significant. Translocation data did not have a normal distribution pattern, so they were subjected to a square root transformation to normalize the data prior to analysis of variance (ANOVA). Duncan's multiple-comparison procedures were employed to separate R and S means in each treatment by using PROC GLM.

Metabolism of [^{14}C]MCPA. The metabolism experiment was organized in a factorial design with (1) biotypes, (2) HAT, (3) plant parts, and (4) metabolites as factors. Metabolism data for [^{14}C]MCPA or ^{14}C-labeled metabolites are expressed as a percentage of total ^{14}C recovered in planta (including root exudation). The data were subjected to square root transformation prior to ANOVA. Duncan's multiple-comparison procedure was used to separate R and S mean values for the concentrations of MCPA, metabolites, or unextracted ^{14}C within each plant part at each harvest time.

Inheritance of MCPA Resistance. Dose−response experiments with R, S, and F_1 seedlings were analyzed as described previously in the dose−response experiment section.

The frequencies of plants that died before setting flowers (designated S phenotype) and plants that set flowers (designated R phenotype) after MCPA treatment (425 g of ai ha^{-1}) within F_2 lines were tabulated. Goodness-of-fit for null hypotheses of 1:3 or 1:15 phenotype ratios was determined using the chi-square (χ^2) test. The Yates correction

term was applied for each analysis of F_2 lines, and heterogeneity tests using χ^2 values were conducted without using the Yates correction term; subsequently, χ^2 tests were conducted on the pooled data using PROC FREQ (*24*).

RESULTS AND DISCUSSION

Dose−Response Experiments. R and S biotypes had different morphologies; the S biotype had a bushier and denser growth pattern with shorter internodes than the R biotype (**Figure 1**). Accumulation of dry matter was not different in untreated R and S biotypes. For example, 7-week-old R and S plants had total dry weights (DW) of 5.0 (±0.6) and 5.4 (±0.5) g (±SE), respectively. However, the proportion of the root DW to plant total DW was significantly lower in R, that is, 0.4 (±0.03) in R versus 0.5 (±0.01) in S. Furthermore, the germination rates of the original seed lots were 2 and 20% for the R and S biotypes, respectively, which improved to 40 and 95% during the breeding program.

Shortly after the application of MCPA, both R and S biotypes showed injury; that is, their petioles bent downward, plant growth was reduced, and affected leaves were curled. S plants treated with 850 g ha^{-1} and R plants treated with 13600 g ha^{-1} MCPA died 14 days after application. Occasionally, adventitious roots developed along the stem of the hemp-nettle plants in response to sublethal doses of MCPA. This pattern of growth may have diverted assimilates to the aerial parts of the plant and may have biased dose−response curves if data were expressed as a percentage of total shoot dry weight. Consequently, data were expressed as a percentage of total plant weight (i.e., root plus shoot).

The R biotype of hemp-nettle was at least 3-fold more resistant to MCPA than the S biotype with respect to GR_{50} comparisons based on total dry weight (438 vs 134 g of ai ha^{-1}) and >7-fold more resistant (1036 vs 110 g of ai ha^{-1}) with respect to GR_{50} comparisons based on root weight (**Figure 1**; **Table 2**). When dicamba was applied, there was no significant difference between GR_{50} values of R and S with regard to total plant DW; however, the root weights of R and S were significantly different and yielded a resistance ratio of 2.4 (**Table 2**). On the basis of total plant DW, R hemp-nettle was 8 times more resistant than S to fluroxypyr (**Table 2**); however, the responses of R and S roots to this herbicide were not different, with GR_{50} values being 68 and 39 g of ai ha^{-1}, respectively (**Table 2**).

