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GLYCOLIPIDS OF THE LEAVES OF A COTTON PLANT OF THE 175-F VARIETY

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The amounts and the quantitative and fatty acid compositions of the classes of lipids from the leaves of a healthy cotton plant of the wilt-resistant variety 175-F and of such a plant artificially infected with Verticillium dahliae *have been determined. Quantitative changes in individual classes of lipids under the action of infection have been revealed.*

The glycolipids of the leaves of the cotton plant [1], like those of the leaves of other plants that have been studied [2-4], include mainly monogalactosyldiacylglycerols (MGDGs), digalactosyldiacylglycerols (DGDGs), and small amounts of sulfoquinovosyldiacylglycerols (SQDGs). There has been a report of a change in the fatty acid composition of the leaves of a cotton plant on its infection by the fungal pathogen *Verticillium dahliae* Kleb. [5], but there has been no information about the influence of infection on the individual classes of glycolipids.

The aim of the present investigation was a comparative study of the glycolipids of the leaves of a healthy cotton plant of the wilt-resistant variety 175-F (sample I) and of one artificially infected with *V. dahliae* (sample II).

Young leaves were taken from the plants in the phase of 3-4 true leaves. Extracts of the crude lipids of samples I and II were first separated by countercurrent distribution into neutral and polar lipids (NLs and PLs), contaminated with accompanying pigments. Then the PLs were chromatographed by the CC method, with the elution of individual fractions of glycolipids containing pigments with mixtures of chloroform and acetone in which the amount of acetone was gradually increased. The fractions were finally purified by TLC, using system 1 for separating the MGDGs and sterol glycosides (SGs), system 2 for the DGDGs and SQDGs, and system 3 for the more polar glycolipids.

Glycolipids were identified with the aid of qualitative reactions, chromatographic behavior [14], and IR spectra [6].

We did not succeed in completely freeing all the classes of glycolipids from the accompanying brown pigments. The amounts of each class in the weighed fractions were therefore determined by a colorimetric method after their acid hydrolysis. The amounts of pigments were determined from the differences between the gravimetric and colorimetric determinations (Table 1).

The absolute amount of glycolipids in sample I was 1.9 times higher and that of pigments somewhat lower than in sample II. The two samples were identical with respect to the set of glycolipid classes. The main glycolipids of the healthy leaves were the MGDGs, and of the infected leaves they were MGDGs and DGDGs, the amounts of which in them were the same. Consequently, infection of the leaves with *V. dahliae* affects the quantitative composition of the glycolipids in the direction of a decrease in the amount of the majority of classes, with a sharper fall in the levels of MGDGs and SGs. An analogous fall in the proportion of MGDGs in leaves is observed when a cotton plant is subjected to water stress [7].

The fatty acid compositions of the acyl-containing glycolipids were determined by the GLC method (Table 2).

The MGDGs and DGDGs were enriched with unsaturated fatty acids (Σ_{unsat} 83-97.5%), mainly the 18:3 acid. As compared with these classes the total amount of unsaturated acids in the SGDGs was smaller by a factor of almost 2, while this class — particularly in sample I — contained 40% more of the 16:0 acid.

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TABLE 1. Amounts of Glycolipids in the Leaves of a Healthy (I) and a *V. dahliae*-Infected Cotton Plant of the 175-F Variety

Class of glycolipid*	Sample I		Sample II	
	mg/g a.d.m.	% by weight of the GLs	mg/g a.d.m.	% by weight of the GLs
Monogalactosyldiacylglycerols	13.0	40.6	5.3	24.2
Digalactosyldiacylglycerols	8.1	25.3	5.3	24.0
Sterol glycosides	1.5	4.6	0.6	2.8
Sulfoquinovosyldiacylglycerols	0.8	2.6	1.1	5.0
Sum of the glycolipids	23.4	73.1	12.3	56.0
Sum of the pigments	8.6	26.9	9.7	44.0

*Sterol glycoside esters — in trace amounts.

