Uptake, Translocation, and Decomposition of Systemic Oxathiin Fungicides in Bean

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ABSTRACT

The systemic oxathiin fungicide, carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) was taken up better by roots of bean than its sulfone analog, oxycarboxin.

Radioautography of plants treated either via the roots or foliage with ^{14}C-labeled carboxin and oxycarboxin indicated apoplastic movement which resulted in marginal accumulation of label in transpiring leaves. Label was not redistributed between organs up to 14 days after cessation of treatment.

Carboxin was readily oxidized in roots to the non-fungitoxic sulfoxide, while oxycarboxin could be detected in acetone extracts of roots and unifoliate leaves up to 21 days after the beginning of the experiment. More than 60% of the label present in roots was acetone-insoluble, irrespective of the oxathiin fungicide used, while less than 5% of the label in unifoliate leaves was acetone-insoluble. Analysis of the root tissue showed that the carboxamide linkage was hydrolyzed producing aniline which was bound to plant polymers and also formed highly water soluble conjugation products. Chemotherapy studies with bean rust (*Uromyces phaseoli typica*) confirmed the data of the translocation, distribution and decomposition studies. Phytopathology 60:1708-1716.

Additional key words: chemotherapy, bean rust.

Oxycarboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide-4,4-dioxide) has been reported by von Schmeling & Kulka (19) to give longer systemic control of bean rust caused by *Uromyces phaseoli typica* Arth. than carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide). But studies on fungitoxicity of oxathiin derivatives to Basidiomycetes (6, 16) indicated that carboxin was significantly more fungitoxic than its sulfone analog, oxycarboxin. Therefore, one would anticipate carboxin to be a better fungicide. Loose smut of cereals is indeed controlled better by the more fungitoxic carboxin (19), while oxycarboxin has been proven to give more satisfactory results than carboxin for diseases requiring a long-lasting chemotherapeutic protection (5, 8, 14, 17, 19).

The aim of the present study was to find an explanation for differences in chemotherapeutic properties of the two oxathiin systemic fungicides. Studies were carried out to gain an understanding of the uptake, translocation, and decomposition of carboxin and oxycarboxin in bean.

MATERIALS AND METHODS.—*Fungicides.*—Oxathiin fungicides were synthesized at UniRoyal Research Laboratory Ltd., Guelph, Ontario, by M. Kulka and coworkers. This laboratory provided us with ^{14}C-labeled carboxin and oxycarboxin, both uniformly labeled in the anilino moiety. Purity of the labeled chemicals was ascertained by means of chromatography, labeled and "cold" carboxin and oxycarboxin were co-chromatographed in a two-dimensional thin-layer chromatography (TLC) system (Solvent 1: benzene-methanol, [9:1, v/v], and solvent 2: chloroform); and by comparison of ultraviolet absorption spectra. The specific activity of ^{14}C-carboxin and ^{14}C-oxycarboxin was 0.2 mc/mmole.

Plant culture.—Seeds of bean (*Phaseolus vulgaris* L.) were germinated in vermiculite. The cultivar, Tendergreen, was used, with exception of the chemotherapy experiments for which Pinto beans were chosen, since the latter cultivar is more susceptible to bean rust than Tendergreen. Preliminary investigations also indicated that there were no significant differences in rate of uptake and translocation patterns of the two oxathiin fungicides in the two bean cultivars. One week after germination, seedlings were transferred to hydroponics. The most suitable nutrient solution proved to be half-strength Knop's solution (10). Experiments were conducted in controlled environment cabinets with a day temp of 21 C, night temp of 18 C, daylength of 16 hr, and a relative humidity of 60%, since preliminary studies had shown that higher wind velocities at edges of benches markedly increased transpiration and consequent uptake of carboxin and oxycarboxin.

Uptake.—Three-week-old plants were placed in 150 ml of nutrient solution with concn of 6.25 and 12.5 μM, respectively, of ^{14}C-carboxin and of ^{14}C-oxycarboxin. The first set of trifoliate leaves were starting to expand at this time. The amount of label present in 150 ml of nutrient solution at these concn was 0.1875 and 0.375 μc, respectively. After 120 hr, the volume of the nutrient solution remaining was measured and compared to untreated controls.

Fungicide taken up over this period was assayed by

determining label remaining in the nutrient solution and subtracting this from the amount of label present in the solution at zero time. Samples of 1 ml of nutrient solution were dispensed into scintillation vials containing 15 ml of scintillation solution. The scintillation solution (toluene:absolute alcohol, 1:1, v/v) contained 6 g of diphenyloxazole (PPO)/liter. Two replicate samples were counted for 10 min each in a Nuclear-Chicago Liquid scintillation spectrometer, Model Unilux II. Quench corrections were made employing the channels ratio method (21). To determine whether the fungicide was chemically altered during the period of application, it was extracted from the nutrient solution by shaking with chloroform ($CHCl_3$:nutrient solution, 1:10, v/v). The $CHCl_3$ fraction was collected and filtered through anhydrous Na_2SO_4, prior to TLC. Eastman Kodak TLC-plates (No. 6061, silica gel without fluorescent dye) were used with chloroform as the solvent system.

