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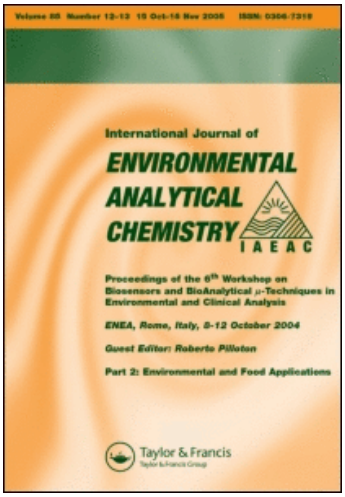

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Comparison of Amino-Compounds Contained in the Needles of Healthy and Damaged *Picea* Trees in Air Polluted Areas. Preliminary Results†

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(*Received November 5, 1985*)

The chromatographic amino-contained profiles of the extracts of needles from healthy and damaged *Picea* trees sampled in two polluted areas of the Vosges region (Massif du Taenchel and Massif du Donon), France are compared. The most interesting differences refer to the content of arginine, the precursor of polyamines. A dramatic difference in arginine content was found between needles from damaged trees as compared to those from seemingly undamaged trees. High levels of arginine, a basic compound, could be required to protect cell metabolism against the acidity of its environment caused by acid rains. High arginine content would thus indicate the resistant-healthy state of the tree. Further research is directed to determine whether titration of arginine content would afford a biochemical diagnostic of metabolic impairment prior to visible damages.

KEY WORDS: Pollution, *Picea*, polyamines, arginine, resistance.

†Presented at the 2nd Symposium on Handling of Environmental and Biological Samples in Chromatography. October 24–25, 1985, Freiburg, FRG.

INTRODUCTION

The present state of knowledge and theories on forest damage and eventual death of the trees observed in air polluted areas are at the basis of the French DEFORPA programme (Dépérissement des Forêts attribué à la Pollution Atmosphérique). Current research in this programme includes analytical work aimed at detecting metabolic modifications ascribable to the mechanisms of action of the pollution on leaf metabolism.

We are involved in this programme with a project dealing with a comparative analytical biochemical study of cellular metabolism between needles of healthy and damaged-polluted *Picea*. In this way we expect to detect metabolic disfunctioning caused by pollution and afford information required for the elaboration of further research axes to be undertaken in a more detailed manner.

The present paper reports preliminary results on polyamine- and their precursors-analysis in needles sampled from *Picea* trees of two polluted areas in the Vosges region (Massif du Taenchel and Massif du Donon).

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MATERIAL AND METHODS

Chemicals

Amino acids, mono-di- and polyamines were obtained from Sigma (St-Louis Mo, USA and Merck (Darmstad, GFR). O-Phthalaldehyde was obtained from Fluka (Buchs, Switzerland). All other chemicals used for the preparation of buffers and reagents were obtained as the highest purity grade from Merck (Darmstad, GFR).

Sample preparation

Shoots of seemingly healthy and damaged *Picea* trees were collected from two air polluted areas in the Vosges region, France (Massif du Taenchel and Massif du Donon). The needles were extracted twice with a pestle and mortar with 5% trichloroacetic acid in 0.05 N HCl (10 needles/1 ml) containing 5 nanomols of 1,7-diaminoheptane/ml as internal standard according to Villanueva *et al.*[1] Proteins were determined by the Bradford's method.[2]

Instrumentations and chromatographic amine analysis

The equipment and the methodology previously described[3] were used for the analysis of amine compounds present in *Picea* needles samples.

A microcomputer "Apple II" along with the logiciel "Apple-plot" was used to obtain the graphics presented in this paper.

RESULTS AND DISCUSSION

The chromatographic amino-contained (picomole/μg protein) profile of extracts of needles from healthy and damaged *Picea* from two polluted area of the Vosges region of France are presented under the form of histogrammes. Data refers to the content of ornithine (Orn) lysine (Lys) arginine (Arg) putrescine (Pu) Spermidine (Sd) and spermine (Sm) of the sampled 1985 and 1984 shoots collected from the same tree at the same time.

