Effects of Oxathiin Systemic Fungicides on Various Biological Systems[1]

by D. E. MATHRE
Department of Botany and Microbiology
Montana State University
Bozeman, Mont. 59715

Most fungicides presently in use are applied to plants to give them a protective coating. Within the last few years, however, fungicides have been developed which will move systemically through plants to eradicate fungal pathogens or to protect the plant internally from invasion (6). The oxathiins were among the first of the "systemic fungicides" to be developed (17). Carboxin, (Vitavax^R) (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) has been shown to be useful as a seed treatment for control of cereal loose and covered smuts (11) and Rhizoctonia seedling blight of cotton (1). It also controls blister blight of tea when applied to the foliage (16). Basidiomycetous fungi and a few Deuteromycetes appear to be the most sensitive fungi to carboxin (13). In sensitive fungi, the mode of action of carboxin is to decrease respiration by inhibiting the oxidation of succinate (8, 9, 10, 18). In barley, Carlson (3) noted that high levels of carboxin sprayed on leaves would inhibit photosynthesis. Seed treatment of barley with carboxin in the absence of disease decreased yields slightly (14).

This study was conducted to determine what effect carboxin and its oxidized products (4) have on non-target organisms, including bacteria, a slime mold, higher plants, rabbit red blood cells, and a human cell line, thus providing information on the environmental impact of this group of new fungicides.

MATERIALS AND METHODS

Chemicals. - Carboxin, its monoxide F831, and its dioxide oxycarboxin (Plantvax^R) were obtained in technical form from Uniroyal Chemical.

Organisms. - The bacteria used were obtained from the stock culture collection of the Dept. of Botany and Microbiology, Montana State University. They were grown in "Difco" yeast nitrogen base media plus 1% glucose at room temperature or 30°C. The fungicide was added to the growth medium along with the inoculum and growth determined by measuring the optical density at 540 nm. The slime mold Dictyostelium discoideum plus its food source Escherichia coli was cultured on "Difco" corn meal agar to which various concentrations of the fungicide had been added before the agar had solidified. The green alga Chlorella pyrenoidosa (211-8b) was cultured as described by Pickett (12). Seeds of soybeans (Glycine max), pinto beans (Phaseolus vulgaris), and corn (Zea mays) were

---

1 Contribution from Montana State University, Agricultural Experiment Station, Bozeman. Journal Series Paper No.367.

Bulletin of Environmental Contamination & Toxicology,
Vol. 8, No. 5, 1972 © by Springer-Verlag New York Inc.

first surface sterilized with 10% NaOCl, washed several times with water, and germinated on blotter paper. When the radical had appeared the germinating seedlings were transferred to boxes filled with vermiculite and watered with Hoagland's nutrient solution in which the fungicide had been suspended. The plants were grown in the greenhouse and alternately irrigated with water and Hoagland's solution.

The human cell line "Hep 2" was obtained from the Montana State Dept. of Health and was cultured according to standard procedures in Earle's (5) or Hank's (7) lactoalbumin media. Whole blood from a rabbit was collected in an equal volume of Alsever's solution (2) and the red blood cells separated by centrifugation. The cells were washed three times in Alsever's solution prior to use.

Metabolic studies. - The effect of the various fungicides on metabolism of ^{14}C-acetate was studied as described previously by Mathre (9). Photosynthesis by Chlorella cells or leaf sections of corn, barley, or pinto beans, was measured by determining oxygen evolution in a Gilson differential respirometer. The flasks contained a CO_2 buffer and light was excluded from some flasks to allow determination of the respiration rate.

RESULTS

In the presence of $10^{-4}M$ carboxin, the following bacteria were inhibited in growth from 0-10%: Proteus vulgaris, Bacillus cereus, Pseudomonas aeruginosa, Nocardia rubra, Lactobacillus casei, and Azotobacter chroococcum. Inhibited from 10-20% were Streptomyces sp., Sarcina lutea, and Mycobacterium phlei. The metabolism of ^{14}C-acetate was somewhat more sensitive to $10^{-4}M$ carboxin in that the release of $^{14}CO_2$ was inhibited by 34% in P. vulgaris and 37% in S. leutea.

