Fatty Acid and Hydrocarbon Constituents of the Surface and Wall Lipids of Some Fungal Spores

By D. J. FISHER, P. J. HOLLOWAY AND D. V. RICHMOND

Long Ashton Research Station, University of Bristol, Bristol, BS18 9AF

(Accepted for publication 17 March 1972)

SUMMARY

Microelectrophoresis has shown surface lipid to be present on conidia of *Alternaria tenuis*, *Botrytis fabae* and *Neurospora crassa* and on sporangiospores of *Rhizopus stolonifer*. Surface lipid is absent from conidia of *Erysiphe cichoracearum*, *E. graminis*, *Nectria galligena*, *Penicillium expansum* and *Verticillium albo-atrum* and sporangiospores of *Mucor rouxii*. The compositions of the fatty acid and hydrocarbon fractions of the surface and wall lipids from the same organisms are different. The fatty acids are mainly straight-chain and even carbon-numbered. Palmitic and stearic acids predominate. Polyunsaturated acids were absent. Surface hydrocarbons consist almost entirely of *n*-alkanes but wall fractions are more complex. An equal distribution of odd and even carbon-numbered alkanes occurs in both the surface and wall lipid fractions.

INTRODUCTION

Many airborne fungal spores have a water-repellent surface which aids dispersal, prevents desiccation and may provide a barrier to the entry of toxicants. This surface may consist largely of waxy material (Emeis, 1958; Laseter, Weete & Weber, 1968*b*) but there are few reports in which the presence of surface lipid has been confirmed chemically. Crystalline spicules of cyclic peptides together with some lipid occur on the surface of *Pithomyces chartarum* spores (Bertaud, Morice, Russell & Taylor, 1963) but the lipids reported from the surface of various smut spores (Oró, Laseter & Weber, 1966) may be intracellular (Weete, Laseter, Weber, Hess & Stocks, 1969).

Surface lipid can be detected by the increase in electrophoretic mobility produced in the presence of anionic surface-active agents such as sodium dodecyl sulphate (SDS) (James, 1965). By means of this technique surface lipid was detected on conidia of *Neurospora crassa*, *Alternaria tenuis* and *Botrytis fabae* (Somers & Fisher, 1967; Fisher & Richmond, 1969).

In the present study other fungal spores are examined for surface lipid. The fatty acids and hydrocarbons of the surface are compared with those in spore walls.

METHODS

Organisms. *Neurospora crassa* Shear and Dodge wild-type 529a and *Mucor rouxii* (Calmette) Wehm. were obtained from the Commonwealth Mycological Institute, Kew, *Penicillium expansum* Link ex Thom from the Bath University culture collection, and *Rhizopus stolonifer* (Ehrenb. ex Fr.) Lind from the Botany Department, University of Bristol. *Alternaria tenuis* Nees, *Botrytis fabae* Sardiña, *Erysiphe graminis* DC ex Mérat, *Erysiphe cichoracearum* DC and *Nectria galligena* Bres. were obtained from naturally infected material at Long Ashton Research Station.

Composition of culture media and isolation of spore walls. *Alternaria tenuis* was cultured on carrot agar, *Botrytis fabae* on peptone medium (Hislop, 1967), *Nectria galligena* on potato-carrot agar, *Neurospora crassa* on modified Fries medium (Richmond & Somers, 1962), *Mucor rouxii* on tomato juice agar (Haidle & Storck, 1966) and *Penicillium expansum* and *Rhizopus stolonifer* on malt agar. All cultures were grown at 25 °C except *B. fabae* (16 °C).

