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FUNGICIDES

Toxic Action of Metal Ions to Fungus Spores

LAWRENCE P. MILLER and S. E. A. McCALLAN

Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.

Quantitative studies have been carried out on the interactions between metal ions and spores of representative fungi. Silver is taken up rapidly by fungus spores, so that germination can be completely inhibited after a contact time of 1 minute or less. Only mercury(I) and (II), and to a lesser extent copper, offer serious competition. The toxicity of silver is unaffected by chloride, but reduced by bromide, and prevented by iodide. Silver has a marked effect on the permeability of spores as measured by the outward movement of phosphorus compounds from cells labeled with phosphorus-32. Copper, zinc, and cadmium reduce germination appreciably only after some hours of contact with the spores. About 75% of the zinc content of spores grown in the presence of zinc-65 is exchanged with nonradioactive zinc within 10 minutes. Materials move inward and outward more readily with fungus spores than was supposed.

METAL IONS have long been of interest because of their fungicidal properties. Copper and mercury are important commercially as components of fungicidal preparations and zinc, cadmium, and silver have either found commercial use or been extensively investigated in laboratory studies.

Laboratory evaluations of the toxicity of metal ions have been concerned chiefly with effects on the germination of fungus spores and prevention of the growth of mycelium on agar plates. Comparisons of toxicity have usually been based on the concentrations in the media required to bring about a certain response. No information is obtained by these procedures on the innate toxicity of the materials, because the quantities required by the spores or mycelium remain unknown.

In these studies toxicity has been expressed, whenever possible, on a spore-weight. The ions investigated differ markedly in their toxicity and in the speed with which they act when brought into contact with spores. There are also

important differences in the degree to which various ions are taken up by spores of different species of fungi.

Materials and Methods

Fungi. Conidia of the following species of fungi were used in these studies: *Neurospora sitophila* (Mont.) Shear and Dodge, *Monilinia fructicola* (Wint.) Honey, *Alternaria oleracea* Milbraith, *Aspergillus niger* van Tiegh, and *Venturia pyrina* Aderh. The methods employed for culturing the fungi, harvesting the spores, determining the spore weights, and the nutrients added for germination tests have been given (*9, 10*).

Toxicants. Silver-110, mercury-203, and cadmium-115 were obtained from the Oak Ridge National Laboratory as the nitrates, zinc-65 as the chloride. To make up solutions of the strength needed for the tests, the metal acetates were added; silver was used as the nitrate. When iodine-131 was employed in conjunction with silver, the iodide was supplied as potassium iodide. Other metals, not in radioactive form, were usually in the form of the acetates; cobalt was used as the nitrate.

Determination of Uptake of Toxicants. Known weights of fungus spores were suspended in solutions containing the toxicant in radioactive form, when available, and the quantity of toxicant removed was determined after appropriate time intervals. The tests were usually carried out in 15-ml. test tubes with conical bottoms. After various time intervals, the tubes were placed in a centrifuge and the spores rapidly spun down into a small area at the bottom of the tubes. In time studies the spores were considered out of contact with the solutions when in the centrifuged condition. There is very little uptake when the spores are tightly packed in the bottom of the tubes during the minute or two required for the sampling procedure. After centrifugation, the radioactivity of an aliquot of the supernatant solution was determined and a small sample of spores taken, after the solution had been mixed again, to determine the germination capacity of the spores. The spores remaining were again in contact with the toxicant and uptake was permitted to proceed until the next time interval, when the procedure was repeated. It is necessary in this method to have all the toxicant in solution. Spore constituents leaching out into the ambient solution and forming insoluble compounds with the toxicant cause high results. Toxicant that enters the spores and unites with a spore constituent to form a soluble compound which in turn passes into the ambient solution influences the results by giving low values. It is not believed that these factors play much of a role with the metal ions studied, except perhaps mercury. When spores are suspended in water, there is a considerable outward movement of spore constituents (*4*). Even after the spores have been washed twice in distilled water, there is still further outward movement of some materials. Tests with the supernatant from the spores used here indicate very little precipitation of any toxicant ions except mercury. Results previously reported for ED_{50} values for mercury are probably somewhat high because of this factor (*7*).

