Lipid Composition of *Entomophthora obscura* Hall & Dunn

By JEAN-PAUL LATGÉ¹* AND CLAUDE DE BIÈVRE²

¹ Unité de Lutte Biologique contre les Insectes and ² Unité de Mycologie,
Institut Pasteur, 25 Rue du Dr Roux, 75015 Paris, France

(Received 12 February 1980; revised 9 May 1980)

The composition of neutral, polar and sphingolipids of *Entomophthora obscura* Hall & Dunn during resting spore formation has been investigated. Sporogenesis was accompanied by an increase in the total lipid concentration and a decrease in the polar/neutral lipid ratio. The most prevalent neutral lipid fatty acids were C_{10:0}, C_{12:0}, C_{16:0} and C_{18:0}; the concentration of C_{18:0} increased greatly during sporogenesis. Polar lipids were characterized by high concentrations of C_{16:0}, C_{20:4} and C_{20:5}; the concentration of C_{20:5} decreased and those of C_{18:1} and C_{20:4} increased during resting spore formation. The fatty acid composition of neutral and polar lipids was similar in several strains of *E. obscura* and proved their conspecificity irrespective of the facility with which each strain produced resting spores.

INTRODUCTION

Entomophthora obscura is an aphid pathogen morphologically characterized by spherical conidia (diam. 32 to 44 µm) with a papillate base, spherical azygospores (diam. 35 to 55 µm) and the absence of rhizoids on the insect host (Hall & Dunn, 1957). Collections in Europe and North America have led to the isolation of over 100 strains of *E. obscura*, only about 20 of which produce resting spores in culture. In addition, strains that produce spores differ in their growth on artificial media and development in the aphid host from those that fail to produce them (Remaudière *et al.*, 1979). These morphological and physiological characteristics, however, are insufficient to determine whether or not these two types of strains should be considered conspecific.

Chemotaxonomic techniques provide a way to clarify further the classification of Entomophthorales species. Among the chemotaxonomic criteria used in mycology, fatty acid composition seems to be the most reliable. Previous studies (Tyrrell, 1967, 1968, 1971; Tyrrell & Weatherston, 1976) demonstrated that species of *Entomophthora* and *Conidiobolus* could be characterized and classified from the composition of their fatty acids. Among all the species, *E. coronata* has been most extensively studied (Mumma & Brzuszewski, 1970, 1973; de Bièvre, 1974*a, b*).

During growth and resting spore development of *E. obscura* *in vitro*, changes occur in the size and abundance of oil globules (Latgé, 1980). Mycelial and young spore stages contain numerous oil droplets about 2 µm in diameter; the number decreases as the spores age and the mature spore contains a single large oil globule, 20 µm in diameter. This paper describes how these morphological changes are associated with changes in lipid metabolism. Firstly, the composition of lipids was examined at different stages during morphogenesis of a spore-producing strain. Secondly, corresponding analyses of the mycelium of strains that produce resting spores were compared with those of some that fail to produce them.

METHODS

Strains and culture conditions. The strains used in this study (455, 542, 637, 766, 879, 940; Lutte Biologique contre les Insectes, Institut Pasteur, Paris, France) were maintained on agar medium containing (per litre) 100 ml egg yolk, 130 ml milk, 20 g glucose and 10 g peptone. However, these strains also developed rapidly on yeast extract/glucose medium (Latgé *et al.*, 1978*b*; Latgé, 1980). In this study, the strains were cultivated at 20 °C in 2 l fermenters (Latgé, 1980) in a medium containing 4 % (w/v) glucose, 1 % (w/v) yeast extract (Difco) and 1 % (v/v) Rhodorsil 426 R (dimethylpolysiloxane oil) antifoam (Prolabo). The pH was initially 6.2 ± 0.1 and remained between 6 and 7 for all strains throughout their growth.