Time-course study of distribution and decomposition.—Three-week-old plants were treated with ^{14}C-carboxin and ^{14}C-oxycarboxin by transferring them to a nutrient solution with a fungicide concn of 6.25 μM. We preferred to analyze plants which had taken up approximately the same amount of ^{14}C-carboxin and ^{14}C-oxycarboxin. In this particular experiment, 7 days were required to take up an amount of the labeled sulfone analog equivalent to the amount of ^{14}C-carboxin taken up over a 3-day period. Analyses of plant samples were carried out immediately after termination of the application which is referred to as cessation of treatment time (CTT). The remaining plants were transferred to nutrient solution without fungicide. Plants treated with ^{14}C-carboxin were sampled 3 (CTT), 7, 14, and 21 days after start of treatment, while plants treated with ^{14}C-oxycarboxin were sampled 7 (CTT), 14, 21, and 35 days after start of treatment.

Plants were divided into roots, stem, unifoliate, and trifoliate leaves. Plant parts were ground for 4 min in an Eberbach semi-micro blender in 5 ml of acetone at 0 C/g fresh wt of plant tissue. The homogenate was collected quantitatively over a Whatman No. 1 filter paper and washed with acetone. The final volume of acetone extract was 10 ml of extract/g of tissue. The extract will be referred to as the acetone-soluble fraction. The acetone-insoluble fraction, which appeared completely free of chlorophylls and other pigments, was dried at room temp and weighed. Aliquots of 1 ml of the acetone-soluble fraction were pipetted into scintillation vials containing 0.5 ml of Nuclear-Chicago Solubilizer (a 0.6 N mixture of quaternary ammonium bases, allowing direct solubilization in toluene), and 0.5 ml of 20% benzoyl peroxide in toluene (w/v) to bleach chlorophylls (7). The samples were incubated in this mixture for 30 min at 50 C before 15 ml of the toluene-absolute alcohol scintillation solution were added.

Fifty mg of the acetone-insoluble fraction was combusted in a Nuclear-Chicago semi-automatic sample combustion apparatus, Model 3151. Labeled CO_2 thus formed was captured in 15 ml of an ethanolamine-absolute alcohol mixture (1:2, v/v) of which 5-ml samples were counted in the scintillation counter. In all instances, samples were counted for 20 min and corrected for quenching. For counting of the background, samples from untreated controls, processed similarly as treated material, were used.

To follow the decomposition of labeled oxathiin fungicides in bean, 200 μliter samples of the acetone-soluble fractions were spotted on TLC-plates and developed in the two-dimensional solvent system mentioned earlier. Samples were cochromatographed with a mixture of "cold" carboxin, oxycarboxin, and the sulfoxide of carboxin; and with each of the following compounds: aniline, crotonanilide, butyranilide, acetoacetanilide, and α-(β-hydroxyethyl) mercaptoacetoacetanilide. Developed chromatograms were placed on Kodak No-Screen Medical X-ray film, which was then exposed for 21 days.

Chemical nature of labeled material in acetone-insoluble fractions of roots.—To determine the chemical nature of oxathiin decomposition products in the acetone-insoluble plant material, we proceeded as follows: 1 g of the acetone-insoluble fraction of roots was extracted in 50 ml of glass-distilled water at 100 C for 4 hr. The water-insoluble fraction was recovered by centrifugation and extracted in 2 N HCl for 4 hr at 100 C. The acid-extractable fraction was separated by centrifugation and the pH adjusted to 7, followed by partitioning in 20 ml of chloroform. The chloroform fraction was concd 4-fold in vacuo and cochromatographed with "cold" aniline in benzene-methanol (9:1, v/v) as well as in chloroform. Bands of silica gel were scraped from the developed TLC-plates, and ^{14}C was quantified. A control sample of labeled carboxin was processed similarly to determine decomposition of the fungicide during this procedure.

Biological nature of labeled material in acetone-insoluble fractions of roots.—Data of the study of the decomposition of ^{14}C-carboxin and ^{14}C-oxycarboxin indicated that more than 60% of the labeled material present in the roots was acetone-insoluble (15).

To determine whether the behavior of the acetone-insoluble material derived from the oxathiins was similar to that reported on streptomycin by Pramer et al. (13), the following experiment was designed. One-hundred mg of the acetone-insoluble fraction of roots of treated plants was incorporated at 50 C in 10 ml of malt extract agar (Difco) containing 0.1% of the antibiotic aureomycin (w/v). To allow possible diffusion of fungicide to take place, the samples were incubated at 50 C for 12 hr before pouring plates. Agar discs (7 mm) with mycelium of *Rhizocotonia solani* Kuehn were placed on the center of the plates. The diam of the mycelium was measured after incubation for 7 days at 25 C. Acetone-insoluble fractions of roots of plants harvested at CTT, 7, 14, and 21 days after start of treatment were tested for fungitoxic properties, using material from each of four replicate plants.

Time-course study of translocation.—Plants used in this experiment were treated via the roots and har-

vested similarly as in the time-course study of decomposition. Techniques for radioautography used in this study have been described extensively by Crafts & Yamaguchi (1).