In the case of samples from the massif of Taenchel, Figure 1 shows the amino-contained profile of needles from healthy (1A) and damaged (1B) young *Picea.* Figure 1C compares arginine and total polyamines (Pu, Sd and Sm) contain of needles of these same samples. Figure 2 shows the amino profile in damaged (2A) and very damaged (2B) adult *Picea.*

Representative results obtained with needles of old *Picea* trees from the Massif of Donon are shown in Figure 3: healthy (3A) damaged (3B) and very damaged (3C). Figure 4 compare arginine and total polyamines from these samples.

As can be observed in both groups of samples (Taenchel and Donon) marked differences, in the amino profile of needles, can be correlated to the apparent physiological state of the tree (healthy, damaged and very damaged) and this holds for young, adult and old trees. Nevertheless in these preliminary results, the most interesting differences between samples from healthy and damaged organism, concern the content in arginine, the precursor of polyamines. Needles of healthy *Picea* contain high levels of arginine contrasting to the low level observed in damaged *Picea.* This relatively high content in arginine, which is a basic compound, in the apparent healthy tree could correspond to a resistance metabolic process of the tree by counteracting the tendency towards local acidification of the cells

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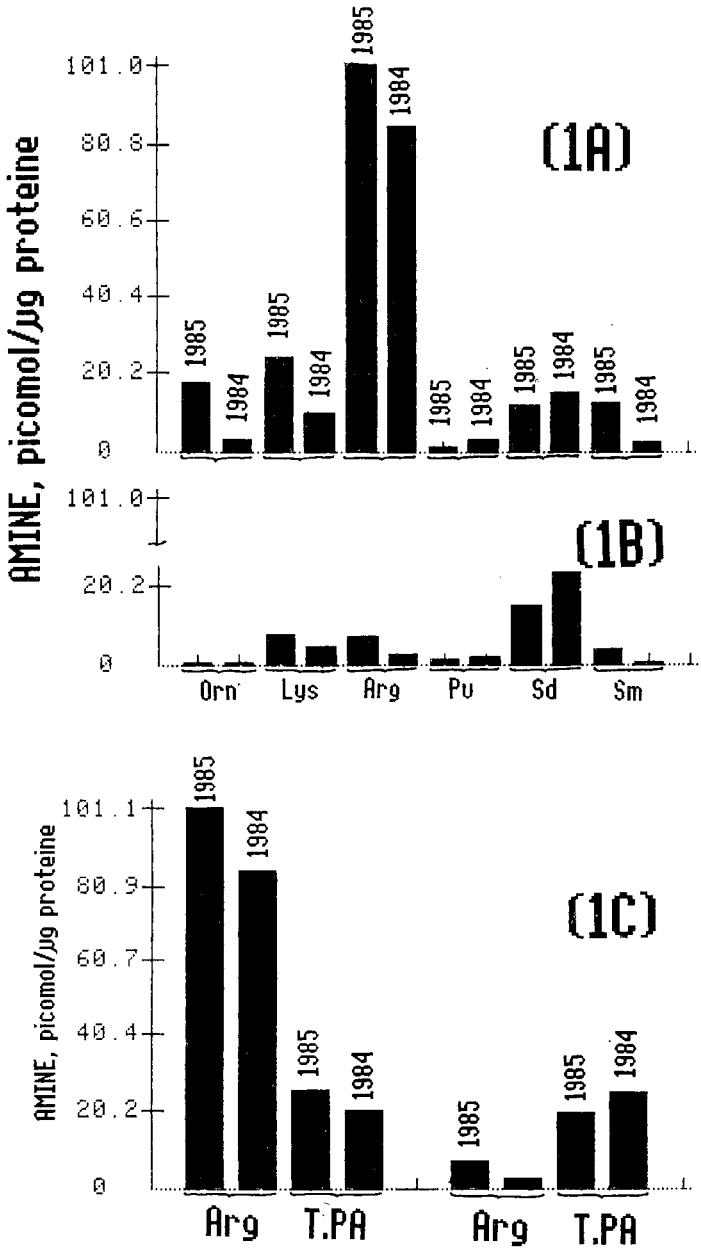

FIGURE 1 Amino-chromatographic profile of needles from healthy (1A) and damaged (1B) young *Picea* trees. (1C) compares Arg and total polyamines (T.PA) from the same samples.

