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METABOLISM OF LIPIDS IN FIR SEEDS (*ABIES ALBA* MILL.)

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Introduction

One of the initial and very important processes involved in the germination of fat-storing seeds is the decomposition of triglycerides into diglycerides, monoglycerides and finally into fatty acids and glycerol. Further breakdown of glycerol and fatty acids furnishes energy and substances (such as carbohydrates) required for the growth of a new embryo (Hitchcock and Nichols 1971).

Although the major contribution in sugar synthesis of lipids is from fatty acids, glycerol moiety also provides a ready source for sucrose production by way of glycerol phosphate, dihydroxyacetone phosphate and gluconeogenesis. On the other hand, dihydroxyacetone phosphate may enter the TCA cycle by way of pyruvic acids. In 1955 Stumpf already succeeded in providing evidence for the existence of enzyme systems which cooperate in converting glycerol to CO_2 by a pathway including glycerol phosphate and pyruvic acid and the subsequent oxidation in the TCA cycle.

Glycerol and fatty acids are also utilized in the synthesis of new lipids, reserve triglycerides as well as complex lipids. The presence of complex lipids is especially observed in membranes, where they play a structural and physiological role (Hitchcock and Nichols 1971).

Lipids are the main reserve substance of fir seeds, which was demonstrated in the present study. Therefore, it was assumed that they play an important role in the germination of seeds. Fir seeds do not seem to be deeply dormant since they germinate even without stratification. However, their germination rate and capacity are lower in comparison with stratified seeds. Since the decomposition and synthesis of lipids ref-

lect the processes proceeding in the seed, examination of their metabolic pathway may clarify the processes which occur in non-stratified and stratified fir seeds prior to and during germination.

Materials and Methods

Fir seeds (*Abies alba* Mill.) collected in 1973 in the Snežnik region, 1020 m above sea-level (provenience 9) and in 1975 in the Rog region, 650 m above sea-level (provenience 7), were used in the experiments.

After 24 hrs imbibition in distilled water at room temperature (25° C) the seeds were treated with distilled water at the same temperature for a definite period of time (non-stratified seeds).

Another set of seeds were imbibed in water for 24 hrs at +3° C and subsequently stored for 21 days in constantly moist vermiculite at the same temperature. After stratification the seeds were transferred to room temperature and treated with distilled water for a definite period of time (stratified seeds).

The extraction of lipids was carried out with isopropanol and chloroform (1 : 2) followed by washing with 0.7% aqueous solution of NaCl (Folch et al. 1957). From the washed lipid extract solvent was subsequently removed under vacuum at 35° C. The amount of total lipids was measured gravimetrically. Lipids were then dissolved in 5 ml of chloroform containing 0.005% butyl hydroxytoluene (BHT) and stored at —20° C for further analysis.

The remaining tissue was refluxed with 95% aqueous methanol for 6 hours. The aqueous solution, with which the lipid extract had been rinsed (0.7% NaCl), was added to the methanol extract and the volume was raised up to 50 ml.

The tissue was dried for 24 hrs at 90° C and weighed. The separation of lipids into simple and complex was carried out by using 95% aqueous methanol and petroleum ether (B. P. 60—80° C) (Nichols 1964). Further separation was achieved by TLC (Silica Gel G, thickness of layers — 0.25 mm). For the separation of simple lipids petroleum ether (B. P. 40—60° C) (diethyl ether) acetic acid (70 : 20 : 4) was used as a solvent, whereas for the separation of complex lipids chloroform/methanol/aqueous 7 N ammonium hydroxide (65 : 30 : 4) was used.

For the detection of spots the following reagents were used: 2,7 dichlorofluorescein and 50% aqueous solution of H_2SO_4 as general reagents, 0.5% solution of ninhydrin in butanol saturated with water for the detection of phospholipids with a free amino group, Dittmer's reagent (Dittmer and Lester 1961) for all phospholipids, and periodate Schiff's reagent (Baddiley et al. 1956) for glycolipids. For the identification of spots the following standards from Sigma were used: oleic and linoleic acids, 1,2 dipalmitin, 1,3 dipalmitin, tristearin, triolein, trilinolein, L-α-phosphatidyl choline, phosphatidyl ethanolamine, cholesterol and cholesteryl oleate. Identity and quantitative percentage of each fatty acid were determined by gas liquid chromatography and mass spectrometry. The fatty acids were methylated according to Jankowski and Garner (1965). Methyl esters were analyzed on a Perkin Elmer F 11 gas chromatograph equipped with a flame ionisation detector. The stationary phase was diethylenglycol succinate on Chromosorb W HMS, 80—100 mesh (Perkin Elmer). Chromatographic analyses were carried out at 180° C with nitrogen as carrier gas. The mass-spectrometer was a Kratos MS 25. The

