ARBORETUM KÓRNICKIE
Rocznik XXIV — 1979

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Changes in chlorophyll a and b content in leaves of the poplar *Populus 'Hybrida* 275' * subjected to action of SO_2 and in the needles of European larch treated with HF**

INTRODUCTION

The studies conducted so far on the effect of injurious gases to changes in the content of chlorophyll a and b in leaves concern the final stage of a plant's reaction to these gases, when their injuries are already visible (Ilkun, 1971). However data is lacking on the changes in chlorophyll composition and other pigments of chloroplasts after short periods of treatment with these gases, when injuries to leaves are not yet externally visible.

The studies conducted by us on the composition of chlorophylls in leaves of Tabacco (*Nicotiana tabacum* L. 'Samson') treated for 3 hours with SO_2 at a concentration of 2.0 ppm have shown that the content of chlorophyll increases before injuries to leaves are visually observable. At the same time with an increase in the content of chlorophyll there was a corresponding increase in tabacco leaves of phaeophytins, which in control plants have remained in trace quantities.

The purpose of the present study was to establish whether the observations made on tobacco can be confirmed on woody plants.

LITERATURE REVIEW

Plants undergo characteristic injuries under the influence of injurious gases depending on their concentration, duration of exposition and ecological conditions under which the plants grow. These injuries will be

* This poplar is known in North America and in Western Europe under the name *Populus 'NE 42'*.

** This work has been partially supported by grant FG-PO-326 from the US Departament of Agriculture ander PL-480.

discolourations and necroses. The injuries observable to the naked eye are the final stage of the action of injurious agents on plants.

Thomson (1975) belongs to the few investigators who have studied ultrastructural changes in plant cells in the first periods of the action of gases, when no visual changes were yet to be observed. He has established that HF already in the first period causes an aggregation of the endoplasmic reticulum and injuries to cellular membranes which lead to profound changes in the functioning of the cells. Inside chloroplasts phytoferritine structures were seen which were the products of sedimentation of the porphyro-protein complexes of the chloroplast grana (Mühlenthaler, 1977). Bligny et al. (1973) have established that the action of HF causes chloroplast injuries in which the swelling and degradation of tyllacoides takes place. The ultrastructural changes observed by Fischer et al. (1973) in the leaves of broad beans (*Vicia faba*) treated with SO_2 occurred primarily within the grana. Similar observations were made by Młodzianowski and Młodzianowska (1976) in larch needles. Structural changes in the chloroplasts cause aberrations in their functioning. The chlorophylls are associated with the grana, the parts of chloroplasts that are most sensitive to the action of toxic substance (Fisher et al., 1973). Rao and Le Blanc (1966) have proposed the following scheme for the alteration of chlorophylls under the influence of SO_2:

$$SO_2 + H_2O \rightleftharpoons H_2SO_3$$

$$H_2SO_3 \rightleftharpoons HSO_3^- + H^+ \quad \text{Chlorophyll a} + 2H^+ \rightleftharpoons \text{Pheophytine a} + Mg^{2+}$$

Coker (1967) suggests that atmospheric oxygen further intensifies the reaction.

$$2HSO_3^- + O_2 \rightleftharpoons 2SO_4^{2-} + 2H^+$$

The process of disintegration of chlorophyll depending on the exchange of central magnesium atom in the pigment molecule for two hydrogen atoms takes place particularly readily in an acid milieu (Krasnowskij, 1974). Such gases as SO_2, HF and NO_x cause the acidification of plant tissues. Resistance to acidification of plants depends on their buffering capacity, that is on the ability of plants to neutralize the acidifying effect of the gases (Czuchajowska, 1978).

Pucket et al. (1973) working with chlorophyll isolated from algae have found that SO_2 in low concentrations hastens the transformation of chlorophyll into phaeophytine at a pH of 2.2, but at a pH of 3.2 or higher this change no longer takes place. A considerable role in the transfer of chlorophyll into phaeophytine is played by light. This confirms the observation that the greater is the light intensity the greater is the damage to plants under the influence of injurious gases.

MATERIALS

Experiments on the effect of gases have been conducted in controlled conditions using fumigation chambers and the apparatus described earlier (Białobok et al., 1978). Conditions of the experiments performed and a describtion of the material are presented in Table 1.

