Interactive Effects of Exogenous Growth Regulators and Artificial Acid Rain on Cytokinins and Gibberellins in Maple Seedlings

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Abstract. Vegetation experiments were conducted under greenhouse conditions in which long-term effects of simulated acid rain on the shoots, and short-term effects of acidified root medium on the roots of two maple species (*Acer pseudoplatanus* L. and *Acer negundo* L.) were studied. Both alternatives of the acidification stress were studied with respect to exogenous applications of auxins and gibberellins. Changes in seedling growth caused by acid rain were not marked, but changes in the levels of endogenous growth regulators were detected. both the long-term exposure of *Acer pseudoplatanus* L. seedling shoots to acid rain and the short-term acidification of root environment resulted in enhanced cytokinin activity and in decreased gibberellin activity. But when *A. pseudoplatanus* L. seedlings were pretreated with gibberellins, their activity was further increased by increased acidity. *Acer negundo* L. seedlings responded to the acidification of their root environment with enhanced cytokinin activity only when pretreated with exogenous auxins. The differences in the responses of *A. pseudoplatanus* L. and *A. negundo* L. to the acidification can be explained in terms of their distinct physiological properties, including changes recorded in chlorophyll content. The results obtained show that a pretreatment of maple seedlings with exogenous growth regulators can modify the effects of increased acidity in the environment on their physiological status as characterized by the level of endogenous cytokinins and gibberellins.

The relationships of forest trees to air pollution factors, including acid rain, can be studied from the viewpoint of total growth and phytopathological, physiological, and biochemical changes (Lendzian and Unsworth 1983, Mudd 1973). As far as physiological processes are concerned, little attention has been devoted to the study of the role of growth regulators (Meyer *et al.* 1987). Phytohormones can modify metabolic and enzymic activities relative to ecological influences. Thus information on phytohormonal changes can also be useful for the assessment of the acidity of the environment. The results obtained in short-term experiments showed a dependence

of the content of endogenous cytokinin-like and gibberellin-like substances in oak seedlings on the severity of the acidification of the environment (Čížková 1990). However, it is the long-term effect of acidity and the character of changes induced in the levels of endogenous growth regulators which are decisive for forest tree vitality under natural conditions. For this reason, changes in the levels of endogenous cytokinin-like and gibberellin-like substances caused by long-term exposure to simulated acid rain were recorded in this study. Besides, the effect of the pretreatment of maple seedlings with exogenous growth regulators on changes in the levels of endogenous cytokinin-like and gibberellin-like substances caused by environment acidification were studied to find out if these changes can be altered in this way. The findings might be applied in forest tree nurseries. Growth regulator applications to forest tree seedlings might result not only in growth rate enhancement but also in a suitable regulation of the phytohormonal status of seedlings grown in regions exposed to acid rain.

MATERIAL AND METHODS

Responses of seedlings of two maple species (field maple *Acer pseudoplatanus* L. and ash-leaved maple *A. negundo* L.) to the acidification of the environment were studied. Maple seeds were stratified during winter season, and germinating seeds with 1 to 2 cm long primary root were transferred to a 1 : 1 (v/v) diluted nutrient solution according to Richter (1926), pH 5.5, supplemented with the A-Z microelement solution (Hoagland and Snyder 1933). Iron was supplied in the form of EDTA. This nutrient solution was renewed once a month. The seedlings were grown for two years in a greenhouse under natural light conditions, with fluctuating air temperatures both during the day and the year. Average air temperature from March to September was 21 °C during the day and 16 °C during the night. From October to February, average (24 h) temperature was 7 °C. From the stage of the first leaf seedlings were sprayed each year five times in one-week intervals with aqueous solutions of auxin (indole-3-acetic acid, IAA, Fluka AG CH-9470 Buchs), or of gibberellin (gibberellic acid, GA$_3$, Phylaxia, Budapest), at a concentration of 10 mg l^{-1}. Control seedlings were sprayed with distilled water.

Acid rain was simulated by spraying seedling shoots with sulphuric acid solution, pH 3.5, and root environment acidification was also simulated by the addition of sulphuric acid solution, pH 3.0. Control plants were treated with distilled water, pH 5.6. Acid spray was applied to *A. pseudoplatanus* seedlings every other day during the growing season at an intensity that resulted in complete moistening of all leaves, but did not cause pollution of the nutrient solution. The total simulated acid rain dose amounted to 60 mm a year. *A. negundo* seedlings were sprayed with sulphuric acid solution only once.

