JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Investigation of MCPA (4-Chloro-2-ethylphenoxyacetate) Resistance in Wild Radish (*Raphanus raphanistrum* L.)

Mithila Jugulam

Department of Agronomy, Kansas State University, Manhattan, Kansas 66506, United States

Natalie DiMeo

School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Linda J. Veldhuis

School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Michael Walsh

School of Plant Biology M086, University of Western Australia, 35 Stirling Highway, Crawley, Western Australia 6009, Australia

J. Christopher Hall*

School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

ABSTRACT: The phenoxy herbicides (e.g., 2,4-D and MCPA) are used widely in agriculture for the selective control of broadleaf weeds. In Western Australia, the reliance on phenoxy herbicides has resulted in the widespread evolution of phenoxy resistance in wild radish (*Raphanus raphanistrum*) populations. In this research the inheritance and mechanism of MCPA resistance in wild radish were determined. Following classical breeding procedures, F_1, F_2, and backcross progeny were generated. The F_1 progeny showed an intermediate response to MCPA, compared to parents, suggesting that MCPA resistance in wild radish is inherited as an incompletely dominant trait. Segregation ratios observed in F_2 (3:1; resistant:susceptible) and backcross progeny (1:1; resistant to susceptible) indicated that the MCPA resistance is controlled by a single gene in wild radish. Radiolabeled MCPA studies suggested no difference in MCPA uptake or metabolism between resistant and susceptible wild radish; however, resistant plants rapidly translocated more ^{14}C-MCPA to roots than susceptible plants, which may have been exuded from the plant. Understanding the genetic basis and mechanism of phenoxy resistance in wild radish will help formulate prudent weed management strategies to reduce the incidence of phenoxy resistance.

KEYWORDS: *phenoxy herbicides, backcross, herbicide resistance, herbicide uptake/translocation, introgression, single incompletely dominant gene*

INTRODUCTION

Wild radish is a prevalent annual weed in Australian cropping systems. This weed is remarkably successful, due to its biological characteristics of flexible life cycle, prolific seed production, and seed dormancy.[1] In particular, considerable genetic diversity within this weed species[1,2] has enabled wild radish to be successful across several agro-ecosystems of the southern Australian dry-land-crop-production region. A number of herbicides with different modes of action have been used to selectively control this weed in Australian cereal cropping systems. However, for many years, auxinic herbicides (e.g., 2,4-D, MCPA, dicamba) have remained the herbicides of choice of growers to control wild radish populations in these systems. This reliance has resulted in the evolution of resistance to these herbicides in wild radish populations.[3] Specifically, continuous exposure to phenoxy herbicides (e.g., 2,4-D and MCPA) in rotation for over 17 years in Western Australia resulted in evolution of phenoxy-resistant populations of wild radish.[3]

2,4-D and MCPA were the first group of selective organic herbicides developed during World War II, and their discovery led to a significant increase in 2,4-D production. Consequently, the use of 2,4-D in cereal crops revolutionized agriculture production throughout the world. Because of their selectivity, efficacy, wide spectrum of weed control, and low application costs, the demand for auxinic herbicides has remained, despite the introduction of more efficient herbicides (e.g., glyphosate, triazines, and acetolactate synthase (ALS) inhibitors). More

Received: September 12, 2013
Revised: November 29, 2013
Accepted: December 3, 2013
Published: December 3, 2013

dx.doi.org/10.1021/jf404095h | J. Agric. Food Chem. 2013, 61, 12516−12521

recently, this demand has increased with the widespread and increasing evolution of resistance in weed species to triazine and ALS inhibitors.[4]

Numerous studies were conducted to understand the mode of action of auxinic herbicides; nonetheless, the precise mechanism is yet to be uncovered. Physiological and biochemical evidence indicates that, in sensitive dicots, auxinic herbicides affect cell division, growth, and differentiation of meristematic tissues as a result of excessive loss of proteins and carbohydrates, leading to damage to the vascular system.[5] Furthermore, it has also been reported that the sensitivity to auxinic herbicides is also due to unregulated auxin response and lethality is due to hyperaccumulation of ethylene, abscisic acid (ABA), and reactive oxygen species (ROS).[6]