Movement of label after foliar application of ^{14}C-carboxin and ^{14}C-oxycarboxin was investigated by making radioautographs of intact plants of which either the apical or basal half of the upper-surface of one of the unifoliate leaves was treated with 0.5 ml (0.05 μc) of an aqueous 0.5 mM solution of labeled fungicide in 0.1% Tween 20 (v/v) (polyoxyethylene sorbitan monolaurate). The X-ray film was exposed for 21 days. Two replicate plants were used at each time for each chemical in the translocation study.

Chemotherapy of bean rust.—Decomposition of the labeled systemic oxathiins may lead to nonchemotherapeutic chemicals, which will nevertheless produce an image in radioautographs. Therefore, a study was made of the chemotherapy of bean rust with "cold" carboxin and oxycarboxin applied at the same concn as in the time-course studies of distribution, decomposition, and translocation. Four replicate plants were inoculated with uredospores of *U. phaseoli typica* at similar time intervals as in the studies mentioned above. In the latter experiment, only the lower surfaces of the leaves were inoculated to prevent spores from coming into direct contact with the fungicide.

RESULTS.—*Uptake.*—The systemic oxathiin fungicide ^{14}C-carboxin was taken up more readily by bean plants than the sulfone analog, ^{14}C-oxycarboxin, at both concn tested (Table 1). It should be noted that although no visible phytotoxicity symptoms occurred, viz, burning of the margins of the leaves, transpiration of plants grown in 12.5 μM of ^{14}C-carboxin was reduced as compared to the untreated control. The rate of transpiration was similar to that of the untreated control in all other treatments. The plants transpired approximately 50 ml of nutrient solution over the 5-day period, which was 33% of the total volume of nutrient solution. At a concn of 6.25 μM, the plants took up 79.7% of ^{14}C-carboxin and 60.1% of ^{14}C-oxycarboxin, indicating that the concn of fungicide in the nutrient solution decreased significantly. Considering the concn of label in the plant tissue on a fresh wt basis, the chemical was taken up against an apparent concn gradient.

Using TLC, it was ascertained that during the period of application of the oxathiin compounds, decomposition of the fungicides in the nutrient solution did not occur. Insignificant amounts of label "leached" from the roots after transfer to nutrient solution without fungicide.

Time-course study of distribution and decomposition.—The uptake of labeled oxathiin fungicides from the nutrient solution by bean plants at CTT in the time course study amounted to 74.8% with a S.E. of 4.21 (± 4.21) and 74.1 ± 3.80% of the chemical present in the nutrient solution, for ^{14}C-carboxin and ^{14}C-oxycarboxin, respectively. Recovery of labeled material from the various parts of plants treated with either labeled fungicide was similar up to 21 days after start of treatment and ranged from 63 to 70%, but recovery decreased to 48% in ^{14}C-oxycarboxin-treated plants at day 35. The rather similar rates of recovery allow direct comparison of the distribution of label in the various organs of the plant at CTT (Fig. 1). Striking features are the high amounts of label present in the roots and unifoliate leaves and the low amounts in trifoliate leaves. At zero time, the first set of trifoliate leaves had just begun to expand. Significant differences in distribution of label in plants treated with ^{14}C-carboxin as compared to plants treated with ^{14}C-oxycarboxin existed in the roots and in the trifoliate leaves. Redistribution of labeled material between various plant organs, with time, did not appear to be significant (Fig. 1). The amount of ^{14}C, however, in the unifoliate leaves of plants treated with ^{14}C-oxycarboxin decreased 2-fold between day 21 and day 35.

At CTT, 67.0 and 72.1% of the total amount of label present in the roots of plants treated with ^{14}C-carboxin and the labeled sulfone analog was acetone-insoluble, while only 2.9 and 5.5%, respectively, of the labeled material in the unifoliate leaves was non-extractable in acetone. More than 35% of the label present in the stem at zero-time was in an acetone-insoluble form, irrespective of the ^{14}C-oxathiin fungicide used. With time, a significant increase in acetone-insoluble labeled material was observed in all fractions of plants treated with either labeled fungicide, as shown for roots and unifoliate leaves of plants treated with ^{14}C-oxycarboxin in Fig. 3. On day 35,

TABLE 1. Uptake[a] of ^{14}C-carboxin and ^{14}C-oxycarboxin by bean

	Concn in nutrient solution of			
	^{14}C-carboxin		^{14}C-oxycarboxin	
	6.25 μM	12.5 μM	6.25 μM	12.5 μM
% Chemical taken up from nutrient solution	79.7 ± 1.03[b]	71.0 ± 1.57	60.1 ± 6.89	66.7 ± 1.73
ml nutrient solution transpired/g fresh wt of leaf tissue[c]	16.6 ± 1.24	14.8 ± 0.15	18.3 ± 1.12	16.1 ± 1.27
Concn of label (μM) in plant tissue (fresh wt basis)	132.1 ± 0.04	270.5 ± 1.57	97.4 ± 0.03	248.6 ± 1.58

[a] Measured over a period of 120 hr at a day temp of 21 C, night temp of 17, day length of 16 hr, and a relative humidity of 60%.
[b] Mean of six replicates with S.E.
[c] Untreated control transpired 17.2 ml ± 0.37/g fresh wt of leaf tissue.