In the first year of seedling cultivation, the levels of endogenous cytokinin-like and gibberellin-like substances and seedling growth parameters were determined in *A.*

pseudoplatanus seedlings four months after transferring the seedlings to nutrient solutions (9 weeks after the last application of exogenous growth regulators and 40 h after the last application of simulated rain). In *A. negundo* the levels of endogenous cytokininelike and gibberellin-like substances were also determined in the first year of cultivation four months after seedling transfer, but only in those which were not treated with growth regulators. Chlorophyll content was determined in control seedlings of both maple species, that were not exposed to any treatment with sulphuric acid or growth regulators.

Simultaneously with seedlings in which during the first year the effect of simulated acid rain was followed, other seedlings grown and treated with exogenous growth regulators were exposed during the second year of cultivation to the effect of acidified root environment. The application of growth regulators and the time interval between the last application and the determination of the levels of endogenous cytokinin-like and gibberellin-like substances were the same as during the first year of cultivation. Seedlings were exposed to the effect of the acidified nutrient solution for three days and then the levels of endogenous cytokinin-like and gibberellin-like substances were determined in both the shoots and roots of *A. pseudoplatanus* and *A. negundo* seedlings.

Besides, growth parameters were determined of those *A. pseudoplatanus* seedlings which were exposed during the first year to the action of exogenous growth regulators and of simulated acid rain. Seedling fresh mass and dry mass were determined, percentage changes in seedling dry mass caused by growth regulator applications and simulated acid rain treatments were calculated, and also relative growth rate (RGR) expressed in terms of mass gain related to total mass of 100 seedlings per time unit, the ratio of root dry mass to total seedling mass (RMR) in percentages, net assimilation rate (NAR) expressed as the increase in total dry mass of 100 sedlings related to leaf dry mass per time unit for the interval of 90 d were calculated (Šesták *et al.* 1971).

Chlorophyll content in leaves was determined spectrophotometrically by measuring the absorbance of acetone extracts and by calculating chlorophyll content according to formulas devised by Vernon (1960).

Endogenous cytokinin-like and gibberellin-like substances (further endogenous cytokinins and gibberellins) were extracted, purified, and quantified as earlier (Čížková 1990). Cytokinin activity was determined after seedling homogenization, exctraction, sample purification, and TLC, by means of the *Amaranthus*-betacyanine bioassay, gibberellin activity by means of the *Lactuca* bioassay.

RESULTS

Changes in growth parameters of *A. pseudoplatanus* seedlings exposed to simulated acid rain were recorded (Table 1). The action of the acidification of nutrient solution was relatively short (three days), and growth changes were

Table 1

Average fresh and dry mass, variation in dry mass percentage, relative growth rate (RGR), root matter ratio (RMR), and net assimilation rate (NAR) of *Acer pseudoplatanus* L. seedlings as affected by rain acidity and exogenous growth regulators.

Year	Rain [pH]	Growth regulator	Fresh mass per 10 seedlings [g]	Dry mass per 10 seedlings [g]	Variation in dry mass [%]		RGR [g g^{-1} d^{-1}]	RMR [%]	NAR [g g^{-1} d^{-1}]
1	5.6	control	14.09	3.13	100	100	1.73	25.88	3.10
		IAA	15.86	3.50	112	100	1.83	24.86	3.21
		GA$_3$	18.10	3.91	126	100	1.92	27.88	1.92
	3.5	control	14.22	2.98	100	95	1.69	26.51	3.04
		IAA	13.49	2.71	91	77	1.62	28.41	3.06
		GA$_3$	16.47	3.87	130	99	1.91	24.81	3.34
2	5.6	control	38.75	8.54	100	100	1.28	24.00	1.63
		IAA	49.91	10.94	128	100	1.34	25.23	1.84
		GA$_3$	52.81	11.51	135	100	1.35	26.93	1.80
	3.5	control	36.35	7.97	100	93	1.27	24.97	1.63
		IAA	36.25*	7.98*	100	73	1.27	24.31	1.60
		GA$_3$	40.98*	9.02*	113	78	1.29	24.17	1.10

