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Phenols and Flavonoids in Aleppo Pine Needles as Bioindicators of Air Pollution

Christine Robles, Stéphane Greff, Vanina Pasqualini, Suzanne Garzino, Anne Bousquet-Mélou, Catherine Fernandez,* Nathalie Korboulewsky, and Gilles Bonin

ABSTRACT

The aim of the present study is to assess whether certain ecophysiological responses (contents of total phenols, total proanthocyanidins, and total and simple flavonols), in the needles of Aleppo pines (*Pinus halepensis* Mill.) may be valid bioindicators for the assessment of the air quality. Samples were taken at five natural sites polluted by various pollutants (NO, NO_2, other NO_x, SO_2, and O_3). The results show a decrease in total phenol concentrations with levels of nitrogen oxide pollutions (significant negative correlations between the total phenol concentrations and concentrations of NO, NO_2, and other NO_x). Total flavonoids (total flavonols and proanthocyanidins) are useful bioindicators for ozone pollution (significant negative correlations between total proanthocyanidins and the concentrations of ozone and significant positive correlations between total flavonols and the ozone pollution). Sulfur dioxide pollution is distinguished by low concentrations in quercetin, isorhamnetin, and kaempferol (significant negative correlations between these simple flavonols and the concentrations of SO_2). This work confirms the strong interest of using the phenolic compounds of *Pinus halepensis* as biological indicators of air quality.

The quality of the air is now monitored daily in sensitive areas by numerous organizations specializing in air quality control (Bortnick and Stetzer, 2002). However, the impact of the various atmospheric pollutants on living organisms remains a poorly known phenomenon. Observation of the reactions of a living organism exposed to air pollution under natural conditions is necessary and some compounds formed in these reactions may be used as bioindicators. Bioindication is defined as the demonstration of the impact of environmental factors on animal or plant organisms. The response of the organisms reflects the complex effects of harmful substances, not only by showing the synergic effects of these substances, but also by integrating the time factor (Rossbach et al., 1999).

The bioindication of air quality has been described in numerous studies on plants (Zobel and Nighswander, 1991; Rautio et al., 1998; Rossbach et al., 1999; Rahman et al., 2000; Vassileva et al., 2000; Conti and Cecchetti, 2001; Godefroid, 2001). A wide range of diagnostic methods is used and may be applied at variable levels of observation: absence or presence of species (Giordani et al., 2002), morphological variations, presence of necroses or growth and development disturbances (Impens and Delcarte, 1995; Manning et al., 2002), or the occurrence of internal physiological alterations (Rossbach et al., 1999). In the latter case, analysis involves the measurement of certain chemical compounds contained in the plants as revealing a particular environmental state. In particular, the impact of air pollutants is often studied on the basis of experimentation under controlled conditions, each pollutant being generally tested in isolation (Peñuelas et al., 1996; Rodrigues et al., 1996). Studies performed under natural conditions are rare and sometimes only deal with the impact of a single type of pollutant (Karlsson et al., 1995; Oleksyn et al., 1999; Chappelka et al., 1999). However, the validation of a bioindicator requires the undertaking of experiments under natural conditions.

Among the chemical compounds in plants, secondary metabolites are of great importance in plant–environment relationships in particular the phenolic compounds (e.g., phenols and flavonoids; Haslam, 1989; Rhodes, 1994). These phenolic compounds are of particular interest because of their involvement in the response of the plant to environmental stress, such as a deficit in nutrients, the impact of ultraviolet rays (UV) or air pollution (Vogt et al., 1991; Chaves et al., 1993; Muzika, 1993; Peñuelas et al., 1996; Cooper-Driver and Bhattacharya, 1998; Pisani and Distel, 1998). The phenolic compounds include a wide range of substances possessing one or several hydroxyls, bound to at least one aromatic ring (Waterman and Mole, 1994). While numerous studies have investigated the impact of air pollutants on concentrations of total phenols (Karolewski, 1990; Giertych and Karolewski, 1993; Karolewski and Giertych, 1994; Karolewski and Giertych, 1995; Peñuelas et al., 1996; Giertych et al., 1999), no research has been done on flavonols and flavonoids except a recent study on the impact of heavy metal air pollution on *Betula* (Loponen et al., 2001).

