Original Article

The Uptake, Metabolism and Phytotoxicity of MCPA in Plants*

Nagi R. ACHHIREDDY,** Ralph C. KIRKWOOD and William W. FLETCHER

Department of Biology, University of Strathclyde, Glasgow, Scotland

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The phytotoxicity of MCPA (2-methyl-4-chlorophenoxyacetic acid) as related to the translocation and metabolism in maize (tolerant), chickweed (moderately sensitive), and broad bean (sensitive) were examined under controlled environmental conditions. The amount of ^{14}C -MCPA translocated in broad bean and chickweed was significantly greater than in maize. However, retained by the treated leaf was greater in maize than in the other two species. The ^{14}C -MCPA retained by the cuticle wax was less than 1% (of the applied) and the test species did not differ significantly in this respect. The release of $^{14}\text{CO}_2$ from ^{14}C -MCPA was significantly greater in broad bean than in maize and chickweed. The foliar application of MCPA resulted in greater inhibition of $^{14}\text{CO}_2$ fixation and ^{14}C -assimilate translocation in broad bean and chickweed than in maize.

INTRODUCTION

The selective control of broad-leaf weeds in grass or cereal crops has been achieved through the foliar application of 2-methyl-4-chlorophenoxyacetic acid (MCPA). There are a few reports which suggest that differences in phytotoxicity of MCPA are due to differentials in absorption, translocation and metabolism of the herbicide.^{1–3)} Most of these studies were conducted on a single sensitive plant or plants selected from a single group (dicotyledons), which differed in sensitivity to MCPA. Very little attention was given to compare the performance and selectivity with regard to uptake, translocation and metabolism of MCPA in different plants. We report in this paper,

the phytotoxicity of MCPA as related to translocation and metabolism in maize (tolerant), chickweed (moderately sensitive), and broad bean (sensitive) under controlled environmental conditions.

MATERIALS AND METHODS

Maize (*Zea mays* L.), chickweed [*Stellaria media* (L.) Vill.], broad bean (*Vicia faba* L.) were grown in John Innes No. 3 compost in 10 cm polystyrene pots in heated greenhouse ($22 \pm 2^\circ\text{C}$) under white fluorescent lights (14 hr photoperiod in a 24 hr cycle). After germination, four uniform plants were allowed to establish per pot. The plants were sprayed just to runoff at four-leaf stage with various concentrations of herbicide (Na salt). Polyoxyethylene sorbitan monolaurate (Tween-20) at 0.1% (v/v, final concentration) was added to the herbicide solution before spraying. The herbicide treated plants were then placed in a growth chamber which was maintained at $20 \pm 0.5^\circ\text{C}$ (air temperature), $150 \mu\text{E cm}^{-2} \text{ hr}^{-1}$ for 14 hr in a 24 hr cycle. The relative humidity was maintained at $70 \pm 5\%$. Seven days after treatment, visual ratings at 0 to 5 (0: no effect; 5: severe wilting

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** Present address: Agricultural Research and Education Center, University of Florida, Institute of Food and Agricultural Sciences, Lake Alfred, FL 33850, USA.

and blackening of leaves) were made on the test species.

For experiments involving the uptake and metabolism of MCPA, the plants were raised in the greenhouse as described above. There were two plants per pot. The specific activity of MCPA [2-methyl-4-chlorophenoxy(^{14}C)-acetic acid] was 6.0 mCi/mmol. ^{14}C -MCPA (2.4 μg , 0.06 μCi) was applied to the fully expanded second leaf at four-leaf stage as two 2 μl droplets to the adaxial surface in the middle on either side of the midrib. The surfactant Tween-20 at 0.1% (v/v) was added to the labelled herbicide. The ^{14}C -herbicide treated plants were then transferred to a growth chamber. The growth chamber conditions were similar to the ones described previously. On the 7th day after treatment, the plants were harvested for ^{14}C analysis. The treated leaves were cut and washed by gentle shaking with 10 ml distilled water held in 125 ml flasks for 2 min to remove surface residues of the herbicide and then in 10 ml chloroform held in liquid scintillation vials for 15 sec to remove the cuticle wax fraction. A 1 ml aliquot of water wash was transferred to 10 ml dioxan-based scintillation liquid⁴⁾ and radioassayed using a Packard Tricarb model 3375 liquid scintillation spectrometer. The chloroform extracts were evaporated to dryness, 10 ml scintillator added and the sample radioassayed as before. The treated leaves, shoots, and roots were then separately homogenized in 20 ml of 40% acetone using an Ultra-turrox model TP-1810 homogenizer and 0.5 ml aliquot of the homogenate radioassayed. The degree of quenching was determined by internal standardization and all the values were corrected for quenching and counting efficiency.