There was no difference between the responses of the two biotypes to 2,4-D, quinclorac, chlorsulfuron, or glyphosate

Table 2. GR_{50}[a] Values, Their Minimum and Maximum 95% Confidence Limits in Parentheses, and Resistance Ratios (R/S) Based on Dry Weight of the Whole Plant[b] or Root[c] of Resistant and Susceptible Hemp-nettle Biotypes Treated with Various Herbicides and Harvested 21 Days after Treatment

herbicide	GR_{50} whole plant, g of ai ha^{-1}			GR_{50} root DW, g of ai ha^{-1}		
	R	S	R/S	R	S	R/S
MCPA	438[d] (347, 528)	134[d] (116, 152)	3.3	1036[d] (374, 1698)	110[d] (78,143)	9.4
2,4-D	1556 (1004, 2107)	976 (247, 1704)	1.6	NA[e]	NA	
dicamba	462 (320, 603)	271	1.7	1115[d] (950, 1280)	456[d] (345, 567)	2.4
fluroxypyr	185[d] (113, 258)	22.5[d] (14, 31)	8.2	68 (NA)	39 (32, 48)	
quinclorac	>2000[f]	>2000[f]		NA	NA	
chlorsulfuron	0.10 (0.08, 0.12)	0.14 (0.12, 0.16)	0.7	NA	NA	
glyphosate	34 (24, 44)	38 (27, 49)	0.9	55 (0, 152)	45 (0, 97)	1.2

[a] GR_{50} dose in grams of active ingredient per hectare that reduced growth by 50%. Values were calculated using SAS PROC NLIN to solve the log-logistic model at 95% level. [b] The whole plant parameter refers to the DW of the shoot plus the roots expressed as percentage of the mean of nontreated control plants. [c] The root parameter refers to the DW of the root expressed as a percentage of the DW of the plant. [d] GR_{50} values of R and S are significantly different because the 95% confidence limits of R and S do not overlap. [e] Not attainable; refers to dose responses that failed to meet the criterion for a sigmoidal curve, hence log-logistic analyses could not be conducted (NA). [f] GR_{50} values could not be computed with certainty.

(**Table 2**). This was expected for the two auxinic herbicides, because wild-type hemp-nettle populations are tolerant of 2,4-D and quinclorac. The GR_{50} values for 2,4-D were higher than the recommended field dose (**Tables 1** and **2**), and the total amounts of DW accumulated in R and S biotypes were approximately 80% of those of nontreated plants after treatment with 125 g of ai ha^{-1} quinclorac (data not shown). Conversely, both biotypes were susceptible to chlorsulfuron and glyphosate (**Table 2**).

Resistance ratios for R and S biotypes of 3.3 and 8.2 for MCPA and fluroxypyr, respectively, were similar to the 5.7 resistance ratio derived for dicamba-resistant kochia biotypes (*8*), but were quite low compared to resistance ratios for most other auxinic herbicide-resistant species. For example, dicamba resistance in a wild mustard biotype (*26*), quinclorac resistance in a false cleavers biotype (*16*), and picloram resistance in a yellow starthistle biotype were 104-, 43-, and 21-fold greater than in their respective S biotype (*9*).

Although the GR_{50} values for the R biotype treated with MCPA or fluroxypyr were lower than the field dose (850 and 200 g of ai ha^{-1}, respectively) for these herbicides (**Figure 1**), we observed that R plants that received the field dose of these herbicides were still able to reproduce. We have repeatedly observed that under controlled environment conditions herbicides are generally efficacious at lower doses than those used under field conditions. We suggest that this occurs because plants grown under these conditions have less-developed cuticles than do plants grown in the field and/or herbicide application is ideal when applied in a spray chamber, for example, no wind.

Uptake and Translocation of [^{14}C]MCPA. Similar to the results from the MCPA dose−response experiments, several hours after hemp-nettle plants were treated with 10 μL of 7.86 mM MCPA (i.e., equivalent to 212.5 g of ai ha^{-1} in 110 L of H$_2$O), which included [^{14}C]MCPA, stems bent, petioles of treated leaves bent downward, and leaves curled toward the abaxial side of the leaf. These symptoms were evident throughout the 72-h experiment.