Spores were harvested after 7 days of growth (Richmond & Somers, 1963). Conidia of *Erysiphe graminis* and *E. cichoracearum* were washed from infected oat seedlings (var. Black Supreme) and marrow plants (var. Green Bush) respectively. The collected spores were freeze-dried. Spore walls were prepared from spores from which the surface lipid had been removed (see below). Dense spore suspensions were shaken with an equal volume of Ballotini (no. 12) beads in a Mickle disintegrator at 4 °C for 15 min (Somers & Fisher, 1967). The centrifuged spore walls were freed from cytoplasmic contaminants by washing ten times with 10 % (w/v) sucrose, five times with 0.9 % (w/v) NaCl and five times with water (Dyke, 1964). The walls were then dried over P₂O₅ under vacuum at room temperature.

Electrophoretic measurements. Mobilities were measured in a laterally mounted rectangular microelectrophoresis cell enclosed in a water bath at 25 °C ± 0.2 °C (Fisher & Richmond, 1969). Spores were suspended in phosphate buffer (pH 7.0, *I*: 0.01) or in buffer containing 1 µM, 10 µM or 100 µM-SDS (Hill, James & Maxted, 1963). These concentrations of SDS were not high enough to alter significantly the pH value, ionic strength or conductivity of the buffer solution. Each mobility was the mean of at least 20 observations; the standard error of the mean was less than 4 %.

Extraction and separation of fungal lipid. Surface lipids were removed from freeze-dried spores (about 2 g) by suspension in light petroleum (boiling range 40 to 60 °C) for 1 min at 25 °C. Wall lipids were extracted with chloroform + methanol (2 + 1, v/v) for 18 h in a Bolton extractor. The suspensions were centrifuged and the supernatant fractions were filtered through ether-extracted cotton wool. The solvents were removed in a rotary evaporator at 40 °C. Lipids were separated into fatty acid and hydrocarbon fractions by thin-layer chromatography (t.l.c.) on silica gel G (Merck Ag., Darmstadt, Germany) with light petroleum (40 to 60 °C) + diethylether + acetic acid (90 + 10 + 1, v/v) as solvent (Malins & Mangold, 1960). The fractions were detected by spraying the plate with chlorosulphonic acid + acetic acid (1 + 2, v/v) and charring in an oven at 200 °C (Krebs, Heusser & Wimmer, 1969). Fatty acids were methylated by the addition of freshly prepared diazomethane in ether before gas-liquid chromatography (g.l.c.).

Gas-liquid chromatography (g.l.c.). A Hewlett-Packard 5750 gas chromatograph fitted with dual columns and flame-ionization detectors and 0 to 1 mV recorder was used. Retention data were determined by means of both OV210 (3 % loading) and E301 (5 % or 8 % loading) on 80- to 100-mesh Chromosorb W-HP. The chromatographic columns were of stainless steel 2 mm internal diameter and 1.8 m in length. The carrier gas was nitrogen, flow rate 40 ml/min (70 p.s.i.). The detectors were maintained at 300 °C and the injection inlet at 250 °C. Analyses were made with temperature programming at a rate of 2 °C per min from 150 to 300 °C (E301) and 100 to 230 °C (OV210). The principal peaks were identified by comparison of retention times with those of known standards. Minor constituents were identified by use of a plot of the log of the retention time versus carbon number. Relative peak areas were measured from the chart by the triangulation method.

Table 1. *Effect of sodium dodecyl sulphate (SDS) on the electrophoretic mobility of spores and isolated spore walls*

Suspension medium: phosphate buffer solution (pH 7.0, I: 0.01)

Material	Electrophoretic mobility, $10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ S}^{-1}$	Percentage increase in negative mobility. Concentration of SDS (μM)		
		1	10	100
Intact spores				
<i>Alternaria tenuis</i> *	-2.62	15	16	24
<i>Botrytis fabae</i>	-1.54	5	6	15
<i>Erysiphe cichoracearum</i>	-0.80	0	0	0
<i>E. graminis</i>	-3.54	0	1	2
<i>Mucor rouxii</i>	-1.72	6	6	4
<i>Nectria galligena</i>	-3.75	0	0	0
<i>Neurospora crassa</i>	-0.45	7	9	24
<i>Penicillium expansum</i> *	-1.54	17	14	14
<i>Rhizopus stolonifer</i>	-1.78	3	5	33
<i>Verticillium albo-atrum</i>	-1.27	0	0	0
Spore walls				
<i>Alternaria tenuis</i>	-1.55	2	5	112
<i>Botrytis fabae</i> *	-1.41	3	6	9
<i>Neurospora crassa</i> †	-0.69	—	78	130
<i>Penicillium expansum</i> *	-1.52	—	3	3

* Recalculated from Fisher & Richmond (1969).