Herbicide resistance results from repeated selection for resistant individuals in field populations, and the resistant plants slowly increase in the population in the presence of continued herbicide application, i.e., selection pressure.[7] Herbicide-resistant weed populations offer excellent model species for investigation of the genetic basis and mechanism of herbicide resistance to these compounds. Understanding the genetic basis (i.e., inheritance) of herbicide resistance will help further our understanding of the evolution as well as spread of resistance alleles in a population. It was demonstrated that the inheritance of herbicide resistance to the majority of herbicide families, including for auxinic herbicides, is controlled by a single gene.[8] For example, dicamba, picloram, and 2,4-D resistance in *Sinapis arvensis*,[9,10] paraquat resistance in *Conyza bonariensis*,[11] and paraquat resistance in *Erigeron canadensis*[12] are determined by a single dominant nuclear gene.

In this research, inheritance as well as mechanism of wild radish resistance to MCPA was investigated following classical genetic approaches as well as using ^{14}C-MCPA. The specific objectives of this research were to (1) develop homozygous MCPA-R and -S lines and generate F_1, F_2, and backcross progeny, and (2) conduct ^{14}C-MCPA uptake, translocation, and metabolism studies.

MATERIALS AND METHODS

Development of Wild Radish Parental MCPA-Resistant (R) and -Susceptible (S) Homozygous Lines. MCPA-R and -S wild radish plants were grown from seeds collected from fields (<20 km apart) in the northern wheat-belt region (latitude 28.33°S, longitude 114.96°E) of Western Australia. The R (WARR-6) biotype was collected from a field where it was exposed to frequent auxinic herbicide applications over at least a17-year period during which lupine (*Lupinus angustifolius* L.) and wheat (*Triticum aestivum* L.) crops were grown continuously in rotation.[3] The MCPA-S (WARR-7) wild radish population was collected from a field with no known herbicide selection. The seeds were sown in 10-cm plastic pots (one seed per pot) containing Promix and were placed in a growth chamber with a 16-h photoperiod and 22/15 °C day/night temperature. The light intensity and relative humidity were maintained at 350 μmol·s^{-1}·m^{-2} and 65−75%, respectively. Plants were irrigated when required and were fertilized weekly with 20:20:20 (N:P:K).

Ten plants each of WARR-6 and WARR-7 field populations were selected to perform self-pollinations. At the commencement of the reproductive phase, WARR-6 and WARR-7 populations were separated and isolated to prevent external cross pollination. Bud pollination was performed to overcome self-incompatibility.[10] Emasculation (i.e., removal of immature anthers before pollen dehiscence) and pollination were performed at various times of the day (from 8 a.m. to 5 p.m.). Four to five unopened flower buds (3−4 mm in size and ∼3 days prior to complete blooming) on four to five racemes (inflorescences) on each of wild radish R and S plants were chosen for self-pollinating. The flower buds were emasculated without damaging the stigma by using sharp-edged forceps. All other flower buds on the racemes were removed. The dehisced anthers from other flowers of the same plant were collected and the pollen was transferred onto the stigmas of the emasculated flower using sterile forceps. Immediately after pollination, the racemes were covered with 20 × 8 cm pollination bags (Lawson 217, IL, USA) to avoid pollination from other plants. The self-pollinated flowers were labeled appropriately. Silique formation from successful self-pollination could be observed a week after pollination, and subsequently, the bags were removed from the racemes. Four to five weeks after pollination, mature siliques were harvested and seed was collected. The seed was planted (∼1−2 weeks after harvest) and seedlings were grown in a growth chamber as described previously. When the seedlings were at the three to four leaf stage of development, they were sprayed with 500 g ae (acid equivalence)/ha MCPA (recommended field rate) to identify the genotype of the original plant based upon the segregation of plants to R or S. Only homozygous plants were used for genetic crosses and resistance mechanism studies.