Regardless of harvest time, the rates of [^{14}C]MCPA absorption were the same for R and S, with 54% of the applied [^{14}C]MCPA being absorbed 72 HAT (**Table 3**). The quantity of ^{14}C in TL decreased with time, with absorption being the greatest during the first 6 HAT. Between 6 and 72 HAT, the rate of ^{14}C exported out of the TL was greater than the rate of absorption of [^{14}C]MCPA by the TL. Approximately 20% less ^{14}C was exported from the TL of R than S plants 72 HAT (**Table 3**). Furthermore, acropetal movement of ^{14}C was 50% less in R

Table 3. Uptake, Distribution, and Total Recovery of ^{14}C in Resistant and Susceptible Hemp-nettle Treated with [^{14}C]MCPA[a]

plant part	biotype	time after treatment				
		6 h[b]	12 h	24 h	48 h	72 h
		% of ^{14}C applied to plant				
uptake[c]	R	26.4 (2.3)	29.3 (2.0)	41.0 (2.1)	48.5 (1.6)	54.4 (1.4)
	S	21.7 (2.2)	29.7 (2.2)	39.1 (2.6)	49.5 (1.9)	54.4 (1.8)
		% of ^{14}C recovered in planta				
treated leaf[d]	R	85.8[e] (1.2)	76.7[e] (1.8)	62.6 (1.5)	53.0 (1.9)	54.8[e] (2.1)
	S	79.7[e] (1.4)	70.2[e] (2.0)	55.1 (3.5)	48.2 (2.1)	41.8[e] (2.4)
shoot above TL	R	2.4[e] (0.6)	3.4[e] (0.6)	5.9[e] (0.3)	6.2[e] (0.6)	5.6[e] (0.6)
	S	5.7[e] (0.9)	8.3[e] (0.6)	13.7[e] (1.6)	12.1[e] (0.9)	12.9[e] (1.0)
shoot below TL	R	5.1 (0.5)	5.5 (0.6)	7.6 (0.9)	8.4 (0.8)	7.8 (1.0)
	S	3.9 (0.2)	5.5 (0.7)	6.8 (0.5)	7.6 (0.9)	7.0 (0.2)
roots	R	6.8[e] (1.0)	13.6 (1.1)	23.9 (1.1)	32.4 (1.7)	31.8[e] (1.2)
	S	10.7[e] (0.9)	16.0 (1.5)	24.4 (1.9)	32.1 (2.0)	38.3[e] (2.0)
		% of ^{14}C applied to plant				
recovery efficiency	R	90.7 (0.4)	89.0 (2.0)	85.8 (1.6)	80.9[e] (1.8)	80.5[e] (1.8)
	S	89.6 (1.1)	87.6 (1.9)	82.8 (0.8)	76.3[e] (0.6)	72.1[e] (0.8)

[a] Data (*n* = 10) are means with SE in parentheses of two experiments that were pooled. [b] Six-hour experiments were conducted only once with five replicates. [c] Statistical analyses were performed separately on uptake, translocation, and total recovery data. [d] Translocation data were subjected to square root transformation prior to ANOVA; however, nontransformed data are presented in the table. [e] Indicates significant difference between R and S within plant part at a particular harvest time based on Duncan's multiple-range test (α = 0.05).

than in S plants, with approximately 6 and 12% of the ^{14}C being recovered in the shoot above the TL of R and S plants, respectively, 72 HAT (**Table 3**). Regardless of the biotype, the root zone appeared to be the major sink for ^{14}C exported from the TL. The concentration of ^{14}C in roots of both biotypes was not different until 72 HAT, when 32 and 38% of the recovered ^{14}C accumulated in the roots of R and S plants, respectively (**Table 3**).

There may be some bias in the absorption and translocation results because the recovery of ^{14}C declined throughout the experiment from 90% of total [^{14}C]MCPA applied 6 HAT to 80 and 72% of applied 72 HAT of R and S plants, respectively (**Table 3**). Using similar application and rinsing methods, Van Eerd et al. (*11*) recovered >90% of [^{14}C]quinclorac applied to false cleavers at similar harvest times. Loss of [^{14}C]MCPA from the TL surface due to volatility is unlikely because [^{14}C]MCPA was applied as an acid (or salt) and has a low vapor pressure of ca. 2.3×10^{-5} Pa at 20 °C (*27*). It is more reasonable to believe

