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SEASONAL VARIATION IN TRIACYLGLYCERIDE FATTY ACIDS OF DWARF MISTLETOES

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Key Word Index—*Arceuthobium* species; Viscaceae; dwarf mistletoes; glyceride fatty acids; seasonal variation.

Abstract—Fatty acids in the triacylglyceride fraction of three species of dwarf mistletoe (*Arceuthobium*, Viscaceae) showed considerable seasonal variation in proportions of saturated acids and some differences in chain length for those fatty acids. Early summer aerial shoot tissue contained 34–49% saturated fatty acids, while the same species in late October had only 8–15% saturated fatty acids. Fatty acids with a chain length of C_{20} or greater comprised 18–25% of the total in late June but only 1.5–6.4% in the autumn. Three other species analysed in only one season showed similar saturated fatty acids, and chain length patterns were also parallel to the seasonally varying species analysed in the same season. The ratio of linolenic to oleic acid remained constant with season but linolenic acid increased from less than 1% in June to 7–8% in October samples.

INTRODUCTION

Triacylglycerides represent the most energy-rich form of stored food in plants and animals. Their insolubility in water suggests that they are assembled in the cells which contain them, possibly from water soluble materials derived elsewhere. Several electron microscopic observations (Alosi, M. C., personal communication) have shown dwarf mistletoes to be particularly rich in lipids [1, 2], both in the tissues embedded in the conifer host and in the aerial shoots. During $^{14}CO_2$ feeding experiments in the light, Miller and Tocher [3] reported that after 1 hr, 10% of the ^{14}C incorporated in detached aerial shoots was chloroform-soluble. A similar level of lipid synthesis also occurs during photosynthesis by newly germinated seedlings exposed to $^{14}CO_2$ [4].

The unsaturation of fatty acids and resultant lowered melting points is thought to be a cold adaptation [5] and may keep lipids in a liquid form. Lower temperatures also increase oxygen solubility in cells [6]. Oxygen is required for desaturase enzymes which insert double bonds into fatty acids. Comparison of fatty acids of summer and fall triacylglycerides of dwarf mistletoe species provides a starting point for studies on the effect of temperature on lipids of these parasitic plants.

RESULTS AND DISCUSSION

Seasonal variation was very marked in the total lipids of the three *Arceuthobium* species (*A. tsugense*, *A. laricis*, and *A. douglasii*) analysed in both June and October (Tables 1 and 2). The summer collections contained more (3–7%) total lipid than the autumn collections (1–4%). Triacylglycerides were typically *ca* 12% total lipids in all samples with one lower value in each of the summer and autumn samples. The triacylglyceride fatty acids showed a higher proportion of saturated acids and a tendency toward longer chain lengths in summer (Tables 3 and 4). Both characteristics increase the melting points of storage fats and represent adaptation to seasonal variations in ambient temperatures. The lower lipid content in autumn may reflect senescence of the mistletoes or dormancy of the hosts on which they are dependent.

An earlier report [3] on *A. tsugense* described a seasonal variation in respiration rates, 20% higher in the spring compared to the autumn. Lipids represent only 2–4% fr. wt of *A. tsugense* but 10% of the radioactivity incorporated in $^{14}CO_2$ feeding experiments for 1 hr is lipid [3]. Clearly, lipids in *A. tsugense* are rapidly synthesized. In October when the lipids are at a low level, Miller and Tocher [3] found that the respiratory quotient was 1.1, indicating that lipids were not the main respiratory substrate.

In a preliminary report [7], Li and Knutson analysed *A. abietinum* growing on *Abies concolor* collected in the spring. They found that total lipids comprised 2.6% fr. wt in female dwarf mistletoes and 3.2% in male aerial shoots. This sex difference was statistically highly significant ($P < 0.001$). The degree of unsaturation of the fatty acids reported by Li and Knutson was somewhat higher than in any of our June or October material (Tables 1 and 2). A particularly striking difference occurred in the percentage of linolenic acid. They found that $C_{18:3}$ made up 23% of the fatty acid content while our analyses of different species gave results of less than 1% in June and 7–8% in late October. Since we have no data on *A. abietinum* it is impossible to rule out species differences although seasonal variation is a strong possibility. Li and Knutson also found that 22% of the fatty acids in *A. abietinum* were saturated, an amount intermediate between our October and June values. Fatty acid chain lengths of C_{20} and greater made up *ca* 6.5% of the fatty acids as compared with

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Table 1. Lipid constituents in late June collections of aerial parts of dwarf mistletoes

