Mode of Action of Oxathiin Systemic Fungicides. I. Effect of Carboxin and Oxycarboxin on the General Metabolism of Several Basidiomycetes

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ABSTRACT

Carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) and oxycarboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide-4,4-dioxide) at concentrations inhibitory to growth also inhibited the respiration of the sensitive Basidiomycetes *Rhizoctonia solani, Ustilago nuda,* and *Ustilago maydis*. When glucose-U-^{14}C was administered to these organisms, both fungicides caused an increased release of $^{14}CO_2$ when compared to a control. Time course studies with glucose-1-^{14}C or glucose-6-^{14}C indicated that there was an initial inhibition of appearance of $^{14}CO_2$ from carboxin-treated cells as compared with nontreated cells, but after 1 hr there was a greater appearance of $^{14}CO_2$ from the carboxin-treated cells as compared with nontreated cells, suggesting that a block in the metabolism of pyruvate causes a recycling of carbon through pathways of glucose metabolism. The appearance of $^{14}CO_2$ when carboxin-treated cells were administered pyruvate-2-^{14}C, acetate-1-^{14}C, or acetate-2-^{14}C was inhibited 75-95% compared with a control. A buildup of label in succinate with a decreased amount of label in citrate, fumarate, and malate occurred in carboxin-treated cells that were administered glucose-U-^{14}C, acetate-1-^{14}C, or acetate-2-^{14}C. Carboxin inhibited the incorporation of phenylalanine into protein about 30% and the incorporation of uracil into RNA about 75%. Phytopathology 60:671-676.

Following the discovery of the systemic fungicidal nature of the oxathiins by von Schmeling & Kulka (25), numerous reports appeared concerning their potential value in controlling various plant diseases (4, 6, 8, 18, 24). A unique feature of the oxathiins carboxin (Vitavax) (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) and oxycarboxin (Plantvax) (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide-4,4-dioxide) is their specificity for members of the fungal class Basidiomycetes (7). Mathre (12) reported that sensitive fungi absorb much more of these two materials than do resistant fungi, suggesting that specificity of the oxathiins is related to the amounts that enter the cell.

Since fungi have many metabolic pathways similar to or the same as those of higher plants, it is of special interest to determine why systemic fungicides can move through plant tissue without adversely affecting it, and at the same time inhibit or kill the fungal pathogen. While the modes of action of some systemic fungicides such as cycloheximide, griseofulvin, and chloroneb have been studied (3, 9, 10, 19, 20, 21), the oxathiins have not been studied in this respect. This report concerns the effect of the oxathiins on the general metabolism of sensitive fungi. A preliminary report has been presented (13).

MATERIALS AND METHODS.—*Organisms.*—Teliospores of *Ustilago maydis* (DC.) Cda. and *Ustilago nuda* (Jens.) Rostr. were collected from their naturally infected hosts, sieved through fine-mesh wire screens, air-dried to a moisture content of 8-11% (dry wt basis), and stored at 4 C. Germination percentage of the teliospores was 90% or greater. *Rhizoctonia solani* Kühn was grown for 5-7 days on a medium previously described (12). A cork borer was used to punch 8.5-mm discs from the colony. The discs or teliospores were washed three times with sterile deionized water before they were used.

Chemicals.—Technical material of carboxin and oxycarboxin was supplied by UniRoyal Chemical, Division of UniRoyal, Inc. The purity of these materials was determined as previously described (12).

Glucose-U-^{14}C, glucose-1-^{14}C, glucose-6-^{14}C, acetate-1-^{14}C, acetate-2-^{14}C, pyruvate-2-^{14}C, L-phenylalanine-U-^{14}C, and uracil-2-^{14}C were purchased from Amersham/Searle.

Respiration.—Respiration rates were determined by conventional manometric procedures with a Gilson differential respirometer (23). Two or four mycelial discs of *R. solani* weighing 2-3 mg each or 30 mg dry wt of *Ustilago* spp. teliospores were used in each flask. All values were corrected to standard conditions of temperature and pressure.