Generation of F_1, F_2, and Backcross Wild Radish Progeny. Plants to generate F_1, F_2, and backcross progeny were raised in a growth chamber (as described previously). The F_1 plants were generated from reciprocal crosses (R × S and S × R) between homozygous MCPA-R and -S plants. Procedures (emasculation and pollination) similar to those described previously were followed to perform reciprocal crosses. Although emasculation and pollinations were performed at various times of the day, the time of pollination did not affect the success of the crosses (data not shown). Four to five weeks after pollination, mature siliques were harvested and the F_1 seed from each cross was harvested separately from R and S plants. After screening the F_1 progeny, the F_2 progeny was generated by self-pollinating the F_1 plants. Backcross progeny were developed by performing crosses between a heterozygous MCPA-R, F_1 hybrid, and a homozygous recessive S parent. Mature seed was harvested from the S plants.

F_1 Dose−Response Experiments To Test for MCPA Resistance. MCPA dose−response experiments were conducted using seeds of parental MCPA-S, MCPA-R, and F_1 hybrids derived from both R × S and S × R crosses. Seeds were planted and seedlings were raised as described previously. When the seedlings were at the three to four leaf stage of development, 10−15 seedlings were treated with MCPA with a range of doses using a motorized hood sprayer: 0, 50, 100, 250, 500, 750, and 1000 g ae/ha. The sprayer was equipped with a flat-fan nozzle (8002 E) and calibrated to deliver 200 L/ha at 276 kPa. One and two weeks after MCPA treatment, the seedlings were visually rated for injury, and classified as R or S by comparing the injury response with those of seedlings from the R and S parental populations. Susceptibility of plants to MCPA was assessed based on mortality and the severity of symptoms (epinasty) followed by death of the plants. Furthermore, 3 weeks after treatment (WAT) four plants from each dose were harvested (above ground). The samples were dried at 60 °C for 48 h, and the dry weights were determined. The remaining plants from each dose were maintained for self-pollination to generate F_2 progeny.

Dose−Response Experiments To Screen F_2 and Backcross Progeny for MCPA Resistance. The F_2 progeny generated by self-pollinating the F_1 plants, and backcross progeny generated by crossing the F_1 plants with S plants, were screened for MCPA resistance. Experimental and screening procedures similar to those described for screening of F_1 progeny were used. The F_2 progeny were treated with 0, 50, 100, 250, and 500 g ae/ha doses of MCPA, whereas the backcross progeny were treated with the field dose (i.e., 500 g ae/ha) of MCPA.

MCPA Uptake and Translocation Experiments. Radiolabeled MCPA was used for uptake, translocation, and metabolism experiments. The ring labeled [^{14}C]MCPA ([ring-U-^{14}C]MCPA, CC-382, Institute of Isotopes Co., Ltd., Budapest, Hungary), with a specific activity of 1000 MBq/mmol, was dissolved in acetone and kept at −20 °C. All chemicals used were of reagent quality or better. Homozygous MCPA-R and -S were used in these experiments. R and S plants were

raised under growth room conditions, as described previously except that the plants were grown in Turface (a porous plant growth medium; Plant Products Co. Ltd., Brampton, ON, Canada). When seedlings were at the three to five leaf stage of development, they were treated with 250 g ae/ha technical grade MCPA. Subsequently, 5 μL of ^{14}C-MCPA containing 3.3 kBq was applied as 1 μL droplets with a 10 μL micropipet to the adaxial surface of the fourth leaf. Plants (four replicates) were harvested 6, 24, 48, and 72 h after treatment (HAT).

