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Short-term effects of cytokinins on the lipid fatty acids of green leaves

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Isolated shoots of *Coleus blumei* and isolated leaves from this species and from *Impatiens sultani* were fed zeatin for different periods. High doses of the hormone (100 μg/ml) caused a measurable effect on lipid fatty acid composition after a two-hour feeding period in isolated leaves of *Coleus*. The maximum effects were at 20–30 μg zeatin taken up per g fresh weight. With the leaves of *Impatiens*, higher doses of the hormone were needed to obtain optimal effects. In both species, the proportion of linolenic acid increased and that of palmitic acid decreased. For higher concentrations, the reverse was true. The effects of the hormone on the fatty acids of glycolipids were more apparent than on those of phospholipids. The results are discussed in view of the general importance of dose-response curves for cytokinin effects and the possible effects on cell membranes.

Key words: *Coleus blumei* — Cytokinins — Dose-response curve — Fatty acids — *Impatiens sultani*.

The effects of cytokinins on leaf lipids are well known. In spinach chloroplasts, the degradation of fatty acids is inhibited by kinetin (*6*). In five out of seven species investigated, an increase in lipid concentration after kinetin application has been observed (*9*). Treatment of leaves from intact plants with kinetin and zeatin for a period of four weeks mostly leads to an increase of linolenic acid, and in some experiments, a decrease of palmitic acid. After application of high doses of zeatin, however, reverse alterations occur (*11*). Application of kinetin to soybean callus causes an increase in linolenic acid and a decrease in palmitic acid percentages (*25*). In some investigations, responses to cytokinins could be observed after a short time. The water permeability of cells is affected after less than 2 min (*13*), and the ion permeability of senescing leaves is affected after 5 hr (*17*). Effects on lipid synthesis occur in tobacco buds after 6 hr (*22*) and in etiolated *Cicer* buds as early as 1 hr after application of benzylaminopurine (*29*). Several investigations show that cytokinins have diverse effects on different cell organelles (*5, 27*). Therefore, different effects of cytokinin application on glyco- and phospholipids seem possible and some preliminary experiments indicated this (*11*).

Most of the research hitherto was done with kinetin. We used the naturally occurring cytokinin zeatin in most experiments. Its effects are not in all cases identical to those of kinetin (*3, 31*); probably the translocation rates in tissues are different (*3*).

Abbreviations: GLC, gas-liquid chromatography; TLC, thin-layer chromatography.

In this investigation, after scrutinizing earlier described long-term effects of zeatin on intact plants, the effect of a short-term application of cytokinin on the fatty acid composition of total saponifiable lipids and of glyco- and phospholipids of green leaf tissues was determined. First, isolated shoots of *Coleus* were used. To find out how soon effects are measurable, further experiments were done with isolated leaves. With high doses of zeatin, an effect was found after 2 to 4 hr. In order to rule out the possibility that the results obtained with *Coleus* are species specific, some similar experiments were carried out with leaves of *Impatiens*.

Materials and methods

Plants of *Coleus blumei* Benth. and *Impatiens sultani* Hook. were propagated vegetatively from clones as done previously (*11*).

Isolated shoots of *Coleus* and isolated leaves of both species were placed in cytokinin solutions (1, 10 and 100 μg/ml) from their stalk bases and petioles, respectively. Controls were placed into water in an identical manner. In some experiments with *Impatiens*, the cytokinins were vacuum-infiltrated into the leaves. Only adult, fully expanded leaves were used throughout. In some supplementary long-term experiments, zeatin was applied with a pencil as described earlier (*11*).