† Recalculated from Somers & Fisher (1967).

— Not determined.

Table 2. *Surface and wall lipids of some fungal spores*

Spores were grown and harvested as described in the text. Surface lipid was removed from freeze-dried spores by suspension in light petroleum (boiling range 40 to 60 °C) for 1 min at 25 °C. Wall lipids were extracted from purified cell walls free of surface lipid (for details see text) with chloroform + methanol (2 + 1, v/v) for 18 h in a Bolton extractor. Fatty acids were determined by extraction from preparative t.l.c. plates with chloroform + methanol.

Organism	Surface lipid as percentage spore dry weight	Wall lipid as percentage wall dry weight	Fatty acid as percentage wall lipid
<i>Alternaria tenuis</i>	0.15	1.67	18
<i>Botrytis fabae</i>	0.19	5.08	31
<i>Neurospora crassa</i>	0.17	8.95	6
<i>Penicillium expansum</i>	Not detected	1.10	31
<i>Rhizopus stolonifer</i>	0.20	3.97	12

RESULTS

Electrokinetic measurements. The electrophoretic mobility of spores of *Alternaria tenuis*, *Botrytis fabae*, *Neurospora crassa* and *Rhizopus stolonifer* rose progressively in increasing concentrations of SDS, indicating the presence of some surface lipid (Table 1). The mobility of *Penicillium expansum* conidia in 1 μM -SDS also increased but the absence of any further rise at higher SDS concentrations indicated the probable absence of surface lipid. The mobilities of spores of other species were unaffected by the presence of SDS. Mobilities of isolated spore walls confirmed the results obtained with whole spores (Table 1).

Analysis of lipid fractions. Only small amounts of surface lipid were present on freeze-dried spores (Table 2) and lipid was absent from surfaces of *Penicillium expansum* conidia,

Table 3 *Relative composition of surface and wall fatty acids*

The lipid extracted from surface and walls of fungal spores (see text for details of culture media and extraction procedures) was esterified with diazomethane, separated by t.l.c. and analysed by g.l.c. The percentage (w/w) of each fatty acid was calculated from peak areas.