Tracer studies.—Radioactive glucose, acetate, pyruvate, phenylalanine, and uracil were usually administered to the test organism after it had been in contact with the fungicide for 45-60 min, since this is the time required for maximum fungicide uptake to occur (12). All glassware and solutions were sterilized prior to use, and aseptic conditions were maintained as nearly as possible. Teliospores of *Ustilago* spp. were suspended in 250 μg/ml streptomycin sulfate and 100 μg/ml penicillin G after they had been washed with sterile water to prevent any effect from bacteria on the surface of the teliospores. Tests in which respired $^{14}CO_2$ was trapped were carried out in 16-ml Warburg flasks. $^{14}CO_2$ was trapped and counted by the method of Buhler (5).

At the end of a test, the fungal tissue was killed by immersion in boiling 80% ethanol. The tissue was further extracted three times with hot 80% ethanol and two times with hot water, and the extracts were then combined. The tissue was next extracted twice with hot 5% trichloroacetic acid for nucleic acids. The teliospore residue was either counted directly by

adding a 0.1 ml aliquot of the extracted spore suspension to scintillator solution or was hydrolyzed for 3 hr in 6 N HCl in a boiling water bath. The hydrolyzed residue was then taken to near dryness by flash evaporation and then made up to a known volume with deionized water from which 0.1-ml aliquots were taken for radioactivity determination. Residue of *R. solani* mycelium was hydrolyzed and counted in the same manner as given for teliospores.

The combined ethanol extract was reduced in volume by flash evaporation and then passed through a 5×1 cm column of Dowex-50 (H^+ form, 100-200 mesh) followed by passage through a 5×1 cm column of Dowex-1 (formate form, 100-200 mesh). Cations were eluted from the Dowex-50 resin with 10% NH_4OH and anions eluted from the Dowex-1 resin with 6 N formic acid. The neutral, cation, and anion fractions were taken to near dryness by flash evaporation and then made up to a known volume (usually 3 ml) with deionized water.

Radioactivity of various fractions was determined by adding 0.1 ml to a scintillation solution described by Strobel (22). Samples were counted for 10 min in a Model 6804 Nuclear Chicago scintillation counter. All counts were corrected for background and for quenching by use of the channels-ratio method. Radioactive spots on chromatograms were located with a Packard radiochromatogram scanner. Activity of spots on chromatograms was determined by cutting out the spot and counting directly in the scintillator solution.

Organic acids were chromatographed on Whatman No. 1 paper in either (A) *n*-propanol:cineole:formic acid (5:5:2, v/v) or in (B) ethanol:NH_3:water (160:10:30, v/v). Tentative identification of unknowns was accomplished by comparison of R_F values of authentic compounds in both solvent systems and by cochromatography with authentic compounds.

All tests were repeated at least twice, with each treatment replicated at least twice.

RESULTS.—When actively growing mycelium of *R. solani* came in contact with 1.87×10^{-4} M carboxin, microscopic examination revealed that linear extension of the hyphae was inhibited within 3-4 hr and had ceased within 8 hr. No other morphological effects, such as hyphal curling, were observed.

Respiration of mycelial discs of *R. solani* was inhibited in the presence of 74.8 μM carboxin. The discs were in contact with the fungicide for 60 min prior to determination of gas exchange (Table 1). The respiratory quotient (RQ) of treated mycelium was also slightly decreased. The exogenous respiration of *U. nuda* and *U. maydis* teliospores was also inhibited by carboxin and oxycarboxin (Table 2). However, low concentrations of oxycarboxin stimulated respiration of all three test fungi.

When glucose-U-^{14}C was administered to mycelial discs of *R. solani,* an increased release of $^{14}CO_2$ was noted in the cells treated with fungicide concomitant with a reduction in oxygen uptake. For discs incubated in 75 μM carboxin for 0.1 hr, 24.28 mμc ^{14}C appeared as $^{14}CO_2$ over a 1-hr period as compared to 23.52 mμc ^{14}C in the control. Oxygen uptake was 29 μliters for the carboxin treatment, and 45 μliters for the control. When the discs were incubated in 75 μM carboxin for 4 hr, 111.17 mμc ^{14}C appeared as $^{14}CO_2$ over a 1-hr period as compared to 28.86 mμc in the control. In this case, the oxygen uptake was 27 μliters for the control and 0 μliters for the carboxin treatment. The release of $^{14}CO_2$ from glucose-U-^{14}C was related to the concentration of fungicide used, with carboxin being more effective than oxycarboxin (Fig. 1).

