Reactions of Cloned Poplars to Air Pollution: Premature Leaf Loss and Investigations of the Nitrogen Metabolism

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Populus nigra L. *cv.* Loenen, *P. maximowiczii* Henry × *P. nigra* L. *cv.* Rochester, Air Pollutants, Nitrogen Metabolism, Premature Leaf Drop

Young poplar cuttings (*Populus nigra* L. *cv.* Loenen and *P. maximowiczii* Henry × *P. nigra* L. *cv.* Rochester) were exposed for six weeks in open-top chambers to realistic concentrations of pollutant mixtures: 1) control; 2) SO_2/NO_x; 3) O_3/NO_x and 4) $SO_2/O_3/NO_x$. In this sequence of fumigation variants, the degree of influence of the various parameters of the nitrogen metabolism and of premature leaf drop increased very frequently compared to the control plants, *P. nigra* L. proving to be the more sensitive species.

The elevated Kjeldahl nitrogen content of the fumigated leaves was accompanied by either an increase in free amino acids or in total protein or, in the case of particularly large rises ($SO_2/O_3/NO_x$ variants), by increases in both substance groups. Proteolytic processes as a cause of the elevated content of free amino acids could be excluded to a large extent. A diminished *de novo* synthesis of proteins obviously led to a shift in the amino acid/protein relationship. In the younger fumigated leaves, the total concentration of free amino acids exceeded the values of the older leaves. The elevated amino acid content of the fumigated leaves was produced to a high degree by the glycolate pathway and the Krebs cycle. The increased turnover of the carbon skeletons was connected with a drastic starch degradation, especially in the older leaves.

The interaction of the amino acid and carbohydrate metabolisms is probably an important regulator in the promotion of rapid growth of young leaves in order to compensate premature leaf loss.

Introduction

It has been known for some years that cloned poplars are suitable objects for investigation in environmental impact research. They have been used in controlled fumigation experiments [1–4], for active monitoring in areas of forest damage [5] or as accumulation indicators for heavy metals [6]. The extent of premature leaf loss proved in our investigations to be a reliable criterion for the degree of injury to fumigated poplar cuttings.

The aim of our studies was to determine the reactions of poplar clones of varying sensitivity as related to premature leaf loss. To this end, various biochemical, physiological and anatomical investigations were carried out. In the following, the influence of 1) 23.0 ppb O_3 + 8.9 ppb NO + 23.0 ppb NO_2; 2) 21.0 ppb SO_2 + 15.3 ppb NO + 26.7 ppb NO_2 and 3) 19.9 ppb SO_2 + 22.0 ppb O_3 + 9.7 ppb NO + 35.6 ppb NO_2 on various parameters of the nitrogen metabolism in younger and older poplar leaves compared to control plants is described.

Material and Methods

Cultivation of the plants

Ten-centimetre-long cuttings of *Populus nigra* L. *cv.* Loenen and *P. maximowiczii* Henry × *P. nigra* L. *cv.* Rochester were obtained from the General Netherlands Inspection Service for Arboricultural Products. The cultivation of the plants was carried out for six weeks in a peat/sand mixture

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Abbreviations: FMOC, 9-fluorenylmethylchloroformate; 3-MPA, 3-mercaptopropionic acid; OPA, *o*-phthaldialdehyde.

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(17:3 v/v) with a pH of 6.0. It was possible to produce reliable control plants by cultivation in a greenhouse whose air was filtered through charcoal. For further details of the plant cultivation, see [7].

Exposure system, dosage and measurement of the air pollutants

For six weeks, from 25.5. to 6. 7. 1988, the fumigation of the poplar cuttings was carried out in the open-top chambers of the Institute for Plant Protection, Wageningen. The pollutant concentrations and further explanations of the fumigation variants used are given in Table I. The measurement and control devices were housed in a container. For sequential concentration measurements, the pollutants from the open-tops were passed to the analysers: (Monitor Labs, SO_2-8850, NO_x-8840, O_3-8810) by Teflon tubes and directed by a random stream selector. For gas-supply regulation, mass-flow controllers (Brooks) were used. The whole system was run and protected with a personal computer (HP 85-B) and a data acquisition system (HP 3497-A). A detailed description of the installation is given by [8].

Investigation of the plant material

Sample removal and storage

From the eight plants of each species, two of each were combined to form a mixed sample, so that there were four repetitions per fumigation variant and clone, whereas the older leaves (from the lower end up to the middle of the shoot) and the younger ones (from 5 cm below the tip down to the middle of the shoot) were harvested separately. Immediately after harvest (between 4 PM and 5 PM), liquid nitrogen (−196 °C) was poured over the leaves, which were stored at −70 °C until they were analyzed.

Analysis of the free amino acids

The free amino acids were extracted from the poplar leaves (1.5 g fresh weight in 20 ml of methanol:chloroform:7 M formic acid = 12:5:3 v/v/v) using a slight modification of the method of Dahlbender and Strack [9].

After the amino acid extracts had been centrifuged, the concentration to dryness was carried out at 35 °C in a rotary evaporator. The samples were absorbed in 50% aqueous methanol, filtered through a 0.45 μm membrane filter (Millipore) and stored at −70 °C until analyzed. The chemicals used were of analytical grade.

The derivatization of the amino acids was carried out in two steps. The mixture A) comprised 25 mg OPA (Fluka) + 1 ml acetonitrile (Baker, HPLC quality) + 4 ml borate buffer (Hewlett Packard) + 25 μl 3-MPA (Fluka). The reagent B) contained 1 mg FMOC (Sigma) + 2 ml acetonitrile (Baker).