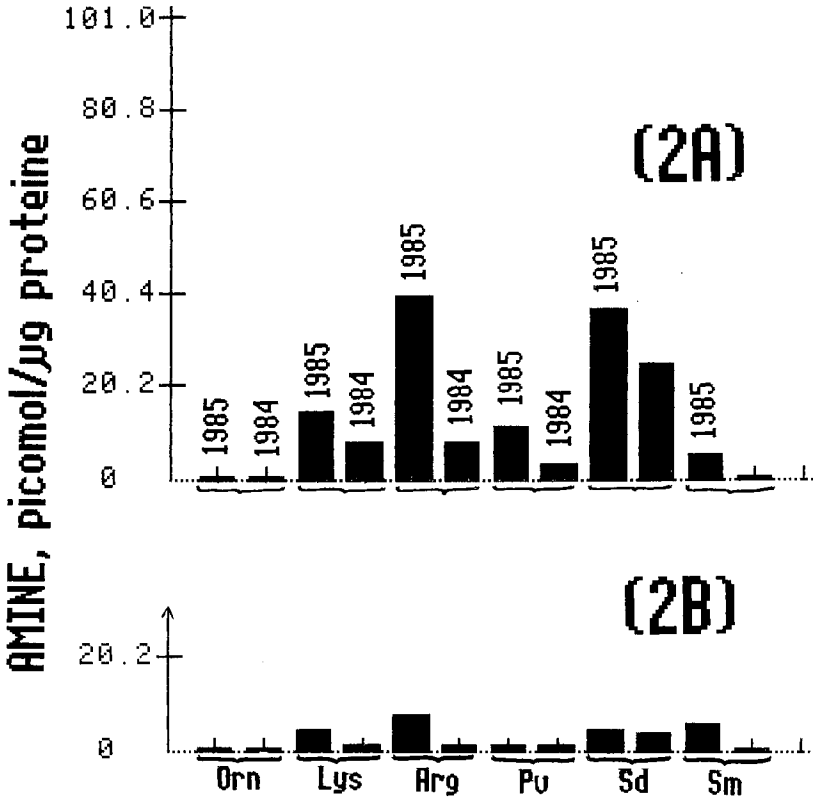

FIGURE 2 Amino-chromatographic profile of needles from damaged (2A) and very damaged (2B) adult *Picea* trees.

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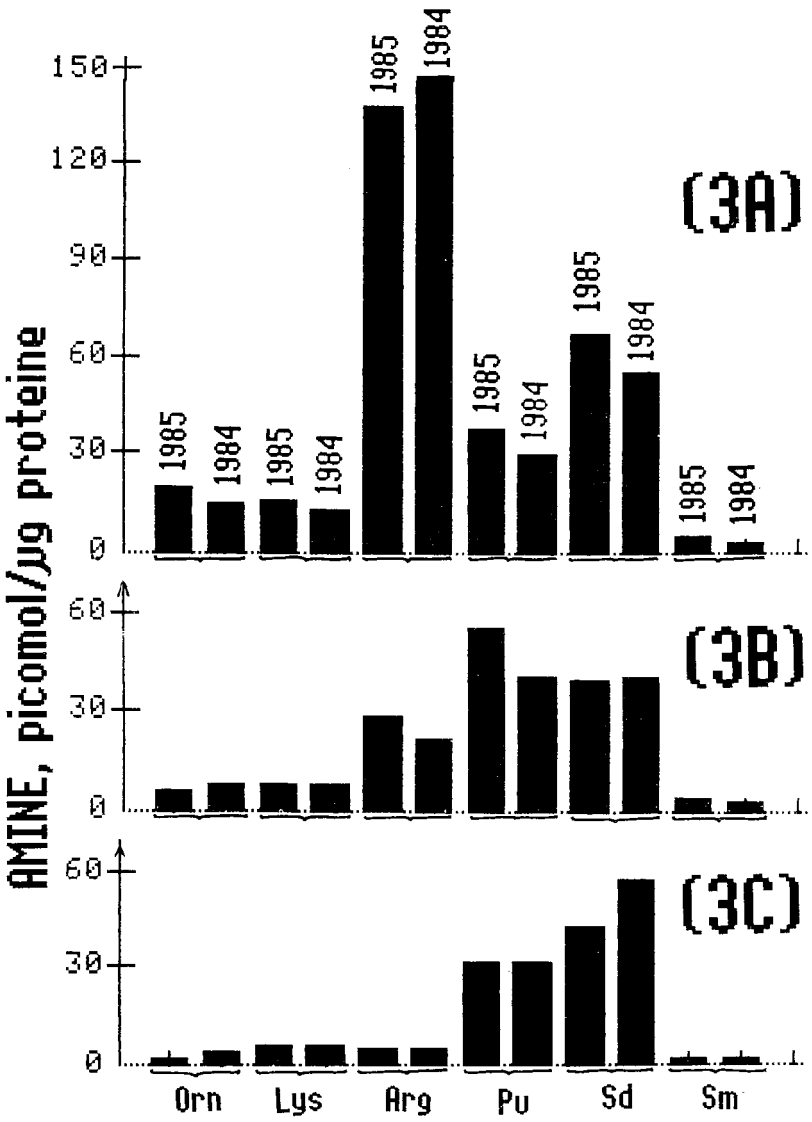