The development of sporangia by the slime mold D. discoideum was not affected by $10^{-4}M$ carboxin, F831, or oxycarboxin.

The formation and stability of cell sheets of the Hep 2 cell line were sensitive to oxycarboxin but not to carboxin or F831 as shown in Table 1.

TABLE 1

Effect of oxathiin fungicides on Hep 2 cell sheet formation and stability.

Compound	Concentration (M)	Sheet Formation[a]	Sheet Stability[b]
Control	0	0	0
Carboxin	10^{-4}	0	0
F831	10^{-4}	0	0
Oxycarboxin	10^{-4}	4	4

[a] Fungicide added at same time as inoculum. Sheet formation was read 2 days later on a scale of 0 = sheet complete, 2 = 50% of cells free, 4 = 100% sheet destruction.

[b] Fungicide added 2 days after inoculum at which time the cell sheet was complete. The integrity of the sheet was read 2 days after addition of the fungicide using the same scale as given above.

When studies of Hep 2 cell proliferation were made by analysis of cell protein content, 10^{-4}M oxycarboxin did cause a 50% inhibition while 10^{-5}M oxycarboxin was not inhibitory. Furthermore, 10^{-3} M carboxin and 10^{-3}M F831 were not inhibitory to cell proliferation.

Another animal system, rabbit red blood cells, was exposed to the three oxathiins at 10^{-4}M and no lysis of these cells was observed as determined by assaying the release of hemoglobin at 577 nm. However, at this same concentration a 15% inhibition in the metabolism of ^{14}C-acetate was detected.

Metabolism of ^{14}C-acetate by Chlorella cells was not inhibited by 10^{-4}M carboxin, F831, or oxycarboxin. However, photosynthesis was inhibited by 52% with 10^{-4}M carboxin but not with 10^{-4}M F831 or oxycarboxin. Lower concentrations of carboxin were not inhibitory to photosynthesis.

The growth of higher plants in nutrient solutions containing the oxathiins is given in Table 2.

TABLE 2

Effect of oxathiin systemic fungicides on the growth of barley, pinto beans, and soybeans.

Plant	Compound	Concentration (M)	Plant Dry Wt. % of Control[a/]
Barley	Carboxin	10^{-3}	6.0 f
		10^{-4}	10.7 f
		10^{-5}	61.4 c
	Oxycarboxin	10^{-3}	8.8 f
		10^{-4}	49.0 d
		10^{-5}	71.9 b
	F831	10^{-3}	9.4 f
		10^{-4}	24.5 e
		10^{-5}	81.8 a
Pinto beans	Carboxin	10^{-3}	29.7 d
		10^{-4}	48.6 c
		10^{-5}	99.2 a
	Oxycarboxin	10^{-3}	43.5 c
		10^{-4}	77.0 b
		10^{-5}	111.0 a
	F831	10^{-3}	26.4 d
		10^{-4}	73.0 b
		10^{-5}	106.6 a
Soybeans	Carboxin	10^{-3}	30.2 e
		10^{-4}	42.2 d
		10^{-5}	90.3 a
	Oxycarboxin	10^{-3}	32.1 e
		10^{-4}	61.7 b
		10^{-5}	84.4 a
	F831	10^{-3}	30.1 e
		10^{-4}	52.6 c
		10^{-5}	70.2 b

[a/] Plants 3 weeks old were removed and dried at 100°C for 24 hrs. Means in the vertical column for each plant followed by the same letter are not significantly different at the 5% level of probability - Duncan's multiple range test.

Photosynthesis in higher plants was also inhibited by the oxathiins. When the fungicide was applied to leaf surfaces concentrations of 0.1 mM or higher were inhibitory (Table 3).

TABLE 3

Effect of leaf application of oxathiin fungicides on photosynthesis.