In the fermenters, only strains 542 and 879 produced azygospores, though strain 455 produced a few on solid media. Changes in fatty acid composition during morphogenesis were determined in strain 542 (see Table 1). The mycelium of strains 455, 637, 766, 879 and 940 was harvested after 22 to 48 h growth depending on the strain (see Table 2).

After harvesting by filtration, the fungal biomass was washed three times with distilled water, freeze-dried and stored at -20 °C until required. The spore stage was washed in sodium hypochlorite solution (2 %, w/v) for 2 min to eliminate residual mycelium and aberrant azygospores.

Extraction procedures. The fungus material was homogenized in a MSK Braun cell disintegrator for 2 min at maximum speed with 1 mm glass beads in chloroform/methanol (2:1, v/v) or trichloroethane/ethanol (2:1, v/v). The suspension was made to 150 ml solvent per gram freeze-dried material, maintained for 16 h at room temperature and then filtered. The residue was refluxed for 1 h at 60 °C in an equal volume of solvent, filtered at room temperature and combined with the original filtrate. The extracts were evaporated under vacuum in a rotary evaporator. Non-lipid material was then removed (Folch *et al.*, 1957) and residual water was removed with anhydrous Na_2SO_4 . After concentration in a rotary evaporator, the lipids were stored in 10 ml chloroform or trichloroethane at -20 °C.

Fractionation procedures. The fungal lipids were fractionated into three classes in an activated 1 g silicic acid column (2.5 × 8 cm) using 100 ml trichloroethane, 100 ml acetone and 50 ml methanol to elute neutral lipids, sphingo- and glycolipids, and polar lipids, respectively. The sphingolipids were further purified by a 12 h hydrolysis of the ester linkages in 0.1 M-KOH in methanol/water (9:1, v/v) under nitrogen (Karlsson *et al.*, 1973). After concentration, the lipids were stored in 10 ml chloroform at -20 °C and the lipid content was determined by weight. The total lipid concentration was estimated on two or three complete extractions for each stage and each strain.

Lipid identification. Lipids were identified by thin-layer chromatography (t.l.c.). Polar lipids were chromatographed on Merck PF₂₅₄ silica gel plates developed with chloroform/methanol/water/acetic acid (65:43:3:1, by vol.); spots were detected with the molybdc reagent of Dittmer & Lester (1964) and the iodo-bismuthic-acetic reagent of Munier & Macheboeuf (1951) for choline. Neutral lipids were chromatographed on Merck 60 F₂₅₄ silica gel plates developed with petroleum ether (b.p. 40 to 65 °C)/diethyl ether/acetic acid (10:9:1, by vol.); spots were detected using a 20 % HClO_4 spray after 1 h at 110 °C. Appropriate authentic standards were chromatographed on each t.l.c. plate. Experimental lipids were identified by comparing their R_f values with those of the standards.

Fatty acid identification. Methyl esters of fatty acids were prepared by hydrolysing the lipids in 0.5 M-methanolic KOH at 100 °C for 5 min, then methylating the fatty acids in methanolic BF_3 (14 %, w/v) for 2 min at 100 °C. The methyl esters obtained were analysed by gas-liquid chromatography (g.l.c.) in a 2 m × 3 mm stainless steel column packed with 15 % (w/w) diethylene glycol succinate (DEGS) on a 100 to 120 mesh Chromosorb Q. The column was operated at 170 °C with N_2 as the carrier gas at a flow rate of 30 ml min⁻¹. A similar column filled with 3 % (w/w) silicone GE-SE-30 on the same support and operated under the same conditions was used for unsaturated fatty acids. The esters were identified by comparing their retention times with those of standards and comparing the logarithmic plots of retention ratios. Quantitative estimates of each fatty acid were based on the area under the peak.

Some of the unsaturated fatty acids were identified by mass spectrometry. An LKB 2091 mass spectrometer was directly connected to a g.l.c. apparatus (column of 10 % SP2340 on Chromosorb WAW, 100 to 120 mesh; helium carrier gas, 30 ml min⁻¹). The operating conditions were: filament intensity, 4 A; electron flux, 50 μA ; electron energy, 20 to 70 eV; accumulation voltage, 3500 V; injector temperature, 270 °C; separation temperature, 265 °C.

