Phytochemistry, 1973, Vol. 12, pp. 383 to 385. Pergamon Press. Printed in England.

FATTY ACIDS OF FUNGI MUTUALISTIC WITH *XYLEBORUS FERRUGINEUS*

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(*Revised Received* 22 *August* 1972. *Accepted* 1 *September* 1972)

Key Word Index—*Xyleborus ferrugineus*; Ambrosia beetle; *Fungi Imperfecti*; mutualistic ambrosial fungi; symbiosis; fatty acid composition of neutral lipids.

Abstract—Neutral lipids of ambrosial fungi, nutritionally important to development of *Xyleborus ferrugineus* beetle, were analyzed for fatty acid composition. Predominant components were 16:0, 18:0, 18:1 and 18:2 in all three mutualistic fungi, *Fusarium solani*, *Cephalosporium* sp. and *Graphium* sp. *F. solani* had nearly twice the total content of the other two species; age of mycelia did not significantly affect the qualitative composition and total yield.

INTRODUCTION

CONTINUING studies on the ambrosia beetle, *Xyleborus ferrugineus*, have shown its nutritional dependency on three mutualistic fungi, *Fusarium solani*, *Cephalosporium* sp. and *Graphium* sp.[1-5] Because of the nutritional importance of the fungal neutral lipids to the beetle, we present here the results of a comparative study of the neutral lipid fatty acids of the three ambrosial fungi.

TABLE 1. LIPID CONTENT* OF AMBROSIAL FUNGI†

Lipid	Ambrosial fungi *Fusarium solani*	*Cephalosporium* sp.	*Graphium* sp.
Fatty acids in neutral lipids	10·93	5·33	6·33
Sterols	0·24	0·15	0·12
Other neutral lipids	1·07	1·40	1·75
Polar lipids	2·80	3·60	4·53
Total lipids	15·04	10·48	12·73

* % of dry fungal weight (mean of three replications).
† Cultured in Neutral-Dox-Yeast medium for 15 days; see Ref. 13.

RESULTS

Fatty acids in the neutral lipids constituted the major lipid component of the ambrosial fungi; *F. solani* had about twice the content of *Cephalosporium* sp. and *Graphium* sp. (Table 1). GLC of methyl esters of the neutral lipid fatty acids revealed the presence of

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14:0, 16:0, 18:0, 18:1, 18:2, 18:3 and 20:0 or 22:0 in all three species. Major components were 16:0, 18:2, 18:1 and 18:0. Although certain similarities in the relative proportions of individual fatty acids among the fungi are evidenced in Table 2, differences in 14:0 and 18:1 in *F solani* as compared to the other two species existed. Also, a trace of 20:0 was detected in *F. solani*, but each of the other two species had a small portion of 22:0.

The composition and quantity of fatty acids were generally unaffected by the period of incubation of the fungi. The same components were present in *F. solani* incubated for 5, 10 or 15 days (Table 2), and the total content of 11% was unchanged with age. However, some changes in the level of individual acids did occur with age. There were decreases in 18:1 (32 → 24%) and 18:0 (21 → 19%), but increases in 18:2 (21 → 26%), 16:0 (21 → 25%) and in 14:0 (1 → 3%). The 18:3 level remained around 4%.

TABLE 2. FATTY ACIDS* FROM NEUTRAL LIPIDS OF AMBROSIAL FUNGI†

	Ambrosial fungi (mycelial age in days)				
	Fusarium solani			*Cephalosporium* sp.	*Graphium* sp.
Fatty acid	(5)	(10)	(15)	(15)	(15)
14:0	1·35	0·90	3·32	8·07	10·30
16:0	20·96	19·87	24·60	24·03	26·50
18:0	20·50	19·97	18·58	16·27	15·07
18:1	31·79	30·44	23.72	19·20	19·93
18:2	21·14	25·29	25·61	27·00	23·89
18:3	4·25	3·52	4·20	3·20	2·80
20:0	Trace	Trace	Trace	0·00	0·00
22:0	0·00	0·00	0·00	2·23	1·53
Total	99·99	99·99	100·03	100·00	100·02

* % of total neutral lipid fatty acids (mean of three replications).
† Cultured in Neutral-Dox-Yeast medium; see Ref. 13.

DISCUSSION

The fatty acid compositions of neutral- and total-lipid fractions were shown to be similar in Phycomycetes,[6] but different in Basidiomycetes;[7] and age of culture did not significantly change the composition in *Rhizopus arrhizus*.[6] Our study of the three ambrosial fungi showed that 16:0, 18:2, 18:1 and 18:0 acids were the predominant components in all three species. These compositions were similar to those previously reported in a number of *Fungi Imperfecti*[8] except for a higher 18:0 level in the ambrosial fungi. A small amount of 16:1 (2·7%) was reported in *Cephalosporium subverticullatum*,[9] but this was not detected in our three ambrosial fungi. Age of mycelia up to 15 days had no quantitative effect on the total content in *F. solani*; the 11% of dry fungal wt compared favorably with the 10% reported in *Penicillium griseofulvum*[10] and 9·8% in *Penicillium pulvillorum*.[11] *Cephalosporium*

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sp. (5·3%) and *Graphium* sp. (6·3%) had about half the amount in *F. solani*, but all the three ambrosial fungi had higher amounts than that reported in other *Fungi Imperfecti: Pithomyces chartarum*, *Stemphylium dendriticum* and *Cylindrocarpon radicicola*,[12] and in a number of yeast-like forms.[8]

EXPERIMENTAL

The technique for axenic culture of the ambrosial fungi and their lipid extraction was similar to that described earlier.[13] 5 ml portions of the effluent $CHCl_3$ were collected by means of an automatic fraction collector and the contents further investigated by TLC on silica gel G (Merck) plates, developed in light petrol.–Et_2O–HOAc (8:2:1). Spots were visualized by spraying with 50% H_2SO_4 and 5% phosphomolybdic acid in EtOH. All portions containing fatty acids were combined, evaporated to dryness, weighed and analyzed by GLC.

GLC. Methyl esters of the fatty acids were prepared with fresh stocks of BF_3–MeOH reagent[14] and separated on 6% DEGS coated on acid-washed DMCS, 80–100 mesh Chromosorb W (Applied Science Labs, Inc.) isothermally at 160 or 180°. N_2 flow rate was 30 ml/min. Identification was by comparison against authentic knowns using a GC with dual flame ionization detectors and 183 cm × 2 mm i.d. coiled stainless steel columns. The relative amounts of fatty acids were calculated from peak areas.[15]

Acknowledgements—Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison; and in part by funds from the Schoenleber Foundation, Milwaukee; and from the Wisconsin Department of Natural Resources.

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