Tests with glucose-^{14}C labeled in the 1 or 6 position revealed that both *R. solani* and *U. nuda* have extremely low $C_6:C_1$ ratios (0.05-0.1). As shown in Table 3, the evolution of $^{14}CO_2$ from carboxin-treated cells is initially less than that from nontreated cells, but after a 1-hr exposure to the labeled glucose there is a reversal in this pattern with an increased amount of label in CO_2 from glucose-1-^{14}C or glucose-6-^{14}C. Furthermore, the increased release of $^{14}CO_2$ from carboxin-

TABLE 1. Effect of carboxin on the respiration of *Rhizoctonia solani*

	Gas exchange[a]				
	Q_{O_2}[b]		Q_{CO_2}[c]		RQ
Treatment	Exogenous	Endogenous	Exogenous	Endogenous	Exogenous
Check	32.8	7.7	27.6	6.4	0.84
Carboxin	12.2	4.3	9.2	3.1	0.75

[a] Flasks contained in a volume of 2.5 ml the following in μmoles: K phosphate 100, pH 6.5; glucose 30, carboxin 0.187. Endogenous rates were determined in flasks in which the glucose was omitted. The mycelial discs were equilibrated for 1 hr before the system was closed and gas exchange determined. The test was run for 3 hr at 30 C.

[b] Q_{O_2} = μliter O_2/hr/mg dry wt.

[c] Q_{O_2} = μliter CO_2/hr per mg dry wt.

TABLE 2. Effect of various concentrations of oxycarboxin and carboxin on respiration of teliospores of *Ustilago nuda* and *U. maydis* and mycelium of *Rhizoctonia solani*

		Oxygen uptake[a]		
		Fungus		
Fungicide	Amount μmoles	*U. maydis*	*U. nuda*	*R. solani*
		Q_{O_2}[b]	Q_{O_2}	Q_{O_2}
Control		0.74	10.6	10.1
Carboxin	0.006	0.75	7.6	
	0.022	0.69		
	0.225	0.66	7.4	
Oxycarboxin	0.006			10.0
	0.022	0.98	11.0	12.2
	0.110	0.91	10.8	8.8
	1.100	0.71	7.0	

[a] Flasks contained in a volume of 3.0 ml the following in μmoles: K phosphate 100, pH 6.5; glucose 300; fungicide as indicated. Thirty to 45 min elapsed between the time the fungal tissue was placed in contact with the fungicide and the time the system was closed and gas exchange determined. The test was run at 30 C for 1 hr with *U. nuda,* 7 hr with *U. maydis,* and 2.5 hr with *R. solani.*

[b] Q_{O_2} = μliter O_2/hr per mg dry wt.