Identification of fatty acids was confirmed using a capillary glass column (48 m × 0.5 mm) with a Carbowax 20M stationary phase. The column temperature was 180 °C, injector and detector temperatures were 210 °C and the N_2 pressure was 30 kPa (Prévôt & Mordret, 1976).

The degree of unsaturation of the total fatty acids is expressed as the number of double bonds per molecule calculated by the formula: $\Delta/\text{mol} = (1 \times \% \text{ monoenes}/100) + (2 \times \% \text{ dienes}/100) + (3 \times \% \text{ trienes}/100) + (4 \times \% \text{ tetraenes}/100) + (5 \times \% \text{ pentaenes}/100)$.

In replicate lipid extractions, fatty acid methylation and g.l.c. of the fatty acid mixture made from samples of the same freeze-dried material, quantities of fatty acids that comprised more than 10 % of the total varied by up to 10 %. This variation was sometimes greater for those present in quantities less than 5 % of the total, but this was unimportant since only qualitative data on these specific minor fatty acids were used in characterizing the strains.

RESULTS

Changes in lipid content and fatty acid composition during morphogenesis of strain 542

The lipid content increased as the fungus developed from the young mycelial stage to the sporulating stage (Table 1). However, the lipid concentration of the mature spores was lower than that of the sporulating mycelium. The mature spore is characterized by a unique and large oil globule which occupies over 20 % of the spore volume. At this stage, the highest lipid concentration would be expected, but our analyses showed that the coalescence of the oil globules is not accompanied by an increase in the total lipid content. The neutral lipids always represented more than 80 % of total lipids, except in the young mycelial stage where the polar lipid comprised 35 % of the total lipids; thus the neutral/polar lipid ratio increased as the mycelium aged (Table 1). There was no difference in the components of the polar and neutral lipids at different stages of the fungus life cycle. Triacylglycerols predominated in the neutral lipids but there were also 1- and 2-monoacylglycerols, 1,3- and 1,2-diacylglycerols, free fatty acids, sterol esters and hydrocarbons. The polar lipids contained mainly lecithin but also cardiolipin, phosphatidylethanolamine and traces of lysophosphatidylethanolamine, phosphatidylinositol and probably lysophosphatidylcholine and sphingomyelin. Sphingolipids represented 0.5 to 2.0 % of the total lipid content, no matter what the stage of sporogenesis.

Capric ($C_{10:0}$), lauric ($C_{12:0}$), palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids were the most abundant of the neutral lipid fatty acids at the different stages of the strain 542 growth cycle (Table 1). The concentration of $C_{10:0}$ and $C_{12:0}$ acids increased as the mycelium sporulated and then decreased as the spores matured. $C_{16:0}$ was predominant in the early stages of mycelial growth (I). The concentration of $C_{18:0}$ increased greatly during the sporulating stages and reached 50 % of the total fatty acids at the mature spore stage (V). The degree of unsaturation was always very low, decreasing as the mycelium aged from stage I to IV, then increasing at the more mature spore stages (IV and V).

The major fatty acids of the polar lipids were $C_{16:0}$, $C_{18:0}$, $C_{18:1(9)}$ plus $C_{18:1(11)}$, $C_{20:4(5,8,11,14)}$ and $C_{20:5(5,8,11,14,17)}$ (Table 1). As the spores matured, the proportions of $C_{18:0}$ and $C_{18:1}$ increased and those of $C_{20:5}$ and $C_{16:0}$ decreased. The degree of unsaturation was highest (3.4) at mycelial stage I and decreased, as the mycelium aged and sporulated, to a value of 2.3 at spore stage V.

