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Influence of Temperature and Seed Ripening on the *in vivo* Incorporation of $^{14}CO_2$ into the Lipids of Oat Grains (*Avena sativa* L.)[1]

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ABSTRACT

To elucidate the influence of growth temperature and of stage of maturity on lipid synthesis in seeds, oat plants (*Avena sativa* nuda L., variety NOS) were fed with $^{14}CO_2$ at different stages after flowering, and the ^{14}C-incorporation into the grain lipids was determined at 2, 24, and 48 hours after the end of $^{14}CO_2$-application. By changing growth temperature from 12 C to 28 C after the application of $^{14}CO_2$ to intact plants, a higher ^{14}C-labeling of saturated fatty acids was found at the higher temperature. At 28 C, palmitic and stearic acids contained 23% and 9% respectively of total fatty acid-^{14}C shortly after the $^{14}CO_2$-application, whereas at 12 C the corresponding values were 19% and 4%, respectively. Within 2 days ^{14}C-activity of saturated fatty acids decreased at both temperatures, but to a lesser degree at 28 C. The higher ^{14}C-labeling of saturated fatty acids and its lower decrease within 2 days at 28 C clearly show a direct influence of temperature on fatty acid biosynthesis in oat grains.

At all stages of grain growth, oleic acid had the highest ^{14}C-activity of all fatty acids shortly after the $^{14}CO_2$-application. However, ^{14}C activity of oleic acid rapidly decreases in favor of linoleic acid. With increasing maturity, the intensity of lipid synthesis in the grains decreases; simultaneously, the relative amount of ^{14}C-saturated fatty acids increases primarily at the expense of ^{14}C-oleic acid. These tendencies, which were observed in oat plants grown at day temperatures of 12 C during seed development, seem to be paralleled by lipid synthesis in younger grains grown at day temperatures of 28 C. This indicates an indirect influence of growth temperature on lipid synthesis in oat grains during maturation.

Previous work has shown that a higher percentage of unsaturated fatty acids accumulates in oat grains grown at day temperatures of 12 C during seed development compared to those grown at 28 C (1), thus indicating that fatty acid biosynthesis in oat grains responds to growth temperature in a similar way as in oil-accumulating seeds (4, 8). Interpretation of the sites of influence of temperature on lipid synthesis in seeds grown at different climates is always difficult, because temperature changes may affect lipid metabolism directly as well as indirectly. Studies of the fatty acid composition in different tissues as well as in lipid classes of oat grains grown at different temperatures during maturation suggest a direct effect of growth temperature on lipid synthesis in intact grains (2, 3).

To clarify the nature of these temperature effects, the incorporation of $^{14}CO_2$ into the grain lipids was studied at different stages of grain maturation—grain growth at day temperatures of 12 C and 28 C respectively—to determine whether different growth temperatures influence grain lipids by acceleration or retardation of maturity. As a second objective, a $^{14}CO_2$ feeding experiment with intact plants was undertaken to indicate whether there is a direct effect of growth temperature on fatty acid synthesis in grains.

MATERIALS AND METHODS

Oat plants (*Avena sativa* nuda L., var. NOS) were cultivated until flowering in pots in the greenhouse on a soil-sand mixture with normal fertilization. At this stage the plants were transferred to climate rooms at day temperatures of 12 C and 28 C, respectively. All other growth factors were held constant: 16 hr at 20,000 lux using mercury high vapor lamps, 70% relative humidity/day and night, and night temperatures of 12 C in both rooms.

Plants were exposed to $^{14}CO_2$ at different stages after flowering. For this purpose a Plexiglas chamber (150 × 30 × 90 cm) was used. The evening before ^{14}C-feeding three pots were transferred into this chamber for adaptation at 12 C and 28 C, respectively. One hour before $^{14}CO_2$-generation, CO_2-free air was pumped through the chamber. While still in the dark, $^{14}CO_2$ was generated from 2 mc $Ba^{14}CO_3$ (51 mc/mmole). After $^{14}CO_2$ had become distributed uniformly within the chamber, the light source (20,000 lux) was switched on. After 2 hr of $^{14}CO_2$-application, the nonassimilated $^{14}CO_2$ was evacuated from the chamber, and the plants were kept in the chamber for additional 2, 24, or 48 hr. Immediately after harvest, the grains were lyophilized or put in a deep freeze (−20 C) prior to lyophilization.