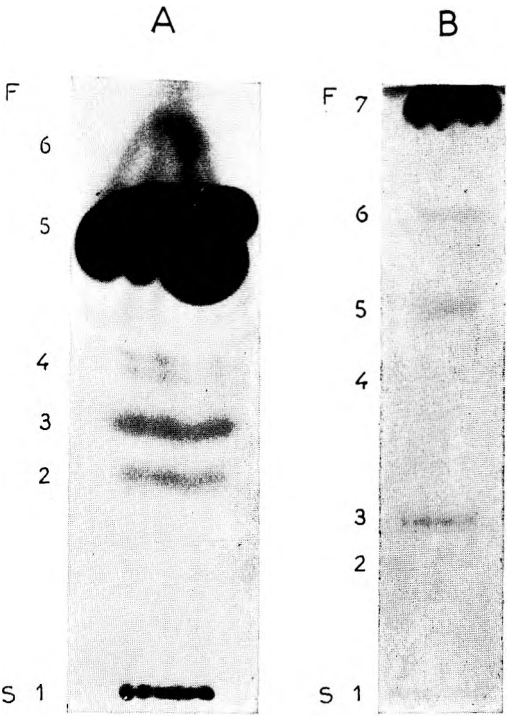


Fig. 1. A: Thin-layer chromatogram of simple lipids;
Solvent: petroleum ether/diethyl ether/acetic acid (70:20:4);
Reagent: 50% H_2SO_4;
1: complex lipids; 2: 1,2 diglycerides; 3: 1,3 diglycerides; 4: fatty acids; 5: triglycerides; 6: cholesteryl esters;
B: Thin-layer chromatogram of complex lipids;
Solvent: chloroform/methanol/7N aqueous ammonia (65:30:4);
Reagent: 50% H_2SO_4;
1 and 2: unidentified components; 3: phosphotidylcholine; 4: the spot which reacted with ninhydrin; 5: phosphatidylethanolamine; 6 and 7: the spots which reacted with Schiff's reagent; S — start; F — front.

gas chromatograph was interfaced using an all-glass single stage jet separator at 250° C. The source was operated at 200° C with ionizing current of 100 μA. All spectra were obtained at 70 eV.

The amount of simple lipids was colorimetrically determined by the method of Fletcher (1968). Absorption measurements were performed with a Beckman, Acta II C Spectrophotometer. The amount calculated in mg in our sample was read from the calibration curve for triolein (Calbiochem).

The relative amount of soluble sugars in aqueous methanol extract was measured according to the method recommended by Gros and Smrekar (1967) and read from the calibration curve for glucose (Kemika).

For experiments with radioactive material, universally labelled ^{14}C-glycerol, spec. activity 494 μCi/mg, obtained from The Radiochemical Centre, Amersham, was used as a substrate.

Twenty intact seeds with testa removed were soaked in 2 ml buffer solution of ^{14}C-glycerol (phosphate buffer 0.1 M, ph 7.4, activity 2 μCi). The results obtained represent mean values of two parallel experiments. After 24 hrs the seeds were removed from the radioactive solution and rinsed with distilled water. For experiments in which also the released $^{14}CO_2$ was measured, the seeds were soaked for 6 hrs in airtight test-tubes (Cuppy and Crevasse 1963). A filter tape (Whatman No. 1, 2 × 0.5 cm) was placed in the vessel in which CO_2 was trapped. One hour prior to the end of soaking 0,2 ml solution of phenethylamine (phenethylamine/toluene/methanol 2 : 1 : 1) was added (Woeller 1961, Duncombe and Rising 1969).

Radioactivity was measured with a Beckman LS 150 liquid scintillation spectrometer. For the measurements of radioactivity of lipid extracts, the counting solvent based on toluene (1 litre toluene, 5 g PPO, 0.3 g POPOP) was used, whereas for the measurements of aqueous substances the counting solvent based on dioxane (1 litre dioxane, 5 g PPO, 0,3 g POPOP) was employed. By means of autoradiography with a Kodak X-ray film the lipids were detected into which radioactive glycerol was incorporated during incubation.

Results and Discussion

Lipids were found to comprise 25.0 ± 0.3% of the fresh weight and 44.0 ± 0.6% of the dry weight of fir seeds, the endosperm containing 89.9 ± 0.7% and the embryo 10.2 ± 0.7% of lipids.

In simple lipids (Fig. 1 A) reserve triglycerides were found in the largest amounts. Chromatographic separation of the extract of complex lipids yielded several substances (Fig. 1 B). In both simple and complex lipids linoleic was found to be present in the largest amounts (18 : 2). The presence of oleic (18 : 1), palmitic (16 : 0), stearic (18 : 0) and palmitoleic acids (16 : 1) was also noted. The constituents X_1 and X_2 were also found to be present in significant amounts (Fig. 2). They were identified as isomers of linoleic and linolenic acids. The MS employed in this study will not provide information on the position or the configuration of the double bonds. However, with knowledge derived from relative retention times and supported by the results obtained by Laseter et al. (1973) the position of the points of unsaturation can be determined with reasonable accuracy as Δ 5,9 and Δ 5,9,12. The minor acids, particularly the unsaturated components, appeared to be characteristic of conifer species in ge-

neral and more specifically the C 18 and C 20 polyunsaturated acids whose double bonds are separated by two methylene units and contain an olefinic bond at the Δ 5 position (J a m i e s o n and R e i d 1972).