To determine whether phenolic compounds may be used as air quality bioindicators, experiments should be performed in the natural environment. The region of Marseille (France) was chosen as experimental base because of the high level of human pressure in the area. The aim of this study was to analyze concentrations of total phenols and flavonoids (total proanthocyanidins, total and simple flavonols) in the needles of *Pinus halepensis* Mill., at various sites exposed to pollution by O_3, SO_2, and NO_x, to determine whether these compounds may be used as bioindicators of air quality. *Pinus halepensis* Mill. was chosen for the study because it is a widespread Mediterranean tree and can be found in both natural and urban environments.

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Abbreviations: NO_x, nitrogen oxides other than NO and NO_2; PCA, principle component analyses; UV, ultraviolet rays.

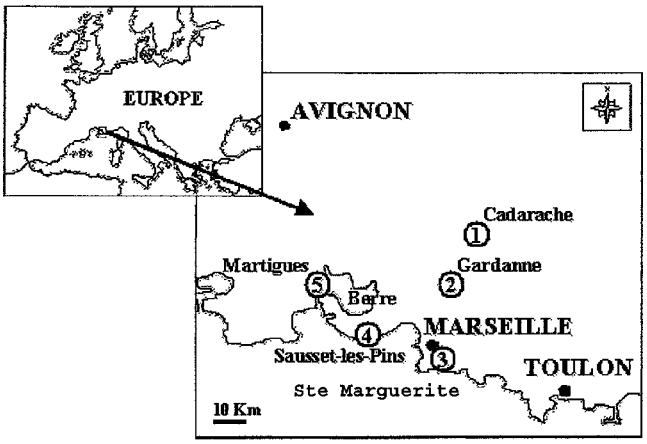

Fig. 1. Localization of study sites.

MATERIALS AND METHODS

Study Sites and Plant Material

Five study sites, situated in the vicinity of Marseille (southern France), were chosen for the experiments [Cadarache (1), Gardanne (2), Ste Marguerite (3), Sausset-les-Pins (4), Martigues (5); Fig. 1]. The sites are located in a geographically limited area with the same bioclimatic and edaphic conditions to minimize variations in these environmental factors. These sites were chosen according to their air pollutant levels measured by AIRFOBEP and AIRMARAIX (the organizations that are responsible for monitoring the air quality in the region). Since industrial activity and road transport represent the major sources of air pollution in this area, five types of pollutants were considered for this study: NO, NO_2, NO_x (excluding NO and NO_2), SO_2, and O_3. Each air pollutant was measured by automatic specific analyzer of each pollutant (AIRFOBEP, 2000). Each atmospheric pollutant was measured every 15 min at each study station over the period between 7 Dec. 2000 and 7 Feb. 2001 (R. Thieleke, personal communication, 2000; Table 1).

Sampling was performed on 7 Feb. 2001 with the objective to obtain current needles that are 1 yr old and because this period corresponds to the winter dry season. For each site, the first year needles from six specimens were randomly collected at all level of the canopy. For each tree, all the current needles collected at all level of the canopy were pooled to obtain one sample per tree. To avoid age-related variations, the samples were taken on specimens of about 30 yr. The age of each specimen was previously determined on cores by means of a Pressler drill (SDMO Quiniou, Haguenau, France). The needles were oven dried at 40°C for 1 wk, then ground and stored in darkness at room temperature.