Part	*A. tsugense*	*A. laricis*	*A. douglasii*	*A. campylopodum*	*A. americanum*
Aerial shoot (g) [freeze-dried wt (g)]	1.50	2.44	2.51	1.49	0.996
Total lipids (%)	7.2	3.2	4.8	4.8	7.2
Ether soluble lipids (mg)					
Triacylglycerides	12.1	4.8	11.5	12.3	12.3
Wax esters	5.4	4.4	13.6	5.4	15.6
Free fatty acids	0.9	2.0	2.1	0.8	1.1
Aliphatic alcohols	2.0	0.8	3.5	1.5	1.7
Other*	35.2	58.0	71.7	39.0	38.1
Ether insoluble lipids (mg)	52.4	7.4	18.5	13.1	3.1

*Other ether soluble lipids represented materials applied to TLC plates but not analyzed in this report.

Table 2. Lipid constituents in late October collections of aerial parts of dwarf mistletoes

Part	*A. tsugense*	*A. laricis*	*A. douglasii*	*A. pusillum*
Aerial shoot [methanol extracted (g)]	6.20	6.70	1.85	4.85
Total lipids (%)	1.7	0.96	4.0	2.1
Ether soluble lipids (mg)				
Triacylglycerides	19.0	13.0	3.0	37*
Wax esters	7.0	2.0	5.0	15
Free fatty acids	5.0	5.0	n.d.	*
Aliphatic alcohols	2.5	7.0	3.4	6.0
Other†	51	34	54	24
Ether insoluble lipids (mg)	20	2.0	8.3	15

*Includes free fatty acids.
†See Table 1.

Table 3. Percent fatty acid composition of the triacylglycerides of dwarf mistletoes collected in late June

Fatty acid	*A. tsugense*	*A. laricis*	*A. douglasii*	*A. campylopodum*	*A. americanum*
12:0	0.11	0.64	0.44	0.34	0.24
14:0	0.15	0.58	0.37	0.18	0.14
16:0	12.6	20.4	15.0	11.7	9.86
16:1	1.83	2.24	2.13	1.01	1.55
18:0	1.73	2.24	2.66	1.61	1.00
18:1	17.0	11.3	15.6	13.2	15.1
18:2	44.4	36.4	44.5	49.3	56.0
18:3	0.91	0.26	0.52	0.34	0.09
20:0	13.2	21.2	17.0	16.2	13.6
22:0	3.03	2.97	0.99	3.91	1.66
24:0	3.27	1.22	n.d*	2.24	0.82
Unidentified	1.65	0.59	0.82	n.d.	n.d.
% saturated	34.1	49.3	36.5	36.2	27.3
chain length $\geq C_{20}$	19.5	2.54	18.0	22.3	16.1

*n.d. = not detected.

Table 4. Percent fatty acid composition of the triacylglycerides of dwarf mistletoes collected in late October

Fatty acid	*A. tsugense*	*A. laricis*	*A. douglasii*	*A. pusillum*
14:0	0.12	0.07	0.18	0.03
16:0	5.49	6.45	7.59	1.93
16:1	0.55	0.32	0.73	0.17
18:0	2.18	2.35	2.20	1.19
18:1	24.8	17.0	14.0	19.2
18:2	56.9	59.8	66.8	67.2
18:3	8.06	7.39	6.83	7.63
20:0	0.63	0.64	0.56	0.33
20:1	0.42	0.32	0.25	0.40
20:2	0.08	0.16	0.17	0.34
22:0	0.13	0.14	0.55	0.07
24:0	0.03	0.05	n.d.*	0.50
26:0	n.d.	5.03	n.d.	n.d.
Odd (C_{13-21})	0.19	0.06	0.00	0.48
%Saturated	8.58	14.7	11.1	4.05
%Chain length $\geq C_{20}$	1.58	6.42	1.53	2.43

*n.d. = not detected.

ca 20% of our June analyses and 1.5–6.5% in October.

In European dwarf mistletoe (*Viscum album*) fatty acids varied depending on the host on which the mistletoe was growing [8]. It was possible to distinguish two subspecies of *V. album* on the basis of their $C_{16:0}$, $C_{18:1}$ and $C_{18:2}$ content. The main acids of *V. album* were $C_{16:0}$, $C_{18:1}$ and $C_{18:2}$ which together made up *ca* 80% of the fatty acids.

