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Effect of NO_2 on photosynthetic pigments in the leaves of *Populus 'Hybrida 280'**

INTRODUCTION

Oxides of nitrogen form characteristic injuries to leaves depending on concentration, duration of exposition, illumination and other ecological conditions. These are leaf necroses or discolourations visible to the naked eye. Before visible symptoms of injury appear it is possible to observe ultrastructural changes in the cells of leaves, primarily in the chloroplasts (Thomson 1975). Wellburn et al. (1972) have studied the effect of NO_2 on the ultrastructure of chloroplasts in the leaves of broad beans. Short expositions to NO_2 (1 hour at a concentration of 1 - 2 ppm) in their experiments have caused reversible swelling of the chloroplast tyllakoids. Irreversible changes in the chloroplasts appear only after the use of higher concentrations of NO_2 (3.0 ppm).

A consequence of the structural changes in the chloroplasts caused by the action of NO_2 is evidenced in physiological changes. Hill and Bennett (1970) have foud that NO and NO_2 at concentrations of 0.4 and 0.6 ppm after 90 minutes of treatment have inhibited the photosynthesis in oat and alfalfa leaves. Transfer of the plants into an atmosphere free of NO_2 eliminated the inhibition. The authors suggest that oxides of nitrogen have acted directly on the photosynthetic systems. A long term exposition (20 days) of bean seedlings and of tomatoes in an atmosphere having a low dose of NO_2 (0.33 ppm) have not led to leaf necroses (Taylor and Eaton 1966).

Kändler and Ulrich (1964) have observed in leaves treated with NO_2 the product of chlorophyll degradation, and they have assumed that it is a specific indicator of pollution with this gas. They have proposed the use of the method of analysis of pigments in order to

* *P.* 'Hybrida 280' is the hybrid *P. maximowiczii*×*P. berolinensis*, known in North America under the name "Oxford Paper 280".

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distinguish injuries caused by NO_2 from those caused by other injurious gases. The studies presented below were aimed at establishing the effect of NO_2 action on photosynthetic pigments in the leaves of the poplar *P. 'Hybrida 280'*.

MATERIALS AND METHODS

The effect of NO_2 on changes in the content of plant pigments has been studied on 2 months old poplar cuttings of the cultivar *P. 'Hybrida 280'*. After 12 hours of adaptation to the illumination used in the experiment the plants were exposed to the action of 2.0 ppm of NO_2 in fumigation chambers using equipment described earlier (Białobok et al. 1978). Nitrogen dioxide was produced by blowing it off a solution of nitric acid held at a temperature of 83°C. The concentration of NO_2 was determined with the help of an analyser type Mast 724-21 calibrated earlier using a colorimetric method based on the Griess-Ilosvay reaction (Hryniewicz and Wielogórska 1970).

Conditions of running the experiment have been presented in Table 1.

Table 1

Condition of experiments on the effect of NO_2 on the content of plant pigments in the poplar *Populus 'Hybrida 280'*

Date	NO_2 conc. ppm	Duration of exposition h	Time between fumigation and extraction h	Illumination klux	Temperature °C
19.04 1979	0	0	0	5 and 50	20 - 22
,,	0	5	0	5 and 50	,,
,,	2	1	0	5 and 50	,,
,,	2	3	0	5 and 50	,,
,,	2	5	0	5 and 50	,,
,,	0	0	24	50	,,
,,	2	1	24	50	,,
,,	2	3	24	50	,,
,,	2	5	24	50	,,

Extraction of chlorophylls and their separation. Chlorophylls were extracted from fresh poplar leaves with 100% acetone and with an addition of the $CaCO_3$ stabilizer. Acetone extracts were filtered quantitatively through Schott's G-3 funnels into volumetric flasks of 50 cm^3 capacity, and from these, aliquots were taken for further studies. Extracts were stored at 4°C in the darkness. All the functions during extraction and separation of the chlorophylls have been performed in a darkened room. An acetone extract corresponding to 40 mg of fresh tissue have been spotted as a 7.5 cm long streak onto a thin

layer plate covered with a neutral silica gel G according to Stahl (from Merck). The chromatograms were developed up to 10 cm in the solvent recommended by Bolliger and König (1969), petroleum ether — acetone — n-propanol (90 : 10 : 2 by volume), at 4°C in darkness. After development the chromatograms were dried and fluorescence of spots was observed in ultraviolet light at 366 nm. The zones visible in natural light as having a green, yellow or bluish-green pigmentation were eluted with 100% acetone. Spektrophotometric measurement of eluates was made with an analyser type UV-Vis (Zeiss). The content of chlorophylls and other pigments has been calculated in units of optical density (OD) converted to 1 gram of fresh weight of the leaf tissue. All analyses have been performed in three independent replications. The results obtained have been verified statistically.