Chemical Processing

The method of extraction of the phenols is based on the work of Peñuelas et al. (1996). One-half gram (dry weight) of needles per sample was extracted with 20 mL of a 70% aqueous methanol solution (v/v) acidified by a few drops of 1 *M* HCl. The mixture is left at ambient temperature for 1.5 h, and then filtered. Quantification of the total phenols is done by colorimetric reaction using Folin-Ciocalteu reagent, following the method of Marigo (1973). After 1 h, the reaction is completed and measured at 720 nm on a Philips PU 8620 spectrophotometer (Philips, Bobigny, France). The quantitative results are expressed with reference to gallic acid as in Peñuelas et al. (1996).

Table 1. Mean concentrations of air pollutants at the study sites.

Stations	NO	NO_x	NO_2	O_3	SO_2
			$\mu g\ m^{-3}$		
1. Cadarache	4.49	13.82	14.27	37.90	2.30
2. Gardanne	3.11	13.55	15.70	31.70	14.53
3. Ste Marguerite	13.09	30.96	27.15	40.24	6.85
4. Sausset-les-Pins	2.14	11.70	13.78	43.59	18.70
5. Martigues	5.81	23.05	26.92	42.76	7.38

For the extraction and the quantification of the flavonoids (total proanthocyanidins, total and simple flavonols), we have used the method described on *Pinus halepensis* needles by Kaundun et al. (1998). Two grams (dry weight) of needles are put into suspension in 100 mL of 2 *M* HCl. The solution is heated to 80°C in a water bath with reflux for 50 min with insufflations of air every 10 min. The acidic treatment generated anthocyanidins from homologous proanthocyanidins and flavonol aglycones from corresponding flavonol glycosides (Kaundun et al., 1998). The solution is left to cool for about 30 min (Solution A), and then except for 2 mL removed for measurement of the proanthocyanidins, the solution is filtered on a no. 3 porosity filter. The flavonols are extracted from the filtered solution and from the filter wash liquid with 60 mL (two times) and then with 40 mL (once) of diethyl ether. These different fractions are then remixed and evaporated. The residue is conserved in the freezer in darkness and then mixed with 10 mL of methanol (Solution B) before analysis of the simple and total flavonols.

A volume of 2 mL taken from the Solution A is adjusted to 5 mL with 2 *M* HCl. The total (pro)anthocyanidins are measured using an absorption spectrophotometer (Philips PU8620) at a wavelength of 530 nm. As shown by Kaundun et al. (1998), the constant ratio between the different (pro)anthocynidins in *Pinus halepensis* needles allows a quantification of the mixture. The results are indicated as measurements of optical density (OD), since there are no standards.

A volume of 100 μL of Solution B is left to react for 20 min in 5 mL of a 1% $AlCl_3$ (w/w) methanol solution. The total flavonols are measured by absorption spectrophotometry at a wavelength of 435 nm (measurement of the formation of colored complex intervening in presence of Al^{3+} ions). The quantitative results are expressed with reference to a standard set made with quercetin.

Separation and quantification of the simple flavonols are performed using the Solution B by high performance liquid chromatography (Hewlett Packard Series 1050, Palo Alto, CA) equipped with a photodiode array detector. The column used is a Nucléosil 100 C18 column (4.6 by 250 mm, 5 μm; Varian, Les Ulis, France) fitted with a stationary same-phase precolumn. The mobile phase consists of a ternary mixture of solvents: A, water with 10% (v/v) acetic acid; B, methanol; and C, tetrahydrofuran. Elution is performed at a flow rate of 1 mL min^{-1} on the following gradient: from 63:10:17 (A/B/C) to 23:60:17 in 40 min. The simple flavonols are detected at 370 nm. The injection loop is 10 μL. Identification of compounds is based on the comparison of their retention times and of their UV spectrum (from 250–600 nm) with those of commercial standards (Roth-Sochiel, Lauterbourg, France), using the photodiode array detector. Calibration is external.

Statistical Analysis

Statistical analysis was performed using the software Statview (Brain Power Inc., Calabasas, CA) and Statitcf (ITCF, SESI, Maisse, France). Concentrations of air pollutants between the different sites were compared by means of a one-way ANOVA. This was based on daily means for each of

the pollutants (roughly 40 measurements per site and per pollutant). Variations in total phenol concentrations, total concentrations in proanthocyanidins and in flavonols, according to site, were determined by non-parametric Kruskal–Wallis tests, followed by a NKS test (six measurements per site for each of the chemical parameters).

