Phytochemistry, Vol. 21, No. 6, pp. 1271–1273, 1982.
Printed in Great Britain.

0031-9422/82/061271-03$03.00/0

FATTY ACID COMPOSITION OF ROOTS AND ROOT NODULES OF *ALNUS* SPECIES

BERNARD MAUDINAS, MICHEL CHEMARDIN and PIERRE GADAL

ERA CNRS No. 799, Laboratoire de Biologie Végétale, Faculté des Sciences, Université de Nancy I, 54037 Nancy Cedex, France

(*Received* 24 *July* 1981)

Key Word Index—*Alnus glutinosa*; *A. incana*; Betulaceae; lipids; fatty acids; roots; root nodules; endophytes.

Abstract—Fatty acid composition of roots and root nodules from *Alnus* species is reported. Differences in total lipid content and in fatty acid composition were observed between these plant organs and with age. The results are discussed in correlation with the growth of pure *Frankia* species in synthetic media.

INTRODUCTION

Information regarding lipid biosynthesis in roots is sparse and nothing is known of the process in tree roots. However, a relationship between adaptation to the environment and lipid composition of plants has been observed on several occasions, and in all cases lipid composition was an important factor in determining the properties of the membranes involved in response to an alteration of the mineral nutrition, especially under 'salt stress' conditions [1–4]. The infection of legume and non-legume roots by nitrogen-fixing micro-organisms is a very important event for plant development and the biochemical basis for specificity of nodule-producing infections is not yet clearly understood. Actinomycete symbiosis in angiosperm nodules is well known, and the infection process has been studied in *Alnus glutinosa* [5, 6], where penetration of hyphae into root hairs has also been described [7]. Nodule formation seems to be restricted to the young parts of the roots just behind the growth region. From the primary nodule, a typical rhizothamnion is induced. Since the recent isolation and *in vitro* cultivation of the *Frankia* endophyte nodulating *Comptonia peregrina* [8], there has been increased interest in the group of actinorhizal woody plants that fix nitrogen. Until now, however, growth of these endophytes has been achieved in complex media and their development is slow even for an actinomycete type [9]. From very recent data, it has been shown that *Frankia* can use some free fatty acids and some Tweens (polyethylene glycol–sorbitol esters) as sole carbon source [10]. As it is not clear which carbon source *Frankia* obtains from the plant when living in the root nodule, the present work describes the fatty acid compositions of roots and root nodules from *Alnus* species grown either in natural conditions or in culture. The results are discussed in relation to *in vitro* pure culture conditions of actinomycete-like diazotrophs.

RESULTS AND DISCUSSION

The lipid composition was studied either in root or in root nodule material. Most of the root material from the natural environment was rather young and part of it was still able to be infected by *Frankia*. However, the root lots collected from nature were heterogenous when compared with the homogeneous root lots from young *Alnus* laboratory cultures.

As shown in Table 1, on a dry wt basis total lipid content in roots increased as trees aged and was rather high in older samples. In contrast (Table 2), the total lipid content from root nodules remained constant. Similarly, although total fatty acid content both in root and in nodules was lower than found in other un-nodulated plant species [2], there were more variations in roots than in root nodules. A net increase in fatty acids was observed in root lipids from the first to the sixth month, which corresponded to the period of growth following the early inoculation of *Frankia* in the rhizosphere. As the trees aged, the proportion of root fatty acids among total lipids seemed to decrease during the period of rhizothamnia

Table 1. Amounts of fatty acids from roots of *Alnus glutinosa* expressed as mg/g root material, dry wt and per g extracted lipid

	Laboratory 1.5 month old	Laboratory 3 month old	Laboratory 6 month old	Field 2–4 years old
Total lipids mg/g dry wt	33	34.5	60	184
Total fatty acids mg/g dry wt	3.2	5.6	17.6	7.9
Total fatty acids mg/g total lipids	97	162	193	43

Table 2. Amounts of fatty acids from root nodules of *Alnus* species expressed as mg/g root nodules dry wt and per g extracted lipid

	A. glutinosa (laboratory) 3 month old	*A. glutinosa* (laboratory) 5 month old	*A. glutinosa* (field) 3–5 years old	*A. incana* (field) 3–5 years old
Total lipids mg/g dry wt	62.7	60	57	57
Total fatty acids mg/g dry wt	4.5	4.7	6.1	5.9
Total fatty acids mg/g total lipids	71.8	78.3	107	104

development, although the culture conditions were different for these samples.

As shown in Tables 3 and 4, large differences exist in the fatty acids composition of *A. glutinosa* between roots and root nodules. In young roots from 1.5 to 6 months old *Alnus* trees grown in the culture chamber, the unsaturated C_{18} fatty acids were the major ones. In contrast, in very young root nodules the saturated fatty acids were the major ones while the content of C_{18}-unsaturated fatty acids increased as the trees aged. As relative anaerobiosis affects the biosynthesis of unsaturated fatty acids [4] and promotes nitrogen fixation by the endophyte, it is important to note that very young root nodules have the highest content of saturated fatty acids. In addition, root nodules from *A. glutinosa* and *A. incana* harvested in the natural environment exhibited the same fatty acid pattern (Table 4).