Spores taken from the spore toxicant mixtures for germination were immediately added to distilled water to give a 10- to 20-fold dilution of the toxicant removed with the spores. On being prepared for the germination test further dilutions are involved as nutrient medium is added when required and the suspension is diluted to yield about 100,000 spores per ml. The test is therefore essentially a fungicidal one, in contrast to one in which spores remain in contact with the toxicant solution while being germinated. Even though spores were often removed after being exposed for a minute or less, with the proper amount of chemical present lethal doses of some ions were taken up in this short time.

Samples to be counted were transferred to 1-inch cupped planchets, of nickel-plated steel, stainless steel, or glass, and dried under a heat lamp. With mercury it was necessary to add 0.5 to 0.1 mg. of sodium sulfide to each planchet before drying to prevent loss of mercury by volatilization. Radioactivity was measured by the use of conventional scaling equipment and end-window-type Geiger-Müller tubes with thin windows. The time required to reach 1024 counts was usually determined three times for each sample by means of automatic equipment. When more than one isotope was present in a sample, the quantities of the individual isotopes were obtained with the use of absorbers or by counting a second time after differences in the half lives of the two isotopes could be utilized.

When values obtained for toxicant taken up per unit weight of spores were plotted against the percentage of nongermination on the usual logarithmic-probability paper, straight lines resulted. With some ions the time required to obtain the particular dose was of minor importance. With others, toxicity was not evident after only short periods of exposure, but became pronounced after longer periods. With these ions, determinations made after various exposure periods gave different ED_{50} values.

Effect of Chemicals on Release of Cell Contents to Ambient Solution. In order to study the effect of various ions in affecting the permeability of the cell walls, spores of *Monilinia fructicola* labeled with phosphorus-32 were grown in 100-ml. tubes containing 25 ml. of medium and about 15 microcuries of phosphorus-32 per tube. On harvesting and after two washings with distilled water, the spores gave about 500,000 counts per minute per 10 mg. of spores. On further suspension in distilled water not more than 2% of the activity was released to the ambient solution.

Labeling Spores with Zinc-65. To study the effect of some ions in replacing zinc in spores of *Aspergillus niger*, in which the zinc had become a spore constituent during its growth and development, the fungus was grown in 100-ml. tubes on the usual nutrient medium with the addition of about 38 microcuries of zinc-65 per tube. On two separate harvests, a few days apart, the spores contained 810 and 910 γ per gram, if it is assumed that no zinc was available to the spores except that added with the zinc-65.

Table I. ED_{50} Values for Silver for Spores of Representative Fungi

Species	ED_{50} Value, γ/G.	Confidence Limits, 95%
Aspergillus niger	560	326–876
Alternaria oleracea	365	335–398
Neurospora sitophila	347	259–465
Monilinia fructicola	283	210–382
Venturia pyrina	89	63–175

Action of Silver

ED_{50} Values for Silver. Silver is of unusual interest because of its great toxicity. It is the most toxic of the metal ions (*10*) and of all the fungicides so far tested on a spore weight basis. Dosage-response curves for a number of species (*10*) and a table of ED_{50} values (*6*) have been published. More recently additional experiments have been carried out and in Table I are listed the ED_{50} values as revised, together with the confidence limits at 95%. Quantities required to inhibit the germination of 50% of the spores range from 89 to 560 γ per gram.

Rate of Uptake. A feature of the action of silver on fungus spores is the rapidity with which the silver is taken out of solution by the spores. Data obtained in an experiment with *Venturia pyrina* are shown in Figure 1. Quantities of silver supplied ranged from 125 to 4000 γ per gram of spores (only the data for 500 to 4000 γ per gram are plotted). Samples were taken after 30 seconds, 5.5 minutes, and 15.5 minutes. At the first sampling period the spores in the highest concentration of silver had taken up 620 γ per gram, or almost seven times the ED_{50} value of 89. At the three lower concentrations shown in Figure 1 the quantities taken up ranged from 2.6 to 6.3 times the ED_{50} value. The corresponding values for the two concentra-

