pares the photodegradation as determined by HPLC and microbiological assay. The data obtained by both methods are comparable. About 50% of the drug was degraded in less than 1 month. Thereafter, the rate of degradation became minimal, probably due to a protective film of degradation products formed around the unreacted drug. The degradation products could not be characterized and are believed to be polymeric. Small amounts of the *anti*-oxime of ceftiofur sodium were also observed, indicating inversion of the *syn*-oxime form of ceftiofur sodium used in this study.

Hydrolysis of Ceftiofur Sodium. The hydrolysis of ceftiofur sodium in pH 5, 7, and 9 buffers at 22 °C is shown in Figure 7. The half-life for the hydrolytic degradation is given in Table V. These data are based on the HPLC analysis of ceftiofur sodium. The potencies as a percent of initial concentration $(\mu g/mL)$ of ceftiofur sodium by HPLC and microbiological assay were similar. The data at 47 °C for HPLC and microbiological assay were also similar (Figure 8) except that degradation was faster compared to 22 °C as expected (Table V). The two methods gave results that were linearly related with y = 0.96. The expected hydrolysis products, desfuroyl ceftiofur and furoic acid, were not present, and there was no indication of the appearance of products that could be detected by HPLC. These products are thought to be polymers that do not elute from the HPLC column until the end of the gradient.

LITERATURE CITED

- AOAC. Official Methods of Analysis, 14th ed.; AOAC: Arlington, VA, 1984; Sections 42.203, 42.204, 42.205, 42.206, 42.207, 42.208, pp 813-815.
- Bartha, R.; Pramer, D. Features of a Flask and Method for Measuring the Persistence and Biological Effects of Pesticides in Soil. Soil Sci. 1965, 100, 68-70.
- Food and Drug Administration. Animal Drugs, Foods and Related Products, Ceftiofur Sterile Powder. Fed. Regist. 1988, 53, 5369-5370.
- Houston, I. K. Effects of Invermectin on Fauna in Dung. Aust. Vet. J. 1987, 64, 4.
- Jaglan, P. S.; Kubicek, M. F.; Arnold, T. S.; Cox, B. L.; Robins, R. H.; Johnson, D. B.; Gilbertson, T. J. Metabolism of Ceftiofur. The Nature of Urinary and Plasma Metabolites in Rats and Cattle. J. Agric. Food Chem. 1989, 37, 1112–1118.
- Patel, I. H.; Kaplan, S. A. Pharmacokinetic Profile of Ceftriaxone in Man. Am. J. Med. 1984, 77 (4C), 17-25.
- UpJohn Co. Environmental Studies Related to Approval of New Animal Health Drugs. Symposium, Brook Lodge, Augusta, MI, May 26, 1988.
- Wall, R.; Strong, L. Environmental Consequences of Treating Cattle with the Anti-Parasitic Drug Ivermectin. 1987, 327, 418-421.
- Yancey, R. J.; Kinney, M. L.; Roberts, B. J.; Goodenough, K. R.; Hamel, J. C.; Ford, C. W. Ceftiofur Sodium, a Broad Spectrum Cephalosporin. Evaluation In Vitro and In Vivo in Mice. Am. J. Vet. Res. 1987, 48, 1050-1053.
- Received for review May 30, 1989. Accepted November 17, 1989. Registry No. Ceftiofur, 80370-57-6.

Effect of Endomycorrhizae on the Bioavailability of Bound ¹⁴C Residues to Onion Plants from an Organic Soil Treated with [¹⁴C]Fonofos[†]

Sherman D. Nelson* and Shahamat U. Khan

Land Resource Research Centre, Research Branch, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6

Uptake of bound ¹⁴C residues from an organic soil treated with radiolabeled fonofos (*O*-ethyl *S*-phenyl ethylphosphonodithioate) by selected *Glomus* endomycorrhiza and onion roots was studied. The hyphae of endomycorrhizal associations were capable of removing ¹⁴C residues from the soil and transporting them to onion plants. Bioavailability of soil-bound ¹⁴C residues, as measured by ¹⁴C residue content in onion, was increased 32 and 40% over that of nonmycorrhizal plants by hyphae of *Glomus intraradices* and *Glomus vesiculiferium*, respectively. The data suggest that under field conditions endomycorrhizal infection may greatly increase the bioavailability of soil-bound pesticide residues to plants.

