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SHORT COMMUNICATION

FATTY ACIDS IN TELIOSPORES AND MYCELIUM OF THE DWARF BUNT FUNGUS, *TILLETIA CONTROVERSA**

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Abstract—Teliospores of the dwarf bunt fungus contain about 35% lipids; free fatty acids accounting for 52% and bound fatty acids 26% of the total lipids. Lipids make up 5·8% of the dry weight of the mycelium, with free fatty acids accounting for 48% and bound fatty acids 14% of the total lipids. The major fatty acids in both the teliospores and the mycelium are C18:2, C18:1 and C16:0.

INTRODUCTION

LIPID components appear to be the chief storage metabolites and are thought to be the primary substrates used during germination of *Tilletia* teliospores. *T. controversa* Kühn teliospores have an unusual requirement of 3–6 weeks at 5° before they can be induced to germinate. Many attempts to shorten this long germination time by the application of exogenous substrates and germination "triggers" have been unsuccessful. It was hypothesized that this inability of dwarf bunt teliospores to germinate under conditions normal for common bunt teliospores may be reflected in the fatty acid patterns of the dormant teliospores compared to the patterns in the vegetative hyphae.

Tulloch and Ledingham[1] studied the total fatty acids in teliospores of *T. foetida*. Laseter *et al.*[2] analyzed the lipids found in the spore walls of *T. caries*, *T. foetida* and *T. controversa*. Graham[3] found pectins, hemicellulose, proteins, melanins and lipids in the complex spore wall of *T. controversa*.

RESULTS AND DISCUSSION

Our results indicate that the total lipids make up 35% of the dry weight of dwarf bunt teliospores and 5·8% of the dry weight of the mycelium. In the teliospores, free fatty acids account for 52% and bound fatty acids 26% of the total lipids. In the mycelium, free fatty acids account for 48% and bound fatty acids 14% of the total lipids. The distribution of the individual fatty acids as a percentage of the total fatty acids is shown in Table 1.

* Cooperative investigations with Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture. Technical Paper No. 2854 of the Oregon Agricultural Experiment Station.

[1] A. P. TULLOCH and G. A. LEDINGHAM, *Can. J. Microbiol.* **6**, 245 (1960).

[2] J. L. LASETER, W. M. HESS, J. D. WEETE, D. L. STOCKS and D. J. WEBER, *Can. J. Microbiol.* **14**, 1149 (1968).

[3] S. O. GRAHAM, *Mycologia* **52**, 97 (1960).

TABLE 1. PERCENTAGE DISTRIBUTION OF THE MAJOR FATTY ACIDS IN TELIOSPORES AND MYCELIUM OF *T. controversa*, RACE D-3

	Teliospores		Mycelium	
Carbon skeleton	Free	Bound	Free	Bound
6:0	6·3	7·6	3·0	4·3
8:0	4·4	8·8	2·1	12·2
10:0	0·2	0·7	0·2	0·7
12:0	0·9	2·9	0·8	7·7
14:0	0·9	1·8	1·7	2·6
15:0			1·7	1·0
16:0	16·0	13·1	9·2	6·8
16:1	2·1	1·8	2·5	2·3
16:2			1·3	
18:0	1·3	1·7	0·2	0·5
18:1	14·4	17·4	10·6	14·7
18:2	49·4	43·8	66·2	46·5
18:3	3·2			
% Unsaturated	69·1	63·0	79·3	63·5

The fatty acids found in dwarf bunt mycelium represent a much smaller percentage of the dry weight than the fatty acids of the teliospores. However, the percentage distribution of fatty acids in the mycelium is very similar to that of the teliospores. The fatty acid distribution in the dwarf bunt teliospores is also similar to the distribution reported by Tulloch and Ledingham[1] for teliospores of common bunt, *Tilletia foetida*, which germinate in 3–5 days.

It is very unusual that free fatty acids account for such a high percentage of the total lipids, approximately 50% in both the teliospores and mycelium of the dwarf bunt fungus. In contrast, free fatty acids make up only 4% of the total spore lipids in wheat stem rust uredospores which germinate readily.[4] In view of the recent report[5] that nonanoic acid is a stable inhibitor of fungus spore germination, it is possible that the high concentration of free fatty acids in dwarf bunt teliospores may be causally related to their extreme dormant nature.

Our results on fatty acid composition are quite different from the values reported by Laseter *et al.*[2] for teliospores of three species of *Tilletia*, including *T. controversa*. Their extraction procedure, however, removed the lipids from only the cell walls as evidenced by the fact that teliospores show excellent germination after that extraction procedure. The results of Laseter *et al.*[2] in relation to our own based on broken teliospores, suggest that C16:0 is concentrated in the cell wall whereas C18:2 is the most abundant fatty acid inside the spore.

EXPERIMENTAL

Teliospores of *T. controversa*, race D-3, were obtained from infected Westmont wheat (CI 12930) near Kalispell, Montana through the courtesy of J. A. Hoffmann, USDA, Pullman, Washington. The teliospores were shaken from the broken sori, air dried, and passed through a series of standard sieves to completely

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[5] M. K. GARRETT and P. M. ROBINSON, *Arch. Mikrobiol.* **67**, 370 (1969).

remove host plant residues. The teliospores were homogenized in a Mickle Shaker (colliding glass beads) for 20 min (90–100 per cent rupture). The ruptured spores were then extracted 3 × Et_2O by stirring for 10 min.

The monocaryotic mycelium of dwarf bunt, *T. controversa*, race D-3, was grown in a chemically defined liquid medium[6] in a fermentor. Excellent growth was obtained and the cells were harvested in the log phase of growth (4 days at 22°), with a yield of 30 g (fr. wt.) of cells 1 l. of medium. The mycelium was homogenized in a Virtis blendor with Et_2O, filtered and the cells rehomogenized twice.

The crude lipid extracts of the mycelium and the teliospores were reduced to near dryness by rotary evaporation. The residues were extracted 3 × light petroleum and any insoluble material or aqueous solution discarded. The petroleum extract was dried (N_2) and weighed.

The free fatty acids were removed from the total lipid fraction by washing 3 × 1% Na_2CO_3. The Na_2CO_3 solution was then acidified, extracted with Et_2O and the solvent evaporated with N_2.

The bound fatty acids were saponified with a 10-fold excess of 1 KOH (10g KOH in 90 ml MeOH) by refluxing for 3 hr.[7] The free and bound fatty acids obtained by these procedures were dried (N_2) and the samples weighed.

Methyl esters of the fatty acids were prepared with CH_2N_2[8] and then analyzed in a Beckman GC-2A gas chromatography apparatus. The 3·6 m chromatography column contained 16% diethylene glycol succinate on chromosorb and was operated at 220° with a corrected flow rate of 80 ml helium/min. The chain length of the fatty acids and their degree of unsaturation was deduced from their retention times.

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