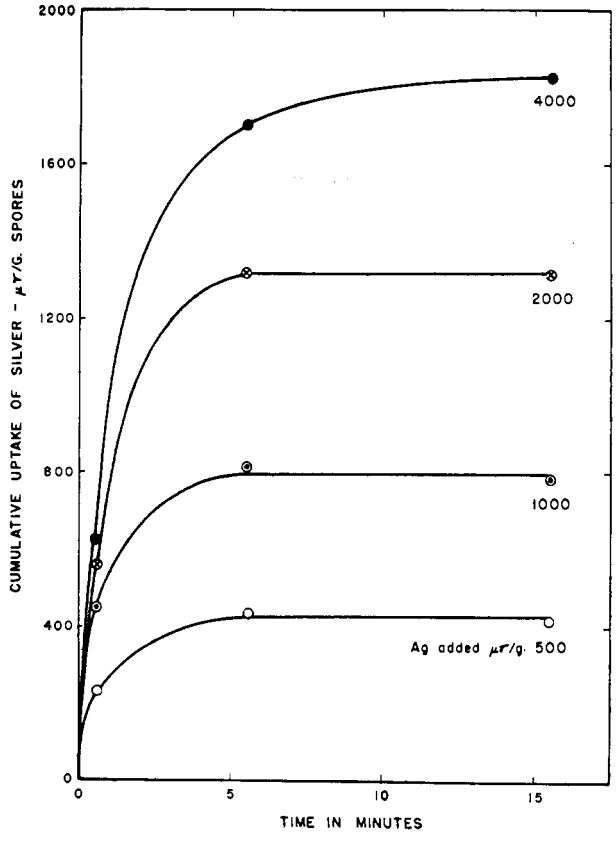

Figure 1. Uptake of silver by spores of *Venturia pyrina* from dilute solutions

tions not plotted amounted to 1.2 and 2.3 times the ED_{50} value. Here the amount of silver available was limiting; 81 and 86% of the silver was removed from the solution in this short time. Additional quantities were taken up in the next 5 minutes at the higher concentrations, but no further uptake of any consequence took place during the following 10 minutes.

Time Required to Inhibit Germination. It is difficult to determine precisely how long is required for a toxicant to inhibit germination, as the germination process requires a number of hours. Samples of spores removed as rapidly as possible, in the experiment described above, after the 30 seconds of exposure to the silver, failed to give any germination at the higher doses. At the lower doses, below those required for complete inhibition and at which the silver present was almost all taken up immediately, the germination in the samples taken at 30 seconds was essentially the same as in those taken later. The action of silver on the cell wall, resulting in the outward movement of cell constituents, is also evident in a few minutes. It would therefore seem that the reactions bringing about inhibition take place in a minute or less.

Effect of Halides on Toxicity of Silver. As silver forms compounds with the halogens of very low solubility, it seemed of interest to determine whether silver in the chloride, bromide, or iodide form was available to fungus spores. The methods used in these studies require that the toxicants not taken up by the spores remain in solution or suspension; difficulties were expected in carrying out these tests. However, in the experiments described, the halides remained in suspension, presumably because they were in a very finely divided state. A series of concentrations of silver was set up ranging from 200 to 3200 γ of silver per gram of spores and equivalent quantities of chloride, bromide, and iodide added before exposure of the spores. The results with chloride and bromide are shown in Table II. The presence of chloride had no effect, but when equivalent quantities of bromide were added considerably less silver was taken up by the spores. The results with iodide, in which the iodine was also radioactive, are shown in Table III. Quantities of silver up to 3200 γ per gram of spores had no effect on germination of spores. Neither the silver nor iodide was taken out of solution by the spores. Apparently the bond between the silver and iodide was stronger than the affinity of the spores for the silver. This effect of iodide in preventing the toxicity of silver has also been verified in experiments in which uptake was not determined but the results were expressed on the basis of the concentration of silver in the applied solution required to inhibit germination.

As iodide prevented the uptake of silver in the above experiment, it seemed of interest to see whether the toxicity of silver could be reversed by adding iodide a short time after the spores had been exposed to silver. Spores were subjected to two series of concentrations of silver ranging from 100 to 3200 γ per gram of spores. In one series equivalent quantities of iodide were added after 15 minutes of exposure of the spores to the silver. After 22 hours, uptake of silver and the germinative capacity of the spores were determined for both series. The addition of iodide had no effect on the subsequent germination. Quantities of silver in the spores were perhaps a little lower in the iodide series at the higher concentrations of silver at the end of this test period, but germination was not increased.

In the experiment in which iodide was added to spores that had taken up silver, determinations were also made of the iodide removed from the solution by the silver in the spores. It is presumed that any iodide removed is the result of the silver present, as spores alone do not remove measurable quantities of iodide under the conditions of these tests. The data in Table IV show that the amount of iodide removed from solution is not influenced by a longer exposure to the iodide than 15 minutes. At low doses of silver practically no iodide was removed and at higher doses the amount of iodide taken up was less than the equivalent amount of silver present by about 600 γ or more of silver per gram of spores. These results suggest that some of the silver in the spores is inaccessible to the iodide or is so firmly held that it cannot react with the iodide ion.