At each harvest time, plants were dissected into root, treated leaf, and nontreated foliage above and below the treated leaf. At the time of harvest, the amount of ^{14}C-MCPA present on the surface of the treated leaf was determined using a foliar rinse treatment (i.e., with a stream of wash solution); 10 mL of aqueous 10% (v/v) ethanol containing 0.5% (v/v) Tween 20 was used to rinse the leaf surface. The rinse solution was collected in two 22-mL scintillation vials containing 5 mL of Ecolite (+) scintillation cocktail. Radioactivity was quantified by liquid scintillation spectrometry (LSS) using a Beckman LS6K-SC scintillation counter. Each plant part was wrapped in tissue paper and dried at 60 °C for 48 h. The quantity of radioactivity in each plant part was determined by combustion of samples to $^{14}CO_2$ using an oxidizer (Model OX-500; R. J. Harvey Instrument Corp., Hillsdale, NJ, USA). $^{14}CO_2$ was trapped in a carbon-14 scintillation cocktail. $^{14}CO_2$ recovery was >97% as determined by combusting known quantities of ^{14}C-D-mannitol (^{14}C was labeled uniformly around the ring structure).

MCPA Metabolism Experiments. R and S plants were grown, and treated with ^{14}C-MCPA (described previously) for uptake and translocation experiments. After a foliar rinse of the treated leaf, the whole plant was harvested 48 HAT and stored individually at −20 °C in aluminum foil until extraction. The MCPA did not break down during storage (because HPLC data indicated no conversion into other compounds). The frozen plant tissue was ground with pestle in a mortar containing acetonitrile/water (7:3, v/v). The ground tissue was filtered through Whatman No. 1 filter paper. The filtrate was transferred into a glass test tube. The filter paper and vacuum flask were rinsed with approximately 5 mL of acetone and added to the filtrate. The filtrate was evaporated under air in a 40 °C water bath until 0.5−1 mL remained in the test tube, prior to being transferred to a 2-mL microcentrifuge tube. The test tube was rinsed with approximately 0.5 mL of acetone two to three times and was added to the microcentrifuge tube. Each microcentrifuge tube was spun at 18 300 rcf for 4 min and the supernatant was transferred to a new microfuge tube and stored at −20 °C until it was analyzed by high-pressure liquid chromatography (HPLC).

A 100-μL portion of plant extract was analyzed by reverse phase HPLC on an Agilent 1100 series HPLC (Agilent Technologies Inc., Alpharetta, GA, USA) equipped with a Thermo Scientific Hypersil BDS 3 μm C18 HPLC column (3 μm particle size; 4.6 × 100 mm; catalog no. 03-050-116, Fisher Scientific Company, Ottawa, ON, Canada). The three mobile phases were A, B and C, where A was 100% acetonitrile, B was 100% methanol, and C was 0.1% formic acid in water. The chromatographic conditions started with 0% A, 20% B, and 80% C and over 15 min pumped in a linear gradient to 90% A, 5% B, and 5% C. From 15 to 18 min, the mobile phases were maintained at 90% A, 5% B, and 5% C. Between 18 and 18.20 min, the mobile phases were returned in a linear gradient to the original conditions of 0% A, 20% B, and 80% C, which was maintained from 18.2 to 25 min. The flow rate was 0.1 mL/min. The column temperature was maintained at ambient room temperature (23 °C). [^{14}C]MCPA and its radiolabeled metabolites were detected and quantified by a Radioflow Detector LB 508 with a type Z cell (LB 508, Berthold Technologies GmbH & Co. KG, Bad Wildbad, Germany) with a cell volume of 1000 μL. The counting efficiency was 80%.

Weed Harvest and Statistical Analysis. *Growth Room, Dose−Response, and Radiolabeled Experiments.* Mortality and shoot dry weight were determined 21 DAT (days after treatment) for wild radish parental R, S, and F_1 (R × S and S × R) progeny. For determining the dry matter production, the above-ground plant material was harvested from at least four plants and dried in paper envelopes at 68 °C for 2−3 days. Furthermore, visual rating and phytotoxic symptoms were determined for R, S, F_1, F_2, and backcross progeny 21 DAT.