Compound	Concentration (mM)	% Inhibition of Photosynthesis		
		Barley	Pinto beans	Corn
Carboxin	15.0	92	90	84
	1.5	82	75	79
	0.1	22	38	56
Oxycarboxin	15.0	--	68	38
	1.5	4	10	0
	0.1	0	--	--
F831	15.0	--	58	33
	1.5	0	27	12
	0.1	0	--	--

When leaves were allowed to take up and translocate 10^{-4}M carboxin for 16 hours, photosynthesis in the leaf tissue of barley, pinto beans, and corn was inhibited by 3, 72, and 60%, respectively.

DISCUSSION

The ED_{50} value for carboxin has been reported to be 1.0 µM or less for many Basidiomycetous fungi (13). The results of this study would indicate that much higher concentrations, usually on the order of 10 or 100 times higher, are necessary for inhibition of growth or metabolism of non-target organisms. In some cases with bacteria and the human cell line Hep 2, concentrations as high as 1 mM were non-inhibitory to growth. With sensitive fungi carboxin is about 10-100 times more effective than oxycarboxin. This relationship was not always observed with the non-target organisms included in this study. For example, only oxycarboxin was effective in destroying the stability of cell sheets of the Hep 2 cells (Table 1).

The effect of the oxathiins on photosynthetic plants is observed in both growth and photosynthesis. Carlson's study (3) on barley indicated that sprays of 15 mM carboxin were highly inhibitory to photosynthesis. This report confirms this observation but it also shows that photosynthesis in other plants reacts similarly to carboxin while oxycarboxin and F831 are less effective. However, at more moderate levels (eg 10^{-4}M) only carboxin would inhibit photosynthesis and then only to the extent of 22-56%.

In conclusion, it appears that carboxin and its oxidized products are not effective in inhibiting the growth or metabolic activities of the various non-target organisms or systems included

in this study until concentrations of 10^{-4}M or higher are reached. In contrast, target species are strongly inhibited at 10^{-6}M. The likelyhood that non-target organisms would have contact with high concentrations of these compounds in nature would appear to me to be extremely remote except where the materials are misused, such as the feeding of treated seed to livestock. The reason for the low sensitivity of most organisms to the oxathiins is not known but may be related to the uptake of the compounds into cells and/or mitochondria.

ACKNOWLEDGEMENTS

Supported in part by Public Health Service Grant No. EP-00827 from the Environmental Protection Agency, Washington D.C.

REFERENCES

1. BORUM, D. E., and SINCLAIR, J. B., Phytopathology 57, 805 (1967).
2. CAMPBELL, D. H., GARVEY, J. S., CREMER, N. E., and SUSSDORF, D. H., Methods in Immunology (1964) W. A. Benjamin, Inc., New York.
3. CARLSON, L. W., Can. J. Pl. Sci. 50, 627 (1970).
4. CHIN, W., STONE, G. M., and SMITH, A. E., J. Agr. Food Chem. 18, 731 (1970).
5. EARLE, W. R., J. Nat. Cancer Inst. 4, 165 (1943).
6. ERWIN, D. C., FAO Pl. Prot. Bull. 18, 73 (1970).
7. HANKS, J. H., and WALLACE, R. E., Proc. Soc. Exper. Biol. & Med. 71, 196 (1949).
8. LYR, H., LUTHARDT, W., and RITTER, G., Zeit. f Allg. Mikrobiol. 11, 373 (1971).
9. MATHRE, D. E., Phytopathology 60, 671 (1970).
10. MATHRE, D. E., Pest. Biochem. & Physiol. 1, 216 (1971).
11. MOSEMAN, J. G., USDA CR 42-68 (1968).
12. PICKETT, J. M., Plant Physiol. 48, 265 (1971).
13. SNEL, M., VON SCHMELING, B., and EDGINGTON, L. V., Phytopathology 60, 1164 (1970).
14. STOKER, G. L., and DEWEY, W. G., Utah Sci. 31, 46 (1970).
15. ULRICH, J. T., and MATHRE, D. E., J. Bact. In Press (1972).
16. VENKATA RAM, C. S., Phytopathology 59, 125 (1969).
17. VON SCHMELING, B., and KULKA, M., Science 152, 659 (1966).
18. WHITE, G. A., Biochem. Biophys. Res. Comm. 44, 1212 (1971).