Total lipids were extracted by the method of Bligh and Dyer (*1*). Saponification and preparation for GLC were performed as described elsewhere (*10*). GLC data: Varian Aerograph 2740; column: 20′ × 1/8″ stainless steel, 10% EGSS-X on 60–80 Chromosorb W/AW-DMCS; column temperature: 180°C; detector: FID, 280°C; injector temperature: 210°C; carrier gas: N_2, 30 ml/min. All analyses were repeated at least twice and the average value was taken. The separation of glycolipids, phospholipids and neutral lipids by TLC (acetone-benzene-water, 91 : 30 : 8, v/v) and the following transmethylation with sodium methylate were done according to the procedure of Pohl et al. (*20*).

The standard deviations, SD, are shown in the figures. With regard to the major components of total fatty acids (palmitic, linoleic and linolenic acids), in most cases the differences between controls and treated leaves in the range of 1.0 to 1.8% are significant to $p=0.05$. In the fraction of neutral lipids, no significant differences ($p<0.1$) were observed, and their behavior is not discussed further. In the different controls of the separate groups of experiments, differences appear in the percentages of the fatty acids. This may be traced back to seasonal and, according to Chapman et al. (*4*), to daily variations. Therefore, only from one group of experiments are the percentages of all fatty acids for the different controls and experiments shown as an example in Table 1.

The ratio of the principal multiunsaturated acids (linolenic and linoleic acids) to palmitic acid as the principal saturated fatty acid is called the molar quotient. If this ratio becomes greater it indicates an increase of multiunsaturated fatty acids.

Results

Long-term experiments. Some long-term experiments were performed in the same manner as those carried out earlier (*11*) to ensure that the effects would be

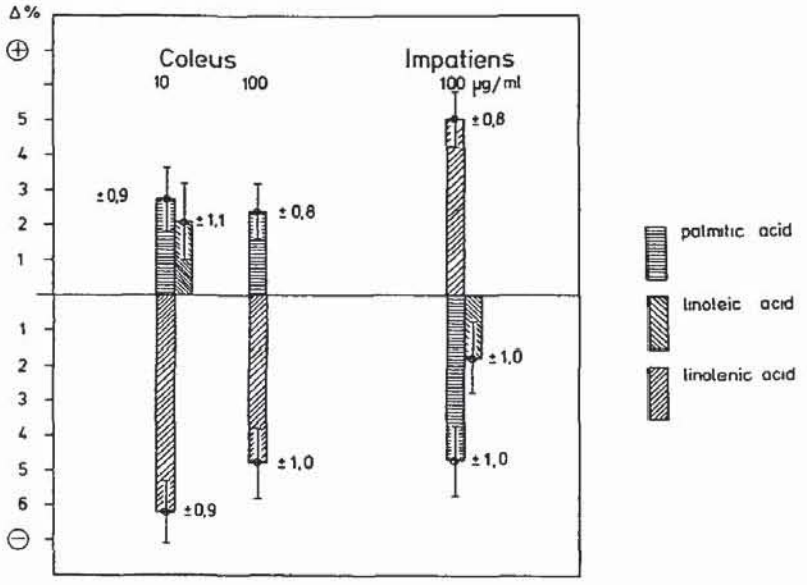

Fig. 1. *Effect of zeatin on fatty acid composition of leaves; long-term experiments.* Zeatin was applied four times at weekly intervals to intact plants of *Coleus blumei* and *Impatiens sultani*; plants were harvested one week after the last application. The diagrams show differences (as % of total fatty acids) between controls and hormone-treated leaves.

as described and to investigate the fractions of phospho- and glycolipids. Fig. 1 shows that in *Coleus*, when 10 and 100 µg/ml zeatin were used, the content of linolenic acid decreased and that of palmitic acid increased. Therefore, the molar quotient is decreased by zeatin in *Coleus*. In *Impatiens*, using 100 µg/ml zeatin, the reverse occurs (Fig. 1).

Total lipids from the *Coleus* experiments were fractionated. When 100 µg/ml of the cytokinin was used, in phospholipids the proportion of linolenic and linoleic acids decreased by 8.5 and 4%, respectively. The relative content of linolenic and linoleic acids in glycolipids increased by 6.5 and 7.1%, respectively. Using 10 µg/ml of hormone, similar results were obtained.