Lipids were extracted from dried intact grains by chloroform:methanol:water (8:4:3 v/v) by the method of Winter (26). After weighing the crude lipids they were esterified with BF_3-CH_3OH (20). The analysis of the ^{14}C-fatty acids was done by gas liquid chromatography with the Fraktometer F 6/4 (Bodenseewerk Perkin Elmer). A combustion oven for the oxidation of the ^{14}C-acids to $^{14}CO_2$ was installed between the column (2 m, 15% ethylene glycol succinate on Celite 454) and the thermal conductivity detector. The $^{14}CO_2$ was collected manually in scintillation vials which contained 10 ml of scintillator solution (5.5 g diphenyloxazole + 50 ml ethanola-

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[3] Abbreviation: DAF: days after flowering.

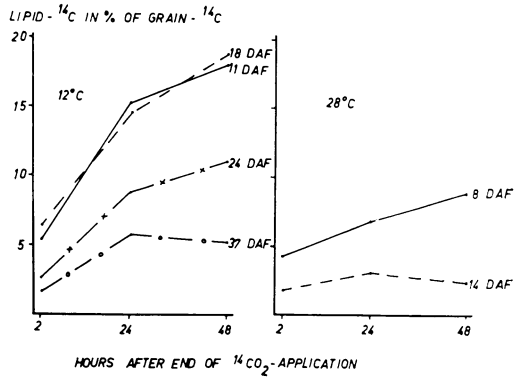

FIG. 1. Incorporation of ^{14}C into oat grain lipids within 48 hr at different stages of seed growth. Left: plants grown from flowering till end of $^{14}CO_2$-experiment at 12 C; right: plants grown from flowering till end of $^{14}CO_2$-experiment at day temperatures of 28 C.

mine + 500 ml toluene + 400 ml ethylene glycol monomethyl ether, [9]). A Packard Model 3310 liquid scintillation counter was used for measurement of ^{14}C. Lipid extractions were done in duplicate and from each extract three fatty acid analyses were made. The total ^{14}C in grains and lipid extracts was determined by Schoeniger digestion (17).

RESULTS

Influence of Seed Development at Different Temperatures on Fatty Acid Synthesis in Grains. Lipid synthesis in oat grains at different stages of grain development and growth temperatures is given in Figure 1. As measure of lipid synthesis, the lipid-^{14}C is expressed as a percentage of total grain-^{14}C. This was necessary because in *in vivo* experiments the absolute amounts of labeled lipids in the grains differ according to the physiological state on the day of $^{14}CO_2$ application, according to the rate of $^{14}CO_2$-assimilation and to the translocation of labeled substances to the grain.

Two important conclusions may be drawn from Figure 1: (*a*) the ^{14}C-incorporation in lipids decreased with increasing maturity. This occurred at both temperatures. (*b*) Incorporation of $^{14}CO_2$ or the transformation of labeled assimilates into grain lipid was rapid. Although all curves are based on only three points, the higher slopes in the plants grown at 12 C indicate a higher rate of lipid synthesis.

The ^{14}C-fatty acid patterns obtained at different stages of seed development are shown in Figure 2.

The salient feature of the results with plants of the 12 C-maturation series (the four upper graphs in Fig. 2) is that at all stages of seed development subsequent to the cessation of $^{14}CO_2$-application, more than 55% of total fatty acid-^{14}C was located in oleic acid. This percentage was still higher at earlier days after flowering. Within 2 days the activity in oleic acid dropped sharply, while the activity in linoleic acid increased concomitantly. Besides oleic acid-^{14}C the ^{14}C-content of the two saturated fatty acids within the 2 days of each experiment also decreased, although to a much lesser degree. Considering the question of the ^{14}C-incorporation into the lipids at different stages of grain development, it is important to note that the ^{14}C-label in the saturated fatty acids increased from 11 to 37 DAF.[3] This clearly indicates that at later stages of maturity the relative synthesis of saturated fatty acids is slightly preferred.