Competition between Silver and Other Ions for Receptor Sites in Fungus Spores. Competition for receptor sites has been studied by determining the effect of other ions on the uptake of silver when present in considerable excess of the silver at the time of exposure of the spores. Ability of silver to replace other ions previously taken up or to occupy receptor sites not utilized by these ions has been determined by adding silver after pretreatment with the other ions. Finally, the ability of other ions to release silver from spores which had previously taken it up was also investigated (*11*). The quantity of silver used was 2×10^{-7} equivalent for 10 mg. of spores (2160 γ per gram) and the other ions were used at concentrations 50 times higher on an equivalent basis. In the simultaneous treatments the uptake of silver was retarded by the other ions in the following order of decreasing effect during the first 10 minutes of exposure: mercury(I), mercury(II) > copper > cadmium > cobalt > nickel > zinc; zinc had no retarding effect and mercury(I) and (II) prevented all uptake during this

period. On a further exposure period of 15 hours zinc remained without effect and the quantities of silver taken up in the presence of copper, cadmium, cobalt, and nickel were not much less than in the control, while the uptake was still reduced 85% by mercury(II) and 75% by mercury(I). When spores were pretreated for 1.5 hours before the addition of silver (the pretreating solution being removed before exposure to silver) the uptake of silver was retarded in the following order of decreasing effect during the first 10 minutes: mercury(II) > mercury(I) > copper > zinc, cadmium, nickel. After another 19 hours the uptake of silver was still less by 54 and 32%, respectively, with spores pretreated with mercury(II) and mercury(I) ions. The retarding effect of the other ions had been overcome during this interval. The subsequent addition of various ions after treatment with silver resulted in complete exchange of the silver that had been taken up when nonradioactive silver was added; with mercury(I) 48% of the silver was displaced [the mercury(II) ion was not included in this test], and the other ions had no significant effect over that of the water control.

Only mercury(I), mercury(II), and to a lesser degree copper, provided serious competition for receptor sites for silver. Silver was able to replace with time any of the other ions previously taken up. The effect of the mercury(I) and (II) ions, when presented in concentrations 50 times that of silver, persisted and was also evident in the ability of at least the mercury(I) ion to replace some of the silver previously taken up.

In view of the definite interference between silver and mercury, it was surprising that when the amount of mercury taken up under various combinations of exposure to mercury and silver was determined, pretreatment or simultaneous treatment with silver markedly increased the apparent uptake of mercury (*11*). Later studies carried out with spores labeled with phosphorus-32 have shown a pronounced effect of silver on the cell walls leading to leaching out of cell constituents (*6–8*). The action of silver in increasing the apparent uptake of mercury is no doubt connected with this effect.

Table II. Effect of Equivalent Quantities of Chloride or Bromide on Uptake of Silver and Germination of Spores of *Neurospora sitophila*

Quantity of Silver, γ/G. Spores	Uptake, γ/G., and % Germination after 10 Minutes					
	Ag		Ag + Chloride		Ag + Bromide	
	Uptake	Germination	Uptake	Germination	Uptake	Germination
200	155	79	160	63	91	100
400	270	55	270	49	151	85
800	535	44	550	50	340	52
1600	1330	11	1375	9	380	10
3200	2930	0	2850	0	1180	0

Table III. Effect of Equivalent Quantities of Iodide in Preventing Toxic Action of Silver to Spores of *Neurospora sitophila*

Silver Added, γ/G. Spores	Activity, C/M per Ml. after Spores Added			C/M after 10 Min.	Germination, %
	Ag^{110}	I^{131}	Total		
200	172	177	349	331	98
400	344	354	698	645	100
800	80	708	788	735	98
1600	161	1416	1577	1424	100
3200	322	2832	3154	3186	97
800	80	...	80	23	47
1600	161	...	161	17	31

Table IV. Uptake of Iodide by Spores of *Neurospora sitophila* Treated with Silver

15-Minute Contact		1320-Minute Contact	
Silver Present, γ/G.	Iodide Equiv. Taken Up	Silver Present, γ/G.	Iodide Equiv. Taken Up
85	Nil	80	12
175	23	150	Nil
335	34	290	25
1285	508	1250	667
2870	1650	2160	1500

