Changes in fatty acid composition of sulfolipid and phospholipids during maturation of alfalfa

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ABSTRACT Lipids were extracted from alfalfa samples collected at intervals over the growing season and were fractionated to yield pure sulfolipid. In the sulfolipid and in a phospholipid fraction the major fatty acids were palmitic, linolenic, and linoleic, of which the palmitic acid increased in proportion during the season while the proportion of linolenic acid dropped. The sulfolipid contained more linolenic acid and less palmitic and linoleic acids than the phospholipids, and had a greater rate of change of fatty acid composition.

KEY WORDS plant sulfolipid · phospholipid · 6-sulfo-α-D-quinovopyranosyl-(1–1′) diglyceride · fatty acid composition · fatty acid variation · alfalfa

PLANT SULFOLIPID was discovered by Benson, Daniel, and Wiser in 1959 (1). Its structure was later shown by this group to be a glyceride of 6-sulfoquinovosyl glycerol (2). The fatty acid composition of alfalfa sulfolipid determined by O'Brien and Benson (3) indicates that palmitic and linolenic acids predominate. These authors observed no marked changes in fatty acid composition between July and August.

Studies of fatty acid composition of lipids from plant tissue at different stages of maturity are not numerous. Hawke (4) has studied the difference in the composition of the lipids of "new growth" and mature ryegrass. The fatty acids of the new growth were linolenic (75%), palmitic (12%), and linoleic (8.4%). In the mature grass the composition was linolenic (65%), palmitic (16%), and linoleic (12%). Newman (5) found that the fatty acid composition of the leaves from the different nodes of bush beans varied, the young leaves containing more C_{18} unsaturated acids and the older ones more palmitic acid.

MATERIALS AND METHODS

Samples of alfalfa (*Medicago sativa*, var. Ranger) were collected from an established field near Reading, Pa. from April until October 1963. Collections were made at intervals of approximately 2 wk. The samples were obtained at several times on each collection day to check for daily variations.

The lipids were Soxhlet-extracted with ethanol, and the extracts were fractionated (6) by column and thin-layer chromatography. Components on the plates were detected in UV light of short wavelength. The sulfoquinovosyl diglyceride, R_f 0.7, and a phospholipid fraction composed of phosphatidyl ethanolamine, phosphatidic acid, and phosphatidyl inositol, R_f values of 0.5–0.65, were obtained by scraping the appropriate areas from the thin-layer plates. The lysosulfolipid, had it been present, would have exhibited an R_f of 0.35.

Lipids were methanolyzed by heating the silica gel–lipid mixture with 10 ml of 0.5% H_2SO_4 in methanol at 70°C for 2 hr. The esters were recovered by extraction with petroleum ether. The ether solution was washed with water, dried with anhydrous sodium sulfate, and evaporated almost to dryness under a stream of nitrogen.

The ester mixtures were analyzed by gas chromatography on a column of 15% ethylene glycol succinate–silicone polyester on 80–100 mesh Gas-Chrom P (Applied Science Laboratories Inc., State College, Pa.). An argon ionization detector was used. Quantitative results with National Heart Institute fatty acid standards B and D agreed with the stated composition data with a relative error less than 10% for components comprising more than 5% of the total mixture and less than 20% for components comprising less than 5% of the total mixture. The

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quantities of esters were determined by disc integration without use of correction factors.

A secondary standard, which corresponded closely in composition to the mixtures being analyzed, was prepared from linseed oil with added palmitic acid. This secondary standard was analyzed periodically as a check on the behavior of the instrument.

Only methyl palmitate, stearate, oleate, linoleate, and linolenate were considered in this study as these esters generally accounted for 90–95% of the total esters present. Since more collections were made at 2:00 p.m. than any other time, the fatty acid compositions of the sulfolipid and the phospholipid fractions at this time were plotted with respect to day of collection. When collections were not made at exactly 2:00 p.m., the compositions were assessed by interpolation. The fatty acid composition of the sulfolipid collected at 8:00 a.m. was similarly plotted.

