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Effect of Actidione on Growth and Respiration of *Myrothecium verrucaria*.* (19939)

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Actidione produced by *Streptomyces griseus*(1) is one of the most active antibiotics toward yeasts and filamentous fungi(2,3). As with other fungicides, however, there is little or no evidence on the nature of its action. Since penicillin and streptomycin are known to interfere with respiratory metabolism(4), it was desirable to compare the effects of actidione on fungus growth and respiration. This was done by measuring the inhibition of germination and of spore and mycelial respiration of *Myrothecium verrucaria* under various conditions.

Methods. Strain QM460 of *Myrothecium verrucaria* from the Quartermaster General Laboratories was cultured at 30°C on Whatman No. 2 filter paper on a modified Fries medium of Sinden, Mix and Siu(5). Point inoculation was used for routine transfer to avoid sector variants and spore suspension inoculation was used for rapid production of spores of uniform age (Mandels and Norton)(6). Mycelial pellets, 0.2 to 0.5 mm in diameter were produced in a 24-hour growth period at 30°C on a rotary shaker, essentially by the technic of Darby and Goddard(7). Percentage germination was measured at 30°C on glass slides in drops of the Mandels and Norton(6) nutrient solution. Counts of 300 to 500 spores were taken after 4 to 5 hours from 4 fields in each of 3 drops. Controls regularly germinated 99 to 100% so that corrections for natural mortality were not required. Actidione† solutions were prepared in serial dilution with a dose ratio of 1.1 which provided 4 or 5 levels of inhibition between 1 and 99%. LD50 values were determined graphically from dosage response curves on log-probability coordinates. *Respiration measurements* were made in air in standard Warburg apparatus at 30°C using the "direct" method(8) for CO_2 with correction for bound CO_2. Two to 4 mg dry weight of washed spores or mycelium was used per flask. Spores were suspended in nutrient solution(6) containing

TABLE I. Effect of Actidione on Germination and on Respiration of Spores and Mycelium of *M. verrucaria*.

	No. of exp.	LD50 values in p.p.m. Mean ± S.E.
Germination	5	2.71 ± .20
Spore resp.* (O_2 consump.)	14	.74 ± .14
Mycelium resp.* (O_2 consump.)	4	8.03 ± .17

* Period of maximum sensitivity: ca. 30-60 min. for spores, 2nd or 3rd hr for mycelium.

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† Actidione (reagent grade) was kindly supplied by J. H. Ford, The Upjohn Co., Kalamazoo, Mich.

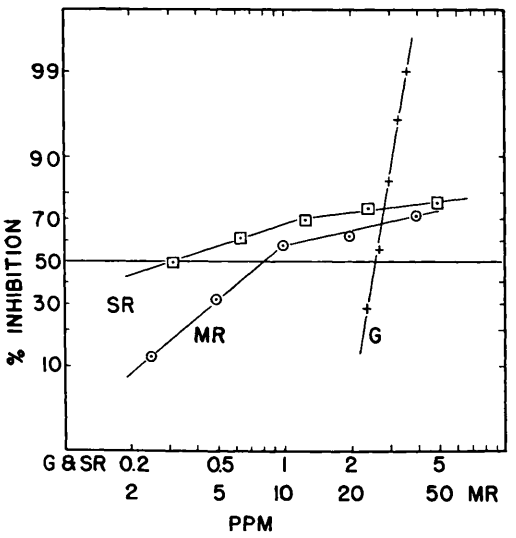

FIG. 1. Typical dosage-response curves on log-probability coordinates for spore O_2 consumption (SR), mycelial O_2 consumption (MR) and germination (G).

mineral salts, glucose and yeast extract and the mycelial pellets in pH 6.2 phosphate containing Mg and glucose(7). Actidione concentrations were chosen to give 3 to 5 levels of inhibition and LD50 values were estimated as in the case of germination.