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Table 4. Metabolism of [^{14}C]MCPA in Various Parts of Resistant and Susceptible Hemp-nettle Plants Grown in Hydroponic Culture[a]

plant part	^{14}C category	biotype	12 HAT	24 HAT	48 HAT	72 HAT
			% of ^{14}C recovered in planta[b]			
treated leaf	parent	R	52.1 (2.2)	66.8 (4.7)	62.0[c] (3.6)	58.0 (3.5)
		S	47.7 (3.7)	56.2 (6.0)	47.7[c] (4.5)	50.0 (3.0)
	metabolites	R	8.2 (1.0)	6.1[c] (0.8)	6.4[c] (0.6)	6.5 (0.8)
		S	11.1 (1.2)	8.0[c] (0.2)	9.3[c] (0.9)	7.8 (0.5)
	unextracted	R	7.8 (0.4)	8.4[c] (0.8)	8.1 (0.7)	7.7 (0.2)
		S	7.7 (0.6)	6.0[c] (0.3)	7.0 (0.2)	7.0 (0.2)
shoot	parent	R	5.7[c] (0.7)	2.6[c] (0.5)	1.4[c] (0.1)	1.3 (0.1)
		S	10.2[c] (1.2)	5.8[c] (1.2)	3.3[c] (0.4)	1.5 (0.1)
	metabolites	R	2.1 (0.3)	1.3 (0.3)	2.2 (0.5)	2.2 (0.4)
		S	1.9 (0.2)	2.3 (0.5)	3.1 (0.6)	2.9 (0.4)
	unextracted	R	2.4[c] (0.2)	1.3 (0.2)	2.2 (0.4)	4.7 (0.7)
		S	1.5[c] (0.2)	1.4 (0.3)	2.7(0.5)	3.8 (0.3)
root	parent	R	11.7(1.0)	5.3 (1.1)	3.3[c] (0.4)	3.0[c] (0.3)
		S	10.5 (1.4)	5.5 (0.5)	6.9[c] (0.5)	5.1[c] (0.4)
	metabolites	R	6.0[c] (0.7)	3.7 (0.6)	4.8 (0.7)	10.1 (1.2)
		S	2.3[c] (0.4)	3.5 (0.8)	5.4 (1.0)	9.3 (1.0)
	unextracted	R	1.1 (0.1)	0.6 (0.1)	0.7[c] (0.1)	0.8 (0.1)
		S	1.3 (0.1)	0.9 (0.2)	1.3[c] (0.2)	1.1 (0.1)
root solution (exuded)	parent	R	6.7 (2.0)	6.3 (1.0)	6.4[c] (1.3)	4.9[c] (0.5)
		S	5.4 (0.7)	10.5 (2.0)	12.3[c] (0.6)	9.5[c] (1.3)
	metabolites	R	0.4 (0.1)	0.6 (0.2)	0.7 (0.2)	0.8[c] (0.1)
		S	0.4 (0.1)	1.0 (0.3)	1.3 (0.3)	2.1[c] (0.1)
recovery efficiency[d]	% of recovered ^{14}C	R	84 (3.2)	84 (3.2)	84 (3.2)	84 (3.2)
		S	84 (3.2)	84 (3)	84 (3.2)	84 (3.2)

[a] Prior to ANOVA, data were subjected to square root transformation, and analyses were conducted on each plant part separately. [b] Data are nontransformed means of five observations with standard errors in parentheses. [c] Indicates significant difference between R and S within a plant part based on Duncan's multiple-range test (α = 0.05). [d] Total recovery of [^{14}C] was consistent across harvest times and averaged 84% of applied [^{14}C]MCPA for both biotypes.

that root exudation accounted for the loss of ^{14}C. Using hydroponically grown plants, Van Eerd et al. (*11*) showed that R and S false cleavers exuded 8 and 17% of the absorbed [^{14}C]quinclorac, respectively, 96 HAT. Because the hemp-nettle plants of this study were grown in a soil medium, root exudation was not measured.

