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Original Article

The Uptake and Distribution of MCPA and Oxadiazon in Excised Leaves*

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By using excised leaves or leaf-discs, the uptake and translocation of ^{14}C-MCPA (2-methyl-4-chlorophenoxyacetic acid) in maize (resistant), chickweed (moderately susceptible) and broad bean (susceptible) and ^{14}C-oxadiazon [2-*tert*-butyl-4-(2,4-dichloro-5-isopropoxy-phenyl)-1,3,4-oxadiazoline-5-one] in rice (resistant) and barnyardgrass (susceptible) were examined. Translocation of ^{14}C-MCPA was significantly greater in broad bean and chickweed than in maize. Similarly, greater translocation of ^{14}C-oxadiazon was recorded for barnyard-grass than for rice. Microautoradiographic studies of leaf discs revealed high absorption of ^{14}C in maize and barnyardgrass but vein accumulation was limited in the former. Unlike ^{14}C-MCPA, movement of ^{14}C-oxadiazon was not significantly influenced by sink creation.

INTRODUCTION

The selective control of broadleaf weeds in cereal crops has been achieved through the foliar application of MCPA (2-methyl-4-chloro-phenoxyacetic acid). 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 on a few plants selected from a single group (dicotyledons) which differed in sensitivity to MCPA. Very little attention was given to compare selectivity with regard to uptake and translocation of MCPA in different plants.

Oxadiazon is used as a pre- and post-emergence herbicide on grasses and broadleaf weeds in rice, cotton, soybean and groundnut.[4] The absorption, translocation and metabolism of oxadiazon was examined extensively in rice plants by Ishizuka *et al.*[5] A limited amount of work, however, has been conducted on oxadiazon in other plants. The objectives of this study were to examine the uptake, localization within the treated leaves and pathways of movement of MCPA in maize, chickweed and broad bean or oxadiazon in rice and barnyardgrass, using excised leaves or leaf-discs.

MATERIALS AND METHODS

1. *Growth of Plants and Preparation of Explants*

Maize (*Zea mays* L.), chickweed [*Stellaria media* (L.) Vill.], broad bean (*Vicia faba* L.), rice (*Oryza sativa* L.) and barnyardgrass [*Echinochloa crusgalli* (L.) Beauv.] were grown in John Innes No. 3 compost in 10 cm polystyrene pots under greenhouse conditions. The greenhouse temperature was maintained at 22±2°C under white fluorescent lights with 14 h photoperiod. The greenhouse was not heated during the summer. The leaves were excised from the plants of uniform age (usually at the 4 leaf stage). The excised leaves were then inserted in glass tubes containing 7 ml sterilized Nitsch's H medium.[6] Sink-demand

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in excised leaves was induced by covering the lower half of the leaf (or the righthand leaflet in the case of broad bean) with aluminum foil. In the case of leaf disc/agar system, leaf discs (5 mm diameter) were cut using a corkborer from fully expanded leaves and placed on 1.2% (w/v) agar discs (Oxoid agar No. 3) of 5 mm diameter and 3 mm thickness in planchettes. The planchettes were then placed in petri dishes (5 per dish) containing a layer of moist filter paper. The ^{14}C-herbicide treated samples were then placed in a growth chamber at 20±0.5°C and relative humidity 80±5%, illumination being 27,000 lux, 45 cm from the light source. There were four replicates for each treatment and all experiments were repeated with the similar results.

2. *Herbicide Treatment Procedure*

The labelling positions and specific activities of the two herbicides were: 4-chloro-2-methylphenoxy (^{14}C)-acetic acid (6.0 mCi/mmol) and 2-*tert*-butyl-4-(2,4-dichloro-5-isopropoxyphenyl-(^{14}C) 1,3,4-oxadiazolin-5-one (25.87 mCi/mmol). The radioactive doses used are specified in Table 1. The surfactant Tween-20 (polyoxyethylene sorbitan monolaurate) at 0.1% (v/v) was added to the labelled herbicides. The ^{14}C-herbicides were applied in two 2 μl droplets (one 2 μl droplet for leaf-disc systems) to the adaxial surface in the middle on either side of the midrib.