Experiments with whole shoots. The experiments were carried out with *Coleus* shoots bearing six to eight expanded leaves. First, different concentrations of zeatin were fed for 24 hr. The effect of zeatin decreased remarkably with increasing hormone concentrations (Fig. 2a). In the next set of experiments, the zeatin concentration was kept constant at 1 µg/ml and the feeding time was increased from 24 hr to 48 hr and 96 hr (Fig. 2b). The results show that after 96 hr, a situation inverse to that after 24 hr occurs. A rather small effect occurs after 48 hr; there is only one significant difference in the palmitic acid content between control and hormone-treated leaves. Further shortening of the application period with *Coleus* shoots led to that a clear effect on leaf fatty acids after 4 hr (using 100 µg/ml zeatin; Fig. 2c). Fractionation of total lipids of this experiment showed that in the phospholipids, only the percentage of linolenic acid was altered significantly (decrease by 5.1%; $p=0.1$), whereas in glycolipids, the percentage of linolenic and linoleic acids increased by 10.4 and 6.9%, respectively. Palmitic acid decreased by 9%.

Comparison of the treatments of whole shoots with 1 µg/ml for 24 hr and with

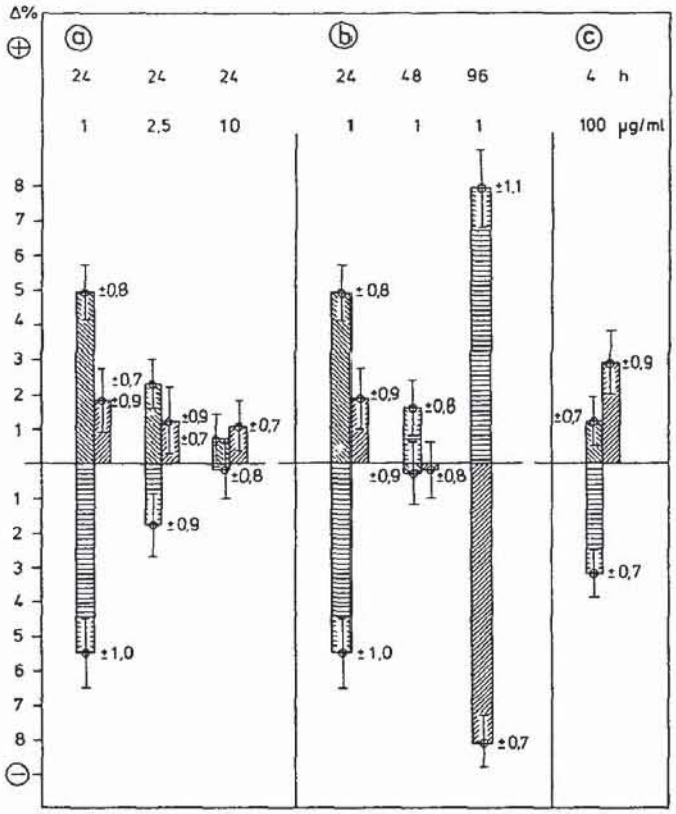

Fig. 2. *Effect of zeatin on fatty acid composition of leaves after shoots of* Coleus *were placed in hormone solutions from their stalk bases.* (a) Different hormone concentrations fed for 24 hr. (b) A hormone solution of 1 µg/ml fed for different periods. (c) A hormone solution of 100 µg/ml fed for 4 hr. When a significant difference of a distinct fatty acid was measured in some experiments from a group and the alterations were not significant in others, the results of both are shown [as in (a) 10 µg/ml and (b) 48 hr]. Symbols as in Fig. 1.

100 µg/ml for 4 hr with the treatment with 1 µg/ml for 96 hr indicates that high amounts of zeatin have an opposite effect on leaf fatty acids to that of low amounts from more dilute solutions or very short application periods.