These results are in agreement with those obtained by N y m a n (1966) and L a s e t e r et al. (1973) in their investigation of fatty acids of conifers *Pinus sylvestris* and *Pinus elliottii* as well as those obtained by C h i n g

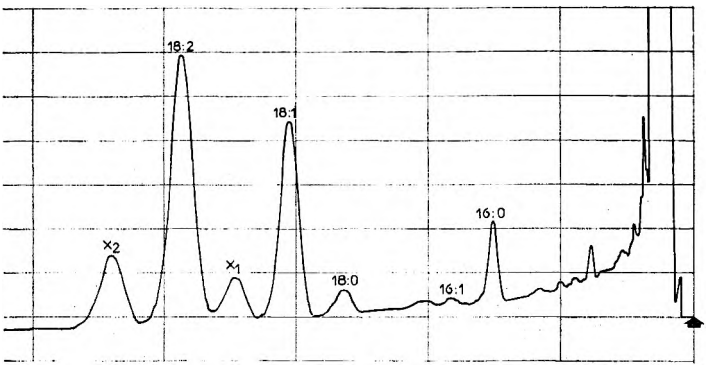


Fig. 2. Gas chromatographic analysis of fatty acids from fir seeds. Gas-liquid cromatograph, Perkin Elmer Fll. Liquid phase: 20% diethyleneglycol succinate on Chromosorb W HMDS. Temperature: 180 °C.

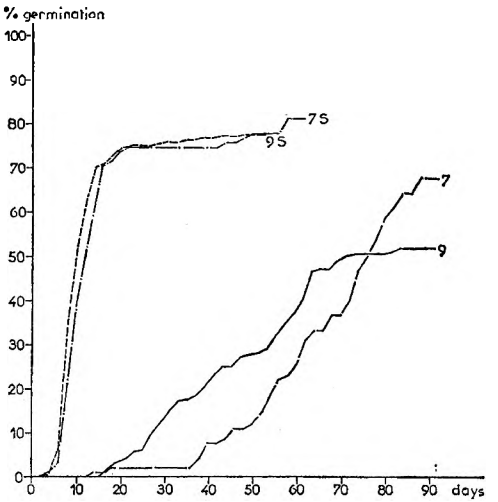


Fig. 3. Germination curves for the 1973 seeds, provenience 9, and the 1975 seeds, provenience 7, subjected to germination in 1976; 9: non-stratified seeds of provenience 9; 9S: 21 days stratified seeds of provenience 9; 7: non-stratified seeds of provenience 7; 7S: 21 days stratified seeds of provenience 7.

(1963) in his study on Douglas fir seeds. This conclusion seems to prove the assumption that similar species of plants have a similar composition of fatty acids (M e a r a 1957). Data in Table 1 illustrate a similar quantitative composition of fatty acids in simple and complex lipids.

Table 1. Percentage occurrence of each fatty acid of simple and complex lipids

Fatty acids	Simple lipids	Complex lipids
16 : 0	4.1	4.9
16 : 1	0.7	0.7
18 : 0	2.6	3.0
18 : 1	30.0	26.8
X_1	5.1	6.4
18 : 2	43.7	44.7
X_2	13.8	13.5