3. *Harvesting and Liquid Scintillation Spectrometry*

On the 7th day (3rd day in the case of leaf-disc experiments) the excised leaves were harvested. The treated leaves or leaf discs were washed 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 of chloroform held in liquid scintillation vials for 15 s to remove the cuticle wax fraction. A 1 ml aliquot of wash water was transferred to 10 ml dioxan-based scintillation liquid[7] and radioassayed using Packard model 3375 Liquid Scintillation Spectrometer. The degree of quenching was determined by internal standardization and all the values were corrected for quenching and counting efficiency. The chloroform extracts were evaporated to dryness, 10 ml scintillation liquid added and the samples radioassayed as before. The treated and untreated portions were then separately homogenized in 10 ml of 40% acetone and a 0.5 ml aliquot of the homogenate radioassayed. In the case of leaf disc experiments, the leaf-discs were radioassayed directly without homogenization.

Table 1 The radioactive doses used in experiments.

	Herbicide and radioactive dose Experiments			
	Leaf		Leaf-disc	
	(μg)	(μCi)	(μg)	(μCi)
MCPA	2.40	0.06	1.20	0.03
Oxadiazon	0.56	0.04	0.42	0.03

4. *Autoradiography*

For autoradiography, the excised leaves were freeze-dried following the water wash of the treated leaf, mounted on paper and autoradiograms prepared using the technique of Yamaguchi and Crafts.[8] Where microautoradiography of the treated leaf discs was carried out, the surface residues were removed by washing in water, the discs freeze-dried and sandwiched between slides precoated with Kodak A. R. 10 stripping film. After an exposure period of 15 days, the slides were developed using Kodak D-19, fixed, examined under the light microscope (Olympus Vanox) and photographed.

5. *Statistical Evaluation*

The results are expressed as percentages of applied activity. The percentage values were converted to arcsines and subjected to analysis of variance according to Snedecor and Cochran[9] and significant differences between means determined by Duncan's multiple range analysis.

RESULTS AND DISCUSSION

The present work on the absorption and translocation of ^{14}C following the application of ^{14}C-MCPA and ^{14}C-oxadiazon to excised leaves or leaf-discs revealed greater absorption and translocation in susceptible species than in

Table 2 The absorption and translocation of ^{14}C-MCPA in maize (M), chickweed (C) and broad bean (B).

Regions	^{14}C-Distribution[a] (%) Experiments					
	Leaf			Leaf-disc		
	M	C	B	M	C	B
Surface residue	1.3 a	53.4 b	18.0 c	0.0 a	5.2 b	1.9 c
Wax residue	0.3 a	0.6 a	1.4 b	0.2 a	2.5 b	1.5 b
Treated leaf	7.3 a	2.6 b	3.2 b	22.3 a	11.8 b	40.0 c
Translocation[b]	3.0 a	4.7 b	5.6 b	0.4 a	34.7 b	1.2 a
Analysis of variance (*P*)	Species=0.01 Regions=0.001 Spp/regs=0.001			Species=0.01 Regions=0.001 Spp/regs=0.001		

a) Duncan's multiple range test: for each experiment, values within the same row (horizontal) with common letter postscripts are not significantly different at 5% level.
b) Covered leaflet or covered lower portion of the leaf and agar block in the case of leaf and leaf-disc systems, respectively.

Table 3 The absorption and translocation of ^{14}C following the application of ^{14}C-oxadiazon to rice (R) and barnyardgrass (B).

Regions	^{14}C-Distribution[a] (%) Experiments			
	Leaf		Leaf-disc	
	R	B	R	B
Surface residue	8.4 a	4.3 b	14.6 a	11.7 a
Wax residue	20.5 a	13.9 b	32.1 a	25.7 b
Treated leaf	19.4 a	17.3 a	8.4 a	25.4 b
Translocation[b]	0.3 a	2.5 b	2.9 a	1.8 b
Analysis of variance (*P*)	Regions=0.01 Spp/regs=0.001		Regions=0.001 Spp/regs=0.001	

a) Duncan's multiple range test: for each experiment, values within the same row (horizontal) with common letter postscripts are not significantly different at 5% level.
b) Covered lower portion of the leaf and agar block in the case of leaf and leaf-disc systems, respectively.

resistant species (Tables 2 and 3). Microautoradiograms of leaf discs indicated heavy accumulation of ^{14}C at the point of application in maize than in chickweed or broad bean (Fig. 1). In the latter two species, most of the absorbed ^{14}C-MCPA was translocated into the major veins away from the point of application. There is evidence from isolated leaf experiments that MCPA was translocated in the assimilate stream. Artificial sink creation significantly increased translocation in all 3 test species (Fig. 2). However, chickweed and broad bean translocated greater levels of ^{14}C-MCPA into the sinks than did maize (Table 2).