The high proportion of saturated acids in the triacylglycerides of the June samples could be functional in terms of the physical properties of the lipid deposits. However, the disappearance of the longer-chain acids in the October samples, as well as a reduction by half for 16:0 but not 18:0, strongly suggests that these acids are temporarily stored in the triacylglycerides and then mobilized for another purpose, possibly for incorporation into wax esters or even for modification to hydrocarbon. The ratios of 18:2 to 18:1, averaged for all samples in Tables 3 and 4, are 3.2 and 3.3 respectively. These acids are likely to be distributed in specific positions on the glycerol moiety [9], as part of the biosynthesis and assembly process for the triacylglycerides. In any case the balance of the triacylglyceride position can be filled with the saturated acids in the summer samples. The role of the linolenic (18:3) acid is evidently more important, possibly in the context of over-wintering of the plant, but more likely as provision of a fatty acid base essential for future phospholipids in spore lipids [10]. A high linolenic acid content is not only necessary for membrane cellular function, but work with wheat [11] suggests that 18:3 may confer frost resistance in germinating seeds. If biosynthesized by a route separate from that of 18:1 and 18:2, the formation of the 18:3 would not disturb their proportion.

EXPERIMENTAL

The western dwarf mistletoes were collected in either late June or late October in the vicinity of Mt. Hood, Oregon, while *A. pusillum* was collected in Halifax County, Nova Scotia near West Jeddore in late October. Host branches bearing *Arceuthobium* aerial shoots were returned to the laboratory in an ice box. Aerial shoots were removed by hand and either freeze-dried (June collections) or placed in MeOH under N_2 (October collections). The latter were air-shipped to Halifax where the analyses were carried out.

Lipid extraction. Freeze-dried tissue (June) was weighed, macerated and extracted (×2) $CHCl_3$–MeOH (2:1) in a blender and filtered. The MeOH-treated tissue (collected in October) was blotted dry, weighed and the original MeOH was mixed with $CHCl_3$ to give an extraction mixture $CHCl_3$–MeOH extracted (×2) in a blender and filtered.

The vol. of each filtrate was measured and phase separation was effected by addition of H_2O. The $CHCl_3$–lipid layer was transferred to a weighed flask and evaporated to dryness at 40° *in vacuo* with a small amount of C_6H_6 added to remove traces of H_2O. When completely dry, the flask was re-weighed to obtain the wt of total extractable lipid. Et_2O was then added to the flask in three batches which were swirled and combined to obtain the Et_2O soluble lipids, and the Et_2O insoluble residue. The Et_2O soluble phase was evaporated to an appropriate vol. and applied as a streak to Si gel-G TLC plates which had been pre-run in EtOAc and activated (105°, 1 hr). The developing solvent was hexane–Et_2O–HOAc (84:15:1). The plates were sprayed with 0.5% 2′, 7′-dichlorofluoresein in EtOH and examined under UV. Generally, four obvious mobile bands were visible, corresponding to wax esters, triacylglycerides and two faint bands tentatively identified as free fatty acids and free aliphatic alcohols on the basis of R_f values similar to octadecanol and palmitic acid applied as standards. The bands were scraped into separate tubes, then the remaining Si gel scraped into a fifth tube and the materials dissolved in Et_2O; equal vols of Et_2O–$CHCl_2$; $CHCl_3$ and hexane. The combined solvents were evaporated under N_2 and the wt of each fraction determined.

Methyl transesterification. Triacylglyceride fatty acids in the October collections were transesterified at room temp. in hexane by the addition of 5% 2 N KOH in dry MeOH [12]. The hexane fraction was used for GC.

The samples collected in June were methylated in C_6H_6 by the addition of 50 ml MeOH and 5 ml of acetyl chloride. The mixture was refluxed 100°, 1 hr. 10 ml H_2O were added and the fatty acid methyl esters extracted into hexane. The hexane phase was evaporated to an appropriate vol. and analysed by GC.

GC. All analyses were carried out using He as carrier gas at 40–60 ml/min in GCs equipped with flame ionization detectors. The October collections were analysed at 180° on a 50 m open-tubular column coated with diethylene glycol succinate (DEGS). This machine was fitted with a 1% injection splitter to reduce the sample size. Quantitation on the recorder charts was by the use of a Disc integrator. The June collections were analysed at 180° with a 2.44 m × 0.32 cm column packed with 15% DEGS. Quantities of components of the fatty acid methyl ester mixes were estimated by multiplying peak height by the width at half height. Identity of major components was verified by peak enhancement when known standards were co-chromatographed with the samples.

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