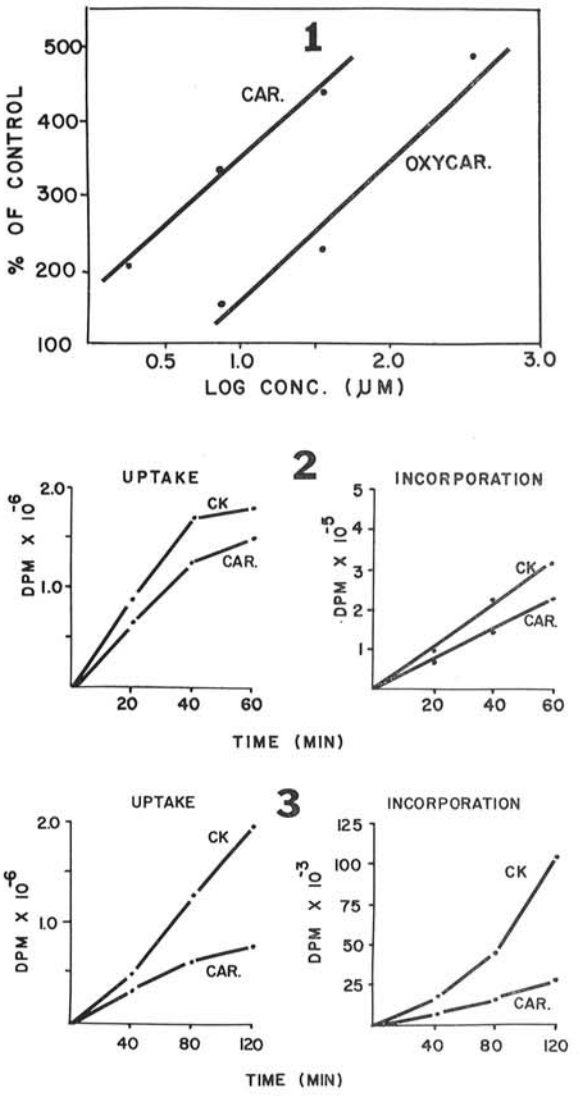

Fig. 1-3. **1)** Effect of carboxin and oxycarboxin concentrations on evolution of $^{14}CO_2$ from glucose-U-^{14}C by mycelium of *Rhizoctonia solani*. Flasks contained 3 mg dry wt mycelium in a volume of 3.0 ml containing the following in μmoles: K phosphate 100, pH 6.5; glucose-U-^{14}C 3.72 (0.273 μc); carboxin or oxycarboxin as indicated. **2)** Effect of carboxin on protein synthesis by mycelium of *Rhizoctonia solani*. Reaction mixture contained 14.7 mg dry wt mycelium in a volume of 3.0 ml containing the following in μmoles: K phosphate 100, pH 6.5; carboxin 0.225; L-phenylalanine-U-^{14}C 0.0654 (1.0 μc). **3)** Effect of carboxin on synthesis of RNA in mycelium of *Rhizoctonia solani*. Reaction mixture contained 15.6 mg dry wt mycelium in a volume of 3.0 ml containing the following in μmoles: K phosphate 100, pH 6.5; carboxin 0.225; uracil-2-^{14}C 0.0182 (1.0 μc).

treated cells occurs from the 6 position of glucose before it does from the 1 position of glucose.

The effect of carboxin on the incorporation of label from glucose-U-^{14}C into cell constituents was studied, using *R. solani* and *U. nuda*. While uptake of the labeled glucose is inhibited 23% to 33% for *R. solani*

TABLE 3. Effect of carboxin on appearance of label in CO_2 when glucose-1-^{14}C or glucose-6-^{14}C was administered to teliospores of *Ustilago nuda*

Treatment	$^{14}CO_2$[a] Exposure time 10 min	30 min	60 min
	mμc	mμc	mμc
Glu-1			
Check	111.6	313.6	329.8
Carboxin	30.3	202.5	400.5
Glu-6			
Check	6.1	23.7	31.1
Carboxin	4.4	45.5	105.0
C_6:C_1 Ratio			
Check	0.06	0.07	0.09
Carboxin	0.15	0.22	0.26

[a] Flasks contained in a volume of 3.0 ml the following in μmoles: K phosphate 100, pH 6.5; carboxin 0.225; glucose-1-^{14}C or glucose-6-^{14}C 0.5 (1.0 μc).

(Table 4) and only 3% for *U. nuda* (Table 5), there is a substantial inhibition of incorporation of label into ethanol-insoluble materials, such as nucleic acids and protein. Additional experiments with *U. nuda* teliospores indicated that the sharp reduction in labeling of the nucleic acid fraction is related to fungicide concentration. Those concentrations of carboxin that completely inhibit spore germination (37.5 and 3.7 μM) also inhibit the incorporation of label from glucose-U-^{14}C into nucleic acids, while a concentration not inhibiting spore germination (0.37 μM) did not inhibit this process.

Inhibitors with a known mode-of-action were compared with carboxin to determine their effects on the metabolism of glucose-U-^{14}C (Table 6). While 10^{-4} M dinitrophenol did cause some stimulation in release of $^{14}CO_2$, it was not nearly so effective in this respect as carboxin, and cycloheximide did not stimulate $^{14}CO_2$ evolution. Carboxin was also more effective in inhibiting incorporation of label into nucleic acids than was dinitrophenol or cycloheximide.