The derivatization was carried out according to the following scheme: 200 μl of the mixture A) reacted with 40 μl sample + 20 μl internal standard

Table I. Mean concentrations of air pollutants (ppb) and growth parameters during the six-week exposure in open-top chambers (25.5.−6.7.1988). The mean values were calculated from 922 hourly measurements. The variance of the air pollutant concentrations was in the order of 2 ppb (SO_2), 6 ppb (O_3), 10 ppb (NO), 7 ppb (NO_2). Variant a = filtered air; b = filtered air + permanent SO_2-dosage; c = unmodified ambient air of Apeldoorn (with diurnal ozone cycles); d = ambient air + permanent SO_2-dosage. The pollutant concentrations of variant d are comparable with those from the Ruhr District, station Essen LIS (mean values from April−September 1985−1987, [57]).

Variant	Control	$SO_2 + NO_x$	$O_3 + NO_x$	$SO_2 + O_3 + NO_x$
Synonym	a	b	c	d
SO_2	0	21.0	1.9	19.9
O_3	4.0	4.0	23.0	22.0
NO	13.7	15.3	8.9	9.7
NO_2	15.2	26.7	23.0	35.6
Light [W/m²]		42.0		
Temperature [°C]		14.6		
Rel. humidity [%]		73.0		

for 60 sec for the derivatization of the primary amino acids. Immediately after the 1 min reaction time, reagent B) was added for the derivatization of proline. After a further reaction time of 45 sec, the immediate injection of a 20 µl sample into the HPLC column was performed, using the method described by Godel *et al.* [10]. Only the gradient conditions were slightly modified.

The HPLC equipment (Waters) comprised the following components: injection system (712 WISP), multisolvent delivery system (600), NEC III computer, column heater, integrator (Shimadzu CR 3A) and spectrophotometer (RF 5000, Shimadzu).

An analytical separation column (Macherey-Nagel, Nucleosil RP 18, 5 µm, 250 × 4 mm) and a precolumn (Macherey-Nagel, Nucleosil RP 18, 5 µm, 30 × 4 mm) were used for separation.

For the measurement of the OPA/3-MPA derivatives of the primary amino acids ($t_0 - t_{60}$), an excitation wavelength of 340 nm and an emission wavelength of 450 nm with a slit width of 5 nm each were chosen.

For the measurement of proline the excitation wavelength was changed to 266 nm and the emission wavelength to 305 nm after 60 min separation time.

The identification of amino acids was carried out by a comparison of their retention times with authentic standard material (Serva). Fig. 1 shows a typical chromatogram of the free amino acids of a leaf from *P. nigra* L. *cv.* Loenen.

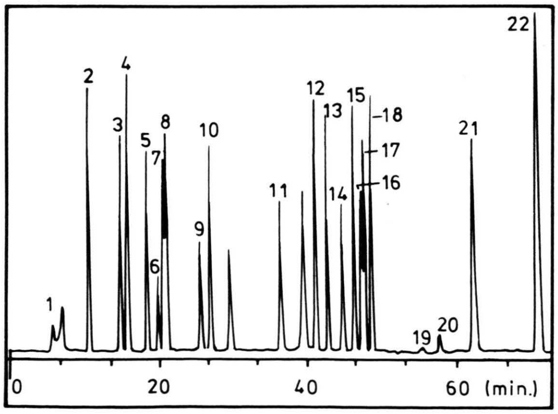

Fig. 1. A typical HPLC profile of the free amino acids from a leaf of *P. nigra* L. *cv.* Loenen. Peak 1 = Asp, 2 = Glu, 3 = Asn, 4 = Ser, 5 = Gln, 6 = His, 7 = Gly, 8 = Thr, 9 = Ala, 10 = Arg, 11 = Tyr, 12 = Val, 13 = Met, 14 = Gaba, 15 = Ile, 16 = Trp, 17 = Phe, 18 = Leu, 19 = Orn, 20 = Lys, 21 = Pro, 22 = FMOC.

Measurement of the Kjeldahl nitrogen and total protein

For the measurement of the nitrogen content of poplar leaves a Kjeldahl apparatus (Büchi, System B-322) was available and the leaf content of total protein was determined using the method of Marks *et al.* [11].

Starch analysis

Enzymatic starch analyses of the poplar leaves were conducted according to the method of Beutler [12].

Electron microscope investigations

The leaf samples were fixed in 4% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2−7.4) for several hours and embedded in epoxy resin (ERL). For ultrastructural investigations a transmission electron microscope (Zeiss 902) was used.

Dry weight measurements

These measurements were performed by lyophilizing portions of 1.5 g fresh leaf material for 10 h.

Results

The increase in leaf loss of the fumigated poplars was greater in *P. nigra* L. *cv.* Loenen than in the "Rochester" clone (Table II). The premature leaf drop was connected to increases in the Kjeldahl nitrogen, the more drastic reactions (increases) occurring again in the more sensitive "Loenen" clone (Figs. 2, 3). In both species there were increases in the nitrogen contents, both in the older and

Table II. Extent of premature leaf loss (relative degree of total leaf number) in *P. nigra* L. *cv.* Loenen and *P. maximowiczii* × *P. nigra cv.* Rochester. The data are means from the 10 plants investigated per fumigation variant.

Clone	"Loenen"	"Rochester"
Variant		
a	2.2%	1.7%
b	10.0%	7.6%
c	29.1%	9.0%
d	25.2%	18.3%

younger leaves. In *P. nigra* L. *cv.* Loenen the leaf content of Kjeldahl nitrogen increased in the following order of the fumigation variants: SO_2/NO_x; O_3/NO_x; $SO_2/O_3/NO_x$. Ozone therefore initiated in combination with NO_x a stronger reaction than sulphur dioxide, as was particularly evident in the case of leaf loss.