Fatty acid	Percentage of total fatty acids									
	<i>Rhizopus stolonifer</i>		<i>Alternaria tenuis</i>		<i>Botrytis fabae</i>		<i>Neurospora crassa</i>		<i>Penicillium expansum</i>	
	Surface	Wall	Surface	Wall	Surface	Wall	Surface	Wall	Surface	Wall
12:1	—	—	0.4	—	—	—	—	—	—	—
12:0	3.1	5.3	2.2	2.8	3.1	4.7	3.1	1.4	1.4	—
13:1	t	—	0.3	—	0.8	—	0.8	—	—	—
13:0	t	—	t	—	0.4	—	0.5	—	—	—
14:1	1.6	—	1.3	—	3.3	0.4	t	1.3	2.7	—
14:0	8.5	6.0	8.4	4.9	8.8	6.3	10.3	4.3	2.0	—
?	t	—	0.4	—	0.8	—	1.1	—	—	—
15:1	1.0	—	1.3	1.4	1.2	0.4	1.2	0.9	—	—
15:0	1.8	3.0	1.7	4.6	1.5	3.9	2.3	2.4	2.3	—
?	3.8	—	3.2	—	5.2	—	1.1	—	—	—
16:1	3.6	—	2.5	5.3	5.5	1.2	—	1.8	t	—
16:0	41.3	33.3	28.7	41.7	27.9	22.7	40.3	47.3	30.6	—
?	2.1	—	1.5	—	1.8	—	3.2	—	—	—
17:1	2.5	3.0	1.8	—	2.6	3.3	2.7	2.8	0.8	—
17:0	2.7	—	2.4	4.9	1.3	5.6	1.6	6.5	1.7	—
?	—	—	—	7.4	—	—	—	t	1.7	—
18:1	2.7	12.0	3.0	4.9	2.1	9.6	t	4.6	38.7	—
18:0	14.9	18.3	14.8	22.2	11.2	10.8	17.5	13.5	11.5	—
19:1	—	6.0	—	—	—	t	—	—	1.0	—
19:0	2.1	8.0	2.6	—	1.6	t	1.4	—	—	—
20:1	—	—	1.5	—	3.1	—	2.2	4.3	—	—
20:0	2.2	1.7	5.5	—	—	1.4	1.5	2.5	—	—
21:1	2.7	—	1.5	—	—	—	—	—	—	—
21:0	t	—	1.0	—	2.2	1.3	1.3	2.0	—	—
?	—	—	0.9	—	—	—	—	—	—	—
22:1	—	1.3	1.0	—	2.7	—	0.7	1.4	2.1	—
22:0	3.4	2.0	6.2	—	1.1	—	2.8	1.4	2.5	—
23:1	—	—	—	—	—	8.6	—	—	—	—
23:0	—	—	1.8	—	2.1	16.3	1.6	1.6	—	—
24:1	—	—	—	—	—	—	1.6	—	—	—
24:0	t	—	2.0	—	1.4	3.5	1.2	—	1.1	—
25:1	—	—	t	—	1.2	—	—	—	—	—
25:0	—	—	1.3	—	3.4	—	—	—	—	—
26:1	—	—	0.8	—	1.6	—	—	—	—	—
26:0	—	—	t	—	2.1	—	—	—	—	—

— = Not detected, t = trace detected, ? = unidentified fatty acids.

confirming the electrophoretic results. Fatty acids, hydrocarbons and phospholipids were present in both surface and wall extracts. The surface extract consisted mainly of fatty acids. The amounts of fatty acids in the walls of different species varied considerably. Walls of *P. expansum* contained only a small amount of lipid, comparable to the amount in mycelial walls of *P. notatum* (Applegarth, 1967).

Surface and wall fatty acid composition. From 10 to 18 different fatty acids were found in the spore-wall lipids and 22 to 31 in the surface lipids (Table 3). The total lipids from fungal spores or mycelium usually contain far fewer fatty acids (Shaw, 1966). The surface acids were mainly saturated with even numbers of carbon atoms. Myristic, palmitic and stearic

Table 4. *Relative composition of surface and wall hydrocarbons*

The lipid extracted from surface and walls of fungal spores (see text for details of culture media and extraction procedures) was separated by t.l.c. and analysed by g.l.c. The percentage (w/w) of each hydrocarbon was calculated from peak areas.