72.6 ± 2.32% of the label in stems of plants treated with ^{14}C-oxycarboxin was acetone-insoluble, implying that during 28 days the amount of acetone-insoluble labeled material doubled. A similar trend was observed in stems of plants treated with ^{14}C-carboxin.

TLC was employed to determine the chemical nature of the labeled compounds in the acetone-soluble fraction. Only traces of ^{14}C-carboxin could be detected in acetone extracts of roots, and none in unifoliate leaves of plants treated with ^{14}C-carboxin at zero time. The major decomposition product was the sulfoxide of carboxin, which constituted 50 to 55% of the label present in the acetone soluble fraction at zero time (Fig. 2-a). Three labeled decomposition

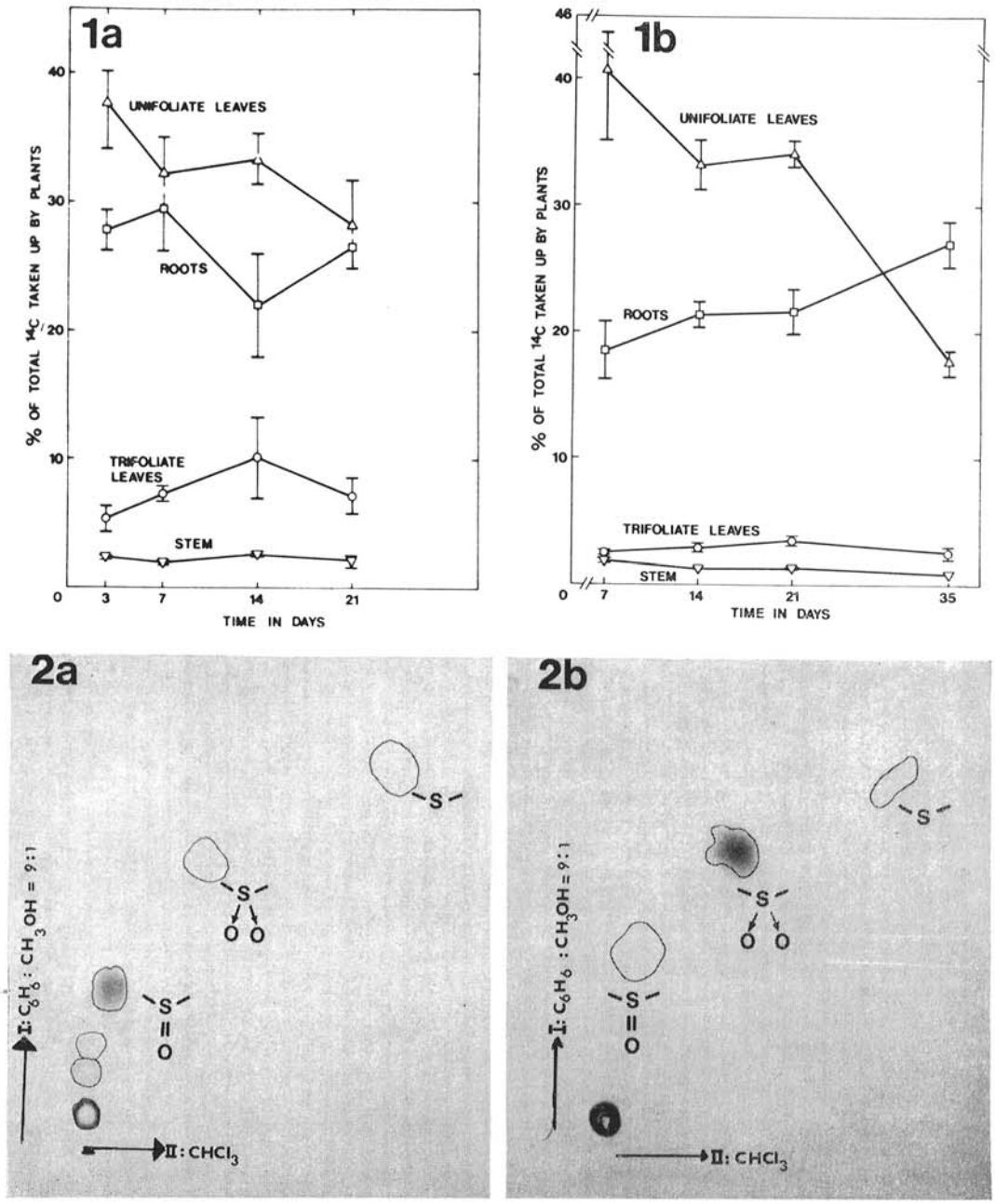

Fig. 1-2. **1)** Distribution of ^{14}C in bean treated via the roots with **a)** ^{14}C-carboxin; **b)** ^{14}C-oxycarboxin. Mean of four replicate plants are given with S.E. **2)** Radioautographs of TLC-plates of acetone extracts of unifoliate leaves of bean plants treated with **a)** ^{14}C-carboxin for 3 days; **b)** ^{14}C-oxycarboxin for 7 days. Location of carboxin (\S/), oxycarboxin (\S/), and the sulfoxide of carboxin (S) was determined by means of cochromatography.

products of carboxin other than the sulfoxide appeared on the radioautographs. The R_F values of these unidentified compounds were lower than the R_F of the sulfoxide in both solvent systems. It was ascertained by TLC that these decomposition products were none of the following compounds: aniline, crotonanilide, butyranilide, α-(β-hydroxyethyl) mercaptoacetoacetanilide, or acetoacetanilide.