It is now well established that many commonly used pesticides form bound residues when applied to agricultural soils (Khan, 1982; Katan et al., 1976). These residues may constitute an environmental hazard as their bioavailability and ultimate fate is generally unknown. Recent studies using radiotracer techniques have indi-

cated that bound pesticide residues in soil may become bioavailable to plants (Racke and Lichtenstein, 1985; Khan, 1980; Helling and Krivonak, 1978) and microbes (Khan and Ivarson, 1981, 1982). In general, plants grown in soil containing only ¹⁴C-bound residues of pesticides were found to take up 0.1-1.0% of these residues (Khan, 1982). The role of plant roots and soil microbes and the mechanisms of release and removal of these bound pesticide residues remain unclear.

[†] LRRC Contribution No. 89-105.

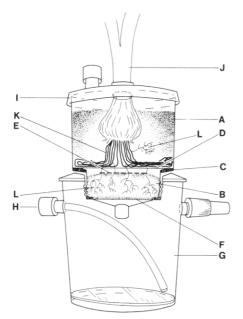


Figure 1. Treatment vessel used to study uptake of bound ¹⁴C residues by roots and endomycorrhizae. Modified 150-mL disposable Falcon filter unit: A, upper soil compartment containing non-¹⁴C-labeled soil; B, lower soil compartment containing ¹⁴C-labeled soil; C, Viceroy rubber fruit jar ring; D, polyester screen with either 43- or 1000- μ m mesh; E, 1000- μ m mesh polyester screen; F, 0.45- μ m membrane filter; G, receptacle for leachate; H, siphon port for leachate removal; I, funnel lid; J, onion; K, onion roots; L, hyphae of endomycorrhizae.

The role of endomycorrhizal associations in determining the fate of bound pesticide residues in soil has not been investigated. These specialized associations between soil fungi and plant roots are known to improve the uptake of several soil components into the plant (Cooper, 1984). This improved uptake is understood to result from an extensive network of fungal hyphae exploring the soil mass more thoroughly than roots and forming an extended solute capture system for the plant. In addition, hyphae contain enzymes that solubilize various soil nutrients facilitating their uptake by the host plant (Cooper, 1984; Mac-Donald and Lewis, 1978). This study was undertaken to determine whether selected endomycorrhizal soil fungi could influence the fate of bound residues of fonofos (Oethyl S-phenyl ethylphosphonodithioate) in an organic soil. Fonofos, an organophosphorus insecticide, is used in onion cultivation to control onion maggots [Hylenga antigua (Meig)] and is known to form bound residues in organic soils (Khan et al., 1976).

MATERIALS AND METHODS

Soil. A Humic Mesiosol organic soil previously described (Khan et al., 1976) was used. The soil was treated in the field with [ring-¹⁴C]fonofos (10% granular formulation, 7.6 kg·ha⁻¹) and collected after a cropping season (Khan and Belanger, 1987). This soil was exhaustively extracted, until further extraction did not remove any measureable ¹⁴C residues, with hexane-acetone (Khan and Belanger, 1987). The ¹⁴C-bound (nonextractable) residue content of the soil was 3.213 × 10⁴ dpm/g (dry weight), or 2.1 ppm fonofos equiv. Control soil consisted of soil collected from nearby plots under the same cultural practices that had not received any [¹⁴C]fonofos treatment (Khan and Belanger, 1987).

Treatment Vessel. The treatment vessels were 150-mL disposable FALCON filter units (7102; Becton Dickinson and Co., Crockeysville, MD 21030) that had been modified to allow the insertion of a screen into the funnel to form two soil compartments (Figure 1). The compartments were separated by various mesh polyester fiber screens: 43-µm plain mesh (HD7-43, Tetko Inc., 420 Saw Mill River Road, Elmsford, NY 10523) to