Hydrocarbon	Percentage of total hydrocarbons								
	<i>Rhizopus stolonifer</i>		<i>Alternaria tenuis</i>		<i>Botrytis fabae</i>		<i>Neurospora crassa</i>		<i>Penicillium expansum</i>
	Surface	Wall	Surface	Wall	Surface	Wall	Surface	Wall	Wall
<i>n</i> -C ₁₆	—	0.5	—	1.0	—	0.6	—	—	t
?	—	0.5	—	—	—	1.3	—	t	—
<i>n</i> -C ₁₇	—	3.3	—	2.1	—	2.1	—	3.2	—
?	—	1.6	—	1.5	—	0.8	—	0.7	3.6
?	—	—	—	t	—	—	—	0.7	—
<i>n</i> -C ₁₈	t	5.5	3.2	2.0	1.1	1.2	—	3.2	3.3
?	—	3.3	—	1.0	—	2.1	—	2.8	6.7
<i>n</i> -C ₁₉	0.2	2.5	11.2	3.1	2.3	1.7	8.1	2.1	2.7
?	—	1.1	—	4.6	—	2.9	—	2.8	1.8
?	—	—	—	—	—	0.3	—	—	—
?	—	—	—	—	—	—	1.9	—	—
<i>n</i> -C ₂₀	5.2	1.6	14.5	2.0	13.6	0.8	28.1	2.1	2.0
?	—	5.7	—	6.1	—	6.7	—	10.6	8.9
<i>n</i> -C ₂₁	12.4	1.4	12.1	4.6	22.7	2.1	21.0	2.1	1.8
?	—	—	—	—	—	16.3	—	—	—
?	—	7.7	—	13.8	—	12.9	—	0.5	9.4
<i>n</i> -C ₂₂	15.5	2.5	4.8	2.6	19.3	2.0	10.9	11.2	8.0
?	—	13.1	2.4	26.8	—	—	—	2.4	21.4
<i>n</i> -C ₂₃	14.5	4.9	8.0	4.6	11.4	3.4	6.2	4.2	t
?	—	t	—	1.0	—	5.5	—	0.9	2.7
<i>n</i> -C ₂₄	15.6	6.6	6.4	14.3	10.2	7.3	5.0	9.3	8.9
<i>n</i> -C ₂₅	13.1	4.9	10.0	2.3	5.7	2.5	4.4	3.5	2.7
<i>n</i> -C ₂₆	8.3	6.8	8.0	3.8	4.5	4.2	3.1	5.8	2.7
<i>n</i> -C ₂₇	6.2	4.9	1.6	1.5	2.3	3.9	3.1	5.6	2.7
?	—	—	—	—	—	2.1	—	6.9	—
<i>n</i> -C ₂₈	4.2	2.7	8.0	1.0	6.8	3.4	3.9	0.7	—
?	—	—	—	—	—	0.6	—	—	—
<i>n</i> -C ₂₉	2.6	12.3	4.8	—	t	4.9	1.9	7.5	2.7
?	—	—	—	—	—	2.2	—	2.9	—
<i>n</i> -C ₃₀	1.3	3.3	2.4	—	—	2.8	1.3	1.8	4.7
<i>n</i> -C ₃₁	0.6	3.3	2.4	—	—	2.1	0.7	5.2	1.3
?	—	—	—	—	—	0.8	—	t	—
<i>n</i> -C ₃₂	0.3	—	—	—	—	0.6	0.5	1.4	2.0
Carbon preference index	0.99		1.06		0.80		0.86		

— = Not detected, t = trace detected, ? = unidentified hydrocarbon.

were the most abundant acids. Although spore walls had similar fatty acid components to surface fractions there were numerous quantitative differences. The C_{23:0} and C_{23:1} acids were major components of *Botrytis fabae* conidial walls. Walls of *Penicillium expansum* contained a relatively high proportion of unsaturated acids; oleic was the main acid present.

Surface and wall hydrocarbon components. Surface hydrocarbons consisted almost entirely of *n*-alkanes which ranged from C₁₈ to C₃₂ (Table 4). Carbon-preference ratios (ratio of C odd to C even compounds) approximated unity. Surface hydrocarbons from *Alternaria tenuis* showed a random distribution with major peaks corresponding to C₁₉, C₂₀, C₂₁, C₂₃, C₂₅, C₂₆ and C₂₈. Hydrocarbons from the surface of *Botrytis fabae* and *Neurospora crassa*

showed maxima at the lower end of this series with main peaks at C_{20} , C_{21} and C_{22} . *Rhizopus stolonifer* had a maximum distribution of surface alkanes in the C_{21} – C_{25} range. Wall alkanes ranged from C_{16} to C_{32} . The distribution patterns of *n*-alkanes in the walls (Table 4) differed widely from those of the corresponding surface fractions and the major peaks did not coincide.

