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RESPONSE REACTIONS OF CONIFERS TO ALKALINE DUST POLLUTION. CHANGES IN THE CONTENT OF SOLUBLE PHENOLIC SUBSTANCES IN *PICEA ABIES* L. NEEDLES

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Abstract. The content of soluble phenolic substances in Norway spruce needles in sample plots at different distances from a cement plant was examined. It was established that the total amount of phenolic substances and the content of nontannins and tannins increase generally with the shortening of the distance from the cement plant, i.e. with increasing pollution level. This pollution effect, which is detectable as far as 15 km from the emission centre, does not depend monotonously on the distance (pollution load), but has a local minimum near the emission centre in the case of bulk phenolics, tannins, and catechins.

Key words: *Picea abies* L., needles, phenolics, air pollution, cement dust, North Estonia.

INTRODUCTION

Changes in the content of phenolic compounds in plants as a response to gaseous phytotoxic pollutants such as SO_2, NO_x, O_3, etc. have been repeatedly observed. A certain specificity of the response on the level of bulk phenolics and individual phenolic compounds to a particular pollutant as well as the dependence of the direction and degree of the response on the plant species have been pointed out (Yee-Meiler, 1974, 1978; Grill et at., 1975; Гетко, 1989). Changes in the content of phenolic compounds have been proposed as a parameter of the indication and estimation of air pollutant effects (Darrall & Jäger, 1984).

However, research into the effects of dust pollution on plants has never received the same attention as that into gaseous phytotoxic pollutants, and no information is available on the effects of industrial dust on the metabolism of phenolic compounds. Research results together with repeated observations of dust deposits on vegetation demonstrate that the effects of dust on plant growth, yield, and several physiological and biochemical processes are remarkable (Farmer, 1993).

Long-term observations (1982—94) in forests situated in the dust fallout area of a cement plant in Kunda, Northeast Estonia, demonstrate the occurrence of a strong injurious impact of alkaline dust on conifers. A number of morphological and growth parameters, such as needle and shoot length, radial increments, mass and density of needles on the shoots, are substantially reduced and the reduction correlates with the pollution degree (Mandre et al., 1994). In the zone of 0.5—2 km from the emission centre the decrease in the content of chlorophylls and carotenoids as well as a reduced value of the ratio of Chl *a* to Chl *b* were observed (Mandre et al., 1992).

The aim of the present work was to find out whether long-term cement dust pollution has an effect on the phenolic complex of Norway spruce needles and, if present, how it depends on the distance from the source of dust emission, i. e. on the pollution load. This investigation is the first step toward designing an assay for diagnosing dust pollution stress in plants by the content of phenolic compounds.

MATERIAL AND METHODS

The content of water- and ethanol-soluble phenolic substances was measured in one-year-old needles gathered from spruce trees growing on permanent sample plots selected in natural forests of similar forest site and soil types. Thirteen sample plots were located on a transect in west—east direction within a distance of 38 km to the west and 12 km to the east from the emission source. In the area southeasterly winds prevail. A detailed characterization of the transect and sample plots is given elsewhere (Mandre et al., 1994). Needles were taken from branches on the southern side of at least five 60—80-year-old trees on 20 July 1993. The seasonal dynamics of phenols was determined in needles of spruces growing 0.5 km from the emission centre from March 1992 till March 1993. Average needle samples (0.5 g) were frozen and stored in liquid nitrogen.

Before extraction the needles were fixed in boiling ethanol and ground in mortar with glass rubble. The ground material was extracted thrice with 70% ethanol. Combined extracts were dried in air stream at room temperature. The dry residue was dissolved in hot water. From the water solution tannins were precipitated with gelatin (Marigo, 1973; Александрова & Осипов, 1985). 0.5 ml of 1% gelatin solution in 10% NaCl solution was added by drops to 4 ml of the solution examined. Phenolic compounds in the supernatant obtained after centrifugation (supernatant I) are denoted as nontannins. From supernatant I flavonoids were precipitated with formaldehyde (Marigo, 1973; Александрова & Осипов, 1985). Hydrochloric acid and water solution of formaldehyde were added to the supernatant to the final concentration 0.6 N and 0.16%, respectively. The precipitation was accomplished at room temperature in nitrogen atmosphere during 24 h. Phenolic compounds remaining in supernatant II are denoted as simple phenols.

The content of phenolic compounds in the original water solution and supernatants I and II was determined by the colorimetric method using the Folin—Denis reagent (Swain & Hillis, 1959; Александрова & Осипов, 1985). Chlorogenic acid was used for calibration. Catechins were determined in water solution according to Swain & Hillis (1959) using catechin (+) for calibration. The amount of tannins was calculated from the difference of phenolic content in the original water solution and supernatant I, and the amount of flavonoids from that of supernatants I and II.

RESULTS

The concentration of water- and ethanol-soluble phenolic compounds in one-year-old spruce needles changes in the course of a year (Fig. 1). Two periods can be distinguished in the seasonal dynamics of total phenolic compounds: a winter decay and a high level in summer, after rapid accumulation in spring. The amplitude of the seasonal variation of different fractions of the phenolic complex differs substantially. The amounts of phenolics in supernatants I and II are more stable than the total amount of soluble phenolic substances and tannins. The similarity of the seasonal dynamics of the total amount of phenolics to that of tannins indicates that the latter is of a cardinal importance for the former.