prevent root but allow hyphal penetration and $1000-\mu m$ plain mesh (H27-100) to allow root and hyphal penetration. Screens were sealed onto Viceroy rubber fruit jar rings with Instant Krazy Glue II. Moist soil containing ¹⁴C-bound residues, 11.56 ± 0.12 g (dry wt) of soil/treatment vessel, was placed in the bottom funnel compartment. One layer of the $1000-\mu m$ mesh screen was placed on top of the soil in all treatments. The screens with attached rubber rings were placed into the funnels and sealed to the funnel lip with 502 epoxy resin and H956 hardener (5:1). Screens with rubber rings were placed so that an air gap equal to the width of the rubber ring (at least 1 mm) separated this screen from the layer of $1000-\mu m$ screen resting on top of the soil. Moistened control soil, $39.15 \pm 0.43 \text{ g} (\text{dry wt})/$ treatment vessel, was then placed above this screen. After insertion of plant material into the vessels, the filter unit caps were replaced and the units were wrapped in aluminum foil.

Plant Material. Surface-sterilized seeds of Allium cepa (L.) cultivar Autumn Spice were germinated in sterile quartz sand. Seedlings were transplanted to the treatment vessels after 10 days. Plants were watered every 2–3 days with sterile distilled water and were kept under a 16-h photoperiod of $250 \,\mu\text{E}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ with a 26–21 °C day–night temperature. Plants were given filter-sterilized Long-Ashton nutrient solution (Hewitt, 1966) once every fourth week to a total of 15-mL spread over 1 week.

Water Use. Soil water contents were maintained at 181.72 \pm 1.21% by injecting measured amounts of sterile distilled water determined from weight loss over 2–3-day periods. This allowed precise monitoring of water use and minimized errors due to capillary rise or variability caused by leaching of released radiolabeled materials from the compartment of soil containing ¹⁴C-bound residues. Leaching of solution from the soil containing ¹⁴C-bound residues to the treatment vessel receptacle was not observed.

Mycorrhizal Inoculum. Fresh chopped leek (Allium porrum L.) root inocula of Glomus intraradices and Glomus vesiculiferium was used for the mycorrhizal treatments. These inocula were produced by the host plant soil-culture system (Menge, 1984) with sterilized turface as the soil medium (Plenchette et al., 1982). Inoculum was surface-sterilized according to Furlan and Fortin (1973) and 0.1 g (dry weight) placed in the rooting zone of control soil either prior to or after γ irradiation of the vessel plus soils. The percent of root infection was determined after roots were stained with acid fuschin (Berch, 1979) by a glass slide transect technique (Plenchette et al., 1982). This technique determines, at 100× magnification, the percent of root sections observed containing visible evidence of root infection by endomycorrhizae. The leek root inocula of G. intraradices and G. vesiculiferium had 78 and 84% levels of infection, respectively.

Treatments. The experimental design was a completely randomized design consisting of six replicates and eight treatments. These treatments and the possible uptake processes that each treatment was designed to represent are summarized in Table I. Soils were γ -irradiated after placement in the treatment vessels with 1.2 MRad to kill soil microfauna and microflora (Mosse 1973). γ -Irradiation was performed by RadioChemical Canada Ltd. (Kanata, ON) using a ⁶⁰Co source. An additional treatment, which did not contain plants, was handled under the same conditions throughout the experiment in order to determine the release of ¹⁴C residues from soil due to abiotic processes.

Plant Harvest. Plant samples were harvested after 90 days of growth. Care was taken to separate leaf and bulb tissues from the root system. Roots were cut just above the variable mesh screen and thereby divided into those in contact with cold vs hot soil. Each of these root samples was separately rinsed in four changes in distilled water to remove adhering soil particles. Dry weights were determined, and then samples were analyzed for ¹⁴C.

¹⁴C Analysis. Combustion of plant tissues to ¹⁴CO₂ was done in a Packard sample oxidizer, Model 306. The ¹⁴CO₂ was absorbed in and mixed with an appropriate volume of oxisorb and oxiprep (Packard Instrument Canada, Ltd.). Samples were analyzed in a Beckman Series 8000 liquid scintillation spectrometer, using an external standard. Samples displayed variable chemiluminescence, which decayed after a 2-week period. Ten consecu-