The "Rochester" clone also exhibited elevated leaf content of Kjeldahl nitrogen and increasing leaf loss after exposure to SO_2/NO_x and to $SO_2/O_3/NO_x$ compared to the control plants (Fig. 3). But the plants of the O_3/NO_x variants differed markedly from those of the "Loenen" clone, since the leaf drop was comparatively very small and the nitrogen content was similar to the values of the control plants.

The total contents of free amino acids and total protein of the older and younger poplar leaves are given in Fig. 4. A comparison of Figs. 2 and 4 shows that the largest increases in the nitrogen content (after exposure to $SO_2/O_3/NO_x$) in the younger and older leaves of both species are connected with marked increases in the protein and

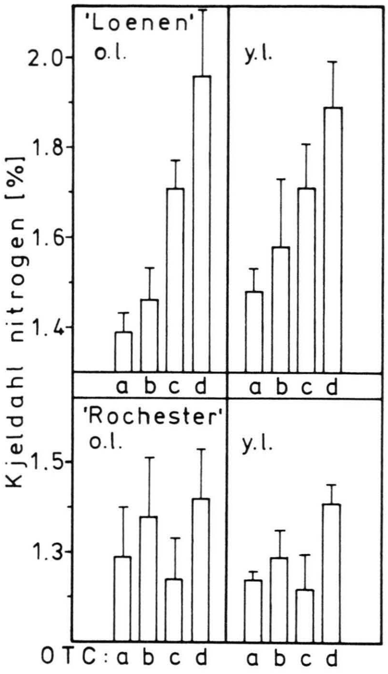

Fig. 2. Influence of a six-week pollutant exposure on the leaf contents of Kjeldahl nitrogen in older and younger leaves of *P. nigra* L. *cv.* Loenen and *P. maximowiczii* Henry × *P. nigra* L. *cv.* Rochester. Explanations: OTC = open-top chamber, a = control, b = SO_2/NO_x, c = O_3/NO_x, d = $SO_2/O_3/NO_x$ variants (the pollutant concentrations are given in Tab. I), o.l. = older leaves, y.l. = younger leaves, s.d. = standard deviation (n = 4).

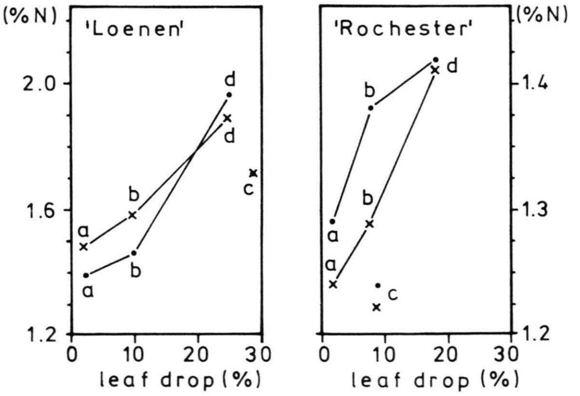

Fig. 3. Pollution-related premature leaf loss and the leaf contents of Kjeldahl nitrogen in both poplar species. For explanations see Fig. 2.

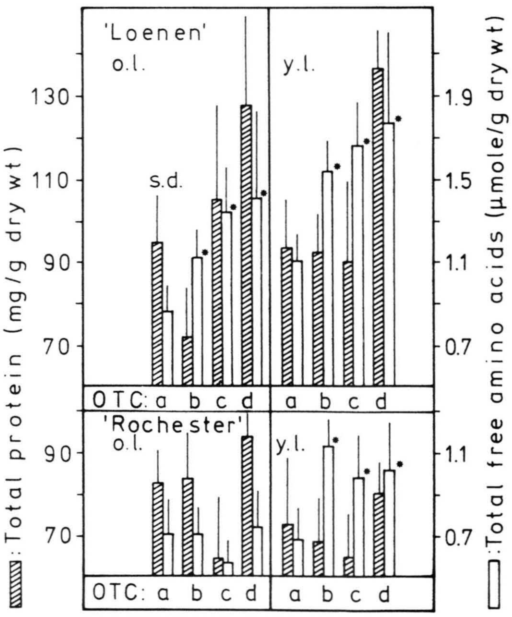

Fig. 4. Pollution-related changes in the leaf contents of free amino acids and total protein in the older and younger leaves of both poplar species. Explanation: * = significant differences (<1%) of amino acid contents between control and fumigated plants (Student's t-test). For further explanation see Fig. 2.

Table III. Relative and absolute content of free amino acids (AA) related to the concentrations of total protein (prot.); $n = 4$. Abbreviations: o.l. = older leaves; y.l. = younger leaves.

Clone	"Loenen"				"Rochester"			
Leaf age	o.l.		y.l.		o.l.		y.l.	
Content of AA	[nmol/ mg prot.]	[%]	[nmol/ mg prot.]	[%]	[nmol/ mg prot.]	[%]	[nmol/ mg prot.]	[%]
Variant								
a	9.1	100.0	11.8	100.0	8.5	100.0	9.4	100.0
b	15.5	170.3	16.7	141.5	8.4	98.8	16.4	174.5
c	12.7	136.6	18.4	155.9	8.8	103.5	15.0	159.6
d	11.0	120.9	12.9	109.3	7.9	92.9	12.6	134.0

amino acid contents. In the other variants, however, the elevated leaf content of Kjeldahl nitrogen was almost without exception connected only with increases in the amino acid contents. An exception are the older leaves of the "Rochester" clone in which there was no elevated leaf content of free amino acids after fumigation.

Table III illustrates that – again with the exception of the older leaves of the "Rochester" clone – all fumigation variants led to shifts in the amino acid/protein relationships in favour of elevated amino acid content.