Statistical Analyses. All experiments were conducted twice. Frequencies of R and S phenotypes were tabulated for F_1, F_2, and backcross progeny. Chi-square (χ^2) tests were performed to determine the goodness of fit to specific genetic ratios. In addition, homogeneity χ^2 tests[13] were performed to determine if data could be pooled. Data from dose−response experiments were analyzed using a nonlinear regression model with the drc package in R[4].[14] Dose−response models were constructed using the following.

$$y = c + \frac{d - c}{1 + \exp\{b[\log(x) - \log(GR_{50})]\}} \quad (1)$$

In the four-parameter logistic model eq 1, b is the slope of the curve, c is the lower limit, d is the upper limit, and GR_{50} is 50% reduction in dry biomass.

Radioactivity was determined by oxidative combustion of all samples and expressed as percent of $^{14}CO_2$ recovered. The data was subjected to an analysis of variance (ANOVA; $P \leq 0.05$).

RESULTS AND DISCUSSION

Progeny from two WARR-6 self-pollinated plants did not show any segregation and all plants survived 500 g ae/ha MCPA application, implying that the genotype of these plants is homozygous for MCPA resistance. Of progeny from the eight remaining self-pollinated WARR-6 plants screened with MCPA (500 g ae/ha) there was segregation of R or S, suggesting that the genotype of these plants was heterozygous. All WARR-7 plants tested were homozygous susceptible. It was observed that the homozygous R and S plants had slightly different morphologies. The R plants were shorter with darker green leaves, whereas S plants had larger, light green leaves. The difference in morphology of R and S plants could be attributed to the extensive genetic variability present within wild radish species.[1,2]

Response of Parental, F_1, F_2, and Backcross Progeny to MCPA. Overall, dose−response studies and the genetic analyses of F_1, F_2, and backcross progeny confirmed that (1) MCPA-R wild radish plants were 10-fold more resistant to MCPA than S plants, (2) resistance is transmitted via nuclear genes, and (3) resistance is an incompletely dominant trait. Specifically, S plants exhibited epinasty 2−3 days after spraying with MCPA and eventually died when treated with 100 g ae/ha or more (Figure 1). However, R plants at doses up to and

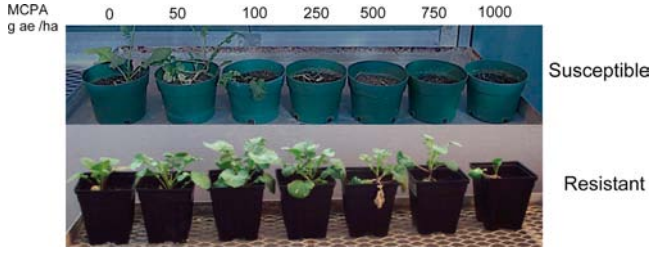
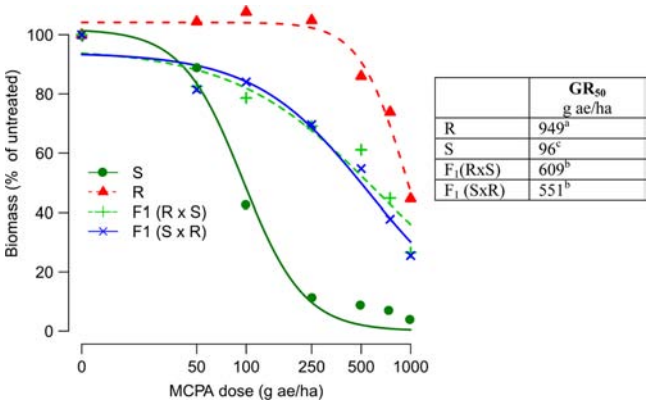


Figure 1. MCPA dose−response of susceptible and resistant wild radish.

including 250 g ae/ha showed no MCPA injury, at 500 and 750 g ae/ha there was moderate injury (Figure 1), and at 1000 g ae/ha there was severe injury; regardless of the dose, all plants lived and were able to produce well-developed flowers and viable seed. Furthermore, in the dose−response experiments with F_1 plants (Figure 2), all plants survived MCPA application. The ratio of GR_{50} values for R and S plants indicates that the R



	GR_{50} g ae/ha
R	949[a]
S	96[c]
F_1(RxS)	609[b]
F_1 (SxR)	551[b]