The major fatty acids of the sphingolipids appeared to be $C_{16:0}$, $C_{18:0}$ and $C_{19:1(13)}$. Significant amounts of $C_{15:0}$, $C_{16:1}$, $C_{17:0}$ and $C_{19:0}$ were noted (Table 1). A large increase in $C_{18:0}$ accompanied by a decrease of $C_{19:1}$ occurred as the mycelium of strain 542 sporulated.

Lipid content and fatty acid composition of mycelium of six strains of E. obscura

The lipid content of the mycelium of the six strains varied from 13.1 to 33.8 % of the dry weight (Table 2) but these differences were not correlated with the ability of the strains to produce resting spores *in vitro*.

In all strains analysed, the major fatty acids of the neutral lipids were $C_{10:0}$, $C_{12:0}$, $C_{16:0}$ and $C_{18:0}$ (Table 2). Strains which did not produce resting spores in egg yolk medium (637, 766 and 940) contained more palmitic acid than stearic acid. The polar lipid compositions of the six strains were similar with high levels of $C_{16:0}$, $C_{18:1(9)}$ plus $C_{18:1(11)}$, $C_{20:4(5,8,11,14)}$ and $C_{20:5(5,8,11,14,17)}$, the latter always predominating (Table 2). Among the sphingolipids, concentrations of $C_{16:0}$, $C_{18:0}$, $C_{18:1}$ and $C_{19:1}$ were high in all strains

Table 1. *Total lipid concentration, polar/neutral lipid ratio and fatty acid composition of neutral, polar and sphingolipids of E. obscura strain 542 during morphogenesis*

	Growth stages*														
	I			II			III			IV			V		
Growth time (h)	16			30			39			45			170		
Growth yield (g dry wt l ⁻¹)	5.4			11.8			16.4			16.0			11.0		
Total lipid content (% cell dry wt)	5.3			17.8			18.5			24.4			19.2†		
Polar/neutral lipid ratio (%)	59			13			6			7			13†		
Fatty acid composition of lipids (% of total in each lipid class)‡	N	P	S	N	P	S	N	P	S	N	P	S	N	P	S
10:0	10	—	2	16	—	tr	23	—	7	26	—	5	12	—	1
12:0	17	tr	tr	22	tr	tr	20	tr	1	21	tr	2	11	tr	1
14:0	8	2	3	7	3	3	7	3	4	4	2	6	3	tr	2
14:1(9)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
15:0	—	—	2	—	—	1	—	—	2	—	—	—	—	—	1
15:1(?)	—	—	—	—	—	1	—	—	tr	—	—	—	—	—	—
16:0	10	18	8	5	22	12	4	20	11	3	18	14	6	13	9
16:1(9)	—	tr	1	—	tr	1	—	tr	2	—	tr	3	tr	tr	2
17:0	—	—	4	—	—	6	—	—	5	—	—	1	—	—	tr
17:1(11)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	tr
18:0	27	3	13	39	2	11	37	4	17	42	2	50	45	20	63
18:1(9)+18:1(11)	5	5	3	2	4	3	2	8	5	1	7	7	7	18	6
18:2(9,12)	1	2	6	1	2	1	tr	1	8	tr	1	1	1	2	1
18:3(9,12,15)	2	3	tr	1	4	tr	tr	1	—	tr	2	1	tr	1	tr
18:3(6,9,12)	2	1	—	1	1	tr	tr	3	—	tr	1	tr	2	2	tr
18:4(6,9,12,15)	1	2	—	tr	1	—	tr	tr	—	tr	tr	—	tr	2	—
19:0	—	—	8	—	—	4	—	—	4	—	—	—	—	—	—
19:1(13?)	—	—	13	—	—	21	—	—	26	—	—	—	—	—	1
20:3(8,11,14)	8	4	—	4	9	—	1	7	—	1	8	—	9	4	—
20:4(5,8,11,14)	1	5	—	1	9	—	tr	6	—	tr	10	—	tr	3	—
20:4(8,11,14,17)	3	5	—	1	5	—	1	6	—	tr	4	—	tr	10	—
20:5(5,8,11,14,17)	6	51	—	3	40	—	1	43	—	2	46	—	5	25	—
Unknowns	—	—	35	—	—	34	—	—	7	—	—	3	—	—	11
Degree of unsaturation§	0.9	3.4	NC	0.4	3.0	NC	0.2	3.1	NC	0.2	3.3	NC	0.7	2.3	NC

* I and II, mycelial stages; III and IV, sporulating stages; V, spore stage.