Table IV shows that elevated total content of free amino acids was present in the younger poplar leaves, compared to the older ones, especially in those of the "Rochester" clone.

In Fig. 5 the alterations in the amino acid families after pollutant exposure in comparison to the control plants are depicted. The different fumigation variants led almost without exception to an increased content of all amino acid families of the "Loenen" clone in comparison to the control plants. The older and younger leaves both revealed largely comparable trends. The fumigation variants in the order SO_2/NO_x; O_3/NO_x and $SO_2/O_3/NO_x$ often led to progressive increases in concentration, which was correspondingly also true for the total content (Fig. 4).

A different situation was present in the case of the "Rochester" clone. Here, in comparison to the controls, either all the fumigation variants (aspartate and shikimic acid families) or only a few of the variants (glutamate, serine and pyruvate families) caused decreases in the older leaves with a simultaneous increase in the younger ones.

The leaf content of the individual amino acids, arranged in families, can be seen in Figs. 6 and 7.

Table IV. Relative content of free amino acids in younger poplar leaves related to the values of the older ones (= 100%; $n = 4$).

Clone	"Loenen"	"Rochester"
Variant		
a	127.7%	97.2%
b	137.5%	159.7%
c	124.1%	171.2%
d	125.1%	137.3%

The HPLC investigations [13] showed that the pollutant-related increases in the amino acid families depended on the more or less large increases in most of the individual amino acids. This is true of the older and younger leaves of the "Loenen" clone and to a lower extent of the younger leaves of the "Rochester" clone.

In the case of *P. nigra* L. "Loenen", both leaf-age classes showed a rise in Gln and Gaba after exposure to various pollutant combinations. In the variants containing O_3, these increases occurred almost without exception accompanied by simultaneous decreases in the leaf content of Glu, from which both amino acids originate.

In the younger exposed leaves of the "Rochester" clone, the content of all three amino acids increased compared to the controls. Gaba, which is produced from Glu by decarboxylation, slightly increased in the older leaves, even in the case of the "Rochester" clone. This shift was also accompanied by a drop in the content of Glu.

In both species compared to the controls, proline showed almost regularly only a slight pollutant-related increase.

Within the serine family, the separation of Gly and Thr was incomplete. Both amino acids taken

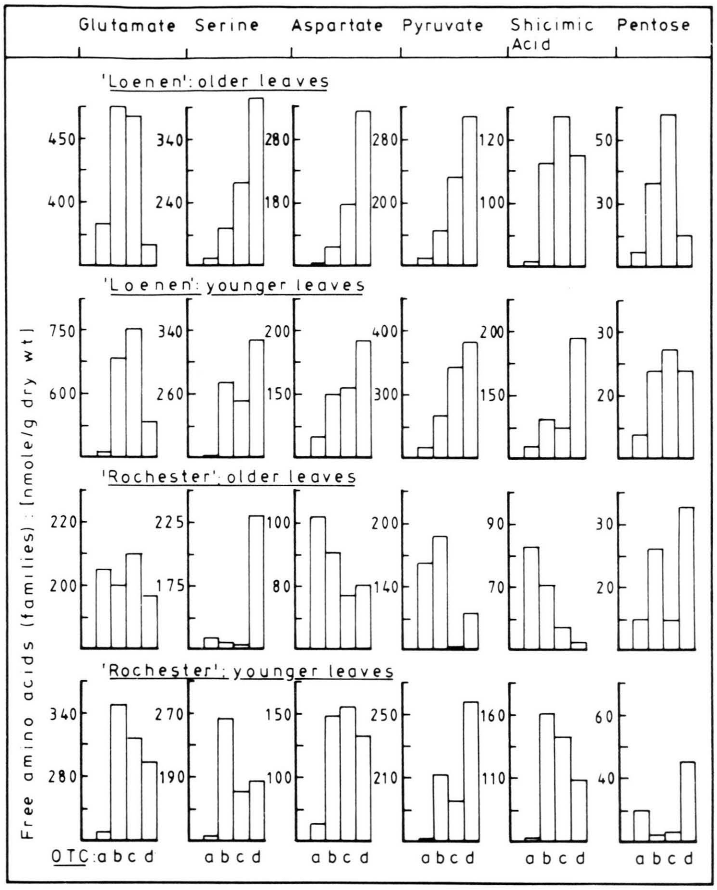

Fig. 5. Content of various amino acid families in the poplar leaves after pollutant exposure. Abbreviations: OTC = open-top chamber, a = control chamber, b = SO_2/NO_x, c = O_3/NO_x, d = $SO_2/O_3/NO_x$ variants.

together, showed no deviations from the general trend. Seen quantitatively, Ser belongs to the main components among the free amino acids of both poplar clones. Especially in the case of Ser, the fumigation with $SO_2/O_3/NO_x$, led in the older leaves of both species to appreciable increases in leaf contents (Figs. 6, 7).

Of the amino acids of the aspartate family, Met showed in the "Loenen" clone the smallest and Asn, Asp and Ile, especially after $SO_2/O_3/NO_x$ exposure, the largest increases. In the older leaves of the "Rochester" clone, as was also the case with many other amino acids, partial decreases in the content was to be observed in these families. The concentrations of Met were comparable in the fumigated leaves to those of the control plants. In the case of Asn, Asp and Ile, however, pollutant-related increases in the younger leaves occurred.

Of the pyruvate family, Ala, Val and Leu were quantitatively determined. All three amino acids showed in the older and younger leaves of *P. nigra* L., especially in the variants containing O_3, increases in concentration. In the "Rochester" clone a similar situation was found in the younger leaves concerning the Ala and Val content. In the older leaves, the content of Ala decreased drastically after fumigation with O_3/NO_x and $SO_2/O_3/NO_x$ and that of Leu did so slightly in plants from all treatments.