Results. Table I gives mean LD50 values and standard errors for germination and for spore and mycelial respiration of *M. verrucaria*. Dosage-response curves for germination (Fig. 1) were linear throughout the measurable range of inhibition and were very steep. Mean values for this range can be taken as from 2 p.p.m. (LD1) to 3.6 p.p.m. (LD99). On the basis of LD50 values spore respiration was almost 4 times as sensitive to actidione as germination during the period of maximum sensitivity. This period was usually from 30 to 60 min. after addition of spores to nutrient and toxicant. Individual LD values for spore respiration varied considerably more than those for germination or mycelial respiration. This was due partly to the effect of the low slopes of the curves (Fig. 1) on the accuracy of estimating LD50 and partly to the rapid change in inhibition of spore respiration with time which is discussed below. It is also apparent that spore respiration was about 10 times as sensitive to actidione as mycelial respiration.

Representative data in Table II show that inhibition of spore respiration decreased rapidly after the first hour and disappeared completely after 3 or 4 hours at actidione levels causing initial inhibitions up to 50 to 60%. Three possible reasons were investigated: 1) decrease in spore sensitivity during the early stages of growth; 2) spontaneous decomposition of actidione; and 3) detoxication of actidione by the spores. The first possibility was tested by comparing the inhibition by 0.7 p.p.m. of actidione added at the start and after 200 min. incubation of the spores in the Warburg. The respective % inhibitions were 35 and 0 indicating a marked decrease in sensitivity during the first 3 hours. Spontaneous decomposition was tested by incubating actidione in nutrient without spores for 4 hours before measuring respiratory inhibition. Results of a typical experiment in Table III show that there was no decomposition. Detoxication was tested as follows: actidione was incubated with spores for a 4-hour period, the spores were removed by centrifugation, a fresh batch of spores was added with additional nutrient, and the respiratory inhibition measured. The results in Table III show that there was extensive detoxication or removal of actidione from the medium during the first 4-hour period in which spore respiration had recovered completely at the two lowest concentrations and about 80% at the highest.

At concentrations of actidione above 1 p.p.m., which gave initial inhibitions of spore respiration greater than 50 to 60%, the inhibition increased with time reaching a maximum of 80 to 85% (Table II and Fig. 1) which remained constant from about 2 to 20 p.p.m. The % inhibition of respiration at 2 p.p.m. where inhibition of germination just began (LD1) ranged from 65 to 80 with an average of about 75% for 9 trials.

The respiratory quotient for untreated spores averaged 0.96 and 1.03 for the first and second hours, respectively. Comparison of inhibition of O_2 consumption and CO_2 evolution of spores is shown in Table IV. No significant differences in % inhibition were found over a range of concentrations and times of exposure.

TABLE II. Change in % Inhibition of O_2 Consumption of Spores and Mycelium of *M. verrucaria* with Time.

Exp.	Conc. of actidione in p.p.m.	Hr after treatment 1st	2nd	3rd	4th
Spores 2-2A	.20	45	17	5	—3
	.40	58	31	25	2
	.60	62	52	36	22
1-11	.63	51	47		
	1.25	57	71		
11-15	1.25	74	81		
	2.50	78	84		
Mycelium 1-12	1.25	26	12	3	
	2.50	51	37	30	
	5.0	45	45	37	
	10.0	50	52	58	
1-24	10.0	42	48	51	49
	25	43	49	63	76
	50	48	51	65	83

TABLE III. Detoxication of Actidione by Spores of *M. verrucaria* Measured by % Inhibition of O_2 Consumption (First Hour).

Conditions	Conc. in p.p.m. .51 %	.34 %	.17 %
Fresh actidione standard	52	46	33
Actidione incub. without spores*	54	48	34
Actidione incub. with spores*	24	6	—6

* Actidione incubated under same conditions, with or without spores, for 4 hr before inhibition measurement.

TABLE IV. Relative Effect of Actidione on O_2 Consumption and CO_2 Evolution of Spores of *M. verrucaria* (First Hour).