METHODS

CHLOROPHYLL EXTRACTION

The chlorophylls were extracted from fresh poplar leaves or from needles of larch short shoots with 100% acetons with a stabilizer in the form of $CaCO_3$ using about 2 g per 1 g of fresh weight of the tissue. Acetone extract has been transferred from a volumetric flask from where appropriate quantities were taken for further studies. The extract was stored at +4°C in the dark. All functions of extraction were performed in a darkened chamber. Chromatographic separation of chlorophylls and their elution from chromatograms.

The acetons extract corresponding to 40 mg of fresh weight was spotted as a 7.5 cm long line on a thin-layer plate covered with a silica gel G according to Stahl (Merck). The plates were developed ascending for 10 cm in the solvent recommended by Bollinger and König (1969) consisting of petroleum ether-acetone-*n*-propanol (90 : 10 : 2) at a temperature of +4°C in the dark. After development the plates were dried in stream of cold air. Fluorescence of chlorophyll spots has been observed in UV light at 366 nm. The zones visible in daylight, which had a green

Table 1

Conditions of running experiments on the effect of SO_2 on leaves of poplar, *P.* '*Hybrida* 275', plants and of HF on needles of detached shoots of larch *Larix decidua*

Plant Material	Gas used and conc.	Date of exposition	Hrs. of exposition	Time of material collection	% leaf injury	Temp. °C	Rel. air humidity	Light intensity in lux
Poplar	SO_2	26.05.78	0	t_0	0	22 - 25°C	60 - 80	5000
P'.Hybrida 275'	2 ppm	26.05.78	4		0	22 - 25°C	60 - 80	5000
(two year old plant)		27.05.78	4		0	22 - 25°C	60 - 80	5000
		28.05.78	4	t_1	0	22 - 25°C	60 - 80	5000
		20.05.78	4		0	22 - 25°C	60 - 80	5000
		30.05.78	4		0	22 - 25°C	60 - 80	5000
		31.05.78	4	t_2	0	22 - 25°C	60 - 80	5000
		1.06.78	4		0	22 - 25°C	60 - 80	5000
		2.06.78	4	t_3	40.0	22 - 25°C	60 - 80	5000
Larch	HF	31.05.78	0	t_0	0	22 - 25°C	60 - 80	10000 - 15000
L. decidua	1 ppm	31.05.78	2.5	t_1	5	22 - 25°C	60 - 80	10000 - 15000
(detached shoots)		31.05.78	4.5	t_2	20.0	22 - 25°C	60 - 80	10000 - 15000

or gray-green colour characteristic for the chloropyhlls and phaeophytine were eluted with 100% acetone. The light absorption of individual eluates was measured in a spectrophotometer UV-VIS (Zeiss).

The content of chlorophylls and of other pigments was expressed in units of optical density (OD) per one gram of fresh weight of the tissue.

RESULTS AND DISCUSSION

A. EFFECT OF SO_2 ON THE CONTENT OF CHLOROPHYLLS IN THE LEAVES OF THE POPLAR *P. 'HYBRIDA* 275'

Plants of *P. 'Hybrida* 275' were placed in a chamber with 2.0 ppm of SO_2 and an illumination of 5.000 lux for 7 days during which the plants were for 32 hours under the influence of SO_2. There were no visual signs of injury. Figure 1 demonstrates the separation of the plant pigments. The quantity of individual pigments is given in Table 2.

In the early part of the experiment after 12 hours of fumigation the content of chlorophyll a increased fivefold. Also chlorophyll b increased twofold. The increase in chlorophyll content was accompanied by a lowering in the content of phaeophytine a, the product of chlorophyll a degradation. The results obtained indicate that in the studied leaves synthesis of new chlorophyll increases and its degradation is reduced.

In our conditions, where there were still no visual injuries to the leaves SO_2 increased chlorophyll content. An increase in the content of many cell components is frequently observed as a defence mechanism against toxic influences. In order to clarify what are the reasons for the increase in chlorophyll content before the appearance of visual injuries, further studies are needed.