The tendencies of ^{14}C-fatty acid synthesis in the 12 C-maturation series, *i.e.*, (*a*) increasing ^{14}C-labeling of saturated acids and (*b*) decreasing ^{14}C-levels of oleic acid with increasing maturation, seem to be continued by the ^{14}C-labeling pattern of the 28 C-maturation series (lower part of Fig. 2). Again the highest label is associated with oleic acid. It decreases quickly in favor of linoleic acid so that after 24 hr ^{14}C-linoleic acid is higher than ^{14}C-oleic acid. In addition, the percentage of ^{14}C-16:0 is higher and of ^{14}C-18:1 is lower in the eight DAF-28 C-grain lipids than the corresponding values in the 37 DAF-12 C grain lipids. As can be seen from the decreasing grain moisture of the eight DAF grains to the 14 DAF grains in the 28 C maturation series, the high growth temperature accelerated the maturation process enormously. This may be the reason not only for the poor transformation of ^{14}C-18:1 to ^{14}C-18:2 and for the still higher labeling of saturated acids, but also for the low intensity of ^{14}C-lipid synthesis in the 14 DAF-28 C grains (Fig. 1).

Evidence for a Direct Effect of Temperature on Fatty Acid Synthesis. The direct regulation of fatty acid synthesis by temperature was demonstrated by another *in vivo* experiment in which the temperature was varied only from the end of $^{14}CO_2$-application till the end of the experiment 2 days later. The results are given in Table I. All plants of this experiment had been in the same physiological state. This conclusion is based upon the comparable quotient of specific radioactivity in the grain to the specific radioactivity in the grain lipids at the corresponding periods after the $^{14}CO_2$-application, although the transport of ^{14}C-assimilates was slightly less within the first 24 hr at 12 C. Furthermore, all grain samples of Table I were of nearly the same size (13.9 mg dry weight/grain), lipid content (12.0 g/100 g dry matter), and ^{12}C-fatty acid pattern.

The relative distribution of ^{14}C on the different fatty acids clearly shows the influence of temperature. At 28 C 2 hr after the end of $^{14}CO_2$-application, 31.6% of the total fatty acid-^{14}C are located in palmitic and stearic acid, whereas at 12 C only 23.1% are found in the saturated fatty acids. These ^{14}C percentages are compensated by lower or higher labeling primarily of oleic acid. The higher labeling of the saturated acids in all three samples at the high temperature is a strong argument that under *in vivo* conditions temperature controls fatty acid synthesis in intact seeds by a direct mechanism.

The relative specific activities of fatty acids, expressed as ratios of radioactivity percentage to mass percentage, are given in Table II. They again demonstrate the influence of temperature; namely at the high temperature both saturated fatty acids are more labeled and oleic acid is less labeled than at 12 C. In addition to this, stearic acid has the highest labeling of all fatty acids at both temperatures. Within 2 days, the relative specific radioactivity of stearic acid drops by 70% at 12 C and by 65% at 28 C.

The labeling of palmitic acid is also higher and decreases with less intensity at higher temperatures. This indicates the regulating effect of temperature between saturated and unsaturated fatty acids and agrees well with earlier results with oat, which have shown that the growth temperature determines primarily the ratio of saturated to unsaturated fatty acids (1, 3).

DISCUSSION

In vitro experiments on the incorporation of radioactive materials into fatty acids are suitable to elucidate biochemical pathways in detail. But the question remains what relevance single reactions may have for the metabolism of an entire organism. Therefore $^{14}CO_2$, which was found more effective in labeling grain lipids than ^{14}C-acetate, was fed to intact plants to learn something about physiological and agriculturally important aspects of lipid synthesis in seeds.