Experiments with isolated leaves. In further experiments, isolated *Coleus* leaves of identical size were fed through the petioles with zeatin. The quantity of the hormone taken up per g fresh weight could be approximately calculated from the amount of solution taken up by the leaves. Using low concentrations of zeatin, we performed experiments with a feeding period of 24 hr (Fig. 3). The amount of linolenic acid increased. Higher zeatin concentrations also led to an increase of linoleic acid. The percentage of palmitic acid decreased; therefore the molar quotient rose. The tendency observed for whole shoots treated with 2.5 µg/ml zeatin for 24 hr (Fig. 2a) was more pronounced in the experiments with isolated leaves treated with roughly the same concentration of 3 µg/ml for the same period (Fig. 3). With high hormone concentrations (using solutions of 100 µg/ml), the feeding time could be reduced to four and in some experiments to two hours, and still a distinct effect was observable (Fig. 4). The inversion of the percentage differences of linolenic and palmitic acids appearing at higher cytokinin concen-

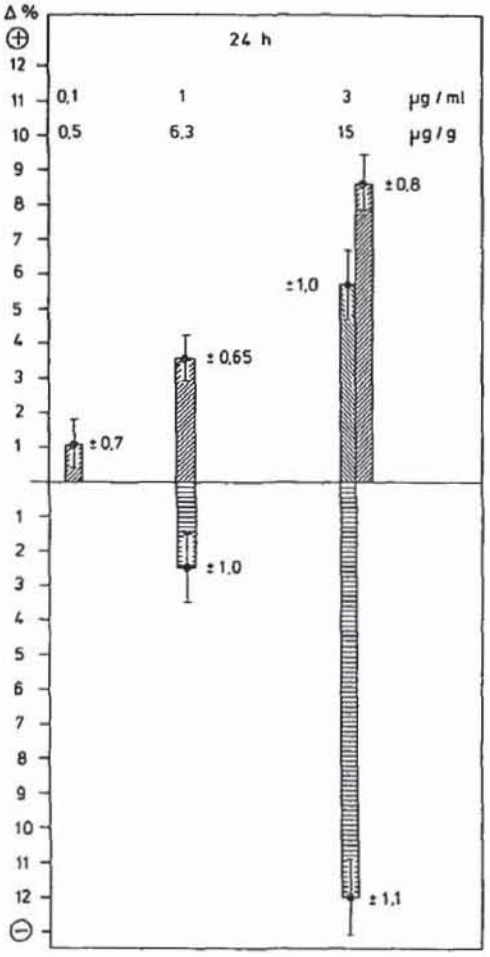

Fig. 3. *Effect of 24-hr zeatin application on fatty acid composition of isolated* Coleus *leaves.* The amount of cytokinin taken up by the leaf tissue is represented by µg/g fresh weight. Symbols as in Fig. 1.

trations may be seen from Fig. 4. As an example, the complete results of this group of experiments are shown in Table 1. (In another set of similar experiments performed six months later, the changes of palmitic and linolenic acids were essentially the same, but quantitatively only about two-thirds of those shown in Table 1). In all the experiments lasting 2 to 4 hr, the most distinct rise of the multiunsaturated acids occurred when concentrations of 20–30 µg zeatin were taken up per g leaf fresh weight; higher concentrations always decreased the proportion of linolenic and often also of linoleic acids. From some of the experiments, the fatty acid composition of phospho- and glycolipids was investigated (Table 2). Especially at the higher zeatin concentrations, that is, when 15 µg hormone or more was taken up per g fresh weight, the glycolipids seemed to be affected more than the phospholipids. However, this is perhaps not true for very high hormone amounts (40 µg zeatin/g).