The symptoms of injuries in the form of necroses in *P. 'Hybrida* 275' appeared after 32 hours of exposition to SO_2. In leaves with signs of the necroses a lowering of the content of the chlorophylls was observed as well as an increase in the content of phaeophytine compared to the situa-

Table 2

Content of plant pigments in SO_2 fumigated leaves of *P. 'Hybrida* 275'. The content of chlorophyll is expressed as optical density per gram of fresh weight. The values given are averages of three replicates

Variable		Duration of exp. in hrs.	Chlorophyll a		Chlorophyll b		Phaeophytin a		Carotene		
			$\lambda=415$	$\lambda=671$	$\lambda=460$	$\lambda=645$	$\lambda=413$	$\lambda=657.2$	$\lambda=430$	$\lambda=452$	$\lambda=478$
Control	t_0	0	16.66	8.67	9.37	2.22	6.17	3.05	9.85	10.31	7.98
SO_2	t_1	72	86.08	52.48	17.18	5.20	4.74	1.25	8.95	10.55	8.90
SO_2	t_2	144	38.12	25.62	21.25	5.62	3.54	1.25	11.35	11.80	9.41
SO_2	t_3	196	36.44	23.88	23.74	7.56	9.70	3.52	11.40	8.85	11.36

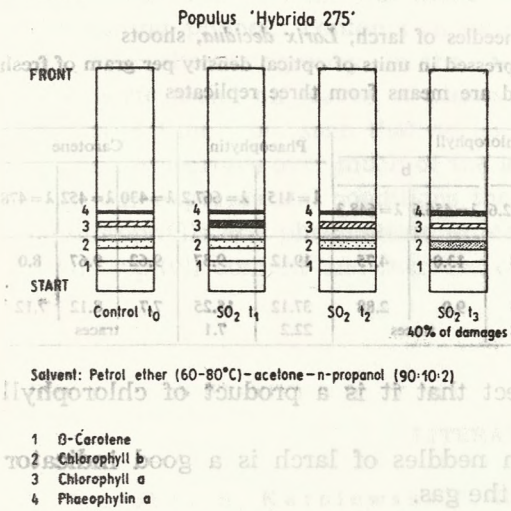

Fig. 1. Chromatographic separation of plant pigments from poplar leaves subjected to the action of SO_2 for various lengths of time

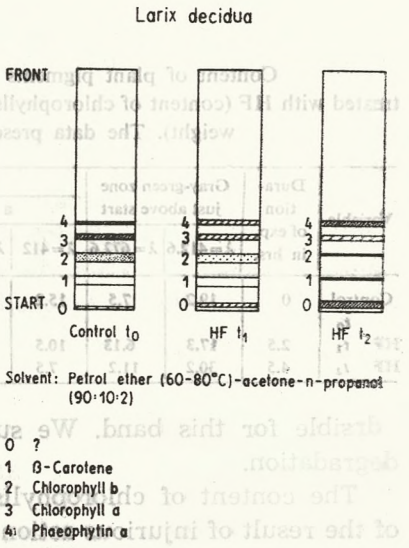

Fig. 2. Chromatographic separation of plant pigments from larch, *Larix decidua*, leaves subjected to the action HF for various lengths of time

tion before necrose formation. This can be explained by the effect of SO_2 on the acidification of cytoplasm in the cells (Rao and Le Blanc, 1966).

The action of sulphur dioxide throughout the study period had no significant effect on the level of β-carotene.

B. EFFECT OF HF ON THE CONTENT OF CHLOROPHYLLS IN THE NEEDLES OF LARCH, *LARIX DECIDUA*, SHORT SHOOTS

Detached shoots of *Larix decidua* have been subjected to the action of 1.0 ppm of HF. After 2.5 hours of treatment with HF when there were still no visual injuries to the leaves a lowering in the level of chlorophyll a and chlorophyll b was observed while at the same time there was a twofold increase in the content of phaeophytine (Fig. 2, Table 3).

Extension of the fumigation period to 4.5 hours has caused injuries to needles. In the composition of the chlorophylls substancial changes resulted. Chlorophyll b was destroyed completely. The content of chlorophyll a declined to half the initial value. Also a reduction was observed in the content of β-carotene. On chromatograms with separated acetone extracts of larch needles there appears above the start at R_f 0.02 an additional greyish-green band. This band increases in intensity as the duration of fumigation is extended. We do not know what pigment is res-

Table 3

Content of plant pigments in needles of larch, *Larix decidua*, shoots treated with HF (content of chlorophylls expressed in units of optical density per gram of fresh weight). The data presented are means from three replicates

Variable	Duration of exp. in hrs.	Gray-green zone just above start		Chlorophyll a		Chlorophyll b		Phaeophytin		Carotene		
		$\lambda=412.6$	$\lambda=672.6$	$\lambda=412$	$\lambda=672.6$	$\lambda=456.6$	$\lambda=648.3$	$\lambda=415$	$\lambda=667.2$	$\lambda=430$	$\lambda=452$	$\lambda=478$
Control t_0	0	19.2	7.5	15.2	8.8	13.0	4.75	19.12	9.37	9.62	9.67	8.0
HF t_1	2.5	17.3	6.13	10.5	4.3	9.0	2.88	37.12	15.25	7.7	8.12	7.12
HF t_2	4.5	30.2	11.2	7.5	1.2	traces		22.2	7.1	traces		

ponsible for this band. We suspect that it is a product of chlorophyll degradation.