† Average of three replicates.

‡ N, Neutral lipid (excluding glycolipid); P, phospholipid (excluding sphingolipid); S, sphingolipid; tr, trace (values of less than 0.5%).

§ NC, Not calculated due to unknown fatty acids.

Table 2. Total lipid concentration and fatty acid composition of neutral, polar and sphingolipids of six strains of *E. obscura* at the mycelial stage

	Strain																							
	542			879			455			940			766			637								
Growth time (h)	30			22			48			48			48			46			24			637		
Growth yield (g dry wt l ⁻¹)	11.8			9.2			10.1			10.1			10.1			10.7			7.0			7.0		
Total lipid content (% cell dry wt)	17.8			13.1			14.1			20.5			20.5			33.7			33.8*			33.8*		
Fatty acid composition of lipids (% of total in each lipid class) [†]	N	P	S	N	P	S	N	P	S	N	P	S	N	P	S	N	P	S	N	P	S	N	P	S
10:0	16	—	tr	22	—	4	29	—	4	30	—	3	22	—	12	23	—	‡	—	—	—	—	—	—
12:0	22	tr	tr	16	tr	1	20	tr	3	16	1	1	16	tr	7	18	1	—	—	—	—	—	—	—
14:0	7	3	3	7	1	6	2	2	4	5	tr	3	5	tr	3	8	2	—	—	—	—	—	—	—
14:1(9)	—	—	—	—	—	1	—	—	—	—	—	tr	—	—	—	—	—	—	—	—	—	—	—	—
15:0	—	—	—	—	—	4	—	—	2	—	—	2	—	—	1	—	—	—	—	—	—	—	—	—
15:1(?)	—	—	1	—	—	1	—	—	—	—	—	tr	—	—	—	—	—	—	—	—	—	—	—	—
16:0	5	22	12	7	20	21	2	14	13	36	17	15	33	21	22	37	33	—	—	—	—	—	—	—
16:1(9)	—	tr	1	tr	1	6	tr	tr	2	—	tr	3	tr	tr	2	—	tr	—	—	—	—	—	—	—
17:0	—	—	6	—	—	1	—	—	4	—	—	6	—	—	1	—	—	—	—	—	—	—	—	—
17:1(11)	—	—	—	—	—	1	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—
18:0	39	2	11	39	1	35	46	2	39	10	1	11	19	1	12	7	tr	—	—	—	—	—	—	—
18:1(9)+18:1(11)	2	4	3	2	9	10	tr	10	3	1	13	4	1	11	5	4	14	—	—	—	—	—	—	—
18:2(9,12)	1	2	1	1	1	3	tr	2	2	tr	2	9	tr	1	1	tr	3	—	—	—	—	—	—	—
18:3(9,12,15)	1	4	tr	tr	2	tr	—	1	tr	tr	2	tr	tr	tr	1	tr	2	—	—	—	—	—	—	—
18:3(6,9,12)	1	1	tr	tr	tr	tr	tr	2	tr	tr	tr	tr	tr	tr	1	tr	tr	—	—	—	—	—	—	—
18:4(6,9,12,15)	tr	1	—	tr	tr	—	tr	1	—	tr	1	—	tr	tr	—	tr	1	—	—	—	—	—	—	—
19:0	—	—	4	—	—	1	—	—	2	—	—	9	—	tr	—	—	—	—	—	—	—	—	—	—
19:1(13?)	—	—	21	—	—	3	—	—	16	—	—	20	—	—	2	—	—	—	—	—	—	—	—	—
20:3(8,11,14)	4	9	—	4	7	—	tr	5	—	2	5	—	3	6	—	tr	3	—	—	—	—	—	—	—
20:4(5,8,11,14)	1	9	—	1	18	—	tr	26	—	tr	14	—	tr	19	—	1	10	—	—	—	—	—	—	—
20:4(8,11,14,17)	1	5	—	1	3	—	—	3	—	tr	5	—	tr	4	—	tr	2	—	—	—	—	—	—	—
20:5(5,8,11,14,17)	3	40	—	3	37	—	tr	32	—	tr	38	—	1	35	—	2	30	—	—	—	—	—	—	—
Unknowns	—	—	34	—	—	4	—	—	11	—	—	10	—	—	30	—	—	—	—	—	—	—	—	—
Degree of unsaturation [§]	0.4	3.0	NC	0.4	3.1	NC	0.1	3.2	NC	0.1	3.1	NC	0.2	3.0	NC	0.2	3.0	NC	0.2	2.3	—	—	—	—