Table I. Treatment of Soil and Experimental Conditions for Studying the Uptake of Bound ¹⁴C Residues from an Organic Soil Treated with [¹⁴C]Fonofos

treatment and contact with ¹⁴ C-labeled soil compartment	mesh size, μm	γ-irrad ^a rel to inoc	inoculum			
(a) Root plus Mycorrhizal Hyphae						
root + G. vesiculiferium	1000	before	G. vesiculiferium			
root + G. intraradices	1000	before	G. intraradices			
(b) Mycorrhizal Hyphae Only						
G. vesiculiferium hyphae	43	before	G. vesiculiferium			
G. intraradices hyphae	43	before	G. intraradices			
indigenous mycorrhizal species hyphae	43	none	none			
(c) Contr	ol Trea	tments				
root only	1000	before	none			
inoculum contaminants	43	before	nonmycorrhizal ^b			

 $^{\alpha}$ γ -Irradiation of assembled vessel and soils either before or after inoculum placement in treatment vessel (1.2 MRad). ^b Nonmycorrhizal inoculum refers to chopped leek roots without any endomycorrhizal symbionts. ^c Abiotic transport processes such as volatilization, capillarity, or bulk flow of soil solution.

43

after

nonmycorrhizal

tive readings were averaged to establish a mean sample reading. Samples from control plants grown without any ¹⁴C-labeled soil were counted to establish background levels for the scintillation counter. Roots in direct contact with ¹⁴C-labeled soil were not analyzed for radioactivity due to potential surface contamination by adhering soil particles. Therefore, total root radioactivity was calculated from total root dry weight (roots in contact with ¹⁴C-labeled soil plus roots in contact with non-¹⁴C-labeled soil) and the radioactivity per gram dry weight determined for roots in the non-¹⁴C-labeled soil. After the experiment was completed, soils were exhaustively extracted with hexane-acetone to determine the amounts of ¹⁴C released and not taken up into plants.

RESULTS AND DISCUSSION

abiotic transport processes

Plant Growth and Water Use. Roots did not show any visible growth abnormality except where the roots had encountered the barriers to downward and horizontal growth. Characteristics of pot-bound conditions were evident in this case. Symptoms of soil toxicity in onion, due to prior extraction of soil with organic solvents (Helling and Krivonak, 1978), were not observed. In our study, plant roots also had access to nonextracted soil in the upper soil compartment in each treatment vessel.

At harvest, 37%, 42%, and 21% of total plant mass were located in the leaf, bulb, and root tissues, respectively. In treatments with roots in both soil compartments, approximately 68% of the total root mass was in the lower ¹⁴C-labeled soil compartment. Treatments in which roots were restricted to the upper soil compartment had significantly less total plant growth and water use than treatments in which roots were allowed to grow into the lower compartment (Table II). A comparison of treatments in which mycorrhizae were present indicates that plants with roots restricted to the upper soil compartment had smaller root systems (Table II). This reduced root growth may relate to the influence of roots encountering the narrow-mesh screen sooner and the resulting decreased growth rate due to becoming pot-bound in the more limiting soil volume of the upper compartment.

Mycorrhizal treatment of onion, under the growth conditions used in this study, did not stimulate plant growth. Growth enhancement has often been observed in onion (Furlan and Fortin, 1973) especially under conditions in which the supply of nutrients and water to roots is limiting plant growth. In this study, the small soil volume in the treatment vessel may have been the most limiting factor, and the potential benefit from mycorrhizal infection, resulting from mycorrhizal exploration of additional soil mass, was not expressed.

Mycorrhizal Infection. Levels of mycorrhizal infection with *G. vesiculiferium* and *G. intraradices* were high, and no significant level of contamination by nonmycorrhizal fungi was noted (Table II). No significant differences were found in the level of mycorrhizal infection for roots in the upper and lower soil compartments, indicating that the extracted soil was not inhibitory to hyphal growth and the infection process. Pesticides are known to have varying effects on endomycorrhizae (Trappe et al., 1984); however, studies with fonofos have not been previously reported. Our data indicate that fonofos residues at these levels have little or no influence on *Glomus* endomycorrhiza. These residues were mostly soilbound and therefore of low bioavailability.

Onion root pieces grown in non- γ -irradiated soil were cultured and fungal isolates similar to *Chrysosporium* pannorum, Fusarium sporotrichoides, and Lasiobolus pulcherrinus (Dalpé, Y., personal communication) were obtained. The relationships between these isolates and residue uptake processes were not determined but were assumed to be insignificant since only rare root infection with Lasiobolus was observed on root preparations. In addition, these fungi are not aggressive root pathogens.