The content of chlorophylls in neddles of larch is a good indicator of the result of injurious action of the gas.

CONCLUSIONS

1. Before visual symptoms of injuries to leaves of poplar *P. 'Hybrida* 275' appear following treatment with SO_2 there occurs an increase in the level of chlorophyll a and b while the levels of paeophytine a and β-carotene decline.

2. In leaves with necroses resulting from the action of SO_2 a high level of chlorophyll was observed, higher than in untreated leaves, and a double quantity of phaeophytine a. An increase in the level of phaeophytine indicates that the process of chlorophyll degradation is taking place. β-carotene is more resistant to the action of SO_2 and its level does not undergo changes.

3. Hydrogen fluoride causes substancial changes in the composition and content of plant pigments. Even a short exposition to HF causes a sudden drop in the content of chlorophyll a and b, the latter being more sensitive. A drop in the chlorophyll level is accompanied by an increase in the content of phaeophytine a and of a grayishgreen pigment that migrates little in the chromatographic system used by us. Reduction in the level of chlorophyll under the influence of HF treatment is probably a results of its degradation primarily through inactivation of chlorophyll into phaeophytine. Also β-carotene undergoes complete degradation.

SUMMARY

In the study changes in chlorophyll content in the leaves of *Populus 'Hybrida 275'* under the influence of SO_2 action and in the leaves of *Larix decidua* under the influence of HF action have been investigated.

In the measurements use was made of the spectrophotometric method of determining pigment contents in extracted samples after separation by thin layer chromatography. In the case of both gases the determinations were performend on leaves of plants exposed to fumigation for various lengths of time from such that do not cause any visible injuries to those that cause necroses over much of the leaf surface.

In our experimental conditions the SO_2 action resulted in an increase of chlorophylls and pheophytine in leaves while HF has caused a decline in chlorophyll content and increase of pheophytine level.

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Zmiany zawartości chlorofili w liściach topoli Populus 'Hybrida 275' traktowanych SO_2 i w igłach Larix decidua traktowanych HF

Streszczenie

W pracy przedstawiono zmiany zawartości chlorofili w liściach *Populus 'Hybrida 275'* pod wpływem działania HF. Przy pomiarach posługiwano się spektrofotometryczną metodą oznaczania zawartości barwników uprzednio wyekstrahowanych i rozdzielonych metodą chromatografii cienkowarstwowej. W przypadku obydwu gazów oznaczenia przeprowadzono na liściach roślin eksponowanych w różnych okresach, od nie powodujących jeszcze widocznych uszkodzeń aż do pojawienia się nekroz na znacznej powierzchni liści.

Działanie SO_2 w naszych warunkach doświadczalnych spowodowało wzrost zawartości chlorofili i feofityny w liściach. W przypadku fluorowodoru stwierdzono znaczny spadek zawartości chlorofili i wzrost zawartości feofityny.

КАЗИМЕЖ КРАВЯЖ, ЯЦЕК ОЛЕКСИН, ПЕТР КАРОЛЕВСКИ

Изменения содержания хлорофиллов в листьях тополя Populus 'Hybrida 275' газированных SO_2 и хвое лиственницы Larix decidua под влиянием HF

Резюме

В работе исследовались изменения содержания хлорофиллов в листьях *Populus 'Hybrida 275'* под влиянием действия SO_2 и в хвое *Larix decidua* под влиянием действия HF. Экстрагированные пигменты разделяли с помощью тонкослойной хроматографии и определяли их содержание спектрофотометрическим методом. Для обоих газов пигменты определялись в листьях растений газированных с различной продолжительностью — от не вызывающей видимых повреждений, до возникновения некрозов на значительной части поверхностей листьев.

Сернистый ангидрид вызывал в листьях рост содержания хлорофиллов и феофитина. В случае, когда действовали на растения HF констатировано значительное уменьшение содержания хлорофиллов и рост содержания феофитина.