On the other hand, 30 to 40% of labeled oxycarboxin was detected with TLC of acetone-soluble fractions of roots and unifoliate leaves of plants treated with this fungicide, at CTT (Fig. 2-b). One or more decomposition products remained at the origin, the percentage gradually increasing from 60-70% of label in the acetone soluble fraction of roots and unifoliates at 7 (CTT), to 90-95% at 21, and to 100% at 35 days after start of treatment.

Aniline, a breakdown product of carboxin and oxycarboxin which would be formed as the result of cleavage of the carboxamide linkage, was not detected in the acetone extracts.

The concn of ^{14}C-carboxin remaining in the nutrient solution at CTT was 0.75 ppm as compared to 1.47 ppm at the beginning of the experiment. Although 27.85% of the label present in the plants at CTT was recovered from the roots, the amount of label present in the acetone-soluble fraction of the roots constituted only 9.37% of the label taken up by the plants. The concn of acetone-soluble labeled chemicals in the roots was equivalent to 3.48 ppm of ^{14}C-carboxin. The true concn of carboxin cannot be determined, since only traces could be detected in radioautographs of thin-layer chromatograms.

The concn of oxycarboxin in nutrient solution decreased during the uptake period from 1.67 to 1.43 ppm. The amount of label in roots of plants treated with oxycarboxin at CTT was 18.5% of total label in plants. On a fresh wt basis, this would be equivalent to 6.55 ppm in roots. Only 5.1% of total label was in the acetone-soluble fraction which would be equivalent to 1.81 ppm. Since only 30 to 40% of the labeled chemical in the acetone-soluble fraction was oxycarboxin, the actual concn ranged from 0.54 to 0.72 ppm.

Chemical nature of labeled material in the acetone-insoluble fractions of roots.—Extraction of the acetone-insoluble fraction of roots of plants treated with ^{14}C-carboxin yielded 31.8% of the total ^{14}C present in the acetone-insoluble fraction with boiling water, while 27.4% could be recovered from the water-insoluble fraction remaining, when extracted with HCl. Very similar figures for recovery of ^{14}C were obtained with material of plants treated with ^{14}C-oxycarboxin.

No labeled aniline was detected in TLC of the water-soluble fraction, although label was present at the origin. In the acid-extractable fraction, 60% of the label was accounted for as aniline, demonstrated with TLC using two separate solvent systems. The remainder of the label appeared as a "tail". Carboxin proved to be stable during acid treatment, for 95% could be recovered chemically unaltered.

Biological nature of labeled material in the acetone-insoluble fractions of roots.—Labeled material in the acetone-insoluble fraction of roots failed to inhibit mycelial growth of the assay-organism, *Rhizoctonia solani,* irrespective of the fungicide used. Interestingly, if the labeled chemical in the acetone-insoluble fraction of roots of plants treated with ^{14}C-carboxin would have retained its fungitoxic properties, the concn of labeled fungitoxic chemical present in the assay medium would have been equivalent to 3 to 4 μM of ^{14}C-carboxin. Complete inhibition of *R. solani* should have occurred at that concn (16).

Time-course study of translocation.—Radioautographs of plants treated via the roots with either labeled oxathiin fungicide, showed that the label moved in the transpiration stream, especially to the fully expanded unifoliate leaves.

DISCUSSION.—*Uptake.*—The more lipid-soluble analog of the two oxathiin fungicides studied, ^{14}C-carboxin, was taken up at a greater rate by bean plants than the sulfone analog, ^{14}C-oxycarboxin (Table 1). Differences in uptake may be accounted for by differences in lipid-solubility as shown for fungi by Mathre (11).

Data presented in the time-course study of distribution and decomposition indicate that, at CTT, the concn of ^{14}C-carboxin in the acetone-soluble fraction of roots appeared to be almost 5-fold higher than in the remaining nutrient solution. This would imply uptake of carboxin against a concn gradient. But only traces of ^{14}C-carboxin could be detected in acetone-soluble fractions of roots at CTT, thus ruling out uptake against a concn gradient. By similar reasoning, the true concn of oxycarboxin in roots is less than half that in the nutrient solution. Therefore, it seems unlikely that oxathiin fungicides are taken up actively. To us, this appears to be in agreement with the apoplastic pattern of movement of oxathiin fungicides.