Effect of Silver on Outward Movement of Cell Constituents. When comparisons are made between the effect of silver and other toxic ions in releasing phosphorus compounds from labeled spores into the ambient solution, it becomes clear that an important characteristic of the action of silver is its interference with cell permeability. In an experiment with spores of *Aspergillus niger* and ions of silver, copper, zinc, cadmium, mercury(II), and cobalt, only silver, and copper after a longer time interval, produced a large release of cell contents. With silver and copper from 35 to 45% of the phosphorus compounds were released into the ambient solution, while killing the spores by heat released only 27%. This effect cannot be explained merely as the result of killing the spores, for ions of zinc, cadmium, and mercury(II) also completely inhibited germination without a comparable effect. Similar results were obtained with spores of *Neurospora sitophila* (*8*). In an experiment with spores of *Aspergillus niger*, in which a range of concentrations of silver from 200 to 25,600 γ per gram was used (*7*), the outward movement of phosphorus compounds was evident at the highest concentration within 10 minutes, and

Table V. Effect of Silver on Germination and Outward Movement of Phosphorus Compounds from Spores of *Monilinia fructicola*

Ag Added, γ/G. Spores	Ag Taken Up, Germination, and Phosphorus Released after Various Time Intervals											
	0.02 Hour			2.02 Hours			4.02 Hours			21.4 Hours		
	Ag, γ/g.	Germination, %	P, %	Ag, γ/g.	Germination, %	P, %	Ag, γ/g.	Germination, %	P, %	Ag, γ/g.	Germination, %	P, %
50	32	100	0.8	42	100	2	43	98	3	42	100	5
100	48	99	1.2	85	94	2	84	89	3	72	100	5
200	106	95	1.0	166	92	3	168	86	5	168	92	8
400	213	31	1.4	256	62	11	340	62	17	345	46	22
800	565	9	3.8	720	20	40	635	18	40	640	13	53
1600	1445	5	14.2	1445	6	44	1295	3	35	...	...	..
3200	2924	0	12.4	2790	0	29	2800	0	32	2805	0	37
Control	...	100	0.7	...	100	1	...	100	2	...	100	2

Table VI. Effect of Copper on Germination and Outward Movement of Phosphorus Compounds from Spores of *Monilinia fructicola*

Copper Added, γ/G. Spores	Germination and P Compounds Released after Various Time Intervals							
	0.02 Hour		2.02 Hours		4.02 Hours		21.4 Hours	
	Germination, %	P, %	Germination, %	P, %	Germination, %	P, %	Germination, %	P, %
50	100	1.2	98	1.1	98	2.1	100	2.3
100	100	1.2	100	1.5	96	2.0	100	2.0
200	99	1.2	98	1.2	97	1.4	100	2.6
400	93	1.0	96	0.7	98	1.9	100	1.9
800	90	3.4	97	3.1	97	2.5	100	2.5
1600	90	1.5	46	1.1	41	1.4	19	3.9
3200	78	2.5	36	2.6	25	4.0	8	10.0
None	100	0.7	100	0.7	100	1.2	100	1.3

after 3 hours had reached about 50% of its maximum except at the lower concentrations of silver. With *Monilinia fructicola* the effect takes place still more rapidly (Table V). Within 5 minutes 12 to 14% of the contained phosphorus was released at the higher concentrations and some release was evident at the concentrations which had little or no effect on germination. After 2 more hours the release of phosphorus compounds was much more pronounced, although the maximum had not been reached. The apparent lesser release at the highest concentration may be the result of precipitation of some phosphorus compounds by the large amounts of silver present.

The effect of copper on germination and on permeability was much slower than that of silver (Table VI). Maximum release was only 10% of the contained phosphorus. Lower concentrations, which had a lesser or no effect on germination, also resulted in release of phosphorus compounds larger than that occurring in the control in distilled water.