Determination of the content of chlorophylls in raw acetone extracts. Chlorophyll a+b has been determined using the UV-Vis spectrophotometer. From the absorption curves obtained for individual extracts the value of extinction was measured at wavelengths of 662 and 644 nm. Concentration of chlorophylls a+b was calculated by the formula of Wettstein (Gavrilenko et al. 1975).

Chlorophyll concentration = $5{,}134 \cdot E_{662} + 20{,}436 \cdot E_{644}$ (mg/dcm³)

The determinations were performed in three independent replicates and the results obtained have been subjected to a statistical analysis.

RESULTS AND DISCUSSION

Effect of NO_2 on the sum content of chlorophylls *a* and *b* in the leaves of poplar cv. *P. 'Hybrida 280'* under conditions of low and high illumination. Intensity of illumination has a significant effect on the degree of injury of leaves by oxides of nitrogen. A 5 hour exposition of leaved poplar cuttings in an atmosphere of 2 ppm NO_2(3.64 mg/m³) and an illumination of 5 klux has not caused any visible injuries to poplar leaves. The content of chlorophyll in the leaves of poplars treated with 2 ppm NO_2 for one hour was significantly higher than in the controls (Fig. 1). The higher level of chlorophylls than in the controls continued to be observable after 3 and 5 hours of exposition to NO_2 in spite of the lack of necrotic injuries.

An exposition of poplar cuttings to the action of NO_2 at an illumination of 50 klux for 3 hours has not caused any injuries to leaves. Necrotic spots appeared on the leaves only after 5 hours of exposition of plants to NO_2. The content of chlorophylls in the leaves of poplar cuttings treated with NO_2 (2 ppm) at an illumination of 50 klux is presented in Fig. 2. After three hours exposition of leaves to NO_2 they held significantly more chlorophyll than control plants.

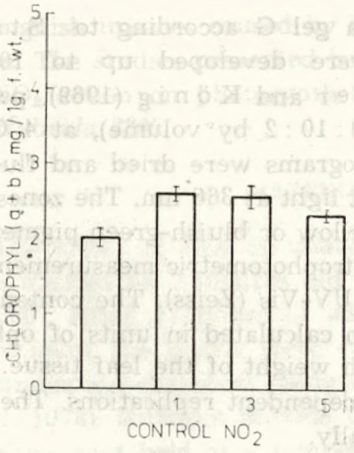

Fig. 1. Content of total chlorophyll in leaves of poplars treated with 2 ppm NO_2 (lighting 5 klux)

Poplar cuttings after being treated with NO_2 for 24 hours have been left in an atmosphere free of this gas. The results of chlorophyll content after that time are presented in Fig. 3. Leaves of cuttings treated with NO_2 have had significantly more chlorophyll than immediately after fumigation.

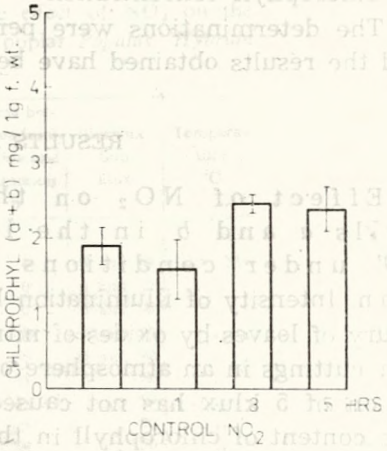

Fig. 2. Content of total chlorophyll in leaves of poplars treated with 2 ppm NO_2 (lighting 50 klux)

Effect of NO_2 on the content of photosynthetic pigments in the leaves of *P. 'Hybrida 280'*. Chlorophyll *a*. Treatment of poplar cuttings with NO_2 (2 ppm) at an illumination of 50 klux has caused visible necrotic injuries to leaves after 5 hours of fumigation. The leaves fumigated with NO_2 have had dark green aeroles on the leaves. The vascular bundles on the other hand were lighter compared to those of control leaves. After a chromatographic separation of acetone extracts it was found that the greatest changes in the content of pigments is visible after 5 hours of treatment with NO_2. When extracts were analysed from the leaves of cuttings after 24 hours from the