Total ¹⁴C Residue in Onion Plants. After 90 days of growth, ¹⁴C residues were found in onion plants (Table III). Plants in the G. vesiculiferium treatment in which both roots and hyphae were in contact with soil containing bound ¹⁴C residues contained 0.11% of the original soil-bound ¹⁴C residue. This amount of ¹⁴C residue in onion was low but similar to that found in other studies. Uptake levels at 0.46-1.06% of bound [14C]dinitroaniline from a soil-sand mixture into soybean (Glycine max) (Helling and Krivonak, 1978) and 0.53% of bound ¹⁴C]prometryne from an organic soil into oats (Avena sativa) (Khan, 1980) have been reported. In comparison to the above noted studies, lower levels of ¹⁴C residue in onion may relate to restricted root growth in the limited soil volume of the treatment vessel, low plant mass, and low level of available ¹⁴C residue for uptake.

On a plant and per gram dry mass basis, the greatest amount of ¹⁴C was found in treatments in which both mycorrhizal-infected roots and mycorrhizal hyphae were located in soil containing bound [14C]fonofos residues (Table III). Treatments to control for transport of ¹⁴C due to either inoculum contaminants or abiotic processes did not contain significant amounts of ¹⁴C residues (Table III). Control treatments for physical processes, such as the capillary rise of soil water from the lower to the upper soil compartment, accounted for only 6.2% of the mean ¹⁴C residues found in plants of treatments in which roots plus mycorrhizal hyphae penetrated the lower ¹⁴C-labeled soil compartment. The mean ¹⁴C residue uptake in hyphae-only treatments accounted for 34% of that in root plus hyphae treatments. The presence of mycorrhizal hyphae on roots increased radiolabeled residues uptake by 40 and 32% over the root only treatments for G. vesiculiferum and G. intraradices, respectively (Table III).

Influence of Mycorrhizal Species on ¹⁴C Residues in Plants. G. intraradices hyphae only treatments contained significantly greater amounts of ¹⁴C residue on a plant and a per gram dry mass basis than either the G. vesiculiferium hyphae only or indigenous species hyphae only treatments. Although differing enzyme sys-

Table II. Plant Mass, Water Use, and Level of Mycorrhizal Infection of Onion after 90 Days of Growth in Organic Soil Containing Bound ¹⁴C Residues of Fonofos⁴

treatment and contact with ¹⁴ C-labeled soil compartment	leaf mass, g	bulb mass, g	root mass, g	plant mass, g	infection, %	water use, g H ₂ O/plant
		(a) Root plus N	Aycorrhizal Hyp	ohae		
root + G. vesiculiferium	0.39 b	0.54 a	0.21 ab	1.14 ab	75.1 b	142.2 bc
root + G. intraradices	0.39 b	0.55 a	0.22 a	1.22 a	87.9 ab	152.2 bc
		(b) Mycorrh	izal Hyphae On	lv		
G. vesiculiferium hyphae	0.30 b	0.36 b	0.16 c	0.81 c	81.2 ab	66.9 e
G. intraradices hyphae	0.32 b	0.35 b	0.17 bc	0.83 c	91.3 a	98.9 d
indigenous species	0.31 b	0.37 b	0.14 cd	0.82 c	47.5 c	118.4 cd
		(c) Contr	ol Treatments			
root only	0.59 a	0.36 b	0.15 c	1.09 ab	0 d	207.4 a
inoculum contaminants	0.38 b	0.32 b	0.10 d	0.76 c	0 d	136.9 bc
abiotic transport processes	0.51 a	0.33 b	0.16 c	1.00 b	0 d	173.2 b

^a Mean separation within a column by Duncan's multiple-range test, P = 0.05; six replicates.