RESULTS AND DISCUSSION

Figs. 1 and 2 are regression curves showing the percentages of three major fatty acids in lipids of leaves collected at 2:00 p.m. over the growing season. As the plants matured these lipid fractions both showed the same trend toward higher concentrations of palmitic acid and lower concentrations of linolenic acid while the linoleic acid concentration remained fairly constant. These figures also show that the sulfolipid initially contained much higher concentrations of linolenic acid than the phospholipid (50 vs. 30%), and lower concentrations of palmitic acid (42 vs. 54%) and of linoleic acid (7 vs. 15%). The fatty acid composition changed faster in sulfolipid than in the phospholipid mixture. The fatty acid composition of the sulfolipid from 8:00 a.m. collections showed the same trends as the 2:00 p.m. collections.

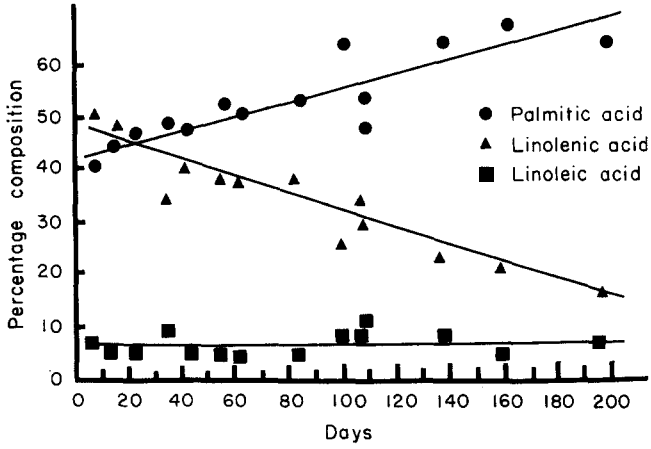

FIG. 1. Fatty acid composition of sulfolipid over the growing season (2:00 p.m. values). Linear regression curves were obtained by the method described by Alder and Roessler (9). Day zero is 11 April 1963.

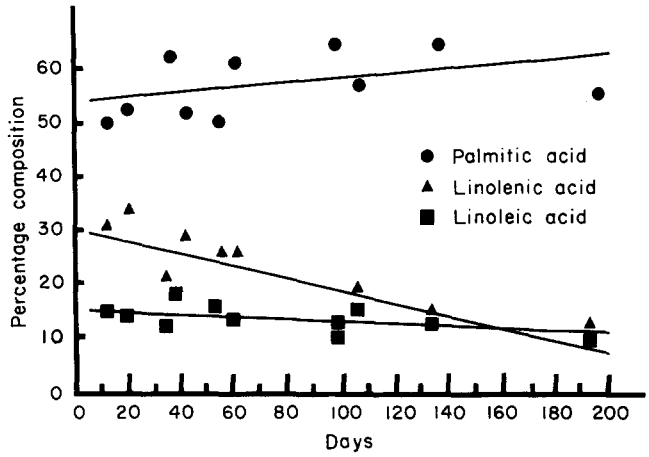

FIG. 2. Fatty acid composition of phospholipid fraction over the growing season (plotted in the same manner as Fig. 1). Phospholipids and the sulfolipid show the same trends, with linolenic acid decreasing and palmitic acid increasing, but the initial compositions are quite different.

These observed changes in composition with age of the plant are in general agreement with the findings of others. Hawke (4) and Brouwer (7) found that as grasses mature, their lipids become more saturated. Newman (5) found that the older leaves of a plant contained higher percentages of saturated acids than the younger leaves. If it is a general property of plants that as they mature, their leaf lipids become more saturated, it will not be possible to assign a characteristic fatty acid composition to a plant lipid on the basis of only one analysis.

The photosynthetic rate has also been observed to decrease with age, in a manner similar to the decrease in the linolenic acid concentration (8). It would be of interest to determine whether the variation in fatty acid composition is the cause or the effect of the change in the photosynthetic rate.

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