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Air pollution effects on flavonoids in pollen grains of some ornamental plants

Farkhondeh REZANEJAD*
Department of Biology, Shahid Bahounar University, Kerman - IRAN

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Abstract: Phenolic compounds function as stress indicators because they accumulate to high levels in many plant tissues in response to a wide range of biotic and abiotic signals. These compounds are involved in pollen development, pollination, pollen germination, and pollen tube growth. The mature pollen grains of *Spartium junceum* L., *Lagerstroemia indica* L., *Thuja orientalis* L., and *Petunia hybrida* L. collected from control (less polluted) and polluted areas [mainly SO_2, NO_2, CO, hydrocarbons (HC), and airborne particulate material (APM)]. The ethanolic aquatic extracts of pollen grains were prepared. The extracts were analysed by high pressure liquid chromatography (HPLC). HPLC analysis demonstrated that air pollution induces flavonoids accumulation to significantly higher levels in the polluted pollen of *Spartium junceum*, *Lagerstroemia indica*, and *Thuja orientalis* than in the controls. In *Petunia hybrida* the flavonoids level increased more slightly in the pollen grains exposed to air pollutants compared to controls.

Key words: Air pollution, flavonoids, ornamental plants, pollen

Introduction

Increase in the levels of air pollution due to the increase in industrial and agricultural technology has prompted investigation of the mechanisms that contribute to air pollution tolerance in plants.

The quality of the air is now monitored daily in sensitive areas by numerous organisations specialising in air quality control (Bortnick & Stetzer, 2002). However, the impact of the various atmospheric pollutants on living organisms remains a poorly understood 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.

Among the chemical compounds in plants, secondary metabolites—in particular the phenolic compounds—are of great importance in plant-environment relationships (e.g., phenols and flavonoids) (Rhodes, 1994). These compounds are of particular interest because of their involvement in the response of the plant to environmental stress, such as a deficit in nutrients and the impact of ultraviolet (UV) rays or air pollution (Chaves et al., 1993; Vogt et al., 1994; Cooper-Driver & Bhattacharya, 1998; Pisani & Distel, 1998; Robles et al., 2003).

Besides providing beautiful pigmentation in flowers, fruits, seeds, and leaves, flavonoids have been proposed to play diverse roles in plant growth and development. Flavonoids are involved in

* E-mail: frezanejad@uk.ac.ir

defence, symbiosis, pollen development and male fertility, pollen germination and tube growth, polar auxin transport, the pigments that attract pollinators and seed dispersers, protection against ultraviolet radiation, and the regulation of the cell cycle (Murphy et al., 2000; Woo et al., 2005)

Flavonoid synthesis is organ and tissue dependent. In flowers, it accumulates in petals, pistils, ovaries, and anthers (Ylstra et al., 1994). According to the current hypothesis, flavonols are synthesised in the tapetum and deposited on the developing pollen after tapetal breakdown at the end of anther development. Their uptake by the pollen cytoplasm results in the presence of flavonols inside the grains (Ylstra et al., 1994). However, there is accumulating evidence that genes encoding enzymes of the flavonoid biosynthetic pathway are also expressed in pollen during early development, which opens the possibility that flavonols are produced in developing microspores as well (Van Tunen et al., 1990). A flavonoid deficient mutant of *Petunia* Juss. is male sterile because its pollen fails to germinate (Woo et al., 2005). However, this is not true for *Arabidopsis* Heynh., in which the lack of flavonoids in flowers and stamens does not affect male fertility (Burbulis et al., 1996). This suggests that flavonoids are not universally required for fertility.

While numerous studies have investigated the impact of air pollutants on concentration of total phenols (Karolewski & Giertych, 1995; Giertych et al., 1999), few studies have been done on the impact of air pollution on leaf flavonoids (Loponen et al., 2001; Robles et al., 2003). Our review of the literature did not show any research measuring the effects of air pollution on pollen flavonoids. The aim of this study was to assess the impact of air pollutants on flavonoids content of plants that are widespread in Iran.