A correlation matrix was devised on the basis of the full set of data on the concentrations (per individual) of total phenols, total proanthocyanidins, and total flavonols and the concentrations of each of the pollutants at the different sites, to assess whether there were significant statistical relations between these groups of data. A correlation matrix was also devised on the basis of concentrations per individual of simple flavonols and concentrations of each of the pollutants.

A principal components analysis (PCA) was performed. This concerned the concentrations of total phenols, total proanthocyanidins and total flavonols, and the air pollutant concentrations, on the basis of the full set of data for the samples, or 30 individual points (six individuals, five stations), and eight variables. On the basis of the coordinates of the individual points in the PCA, a hierarchical ascending classification of momentum of the order two was performed to allow statistical grouping of individuals.

RESULTS

The six study sites had statistically different air pollutant concentrations (one-way ANOVA, $P < 0.05$; Table 1). Cadarache (1) was characterized by low concentrations of all the measured pollutants. Gardanne (2) showed a high SO_2 content. High SO_2 and O_3 concentrations were recorded at Sausset-les-Pins (4). Ste Marguerite (3) and Martigues (5) had high N-based compounds and O_3 concentrations.

Total Phenolic Compounds

The concentrations of total phenols in current needles differ significantly between the various study sites (Kruskal–Wallis Test, $P < 0.0001$; Fig. 2). Results show two groups: the first composed by Cadarache (1), Gardanne (2), and Sausset-les-Pins (4); the second by Martigues (5) and Ste Marguerite (3). The first group present significant higher concentrations than the second (NKS test, $P < 0.05$). There are significant negative correlations between the total phenol concentrations and concentrations of NO ($r = -0.524$, $P < 0.01$), NO_2 ($r = -0.766$, $P < 0.01$), other NO_x ($r = -0.682$, $P < 0.01$) and O_3 ($r = -0.369$, $P < 0.05$).

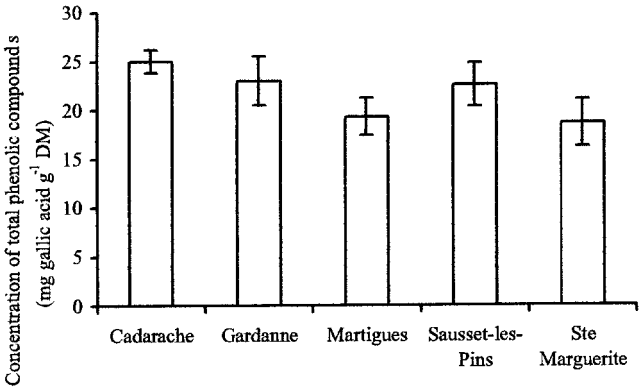

Fig. 2. Mean concentrations of total phenolic compounds in needles of Aleppo pine (mg gallic acid g^{-1} dry matter ± standard deviation) for the various study sites.

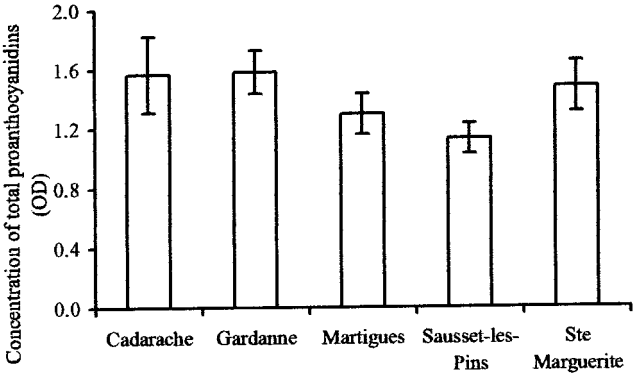

Fig. 3. Concentrations of total proanthocyanidins in needles of Aleppo pine (optical density [OD] ± standard deviation) for the various study sites.