The leaf content of the amino acids Tyr and His also increased in the "Loenen" clone after fumigation. Increases in the Tyr content also occurred in the younger fumigated leaves of *P. maximowiczii* × *P. nigra* while slight decreases were found in the older leaves. In this clone, His showed no uniform trend.

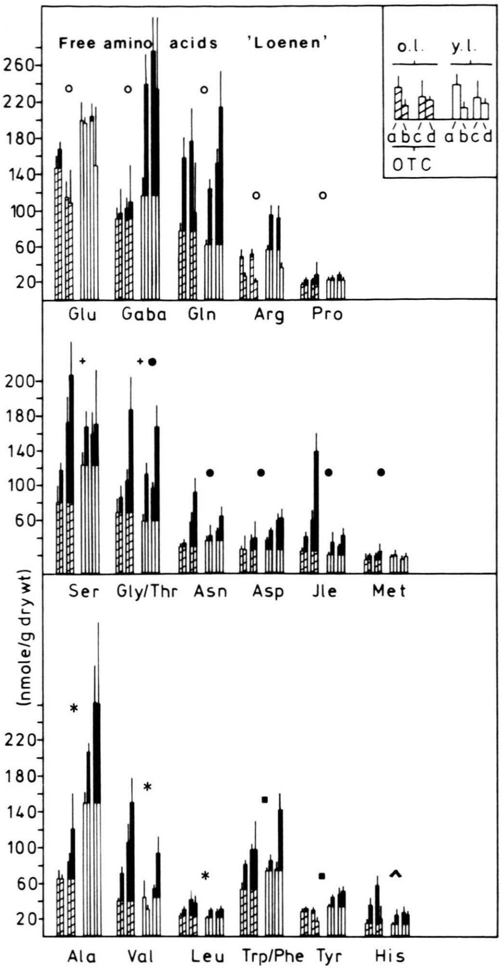

Fig. 6. Influence of various exposure variants on the leaf content of individual amino acids of *P. nigra* L. *cv.* Loenen ($n = 4$). Explanations: ○ = glutamate, + = serine, ● = aspartate, * = pyruvate, ■ = shikimic acid, ∧ = pentose family. Further explanations as in Fig. 2.

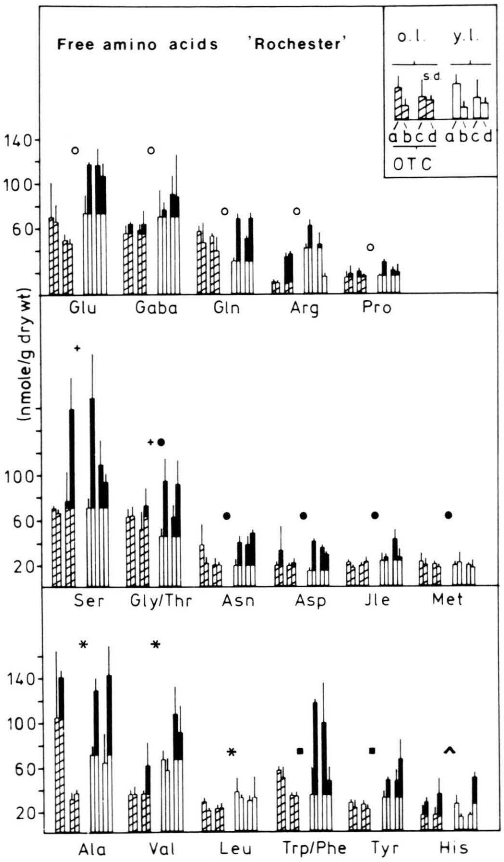

Fig. 7. Changes in the leaf content of free amino acids of *P. maximowiczii* Henry × *P. nigra* L. *cv.* Rochester after pollutant exposure. For explanations, *cf.* Figs. 2 and 6.

The results of the enzymic starch analyses (Table V) and the electron microscope investigations (Fig. 8) show that the activation of the nitrogen metabolism of the fumigated plants of both species was accompanied by a drastic starch reduction, especially in the older leaves.

Table V. Content of starch (mg/g dry weight) in younger (y.l.) and older (o.l.) leaves of *P. nigra* L. *cv.* Loenen and of the "Rochester" clone ($n = 4$).

Clone	"Loenen"		"Rochester"	
Leaf age	o.l.	y.l.	o.l.	y.l.
Control	281.27 ± 14.27	156.58 ± 28.59	121.09 ± 38.86	68.36 ± 11.79
SO_2/NO_x	151.95 ± 9.08	130.46 ± 28.02	37.70 ± 18.28	53.81 ± 9.93
O_3/NO_x	51.08 ± 12.39	109.69 ± 20.45	76.57 ± 12.25	66.37 ± 4.39
$SO_2/O_3/NO_x$	19.30 ± 9.21	95.39 ± 11.67	22.44 ± 4.81	44.83 ± 5.16