Materials and methods

The pollen grains of *Spartium junceum* L., *Lagerstroemia indica* L., *Thuja orientalis* L., and *Petunia hybrida* L. were obtained from plants growing under control conditions (National Botanical Garden, Pykanshahr, Tehran) and polluted areas with heavy traffic (the centre of Tehran). Reports by the air quality centre at the environmental protection agency showed the type and monthly mean of air pollutant concentrations during flowering times at the sampling sites in both control and polluted areas (Table). Pollen grains of *Thuja orientalis* were collected in May and grains from the other species during June. There were few exceptional days during June because short time peaks (3 h) of air pollutants were reported as 0.1 ppm (SO_2), 0.2 ppm (NO_2), 13.7 ppm (CO), 8.1 ppm [hydrocarbons (HC)], and 191 μg m^{-3} [airborne particulate materials (APM)]. A total of 15 plants were randomly selected from each site (control and polluted).

Samples were extracted with ethanol: water (1:1, v/v; 0.5 g pollen in 5 mL solvent) for 4 h at room temperature. Extraction was aided by vortexing in 30 min intervals. The resultant mix was centrifuged at 5000 × *g* for 20 min at room temperature, and the supernatant was used for high pressure liquid chromatographic (HPLC) analysis (Agilent model 1100). Injection volume was 20 μL, and elution

Table. Means of air pollutant concentrations in control and polluted areas.

Type of air pollutant / Month	SO_2 (ppm)	NO_2 (ppm)	CO (ppm)	HC (ppm)	APM (μg m^{-3})
May (polluted area)	0.082	0.064	3.4	1.80	177
June (polluted area)	0.063	0.06	9.1	2.80	162
May (control area)	0.002	0.010	0.6	0.05	46
June (control area)	0.002	0.010	0.6	0.10	54

APM = airborne particulate material; ppm = part per million.

was performed with a flow rate of 0.8 mL min^{-1}. The column used was c18 column (Zorbax 300SB). The solvents comprised water adjusted to pH 2.5 with orthophosphoric acid (A) and acetonitrile (B) mixed with a linear gradient starting with 100% A, decreasing to 91% over the next 12 min, to 87% over the next 8 min, and to 67% over the next 10 min. After holding the solvent at this composition for 2 min, A was decreased to 57% over the next 10 min and then held at this level until the end of the 60 min analysis. Peaks were detected in 340 nm (Campos et al., 1997). The phenolic fingerprints (derived by comparing peaks in each retention time) were found to be sufficient to show the effect of air pollution on flavonoids of pollen grains in the present study. The impact of air pollution on flavonoid content was examined by comparison of number and levels (concentration) of peaks in chromatograms of pollen grains obtained from polluted and control areas. Experiments were performed in duplicate.

Results

The results of HPLC analysis of *Spartium junceum* show that pollen grains exposed to air pollutants exhibited a significant increase (approximately >2-fold in peaks 2 and 5, >5-fold in peak 3, and >3-fold in peak 4) in flavonoids levels (Figure 1). The height of peak 1 did not reveal the obvious difference between the samples obtained from control (unpolluted) and polluted areas (Figure 1). The HPLC pattern of *Lagerstroemia indica* pollen extracts reveals an increase in flavonoids levels for retention times (RTS) 32.73, 36.12, and 39.47 in response to air pollution. RTs 32.73 (peak 1) and 39.47 (peak 3) show an almost 2-fold increase, but the peak of RT 36.12 (peak 2) increases only modestly in response to air pollution (Figure 2). This pattern in *Thuja orientalis* shows that the pollen grains exposed to air pollutants exhibited a 1.5-fold increase in peak of retention time 33 min (peak 1) compared to the same peak in controls (Figure 3). Pollen grains of *Petunia hybrida* plants grown in polluted areas showed a smaller increase at a retention time of 30 min, corresponding to peak 2, than control plants (Figure 4).