Figure 2. Dose−response of susceptible (S), resistant (R), and F_1 (R × S and S × R) wild radish to MCPA (log scale). The inset indicates the GR_{50} values.

biotype has a 10-fold level of resistance (949 vs 96 g ae/ha) when compared to S biotype (Figure 2). F_1 progeny showed an intermediate response to MCPA with GR_{50} values for F_1(R × S) and F_1(S × R) of 609 and 551 g ae/ha, respectively (Figure 2). Since there was a similar level of MCPA resistance observed in the F_1 progeny derived from both the R × S and S × R crosses, it is clear that the gene determining MCPA resistance resides in the nucleus and thus is not maternally inherited. Also, the lower level of resistance of F_1 progeny compared to parental R plants suggests that the MCPA resistance in wild radish is not fully dominant.

The 3:1 (R:S) segregation of the F_2 progeny following MCPA treatment at 100, 250, and 500 g ae/ha suggested that MCPA resistance in wild radish is a single gene trait (Table 1).

Table 1. Segregation of MCPA-Resistant (R) and -Susceptible (S) Phenotypes in F_2 Progeny[a,b,c]

	F_2 progeny response [3:1 (R:S)] to MCPA			
	segregation of plants			
MCPA dose (g ae/ha)	R	S	χ^2	probability
50	22	0	7.33	0.01
100	18	5	0.13	0.719
250	16	6	0.06	0.806
500	182	47	2.44	0.12

[a]Resistance and susceptibility were assessed by comparing the response of F_2 seedlings to response of seedlings from R and S parental populations following MCPA treatment. [b]χ^2 values are the results of tests for goodness of fit to a 3:1 (R:S) segregation model. [c]If χ^2 and probability values are <3.84, and >0.05, respectively (for degree of freedom (df) = 1), accept null hypothesis, as the deviation from expected is due to chance only.

The null hypothesis was stated as "the observed ratios were in accordance with the expected ratios for a 3:1 segregation" (R:S, Table 1). χ^2 tests for goodness of fit to a 3:1 segregation (R:S) supported our null hypothesis; i.e., the observed frequencies (R or S) after herbicide treatment were in accordance with the expected frequencies for a 3:1 (R:S) segregation ratio, at doses of 100, 250, and 500 g ae/ha (Table 1). The majority of the survivors showed an intermediate phenotypic response similar to that of F_1 hybrids, and the S and R plants were clearly distinct among the F_2 progeny. S plants showed epinasty initially followed by chlorosis and complete death. Furthermore, when seedlings of the progeny from backcrosses (resulted from cross-pollination of S plants by F_1 hybrids) were scored for MCPA injury after treatment, all the S parental plants and approximately half of the backcross progeny exhibited epinasty and eventually died. Conversely, the parental R plants as well as the remaining backcross progeny withstood MCPA application. The null hypothesis was stated as "the observed ratios were in accordance with the expected ratios for a 1:1 segregation" (R:S, Table 2). χ^2 tests for goodness of fit to

Table 2. Segregation into Resistant (R) and Susceptible (S) Phenotypes in Backcross Progeny Following Sequential Treatment with 500 g ae ha^{-1} MCPA[a,b,c]

	backcross progeny response [1:1 (R:S)] to MCPA			
	segregation of plants			
MCPA dose (g ae/ha)	R	S	χ^2	probability
500	18	15	2.72	0.0990

[a]Resistance and susceptibility was assessed by comparing the response of F_2 seedlings to response of seedlings from R and S parental populations following MCPA treatment. [b]χ^2 value is the result of test for goodness of fit to a 1:1 (R:S) segregation model. [c]If χ^2 and probability values are <3.84, and >0.05, respectively (for df = 1), accept null hypothesis, as the deviation from expected is due to chance only.

a 1:1 segregation (R:S) supported our null hypothesis; i.e., the observed frequencies (R or S) after herbicide treatment were in accordance with the expected frequencies for a 1:1 (R:S) segregation ratio. Based on the data from F_1 progeny with an intermediate response to MCPA, compared to R and S parents, the MCPA resistance in wild radish is inherited as an incompletely dominant trait. Furthermore, the segregation ratios observed in F_2 and backcross progeny suggest that the MCPA resistance is controlled by a single gene in wild radish.