* Average of two replicates.

† N, Neutral lipid (excluding glycolipid); P, phospholipid (excluding sphingolipid); S, sphingolipid; tr, trace (values of less than 0.5 %).

‡ Sphingolipid of strain 637 was not tested.

§ NC, Not calculated due to unknown fatty acids.

analysed. However, the analyses of the sphingolipids from the 2 m × 3 mm stainless steel column were difficult to interpret and many acids were not identified; these unidentified fractions reached 35 % in the mycelium stage of strain 542. This class of lipids was therefore not considered in the strain characterization.

DISCUSSION

In *E. obscura*, the progressive increase in lipid content of the mycelium as the culture develops is due mainly to the accumulation of triacylglycerols in the neutral lipid fraction of the cell. This has been recorded on many occasions for other moulds (Weete, 1974). Neutral lipids are considered to be principally energy storage materials; the ability of resting spores to germinate without exogenous nutrients (Latgé *et al.*, 1978a) could be related to the abundance of this reserve lipid.

The composition of the neutral lipid fatty acids of *E. obscura* varied as the mycelium aged and sporulated. Neutral lipids are usually restricted to storage lipid vacuoles; therefore, these fatty acid variations could be associated with the important morphological changes of the lipid vacuoles that occur during the growth and sporulation of this species (Latgé, 1980). The most important change occurring during the sporogenesis of *E. obscura* was an absolute increase in C_{18:0}. It is noteworthy that the strains producing resting spores on solid medium had a high concentration of C_{18:0} while the non-sporulating strains contained much C_{16:0} but little C_{18:0}. The metabolism of stearic acid appeared therefore, to be one of the biochemical pathways linked to resting spore formation.

Polar lipid fatty acid composition appeared more constant than that of neutral lipids for mycelia of different ages and for the two types of strains. Phospholipids, being functional membrane components, probably remain constant under any given growth condition, while neutral lipids, being storage products, may vary without interfering in metabolic pathways.

Both α -linolenic acid [C_{18:3} (9,12,15)] and γ -linolenic acid [C_{18:3} (6,9,12)] occurred in all the strains of *E. obscura* grown on yeast extract medium. In contrast, α -linolenic acid was not found in several Entomophthorales species (including *E. obscura*) grown on Sabouraud medium (Tyrrell, 1967, 1971). Culture media influence the lipid characteristics of fungi (Weete, 1974) and yeast extract (extracted from a yeast rich in α -linolenic acid) could have induced α -linolenic acid synthesis. Phycomycetes characteristically produce γ -linolenic acid (Weete, 1974). However, the simultaneous presence of α - and γ -linolenic acids has been detected in a few Phycomycetes species, e.g. *Blastocladiella emersonii* (Sumner, 1970), *Thraustochytrium roseum*, *T. aureum* and *Phlyctochytrium punctatum* (Ellenbogen *et al.*, 1969).