Though the total amount of label from glucose-U-^{14}C in the anion fraction of carboxin-treated mycelium of *R. solani* or teliospores of *U. nuda* was not greatly different from that of the control, the pattern of labeling of the various organic acids was different (Table 7). There was a decreased amount of label in citrate, malate, and fumarate, but an increased amount of label in succinate in carboxin-treated cells as compared with nontreated cells.

To determine if the tricarboxylic acid cycle was involved in the increased labeling of CO_2 in the presence of carboxin, acetate-1-^{14}C, acetate-2-^{14}C, and pyruvate-2-^{14}C were administered to treated and nontreated mycelium of *R. solani* and teliospores of *U. nuda*. Over a 1-hr exposure period, approximately 10 times more $^{14}CO_2$ was evolved from nontreated tissue using acetate-1-^{14}C as compared to acetate-2-^{14}C. This is not unexpected if the tricarboxylic acid cycle is operating. In the presence of carboxin, there is a great inhibition of evolution of $^{14}CO_2$ from either acetate-1-^{14}C or acetate-2-^{14}C (Table 8), as well as an inhibition of

TABLE 4. Effect of carboxin on incorporation of label from glucose-U-^{14}C into cell constituents of mycelial discs of *Rhizoctonia solani*

	Distribution of ^{14}C[a] Time in fungicide					
	30 min			120 min		
Fraction	Control	Carboxin	% of control	Control	Carboxin	% of control
	μc	μc	%	μc	μc	%
Neutral	2.067	1.670	81	0.883	0.676	77
Cation	0.134	0.615	459	0.150	0.202	135
Anion	0.046	0.069	150	0.043	0.040	93
Residue	1.066	0.187	18	2.799	0.461	16
Uptake	5.331	4.815	77	5.552	3.694	67

[a] Flasks contained 12 mycelial discs (39.7 mg dry wt) in a volume of 12.0 ml containing the following in μmoles: K phosphate 400, pH 6.5; carboxin 0.45; glucose-U-^{14}C 0.019 (6.03 μc).

TABLE 5. Effect of carboxin on incorporation of label from glucose-U-^{14}C into cell constituents of teliospores of *Ustilago nuda*

	Distribution of ^{14}C[a]		
Fraction	Control	Carboxin	% of control
	mμc	mμc	%
Anion	22.04	23.37	106
Cation	63.03	72.81	116
Nucleic Acids	60.59	12.39	20
Residue	17.95	11.59	65
CO_2	109.37	320.54	293
Uptake	682.60	659.55	97

[a] Flasks contained 16.7 mg dry wt of teliospores in a volume of 3.0 ml containing the following in μmoles: K phosphate 100, pH 6.5; carboxin 0.225; glucose-U-^{14}C 0.000218 (0.753 μc). Both the control and fungicide treatments contained 33 μg/ml penicillin G and 80 μg/ml streptomycin sulfate. The teliospores were exposed to the fungicide for 60 min prior to the addition of the labeled glucose. The test was run at 27 C and terminated after a 60-min exposure to the glucose.

incorporation of label into all fractions. Analysis of the organic acid fraction also revealed the same type of labeling pattern from acetate-^{14}C as when glucose-U-^{14}C was used.

Carboxin (37.5 μM) also caused a 96% and a 91% inhibition in appearance of label in CO_2 from pyruvate-2-^{14}C administered to *U. nuda* and *R. solani*, respectively.

Uptake and incorporation of phenylalanine-U-^{14}C into mycelial protein of *R. solani* was studied to determine the effect of carboxin on protein synthesis (Fig. 2). While uptake over a 1-hr period was inhibited by 16%, incorporation into protein was inhibited by 29%. By comparison, 100 μM cycloheximide caused a 97% inhibition of protein synthesis. Synthesis of RNA was drastically inhibited by carboxin, as shown in Fig. 3. For the 2-hr period, carboxin caused a 61% inhibition in uptake of uracil and a 73% reduction in incorporation of label into RNA.