Mean total proanthocyanidin concentrations are significantly different between the various study sites (Kruskal–Wallis Test, $P = 0.0005$; Fig. 3). The highest concentrations are observed for specimens from Gardanne (2) and the lowest for specimens from Sausset-les-Pins (4). There are negative significant correlations between this parameter and the concentrations of O_3 ($r = -0.625$; $P < 0.01$) and SO_2 ($r = -0.387$; $P < 0.05$).

Total flavonol concentrations differ significantly between the study stations (Kruskal–Wallis Test, $P = 0.001$; Fig. 4). Martigues (5) shows the highest values, whereas Gardanne (2) has low concentrations. The total flavonol concentration is correlated positively with the O_3 concentration ($r = 0.519$, $P < 0.01$) and negatively with the SO_2 concentration ($r = -0.336$, $P < 0.05$).

Figure 5 represents two-dimensional mapping of the PCA. Axis 1, representing 46.5% of the information, is characterized on the positive side by the N compound concentrations (NO_x, NO_2, and NO in decreasing order of importance) and on the negative side by total phenol concentrations. Axis 2, representing 25.3% of the information, is characterized on the positive side by O_3 concentrations and on the negative side by proanthocyanidin concentrations. The distribution of individual points in Plan 1-2 shows homogeneity between individuals from the same study site. The hierarchical ascending classification distinguishes statistically two main groups of sites:

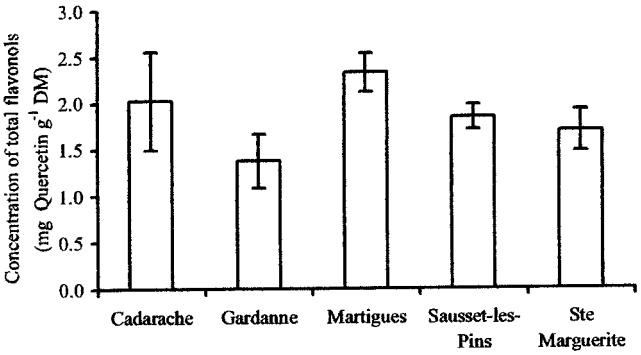

Fig. 4. Mean concentrations of total flavonols in needles of Aleppo pine (mg of quercetin g^{-1} dry matter ± standard deviation) for the various study sites.

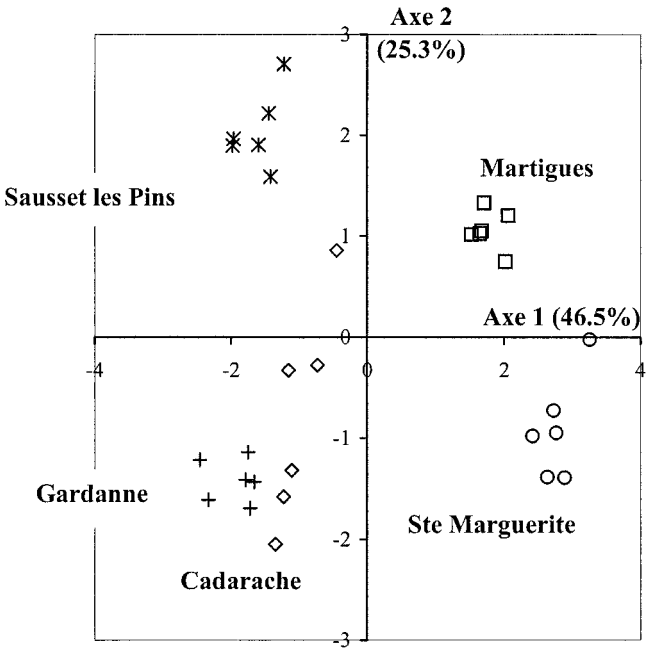

Fig. 5. Two-dimensional mapping of the principal component analysis performed for total phenols, total proanthocyanidins, total flavonols, and air pollutants.