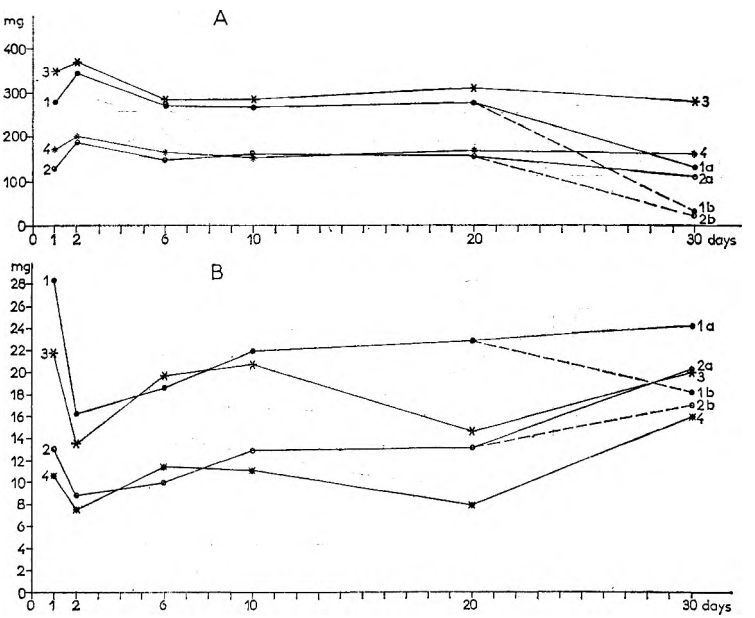


Fig. 4. A: the amount of simple lipids in non-stratified seeds of provenience 9 and 7 with testa removed;
B: the relative amount of sugars culculated for glucose in non-stratified seeds with testa removed;
1: the amount in provenience 9 expressed in mg/g of fresh weight;
2: the amount in provenience 9 expressed in mg/0.1 g of dry weight;
3: the amount in provenience 7 expressed in mg/g of fresh weight;
4: the amount in provenience 7 expressed in mg/0.1 g of dry weight;
a: germinating seeds, length of roots from a few mm to 2 cm;
b: germinating seeds, length of roots from 2.5 to 10 cm.

Germination curves indicate the rate of germination of stratified and non-stratified seeds of provenience 7 and 9 (Fig. 3).

The amount of simple lipids was measured at different intervals at room temperature. Figure 4 A indicates that simple lipids are utilized 20 days after imbibition in germinating seeds only. In the seeds with longer roots a decrease of 80% od the dry weight, from the value measured after imbibition, was observed. Figures of the two proveniences differ essentially only as far as the rate of germination of the proveniences is concerned.

Stratified seeds of provenience 7 germinated already after the sixth day. After that time an increased decomposition of simple lipids was observed (Fig. 5 A).

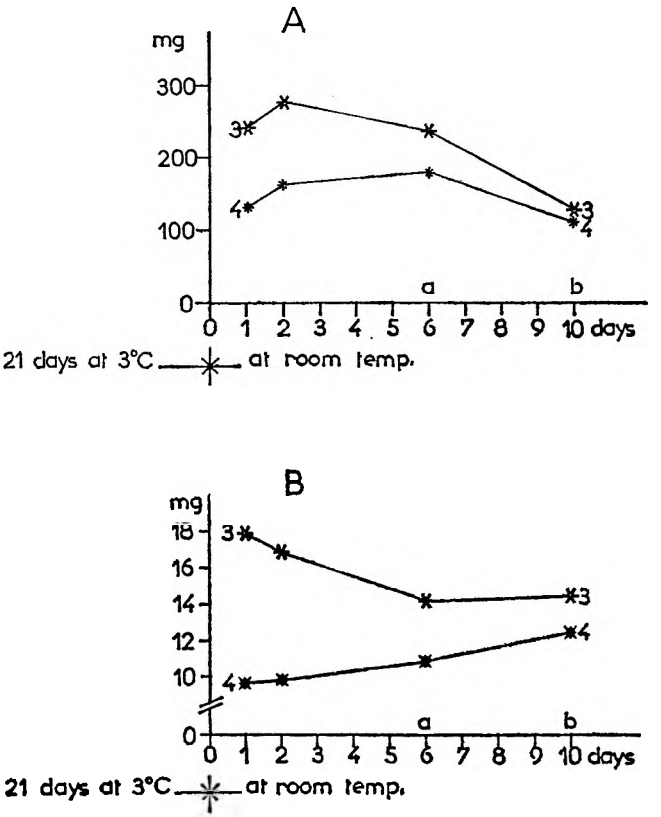


Fig. 5. A: the amount of simple lipids in stratified seeds of provenience 7 with testa removed;
B: the relative amount of soluble sugars calculated for glucose in stratified seeds of provenience 7 testa removed;
3: the amount expressed in mg/g of fresh weight;
4: the amount expressed in mg/0.1 g of dry weight;
a: germinating seeds, length of roots a few mm;
b: germinating seeds, length of roots from 1 to 2 cm.

Measurements of the relative amount of soluble sugars indicate a trend contrary to the measurements of the amount of lipids (Fig. 4 B). On the second day of imbibition a considerable decrease in sugars (the amount fell 32.3% of the dry weight) was noted followed by a gradual increase, which corresponds to the increase of α-amylase activity (Jurc 1977). The activity of this enzyme was observed to decrease after 21 days, that is, at the time when seeds were already germinating and an intensive utilization of reserve triglycerides had commenced. The increase of the amount of soluble sugars despite a low α-amylase activity in germinating seeds may be ascribed to their synthesis from fats. A similar tendency was noted in stratified seeds (Fig. 5 B). The conversion of fats into sugars has also been demonstrated in Douglas fir seeds (Ching 1966), hazel seeds (Bradbeer and Colman 1967, Stobart and Pinfield 1970), castor bean (Beevers 1956, Kornberg and Beevers 1957), *Manihot esculenta* (Narty et al. 1974) and even in those seeds in which carbohydrates and not fats are the main reserve substance as in barley (Newman and Briggs 1976). For cotyledons of *Cucumis sativus* it has even been demonstrated that the addition of sucrose inhibits the decomposition of lipids (Slack et al. 1977).