Discussion

Pollen may be used as a sensitive biological indicator of pollution (Wolters & Martens, 1987). In this report the accumulation of high levels of flavonoids is characterised in *Spartium junceum*, *Thuja orientalis*, and *Lagerstroemia indica* in response to air pollution. The type and amount of these compounds depends on the plant species. Our results are consistent with the findings reported

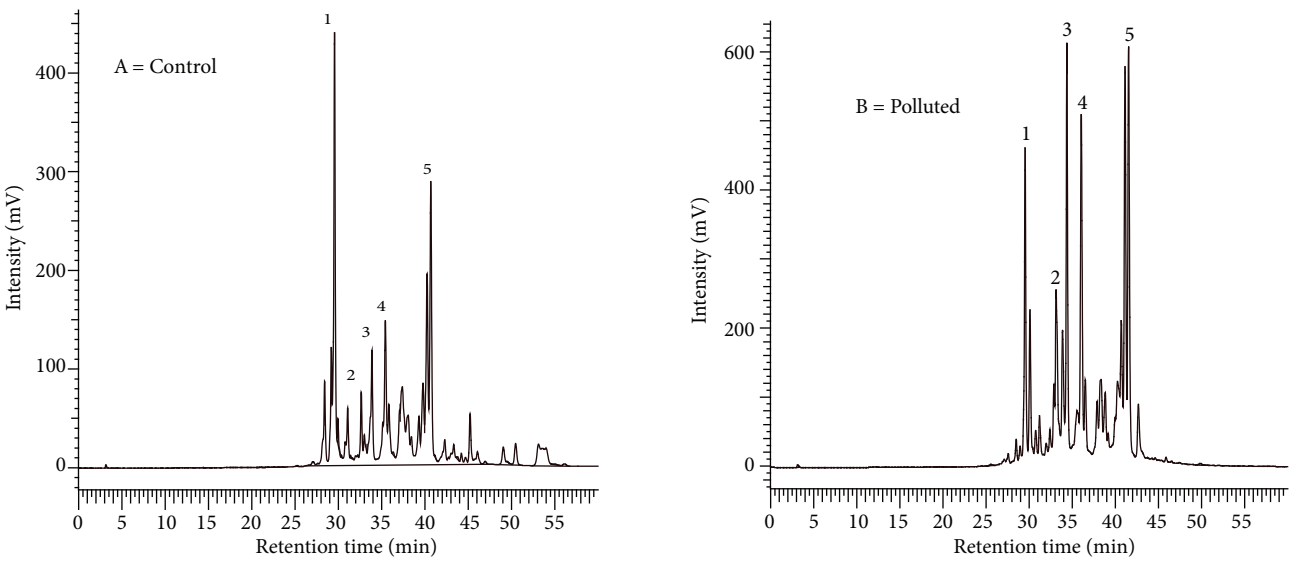

Figure 1. HPLC chromotograms of flavonoids from pollen grains in *Spartium junceum* grown in control and polluted areas, respectively. Key components are designated by their peak retention times (min).