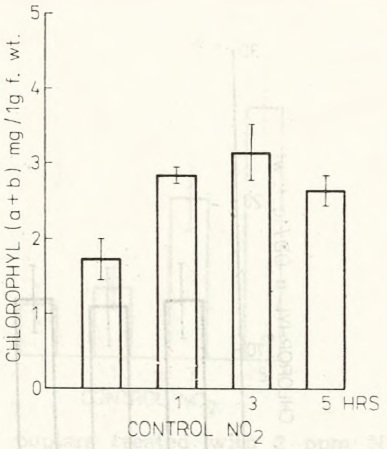

Fig. 3. Content of total chlorophyll in leaves of poplars treated with 2 ppm NO_2, 24 hours after treatment (lighting 50 klux)

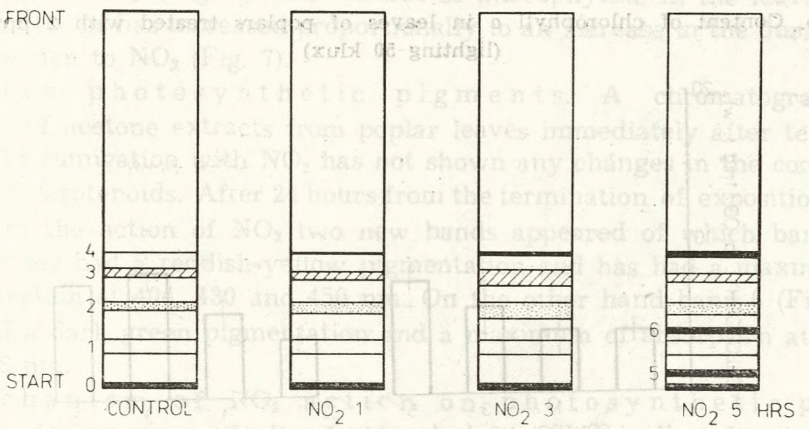

Fig. 4. Chromatographic separation of plant pigments from poplar leaves subjected to the action of NO_2 for various lengths of time

Solvent: petrol ether (60 - 80°C) — acetone — n-propanol (90 : 10 : 2) *1* — carotenoid, *2* — chlorophyl *a*, *3* — chlorophyl *b*, *4* — phaeophytin *a*, *0*, *5*, *6* — ?

moment of termination of fumigation the changes were even greater (Fig. 4).

The content of chlorophyll *a* in poplar cuttings treated for 3 hours with NO_2 does not change. A significant increase in the content of chlorophyll *a* relative to the control occurred only after 24 hours from the moment of terminating exposition (Fig. 5).

Chlorophyll *b*. In the leaves of poplar cuttings treated with NO_2 at an illumination of 5 klux the content of chlorophyll *b* increases already after 3 hours (Fig. 6), while a similar increase in chlorophyll *a* was observed only after 5 hours (Fig. 5). After 24 hours from the moment of

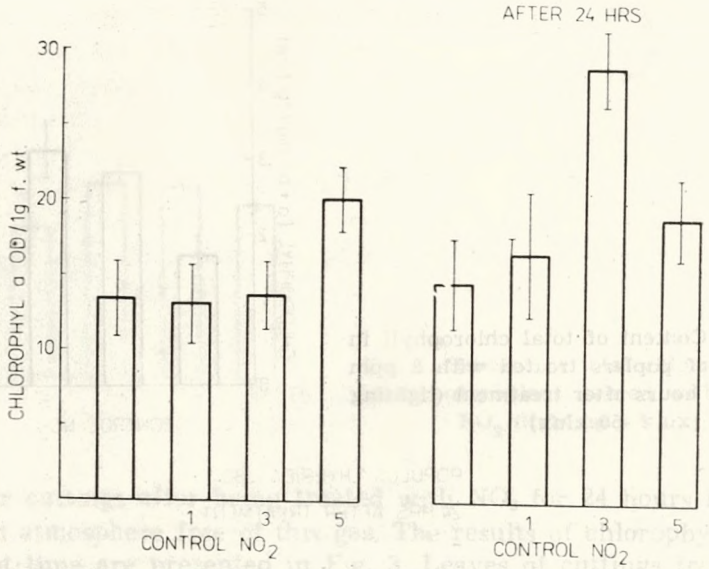

Fig. 5. Content of chlorophyll *a* in leaves of poplars treated with 2 ppm NO_2 (lighting 50 klux)