Although it is difficult to conclude whether the amount of metabolites is due to decomposition or synthesis, it may be assumed, according to the above observations, that during induction of seed germination mainly the decomposition of carbohydrates proceeds although they are not the main reserve substance. The decomposition of reserve triglycerides, however, appears to initiate only later when the seeds already germinate to make possible the rapid growth of the embryo.

Since the synthesis of metabolites reflects the processes in the seed prior to and during germination, fir seeds were incubated with radioactive glycerol, which is not only a lipid and sugar substrate but also a respiratory substrate.

The experiments were first focused especially on the incorporation of ^{14}C-glycerol into lipids. Germinating experiments of provenience 9 were carried on for 16 days at room temperature. During this time the seeds did not germinate yet.

The radioactivity of complex lipids was higher than that of simple lipids during the whole period of observation (Fig. 6). Since complex lipids

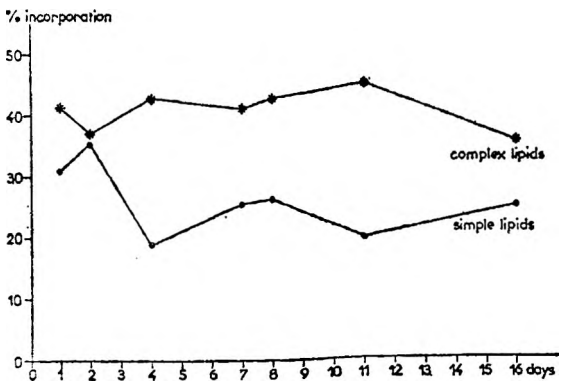


Fig. 6. Percentage of the incorporation of ^{14}C-glycerol into simple and complex lipids. Twenty intact peeled seeds of provenience 9 were soaked for 24 hrs.

are present especially in membranes, it is likely that their synthesis may be due to their development. This is in agreement with the observation that in soybean seeds (Kagawa et al. 1973, Negishi 1976), Douglas fir seeds (Ching 1973), wheat (Varty and Laidman 1976), corn (Hawke et al. 1974), hazel seeds (Stobart and Pinfield 1970) and in the seeds of other plants, complex lipids are synthetized to a larger extent than simple lipids before and during germination.

The constituents into which radioactive glycerol was incorporated were determined by means of autoradiography (Fig. 7). Radioactive spots were removed from chromatograms and radioactivity was measured again (Figs. 8 and 9).

Among the simple lipids, diglycerides are radioactive and not triglycerides although the latter are the main reserve substance. However, for ripe seeds of the Douglas fir it has been reported that radioactive acetate and glucose are incorporated into all groups of simple lipids, that is, in triglycerides and diglycerides, monoglycerides, free fatty acids, sterols and steryl esters (Ching and Fang 1969, Ching 1973). It should be pointed out that not only 1,2 diglycerides, which are well-known in the literature as precursors of plant lipids (Hitchock and Nichols 1971, Hawke et al. 1974, Wilson and Rinne 1976) but also 1,3 diglycerides were found to be radioactive. The synthesis of 1,3 diglycerides has also been observed in the cotyledons of cucumber during germination (Macher et al. 1975). Acyl groups in diglycerides are often transferred to the adjacent free hydroxyl groups. Thus 1,3 diglycerides may also result from interesterification (Gunstone 1975). 1,2 and 1,3 diglycerides may also be considered as the product of lipid hydrolysis as has been demonstrated for castor bean seeds (Noma and Borgström 1971).

In complex lipids, nearly all spots were found to be radioactive. The spot travelling with the solvent was, however, the most radioactive. The next spots following were phosphatidyl choline and phosphatidyl ethanolamine, which are important constituents of plant and animal membranes.

It may be concluded from the results that the synthesis of complex lipids prior to germination proceeds also by way of those diglycerides which are de novo synthetized with radioactive glycerol during incubation. Biosynthesis of complex lipids, then, is an early anabolic process.

Donaldson (1976) has also reported that membrane lipids of castor bean seeds are synthetized de novo to a larger extent and not from reserve triglycerides. The synthesis of complex lipids is, however, a very dynamic process (Roughan 1970); therefore, it may be assumed that radioactive diglycerides also result from the decomposition of lipids and may be again acylated into complex lipids or triglycerides, as has been found for the cotyledons of soybeans (Wilson and Rinne 1976).

The second part of the study on the synthesis from ^{14}C-glycerol also included the measurement of radioactive CO_2 and the comparison of the synthesis of stratified and non-stratified seeds (Fig. 10). For these experiments the seeds of provenience 7 were soaked in radioactive solution for 6 hrs. It was observed that the incorporated glycerol was mainly consumed as a respiratory substrate since 70—85% of radioactive glycerole was oxidized into CO_2 in stratified as well as non-stratified seeds. They differed essentially only in the rate of incorporation, since the incorporation proceeded at the lowest rate in the first days after imbibition of non-stratified seeds and then slowly increased, whereas in stratified seeds the rate of incorporation was reverse.

