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LIPIDS OF OAK GALLS

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Abstract—The nutrient tissues of oak galls accumulate a great amount of lipids. The neutral lipids (essentially triacylglycerol) are the most abundant storage form but some membrane lipids (mainly phospholipids) also occur. However, the galactolipids are very poorly represented. Among the fatty acids, oleic acid is predominant. These data correlate well with the cytological features of gall nutrient cells.

INTRODUCTION

The plant galls induced by Cynipids (Hymenoptera) are the most evolved of the insects cecidia. In the gall-nuts of oak, one or more grubs is completely closed in plant tissues. The first rows of cells around the grub form the nutrient tissue for the grub and it has characteristic cytological and cytochemical properties. Among those, an important accumulation of lipid droplets has often been noticed [1–5]. There is also a great development of the endoplasmic reticulum (ER) profiles, especially smooth ER [4, 6, 7]. Histo- and cytochemical information has been given on the lipid droplets [2, 4] but chemical characterization was not carried out. In this work, data are presented on the lipid accumulation in three 'oak apples' induced by *Biorhiza pallida*, *Neuroterus quercusbaccarum* and *Andricus curvator*.

RESULTS AND DISCUSSION

As shown in Table 1, the fatty acid content of the nutrient tissues of the galls varied from 7.5 to 35 mg/g (0.75–3.5%) fr. wt and this was much higher than that found in normal leaves (1.6 mg/g—0.16%). The fatty acid composition was different in gall tissues and normal leaves; the former contained a greater amount of saturated acids (16:0, 18:0) and oleic acid (18:1ω9) and smaller amounts of linoleic (18:2ω6) and α-linolenic (18:3ω3) acids. This specific modification of the fatty acid composition was observed in the three galls studied, but was slightly less significant in *Neuroterus quercusbaccarum*. No marked variation was observed in *Biorhiza pallida* between the two stages of maturation except a 17% increase of the total fatty acid content. The high percentage of oleic acid still increased and reached 46% for the oldest stage of maturation.

Considering the total quantities of fatty acids/g fr. wt, it could be seen that all fatty acids were more important in the gall tissues, even those which decreased in percentages, such as poly-unsaturated acids.

Neutral lipids were the most abundant class among lipids in the gall tissues, and were essentially triacylglycerols. Diacylglycerol and free fatty acids were less important. Polar lipids were qualitatively analysed

Table 1. Fatty acid composition of lipids from the nutrient tissues of galls and in the normal leaves of oak*

% of total fatty acids	*B.p.* gall 1st stage			*B.p.* gall 2nd stage			*N.q.* gall			*A.c.* gall			Normal oak leaf
	TL	TG	PL	TL	TG	PL	TL	TG	PL	TL	TG	PL	TL
$C_{16:0}$	21.4	30.3	25.6	22.6	30.2	25.0	20.8	28.6	32.0	19.2	26.2	35.7	15.4
$C_{16:1\omega7}$	—	—	—	—	—	—	—	—		—	—	—	4.3
$C_{16:1\omega13t}$	—	—	—	—	—	—	—		—	—	—		1.8
$C_{18:0}$	2.5	2.0	4.6	1.6	2.0	2.5	1.7	4.1	4.0	3.0	3.5	4.8	0.8
$C_{18:1\omega9}$	43.1	51.5	18.6	45.8	48.8	17.5	34.3	30.6	12.0	40.1	48.9	14.3	4.9
$C_{18:2\omega6}$	13.0	6.6	20.9	11.9	10.0	20.0	11.3	10.2	20.0	8.7	6.9	19.0	16.0
$C_{18:3\omega3}$	20.0	9.6	30.3	18.1	9.0	35.0	31.9	26.5	32.0	29.0	14.5	26.2	56.7
Total mg/g fr.wt	30.6	19.8	4.3	35.0	24.8	4.0	7.5	4.9	1.25	18.4	14.5	2.1	1.6

* *B.p.*: *Biorhiza pallida*; *N.q.*: *Neuroterus quercusbaccarum*; *A.c.*: *Andricus curvator*.