An investigation of the possible release of $^{14}\text{CO}_2$ from ^{14}C -MCPA treated test plants was carried out by determining the release of $^{14}\text{CO}_2$ during a 7-day period. The $^{14}\text{CO}_2$ evolved from the test species was trapped in 14% (v/v) ethanolamine in methoxyethanol. The treated plants were placed in airtight kilner jars, one jar per species. The absorption system set up was similar to that described by Veerasekaran.⁵⁾ The outlet tube led to three glass washing bottles in series, each containing 100 ml of ethanolamine solution. The amount of $^{14}\text{CO}_2$ absorbed

in ethanolamine was determined at 2, 5, and 7 days after treatment by radioassay of 1 ml aliquots from each trap. Extraction of ^{14}C -MCPA and its metabolites from the treated plants was carried out according to the methods described by Fawcett *et al.*⁶⁾ Briefly, the plants were homogenized, acidified, and extracted three times with 50 ml of ethylacetate, further extracted with 20 ml of sodium carbonate, reacidified, and finally extracted three times with ether (50 ml each time). The pooled ether extracts were left overnight to evaporate under lab temperature and redissolved in 2 ml ether, of which 0.05 ml was spotted on precoated silica gel (Merck, Gf 254) and alumina (Eastman 6063) TLC plates. A mixture of liquid paraffin, benzene, acetic acid, and cyclohexane (1 : 3 : 2 : 20, v/v/v/v) was used as a developing solvent. The radioactive spots on the TLC plates were located by autoradiography.⁷⁾ The radioactive spots were removed from the chromatograms by scraping and these samples were then radioassayed by liquid scintillation spectrometry. Quantitative assessments were also made by scanning the TLC plates for radioactive compounds using Packard radiochromatogram scanner model 7201.

The results are expressed as percentage of applied activity. The percentage values were converted to arcsines and subjected to analysis of variance as described by Snedecor and Cochran.⁸⁾ Significant differences between means were determined by Duncan's multiple range analysis.⁹⁾

Experiments were also conducted to see whether MCPA affects differentially $^{14}\text{CO}_2$ fixation and ^{14}C -assimilate translocation in the test species. Uniform plants of each species (two plants per pot) were sprayed just to run off with various concentrations of herbicide. The surfactant Tween-20 at 0.1% (v/v) was added to the herbicide. The treated plants were then transferred to a growth chamber. The growth chamber conditions were similar to the ones described previously. On the 7th day, the second oldest leaf of each species was allowed to assimilate $^{14}\text{CO}_2$ using a modification of the method of Ismail.¹⁰⁾ The leaf was enclosed in a transparent perspex chamber (6 $\times$ 13.5 $\times$ 8 cm). The leaf was placed in a notch cut into the lower half of the chamber,

and after replacing the top, the notch was sealed using silicone grease. $^{14}\text{CO}_2$ (5 $\mu\text{Ci}/\text{leaf}$) was liberated into the chamber from $\text{Na}_2^{14}\text{CO}_3$ solution (specific activity, 59.5 mCi/mmol) by the addition of 1 N HCl. The leaf was allowed to fix $^{14}\text{CO}_2$ for 15 min after which the remaining $^{14}\text{CO}_2$ was extracted by injecting 14% ethanolamine solution in methoxyethanol into a petri dish previously placed in the feeding assembly; the chamber was then removed. The plants were harvested after 48 hr and divided into the treated leaf, shoot and root, freeze-dried, and weighed. Each region was then homogenized in 20 ml of 90% (v/v) ethanol as described previously. A 0.5 ml aliquot was placed in a scintillation vial and allowed to evaporate under lab temperature and radioassayed. The results are expressed in two ways as described by Ismail.¹⁰⁾ (1) Total ^{14}C recoveries were considered equal to dpm/fed leaf + dpm/shoot + dpm root; losses due to respiration were ignored and (2) the ^{14}C recovered in the whole plant, except the fed leaf, is expressed as percentage of the total recovery and regarded as total translocation.

RESULTS AND DISCUSSION

The phytotoxicity of a range of concentrations of MCPA on the test species under controlled conditions was assessed by visual symptoms (Table 1). Broad bean was susceptible, chickweed moderately susceptible and maize tolerant. Phytotoxic symptoms were virtually absent in maize at concentrations less than 5000 ppm. At high concentrations, chlorosis of older leaves and distortion of the roots was noted. Eddowes and Harpur¹¹⁾ reported excellent control of weeds with post-emergence application of MCPA (1.25 kg/ha). But in maize, leaf rolling and distortion of prop roots was evident 3 weeks after spraying. In the present investigation, treatment of broad bean with high concentrations (>1000 ppm) produced epinasty, necrosis, severe wilting, lodging, and blackening of the foliage. In the case of chickweed, low concentrations caused epinasty of the younger leaves and at higher concentrations the older leaves showed necrosis.