FIGURE 3 Amino-chromatographic profile of needles from healthy (3A) damaged (3B) and very damaged (3C) old *Picea* trees.

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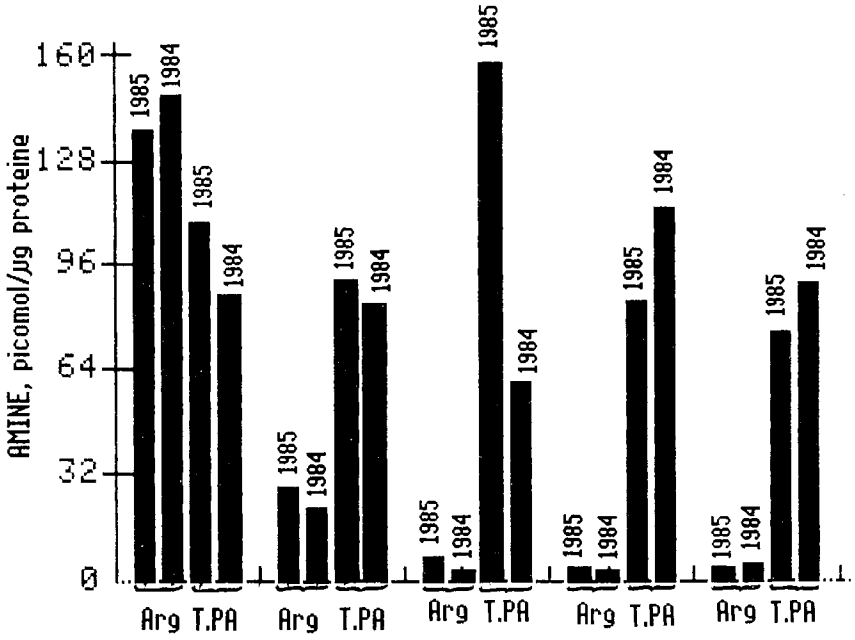

FIGURE 4 Arg and total polyamines (T.PA) profiles comparison of the same samples of Figure 3.

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caused by acid rains in polluted areas. Arginine and Polyamines accumulation are known to occur in cell plants subjected to endogenously[4] or exogenously[5] originated acid stress.

If further results confirm these findings, arginine could serve as a biochemical marker of healthy or damaged state of trees living in polluted areas. The titration of arginine content would afford a biochemical "diagnostic" helping to determine the healthy or damaged metabolic state of the tree in the absence of visible damage.

Work in this area is in progress in this laboratory.

Acknowledgements

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References

1. V. R. Villanueva, R. C. Adlakha and A. M. Cantera-Soler, *J. of Chromatog.* **139,** 381 (1977).
2. M. M. Bradford, *Anal. Biochem.* **72,** 248 (1976).
3. V. R. Villanueva, M. Mardon and M. Th. Le Goff, *Intern. J. Environ. Anal. Chem.* **25,** 115 (1986).
4. C. Morel, V. R. Villanueva and O. Queiroz, *Planta* **149,** 440 (1980).
5. T. A. Smith, *Ann. Rev. Plant Physiol.* **36,** 117 (1985).

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