DISCUSSION

Various extraction procedures have been used by other workers to remove surface lipid from fungal spores. Bertaud *et al.* (1963) extracted fatty acids from the surface of *Pithomyces chartarum* spores by 10 successive benzene washes at room temperature each of 1 min, while Laseter *et al.* 1968*a, b* extracted spores of *Ustilago maydis* and three species of *Tilletia* with chloroform + benzene (3 + 1) for 30 min at 50 °C. Although teliospores of *Tilletia* germinate after the 50 °C extraction with chloroform + benzene (Trione & Te May Ching, 1971) there remains the possibility that some wall or intracellular lipid may have been extracted. Our less rigorous extraction procedure is similar to that employed by Kaneda (1969) in studies of surface waxes on leaves. The usefulness of the electrophoretic technique for detecting surface lipid was demonstrated when lipid was easily removed from the surfaces of those spores which showed a progressive increase in mobility in the presence of SDS.

Surface and wall lipids were mainly saturated. Polyunsaturated acids, such as linoleic which is almost universally present in the cellular lipids of fungi (Shaw, 1966), were not detected in any of the surface or wall lipids examined. This suggests that the spore walls were free from cytoplasmic contamination. Fatty acids in cell walls of the yeast *Nadsonia elongata* are also mainly saturated while unsaturated acids predominate in whole cells (Dyke, 1964). Similarly, large amounts of palmitic acid occur in teliospore walls of *Tilletia controversa* (Laseter *et al.* 1968*a*), while linoleic is the most abundant acid inside the spores (Trione & Te May Ching, 1971). The presence of C_{23} and $C_{23:1}$ acids as major components of *Botrytis fabae* conidial walls is interesting as these acids have not previously been reported in any quantity from living organisms.

The surface lipids had an almost equal distribution of alkanes with odd and even numbers of carbon atoms. A similar alkane distribution occurs in the total mycelial lipids from *Debaryomyces hansenii* (Merdinger & Devine, 1965), *Trichoderma viride*, *Penicillium* sp., *Aspergillus* sp. and *Saccharomyces* sp. (Jones, 1969). In contrast the major peaks in the surface alkanes of smut spores are of the 'odd' series (Oró *et al.* 1966; Laseter *et al.* 1968*a, b*).

In contrast with the surface fractions, a considerable proportion of the wall hydrocarbons including some of the major peaks were not identified, indicating a high degree of unsaturation or branching. Carbon-preference ratios of hydrocarbons in wall fractions cannot therefore be determined accurately. Wall hydrocarbons ranged from C_{16} to C_{32} . The distribution patterns of hydrocarbons in the walls (Table 4) differed widely from the patterns of the corresponding surface fractions and the major peaks did not coincide. In contrast to the fatty acids a larger number of hydrocarbons were found in the wall lipids (21 to 30) than in the surface lipids (12 to 15).

Stránský, Streibl & Herout (1967) found that the ratio of odd to even carbon-numbered alkanes was approximately unity in primitive plants, whereas in cuticular waxes of higher plants the odd number homologues predominated. Herbin & Robins (1969) suggested that in higher plants where *n*-alkanes form only a small proportion of the leaf cuticular wax the dominance of odd over even carbon number chain lengths tends to disappear. As the hydrocarbons in the total leaf lipids have a flat distribution of chain lengths, Herbin & Robins (1969) suggest that the differences which Stránský *et al.* (1967) ascribe to plants of higher and

lower evolutionary levels may be more a reflection of the qualitative differences between internal and external lipids: where 'wax' has been obtained by extraction of total lipids the external lipids will be quantitatively more significant in higher plants. Our results for the surface and wall hydrocarbons of fungal spores support the views of Stránský *et al.* (1967) that in lower plants the ratio of odd to even carbon-numbered alkanes approaches unity.