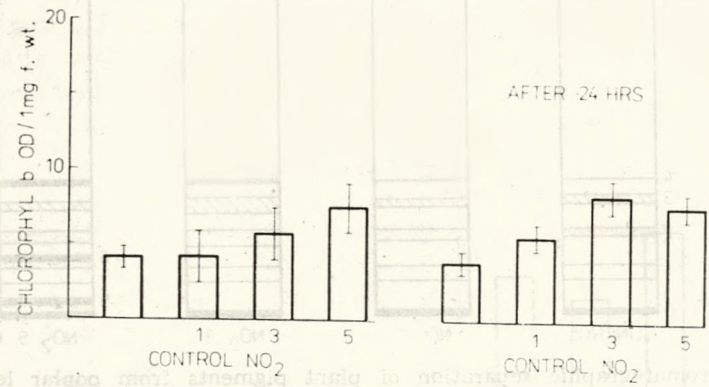

Fig. 6. Content of chlorophyll *b* in leaves of poplars treated with 2 ppm NO_2 (lighting 50 klux)

termination of treating with NO_2 for one hour the content of chlorophyll *b* increased significantly relative to the control. The data presented indicate that chlorophyll *b* is a more sensitive pigment to pollution with NO_2 than chlorophyll *a*. Chorophyll *b* enters besides chlorophyll *a* into the IInd photosystem. Thus one can suspect that photosystem II is also more sensitive to NO_2 than photosystem I, as shown also for SO_2 by Shimazaki and Sugahara (1979).

Phaeophytine. A chromatographic separation of acetone extracts has shown that earliest changes under the influence of NO_2 treatment have appeared in the content of phaeophytine, which occured only in small quantities in leaves of untreated plants. Under the influence of

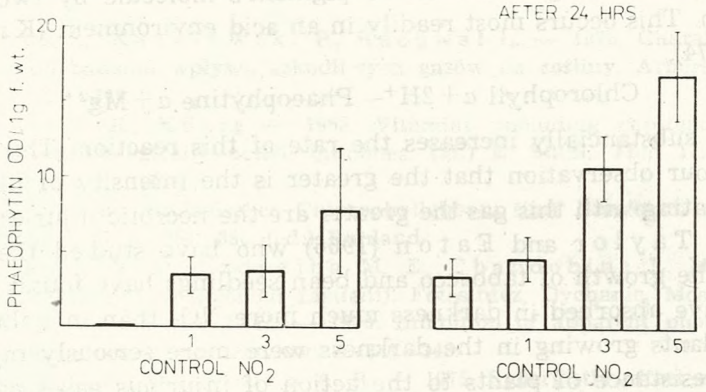

Fig. 7. Content of phaeophytin in leaves of poplars treated with 2 ppm NO_2 (lighting 50 klux)

NO_2 already after 1 hour of treatment the content of this pigment was readily detectable (Fig. 7). The content of phaeophytine in the leaves of poplar cuttings has increased proportionally to an increase in the duration of exposition to NO_2 (Fig. 7).

Other photosynthetic pigments. A chromatographic analysis of acetone extracts from poplar leaves immediately after termination of fumigation with NO_2 has not shown any changes in the composition of carotenoids. After 24 hours from the termination of exposition of plants to the action of NO_2 two new bands appeared of which band 5 (Fig. 4) has had a reddish-yellow pigmentation and has had a maximum of absorption at 404, 430 and 450 nm. On the other hand band 6 (Fig. 4) has had a dark green pigmentation and a maximum of absorption at 420 and 678 nm.

Mechanism of NO_2 action on photosynthetic pigments. Observations of ultrastructural changes in a cell under the influence of NO_2 in the early phases of exposition of plants to the action of this gas have shown that in the first place chloroplasts have been injured (Thomson 1975, Lopata and Ullrich 1975). Structural changes in chloroplasts cause aberrations in their functioning. The grana of chloroplasts which are most sensitive to the presence of toxic compounds are associated with chlorophylls that play a decisive role in the conversion of sun's energy for photosynthesis. Similarily as in our experiments Lopata and Ullrich (1975) have found that NO_2 has caused a transformation of chlorophyll into phaeophytine. The phaeophytinisation of chlorophylls under the influence of NO_2 occurs in leaves always when the plant grows in an environment polluted with this gas. Kändler and Ullrich (1964) have proposed a determination of the phaeophytine content as a measure of air pollution with NO_2.