Table III. Total Amount of ¹⁴C Residue in Tissues of Onion after 90 Days of Growth in Organic Soil Containing Bound ¹⁴C Residues of Fonofos⁴

treatment and contact with ¹⁴ C-labeled soil compartment	total dpm/plant	dpm/g dry mass				
(a) Roots plus Myc	orrhizal Hyphae					
roots + G. vesiculiferium	454.8 a	398.9 a				
roots + G. intraradices	428.6 a	351.3 a				
(b) Mycorrhizal Hyphae Only						
G. vesiculiferium	140.2 d	173.1 c				
G. intraradices	219.2 c	264.1 b				
indigenous species	84.9 de	103.5 c				
(c) Control 7	Freatments					
roots only	325.3 b	298.4 b				
inoculum contaminants	36.9 e	48.6 d				
abiotic transport processes	26.8 e	26.8 d				

^a Mean separation within a column by Duncan's multiple-range test, P = 0.05; six replicates.

Table IV.Allocation of ¹⁴C Residue within Onion after 90Days of Growth in Organic Soil Containing Bound ¹⁴CResidues of Fonofos^a

treatment and contact with	% of total plant ¹⁴ C in				
¹⁴ C-labeled soil compartment	leaf	bulb	root		
(a) Roots plus Mycorrhizal Hyphae					
roots + G. vesiculiferium	81.2 a	11.1 a	7.5 b		
roots + G. intraradices	78.5 a	11.9 a	9.7 b		
(b) Mycorrhizal Hyphae Only					
G. vesiculiferium	40.1 b	3.5 b	56.4 a		
G. intraradices	32.1 b	3.4 b	64.6 a		
indigenous species	38.2 b	5.3 b	56.5 a		
(c) control treatment ^{b}					
root only	77.1 a	8.6 ab	14.4 b		

^a Mean separation within a column by Duncan's multiple-range test, P = 0.05; six replicates. ^b Other control treatments not included due to their low total dpm values.

tem capacities for release of bound residues between species is possible, it is likely that the higher levels of infection and significantly greater total water use in the G. *intraradices* treatment, 13% higher infection and 48% greater water use then G. *vesiculiferium*, relate to these differences in total ¹⁴C residue uptake. Although the indigenous species hyphae only treatment had 20% greater water use than the G. *intraradices* hyphae only treatment, the lower level of ¹⁴C residue in the indigenous treatment was likely due to the significantly lower level of mycorrhizae infection (Table II).

Allocation of ¹⁴C Residue in Onion. Significant differences in allocation of radiolabeled residues were found among the treatments. Treatments in which only mycorrhizal hyphae were responsible for ¹⁴C residue uptake had a much greater allocation to roots, ca. 8 times more

Table V. Onion Tissue ¹⁴C Residue Concentration (dpm·g⁻¹ Dry Weight) after 90 Days of Growth in Organic Soil Containing Bound ¹⁴C Residues of Fonofos⁴

treatment and contact with ¹⁴ C-labeled soil compartment	leaf	bulb	root			
(a) Roots plus M	ycorrhizal	Hyphae				
roots + G. vesiculiferium	960.2 a	100.7 a	125.7 d			
roots + G. intraradices	881.0 a	95.5 a	191.6 cd			
(b) Mycorrhizal Hyphae Only						
G. vesiculiferium	192.1 c	15.7 b	469.7 b			
G. intraradices	212.4 с	21.9 b	842.9 a			
indigenous species	121.8 c	13.7 b	319.6 bcd			
(c) Control Treatment ^b						
roots only	438.9 b	77.9 a	367.3 bc			

^a Mean separation within a column by Duncan's multiple-range test, P = 0.05; six replicates. ^b Other control treatments not included due to their low total dpm values.

Table VI. Bioavailability of Bound ¹⁴C Residues of Fonofos from an Organic Soil⁴

treatment and contact	% orig soil-bou	% ¹⁴ C in				
with ¹⁴ C-labeled soil compartment	onion	soil extract	soil extract onion			
(a) Roots plus Mycorrhizal Hyphae						
roots + G. vesiculiferium	0.11 a	1.19 a	8.73 a			
(b) Mycorrhizal H	Iyphae Only				
G. vesiculiferium	0.04 b	1.36 a	2.59 b			
(c) Control Treatments						
roots only	0.08 a	1.57 a	5.00 a			
abiotic transport processes	<0.01 c	1.73 a	0.46 c			
no plants		<0.01 b				

^a Mean separation within a column by Duncan's multiple-range test, P = 0.05; six replicates. ^b Original soil-bound ¹⁴C residues were 2.1 ppm fonofos equiv (dry weight) of soil.