Time-course study of distribution and decomposition. —Distribution.—Systemic oxathiin fungicides, and/or their breakdown products, did not show extensive accumulation in the roots (Table 2), in contrast to the following fungicides: *n*-alkyl quaternary ammonium compounds with alkyl chains longer than *n*-octyl (4), griseofulvin in tomato (2), and a number of sul-

TABLE 2. Evaluation of carboxin and oxycarboxin as chemotherapeutants of bean rust

	Control of bean rust[a]			
	carboxin[b]		oxycarboxin[b]	
Day of inoculation	Unifoliate leaves	First set of trifoliate leaves	Unifoliate leaves	First set of trifoliate leaves
Day 3	0	0		
Day 7	0	0	95	75
Day 14	0	0	70	55
Day 21			35	20

[a] Control expressed as percentage reduction in pustules per leaf of treated vs. untreated plants.

[b] Three-week-old plants were grown in nutrient solution with a concn of carboxin and oxycarboxin of 6.25 μM, for 3 and 7 days, respectively.

fonamides in broad bean, with exception of sulfanilamide (3), which were taken up readily by roots, but were not translocated, or scarcely, into the shoots and leaves. A certain number of hydrophilic properties are needed for translocation of a chemical within the plant (20). We assume, therefore, that the most hydrophilic oxathiin fungicide tested, ^{14}C-oxycarboxin, would be translocated at a higher rate than ^{14}C-carboxin, and would consequently accumulate at a higher concn in the unifoliate leaves. This trend exists according to our results (Fig. 1), although differences in the amount of label are not significant. Labeled carboxin is decomposed in the roots to the labeled sulfoxide analog of carboxin, which in turn is at least 10 times more water-soluble than the sulfone analog, and will thus be translocated equally well or better than oxycarboxin. The final result is that the amount of label in the unifoliate leaves of plants treated with ^{14}C-oxycarboxin is only slightly higher than in unifoliates of ^{14}C-carboxin-treated plants. The higher amount of label present in trifoliate leaves of plants treated with ^{14}C-carboxin as compared to plants treated with ^{14}C-oxycarboxin can possibly also be explained on the basis of probable differences in translocation of the sulfoxide, the major breakdown product of carboxin, and the sulfone analog.

Redistribution of label between organs in bean plants treated with labeled oxathiins, after cessation of treatment, is not of significance (Fig. 1) until after day 21. At day 35, however, a significant reduction of the amount of label had occurred in the unifoliate leaves of plants treated with ^{14}C-oxycarboxin. The unifoliate leaves showed signs of beginning senescence at this time, which could imply changes in metabolism resulting in decomposition of the labeled anilino moiety of oxycarboxin to ^{14}C-CO_2. Therefore, the decreased amount of label recovered at day 35, which is the result of a reduced amount of radioactive chemicals in the acetone soluble fraction of the unifoliate leaves, could indicate a loss of ^{14}C-CO_2 during respiration (Fig. 3).

Decomposition.—The two most significant findings in our time-course study of decomposition are (i) the rapid rate of oxidation of ^{14}C-carboxin in bean plants to the labeled sulfoxide analog; and (ii) the increasing appearance of labeled chemicals in the acetone-insoluble fractions with time, irrespective of the oxathiin fungicide with which the plants are treated.

We have proposed (15) that oxidation of carboxin to its sulfoxide analog accounts for the earlier loss of chemotherapeutic properties of carboxin as compared to the more stable sulfone analog, oxycarboxin, since Edgington (*unpublished data*) showed that the sulfoxide is not toxic to Basidiomycetes.

Availability of aniline derivatives in plants is greatly influenced by conjugation of decomposition products of these chemicals to plant polymers, viz, lignin, cellulose, and hemicellulose (18, 22, 23). Verloop (18) demonstrated that binding of decomposition products of *p*-aminophenylmethylsulfone is dependent on the number of sites available. With time, increased secondary growth would provide more sites for binding of

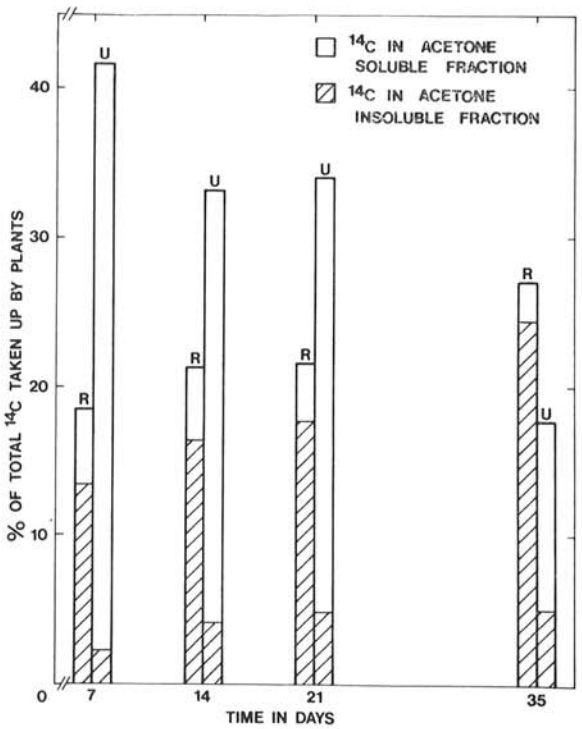

Fig. 3. Distribution of ^{14}C in acetone-soluble and acetone-insoluble fractions of roots (R) and unifoliate leaves (U) of bean treated via the roots with ^{14}C-oxycarboxin. Plants were 3 weeks old at the beginning of the experiment. Four replicate plants were sampled at each time interval.

decomposition product(s) of oxathiin fungicides to plant polymers. Our data (Fig. 3) show that the amount of label in the acetone-insoluble fraction increases significantly with time, thus providing indirect evidence that the acetone-insoluble decomposition products of oxathiins are indeed bound to plant polymers. The percentage of label present in the acetone-insoluble form in the unifoliate leaves is considerably lower than in roots or stems, since secondary growth in leaves is negligible.