- The first includes the specimens from the sites of Sausset-les-Pins (4), Cadarache (1), and Gardanne (2) that are situated on the negative side of Axis 1. They are characterized by very high total phenol concentrations. These sites show fairly low N air pollutant concentrations. This group is subdivided secondarily into two subgroups, according to Axis 2. The Sausset-les-Pins site (4) shows high O_3 concentrations, and is therefore situated on the positive values side of this axis, whereas the specimens from Cadarache (1) and Gardanne (2), situated on the negative side, show high values for proanthocyanidins.
- The second group includes specimens from the sites at Martigues (5) and Ste Marguerite (3). They are situated on the positive side of Axis 1. These sites show very high concentrations of N based air pollutants. This group is subdivided into two subgroups. The Martigues site (5), with the higher O_3 concentrations and lower proanthocyanidin concentrations, is thus placed on the positive values side of Axis 2.

Simple Flavonols

Six simple flavonols: kaempferol, isorhamnetin, quercetin, larycitrin, myricetin, and syringetin have been identified in the needles of *Pinus halepensis*. They are arranged in order of elution (Table 2). All these compounds contain two aromatic rings (A and B) and a heterocycle of three C atoms including a hydroxyl group on the Carbon 3. They differ from each other in the substitution number (methoxyl or hydroxyl groups) on Cycle B. For the whole set of study sites, kaempferol is the major compound (0.475 mg g^{-1} of dry matter $\pm$0.06; Fig. 6). For the five other compounds measured, the mean concentrations range from 0.050 to 0.238 mg g^{-1} dry matter. After kaempferol, the second major compound is isorhamnetin, followed by quercetin, larycitrin, myricetin, and syringetin, except at Cadarache where quercetin concentrations are higher than those of isorhamnetin (Table 2).

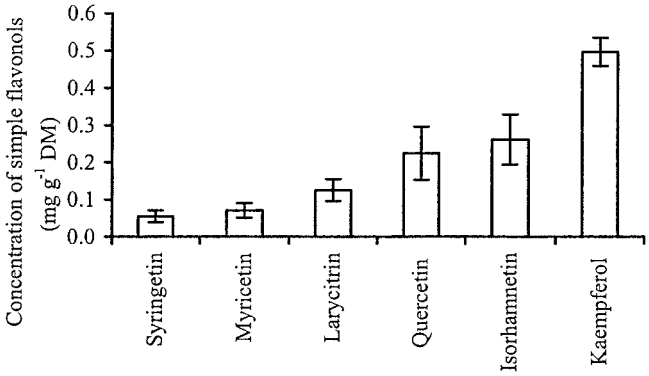

Fig. 6. Mean concentrations of simple flavonols (in mg g^{-1} dry matter $\pm$ standard deviation) for the whole set of study sites.

Correlation matrix shows several significant correlations

(i) negative between the SO_2 and myricetin ($r = -0.423$, $P < 0.01$), quercetin ($r = -0.627$, $P < 0.01$), isorhamnetin ($r = -0.0544$, $P < 0.01$), kaempferol ($r = -0.371$, $P < 0.05$), larycitrin ($r = -0.411$, $P < 0.05$), and syringetin ($r = -0.387$, $P < 0.05$),

(ii) positive between the NO_2, the other nitrogen oxides and the syringetin (respectively $r = 0.520$, $P < 0.01$ and $r = 0.410$, $P < 0.05$), and

(iii) positive between the O_3 and the myricetin ($r = 0.385$, $P < 0.05$), larycitrin and the syringetin ($r = 0.445$ and 0.535 respectively, $P < 0.01$). Only the NO concentration shows no significant relation with concentrations of the various flavonols ($0.003 < r < 0.275$, $P > 0.05$).

DISCUSSION

The amounts of total phenols in the *Pinus halepensis* needles obtained in this study are of the same order as those found in other species of the same genus that

Table 2. Mean concentrations of simple flavonols for the various study sites ($n = 6$).