$C_{16:0}$: palmitic acid; $C_{16:1\omega7}$: palmitoleic; $C_{16:1\omega13t}$: *trans*-hexadecenoic; $C_{18:0}$: stearic; $C_{18:1\omega9}$: oleic; $C_{18:2\omega6}$: linoleic; $C_{18:3\omega3}$: α-linolenic. TL: total lipids; TG: triacylglycerols; PL: polar lipids.

Table 2. Amounts of lipid classes in nutrient tissues from galls

	*B.p.** gall 1st stage	*B.p.* gall 2nd stage	*N.q.* gall	*A.c.* gall
Triacylglycerols (mg/g fr wt)	20.8	26.1	5.1	15.3
Polar lipids (mg/g fr wt)	5.7	5.2	1.7	2.8

* See Table 1.

and phosphatidylcholine and phosphatidylethanolamine were the most important phospholipids. The galactolipid content was very weak in contrast to the normal tissue of green leaves, known to be rich in these typical chloroplast lipids.

The fatty acid composition of the triacylglycerols (Table 1) reflected their importance among the lipid classes, being very close to the total fatty acid composition. Oleic acid was the major acid, comprising up to 51 % of the fatty acids of triacylglycerol. In polar lipids the fatty acid composition was very different (Table 2): 50% of polyunsaturated fatty acids and only 10–20% of oleic acid. This composition was not as unsaturated as in normal tissue but in fact very different from the neutral lipids of gall tissues. It looked like a normal tissue without the chloroplastic compartment, i.e. poor in α-linolenic acid and related galactolipids.

All the galls studied were not only very rich in storage lipids, but also very rich in membrane lipids because of a 2–5 mg/g fr. wt content of membrane lipids (Table 1). In normal tissues the total lipid content is 1 mg/g fr. wt (including few storage lipids) [8].

This work demonstrates the chemical nature of the storage lipids in gall nutrient tissues. As for seed storage lipids and other lipid rich tissues, neutral lipids as triacylglycerols are the accumulation form of lipids. Oleic acid is always the most important among the fatty acids of the lipid droplets of the tissue. On the other hand a typical accumulation of membrane lipids (especially phospholipids) is shown and correlates very well with the density of ER profiles observed in the nutrient cells [4, 6, 9, 10]. The reversion of the chloroplasts to a proplastid form is well shown by the very low galactolipids and α-linolenic acid levels compared with normal leaf tissue. This membranous accumulation is correlated with a higher polyunsaturated fatty acid percentage in polar lipids than in neutral lipids.

In the gall of *Neuroterus quercusbaccarum*, where the increase in fatty acids was the smallest, the linoleic and the α-linolenic acids were 3 times higher than in normal time. Comparatively the oleic acid was *ca* 30 times higher and 200 times higher for the *Biorhiza pallida* gall (second stage). These modifications suggest a very important transformation of the normal metabolism of leaf cell usually turned toward sugar storage.

EXPERIMENTAL

The 3 galls studied were harvested on *Quercus pedunculata* (Ehrh). The gall of *Biorhiza pallida* (Ol.) was studied either as 3 cm dia (harvested early in May) or 5 cm dia (adult, harvested late in May). The galls of *Neuroterus quercusbaccarum* (L.) and *Andricus* curvator (Hart.) were studied at the adult stage only (harvested in the middle of May).

Preparation of material. The nutrient tissue was isolated, care being taken not to take parts containing the grubs and gall cortex tissue; up to 25–100 mg of the nutrient tissue was harvested. The fr. wt of the tissue analysed is certainly not exactly that of the nutrient tissue because some cortex cells (rich in amylaceous storage granules) are still attached to the nutrient tissue (rich in lipid). Normal tissue of the intact leaf harvested at the same time was taken as control.

Quantitative analysis of lipids. The samples were boiled in few ml of H_2O and lipids were extracted according to ref. [11]. GC analyses were performed either with total fatty acids Me esters (prepared according to [12]) or with Me esters of fatty acids from polar lipids separated by TLC [13] and from neutral lipids [14]. Me heptadecanoate was added as an int. standard for quantitative analysis of fatty acids on a 4% DEGS column, 3 m long, at 170° with N_2 as carrier gas (1.3 l./hr).

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