Statistically significant differences (G-test) (Fryer 1957) in fresh and dry mass between seedlings sprayed with distilled water (pH 5.6) and with sulphuric acid solution (pH 3.5), between the effect of IAA at pH 5.6 and 3.5, and between the effect of GA$_3$ at pH 5.6 and pH 3.5 are designated with symbol * (P = 0.05, n = 6).

therefore not recorded in this case. Differences in growth parameters were small, because the acidity of the applied sulphuric acid solution and its doses were selected on the basis of results obtained in earlier experiments in such a range that would not cause visible damage and inhibition of seedling growth. Nevertheless, growth changes caused by previous applications of simulated acid rain were recorded in the second year of cultivation in those seedlings which in the first year of cultivation were treated with exogenous auxin and gibberellin. The interaction of the effect of exogenous growth regulators with simulated acid rain was dependent on time. However, the initial lowest growth reduction caused by simulated acid rain in seedlings treated with GA_3, which was recorded in the first year, was marked in the second year. The auxin application was neither stimulatory in relative to simulated acid rain in the case of seedling growth parameters (Table 1).

The reaction of maple seedlings to simulated acid rain and to exogenous growth regulators expressed in terms of chlorophyll content will be described in an other study. But in this paper I wish to state that control A. pseudoplatanus seedlings had a higher chlorophyll content (4.89 mg chlorophyll a g^{-1} dry mass and 1.46 mg chlorophyll b g^{-1} dry mass) than A. negundo seedlings (2.63 mg chlorophyll a g^{-1} d.m., and 1.25 mg chlorophyll b g^{-1} d.m.).

In the first year of cultivation of A. pseudoplatanus seedlings, cytokinin activity in shoots (roots were not analyzed) was increased by the application of simulated acid rain in seedlings treated with IAA and in control seedlings not treated with any exogenous growth regulator. In seedlings treated with GA_3, acid rain applications resulted in reduced cytokinin activity (Fig. 1.). Opposite relations were assessed in endogenous gibberellins (Fig. 2). In the second year the acidification of nutrient
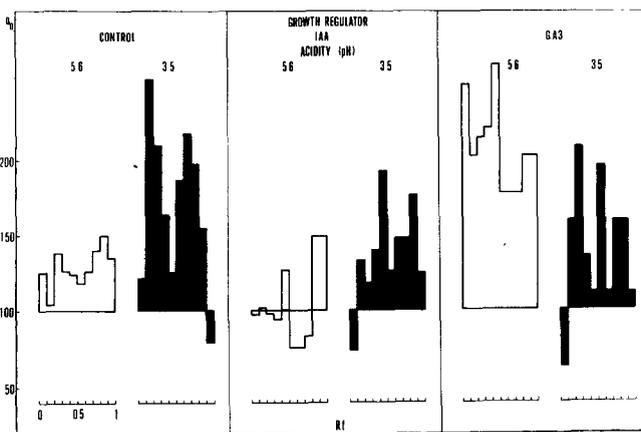