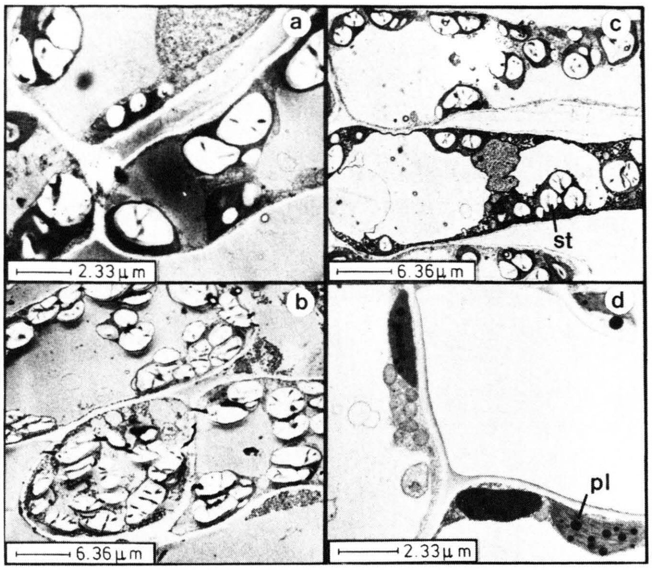

Fig. 8. Ultrastructural changes in the chloroplasts of *P. nigra* L. *cv.* Loenen after a six-week period of a fumigation with 19.9 ppb SO_2 + 22.0 ppb O_3 + 9.7 ppb NO + 35.6 ppb NO_2. The micrographs a, b were taken from control plants and c, d from fumigated poplars. The upper leaf cross sections are from younger leaves and the lower ones from older leaves. The pollutant-induced decrease in starch (st) content and the increase in the number and size of plastoglobuli (pl) of the older fumigated leaves are depicted in Fig. d.

Discussion

The gradually differing resistance of both species, *P. nigra* L. *cv.* Loenen and *P. maximowiczii* × *P. nigra cv.* Rochester, to realistic pollutant mixtures (see explanations of Table I) was shown, for example, by the varying degree of premature leaf loss. This is an easily determined criterion of damage state which, in our experiments under controlled exposure conditions with cloned poplars, showed repeatedly good temporal (begin of leaf drop) and quantitative reproducibility.

Premature leaf loss of fumigated trees has already been described by various authors [14–18]. Our investigations confirm quantitative connections between the extent of leaf loss and the degree of influence of selected parameters of the nitrogen metabolism in comparison to control plants. For example, with progressive leaf drop elevated contents of organic nitrogen in the remaining leaves – caused by increasing NO_x concentrations of the fumigation variants (Table I) – of both clones were observed after SO_2/NO_x and $SO_2/O_3/NO_x$ exposure. These increases were accompanied by elevated leaf content of free amino acids (SO_2/NO_x variants) and additionally of total protein ($SO_2/O_3/NO_x$ variants). It is, however, also known that the concentrations of polyamines increase under the influence of stress [19–21], so that rises in the Kjeldahl nitrogen content should be partially ascribed to this fact. For example, the rise in the N content in the older leaves of the "Rochester" clone after SO_2/NO_x exposure could be explained in this way.

The increases in the leaf content of free amino acids occurring after pollutant exposure and the rise in the amino acid/protein relationship cannot in most cases be explained by proteolytic processes, since the protein content in the exposed leaves compared to the respective control plants was either identical or only slightly lower. Moreover, as can be seen from Figs. 4, 6 and 7, the composition of the amino acids in the fumigated poplars did not change, with reduced protein content, in favour of those which, according to [22], account for a higher percentage of proteins (Lys, Gly, Thr, Met, and Ala).

A disturbed *de novo* synthesis of proteins could have led to the rise in the amino acid/protein relationship in the exposed leaves of both poplar clones, as reported by [23–25] on their plant species.

A reduction in protein synthesis in beans caused by the impairment of polysomes by ozone was observed by Chang [26, 27]. On the contrary, the protein content appreciably rose in our poplar leaves after exposure to SO_2 + O_3 + NO_x. In this context it should be mentioned as a possibility that stress proteins are known to be synthesized by eukaryotic cells exposed to noxious chemicals (*e.g.* [28]).

Numerous studies have determined the influence of air pollution on the contents of free amino acids in various plant species. According to these findings, sulphur dioxide in low concentrations causes decreases in the amino acid contents in *Pisum sativum* [29] and *Fagus sylvatica* [30], whereas higher concentrations of SO_2 leads to increased amino acid contents in *Phaseolus vulgaris* [23], in *Pinus banksiana* [22] or in *Pisum sativum* [31].

In the case of exposure to varying ozone concentrations, both increases and decreases in the amino acid content were found in different plant species. At higher concentrations, Tingey *et al.* [32] found decreases in the amino acid content in glycine and Ting and Mukerji [33] in cotton plants. Joestel and Schaub [34], however, observed decreases in the amino acid content in various plant parts of *Helianthus* at lower ozone concentrations. In contrast, increases in the amino acid concentrations after ozone exposure were also measured in various species [33, 35–38].

On the other hand, far less information is available on the influence of realistic concentrations of complex pollutant mixtures such as were used in our experiments. Joestel and Schaub [34] found that in *Helianthus* the changes in the amino acid concentration after SO_2 exposure was less pronounced than after O_3 and SO_2/O_3 exposure. This trend was also to be observed in *P. nigra* L., although it must be pointed out that all the fumigation variants used by us – especially the $SO_2/O_3/NO_x$ treatment – contained NO_x, which certainly had an influence on the observed increases in amino acid synthesis. Ito *et al.* [39, 40] also found large increases in amino acid concentration after the exposure of experimental plants to O_3/NO_x and Ruffin *et al.* [41] reported increases in contents as a result of an $SO_2/NO_2/CO$ exposure in various plant species.