Experiments with predarkened plants. Plants of *Coleus* were predarkened for 24 hr, then the leaves were isolated and placed into a zeatin solution (100 µg/ml) for 2 hr in the dark and in light, respectively. We tested whether zeatin could be a substitute for the influence of light in short-term effects on lipids. The results showed a greater conformity between the different zeatin-fed leaves than between leaves fed zeatin in the dark and light controls. The effects of total fatty acids, were

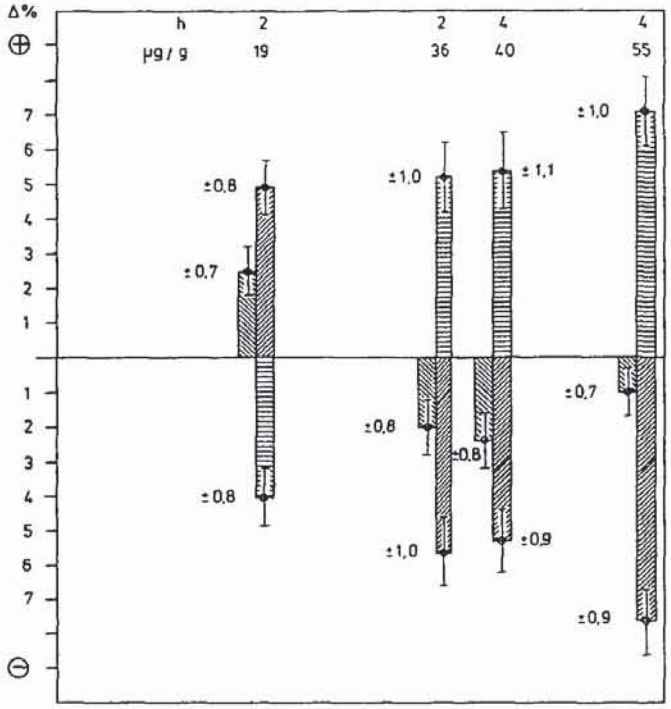

Fig. 4. *Effect of 2- and 4-hr zeatin applications (solution of 100 µg/ml) on fatty acid composition of isolated* Coleus *leaves.* The amount of cytokinin taken up by the leaf tissue is represented by µg/g fresh weight. Symbols as in Fig. 1.

Table 1 *Fatty acids of saponifiable lipids from isolated leaves of* Coleus *(as % of total fatty acids) treated with zeatin (100 µg/ml) for 2 and 4 hr, respectively*

Fatty acid	Zeatin application											
	2 hr			2 hr			4 hr			4 hr		
	Hormone taken up: as µg/g fresh weight											
	19			36			40			55		
	co.	zea.	diff.	co.	zea.	diff.	co.	zea.	diff.	co.	zea.	diff.
Short chain	2.3	1.5	−0.8	1.2	0.4	−0.8	1.2	0.7	−0.5	1.9	0.8	−1.1
14:1	0.7	0.4	−0.3	1.1	1.2	+0.1	0.7	1.1	+0.4	0.5	1.3	+0.8
16:0	22.7	18.7	−4.0 a	22.1	27.3	+5.2 a	21.9	27.3	+5.4 a	21.5	28.6	+7.1 a
16:1	2.3	2.3	0	2.3	3.5	+1.2	1.8	2.7	+0.9	1.6	2.4	+0.8
16:3	0.4	0.3	−0.1	1.0	1.1	+0.1	0.6	0.8	+0.2	0.5	0.8	+0.3
18:0	4.1	2.7	−1.4	4.1	4.9	+0.8	3.8	4.6	+0.8	3.9	4.6	+0.7
18:1	2.0	1.2	−0.8	2.2	3.2	+1.0	2.4	2.9	+0.5	2.6	2.6	0
18:2	22.9	25.4	+2.5 a	23.2	21.2	−2.0	21.9	19.5	−2.4 a	21.5	20.5	−1.0
18:3	42.6	47.5	+4.9 a	42.8	37.2	−5.6 a	45.7	40.4	−5.3 a	46.0	38.4	−7.6 a
m.q.	2.7	3.6		2.7	2.0		2.8	2.0		2.9	1.9	

co. = control (water-treated).
zea. = zeatin-treated.
diff. = difference between controls and zeatin-treated leaves.
m.q. = molar quotient 18 : 2+18 : 3 / 16 : 0.
a = level of significance p = 0.05 or better.