Significant differences were observed between barnyardgrass (susceptible) and rice (resistant) with regard to the translocation of ^{14}C-oxadiazon (Table 3). Translocation of ^{14}C-oxadiazon was significantly greater in barnyardgrass than in rice in the leaf systems. Furthermore, in leaf-disc experiments, a greater amount of ^{14}C was detected within the treated leaf of barnyardgrass than was detected in rice. During the first 2 days, ^{14}C detected in the

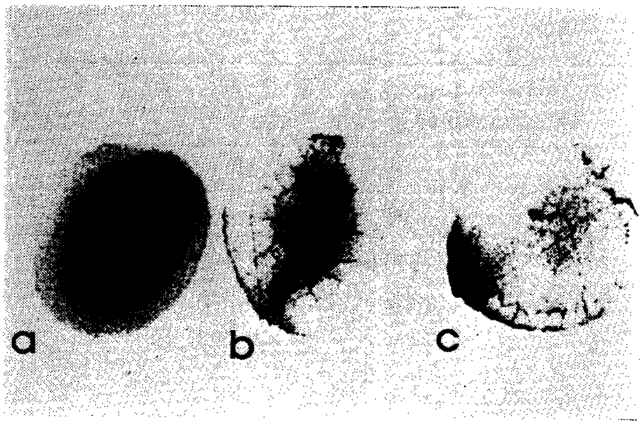

Fig. 1 Leaf disc microautoradiograms of maize (a), chickweed (b) and broad bean (c) treated with ^{14}C-MCPA.

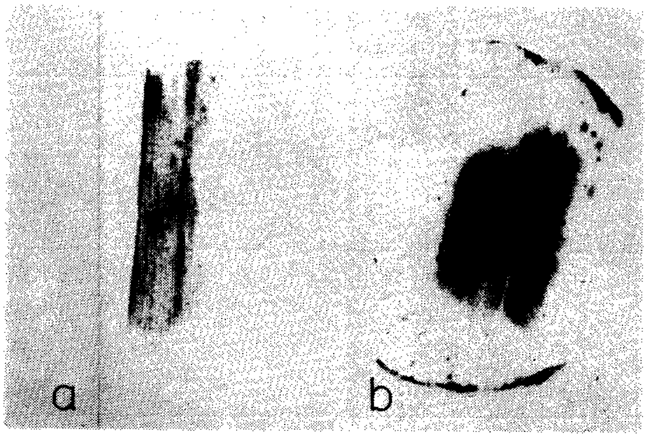

Fig. 3 Microautoradiograms of leaf discs of rice (a) and barnyardgrass (b) treated with ^{14}C-oxadiazon.

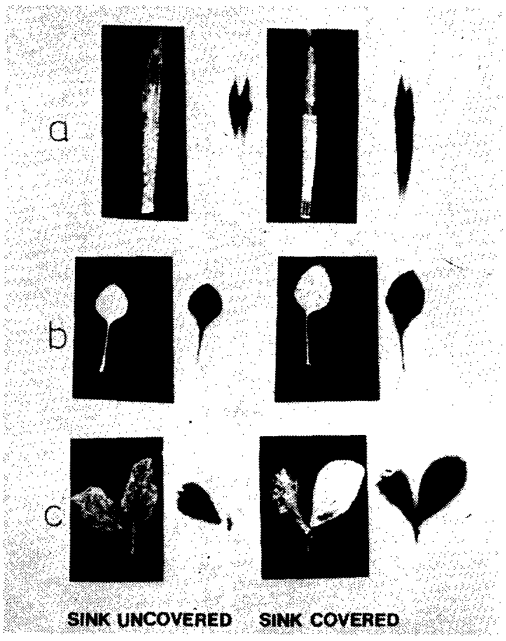

Fig. 2 Translocation of ^{14}C-MCPA in excised leaves of maize (a), chickweed (b) and broad bean (c). Samples on left, autoradiograms right.