Interaction between Silver and Mercury. The effect of pretreatment with silver in increasing the apparent uptake of mercury subsequently supplied is probably largely the result of precipitation of the mercury by some of the released cell contents. The question arises as to whether silver has a demonstrable effect on permeability at concentrations that cause no appreciable outward movement of cell contents. The data presented above indicate that there may be an area of little or no effect on germination and still some increase, even if slight, in the outward movement of phosphorous compounds. Similarly data have been accumulating indicating that low concentrations of silver may increase uptake of mercury more than precipitation of released cell contents can account for (Table VII). A series of concentrations of silver was chosen covering the range from no to only a very slight effect on germination. Experiments with labeled spores indicate that concentrations giving only a few per cent reduction in germination will not bring about a substantial release of cell contents and that release will be delayed. The data in Table VII show that the apparent uptake of mercury can be doubled in the first 10 minutes of exposure, even though previous treatment with silver has reduced germination by only a few per cent. The data suggest a possible effect on permeability, permitting increased inward movement of materials. Definite proof must await experiments using labeled spores and radioactive silver and mercury all in the same experiment.

Penetration of Silver into Spores. Spores of *Neurospora sitophila* (549 mg.) were exposed to silver at a dose of 270 γ per gram. After 5 minutes 180 γ per gram had been taken up. The remaining supernatant was removed and the spores were suspended twice in 10 ml. of distilled water. This procedure removed 24 γ of silver per gram of spores. The spores were now calculated to contain 156 γ per gram of silver, which was confirmed by direct counting of a small aliquot of the spores.

The spores were divided into five lots of 100 mg. each and placed in 5-ounce bottles in 4 ml. of water with 3 grams of glass beads; the bottles were rotated on a rolling machine for various lengths of time. A control was rotated for the maximum time period without glass beads. At the end of the test the samples were examined under the microscope to determine the degree of disintegration. The mixtures were transferred to centrifuge tubes and the proportion of silver remaining in the supernatant was determined after standing for 2 hours, 4 hours, and further after centrifugation for 1 minute. The values obtained were essentially the same in all the tests and only those obtained after centrifugation are given in Table VIII. If it is assumed that in this type of mechanical treatment the cell contents have a tendency to stay in the supernatant while the fragments of cell walls settle or centrifuge down, it is clear that much of the silver which has been taken up actually penetrated into the spores. The proof is not unequivocal, as possible redistribution from the cell surfaces to the cell contents could have taken place during the grinding process.

Table VII. Effect of Pretreatment with Small Quantities of Silver on Subsequent Uptake of Mercury by Spores of *Neurospora sitophila*

Silver Taken up, γ/G.	Germination, %	Cumulative Uptake of Mercury, γ/G., and Effect on Germination		
		10 Minutes		30 minutes, Hg
		Hg	Germination	
0	100	3595	21	4385
5	100	2710	21	3245
15	100	3025	26	3480
36	100	3850	37	4315
59	100	4430	42	5785
70	97	4475	32	6765
138	97	7130	48	...
150	78	6685	44	8805
163	83	9365	34	10780

Action of Copper, Zinc, and Cadmium

In addition to silver, mercury is also taken up very rapidly by fungus spores (*11*), so that toxic doses are reached within a matter of minutes. This is also true of the organic fungicides, 2-heptadecyl - 2 - imidazoline and 2,3-dichloro-1,4-naphthoquinone. In contrast, the action of copper, zinc, and cadium is very slow.

Copper. The uptake of copper has not been quantitatively determined in the present experiments, but work by others (*1, 2, 3, 5*) has shown that large amounts are required to inhibit spore germination. In experiments with *Neurospora sitophila* and copper a bleaching out of the pigment often occurs and the spores assume the color of the copper ion in relatively strong solutions. This indicates a high uptake of copper. That the action of copper on germination is slow is shown by Table VI and data in previous publications in which the effects on germination and outward movement of cell constituents were compared (*7, 8*). In the experiments of Table VI external concentrations of copper of 1600 and 3200 γ per gram inhibited germination only 10 and 22% after 5 minutes, but the inhibition gradually increased with time until it amounted to 81 and 92% after 21.4 hours.

The contrast between the speed of action of silver and copper is further illustrated in Figure 2. Based on the external concentrations, the ED_{50} values for silver as indicated by the dosage-response curves shown do not differ appreciably if determined after 20 minutes or 24 hours of contact between the spores and the toxicant. On the other hand, with copper the ED_{50} values are much affected by the length of time of exposure, about 40-fold between 6 and 150 minutes.

Zinc and Cadmium. Short exposures of spores of *Neurospora sitophila*, *Monilinia fructicola*, or *Aspergillus niger* to zinc or cadmium fail to produce any marked effect on germination. Even when the quantities supplied are increased to what seem to be unreasonably high levels, considerable time is required to bring about inhibition of germination. Spores of *Neurospora sitophila* took up these ions even less readily than the other two species. The results of an experiment with *Neurospora sitophila* and zinc and cadmium are given in Table IX.