A comparison of the content of phenolic substances in the needles of spruces growing at different distances from the cement plant indicates that the pollution effect on the phenolic complex is detectable as far as 15 km to the west from the emission centre (Fig. 2). The stimulatory effect of air pollution on the accumulation of soluble phenolic substances tends to increase with decreasing distance, i. e., as the pollution load increases. Fig. 2 shows that the bulk pollution load, as well as its gradient, is on continuous rise as the distance to the pollution source decreases. This rise is particularly steep within a distance of 2 km. It

Fig. 1. Seasonal course of the content of phenolic substances in needles of Norway spruce growing at a distance of 0.5 km from the cement plant: *1* total phenolics, *2* nontannins, *3* simple phenolics, *4* tannins, *5* catechins.

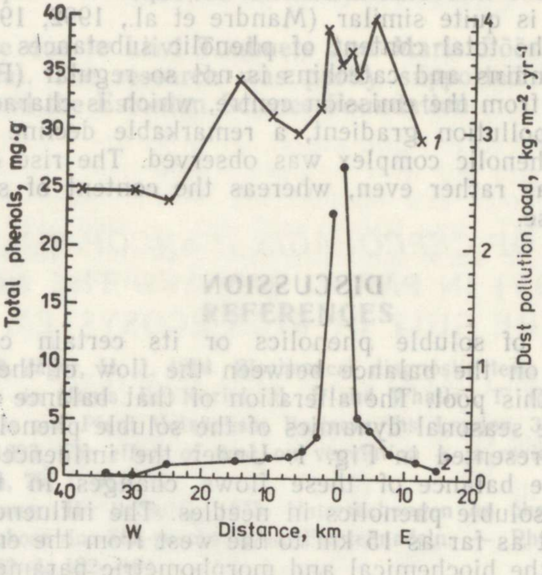

Fig. 2. Total content of phenolic substances in Norway spruce needles and dust fallout at various distances from the cement plant: *1* phenol content, *2* dust fallout.

Fig. 3. Content of different fractions of phenolic substances in Norway spruce needles at various distances from the cement plant: *1* nontannins, *2* tannins, *3* catechins, *4* simple phenolics.

must be noted that the distribution of the separate components of the fallout examined is quite similar (Mandre et al., 1992, 1994). However, the increase in the total content of phenolic substances as well as in the content of tannins and catechins is not so regular (Fig. 3). In the zone of 2—6 km from the emission centre, which is characterized by an increase in the pollution gradient, a remarkable decline of these parameters of the phenolic complex was observed. The rise of the content of nontannins was rather even, whereas the content of simple phenols tended to decrease.

DISCUSSION

The pool size of soluble phenolics or its certain components in needles depends on the balance between the flow of these compounds into and out of this pool. The alteration of that balance during a year is reflected in the seasonal dynamics of the soluble phenolic compounds; an example is presented in Fig. 1. Under the influence of prolonged dust pollution the balance of these flows changes in favour of the accumulation of soluble phenolics in needles. The influence of pollution becomes apparent as far as 15 km to the west from the emission centre. At this distance the biochemical and morphometric parameters of spruce investigated earlier were not influenced, though dust fallout (Fig. 2) and a rise of soil pH were already registered (Mandre, 1993; Mandre et al., 1992, 1994). However, there is no good correlation between the pollution load and the degree of response. In the zone of 2—6 km from the cement plant a marked decline of the content of total soluble phenolics, tannins, and catechins was observed (Fig. 3). Such an anomaly suggests that among the factors connected with air pollution there are those that cause a rise and those that lead to a decrease in the pool of certain phenolics. Obviously, the balance of the effect of different factors alters along the transect so as to cause the observed dependence. For instance, the alkalization of upper horizons of forest soil alters the availability of several nutrients. This is probably the reason why the content of nitrogen in spruce needles on the polluted area has fallen as compared with unpolluted areas by 40%, that of copper by 30%, and manganese by 98%. The sulphur content has risen by up to 100% and boron even up to 1500% (Mandre, 1993). There is evidence that both nitrogen shortage (Margna, 1977) and boron excess (Школьник & Абышева, 1982) stimulate the biosynthesis and accumulation of phenolic compounds, while manganese deficiency may retard it (Rubin & Jensen, 1985).

On the other hand, the rise of the pH value and calcium and potassium content in the cell wall compartment due to the dust on needles favour the oxidation of phenolics, esterification with cell wall carbohydrate polymers, and lignification (Heath & Castillo, 1987). Special investigations should be undertaken to specify these and other possible effects of alkaline dust pollution.

Lack of one-to-one correspondence between the pollution level and the content of soluble phenolics complicates their use as a biochemical indicator of air pollution consequences in plants. However, when used in investigating the dependence of pollution effects on the distance from an emission centre, as in the present work, or on the pollution level, the data of the content of soluble phenolics are quite informative. We hope that including insoluble phenolics into the investigated set of phenolic compounds will help advance the interpretation of pollution effects on phenolics and the development of a suitable diagnostic test on this basis.

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