DISCUSSION.—Carboxin and oxycarboxin are able to inhibit respiration in sensitive fungi with carboxin being more effective than oxycarboxin (Table 2). This difference in effectiveness may be due to the amounts of fungicide that penetrate into the cell, since carboxin enters the cells of the fungi used in this study in greater quantities than oxycarboxin (12). While low quantities of these fungicides appear to stimulate oxygen uptake slightly (Table 2), the effects are different from known uncouplers of oxidative phosphorylation, such as dinitrophenol, since the RQ decreases (Table 1) rather than increases as it does with dinitrophenol (2). Even though the total amount of CO_2 is decreased in the presence of carboxin (Table 1), the amount of $^{14}CO_2$

TABLE 6. Effect of various inhibitors on incorporation of label from glucose-U-^{14}C into cell constituents of teliospores of *Ustilago nuda*

		Distribution of ^{14}C[a]				
Fraction	Check	Carboxin 3.7×10^{-5} M	DNP[b] 10^{-3} M	DNP[b] 10^{-4} M	Cyclohex.[b] 10^{-4} M	Cyclohex.[b] 10^{-5} M
	mμc	mμc	mμc	mμc	mμc	mμc
Cation	43.993	66.950	25.541	87.928	52.808	63.446
Anion	19.437	19.324	6.306	26.869	18.401	19.707
Nucleic Acids	50.788	8.401	0.000	26.520	33.559	35.867
Residue	17.266	7.636	0.092	19.930	7.462	9.335
CO_2	119.325	346.420	23.346	205.728	90.722	101.864
Uptake	750.203	734.811	84.811	742.581	671.595	670.243

[a] Flasks contained 16.7 mg dry wt of teliospores in a volume of 3.0 ml containing the following in μmoles: K phosphate 100, pH 6.5; carboxin 0.225; glucose-U-^{14}C 0.000218 (0.753 μc). Both the control and fungicide treatments contained 33 μg/ml penicillin G and 80 μg/ml streptomycin sulfate. The teliospores were exposed to the fungicides for 60 min prior to the addition of the labeled glucose. The test was run at 27 C and terminated after a 60-min exposure to the glucose.

[b] Abbreviations: DNP = dinitrophenol; Cyclohex. = Cycloheximide.

TABLE 7. Effect of carboxin on incorporation of label from glucose-U-^{14}C into organic acids of *Rhizoctonia solani* mycelium or *Ustilago nuda* teliospores

	Distribution of ^{14}C[a]			
	U. nuda		*R. solani*	
Compound	Check	Carboxin	Check	Carboxin
	mμc	mμc	mμc	mμc
Citrate	3.90	0.91	1.08	0.12
Malate	3.88	2.78	11.58	1.20
Succinate	0.28	16.00	1.47	3.06
Fumarate	0.09	0.06	0.52	0.17

[a] Flasks contained 41.1 mg or 25.6 mg dry wt of mycelium or teliospores, respectively, in a volume of 6.0 ml containing the following in μmoles: K phosphate 200, pH 6.5; carboxin 0.45, glucose-U-^{14}C 0.000436 (1.6 μc). The tissue was exposed to the fungicide for 45 min prior to addition of the labeled glucose. The test was run at 27 C and terminated after a 60-min exposure to the glucose. Uptake (μc) was as follows: *U. nuda,* check 1.49; carboxin 1.35; *R. solani,* check 1.51; carboxin 0.23.

that is evolved when glucose-U-^{14}C is administered in the presence of carboxin is increased. A similar increased release of $^{14}CO_2$ was obtained with dinitrophenol but the magnitude of the response was not as great as that obtained with carboxin (Table 6).

One of the most striking effects of carboxin, even at concentrations as low as 3.7 μM, is an inhibition of the oxidation of acetate and pyruvate (Table 8) which probably accounts for the inhibition of respiration by carboxin. A similar effect has also been reported for other fungicides such as dichlone, several dithiocarbamates, and captan (14, 15, 16, 17). Preliminary evidence suggests that carboxin may inhibit the oxidation of succinate, since the amount of label in succinate in fungicide-treated cells exposed to glucose-U-^{14}C, acetate-1-^{14}C, or acetate-2-^{14}C is higher than that in untreated cells, while label in citrate, fumarate, and malate is less in treated cells (Table 7). Malonate, a known inhibitor of succinic dehydrogenase, has also been reported to cause a buildup of succinate in mitochondria of avocado fruit administered 0.02 M pyruvate sparked by 0.001 M malate (1).