Fig. 1. The activity of endogenous cytokinins (Amaranthus-betacyanin bioassay) in the shoots of Acer pseudoplatanus L. seedlings (control and pretreatment with IAA and GA_3) as influenced by repeated spraying with distilled water (pH 5.6) and simulated acid rain (pH 3.5) in the first year of cultivation. Abscissa: R_f values; ordinate: betacyanin absorbance in relation to control (100 %).
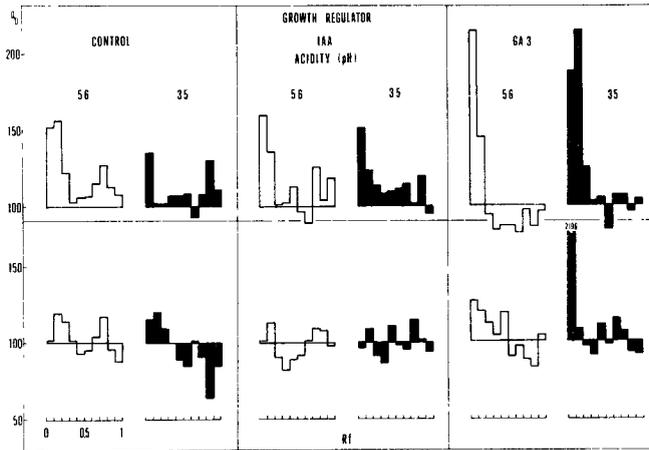
Fig. 2. The activity of endogenous gibberellins (*Lactuca* bioassay) in the shoots (above) and roots (below) of A. pseudoplatanus L. seedlings (control and pretreatment with IAA and GA₃) as influenced by repeated spraying with distilled water (pH 5.6) and simulated acid rain (pH 3.5) in the first year of cultivation. – Abscissa: R_f values; ordinate: length of lettuce seedling hypocotyls relative to control (100 %).
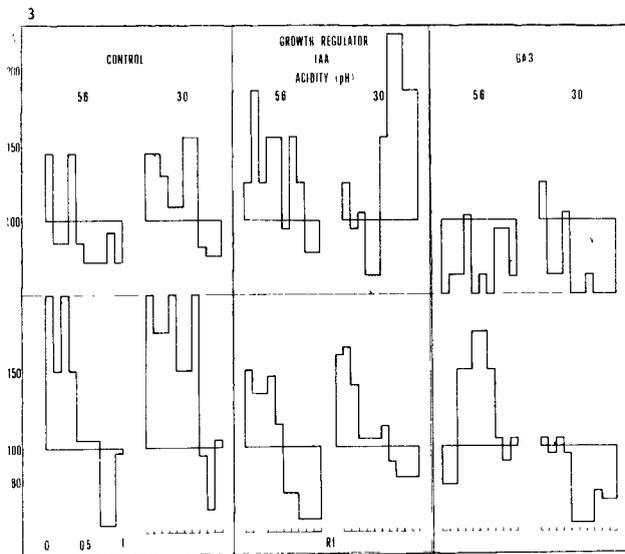

Fig. 3. The activity of endogenous cytokinins (*Amaranthus*-betacyanin bioassay) in the shoots (above) and roots (below) of A. *pseudoplatanus* L. seedlings (control and pretreatment with IAA and GA₃) as influenced by the root environment acidity (distilled water pH 5.6, sulphuric acid solution pH 3.0) in the second year of cultivation. – For other details see Fig. 1.

solution produced increased cytokinin content in both the shoots and roots of seedlings untreated and those treated with IAA. A remarkable attractive action of auxins showed itself after exogenous IAA, application, although not very clearly. Under these conditions, higher amounts of cytokinins were transported to shoots than in other variants. GA_3 application to seedlings resulted in cytokinin content decrease (Fig. 3). Changes in the content of endogenous gibberellins caused by the action of acidified nutrient solution were similar as in the case of simulated acid rain applications during the first year of seedling cultivation (Fig. 4).
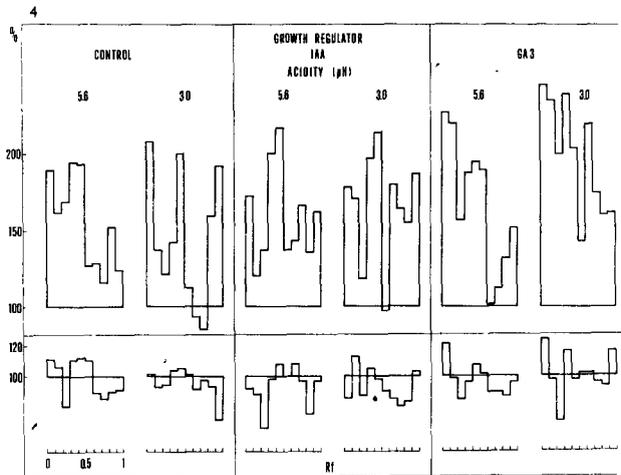

Fig. 4. The activity of endogenous gibberellins (*Lactuca* bioassay) in the shoots (above) and roots (below) of *A. pseudoplatanus* L. seedlings (control and pretreatment with IAA and GA_3) as influenced by the root environment acidity (distilled water pH 5.6, sulphuric acid solution pH 3.0) in the second year of cultivation. ₮ For other details see Fig. 2.