The absence of any correlation between fatty acids and alkanes makes it unlikely that hydrocarbons are formed by the direct decarboxylation of fatty acids. Oró, Tornabene, Noonan & Gelpi (1967) found a similar lack of relationship in a number of algae and bacteria.

The spores on which surface lipid was found are airborne and difficult to wet. The absence of surface lipid from spores which are water-dispersed, such as *Verticillium albo-atrum* and *Nectria galligena*, is not surprising. Other spores, such as *Erysiphe cichoraceorum*, *E. graminis* and *Penicillium expansum* which lack surface lipids, are nevertheless hydrophobic. Douglas, Ruddick & Williams (1970) found a similar lack of correlation between water-repellent properties and the presence of surface lipid in actinomycete spores. Conidia of *Penicillium* (Hess, Sassen & Remsen, 1968) and *Aspergillus* (Hess & Stocks, 1969) and basidiospores of various gasteromycetes (Bronchart & Demoulin, 1971) are covered with a surface rodlet pattern. Wetting of such spores might be prevented by the trapping of air in surface invaginations. Thus the physical conformation of the surface may be sufficient to account for hydrophobic properties (Fisher & Richmond, 1970). Similar factors have been shown to influence the wetting of the leaves of higher plants (Holloway, 1970).

REFERENCES

- APPLEGARTH, D. A. (1967). The cell wall of *Penicillium notatum*. *Archives of Biochemistry and Biophysics* **120**, 471-478.
- BERTAUD, W. S., MORICE, I. M., RUSSELL, D. W. & TAYLOR, A. (1963). The spore surface in *Pithomyces chartarum*. *Journal of General Microbiology* **32**, 385-395.
- BRONCHART, R. & DEMOULIN, V. (1971). Ultrastructure de la paroi des basidiospores de *Lycoperdon* et de *Scleroderma* (Gastéromycètes) comparée à celle de quelques autres spores de champignons. *Protoplasma* **72**, 179-189.
- DOUGLAS, H. W., RUDDICK, S. M. & WILLIAMS, S. T. (1970). A study of the electrokinetic properties of some Actinomycete spores. *Journal of General Microbiology* **63**, 289-295.
- DYKE, K. G. H. (1964). The chemical composition of the cell wall of the yeast *Nadsonia elongata*. *Biochimica et biophysica acta* **82**, 374-384.
- EMEIS, C. C. (1958). Die Gewinnung von Askosporenmassen von Saccharomyces-Arten auf Grund besonderer Oberflächeneigenschaften. *Naturwissenschaften* **45**, 441.
- FISHER, D. J. & RICHMOND, D. V. (1969). The electrokinetic properties of some fungal spores. *Journal of General Microbiology* **57**, 51-60.
- FISHER, D. J. & RICHMOND, D. V. (1970). The electrophoretic properties and some surface components of *Penicillium* conidia. *Journal of General Microbiology* **64**, 205-214.
- HAIDLE, C. W. & STORCK, R. (1966). Control of dimorphism in *Mucor rouxii*. *Journal of Bacteriology* **92**, 1236-1244.
- HERBIN, G. A. & ROBINS, P. A. (1969). Patterns of variation and development in leaf wax alkanes. *Phytochemistry* **8**, 1985-1998.
- HESS, W. M., SASSEN, M. M. A. & REMSEN, G. C. (1968). Surface characteristics of *Penicillium* conidia. *Mycologia* **60**, 290-303.
- HESS, W. M. & STOCKS, D. L. (1969). Surface characteristics of *Aspergillus* conidia. *Mycologia* **61**, 560-571.
- HILL, M. J., JAMES, A. M. & MAXTED, W. R. (1963). Some physical investigations of the behaviour of bacterial surfaces. X. The occurrence of lipid in the streptococcal cell wall. *Biochimica et biophysica acta* **75**, 414-424.
- HISLOP, E. C. (1967). Observations on the vapour phase activity of some foliage fungicides. *Annals of Applied Biology* **60**, 265-279.
- HOLLOWAY, P. J. (1970). Surface factors affecting the wetting of leaves. *Pesticide Science* **1**, 156-163.