agar block was higher in barnyardgrass than in rice (data not shown). However, on the 3rd day, greater levels of ^{14}C were detected in rice than in barnyardgrass, suggesting that the penetration of leaf discs was considerably more rapid in the former than in the latter species. Indeed, microautoradiograms revealed greater absorption and penetration into the minor veins of barnyardgrass than in rice (Fig. 3). Leaf-disc studies also indicated the absorption of ^{14}C-oxadiazon by the minor veins and translocation into the cut vein endings (Fig. 3). In comparison with phloem-mobile MCPA, the appearance of ^{14}C-oxadiazon at the vein endings was rapid suggesting that movement may have occurred in the transpiration stream reflecting water loss at the cut surface; barnyardgrass accumulated more ^{14}C-oxadiazon than did rice. When applied to the foliage, phloem-mobile herbicides are generally thought to move in the assimilate stream from the region of synthesis (source) to the region of utilization (sink).[10] The effect of inducing sink-demand (sinks covered) on the distribution of ^{14}C-MCPA is generally in agreement with the above source-sink concept. The distribution of the predominantly xylem-mobile herbicide ^{14}C-oxadiazon was not, however, significantly influenced by artificial sink creation.

A relatively higher proportion of the radioactivity was detected as surface residue in rice than in barnyardgrass and is consistent with less absorption by the former. In maize most of the absorbed ^{14}C was immobilized in the treated portion of the leaf, while in chickweed most of the ^{14}C was detected as surface residue.

There is evidence that C_4 plants translocate assimilates more readily than do C_3 plants.[11,12] In the present investigation, microautoradiographic studies revealed greater absorption and enhanced vein loading of oxadiazon in the susceptible species barnyardgrass, a C_4 plant. Similar results were also obtained for ^{14}C-MCPA in broad bean (susceptible) and chickweed (moderately susceptible) (both C_3 species) but vein loading did not occur in maize (tolerant) (C_4 species). Based on these studies in which only two C_4 species were used (with varying herbicides), it is difficult to say whether C_4 characters had played any important role in the uptake or translocation in these species.

In the present studies no significant relationships between wax retention of ^{14}C and tolerance were obtained. ^{14}C-MCPA obtained from chloroform washings were limited and represent 0.3 to 2.5%. Generally, species did not differ significantly in this respect. However, greater amounts ranging from 14 to 32% were detected from species treated with ^{14}C-oxadiazon. Rice retained more ^{14}C than barnyardgrass.

From these studies and those of others,[2,13–15] it is apparent that the leaf and leaf-disc systems could be successfully used in examining the uptake and distribution of herbicides. However, it is important to recognize the physiological status of excised systems grown in nutrient solution. In the present experiments, we did not observe any symptoms during the experimental period. Furthermore, if the aim of the experiment is to examine the relative movement of a herbicide in plants with differing sensitivity (as exemplified in this report) under identical conditions, these systems appear to have advantages in terms of the efficient use of the growth chamber facilities and greater uniformity of plant material.

CONCLUSIONS

The translocation of ^{14}C following the treatment with ^{14}C-MCPA or ^{14}C-oxadiazon to leaf or leaf-disc was greater in susceptible species. In the case of ^{14}C-MCPA, the effect on ^{14}C distribution following sink creation was generally in agreement with the source-to-sink concept; distribution of ^{14}C-oxadiazon was not, however, significantly influenced by sink creation.

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要　　約

切除葉における MCPA およびオキサジアゾンの吸収と分布

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MCPA に抵抗性を示すトウモロコシ，やや感受性のハコベ，感受性のソラマメの切除葉および円形切除葉を用いて，^{14}C-MCPA の吸収と移行性について検定した．またオキサジアゾンに抵抗性を示すイネおよび感受性のノビエについても，同様の材料を作り，^{14}C-オキサジアゾン処理による吸収と移行を検定した．^{14}C-MCPA の体内移行性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．同様に ^{14}C-オキサジアゾンではイネに比べてノビエで大きな移行性を示した．円形切除葉のオートラジオグラフによる観察では ^{14}C の吸収はトウモロコシ，ノビエで多く，葉脈への集積は前者だけであった．^{14}C-MCPAと異なり，^{14}C-オキサジアゾンでは sink creation よりあまり影響はうけなかった．