Metabolism of [^{14}C]MCPA. In the metabolism study, plants were grown hydroponically so [^{14}C]MCPA and its metabolites that were exuded from the roots could be quantified. Consequently, the total recovery of ^{14}C was consistent across harvest times and averaged 84% of applied [^{14}C]MCPA for both biotypes (**Table 4**).

The fate of [^{14}C]MCPA in hemp-nettle plants was categorized as either (1) parent compound, (2) metabolite(s), or (3) unextractable. The quantities of MCPA and its metabolites were determined after separation of the extracted radioactivity using normal phase silica gel TLC, whereas unextractable [^{14}C] (3) was determined by combusting the plant residue after extraction. The [^{14}C]MCPA standard was determined to have a relative mobility (R_f) of 0.95, whether it was dissolved in acetone or in an extract from untreated plants. The metabolites of [^{14}C]MCPA were more hydrophilic than the parent and had R_f values of 0.07, 0.35, and 0.7, with the former being the most abundant MCPA metabolite (data not shown). MCPA metabolism in plants occurs via (1) methyl hydroxylation followed by glycosylation to yield the *O*-glucoside of MCPA, (2) direct glucosylation of the carboxyl group to yield the glucosyl ester, and (3) amino acid conjugation to the carboxyl group (*28*). It is likely that the MCPA metabolites with R_f values of 0.35 and 0.7 were phase I metabolites, that is, ring or methyl hydroxylates, whereas the most hydrophilic metabolite (R_f = 0.07) was a result of phase II metabolism, that is, a sugar or an amino acid conjugate. All of these potential metabolites are regarded as being far less phytotoxic than MCPA (*28*). For the sake of the evaluation of MCPA metabolism in R and S hemp-nettle biotypes, the

proportions of the three MCPA metabolites (R_f values of 0.07, 0.35, and 0.70) were pooled and categorized as "metabolites".

The rates of MCPA uptake as determined in both the uptake/translocation and the metabolism experiments were similar. Furthermore, regardless of the harvest time, there was no difference between R and S in the total amount of [^{14}C]MCPA metabolites found in the whole plant (**Table 4**). Approximately 66−78% of the total ^{14}C recovered in planta was [^{14}C]MCPA at all harvest times (**Table 4**). Similarly, there were no differences between R and S biotypes with respect to the proportion of unextracted ^{14}C found in the whole plant (**Table 4**). Over 60% of the ^{14}C recovered in planta was found in the TL of both biotypes, most of it being MCPA. However, there was 18−32% more [^{14}C]MCPA in the TL of R than of S plants (**Table 4**). Conversely, quantities of [^{14}C]MCPA outside the TL were significantly lower in R than in S plants. This was especially true in the shoot from 12 to 48 HAT and in the root from 48 to 72 HAT (**Table 4**).