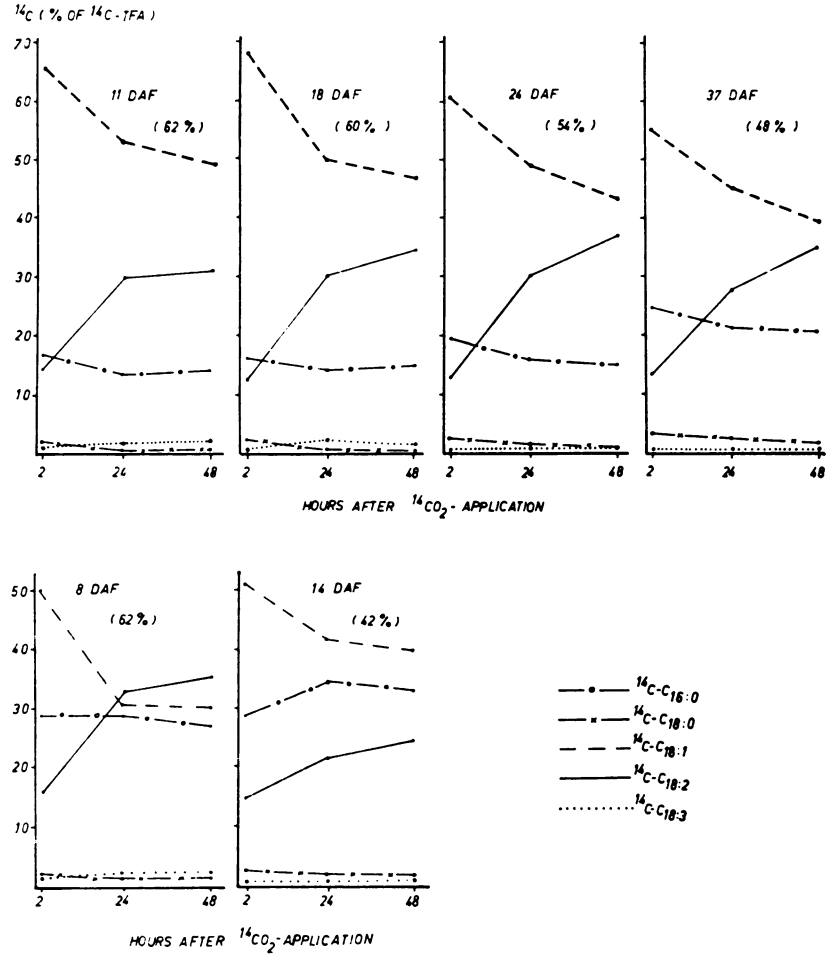

FIG. 2. Influence of grain developmental stage and growth temperature on the distribution of ^{14}C on individual fatty acids. TFA: Total fatty acids; %: grain moisture. Top: Plants grown and fed with $^{14}CO_2$ at 12 C; bottom: plants grown and fed with $^{14}CO_2$ at 28 C.

Table I. *Influence of Temperature on the ^{14}C-Incorporation into the Grain Lipids of Oat during 2 Days*

Plants were grown from flowering till 24 DAF at 12 C. At this day $^{14}CO_2$ was applied for 2 hr at 12 C, and then the temperature was kept at 12 C or changed to 28 C, respectively.

Temperature	Hours after $^{14}CO_2$ Application	Specific Radioactivity		^{14}C in % of Total Fatty Acid ^{14}C						
		Grain	Lipid	<16:0	16:0	18:0	18:1	18:2	18:3	Residue
		μc/g								
12 C	2	2.64	0.57	0.7	19.4	3.7	60.5	13.3	0.5	1.9
	24	11.76	8.84	0.5	16.1	1.4	48.9	30.2	1.1	1.7
	48	15.12	13.87	0.3	15.2	1.1	43.4	37.1	1.2	1.7
28 C	2	5.14	1.21	0.8	22.7	8.9	48.7	16.4	0.7	1.8
	24	12.74	10.37	0.6	21.8	3.4	40.1	31.7	1.0	1.4
	48	14.48	13.58	0.6	19.5	3.1	39.2	34.8	1.2	1.6
				% ^{13}C-fatty acid composition						
					13.5	0.8	43.6	39.8	2.3	