The quantities removed from the solutions were so small that uptake by difference could not be accurately determined. The amount taken up was determined at the end of the experiment by determining the radioactivity of the spores after the treating solutions had been removed. The quantities taken up, while appreciable in one sense, amount to only from 2.4 to 4.3% of that available with zinc, and from 5.0 to 18.0% with cadmium. The effect on germination did not become pronounced until the 20-hour sampling period for cadmium, and germination was inhibited only about 25% with zinc even at the highest concentrations after 25 hours. Because of the differences in atomic weights, equivalent quantities of zinc taken up are actually considerably greater than those of cadmium. The differences in toxicity of the two ions are therefore greater than the data, expressed on a weight basis only, indicate.

Spores of *Aspergillus niger* took up zinc considerably more readily than spores of *Neurospora sitophila*. In an experiment in which both species were exposed to from 2500 to 5000 γ per gram, spores of *Aspergillus niger* took up as much as 3300 γ per gram in 2.4 hours, while the quantities taken up by *Neurospora sitophila* were so small that they could not be determined accurately by difference. The effects on germination were small for both species during this time interval.

Spores of *Monilinia fructicola* also take up zinc and cadmium more readily than those of *Neurospora sitophila* (Table X). Although fairly large quantities are taken up in 10 minutes, effects on germination do not become pronounced until after a longer exposure. ED_{50} values would thus differ, depending upon the length of time involved. Time would seem to be more important than the actual dose on a spore weight basis. When spores of *Monilinia fructicola* were treated with zinc or cadmium for 24 hours and the treating solution was then removed and the spores were suspended in several changes of distilled water, an average of 18% of the cadmium and 46% of the zinc was released into the water. On addition of a large excess of zinc or cadmium to the spores, 60% of the remaining cadmium and 43% of the remaining zinc were brought into the ambient solution. This is in contrast with results with silver taken up by spores, which will exchange practically completely with silver subsequently added (*11*).

Table VIII. Effect of Rolling Treated Spores of *Neurospora sitophila* with Glass Beads on Disintegration and Distribution of Silver between Supernatant and Centrifuged Residue

Time, Hours	Effect on Spores, %				Ag in Supernatant, %
	Whole	Plasmolyzed	Empty	Destroyed	
19 (control)	54	46	0	0	8.8
1	31	44	10	15	16.7
3	11	42	16	31	24.1
8	7	24	45	24	48.6
19	2	7	63	28	62.0

Table IX. Effect of Time of Contact between Spores of *Neurospora sitophila* and Zinc or Cadmium on Germination

Toxicant	γ/G. Spores	Germination Response after Various Time Intervals				Uptake 25.4 Hr., γ/G.
		0.02 hr.	2.08 hr.	20.08 hr.	25.4 hr.	
Zn	12,500	99	100	100	100	257
	25,000	100	100	73	80	930
	50,000	99	100	83	73	2435
	100,000	100	97	82	73	2694
Cd	5,000	95	97	94	64	1010
	10,000	89	82	63	63	1890
	20,000	83	81	40	34	2455
	40,000	87	74	23	11	2245

Experiments with Spores Labeled with Zinc-65

As in general the interaction between fungus spores and various cations and organic fungicides may be extremely rapid, it is of interest to know whether reactions involving normal constituents with externally applied materials also

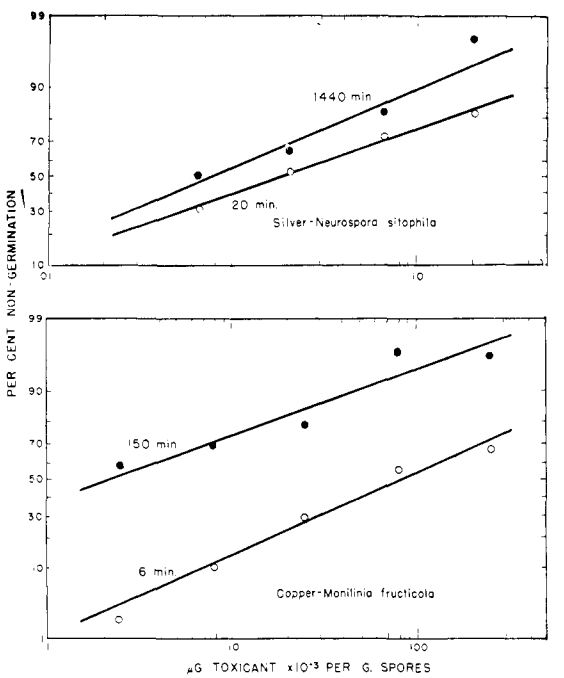

Figure 2. Dosage-response curves for silver and copper as affected by time of contact with fungus spores