(Table IV). The ¹⁴C residue concentrations (dpm·g⁻¹ tissue dry mass) in roots of hyphae-only treatments were greater than in treatments where root uptake was also involved (Table V). These data indicate a selective partitioning of ¹⁴C residues in onion that is dependent upon the method of uptake. Thus, ¹⁴C residue from hyphaeonly uptake is distributed throughout the plant but accumulates in the root, and ¹⁴C residue from root-only uptake results in a greater portion translocated to the shoot. In the root plus hyphae treatments, allocation appears to be dominated by the root-only uptake allocation pattern. This may be a consequence of the limited soil volume in the treatment vessel, reducing the influence of greater exploration of soil mass by hyphae than roots and therefore dominance of allocation by the hyphal pattern. Therefore, the influence of endomycorrhizae on pesticide allocation within onion has important consequences for the potential bioaccumulation into edible portions of plant materials.

Extraction of Bound ¹⁴C **Residues from Soil.** The soil was extracted to determine the level of extractable ¹⁴C residues at the end of the experiment. Significant differences were found among treatments (Table VI). As the amount of extractable ¹⁴C residue in soils was negligible at the start of the experiment and in controls with bound ¹⁴C residues watered throughout the experiment but not containing plants, it is likely that exudates from the root leached into the soil containing bound ¹⁴C residues and released some of the bound ¹⁴C residue. It appears that intimate contact between roots and the soil-bound pesticide residues is not a requirement for bio-availability of pesticide residues.

The amount of 14 C residue extractable with hexaneacetone from soil at harvest exceeded the amount of 14 C residue in the plant by 1 order of magnitude. In this study the soil volume was limited, and root plus hyphal exploration of the soil mass was extensive. Under these circumstances one could expect greater amounts of the extractable 14 C residue to be taken up into onion if it was readily bioavailable. Therefore, either the 14 C residue extractable by hexane-acetone was not bioavailable or onion root and hyphal permeabilities for fonofos and its residues were limiting. The relationship between hexane-acetone extractability and eventual bioavailability remains undetermined.

These data indicate that bound ¹⁴C residues in soil treated with [¹⁴C]fonofos become bioavailable and are taken up into onion plants. Previously, workers had shown negligible (Khan et al., 1976) or limited (Khan and Belanger, 1987) fonofos uptake into onion from soil containing both bound and extractable residues. The mechanism of uptake and the form of ¹⁴C absorbed by hyphae and roots were not elucidated. It remains unclear whether or not the uptake occurs directly from the bound residue reservoir in soil and to what extent soil chemical and/ or biological processes are involved in releasing bound residues. The treatments (Table I) indicated negligible $\rm ^{14}CO_2$ assimilation by onion (Table III). Interestingly, Khan and Belanger (1987) determined by high-temperature distillation procedures that in these soils the parent compound was the major constituent present in the form of bound residues, and Lichtenstein et al. (1983) found that most of the ¹⁴C extractable from soil treated with [¹⁴C]fonofos was the parent compound. Our data indicate that hyphae of Glomus endomycorrhizal fungi are able to take up and transfer to onion ¹⁴C pesticide residues from soil originally containing only bound ¹⁴C residues. The addition of endomycorrhizae to onion roots increased 14 C residue uptake by 32-40%. It appears that under field conditions endomycorrhizal infection would greatly increase the total uptake of bound insecticide residues into plants due to the extensive exploration of soil mass by endomycorrhizal hyphae. The exploration of soil mass by hyphae has been estimated to be at least 1 order of magnitude greater than that by roots. Furthermore, endomycorrhizal infection may also alter the allocation of insecticide within the plant. Consideration of the potential use and manipulation of endomycorrhizae in agriculture will have to include the role of these associations in determining the fate of pesticides in the soil and in plants.

ACKNOWLEDGMENT

Special thanks are expressed to A. Toone for his help with the experimental procedures and plant sampling and to R. McDowell for ¹⁴C analyses. This study was part of a coordinated program of research under the sponsorship of the International Atomic Energy Agency, Vienna, Austria.