TABLE 2. Fatty Acid Compositions of the Glycolipids from the Leaves of a Healthy (I) and a *V. dahliae*-Infected (II) Cotton Plant of the 175-F Variety

Acid	Monogalactosyl-diacylglycerols		Digalactosyl-diacylglycerols		Sulfoquinovosyl-diacylglycerols	
	I	II	I	II	I	II
13:0	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.
14:0	Tr.	Tr.	Tr.	Tr.	0.3	0.4
15:0	Tr.	Tr.	Tr.	Tr.	Tr.	0.4
16:0	2.5	2.7	9.8	14.6	48.5	38.6
16:1	Tr.	Tr.	0.2	0.6	1.0	1.1
17:0	Tr.	Tr.	Tr.	Tr.	Tr.	0.6
18:0	Tr.	0.4	1.6	2.4	3.2	4.6
18:1	0.5	0.4	0.9	2.7	6.4	5.9
18:2	3.9	2.6	3.6	2.7	5.4	4.4
18:3	93.1	93.9	83.9	77.0	35.2	44.0
Σ_{sat}	2.5	3.1	11.4	17.0	52.0	44.6
Σ_{unsat}	97.5	96.9	88.6	83.0	48.0	55.4

Under the action of the wilt infection, the qualitative composition of the fatty acids in the MGDGs scarcely changed, but the quantitative amounts of the 16:0 and 18:3 acids in the SQDGs and DGDGs, and also of the 18:1 acid in the DGDGs, did change appreciably. In the SQDGs of sample II the level of the 26:0 acid fell by a factor of 1.3 and the proportion of the 18:3 acid rose by almost the same amount, while the opposite tendency was observed in the DGDGs of sample II: the level of the 16:0 acid rose by a factor of 1.5 and the amount of the 18:3 acid fell somewhat.

Acid hydrolysis of the MGDGs, DGDGs, and SGs of samples I and II gave water-soluble fractions containing, in each case, galactose and trace amounts of glucose.

According to its mass spectrum, the steroid moiety of the SGs included 4,4-dimethyl-, 4-monomethyl-, and 4-demethylsterols with a predominance of the latter. The qualitative composition of these sterols was identical with that of the free sterols of the neutral lipids from the leaves of a cotton plant of this variety.

Thus, the infection of young leaves of a cotton plant with verticillium wilt causes a substantial fall in the proportions of the main classes of glycolipids in them, and this to a greater degree for the MGDGs and SGs and to a smaller degree for the DGDGs. As the results on the composition of the fatty acids show, the level of dilinolenoyl-containing species of MGDGs and DGDGs most probably decreases, while in the SQDGs the proportion of the same species apparently increases somewhat.

EXPERIMENTAL

IR spectra were taken on a UR-10 instrument in a film, and the mass spectrum on a MKh-1310 instrument with direct injection of the sample at an ionizing potential of 50 V, a collector current of 40 μA, and a temperature of the ionization chamber of 170°C and of the evaporator of 80°C.

The colorimetric determination of glycolipids on the basis of their carbohydrate component with the anthrone reagent was carried out according to [9] on a KFK-2 UKhA-4,2 photocolorimeter at 670 nm.

GLC was performed under the conditions described in [10].

The conditions of growing the cotton plant and isolating the cell lipids were similar to those of [8].

Countercurrent distribution was achieved by the method of [11]. However, in view of the high level of pigments in the total lipids and the consequent difficulty of determining phase separation boundaries, the volume of solvents was increased 5-fold in relation to the weight of the lipids.

CC was conducted on silica gel L 100/250 (Czech Republic) previously washed with the solvent system chloroform–methanol–heptane–acetic acid (20:10:10:1 v/v [12]), using a series of eluents [13].

Thin-layer chromatography was conducted on glass plates (20 × 20 and 10 × 5 cm) with type L 5/40 silica gel (Czech Republic) containing 6.5% of gypsum, in the following solvent systems: 1) acetone–benzene–water (91:30:8 v/v) [14]; 2) chloroform–acetone–methanol–acetic acid–water (65:20:10:10:3) [15]; and 3) chloroform–acetone–methanol–acetic acid (73:25:1.5:0.5) [16].

Glycolipids were revealed with α-naphthol [11].

The acid hydrolysis of the glycolipids was accomplished according to [17]. The PC of the water-soluble fraction of the products of acid hydrolysis was performed by A. Arifkhodzhaev under the conditions of [18]. For PC we used Filtrak FN-12 paper (Germany) and the butan-1-ol–pyridine–water (6:4:3) system. Sugars were detected by spraying the chromatogram with acid aniline phthalate.

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