More of the ^{14}C exported out of the TL of S (33 vs 21% for R) plants was found in the nutrient solution, thus indicating why the recovery of radioactivity was lower in S than in R plants used in the uptake/translocation experiment (**Table 3**). In both R and S biotypes, >80% of the exuded ^{14}C remained as [^{14}C]MCPA (**Table 4**). When the radioactivities recovered from the roots and the hydroponic solution were combined (total root zone), less ^{14}C was found in R than in S, that is, 20 versus 27% of the total ^{14}C recovered in planta, respectively, 72 HAT (**Table 4**). The percentage of [^{14}C]MCPA metabolites in the root zone was significantly greater than the percentage of metabolites in the shoot. For example, 72 HAT, 55 and 43% of total ^{14}C recovered from the root zone were detected as metabolites versus 11 and 15% in the aerial portion (TL plus shoot) of R and S plants, respectively (**Table 4**; **Figure 2**). Furthermore, the quantity of [^{14}C]MCPA metabolites in the root zone of R was, on average, 46% greater than in the S biotype (**Figure 2**).
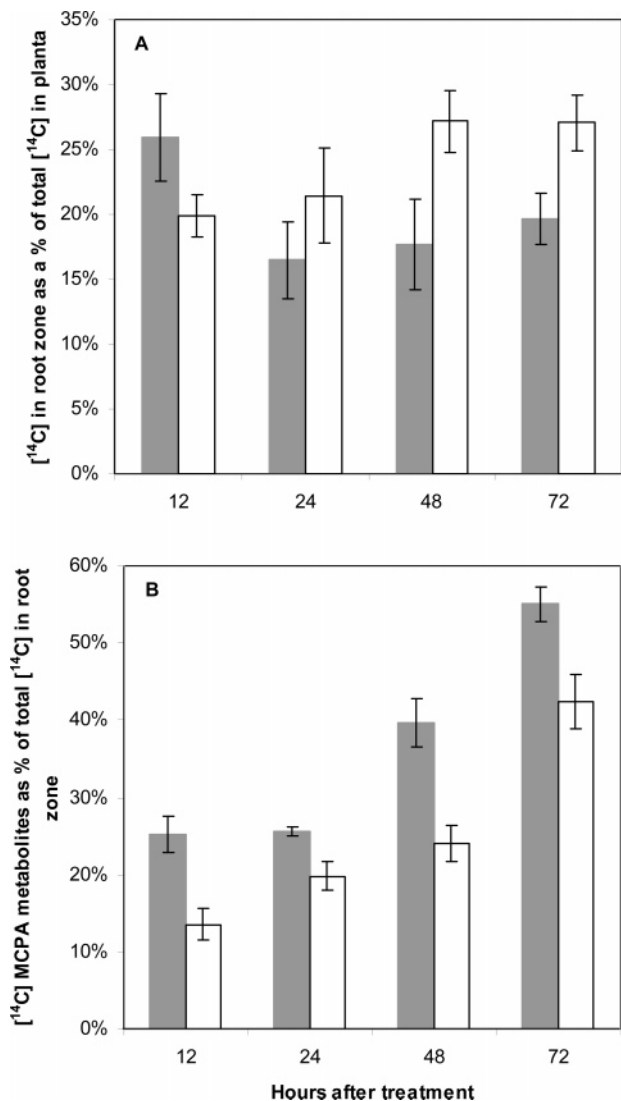
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Figure 2. Accumulation of (**A**) ^{14}C (R, gray bars; S, white bars) and (**B**) [^{14}C]MCPA metabolites (R, gray bars; S, white bars) in the root zone (root plus the root exudates). Data for [^{14}C]MCPA metabolites are expressed as a percentage of total ^{14}C recovered in the root zone, whereas the ^{14}C data are expressed as a percentage of total ^{14}C recovered in planta. Analysis of variance and mean comparison revealed there were significantly more metabolites in the root zone of R than S, 12, 24, 48, and 72 HAT, whereas accumulation of ^{14}C in S roots was significantly greater than in R roots, at only 72 HAT. Bars represent standard error of the means.

Root exudation may be due to membrane leakage of MCPA from injured cells as suggested by Achhireddy et al. (*29*). Thus, the greater exudation of radioactivity from the roots of S versus R plants is probably caused by MCPA injury to root cells of S. This supposition is supported by the fact that more MCPA was found in the hydroponic solution of S than of R plants. Furthermore, <2% of the ^{14}C found in the hydroponic solutions of both biotypes was metabolized MCPA.

Approximately 65% of the total ^{14}C exported out of TL moved to the root system (**Tables 3** and **4**). These results agree with research on MCPA-susceptible species of broad bean, where similar proportions of MCPA translocated to the root, but conflict with results from MCPA-tolerant species of maize and chickweed, where <33% of the [^{14}C]MCPA translocated basipetally to the root (*29*). Furthermore, it appears that a combination of reduced translocation of MCPA to the apical
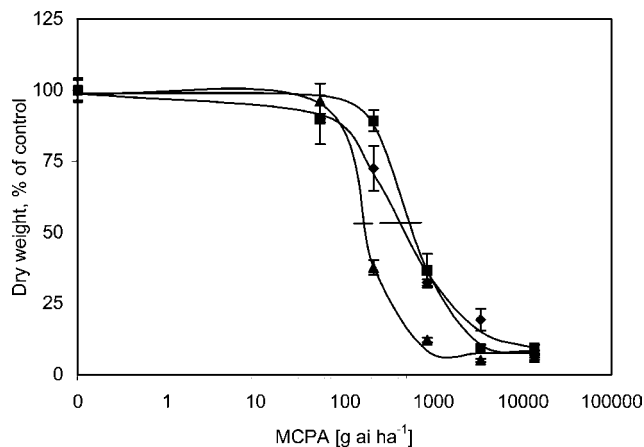