Carboxin-treated cells are able to metabolize glucose, as evidenced by the increased amount of label in CO_2 when glucose-U-^{14}C is administered to treated cells as compared to nontreated cells (Fig. 1). However, much, if not all of the $^{14}CO_2$ must be arising from reactions prior to pyruvate, since metabolism of pyruvate is greatly inhibited. Since the C_6:C_1 ratio of untreated cells is very low (Table 3), pathways other than glycolysis must be involved in the early metabolism of glucose by both fungi used in this study. The data from a time course study with glucose-1-^{14}C or glucose-6-^{14}C showed that there is an initial decreased release of labeled CO_2 in the presence of carboxin, but after 1 hr this phenomenon is reversed (Table 3), thus indicating that the label from glucose is probably being recycled back through pathways, such as the pentose pathway, rather than proceeding through the tricarboxylic acid cycle and then into macromolecules (27).

Carboxin also appears to inhibit the synthesis of nucleic acids (Tables 5, 6, Fig. 3). It is not known whether this is a direct effect or merely a reflection of the lack of high energy compounds resulting from the apparent inhibition of the tricarboxylic acid cycle and oxidative phosphorylation. Other fungicides are also known to inhibit nucleic acid synthesis, including phytoactin (11), chloroneb (9), and cycloheximide (19, 20, 21) but they usually do not affect respiration to any extent.

Protein synthesis is also slightly inhibited (Table 5, Fig. 2). T. Obrig, University of Illinois, also noted that carboxin (140 μM) will inhibit by 25% the incorporation of phenylalanine into polyphenylalanine in a cell-free system of *R. solani* (*personal communication*). This is approximately the same level of inhibition of incorporation of phenylalanine into protein that the author observed using intact cells of the same organism. Carboxin, however, is not nearly as effective in inhibiting protein synthesis in *R. solani* as is cycloheximide, whose mode of action is to inhibit protein synthesis (19, 20, 21). Chloroneb and 2,6-dichloro-4-nitroaniline inhibit protein synthesis in sensitive organisms to about the same degree as carboxin (9, 26).

While more work is needed using cell-free systems and isolated enzymes, it appears that the mechanism of

TABLE 8. Effect of carboxin on metabolism of acetate-1-^{14}C and acetate-2-^{14}C by mycelial discs of *Rhizoctonia solani* and teliospores of *Ustilago nuda*

	Distribution of ^{14}C[a]							
	R. solani				*U. nuda*			
	Acetate-1		Acetate-2		Acetate-1		Acetate-2	
Fraction	Check	Carboxin	Check	Carboxin	Check	Carboxin	Check	Carboxin
	mμc	mμc	mμc	mμc	mμc	mμc	mμc	mμc
Anion	58.78	24.83	55.56	31.34	32.94	4.43	40.66	4.78
Cation	190.36	32.35	214.89	32.94	191.22	4.00	232.49	4.17
Nucleic Acids	15.71	0.79	15.48	0.79	8.34	0.90	8.05	1.06
Residue	30.68	2.95	32.71	2.89	181.74	15.55	180.51	16.71
CO_2	86.14	9.14	7.37	0.68	58.31	3.97	6.28	0.37
Uptake	603.00	118.67	540.19	132.58	737.12	42.16	727.54	39.44

[a] Flasks contained 20 mg dry wt fungal tissue in a volume of 3.0 ml containing the following in μmoles: K phosphate 100, pH 6.5; carboxin 0.225; acetate-1-^{14}C or acetate-2-^{14}C 0.0188 (0.8 μc).

action of the oxathiins may involve several aspects of metabolism. Evidence at present points to at least two major effects, (i) a blockage of the oxidation of pyruvate and acetate and (ii) inhibition of nucleic acid synthesis.

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