There is evidence that after uptake, differences in translocation may be a factor in the selective action of certain herbicides.^{12,13)} Ex-

Table 1 The toxicity of MCPA to maize, chickweed, and broad bean.

Herbicide (ppm)	Visual assessment (0 to 5)		
	Maize	Chickweed	Broad bean
0	0	0	0
50	0	0	0
100	0	0	0
250	0	0	0
500	0	2	4
1000	0	2	4
5000	1	2	4
10000	1	3	5

0: no effect, 1: chlorosis of older leaves and distortion of roots, 2: epinasty of young leaves, 3: chlorosis of older leaves, 4: epinastic bending and wilting, 5: severe wilting and blackening of leaves.

amination of the distribution of ^{14}C -MCPA within the test species revealed differentials in translocation (Fig. 1 and Table 2). The amount of ^{14}C translocated in broad bean and chickweed was about three and two times, respectively, greater than in maize. Most of ^{14}C translocated by broad bean accumulated in the root and shoot apices. The ratios of translocation to absorption were 0.39, 0.21, and 0.08 in broad bean, chickweed, and maize, respectively. Absorption was greater in maize than in the other two species. By using leaf-discs, Sargent and Blackman¹⁴⁾ compared the rates of uptake of 2,4-dichlorophenoxyacetic acid (2,4-D) in six species and found more rapid penetration into *Zea* (most resistant) than into *Pisum*, *Beta*, *Helianthus*, *Gossypium* or *Phaseolus* (all are sensitive to some extent). The relatively high absorption in maize did not result in increased translocation. The surface residue fraction accounts for most of the total recovered ^{14}C in chickweed whereas in maize, most of absorbed ^{14}C was retained by the treated leaf. Sanad⁹⁾ found a clear relationship between the susceptibility of corn cockle to the mobility of MCPA in this plant. Similarly, Collins and Gaunt¹⁾ have reported on the uptake of MCPA by rape, red campion, and pea. They reported that in rape (susceptible), 50% of the label that entered the treated leaves was translocated to the rest

Fig. 1 Autoradiograms of maize (a), chickweed (b), and broad bean (c).
Samples above, autoradiograms below.

Table 2 The uptake and translocation of ¹⁴C-MCPA in maize, chickweed, and broad bean.

Regions	¹⁴ C-Distribution (% of applied) ^{a)}		
	Maize	Chickweed	Broad bean
Surface residue	12.5 a	31.2 b	15.4 c
Wax residue	0.3 a	1.0 a	0.3 a
Treated leaf	30.1 a	21.1 b	11.8 c
Shoot	1.7 a	4.2 b	3.1 c
Root	0.8 a	1.7 b	4.5 c
Recovery	45.4 a	59.2 b	35.1 c
Absorption	32.9 a	28.0 b	19.7 b

Analysis of variance (*P*): Species=0.001; Regions=0.001; Spp/regs=0.001.

^{a)} Duncan's multiple range test: values within the same row (horizontal) with common letter postscripts are not significantly different at 5% level.

of the plant; in pea and red campion (both tolerant) translocation was less extensive. The ¹⁴C retained by the cuticle wax was limited and represented less than 1% (of the applied) and species did not differ significantly in this respect. It is worth noting that most of ¹⁴C recovered in chickweed was detected as surface residue.

Table 3 The relative quantities of ¹⁴C-MCPA or its metabolites in various fractions of the test species.

Fraction	¹⁴ C-Distribution (% of applied) ^{a)}		
	Maize	Chickweed	Broad bean
Ethylacetate fraction	50.8 a	77.0 b	43.6 c
Bound fraction	3.2 a	0.3 b	0.8 b
¹⁴ CO ₂ evolved	5.5 a	3.6 a	15.2 b
¹⁴ C-MCPA leached	1.7 a	0.8 a	6.9 b

^{a)} Duncan's multiple range test: values within the same row (horizontal) with common letter postscripts are not significantly different at 5% level.

The release of ¹⁴CO₂ from MCPA was greater in broad bean than in the other two species (Table 3) indicating faster rates of breakdown of MCPA in broad bean. In the case of broad bean, the loss of ¹⁴C-MCPA through the roots was 6.9% (of the applied dose) and may reflect the rapid accumulation of herbicide in the roots with a consequent increase in cell membrane permeability. A similar exudation into the soil following the foliar application of 2,4-D to Jimsonweed has been reported.¹⁵⁾ Chromatography of ether extracts of the test species revealed some breakdown of the herbicide, but the results are inconclusive. The radioactive spots (*R_f* value 0.0) with 78, 9, and 68% in maize, chickweed, and broad bean, respectively, were regarded as metabolites and were not further resolved. The other spot with *R_f* value 0.10 consisted of 24, 76, and 32% of the total radioactivity in maize, chickweed, and broad bean, respectively, and was identified as ¹⁴C-MCPA.