- JAMES, A. M. (1965). Surface-active agents in microbiology. In *Surface Activity and the Microbial Cell*, pp. 3–22. Monograph No. 19. London: Society of the Chemical Industry.
- JONES, J. G. (1969). Studies on lipids of soil micro-organisms with particular reference to hydrocarbons. *Journal of General Microbiology* **59**, 145–152.
- KANEDA, T. (1969). Hydrocarbons in spinach: two distinctive carbon ranges of aliphatic hydrocarbons. *Phytochemistry* **8**, 2039–2044.
- KREBS, K. G., HEUSSER, D. & WIMMER, H. (1969). Spray Reagents. In *Thin-layer Chromatography*, pp. 854–908. Edited by E. Stahl. London: George Allen & Unwin.
- LASETER, J. L., HESS, W. M., WEETE, J. D., STOCKS, D. L. & WEBER, D. J. (1968*a*). Chemotaxonomic and ultrastructural studies on three species of *Tilletia* occurring on wheat. *Canadian Journal of Microbiology* **14**, 1149–1154.
- LASETER, J. L., WEETE, J. & WEBER, D. J. (1968*b*). Alkanes, fatty acid methyl esters, and free fatty acids in surface wax of *Ustilago maydis*. *Phytochemistry* **7**, 1177–1181.
- MALINS, D. C. & MANGOLD, H. K. (1960). Fractionation of fats, oils and waxes on thin layers of silicic acid. *Journal of the American Oil Chemists Society* **37**, 383–384.
- MERDINGER, E. & DEVINE, E. M. JUN. (1965). Lipids of *Debaryomyces hansenii*. *Journal of Bacteriology* **89**, 1488–1493.
- ORÓ, J., LASETER, J. L. & WEBER, D. (1966). Alkanes in fungal spores. *Science, New York* **154**, 399–400.
- ORÓ, J., TORNABENE, T. G., NOONER, D. W. & GELPI, E. (1967). Aliphatic hydrocarbons and fatty acids of some marine and freshwater micro-organisms. *Journal of Bacteriology* **93**, 1811–1818.
- RICHMOND, D. V. & SOMERS, E. (1962). Studies on the fungitoxicity of captan. I. The structural specificity of captan and six *n*-trichloromethylthio analogues. *Annals of Applied Biology* **50**, 33–43.
- RICHMOND, D. V. & SOMERS, E. (1963). Studies on the fungitoxicity of captan. III. Relation between the sulphhydryl content of fungal spores and their uptake of captan. *Annals of Applied Biology* **52**, 327–336.
- SHAW, R. (1966). The unsaturated fatty acids of microorganisms. *Advances in Lipid Research* **4**, 107–174.
- SOMERS, E. & FISHER, D. J. (1967). Effect of dodine acetate on the electrophoretic mobility of *Neurospora crassa* conidia. *Journal of General Microbiology* **48**, 147–154.
- STRÁNSKÝ, K., STREIBL, M. & HEROUT, V. (1967). Distribution of wax hydrocarbons in plants at different evolutionary levels. *Collection of Czechoslovak Chemical Communications* (English edn) **32**, 3212–3220.
- TRIONE, E. J. & TE MAY CHING (1971). Fatty acids in teliospores and mycelium of the dwarf bunt fungus *Tilletia controversa*. *Phytochemistry* **10**, 227–229.
- WEETE, J. D., LASETER, J., WEBER, D. J., HESS, W. M. & STOCKS, D. L. (1969). Hydrocarbons, fatty acids, and ultrastructure of smut spores. *Phytopathology* **59**, 545–548.