Figure 3. Responses of R (◆), S (▲), and F_1 (■) to ascending doses of MCPA and their respective predicted log-logistic curves. Data points are means of five replicates. GR_{50} values, and their minimum and maximum 95% confidence limits in parentheses, are 424 (252–596) for R, 166 (129–203) for S, and 575 (431–721) g of ai ha^{-1} for F_1. Vertical and horizontal bars represent standard error of the means and 95% confidence limits of GR_{50} values, respectively.

meristem and the roots, coupled with a greater rate of MCPA degradation in the roots of R versus S plants, accounts for the 3.3- and 7.3-fold reductions of MCPA phytotoxicity to the whole R plant and its roots, respectively.

Inheritance of MCPA Resistance in Hemp-nettle. Reciprocal crosses of R and S inbred lines yielded two batches of seed; however, only F_1 seed that originated from the female S parent germinated. Plants from R and S lines as well as the F_1 grew uniformly; however, the F_1 had a more vigorous growth pattern than the R and S lines. The dry weights of untreated R, S, and F_1 plants were 6.3 (±0.2), 5.7 (±0.2), and 7.1 (±0.3) g (±SE), of which 44% (±1), 57% (±1), and 53% (±1) of the total dry weight was attributable to the roots, respectively, 7 weeks after germination. Morphologically, the S line was bushier with shorter internodes along the main stem than the R and F_1 lines. F_1 plants had bigger leaves than did S and R plants. These data indicate that the F_1 exhibited hybrid vigor or heterosis.

R, S, and F_1 plants were compared for sensitivity to MCPA at doses ranging from 53.1 to 13600 g of ai ha^{-1}. On the basis of total plant dry weight, the dose−response curves of R and F_1 plants were similar and overlapped, that is, their GR_{50} values and 95% confidence intervals (in parentheses), which were 424 (252−596) and 575 (431−721) g of ai ha^{-1}, respectively, were not significantly different (**Figure 3**). Conversely, the GR_{50} of the S plants, that is, 166 (129−203) g of ai ha^{-1}, was significantly lower than those for the R and F_1 plants (**Figure 3**). Injury symptoms in hemp-nettle biotypes after MCPA treatment were the same as those described previously. The fact that F_1 seeds were the result of crosses where the R biotype was the pollen donor and F_1 plants were resistant to MCPA indicates that the resistance trait was nuclear-encoded. In addition, because the GR_{50} values of R and F_1 plants were the same, it is likely that the MCPA resistance is a dominant trait.

The inheritance of the MCPA-resistance trait was further evaluated using F_2 progeny. R, S, and F_2 progeny plants from six F2 lines (21, 22, 23, 41, 42, and 45) were treated at the second-leaf stage with 425 g of ai ha^{-1} of MCPA-amine, a dose that caused 50 and 80% reductions in the dry weight of R and S hemp-nettle plants, respectively. This dose was expected to act as a good discriminator, because the largest difference between the responses of R and S lines was observed at this
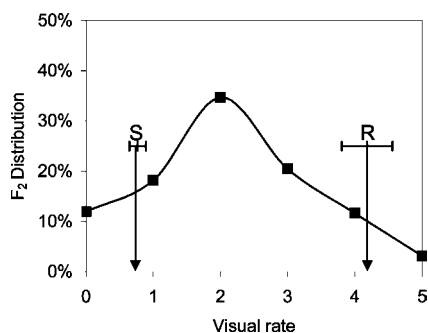
Figure 4. Distribution of F_2 (■) plants based on the combined visual ratings of the shoot plus root, 21 days after MCPA treatment (425 g of ai ha^{-1}). Arrows indicate the mean responses of R and S plants with their standard errors. Plants were rated 0 if they died before they set flowers. R, $n = 11$; F_2, $n = 452$; S, $n = 13$.