MCPA Uptake, Translocation, and Metabolism Experiments. Investigation of ^{14}C-MCPA uptake and translocation in wild radish R and S populations suggests that there were no differences between R and S plants in the quantity of ^{14}C-MCPA absorbed at 6 and 24 HAT; however, the R biotype absorbed more ^{14}C-MCPA than S at 48 and 72 HAT (Table 3). Furthermore, the total amount of MCPA translocated within the plant was significantly greater in R plants at 6, 48, and 72 HAT, with more ^{14}C-MCPA being translocated to the roots of R than of S at 48 HAT (Table 3). Additionally, immediately 6 HAT application MCPA was translocated to roots more rapidly out of treated leaf in R plants than in S plants (Table 3). Furthermore, significantly less amount of the total ^{14}C-MCPA applied was recovered in R plants than in S plants (i.e., only 42.42% of the applied ^{14}C-MCPA was recovered from R plants as opposed to 76.86% from S plants), at 48 HAT (Table 3). These results suggest that the R plants translocated more MCPA rapidly to roots, and also, since in general less amount of total applied ^{14}C-MCPA was recovered in R plants than S, it is likely that the herbicide translocated below the treated leaf possibly exuded out from roots in R plants (Table 3). This may explain why the R plants are able to withstand MCPA application better than S plants. Future research with experiments conducted in a hydroponic system may test this hypothesis. On the other hand, high-pressure chromatography (HPLC) analyses of ^{14}C-MCPA at 48 HAT indicated that there

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

plant part	biotype	time after treatment 6 h	24 h	48 h	72 h
		% of ^{14}C Recovered in Plant			
leaf rinse	R	53 (3.28)	38(0.93)	1.6 (0.26)[b]	1.4 (0.02)[b]
	S	47. (1.45)	35 (0.55)	6.4 (0.15)[b]	3.9 (0.56)[b]
treated leaf (TL)	R	40.46 (3.09)	26.03 (0.93)	9.3 (1.08)[b]	11.1 (0.82)
	S	48.8 (0.89)	32.71 (3.82)	32.40 (2.04)[b]	17.4 (2.51)
shoot above TL	R	0.98 (0.34)	6.87 (0.19)[b]	3.6 (1.48)	7 (0.64)[b]
	S	0.47 (0.12)	4.19 (0.40)[b]	2.2 (0.09)	2.3 (0.00)[b]
shoot below TL	R	2.8 (0.60)	14.66 (2.00)	9.5 (0.50)[b]	9.2 (1.28)[b]
	S	2.8 (0.67)	17.11 (0.86)	29.8 (5.97)[b]	15.7 (1.87)[b]
roots	R	2.32 (0.26)[b]	14.48 (0.06)	18.3 (2.38)[b]	13.2 (0.42)
	S	0.06 (0.01)[b]	10.7 (2.81)	6.1 (0.10)[b]	13.4 (0.32)
MCPA potentially translocated in plant[c]	R	0	0	57.7 (1.71)[b]	58.1 (2.33)
	S	0	0	23.1 (4.0)[b]	47.3 (5.0)
total translocated	R	6.1 (0.61)[b]	36 (1.86)	89.1 (1.32)[b]	87.5 (0.84)[b]
	S	3.33 (0.56)[b]	32 (3.27)	61.2 (2.19)[b]	78.7 (3.07)[b]
total absorbed	R	46.54 (3.27)	62.03 (0.93)	98 (0.25)[b]	99 (0.021)[b]
	S	52.12 (1.00)	64.71 (1.0)	94 (0.001)[b]	96 (1.0)[b]
		% of ^{14}C-MCPA Applied to the Plant			
total recovery	R	93.40 (3.0)	105.55 (3.13)	42.42 (1.72)[b]	41.92 (2.34)
	S	96.86 (1.0)	104.16 (3.0)	76.86 (3.79)[b]	52.66 (4.61)

[a]Data are means with standard errors in parentheses. [b]Indicates significant difference between R and S within plant part at a particular harvest (α at 0.05). [c]MCPA potentially translocated in plant but could not be measured (possibly exuded out).

was no metabolism of MCPA in both R and S plants, suggesting that metabolism is not the basis for MCPA resistance in wild radish (data not shown).