Aniline could not be detected in radioautographs of chromatograms of the acetone-soluble fraction of unifoliate leaves of bean plants treated with ^{14}C-carboxin and ^{14}C-oxycarboxin (Fig. 2). Newby & Tweedy (12) recently reported a labeled compound in exudates of leaves of bean treated with ^{14}C-oxycarboxin, cochromatographing with aniline. Probably, aniline formed by hydrolytic cleavage of the carboxamide linkage of the oxathiins is readily metabolized in bean leaves. Acetone extracts of leaves, unlike leaf exudates which only represent the xylem fluid, may contain conjugation products of aniline, rather than aniline. Three unidentified decomposition products of carboxin and one of oxycarboxin were detected in acetone extracts of unifoliate leaves (Fig. 2). The low rate of movement in the organic solvent systems, as compared to that of the very water-soluble sulfoxide

analog of carboxin, indicates that these decomposition products are even more water-soluble than the sulfoxide. Although further identification of these decomposition products of carboxin and oxycarboxin is needed, we postulate that these oxathiin metabolites are conjugation products of aniline, e.g., *N*-phenyl glucosylamine, similar to decomposition products of the herbicide, 3′,4′-dichloropropionanilide (DPA) (22, 23).

Chemical nature of labeled material in acetone-insoluble fractions of roots.—Since the major labeled decomposition product present in the acid-extractable fraction was aniline, we assume that binding of oxathiin decomposition products can only occur when aniline is formed after hydrolysis of the carboxamide linkage. Yih et al. (23) concluded that DPA complexed with polymers only after hydrolysis to 3,4-dichloroaniline. Verloop (18) proposed that if compounds of the type R-NH_2 are bound to plant polymers, the following type of linkage very probably exists: R-NH-CO-plant polymer.

Biological nature of labeled material in acetone-insoluble fractions of roots.—The lack of antibiotic activity of the chemical(s) in the acetone-insoluble fraction in the assay medium supported our contention