Tyrrell (1967, 1968, 1971) and Tyrrell & Weatherston (1976) have decisively demonstrated the importance of fatty acid composition in the classification of the Entomophthorales. These findings and those presented here demonstrate that strains that are otherwise similar should be considered conspecific, regardless of whether they produce resting spores. This concept is in agreement with King's (1976, 1977) conclusion and is supported by the fatty acid analyses of Tyrrell & Weatherston (1976), indicating that *Conidiobolus polyspermus* is synonymous with *C. megalotocus*, even though the type strain of the former produces resting spores and no microconidia and the type strain of the latter produces no resting spores and abundant microconidia. The non-sporulating strains of *E. obscura* have been induced to form resting spores by incubating the mycelium in soil (Latgé *et al.*, 1979), supporting our contention that all of these strains are referable to *E. obscura*.

Morphologically, the Entomophthorales are usually allied to the 'higher Phycomycetes', e.g. the Zygomycetes and Trichomycetes. However, an examination of fatty acids may enable the position of the Entomophthorales in the Phycomycetes to be reconsidered. Long-chain polyunsaturated fatty acids are typical of many Entomophthorales including *E. obscura*. Among the 65 species of Phycomycetes reviewed by Weete (1974), this potential

for long-chain polyunsaturated fatty acid synthesis would place the Entomophthorales with the Chytridiomycetes and Oomycetes of the 'lower Phycomycetes'. Also, the walls of *E. obscura* (J.-P. Latgé, unpublished results), *E. virulenta*, *E. coronata*, *C. stromioideus* and *B. ranarum* (Hoddinott & Olsen, 1972) are mainly composed of glucan and chitin without chitosan. These are the typical sugars found in Chytridiomycetes walls (Bartnicki-Garcia, 1968). Thus, it is interesting to note that a biochemical classification of the Phycomycetes would place the Entomophthorales nearer the Chytridiomycetes than the 'higher Phycomycetes'.

The authors thank J. M. Ziegler, A. Prévôt and F. Mordret for their help during mass spectrometry and capillary column chromatography analyses. They also thank G. Dunphy, R. Hall, D. King, D. Perry and N. Wilding for their criticisms and English corrections.