In the screening of synthetic culture media it has been shown recently that *Frankia* can use simple fatty acids and fatty acids esterified with polyoxyethylene sorbitol as sole carbon source [10]. The higher growth yields were obtained with Tweens 40, 60, 80 and 85 esterified with palmitic, stearic, oleic and linoleic acids, respectively. Unfortunately, the influence of the degree of fatty acid unsaturation has

Table 3. Fatty acid composition of extractable lipids in roots of *Alnus glutinosa* expressed as a percentage of total fatty acids

Fatty acid	Laboratory 1.5 month old	Laboratory 3 month old	Laboratory 6 month old	Field 2–4 years old
C_{12}	tr	tr	tr	tr
C_{14}	10	27	17.4	15.7
C_{16}	30.2	5.5	20	30
$C_{16:1}$	tr	tr	1.2	4
C_{18}	3.8	tr	1.8	6.2
$C_{18:1}$	7.0	3	3.6	6.0
$C_{18:2}$	45.0	53	43.1	31.5
$C_{18:3}$	tr	11.5	12.3	6.1
Others	4.0	—	0.6	0.5

Table 4. Fatty acid composition of extractable lipids in root nodules of *Alnus* species expressed as a percentage of total fatty acids

Fatty acid	*A. glutinosa* (laboratory) 3 month old	*A. glutinosa* (laboratory) 6 month old	*A. glutinosa* (field) 3–5 years old	*A. incana* (field) 3–5 years old
C_{12}	tr	tr	1.7	1.5
C_{14}	51.6	29	12.1	7.2
C_{16}	7.9	18.5	16.0	15.0
$C_{16:1}$	tr	4.1	2.4	3.0
C_{18}	tr	tr	1.6	0.4
$C_{18:1}$	7.0	6.6	5.3	4.8
$C_{18:2}$	21.0	20.3	35.3	49
$C_{18:3}$	12.1	17.0	23.3	16.3
Others	0.4	4.5	2.3	2.8

not been studied on the growth yield of *Frankia* in pure cultures, but a poisoning effect of free oleate as sole carbon source has been observed [9]. Thus the relative importance of C_{18}-unsaturated fatty acids in root materials could affect the growth of *Frankia* in nodule tissues. Consequently, further studies will be needed to understand the root lipid metabolism and to analyse more precisely the lipid composition of the vesicles, the site of nitrogen fixation in *Alnus* species.

EXPERIMENTAL

Plant material. Young *Alnus* trees were either harvested in the field or grown in culture chambers according to the conditions already described [11]. Roots and root nodules of different ages were carefully washed, separated, freeze-dried and homonogenized. Dry wts were determined and the dehydrated plant materials used for lipid analysis.

Extraction. All operations were carried out under Ar in dim light at room temp. Plant material was first extracted with Me_2CO (10 ml/g dry wt) with continuous stirring. After filtering, the residue was taken up in $CHCl_3$–MeOH (2:1) and this treatment was repeated ×3. The residue was then extracted twice with $CHCl_3$–MeOH (1:1). The filtrates were combined, washed with 1% NaCl soln and allowed to separate either in a separating funnel or in a centrifuge. The lower phase was collected, evaporated under vacuum, dried (Na_2SO_4) and weighed. This fraction was designated as total lipids.

The total lipid extracts were saponified under Ar overnight at room temp. in 10% alcoholic KOH. After elimination of the unsaponifiable fraction by several Et_2O extractions the aq. phase was acidified with 3N HCl and the fatty acids extracted ×3 with petrol–Et_2O (1:1). The Et_2O phases were collected, washed with H_2O and dried (Na_2SO_4). This fraction contained the total fatty acids.

Analysis. Fatty acids were methylated with CH_2N_2 [12]. FID-GC of fatty acid methyl esters was performed on a 3 m × 3 mm column packed with diethylene glycol–succinate (5% w/w DEGS) on Chromosorb G acid washed and silanized (AW-DMCS). The column temp. was 165°, the inlet and detection temps. were 195°. The N_2 flow rate was *ca* 20 ml/min. The R_ts of the fatty acid methyl esters were compared with those of authentic standards. For quantitative analysis, the peak areas of the components were compared with that of a known amount of methyl palmitate [13].

Acknowledgement—We thank the Commissariat à l'Energie Solaire for financial support (Grant No. 80 54 229).

REFERENCES

1. Kuiper, P. J. C., Kähr, M., Stuiver, C. E. E. and Kylin A. (1974) *Physiol. Plant.* **32**, 33.
2. Kuiper, D. and Kuiper, P. J. C. (1978) *Physiol. Plant.* **44**, 81.
3. Kuiper, D. and Kuiper, P. J. C. (1979) *Physiol. Plant.* **45**, 489.
4. Kuiper P. J. C. (1980) in *Biogenesis and Function of Plant Lipids*, (Mazliak, P., Benveniste, P., Costes, C. and Douce, R., eds.) p. 169.
5. Quispel A. (1974) in *The Biology of Nitrogen Fixation*, (Quispel, A., ed.) p. 499.
6. Akkermans A. D. L. (1978) in *Interactions Between Non Pathogenic Soil Microorganisms and Plants*, (Dommergues, Y. R. and Krupa, S. V., eds.) p. 335.
7. Angulo Carmona, A. F. (1974) *Acta Bot. Neerl.* **23**, 257.
8. Callaham O., Del Tredici P. and Torrey J. G. (1978) *Science* **199**, 899.
9. Blom J., Roelofsen, W. and Akkermans A. D. L. (1980) *FEMS, Microbiol. Letters* **9**, 131.
10. Blom J. (1981) *FEMS, Microbiol. Letters* **10**, 143.
11. Pizelle G. (1978) 103rd Congrès national des Sociétés Savantes, Nancy, Sciences fasc. I, 161.
12. De Boer T. J. (1953) Doctoral Thesis, Groningen.
13. Horning E. C., Ahrens E. H., Lipsky S. R., Mattson F. M., Mead J. F., Turner D. A. and Goldwater W. H. (1964) *J. Lipid Res.* **5**, 20.