It is surprising that only a very small amount of radioactive glycerol was incorporated into the lipid fraction. In the aqueous fraction,

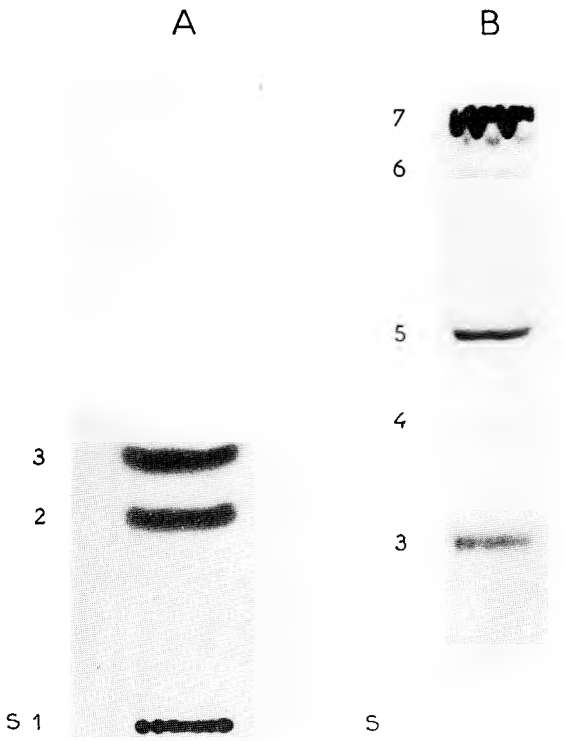


Fig. 7. A: autoradiogram of simple lipids; 1: complex lipids; 2: 1,2 diglycerides; 3: 1,3 diglycerides;
B: autoradiogram of complex lipids; 1: unidentified component; 3 phosphatidylcholine; 4: the spot which reacted with ninhydrine; 5: phosphatidylethanolamine; 6 and 7: the spots which reacted with Schiff's reagent.

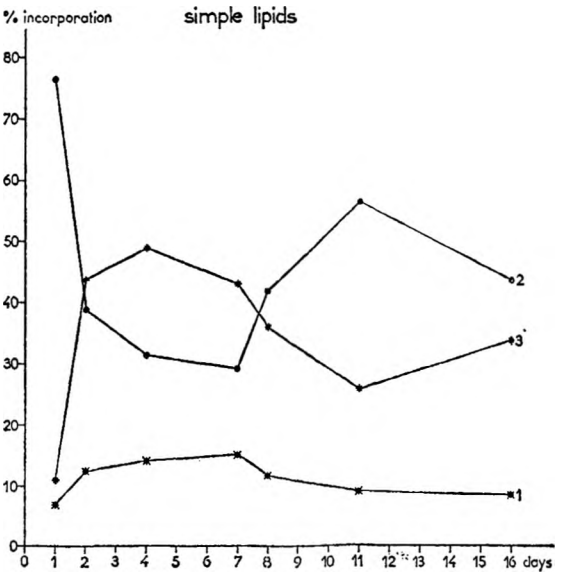


Fig. 8. Percentage of the incorporation of ^{14}C-glycerol into individual spots in thin-layer chromatogram of simple lipids;
1: complex lipids; 2: 1,2 diglycerides; 3: 1,3 diglycerides