REFERENCES

- BARTNICKI-GARCIA, S. (1968). Cell wall chemistry, morphogenesis and taxonomy of fungi. *Annual Review of Microbiology* **22**, 87–108.
- BIÈVRE, C. DE (1974a). Lipides de quelques souches de *Entomophthora coronata* et de *Basidiobolus meristosporus*. *Annales de Microbiologie (Institut Pasteur)* **125A**, 309–321.
- BIÈVRE, C. DE (1974b). Isolement, purification et caractérisation de sphingomyeline chez des souches de *Entomophthora coronata* pathogènes pour l'homme. *Annales de Microbiologie (Institut Pasteur)* **125B**, 471–487.
- DITTMER, S. C. & LESTER, R. L. (1964). A simple specific spray for detection of phospholipids on chromatograms. *Journal of Lipid Research* **5**, 126–128.
- ELLENBOGEN, B. B., AARONSON, S., GOLDSTEIN, S. & BELSKY, M. (1969). Polyunsaturated fatty acids of aquatic fungi: possible phylogenetic significance. *Comparative Biochemistry and Physiology* **29**, 805–811.
- FOLCH, J. M., LEES, M. & SLOANE-STANLEY, G. H. (1957). A simple method for the isolation and purification of the total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 487–509.
- HALL, I. M. & DUNN, P. H. (1957). Entomophthoraceous fungi parasitic on the spotted alfalfa aphid. *Hilgardia* **27**, 159–181.
- HODDINOTT, J. & OLSEN, O. A. (1972). Study of the carbohydrates in cell walls of some species of the Entomophthorales. *Canadian Journal of Botany* **50**, 1675–1679.
- KARLSSON, K. A., SAMUELSSON, B. E. & STEEN, G. O. (1973). The sphingolipid composition of bovine kidney cortex medulla and papilla. *Biochimica et biophysica acta* **316**, 317–335.
- KING, D. S. (1976). Systematics of *Conidiobolus* (Entomophthorales) using numerical taxonomy. II. Taxonomic considerations. *Canadian Journal of Botany* **54**, 1285–1296.
- KING, D. S. (1977). Systematics of *Conidiobolus* (Entomophthorales) using numerical taxonomy. III. Descriptions of recognized species. *Canadian Journal of Botany* **55**, 718–729.
- LATGÉ, J. P. (1980). Sporulation de *Entomophthora obscura* Hall & Dunn en culture liquide. *Canadian Journal of Microbiology* (in the Press).
- LATGÉ, J. P., PERRY, D. F., PAPIEROK, B., COREMANS-PELSENEER, J., REMAUDIÈRE, G. & REISINGER, O. (1978a). Germination des azygospores d'*Entomophthora obscura* Hall & Dunn. *Comptes rendus hebdomadaires de séances de l'Académie des Sciences* **D287**, 943–946.
- LATGÉ, J. P., REMAUDIÈRE, G. & DIAQUIN, M. (1978b). Un nouveau milieu pour la croissance et la sporulation d'Entomophthorales pathogènes d'Aphides. *Annales de Microbiologie (Institut Pasteur)* **129B**, 463–476.
- LATGÉ, J. P., PERRY, D. F., REISINGER, O., PAPIEROK, B. & REMAUDIÈRE, G. (1979). Induction de la formation des spores de résistance d'*Entomophthora obscura* Hall & Dunn. *Comptes rendus hebdomadaires de séances de l'Académie des Sciences* **D288**, 599–602.
- MUMMA, R. O. & BRUSZEWSKI, T. E. (1970). The fatty acids of *Entomophthora coronata*. *Lipids* **5**, 915–920.
- MUMMA, R. O. & BRUSZEWSKI, T. E. (1973). Fungi pathogenic to insects: III. Neutral and polar lipids of *Entomophthora coronata*. *Lipids* **8**, 745–752.
- MUNIER, R. & MACHEBOEUF, M. (1951). Microchromatographie de partage sur papier des alcaloïdes et de diverses bases azotées. *Bulletin de la Société de chimie biologique* **33**, 846–847.
- PRÉVÔT, A. F. & MORDRET, F. X. (1976). Utilisation des colonnes capillaires de verre pour l'analyse des corps gras par chromatographie en phase gazeuse. *Revue française des corps gras* **23**, 409–423.
- REMAUDIÈRE, G., LATGÉ, J. P. & PAPIEROK, B. (1979). Reconsidération taxonomique de *Entomophthora obscura* Hall & Dunn. *Annales de Microbiologie (Institut Pasteur)* **130A**, 151–162.
- SUMNER, J. L. (1970). The fatty acid composition of *Blastocladiella emersonii*. *Canadian Journal of Microbiology* **16**, 1161–1164.
- TYRRELL, D. (1967). The fatty acid composition of 17 *Entomophthora* isolates. *Canadian Journal of Microbiology* **13**, 755–760.
- TYRRELL, D. (1968). The fatty acid composition of some *Entomophthoraceae*. II. The occurrence of branched-chain fatty acids in *Conidiobolus denaesporus* Drechs. *Lipids* **3**, 368–372.
- TYRRELL, D. (1971). The fatty acid composition of

- some *Entomophthoraceae*. III. *Canadian Journal of Microbiology* **17**, 1115–1118.
- TYRRELL, D. & WEATHERSTON, J. (1976). The fatty acid composition of some *Entomophthoraceae*. IV. The occurrence of branched-chain fatty acids in *Conidiobolus* species. *Canadian Journal of Microbiology* **22**, 1058–1060.
- WEETE, J. D. (1974). *Fungal Lipid Biochemistry*. New York & London: Plenum Press.