A single application of simulated acid rain caused only a small increase in the level of endogenous cytokinins (Fig. 5) and a decrease in the level of endogenous gibberellins (Fig. 6) in the shoots of *A. negundo* seedlings in the first year of cultivation. Acidification of the nutrient solution in the second year of seedling cultivation resulted in decreased level of endogenous cytokinins, especially in roots (Fig. 7) of seedlings treated with GA_3. In seedlings treated with IAA, the acidification of root environment resulted in increased cytokinin level in shoots, whereas root cytokinin level was not influenced. Changes in gibberellin activity caused by the interaction of exogenous growth regulator applications with the acidification of nutrient solution showed in *A. negundo* seedlings a similar tendency as in *A. pseudoplatanus* seedlings (Fig. 8).
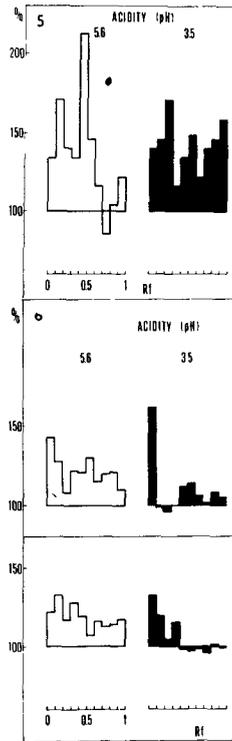
Fig. 5. The activity of endogenous cytokinins in the shoots of *Acer negundo* L. seedlings as influenced by a single application of simulated acid rain (pH 3.0) to a single spraying with distilled water (pH 5.6). For other details see Fig. 1.

Fig. 6. The activity of endogenous gibberellins in the shoots (above) and roots (below) of *A. negundo* L. seedlings as influenced by a single application of simulated acid rain (pH 3.5) to a single spraying with distilled water (pH 5.6). For other details see Fig. 2.

DISCUSSION

With respect to a significant function of phytohormones in plant life and to the important role which phytohormones play in plant growth regulation, cell differentiation, and in the control of metabolic and enzymic activities, it can be expected that exogenous growth regulators might contribute to modification of the effects of air pollution factors on plants by influencing plant growth and metabolism. For this reason, experiments aimed at supporting plant resistance to air pollution by the application of growth regulators were conducted. Growth regulators can be applied either before or during the action of the stress factor of air pollution. In our experiments, the former case was effected by simulating acidification of root environment, and the latter by simulating acid rain. As the uptake of exogenous growth regulators by plants is influenced by pH (Martin and Pilet 1987), spraying of
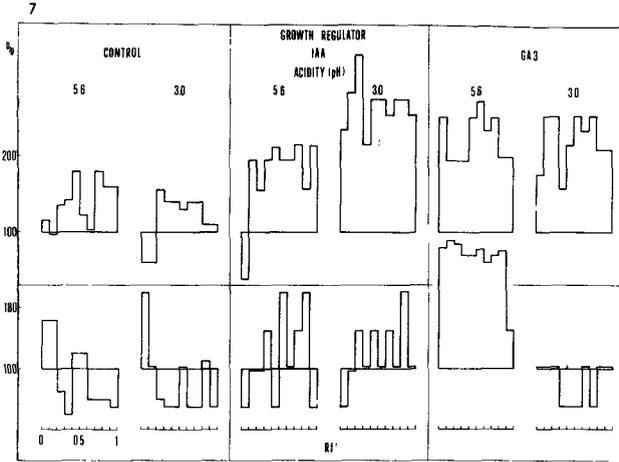
Fig. 7. The activity of endogenous cytokinins in the shoots (above) and roots (below) of *A. negundo* L. seedlings as influenced by root environment acidity (distilled water pH 5.6, sulphuric acid solution pH 3.0). For other details see Fig. 1.
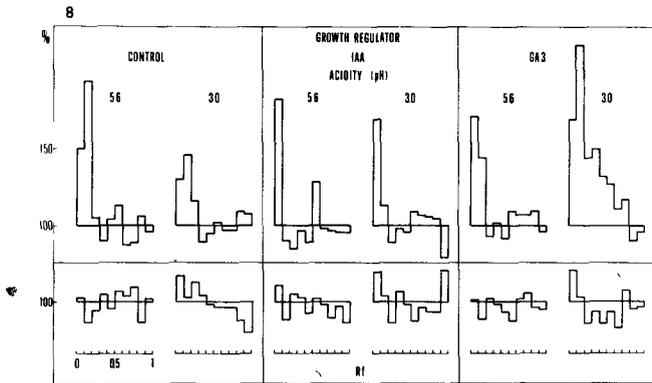

Fig. 8. The activity of endogenous gibberellins in the shoots (above) and roots (below) of *A. negundo* L. seedlings as influenced by the root environment acidity (distilled water pH 5.6, sulphuric acid solution pH 3.0). For other details see Fig. 2.

seedlings with growth regulators and with sulphuric acid solution was carried out at different terms.