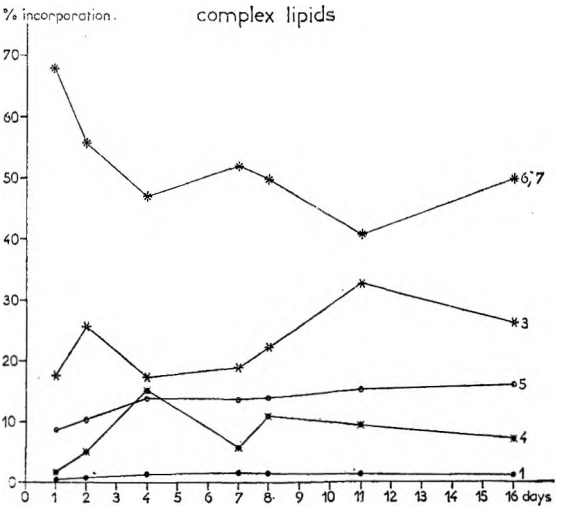
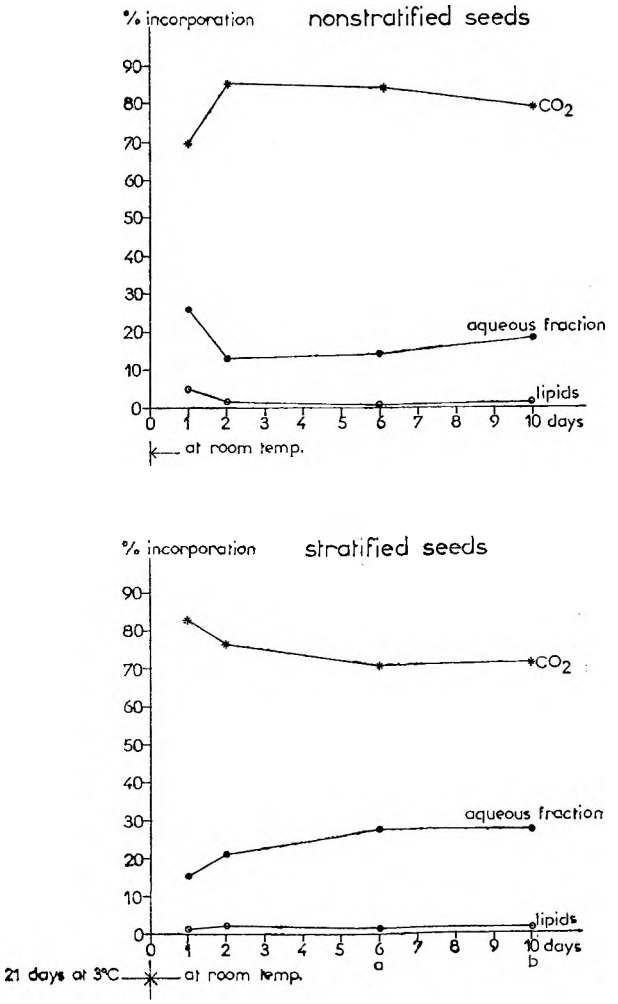


Fig. 9. Percentage of the incorporation of ^{14}C-glycerol into individual spots in thin-layer chromatogram of complex lipids;
1: unindentified component; 3: phosphatidylcholine;
4: the spot which reacted with ninhydrine; 5: phosphatidylethanolamine;
6 and 7: the spots which reacted with Schiff's reagent.

13% to 28% of the whole incorporation of radioactive glycerol was noted. The discrepancy of incorporation of radioactive glycerol between stratified and non-stratified seeds was especially evident in the first days after imbibition. But the incorporation of glycerol in the aqueous fraction of stratified and non-stratified seeds exhibited a trend opposite to the incorporation into CO_2. From this observation it was assumed that in this



a: germinating seeds, length of roots a few mm;
b: germinating seeds, length of roots from 1 to 2 cm.

Fig. 10. A: percentage of the incorporation of ^{14}C-glycerol into CO_2, aqueous and lipid fraction in non-stratified seeds; twenty seeds with testa removed were soaked for 6 hrs;

B: percentage of the incorporation of ^{14}C-glycerol in CO_2, aqueous and lipid fraction in stratified seeds;

case there might be a competition between the oxidation of radioactive glycerol to CO_2 and the metabolism into watersoluble and lipid substances. In this aqueous fraction sugars are likely to be most radioactive as Beevers reported (1956). The rate of change in the relative amount of sugars was, at the time before the germination, similar to that of the incorporation of radioactive glycerol in the aqueous fraction, which may lead to the conclusion that glycerol was converted into sugars. This was observed in stratified and non-stratified seeds.

Beevers (1956) has obtained evidence that, in the incubation of castor bean cotyledons with radioactive glycerol, most of the latter was converted by way of glycerol phosphate into sucrose and CO_2. In the incubation of the cotyledons of hazel seeds with radioactive palmitate and oleate, Shewery (1972) has similarly observed the highest radioactivity in CO_2, less radioactivity in the aqueous fraction and the lowest in lipids. McMachon and Stumpf (1966) have also found a higher percentage of radioactivity in CO_2 than in lipids and aqueous fraction after a 6-hour feeding of the cotyledons of *Carthamus tinctorius* with radioactive acetate. Newcomb and Stumpf (1953) incubated the cotyledons of germinating peanuts with different radioactive precursors of fatty acids for 6 hrs and observed that they were converted into CO_2 to a larger extent than into fatty acids. Conversely, Stobart and Pinfield (1970) found the highest radioactivity in the lipid fraction when they incubated the cotyledons and the embryo of hazel seeds with radioactive glycerol. A similar observation was made by Bradbeer and Colman (1967) when they incubated the seeds of the same kind with radioactive acetate.

The results of our experiments also indicate that stratified and non-stratified fir seeds are metabolically active at the induction of germination since they may synthetize lipids, aqueous substances and oxidize glycerol into CO_2. Measurements of the intensity of respiration in not yet germinating seeds of non-stratified and especially of stratified fir seeds also indicated high values (Hlebš and Vardjan 1974). The most marked changes in the metabolism were already observed in the first days after imbibition. Bradbeer and Colman (1967) have also reported that dormant seeds of hazel exhibit metabolic activity since they have obtained evidence of the activity of the TCA cycle, glutamate dehydrogenase and aspartate aminotransferase as well as that of enzymes involved in the synthesis of lipids, proteins, sucrose, DNA and RNA. The authors suggest that no de novo metabolic pathways occur during stratification. They assume that the germination of dormant seeds may be inhibited by the partial blockage of the metabolic pathway.