	Cadarache (1)	Gardanne (2)	Ste Marguerite (3)	Sausset-les-Pins (4)	Martigues (5)
	in µg g^{-1} dry matter $\pm$ standard deviation				
Myricetin	92.5 ± 25.0	40.8 ± 26.6	62.6 ± 17.0	73.2 ± 13.5	82.3 ± 23.4
Larycitrin	143.0 ± 41.3	81.2 ± 38.5	121.0 ± 25.8	121.1 ± 11.6	161.3 ± 42.5
Quercetin	323.3 ± 75.4	169.3 ± 39.5	194.0 ± 91.3	160.2 ± 61.1	276.3 ± 66.9
Syringetin	54.6 ± 22.8	33.1 ± 7.4	57.7 ± 14.0	49.9 ± 6.1	76.8 ± 13.3
Isorhamnetin	321.0 ± 88.8	212.8 ± 63.1	234.4 ± 63.7	194.4 ± 68.9	344.0 ± 88.8
Kaempferol	542.6 ± 79.3	437.6 ± 102.4	499.0 ± 65.8	493.5 ± 68.4	509.2 ± 72.6

are also exposed to air pollution, such as black pine (*Pinus nigra* Arn.) (Giertych et al., 1999), Scotch pine (*Pinus sylvestris* L.) (Karolewski, 1990), or Eliar pine (*Pinus eldarica* L.) (Peñuelas et al., 1996). The six flavonols identified in the needles of *Pinus halepensis* have already been reported by other authors, which study these compounds in a chimiotaxonomic perspective (Kaundun, 1995; Kaundun et al., 1998). They found also kaempferol as the major compound. In their studies, quercetin and isorhamnetin are the second and third major compounds respectively and whereas we found the opposite. These flavonols are also present in other *Pinus* such as Eliar pine (*Pinus brutia* L. and *Pinus eldarica*) (Kaundun, 1995).

Atmospheric pollution level may be considered as a constant during the whole year with a slight increase during the summer and winter dry seasons (AIRFOBEP, 2000). The monitoring of air pollutants during the study period is thus confirmed by long-term records of air pollution at these sites. A direct concentration-effect relationship between air pollutant concentrations and phenol or flavonoid contents may be assumed. However, other environmental factors can cause variations of phenol and flavonoid contents. That is the reason why the sites have been chosen in a geographically limited area where trees were healthy (no symptom observed in a wide area including the sample site).

Negative correlations between N pollutants and total phenol may be explained by the positive action of these pollutants on the nitrate reductase activity (Krywult et al., 1996). An increase of nitrate reductase activity promotes the N assimilation and several studies have demonstrated the existence of negative correlations between concentrations of N and of phenolic compounds in the leaves of various *Pinus* (Giertych et al., 1999). When the concentration of atmospheric natural coumpounds (CO_2) is high, they may induce similar effects as anthropic pollutant. Indeed, Peñuelas et al. (1996) have observed a decrease in the total phenolic compounds in presence of strong air CO_2 concentrations.

Similarly, with regard to the impact of O_3 pollution on the total phenols, a negative correlation has been demonstrated. Few bibliographical studies have been undertaken on the impact of O_3 on total phenols in plants kept under controlled environmental conditions (Howell, 1970; Louguet et al., 1989; Langerbartels et al., 1990; Jordan et al., 1991; Kainulainen et al., 1994; Booker et al., 1996). According to Howell (1970), high O_3 concentrations could influence the enzymatic activity involved in the metabolism of the phenols. Langerbartels et al. (1990) show an increase in the phenolic compounds in the presence of O_3. However, Kainulainen et al. (1994) suggest that there is no response of the total phenols in the presence of strong O_3 concentrations. Booker et al. (1996) showed an increase in total phenols with chronic exposure to O_3 in previous year's needles of loblolly pine (*Pinus taeda)*. For this same species, no significant changes in total phenols were detected in the current year's needles by the same authors (Booker et al., 1996; Jordan et al., 1991). Ozone impact on total phenol concentrations has given rise to many contradictory results and may be dependent on the species. For *Pinus halepensis*, Anttonen et al. (1995) and Pelloux et al. (2001) clearly showed that O_3 induced a decrease in chlorophyll content and an increase in necrosis, as well as alterations in the C metabolism.