Plant protection against air pollution by means of growth regulator applications can be based on the inhibition of the uptake of the harmful agent, or on a general stimulation of plant metabolism. Inhibition of uptake can be realized especially by the mechanisms of the action of endogenous, but also of exogenously applied abscisic acid. Damage caused by gaseous air pollutants can be reduced when stomata are closed (Kondo and Sugahara 1978). The application of exogenous abscisic acid was effective not only in reducing plant injury caused by SO_2 and O_3 but also the damage

caused by heavy metals (Kobriger and Tibbits 1985). In case the abscisic acid was applied to plants before the action of the stress factor, its effects on different physiological and biochemical processes of plant metabolism (so far not well understood) perhaps were responsible for the protective effects. Acid rain components affecting plant leaves enter the leaves mainly through the cuticula. Rain penetration rate through stomata is low (Evans et al. 1981). Nevertheless, acid rain can cause changes in the activity of stomata and in this way influence the rain penetration rate. Extreme acidification of leaf surface can cause a collapse of epidermal cells, which results in leaching different elements, organic acids, amino acids, sugars, vitamins, substances with allelopatic action, and endogenous growth regulators from affected leaves. This fact indicates a deeper action of acid rain in plant leaves than originally presumed.

As far as the protection of plants against air pollution stress by growth regulator applications is concerned, especially findings on cytokinins are available. Cytokinins have a general antisenescence effect (Thimann 1985) and their role in chlorophyll stabilization is also relevant (Bessonova et al. 1984, 1985). Some findings also indicate that plant protection with cytokinins can be connected with the primary reaction of the harmful agent wit the growth regulator. Pauls and Thompson (1982) demonstrated a direct chemical reaction between cytokinins and O_3 or its reaction product (free radicals) and thus not the effect of changes in cell metabolism induced by the hormone, resulting in enhanced tolerance. Cytokinins can act as binders of free radicals (Leshem et al. 1981, Srivastava 1984), which also are formed during the exposure of plants to SO_2 (Shimazaki et al. 1980), and help to protect chloroplast membrane integrity (Stillwell and Hester 1983, Tomlinson and Rich 1973).

An increased level of endogenous cytokinins was detected in oak seedlings exposed for a short time to acidified root environment (Čížková 1990). These changes could be associated with the enhancement of the rate of plant metabolism and with the induction of defensive reaction. A change in the level of endogenous auxins induced by environment acidification might also contribute to an increase in cytokinin activity.

In earlier experiments the application of exogenous cytokinins resulted in reduction of the negative influence of simulated acid rain on the growth of forest tree seedlings (Čížková 1985, 1988). The results presented in this paper prove the existence of interrelations between the application of exogenous auxins and gibberellins and environmental acidity in connection with endogenous phytohormones. Long-term exposure of maple seedlings to simulated acid rain resulted under given conditions in enhanced cytokinin activity in A. pseudoplatanus seedlings. This result can also be connected with processes controlling the chlorophyll content which was considerably higher in A. pseudoplatanus than in A. negundo. The application of exogenous gibberellin led to a decrease in the activity of endogenous cytokinins due to the action of acid rain. The application of exogenous auxin resulted in a mild enhancement of cytokinin activity at increased acidity of the environment.

Relationships between growth stimulation by exogenous growth regulators and the action of acid rain are time-dependent. For this reason, it is difficult to predict exactly the response of forest tree seedlings grown under natural conditions to growth regulator applications and the effect of environment acidity at different stages of their development. Nevertheless, changes in the activities of endogenous growth regulators and the induction of a defensive reaction expressed in terms of increased cytokinin activity might be one of the criteria in determining the resistance of different forest tree species and ecotypes to the acidification stress of the environment.

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