Exp.	Conc. in p.p.m.	Hr after treatment	Inhibition O_2 %	Inhibition CO_2 %
1-25	1.4	1	75	76
		2	81	82
		3	78	81
1-26	.7	1	67	53
		2	61	63
		3	33	37
	1.4	1	73	69
		2	80	80
		3	73	77
2-14	.5	1	61	61
	1.0	1	70	66
	1.5	1	73	75

Mycelial respiration showed changes in % inhibition with time similar to those of spores, but at higher concentrations (Table II and Fig. 1). Again a maximum inhibition of about 85% was reached, in this case between 50 and 100 p.p.m. of actidione.

Discussion. Although no dosage-response curves or LD50 values for actidione effects on germination of other fungi have been reported, *M. verrucaria* appears to be roughly intermediate in sensitivity as compared with the species tested by Whiffen(2), Wollen, Sutton and Skolko(3) and others. On the other hand, actidione is one of the more active fungicides tested on *M. verrucaria,* although lower LD50 values have been found for ethyl mercury chloride(9) and 2,3-dichloro-1,4-naphthoquinone(10).

In interpreting the action of actidione on *M. verrucaria* it is significant, first, that spore respiration is much more sensitive than mycelial respiration and that both show recovery with time. In the case of spores the recovery is complete in 3 or 4 hours if the initial inhibition is not over 50 to 60%. During this period the sensitivity of spore respiration was shown to decrease, apparently approaching that of mycelium as germination progressed. At the same time spores removed actidione from the medium. Whether this removal was due to actual detoxication and thereby contributed to the decrease in respiratory inhibition, or whether the removal merely paralleled the decrease in respiratory sensitivity, can not yet be decided. Klomparens(11), however, has reported that mycelium of *Poria microspora* will take up actidione from solution and that it cannot be detected in the mycelium by bioassay after drying. It is possible, therefore, that both decreasing sensitivity and detoxication were involved in the recovery.

The most unusual property of actidione, however, is its greater toxicity to spore respiration than to germination. It was the only compound showing this property among 6 organic fungicides tested on *M. verrucaria* (9) including ethyl mercury chloride, 2,4-dinitrophenol, disodium ethylenebisdithiocarbamate, tetramethylthiuram disulfide, and pentachlorophenol. Klöpping(12) also found only one fungicide out of 10 which may have been more toxic to respiration than to growth of *Aspergillus niger* and *Penicillium italicum.* The precision of his methods, however, makes

even this case doubtful.

In the present work about 75% of spore respiration was inhibited by actidione levels (2 p.p.m.) which did not interfere with germination. At higher levels (above 3.6 p.p.m.), where germination was completely inhibited, the maximum inhibition of respiration was about 85%. This may indicate that only roughly 10% of respiration is required for growth. A similar conclusion was reached by Commoner and Thimann(13) for the effects of iodoacetate on *Avena coleoptile*. However, the present manometric data alone can not establish a direct connection between inhibition of growth and respiration. Until this is done other interpretations of the per cent of respiration linked with growth are possible.

Conclusions. The effect of actidione on the germination and the spore and mycelial respiration of *Myrothecium verrucaria* was measured by dosage-response curves and LD50 values and by change in per cent inhibition with time. Spore respiration was about 4 times as sensitive as germination and 10 times as sensitive as mycelial respiration. Only a small fraction of respiration seemed to be required for growth. Inhibition of spore and mycelial respiration decreased with time, in the spores because of decreasing sensitivity and possibly also because of detoxication.

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Effects of 2,4-Dinitrophenol Concentrations on Rates of Respiration and Fermentation of Yeast.* (19940)

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Most of the interest in the action of 2,4-dinitrophenol (DNP) on metabolism has stemmed from its ability to stimulate respiration(1,2), to prevent assimilation and other synthetic processes(3), and to interfere with the coupling of phosphorylation and respiration(4-6). It has also been noted that higher concentrations of DNP inhibit respiration of glucose(1) and acetate(7) and, under anaerobic conditions, the fermentation of glucose(8). However, the observations concerning inhibition of metabolism were incidental to studies pertaining to other actions of DNP. In the present paper quantitative observations are presented concerning marked differences in the inhibitory action of DNP on respiration as compared to fermentation, with glucose as substrate. Comparisons are also made with the stimulating actions of DNP on endogenous and exogenous respiration.

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