Table 2 *Effects of short-term zeatin applications on fatty acid composition of phospho- and glycolipids in isolated* Coleus *leaves*

Fatty acid	Zeatin application			
	1 μg/ml 24 hr	3 μg/ml 24 hr	100 μg/ml 2 hr	100 μg/ml 4 hr
	Hormone taken up: as μg/g fresh weight			
	6.3	15	19	40
Phospholipids:				
16:0	n.s.	n.s.	n.s.	n.s.
18:2	+5.6 b	n.s.	−5.8 b	n.s.
18:3	n.s.	+4.0 c	n.s.	−6.0 b
Glycolipids:				
16:0	−8.9 b	−11.8 a	−9.9 a	+5.2 c
18:2	n.s.	+11.0 b	+6.2 b	n.s.
18:3	+16.0 a	+16.5 a	+12.4 a	−6.1 b

Differences against controls are shown (as % of total fatty acids).
Level of significance: a, p=0.05; b, p=0.1; c, p=0.2; n.s., not significant (in most cases p>0.2).

essentially the same as those shown in Fig. 4. The differences of fatty acid composition of phospho- and glycolipids are shown in Table 3. In the dark, the effects on the phospholipids appeared to be more pronounced, whereas in light they appeared to be more prominent in the fatty acids of the glycolipids. However, we can not fully exclude the possibility that the different effects are caused by the different cytokinin quantities taken up by the leaves.

Experiments with Impatiens. In contrast to the effects on leaves of intact *Impatiens* plants, which showed a prominent response to zeatin treatment (Fig. 1), isolated leaves of this species reacted less distinctly than *Coleus* leaves in the short-term experiments. Therefore, higher hormone concentrations and a longer application period were needed for an effect of the same magnitude as that with *Coleus*.

Table 3 *Effects of zeatin application of 2 hr in light and in darkness on fatty acid composition of phospho- and glycolipids in leaves from predarkened* Coleus *plants*

Fatty acid	Dark	Light
	Hormone taken up: as μg/g fresh weight	
	17	24.5
Phospholipids:		
16:0	−7.3 b	−6.1 b
18:2	+6.0 b	−4.0 c
18:3	+12.5 a	+7.5 b
Glycolipids:		
16:0	−8.0 b	−7.5 b
18:2	n.s.	+5.0 c
18:3	+8.5 a	+12.8 a

Differences against controls are shown (as % of total fatty acids).
Symbols for levels of significance as in Table 2.

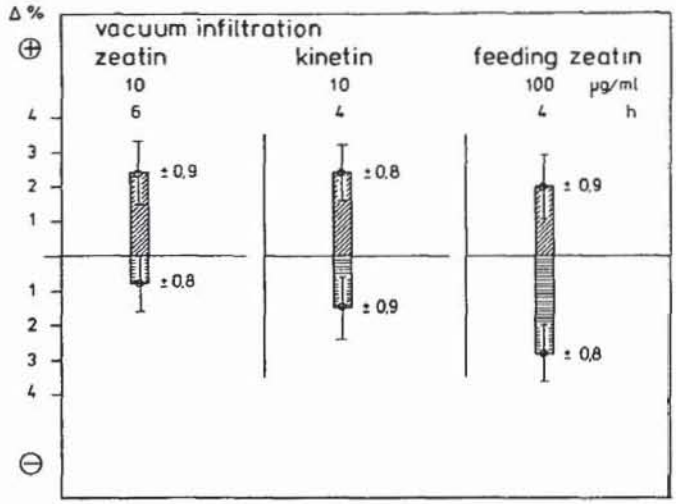

Fig. 5. *Effects of short-term cytokinin applications on fatty acid composition of isolated* Impatiens *leaves.* Application of cytokinin solutions by vacuum infiltration and by feeding through petioles, respectively. Symbols as in Fig. 1.