LITERATURE CITED

- Berch, S. M. Endomycorrhizae of Southern Ontario Ferns. M.S. Thesis, University of Waterloo, Waterloo, 1979.
- Cooper, K. M. Physiology of VA Mycorrhizal Associations. In VA Mycorrhizae; Powell, C. L., Bagyaraj, D., Eds.; CRC Press: Boca Raton, FL, 1984.
- Fuhremann, T.; Lichtenstein, E. P. Release of Soil Bound Methyl Carbon-14 Parathion Residues and their Uptake by Earthworms and Plants. J. Agric. Food Chem. 1978, 26, 605-610.
- Furlan, V.; Fortin, J. A. Formation of Endomycorrhizae by Endogone calospora and *Allium cepa* under Three Temperature Regimes. *Naturalists Can.* 1973, 100, 467-477.
- Helling, C. S.; Krivonak, A. E. Biological Characteristics of Bound Dinitroaniline Herbicides in Soils. J. Agric. Food Chem. 1978, 26, 1114–1172.
- Hewitt, E. J. Sand and Water Culture Used in the Study of Plant Nutrition, 2nd ed. Tech. Commun., Commonw. Bur. Soils 1966, 22.
- Katan, J.; Furhremann, T. W.; Lichtenstein, E. P. Binding of [¹⁴C] Parathion in Soil: A Reassessment of Pesticide Persistance. Science 1976, 193, 892-894.
- Khan, S. U. Plant Uptake of Unextracted (Bound) Residues from Organic Soil Treated with Prometryn. J. Agric. Food Chem. 1980, 28, 1096-1098.
- Khan, S. U. Bound Pesticide Residues in Soil and Plants. *Residue Rev.* **1982**, *84*, 1–25.
- Khan, S. U.; Ivarson, K. C. Microbiological Release of Unextracted (Bound) Residues from an Organic Soil Treated with Prometryn. J. Agric. Food Chem. 1981, 29, 1301-1303.
- Khan, S. U.; Ivarson, K. C. Release of Bound (Nonextractable) Residues by Various Physiological Groups of Microorganisms. J. Environ. Sci. Health 1982, 17, 737-749.
- Khan, S. U.; Belanger, A. Formation of Bound ¹⁴C-Fonofos Residues in an Organic Soil and a Vegetable Crop under Field Conditions. *Chemosphere* 1987, *16*, 167–170.
- Khan, S. U.; Hamilton, H. A.; Hogue, E. J. Fonofos Residues in an Organic Soil and Vegetable Crops Following Treatment of the Soil with the Insecticide. *Pestic. Sci.* 1976, 7, 553-558.
- Lichtenstein, E. P.; Liang, T. T.; Koeppe, M. K. Effects of Soil Mixing and Flooding on the Fate and Metabolism of ¹⁴C-Fonofos and ¹⁴C-Parathion in Open and Closed Agriculture Microcosms. J. Econ. Entomol. 1983, 76, 233-238.
- MacDonald, R. M.; Lewis, M. The Occurrence of Some Acid Phosphatases and Dehydrogeneses in the Vesicular-Arbuscular Mycorrhizal Fungus, *Glomus mosseae*. New Phytol. 1978, 80, 135-142.
- Menge, J. A. Inoculum Production. In VA Mycorrhizae; Powell, C. L., Bagyaraj, D., Eds.; CRC Press: Boca Raton, FL, 1984.
- Mosse, B. Advances in the Study of Vesicular-Arbuscular Mycorrhiza. Annu. Rev. Phytopathol. 1973, 11, 171-196.
- Plenchette, C.; Furlan, V.; Fortin, J. A. Effects of Different Endomycorrhizal Fungi on Five Host Plants Grown on Calcined Montmorillonite Clay. J. Am. Soc. Hortic. Sci. 1982, 107, 535-538.
- Racke, K. D.; Lichtenstein, E. P. Effects of Soil Microorganisms on the Release of Bound ¹⁴C Residues from Soils Previously Treated with [¹⁴C]Parathion. J. Agric. Food Chem. 1985, 33, 938-943.
- Trappe, J. M.; Molina, R.; Castellano, M. Reactions of Mycorrhizal Fungi and Mycorrhiza Formation to Pesticides. Annu. Rev. Phytopathol. 1984, 22, 331-359.

Received for review August 4, 1989. Accepted November 13, 1989.

Registry No. Fonofos, 944-22-9.