Interactions Between DDT and River Fungi

II Influence of Culture Conditions on the Compatibility of Fungi and p,p'-DDT

by

MADELINE HODKINSON and S. A. DALTON*

School of Biology, City of Leicester Polytechnic, P.O. Box 143, Leicester, LE1 9BH

Introduction

The persistent insecticide [1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane] (DDT) is widely distributed throughout the aquatic ecosystem (DALTON, et al., 1970). Microbial communities, by virtue of their contribution to primary production and organic decomposition are important components of aquatic food chains (OPENHEIMER, 1963; WOOD, 1967; SPARROW, 1968). Recent in vitro studies have shown that DDT can alter the metabolism and growth of certain marine (WURSTER, 1968; MOSSER et al., 1972) and freshwater micro-organisms (DALTON et al, 1970; DE KONING & MORTIMER, 1971; BATTERTON et al., 1972). Environmental factors appear to modify the effects of the insecticide (COLLIN & LANGLOIS, 1968; DE KONING & MORTIMER, 1971; BATTERTON et al., 1972), and with some micro-organisms differences in species sensitivity have been detected (MOSSER et al., 1972). The present work describes the effects of DDT on the growth of river fungi and the influence of culture conditions on the interactions between them.

Materials and Methods

Single spore isolates of the following fungi were used:- aquatic phycomycetes, *Saprolegnia* sp., *Isoachlya monilifera* (de Bary) Kauffman, *Isoachlya* sp., *Pythium* sp., aquatic hyphomycetes, *Clavariopsis aquatica* (De Wildeman), *Heliscus submersus* (Hudson), *Tetracladium setigerum* (Grove, Ingold), *Varicosporium elodeae* (Kegal), terrestrial hyphomycetes, *Aureobasidium pullulans* (de Bary) Arnaud, *Cephalosporium acremonium* (Corda), *Cladosporium cladosporioides* (Fres), *Cylindrocarpon orthosporium* (Sacc.) Wollenw. With the exception of *Saprolegnia* sp. the fungi were obtained from river water. *Saprolegnia* sp. was isolated from an infected salmon. Homogenised fungal inocula were prepared in the manner described by DALTON et al. (1970).

Triplicate 250 ml. Erlenmeyer flasks were set up containing 100 ml of basal medium (1g of KH_2PO_4, 0.2 g of $MgSO_4$. $7H_2O$, 0.02 of FeSO4 and 0.5g of Oxoid yeast extract per litre of distilled water) or basal medium to which small volumes of an AR acetone solution of ethanol-recrystallised Puriss grade p,p'-DDT were added to give final concentrations of 2,10, 20 and 60µg/ml. The vessels were seeded with 1 ml aliquots of an homogenised fungal inoculum. The cultures were incubated on an orbital shaker (100 revs/min) for varying periods of time (24-1600 hrs.) All twelve fungi were cultured in this manner at 20°C. Additional cultures of *C.cladosporioides*, *Isoachlya* sp. and *H. submersus* were incubated at 5°C. The mycelium was recovered by filtration and dried to constant weight at 80°C.

* Present address - Didsbury College of Education, Manchester.

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C.cladosporioides, Isoachlya sp. and H. submersus were also grown at 5° and 20°C in shake culture in double glass distilled water, a mineral salts solution (basal medium minus yeast extract), a 0.05% (w/v) solution of yeast extract, basal medium and these media containing 60µg/ml of DDT. The experiment was triplicated and the mycelial yields were determined at various time intervals as described above.

Results and Discussion

The results presented in Table 1 show that the aquatic phycomycetes, the aquatic hyphomycetes and the terrestrial hyphomycetes used in this study, exhibit a similar in vitro response to DDT. In general the growth rates of the twelve fungi were enhanced when the insecticide was included in the basal medium. However, only 50% of the fungi were sensitive to 2µg/ml of DDT, and of these, half responded after prolonged incubation. Normally 10 µg/ml and higher insecticide concentrations induced marked changes in fungal growth within 2 to 4 days at 20°C.

TABLE 1

Percentage increase in fungal growth in medium containing 2, 10, 20 and 60 µg/ml of DDT*

Fungus	20°C				5°C			
	2	10	20	60	2	10	20	60
Isoachlya sp.	1	2	10	39	3	5	7	12
I.monilifera	0	9	58	138				
Saprolegnia sp.	0	2	10	43				
Pythium sp.	0	4	9	41				
H.submersus	5	9	14	65	1	5	14	41
C. aquatica	0	5	12	29				
T. setigerum	0	6	20	48				
V.elodeae	0	2	11	48				
C.cladosporioides	0	4	10	36	4	7	16	46
A. pullulans	0	25	35	74				
C. acremonium	2	4	17	54				
C. orthosporium	0	7	12	57				

* Mycelial yields determined after 100 hrs at 20°C and 400 hrs incubation at 5°C.

Incubation temperature affected the growth rates of the fungi but did not alter their responses to the insecticide. The growth rate of each fungus increased with increased DDT concentration but the form of the growth curves remained the same. The results confirm the observations of DALTON et al. (1970).

When C.cladosporioides, Isoachlya sp. and H. submersus were cultured in distilled water and mineral salts solution, small amounts of growth were recorded. This presumably was due to organic materials introduced with the homogenised inocula. The mycelial yields increased several fold in media containing yeast extract. The media can be arranged in order of their decreasing ability to support fungal growth viz. basal medium, yeast extract, mineral salts and distilled water. The addition of 60 µg/ml of DDT to each medium evoked a similar response from the fungi and significant increases in growth were recorded (Table 2). The greatest mycelial yields were obtained in semi-synthetic media (YE and BM) containing DDT, but much higher percentage increases in yield were detected in DDT-enriched water and mineral salts solution.

TABLE 2

Percentage increases in fungal growth in four media containing 60 µg/ml of DDT*.

Fungus	20°C				5°C			
	DW	MS	YE	BM	DW	MS	YE	BE
Isoachlya sp.	500	300	40	33	1000	575	37	24
H. submersus	600	240	94	45	600	250	19	38
C. cladosporioides	100	160	40	45	100	128	20	36

* Mycelial yields determined after 100 hrs at 20°C and 400 hrs incubation at 5°C.

DW = Distilled water MS = Mineral salts
YE = Yeast extract BM = Basal medium

The results suggest that the nutritional status of the environment in which fungi and DDT interact, governs the extent to which the insecticide affects growth. This observation contrasts with reports by COLLINS & LANGLOIS (1968), DE KONING & MORTIMER (1971), and BATTERTON et al., (1972) that quantitative and qualitative differences in the composition of culture media change the nature of bacterial and algal responses to DDT. It has been suggested by BATTERTON et al., (1972) that DDT interferes with the role of adenosine triphosphatase in ionic transport across cell membranes. An increase in the permeability of hyphal membranes to essential nutrients in the presence of the insecticide might explain the growth increases reported here. Alternatively, the DDT provides the fungi with an additional source of organic carbon and energy, or acts upon their energy-yielding reactions as a growth factor.

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