This study did not reveal any correlation between concentrations of SO_2 and of total phenols. However, significantly positive correlations between these two parameters are found in controlled environment, for *Pinus sylvestris* (Karolewski, 1990; Giertych and Karolewski, 1993) and even for a distant species such as fava-bean (*Vicia faba* L.) (Nandi et al., 1990). High SO_2 concentrations induce an increase in necrosis and a decrease in respiration, as well as other disorders of the vital functions (Pierre and Queiroz, 1981; Nandi et al., 1990). These alterations thus favor and facilitate the biosynthesis of phenols, since these compounds play a role in respiration (Rubin and Arcichowska, 1971 in Giertych et al., 1999). Similarly, the impact of sulfuric acid, in controlled environment, on *Pinus nigra* and on *Pinus resinosa* Ait. engenders an increase in the phenolic compounds (Zobel and Nighswander, 1991). The defense mechanisms would thus appear to be the same as in the presence of SO_2. The results that we have obtained are thus different and show that it is difficult to offer evidence of the impact of SO_2 on phenolic compounds in natural environment. Other environmental parameters may interact more strongly.

The impact of air pollutants on the total flavonoids, O_3, and SO_2 concentrations are negatively correlated with the total proanthocyanidins and positively correlated with the total flavonols. These results on the impact of O_3 might be set alongside those of Vogt et al. (1991) and Chaves et al. (1993), who have shown that the flavonoids increase with strong UV levels. The contrasting behavior of these two chemical families might be explained by their competition in the metabolic pathways of biosynthesis (Cooper-Driver and Bhattacharya, 1998). The defense mechanisms of *Pinus halepensis* against these air pollutants may favor the increase in total flavonol, perhaps at the expense of the flavonoids. Measurement of these two parameters thus produces the same information with regard to O_3 and SO_2 pollution. Loponen et al. (2001) have studied impact of heavy metal pollution on several phenolic compounds in *Betula pubescens* leaves. They have shown inverse results: an increase of (+)-catechin content and a decrease in flavonol content with high level of pollution. The (+)-catechin monomers (flavan-3-ols) serve as a precursor in a chain of steps toward proanthocyanids, and dihydroflavonols can be the common precursors for flavonols and proanthocyanidins (Loponen et al., 2001). According to the air pollutant, certain enzymes may be activated and thus promote some metabolic pathways. This suggests that heavy metal and gaseous pollutant have antagonist impacts on the dihydroflavonol transformation into flavonols or proanthocyanidins.

All the simple flavonols show significant correlations with one or several pollutants. It appears that syringetin reacts to numerous pollutants (SO_2, NO_2, NO_x, and O_3), but in opposite ways for different compounds. Thus, it

cannot be called an easily interpretable bioindicator. Similarly, myricetin and larycitrin react positively to pollution by O_3 and negatively to pollution by SO_2. However, quercetin, isorhamnetin, and kaempferol contents present significant correlation only with SO_2. They can be characterized in a highly significant manner pollution by SO_2.

CONCLUSIONS

Changes in concentrations of total phenols, total flavonoids, and simple flavonols in needles of *Pinus halepensis* must be considered in relation to changes in concentrations of air pollutants under natural conditions. Total phenol concentrations characterize NO pollution, since they show highly significant negative correlations with this parameter. Total flavonoids (total proanthocyanidins and total flavonols) may be used as bioindicators of O_3 pollution and to a lesser extent to SO_2 pollution. Sulfur dioxide pollution is indicated by low concentrations of quercetin, isorhamnetin, and kaempferol in the plant. Other researches will be performed to characterize the level of a given pollutant, and to check these first field results.

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