The findings of this research demonstrated that a single incompletely dominant gene controls MCPA resistance in wild radish and also that the R plants translocate more herbicide rapidly to roots than the S plants. Previously, it was reported that in general auxinic herbicide resistance is controlled by a single gene and, in a few cases, by two major genes. For example, dicamba, 2,4-D, and picloram resistance in wild mustard is determined by a single dominant gene[9,10] and dicamba resistance in kochia (biotypes from Henry, NE, USA) is determined by a single allele with a high degree of dominance.[15] Conversely, a single recessive gene controls clopyralid and picloram resistance in yellow star thistle[16] and quinclorac resistance in false cleavers.[17] However, two additive genes control MCPA resistance in common hemp nettle.[18] Although Gressel and Segel[19] suggested that the low incidence of auxinic herbicide resistance may be due to the need for occurrence of mutations at several loci in order to impart resistance, it appears that this may not be the case because, as described above, in many auxinic herbicide resistant weeds including wild radish, resistance is determined by a single gene. Based on the dose−response data, the wild radish F_1 (heterozygous) plants survived a field dose (500 g ae/ha) of MCPA and responded similarly to homozygous dominant R parent. These results suggests that expression of incomplete dominance in wild radish provides enough resistance (with no injury or reduced fitness) in response to MCPA selection at 500 g ae/ha (i.e., a field dose of MCPA). Thus, the incompletely dominant trait appears to behave exactly like a completely dominant trait, and it can, potentially, spread relatively quickly throughout a population.

Genetic mutations causing herbicide resistance will influence the mechanism by which resistance is imparted via altering one or more of the following processes: (a) uptake and translocation of the herbicide, (b) metabolism of herbicide to nontoxic compounds, (c) sequestration or exudation away from the target site, and/or (d) an altered target site (DNA sequence or expression level). In this research the mechanism of MCPA resistance was investigated by determining the ^{14}C-MCPA uptake, translocation, and metabolism for R and S wild radish plants. R plants translocated more MCPA rapidly to roots than S plants and also significantly less applied MCPA was recovered in R plants than in S plants at 48 HAT (Table 3), suggesting that S plants were unable to translocate MCPA to the roots and possibly exude it as efficiently as R plants. In contrast, previously, we found R plants of hemp nettle translocated less ^{14}C-MCPA to apical meristem and roots than S plants.[18] Additionally, in hemp nettle, more MCPA metabolites were recovered in roots of R plants than in those of S plants.[18] However, it was found that auxinic herbicide resistance in wild mustard,[20] false cleaver,[21] kochia,[22] and yellow star thistle[23,24] were not due to differences in herbicide absorption, translocation, and/or metabolism and, thus, by inference may be due other mechanisms, e.g., altered target site.

In conclusion, the incomplete dominance inheritance of MCPA-resistance trait will have several implications to agriculture, in terms of evolution and spread of herbicide resistance across geographies, which will influence development of effective weed management strategies. As suggested by Jasieniuk et al.,[25] a dominant trait spreads much faster in populations as both homozygous dominant and heterozygous individuals will carry the resistance trait. Thus, to combat the spread of phenoxy resistance of wild radish, viable management strategies including integrated weed control are required. In any case it is clear that knowledge about the inheritance and mechanism of herbicide resistance will help us better understand the evolution and incidence of resistance and methods to manage herbicide-resistant weed species.

AUTHOR INFORMATION

Corresponding Author

*E-mail: jchall@uoguelph.ca.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

2,4-D, [(2,4-diclorophenoxy)acetic acid]; MCPA, [(4-chloro-2-methylphenoxy)acetic acid]

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