The total content of free amino acids in the young poplar leaves exceeded the values of the corresponding older ones (Fig. 4). It is well-known that different amino acids may serve as storage forms of nitrogen. But an increased demand for free amino acids has to be taken into account because of the rapid growth of young leaves. In this context, it is interesting to compare the varying resistance in the two species. After exposure, the less sensitive "Rochester" clone revealed a higher percentage rise in the younger leaves (Table IV), although, on the basis of greater leaf loss, more marked compensation reactions would have been expected from *P. nigra* L. *cv.* Loenen. It is conceivable that in the "Loenen" clone pollution-related disturbances of the amino acid transport from the older to the younger leaves occurred. This assumption is supported by those changes described in the content of the amino acid families (Fig. 5) and also in the content of individual amino acids such as the amides Gln and Asn (Figs. 6, 7) which according to [42, 43], can be regarded as the preferred N transport forms in many plants because of the favourable C:N relationship. While in *P. nigra* rises in both amino acids in the older and younger leaves were present after fumigation, in the "Rochester" clone the content in the younger leaves increased with a simultaneous decrease in the older leaves. Glu is also taken to be a transport form for organic nitrogen compounds [44] and it decreased in the older fumigated leaves of *P. maximowiczii* × *P. nigra* in comparison to the controls, increases in the young leaves again occurring. In contrast, decreases in the Glu content in the older leaves of the "Loenen" clone were not connected to increases in the younger leaves.

Besides the disturbances in the amino acid transport, a number of different causes can be found for pollutant-related changes in the amino acid content. Among these are changes in the photosynthesis activity and the turnover rates of the oxidative pentose phosphate pathway, in glycolysis and the Krebs cycle, as well as changes in the activity of nitrogen-fixating enzymes.

The close connection between the carbohydrate and the amino acid metabolism results, for example, from the origin of the carbon skeletons for amino acid synthesis. The elevated amino acid content of the fumigated leaves was produced to a high degree by the glycolate pathway and the Krebs cycle. One indication of the validity of the connections between the amino acid and the carbohydrate metabolism is provided by the regularly found starch reduction (Table V), which was accompanied by decreases of the total carbohydrate content, especially in the older fumigated leaves and, to a lower extent, also in the younger leaves (Bücker and Ballach, in preparation).

The promotion of the growth of younger leaves to compensate for premature leaf loss and, additionally, the detoxification of NO_x pollution are evidently achieved by the interaction between the carbohydrate and the amino acid metabolisms.

In the older and younger leaves of the "Loenen" clone, in comparison to the controls, increasing Gln content with simultaneously decreasing Glu concentration in the fumigation variants containing O_3 were to be observed (Fig. 6). This finding could indicate an elevated activity of the glutamine synthetase (GS) for the detoxification of the NO_x pollution. According to Treshow [45], the incorporation of NO_2 mainly occurs in the amino group of Gln. Givan [46] emphasizes that the high GS activities have a great degree of importance in the prevention of rising NH_4 concentrations. Düball and Wild [47] conclude from the high activity of GS in damaged spruce needles that the activation of this enzyme should be considered as a general stress syndrome.

The possible breakdown of Glu by decarboxylation to Gaba is indicated by the reversal in their concentrations. Especially in the younger exposed leaves of *P. nigra,* the increase in Gaba in comparison to the controls was among the largest of all those amino acids investigated. The carbon skeleton of Gaba can be channelled back into the Krebs cycle, making a reduction in the consumption of carbon compounds possible. An increased decarboxylation of Glu is not a specific reaction and can be caused by water stress [48], by SO_2 [34], and by H_2S or NO_x pollution [49]. The changes in the Glu content are to be further seen in connection with the fact that it is the primary substance for the synthesis of additional amino acids. An increased GS activity in the older fumigated leaves of the "Rochester" clone could not be deduced from the concentration relationships of Glu and Gln but, because of the expected transport, cannot be completely excluded without further investigations. In the younger leaves there was possibly a pollution-related increase in the enzymatic activities of GS and GOGAT (glutamate synthase), since both Gln and Glu showed an elevated content in comparison to the controls.

Serine plays a number of specific roles in plant metabolism, *e.g.* as a precursor of different phospholipids [50]. The findings of the elevated Ser content in the older leaves of both poplar species in the O_3-rich variants may be an indication for the damage of the cell membranes by ozone. The concentration of Met was hardly influenced in the fumigated poplar leaves compared to the control plants. Methionine is known as a precursor of the stress hormone ethylene whose production is stimulated by different stresses, like ozone [51–53], or SO_2 [54–56]. Consequently, after a three-week exposure of the "Loenen" clone with 37 ppb O_3 for 11 h daily the ethylene production of the younger fumigated leaves was 4 times higher and that of the older leaves was 2 times higher than the production of the corresponding leaves of the control plants (Ballach and Woltering, in preparation).

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[1] L. S. Dochinger and K. F. Jensen, Environ. Pollut. **9,** 219–229 (1975).

[2] J. Mooi, Aquilo Ser. Bot. **19,** 189–196 (1983).

[3] T. Keller, Environ. Pollut. **52,** 183–192 (1988).

[4] H.-J. Ballach and J. Mooi, Internat. Kongr. Waldschadensforsch., Wissensstand und Perspektiven, Friedrichshafen, 2.–6. 10., Forschungsbeirat Waldschäden/Luftverunreinigungen I, 158 (1989).

[5] C. Pilgram and U. Arndt, VDI-Ber. **609,** 513–524 (1987).

[6] G. Wagner, Fortschr. atomspektr. Spurenanalytik **1,** 609–620 (1984).

[7] H.-J. Ballach, J. Mooi, and J. Bücker, Acta Biol. Benrodis **1,** 69–79 (1988).

[8] E. M. J. Meijer and J. Mooi, Research Institute for Plant Protection, Wageningen, The Netherlands, R 353 (1987).

[9] B. Dahlbender and D. Strack, Planta **169,** 382–392 (1986).

[10] H. Godel, T. Graser, P. Pfaender, and P. Fürst, J. Chromatogr. **297,** 49–61 (1984).

[11] D. L. Marks, R. Buchsbaum, and T. Swain, Anal. Biochem. **147,** 136–144 (1985).