Table X. Uptake of Cadmium and Zinc by Spores of *Monilinia fructicola* and Effect of Germination

Treatment		Cumulative Uptake		Germination, %	
Toxicant	γ/g. Spore wt.	0.17 hr.	4.17 hr.	0.17 hr.	4.17 hr.
Zn	2000	235	625	98	22
	5000	445	760	100	24
	10000	1595	1490	100	15
	20000	1230	1725	94	14
Cd	1000	440	625	96	2
	2000	960	1175	95	18
	4000	1130	1415	84	7
	8000	1655	2235	71	1

Table XI. Release of Zinc on Addition of Various Ions to Spores of *Aspergillus niger* Grown in Presence of Zinc-65

Ion Added	Quantity to 5.0 Mg. Spores, γ	Zn Released, %		Spore Germination, 24 Hr., %
		10 min.	24 hr.	
Zn	100	70	57	100
	200	58	58	100
	400	65	64	100
	800	66	89	99
	1600	75	95	98
Cd	100	14	9	96
	200	24	17	84
	400	30	29	68
Ce	100	75	85	100
	200	69	71	100
	400	75	85	100
None	...	4	5	100

Table XII. Release on Zinc on Addition of Various Ions to Spores of *Aspergillus niger* Grown in Presence of Zinc-65

Ion Added, 100 γ to 5 Mg. Spores	Zinc Released, %		Spore Germination, %, 24 Hr.
	10 min.	24 hr.	
Cu	70	78	89
Ag	19	20	0
Zn	45	40	97
Cd	24	28	100
Hg^{++}	10	11	62
Ce	72	73	90
None	3	3	100

take place readily. Experiments with spores labeled with phosphorus-32 have shown that various factors may bring about some outward movement of phosphorus compounds. The distilled water in which spores are placed when first harvested contains a considerable percentage of the total present and a second washing, while containing less, still contains considerable quantities. Whether the phosphorus-32 in the washings all comes out of the spores, or in part is derived from isotope present on the spore surfaces is not completely clear, but the indications are that spores, freshly harvested, release some cell contents into distilled water (*4*). In the experiments with zinc-65 a considerable percentage of the total zinc-65 associated with the spores is found in the first washing, much less in the second. On further suspension in water the remaining zinc is firmly held as far as its release into water is concerned, but is replaced rather readily, at least in part on the addition of various ions. These reactions occur under conditions in which germination remains essentially unaffected.

Spores of *Aspergillus niger* were grown with zinc-65 in the medium described. Two harvests were made, a few days apart, and the spores were found to contain 810 and 910 γ of zinc per gram, respectively, if it is assumed that all the zinc present was derived from that added to the medium. The results of an experiment on the effect of adding various quantities of nonradioactive zinc, cadmium, and cerium ions to spores suspended in distilled water, on the release of zinc-65 into the ambient solution, are given in Table XI. Cerium was included in the tests because it was known to be taken up readily in large amounts by spores of *Aspergillus niger* (*10*).

Table XI shows that considerable exchange occurred with the nonradioactive zinc added within 10 minutes—from 58 to 75% release of the zinc-65 originally in the spores. As would be expected, cadmium was much less effective, although it replaced some of the zinc. Cerium was as effective as zinc itself in bringing about a release of zinc-65 into the ambient solution. In general, little additional release occurred during the interval between 10 minutes and 24 hours. All responses took place rapidly and without affecting spore germination, except with the higher amounts of cadmium.

In another experiment equal absolute quantities of the ions of copper, silver, zinc, cadmium, mercury(II), and cerium (III) were compared as to their ability to displace the zinc-65 in the spores (Table XII). Cerium and copper were the most effective, mercury(II) and silver the least. Here again the action took place within 10 minutes and little additional change occurred up to 24 hours.

These results with spores that contained zinc as supplied, during the period of their formation by the nutrient medium, add to the evidence that inward and outward movement of various materials occurs much more readily with fungus spores than was previously supposed.

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