In accordance with experiments with plants having oil-accumulating seeds (5, 7, 25), oleic acid is the predominant fatty acid labeled in oat grains. This could support the suggestion of separate pathways for the synthesis of saturated and unsaturated fatty acids in higher plants (14, 18, 19). If this were the case, the relative specific radioactivity of stearic acid would be expected to increase rather than to decrease (Table II) because labeled substrates were translocated to the grain steadily and stearic acid would be the terminal saturated fatty acid (Table I). So the *in vivo* incorporation of ^{14}C in the fatty acids of oat grains favors the direct desaturation of stearic acid as the mechanism of oleic acid synthesis (2, 13, 15, 16, 22). Oleic acid may then be converted into a special structure before it can be desaturated to yield linoleic acid (10, 23).

Feeding $^{14}CO_2$ to oat plants at different stages of grain maturation at 12 C and 28 C respectively (Fig. 1, 2) shows that at both temperatures lipid synthesis and the relative amount of ^{14}C-oleic acid decrease with increasing maturity, whereas percentages of ^{14}C-saturated acids increase. This in-

Table II. *Influence of Temperature on the Relative Specific Radioactivity of Fatty Acids*

Temperature	Hours after $^{14}CO_2$-Application	Fatty Acid				
		16:0	18:0	18:1	18:2	18:3
12 C	2	1.44	4.63	1.39	0.33	0.22
	24	1.19	1.75	1.12	0.76	0.48
	48	1.13	1.38	0.95	0.91	0.52
28 C	2	1.68	11.11	1.12	0.41	0.30
	24	1.62	4.25	0.92	0.80	0.44
	48	1.45	3.88	0.90	0.88	0.52

dicates a relatively stronger synthesis of saturated fatty acids in older than in younger oat grains, and points to the fact that patterns of newly synthesized fatty acids vary during grain growth. Probably in young grains with an intense lipid synthesis a relatively higher amount of synthesized ^{14}C-18:1 is separated from the synthesizing enzyme system, incorporated into lipids, and deposited in a "storage pool." Consequently, the percentage of ^{14}C-18:1 would be lower if $^{14}CO_2$ is applied to older plants. Synthesis of ^{14}C-lipid and the distribution of ^{14}C in fatty acids in the 28 C-maturation series seem to be only the continuation of the tendencies observed in the different stages of grain growth at 12 C. Such an assumption might imply that the climate, *i.e.*, the growth temperature, controls fatty acid synthesis primarily via the control of the intensity of grain ripening. However, the comparison of the ^{14}C-distribution between individual fatty acids in the 12 C and 28 C series at comparable stages of maturity (as indicated by moisture content) strongly indicates a more specific effect of temperature on lipid metabolism. This specific effect may be related to a different enzyme synthesis or enzyme pattern at the two temperatures (21) or with different enzyme activities (Table II).

A direct attack of growth temperature on fatty acid synthesis in the grains could be demonstrated by variation of temperature after cessation of $^{14}CO_2$ application (Table I and II). This result with intact plants is in agreement with *in vitro* studies on the influence of temperature on lipid synthesis in seeds of other plant genera (11, 12, 24). Taking stearic acid as a precursor of oleic acid synthesis and considering the importance of dissolved oxygen for the activity of desaturases (6, 11, 12), temperature seems to control primarily the desaturation of stearic acid. Two hours after the end of the $^{14}CO_2$ application the relative specific activity of this acid at 28 C is 2.4 times higher, and the decrease within 48 hours is less than at 12 C.

The reported results indicate that the temperature during grain development modifies fatty acid synthesis directly as well as indirectly. Higher growth temperatures shorten the time of grain growth, thereby pushing the relatively stronger synthesis of saturated fatty acids, observed in the later stages of maturation at 12 C, into the period of intense lipid synthesis. This indirect effect is amplified by a direct temperature effect, because at higher temperatures the transformation of saturated to unsaturated fatty acids is decreased.

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