In experiments where isolated leaves were fed 1 µg/ml zeatin for 24 hr, no significant effects were found. When 10 µg/ml zeatin was used, faint but unequivocal effects could be measured (linolenic acid+2.0%; palmitic acid−1.9%). After reducing the reaction time of the hormone, at least 4 hr was necessary to get significant effects. They could be obtained either by vacuum infiltration of the leaf disks (then 10 µg/ml of zeatin or kinetin was sufficient) or by feeding through the petioles in which application of 100 µg/ml zeatin was necessary (Fig. 5).

Discussion

The effects of cytokinins on lipid fatty acid proportions of green leaves follow a dose-response curve with an optimum, which can be recognized from a maximal increase of linolenic acid and in most cases a decrease of palmitic acid. In earlier experiments, optimum curves were found for the influence of zeatin on respiration rates of leaves (*14*) and isolated mesophyll protoplasts (*12*), on the rate of photosynthesis of protoplasts (*12*), on the permeability of membranes (*18*), in some experiments on steroid levels in tissue cultures (*2*), and in the form of a more complex biphasic dose-response, on K-transport (*24*). In the experiments on leaf respiration, quantitative differences between different species were also found. The effects on fatty acids are distinct with linolenic and palmitic acids, and more variable with linoleic acid. If the same total amounts of hormone are taken up, the application of lower concentrations of cytokinins for a longer period (24 hr, Fig. 3) seems to have a more pronounced effect on the increase of multiunsaturated acids than higher concentrations applied within a few hours (Fig. 4). This agrees with the data of Manos and Goldthwaite (*15*), in which a daily replacement of zeatin solution caused a definite increase in effective concentrations for retarding senescence. The sensitivity of *Coleus* and *Impatiens* is not the same; with *Impatiens* leaves, higher doses of the hormone are needed to obtain optimal effects. The

variable sensitivity of *Coleus* plants with the same zeatin concentrations in different sets of experiments at different times is perhaps caused by a different endogenous cytokinin level of the leaf tissues. Current investigations favor such an explanation.

The influence of cytokinins on the lipid fatty acid composition of green leaves is recognizable after a few hours. This effect may be important in regard to cellular membrane systems. An influence of cytokinins on membrane permeability has been described (*7*, *13*, *17*, *18*) and control of permeation processes by their action on membrane metabolism has been reported (*18*, *19*).

The effect of optimal or near optimal concentrations of cytokinins on fatty acids may be stabilizing, which means an inhibition of degradation especially of multiunsaturated fatty acids (*6*), although a promotion of synthesis cannot be excluded. Our current labelling experiments (*23*) indicate a stimulation of fatty acid synthesis, except of linolenic acid. In experiments with benzylaminopurine, the lipid synthesis in buds of tobacco (*21*, *22*) and *Cicer* (*29*) was stimulated. Very high concentrations of the cytokinin apparently promote degradation of multiunsaturated fatty acids.

The distinct effects on the glycolipid fraction are probably connected with the well-known influence of cytokinins on plastids. The described effects of cytokinins on the stabilization of thylakoid stacking (*16*) may be connected with their influence on glycolipid metabolism. The optimal effects for glycolipids appear to occur at higher concentrations of cytokinin than those for the phospholipids. With this information, it seems interesting that in senescing leaves (*26*) and in cotyledons (*8*), the degradation of glycolipids and some of the phospholipids starts earlier than for some major phospholipids, and a continuous reduction of the cytokinin level in senescing leaves is well known. An alternative explanation for the different sensitivity of phospho- and glycolipids may be an intensified transfer of lipid components to the chloroplasts. Such an increase of chloroplast lipids at the expense of extrachloroplastic components has been demonstrated (*28*, *30*).

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