The phaeophytinisation process depends on the replacement of the

central atom of magnesium in the pigment's molecule by two atoms of hydrogen. This occurs most readily in an acid environment (K r a s n o w s k i j 1974).

$$\text{Chlorophyll } a + 2H^+ \rightarrow \text{Phaeophytine } a + Mg^{2+}$$

Light substancially increases the rate of this reaction. This is confirmed by our observation that the greater is the intensity of illumination when treating with this gas the greater are the necrotic injuries to leaves. However T a y l o r and E a t o n (1966) who have studied the effect of NO_2 on the growth of tabbacco and bean seedlings have found that these plants have absorbed in darkness much more NO_2 than in light. For this reason plants growing in the darkness were more seriously injured.

The resistance of plants to the action of injurious gases agrees with their resistance to acidification, which in turn depends among other factors on the buffer capacity of the cell sap. The increase in the content of chlorophylls observed by us in the first period of fumigation with NO_2 is difficult to explain. This was also observed by H o r s m a n and W e l b u r n (1975) who have exposed 9 days old seedlings of peas to the action of 0.1 and 1.0 ppm of NO_2.

N i k o l a j e v s k i j (1979), who has also observed the increase in the content of chlorophylls under the influence of treatment with injurious gases explains this phenomenon in the differences in the ability of chlorophylls to become extracted under the influence of acidification. The absorbed NO_2 is most probably included in the cell metabolism. It is known that mineral substances, particularily nitrates stimulate the biosynthesis of chlorophyll (E g l e 1960). It is not unlikely that this is the cause why on increase in the content of pigments after a certain period of exposition to NO_2 is observed.

SUMMARY

In controlled laboratory conditions the effect of nitrogen dioxide on changes in the levels of some plant pigments in leaves of poplar cv. *P. 'Hybrida 280'* was observed. The content of pigments was determined after 1, 3 and 5 hours of fumigation with 2 ppm of NO_2, immediately after the fumigation period or one day later. Until the appearance of visible necroses an increase in the levels of chlorophylls *a* and *b* and of phaeophytine was observed proportionately to the duration of the fumigation treatment. Since necroses started to appear the increase in phaeophytine levels continued while the chlorophyll levels began to decline. One day after termination of the fumigation treatment further products of the degradation of pigments began to appear.

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KAZIMIERZ KRAWIARZ, JACEK OLEKSYN, PIOTR KAROLEWSKI

Wpływ NO_2 na barwniki fotosyntetyczne liści topoli Populus 'Hybrida 280'

Streszczenie

W kontrolowanych warunkach laboratoryjnych badano wpływ działania dwutlenku azotu na zmianę zawartości niektórych barwników roślinnych w liściach topoli *P. 'Hybrida 280'*. Zawartość barwników określano po 1, 3 i 5 godzinach działania 2 ppm NO_2, bezpośrednio po gazowaniu oraz po upływie doby od momentu ukończenia ekspozycji roślin. Do pojawienia się nekroz obserwowano pro-

porcjonalny do czasu działania gazu wzrost zawartości chlorofili *a* i *b* oraz feofityny. Od momentu pojawienia się nekroz dalszemu wzrostowi zawartości feofityny towarzyszyło zmniejszenie się zawartości chlorofili. Po upływie doby od ukończenia gazowania stwierdzono pojawienie się dodatkowych produktów degradacji barwników.

КАЗИМЕЖ КРАВЯЖ, ЯЦЕК ОЛЕКСЫН, ПЕТР КАРОЛЕВСКИ

Влияние NO_2 на фотосинтетические пигменты листьев тополя Populus 'Hybrida 280'

Резюме

В контролированных лабораторных условиях исследовали влияние действия двуокиси азота на изменение содержания некоторых пигментов листьев тополя *P. 'Hybrida 280'*. Содержание пигментов определяли после 1, 3, и 5 часов действия 2,0 ч. на млн NO_2, непосредственно после газации и 24 часа спустя от мемента окончания газации. До появления некрозов отмечено пропорциональное ко времени действия газа возрастание содержания хлорофиллов *а* и *б* и феофитина. После появления некрозов дальнейшему возрастанию содержания феофитина сопутствовало уменьшение содержания хлорофиллов. Спустя 24 часа после окончания газации отмечено появление новых продуктов разрушения пигментов.