Summary

Triglycerides are the main reserve substance of fir seeds (*Abies alba Mill.*). Especially, unsaturated fatty acids are present in fir seeds. Among determined fatty acids the presence of linoleic acid was particularly noted next to oleic, palmitic and stearic acids, which were found to be present in smaller amounts in stratified and non-stratified seeds at different intervals prior to and during germination. The results indicate that before germination, especially sugars are utilized although they are not the main reserve substance. The decomposition of reserve triglycerides initiates in already germinating seeds and is associated with an increase in the amount of soluble sugars.

By using incubation with radioactive glycerol, a synthesis of lipids was observed during the induction of germination. The synthesis of complex lipids was more pronounced than that of simple lipids in all measurements. By the use of autoradiography it was demonstrated that nearly all spots of complex lipids were radioactive, whereas from simple lipids only diglycerides but not triglycerides, exhibited radioactivity.

When the incorporation of radioactive glycerol into the lipid fraction, the aqueous fraction and CO_2 were examined, the highest radioactivity was found in CO_2, whereas the aqueous fraction was less radioactive and the lipid fraction exhibited the lowest radioactivity. Quantitative incorporation was similar in all three fractions in stratified as well as non-stratified seeds. However, the rate of incorporation was found to be exactly reverse.

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IZVLEČEK

METABOLIZEM LIPIDOV V SEMENIH JELKE (*ABIES ALBA* MILL.)

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Lipidi so glavna rezervna substanca v semenih jelke (*Abies alba* Mill.). Največji odstotek maščobnih kislin predstavljajo nenasičene maščobne kisline. Od določenih maščobnih kislin je največ linolne, nato oleinske, palmitinske in stearinske. To velja za stratificirana in nestratificirana semena v različnih obdobjih pred in med kalitvijo. Iz rezultatov je razvidno, da se pred kalitvijo porabljajo predvsem sladkorji, čeprav niso glavna rezervna substanca. Razgradnja rezervnih trigliceridov se prične šele pri kalečih semenih. V tem času opazimo povečanje količine topnih sladkorjev.

Pri semenih induciranih h kalitvi smo ugotovili sintezo lipidov z inkubacijo v radioaktivnem glicerolu. Pri vseh poskusih se je sintetiziralo več kompleksnih kot enostavnih lipidov. Z avtoradiografijo smo ugotovili, da so pri kompleksnih lipidih skoroj vse lise radioaktivne, medtem ko so pri enostavnih samo digliceridi.

Pri ugotavljanju vgrajevanja radioaktivnega glicerola v lipidno frakcijo, vodotopno in CO_2, smo dobili daleč največjo radioaktivnost v CO_2, nato v vodotopni frakciji in najmanjšo v lipidni. Vgrajevanje pri stratificiranih in nestratificiranih semenih pred kalitvijo je količinsko podobno v vseh treh frakcijah, potek vgrajevanja pa je prav nasproten.

SAŽETAK

METABOLIZAM LIPIDA U SJEMENKAMA JELE (*ABIES ALBA* MILL.)

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Lipidi su glavna rezervna tvar u sjemenkama jele (*Abies alba* Mill.). Najveći postotak masnih kiselina čine nezasićene masne kiseline. Od određenih masnih kiselina ima najviše linolne, zatim oleinske, palmitinske i stearinske kiseline. To vrijedi i za stratificirane i nestratificirane sjemenke

u različitim razdobljima prije i za vrijeme klijanja. Iz rezultata je vidljivo da se prije klijanja troše prije svega šećeri, iako oni nisu glavna pričuvna tvar. Razgradnja rezervnih triglicerida počinje tek u sjemenaka koje kliju. U to vrijeme primjećujemo povećanje količine topivih šećera.

Kod sjemenki pobuđenih na klijanje utvrdili smo sintezu lipida s pomoću inkubacije u radioaktivnom glicerolu. Pri svim pokusima sintetiziralo se više kompleksnih nego jednostavnih lipida. S pomoću autoradiografije utvrdili smo da su u komplesnih lipida gotovo sve mrlje radioaktivne, dok su u jednostavnih lipida radioaktivni samo digliceridi.

Pri utvrđivanju ugrađenog radioaktivnog glicerola u lipidnu frakciju, frakciju topivu u vodi i CO_2, najveća je bila radioaktivnost u CO_2, zatim u frakciji topivoj u vodi, a najmanja u lipidnoj frakciji. Ugrađivanje kod stratificiranih i nestratificiranih sjemenki prije klijanja je kvantitativno slično u svim trima frakcijama, dok je tijek ugrađivanja upravo suprotan.

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