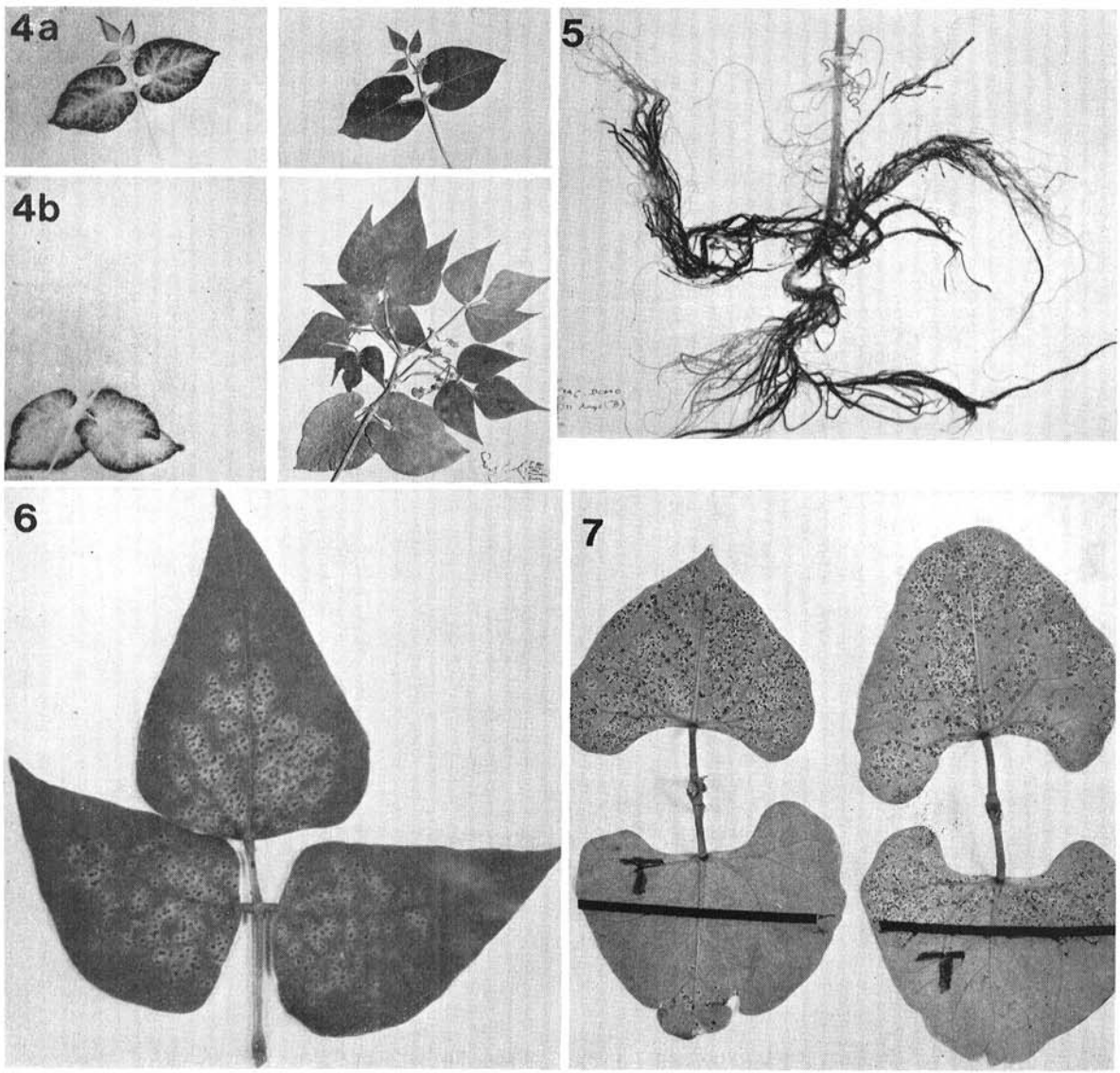

Fig. 4-7. **4)** Distribution of ^{14}C in bean treated via the roots with ^{14}C-oxycarboxin. **a)** Plants immediately after treatment for 7 days; **b)** 40 days after treatment ended. Freeze-dried plants on the right, radioautographs on the left. **5)** Radioautograph of roots of bean plant treated with ^{14}C-carboxin for 3 days, followed by transfer to nutrient solution without fungicide for 11 days, superimposed on freeze-dried root system. **6)** Distribution of bean rust on trifoliate leaves of plants treated via the roots with oxycarboxin for 7 days, followed immediately by inoculation. **7)** Chemotherapy of bean rust with oxycarboxin applied to the upper surface of a unifoliate leaf, either on the apical or the basal half. Plants were inoculated 48 hr after treatment on the lower surface. Therapy verifies acropetal transport.

that no intact carboxin was adsorbed by the plant material. We have earlier reported on the low fungitoxicity of logical decomposition products of carboxin (16).

Time-course study of translocation.—Beans treated via the roots with carboxin and oxycarboxin showed marginal accumulation in leaves. This is characteristic for apoplastic movement (1). Redistribution between organs does not occur after cessation of treatment, as confirmed in our time-course study of distribution (Fig. 6). With time, marginal accumulation in leaves becomes more pronounced, while in the roots the labeled material seems stationary (Fig. 5), probably because of binding of labeled aniline to plant polymers. Kirk et al. (9) showed a similar translocation pattern for ^{14}C-carboxin and its decomposition products following root uptake by cotton seedlings. We found that, following foliar application, the label moved exclusively acropetally. Reduced movement of oxathiins into a covered leaf demonstrated the importance of transpiration for translocation.

Chemotherapy of bean rust.—Experiments on chemotherapy of bean rust with carboxin and oxycarboxin following root and foliar application are in agreement and reconfirm the results of the experiments discussed earlier. Following root application, oxycarboxin was found in decreasing concn until day 21. It also gave decreasing control of bean rust during this period (Table 2). Control of bean rust in carboxin-treated plants did not prove feasible on day 3. This fact agrees with the observation that on day 3, carboxin could not be detected in acetone extracts of leaves and only the nontoxic sulfoxide could be found. These data support a statement made earlier by Snel et al. (16); namely, that the chemotherapeutic properties of the oxathiins should be attributed to the fungitoxicity of oxathiin fungicides rather than to changes in the metabolism of the host.

Marginal accumulation, a result of apoplastic movement of the fungicides, explains the perfect control of bean rust on the margins of the leaves rather than on the entire leaves (Fig. 6). Unlike chemicals which move in the symplast, e.g., the herbicide amitrole (3-amino-1,2,4-triazole) (1), oxathiins do not accumulate in shoot apices. Thus control of bean rust on leaves that do not transpire at time of treatment is ruled out. The fact that systemic oxathiin fungicides move only in the transpiration stream seems a serious limitation to the use of these chemicals as chemotherapeutants.

The distribution pattern of bean rust following foliar application was inversely related to the translocation pattern of the chemicals (Fig. 7). The experiment showed further that the chemicals were translocated systemically through the leaf, since the oxathiins were applied to the upper surface while the lower surface was inoculated. Carboxin seems to be decomposed rapidly in leaves as well as in roots, for disease control did not prove feasible when leaves treated with carboxin were inoculated 7 days after treatment.

A direct comparison with the chemotherapy experiment of von Schemling & Kulka (19) is not possible. They planted treated seed in soil, a technique which does not allow determination of the amount and time over which the chemical is taken up. Therefore, von Schmeling & Kulka (19) obtained nearly perfect control of bean rust with carboxin 7 days after planting. But the chemotherapeutic activity of oxycarboxin for a longer period than carboxin agrees with our data on chemotherapy of bean rust and studies on decomposition of carboxin and oxycarboxin.

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