[12] H. O. Beutler, Stärke **30,** 309–312 (1978).

[13] S. Oppenheimer: Wirkungen von Kombinationen gasförmiger Luftverunreinigungen auf primäre und sekundäre Aminosäuren von Forstpflanzen. Staatsexamen, Universität – GHS Essen (1989).

[14] P. R. Miller, J. R. Parmeter, Jr., and C. W. Martinez, J. Air Pollut. Control Assoc. **19,** 435–438 (1969).

[15] K. F. Jensen, Plant Diss. Rep. **57,** 914–917 (1973).

[16] W. Flueckiger, J. J. Oertli, H. Flueckiger-Keller, and S. Braun, Environ. Pollut. **19,** 171–176 (1979).

[17] W. T. Williams, Environ. Sci. Technol. **14,** 179–182 (1980).

[18] J. Mooi and H.-J. Ballach, in: Mechanisms and Effects of Pollutant Transfer into Forests (H. W. Georgii, ed.), pp. 323–331, Kluwer Academic Publishers, Dordrecht 1989.

[19] A. Priebe, H. Klein, and H.-J. Jäger, J. Exp. Bot. **29,** 1045–1050 (1978).

[20] T. A. Smith, Annu. Rev. Plant Physiol. **36,** 117–143 (1985).

[21] R. Pistocchi, F. Keller, N. Bagni, and P. Matile, Plant Physiol. **87,** 514–518 (1988).

[22] S. S. Malhotra and S. K. Sarkar, Physiologia Plant **47,** 223–227 (1979).

[23] S. Godzik and H. F. Linskens, Environ. Pollut. Ser. A Biol. Ecol. **7,** 25–38 (1974).

[24] H.-J. Jäger, E. Pahlich, and L. Steubing, Angew. Bot. **46,** 199–211 (1972).

[25] A. A. Khan and S. S. Malhotra, Phytochemistry **22,** 1325–1328 (1983).

[26] C. W. Chang, Phytochemistry **10,** 2863–2868 (1971).

[27] C. W. Chang, Biochem. Biophys. Res. Commun. **44,** 1429–1435 (1971).

[28] R. J. Deshaies, B. D. Koch, and R. Schekman, TIBS **13,** 384–388 (1988).

[29] L. Grünhage and H.-J. Jäger, Angew. Bot. **56,** 167–178 (1982).

[30] C. Brunhold, W. Landolt, and P. Lavanchy, Physiologia Plant **59,** 313–318 (1983).

[31] H.-J. Jäger, Phyton (Austria) **18,** 85–94 (1977).

[32] D. T. Tingey, J. A. Dunning, and G. M. Jividen, in: Proc. 3rd Int. Clean Air Congr., VDI, Düsseldorf, pp. 154–156 (1973).

[33] I. P. Ting and S. K. Mukerji, Am. J. Bot. **58,** 497–504 (1971).

[34] A. Joestel and H. Schaub, Berichte d. Zentrums für Umweltforschung 9, Johann Wolfgang Goethe-Universität, Frankfurt/Main 1988.

[35] H. Tomlinson and S. Rich, Phytopathology **57,** 972–974 (1967).

[36] R. K. Howell, Phytopathology **60,** 1626–1629 (1970).

[37] L. E. Craker and J. S. Starbuck, Can. J. Plant Sci. **52,** 589–597 (1972).

[38] D. T. Tingey, R. C. Fites, and C. Wickliff, Physiologia Plant **29,** 33–38 (1973).

[39] O. Ito, F. Mitsumori, and T. Totsuka, J. Exp. Bot. **36,** 281–289 (1985).

[40] O. Ito, K. Okano, and T. Totsuka, Soil Sci. Plant Nutr. **32,** 351–363 (1986).

[41] J. Ruffin, M. Y. G. Liu, R. Sessons, and S. Banerjee, Cytobios **46,** 119–129 (1986).

[42] C. F. Higgins and J. W. Payne, in: Microorganisms and Nitrogen Sources (J. W. Payne, ed.), Wiley, Chichester 1980.

[43] I. Rosnitschek-Schimmel, Plant Cell Physiol. **26,** 215–219 (1985).

[44] K. Mengel and R. Simic, Physiologia Plant **28,** 232–236 (1973).

[45] M. Treshow: Air Pollution and Plant Life (M. Treshow, ed.), Wiley, Chichester 1984.

[46] C. V. Givan, Phytochemistry **18,** 375–382 (1979).

[47] S. Düball and A. Wild, J. Plant Physiol. **123,** 491–498 (1988).

[48] D. Rhodes, S. Handa, and R. A. Bressom, Plant Physiol. **82,** 890–903 (1986).

[49] M. Pierre and O. Queiroz, Environ. Pollut. Ser. A Ecol. Biol. **25,** 41–51 (1981).

[50] L. A. Kleczkowski and C. V. Givan, J. Plant Physiol. **132,** 641–652 (1988).

[51] N. O. Adedipe and D. T. Tingey, Z. Pflanzenphysiol. **93,** 259–264 (1979).

[52] H.-J. Stan, S. Schicker, and H. Kassner, Atmos. Environ. **15,** 391–395 (1981).

[53] K. D. Rodecap and D. T. Tingey, Z. Pflanzenphysiol. **110,** 419–427 (1983).

[54] G. D. Peiser and S. F. Yang, Plant Physiol. **63,** 142–145 (1979).

[55] J. B. Bucher, Eur. J. For. Path. **11,** 369–373 (1981).

[56] T. W. Kimmerer and T. T. Kozlowski, Plant Physiol. **69,** 840–847 (1982).

[57] Landesanstalt für Immissionsschutz NRW, TEMES Monatsberichte 1985–1988.