dose (**Figure 3**). On the basis of visual ratings conducted 21 DAT, the mean responses of the six F_2 lines were uniform (across lines) and fell between the means of R and S lines (data not shown). In addition, after treatment with MCPA (425 g of ai ha^{-1}), approximately 0, 30, and 12% of R, S, and F_2 plants died before setting flowers, respectively. The proportion of dead F_2 plants (12% of total 452 tested plants) was between classical Mendelian segregation ratios of 1:3 for a single, dominant locus and 1:15 for two unlinked, dominant loci. Nevertheless, χ^2 analyses under one- and two-gene models (i.e., 1:3 and 1:15 ratios for F_2 plants that died before setting flowers vs those that set flowers, respectively) could support neither of these hypotheses. In addition, the response of F_2 plants to MCPA treatment resulted in a unimodal bell-shaped curve that skewed slightly toward the left (i.e., the S side) (**Figure 4**). The shape of this F_2 distribution suggested that multiple genes with additive effects governed MCPA resistance in hemp-nettle (*30*).

The MCPA dose−response curves and GR$_{50}$ values were not significantly different between F_1 and R plants (**Figure 3**) and suggested that MCPA resistance in hemp-nettle was a dominant trait. However, if MCPA resistance was a qualitative dominant trait, F_2 plants should fall into a bimodal distribution with a major cluster close to the response of the R biotype and a minor cluster close to the S biotype (*31*). Alternatively, if a single gene with an incomplete dominance mechanism governs MCPA resistance, then a sufficiently large F_2 population should be trimodal with peaks representing a 1:2:1 distribution of S, intermediate, and R phenotypes, respectively (*30*). However, because ca. 70% of the S plants set flowers and R plants had a high degree of variability after treatment with MCPA (425 g of ai ha^{-1}) (**Figure 4**), the interpretation of the results of the segregation of F_2 plants was difficult. Nevertheless, the visual ratings of the F_2 plants revealed a unimodal distribution (**Figure 4**), which suggests that MCPA resistance was likely governed by a quantitative genetic mechanism.

Both the physiological and inheritance data supported the conclusion that the relatively small increase in resistance (3 times) was due to two separate physiological mechanisms, that is, reduced translocation and enhanced root metabolism of MCPA, and suggest that more than one genetic locus with additive effects was involved. Furthermore, the dry weight of F_1 plants was greater than both parental biotypes and indicates heterosis. Evidence linking heterosis with enhanced herbicide resistance is rare; nevertheless, it was found among maize hybrids with regard to increasing atrazine resistance (*32*). Furthermore, the inheritance of pendimethalin resistance in soybean cultivars was found to be under quantitative genetic control (*33*). These studies (*32, 33*) support the notion that

MCPA resistance in hemp-nettle may be a quantitative trait. Such polygenic resistance mechanisms likely evolve under moderate selection pressures (*34*). Indeed, the R hemp-nettle was subjected to a moderate MCPA selection pressure because MCPA had been applied in three of five years prior to the discovery of the R biotype in 1998.

In conclusion, this is the first study of auxinic herbicide resistance in hemp-nettle. The work presented here suggests that differences in translocation and metabolism account for the marginal MCPA resistance of the R biotype, making this the first example of low-level herbicide resistance that has a complex mechanism, that is, one governed by two or more nuclear genes.

ABBREVIATIONS USED

ABA, abscisic acid; 2,4-D, 2,4-dichlorophenoxyacetate; DAT, days after treatment; DW, dry weight; GR$_{50}$, dose (g of ai ha^{-1}) that results in 50% growth reduction, equivalent to I_{50}, the midpoint between the upper limit (*D*) and the lower limit (*C*) of the log-logistic model (eq 1); HAT, hours after treatment, LSS, liquid scintillation spectrometry; MCPA, 4-chloro-2-ethylphenoxyacetate; R, MCPA-resistant hemp-nettle biotype; S, MCPA-susceptible hemp-nettle biotype; TL, treated leaf.

ACKNOWLEDGMENT

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