The foliar application of MCPA resulted in inhibition of ¹⁴CO₂ fixation and ¹⁴C-assimilate translocation, the effect being particularly greater in sensitive species (Table 4). At relatively low concentrations (non-toxic levels) (500 ppm) MCPA increased the export of photosynthates to the root in the test species, while at higher concentrations, there was a decrease in the level of export and shoot particularly in the sensitive species, broad bean and chickweed. The reduction in trans-

Table 4 The effect of MCPA on $^{14}\text{CO}_2$ fixation and ^{14}C -assimilate distribution in maize (M), chickweed (C), and broad bean (B).

Concentration (ppm)	Species	^{14}C -Distribution (% of total recovery) ^{a)}			Total $^{14}\text{CO}_2$ fixation (dpm $\times 10^3$) ^{a)}
		Fed leaf	Shoot	Root	
0	M	36.3 a	39.5 h	24.2 f	269.5 j
	C	51.8 cd	38.8 h	9.4 c	41.6 b
	B	49.8 c	19.8 d	30.4 h	192.9 g
500	M	42.4 b	30.8 fg	26.8 g	248.0 k
	C	62.0 e	23.8 e	14.2 d	35.2 ab
	B	54.9 d	11.6 b	33.5 i	150.0 f
1000	M	49.9 c	28.6 f	21.5 e	228.4 i
	C	64.8 f	22.6 e	12.8 cd	32.5 ab
	B	60.0 e	9.9 b	30.1 h	135.9 e
5000	M	54.7 d	30.4 g	14.9 c	220.5 i
	C	70.1 g	19.9 d	10.0 c	30.5 a
	B	69.9 g	9.0 b	21.1 e	120.5 d
10000	M	60.1 e	19.3 d	20.6 e	202.0 h
	C	75.0 h	14.7 c	10.3 c	28.9 a
	B	81.5 i	7.9 b	10.6 e	100.5 c

Analysis of variance (*P*): species, concentrations, regions (all=0.001), spp/concs, spp/regs, conc/regs, spp/conc/regs (all=0.001).

^{a)} Duncan's multiple range test: values within the same column (vertical) with common letter postscripts are not significantly different at the 5% level.

location of assimilates may be due to the creation of new sinks in the treated leaves¹⁶⁾ and/or to an inhibition of the loading process. It is noteworthy that the levels of $^{14}\text{CO}_2$ fixed and the rates of export of ^{14}C -assimilates in maize controls were greater than broad bean and chickweed. These findings are in close agreement with the suggestion that C_4 plants, such as maize, have reduced photorespiration, special leaf anatomy, biochemical pathways, and greater translocation capacities than C_3 plants such as broad bean and chickweed.¹⁷⁻¹⁹⁾

CONCLUSIONS

The absorption of foliar applied ^{14}C -MCPA was significantly greater in tolerant maize than sensitive broad bean or chickweed. However, the amount of ^{14}C -MCPA translocated in broad bean or chickweed was greater than in maize. The foliar application of MCPA resulted in greater inhibition of $^{14}\text{CO}_2$ fixation and ^{14}C -assimilate translocation in broad bean and chickweed than in maize.

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要 約

植物体内における MCPA の吸収、代謝および葉害

Nagi R. Achhireddy, Ralph C. Kirkwood,
William W. Fletcher

制御された環境条件下で、MCPA に抵抗性を示すトウモロコシ、やや感受性のハコベ、感受性のソラマメに対し、MCPA の植物毒性を体内移行、代謝と関係づけて検討した。ソラマメ、ハコベおよびトウモロコシにおける ^{14}C -MCPA の移行量は、それぞれ処理総量の 7.6, 5.9 および 2.5% であった。しかし処理葉における ^{14}C -MCPA の残量はソラマメ、ハコベに比べてトウモロコシで多かった。葉部残量のうちクチクラワックス内には処理量の 1% 以下にすぎず、この数値は 3 植物間に有意な差は認められなかった。 ^{14}C -MCPA 処理による $^{14}\text{CO}_2$ の発生はトウモロコシ、ハコベに比べてソラマメにおいて明らかに多かった。MCPA の葉面処理により、 $^{14}\text{CO}_2$ の固定およびその同化生成物の体内移行阻害はトウモロコシに比べてソラマメ、ハコベで明らかに大きかった。