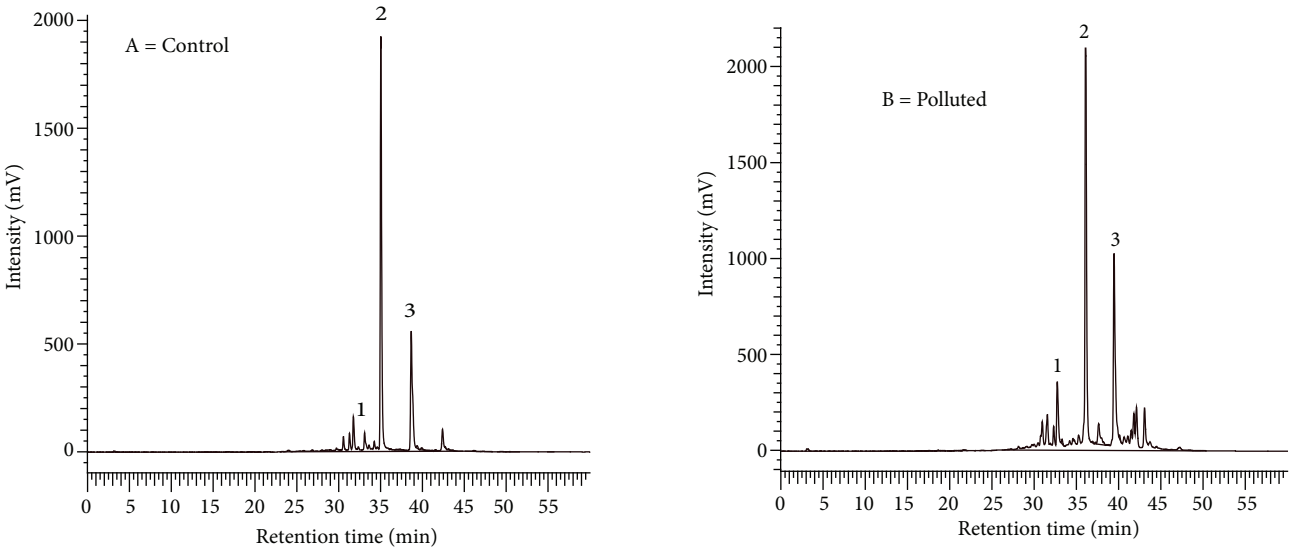

Figure 2. HPLC chromotograms of flavonoids from pollen grains in *Lagerstroemia indica* grown in control and polluted areas, respectively. Key components are designated by their peak retention times (min).

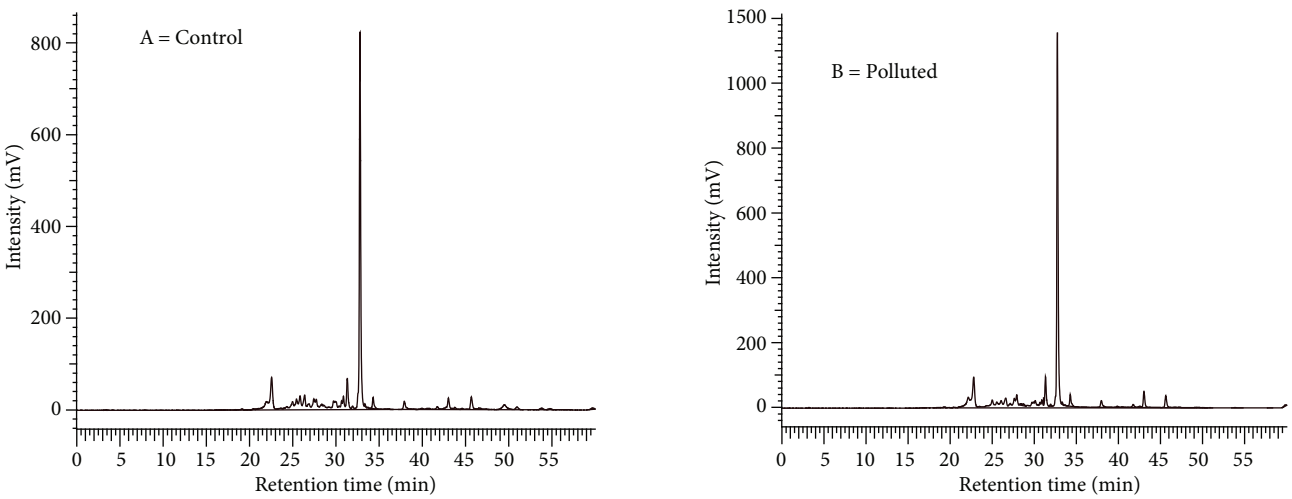

Figure 3. HPLC chromotograms of flavonoids from pollen grains of *Thuja orientalis* grown in control and polluted areas, respectively. Key components are designated by their peak retention times (min).

by Dixon and Paiva (1995), which showed higher quercetin content in samples from a polluted site. They suggested that the content increase could be associated with the defensive role of flavonoids under conditions of environmental stress. The studies by Robles et al. (2003) in *Pinus halepensis* L. leaves did not reveal any correlation between concentrations of SO_2 and total phenols. However, a significantly positive correlation between these 2 parameters was found in a controlled environment in *Pinus sylvestris* L. (Giertych & Karolewski, 1993) and even in the distant species, *Vicia faba* L. (Nandi et al., 1990). Similarly, the impact of sulphuric acid on *Pinus nigra* Arnold—in a controlled environment—and *Pinus resinosa* Ait. shows an increase in phenolic compounds (Zobel & Nighswander, 1991). Robles et al. (2003) showed a decrease in total phenol concentrations with levels of nitrogen oxide pollution. However,

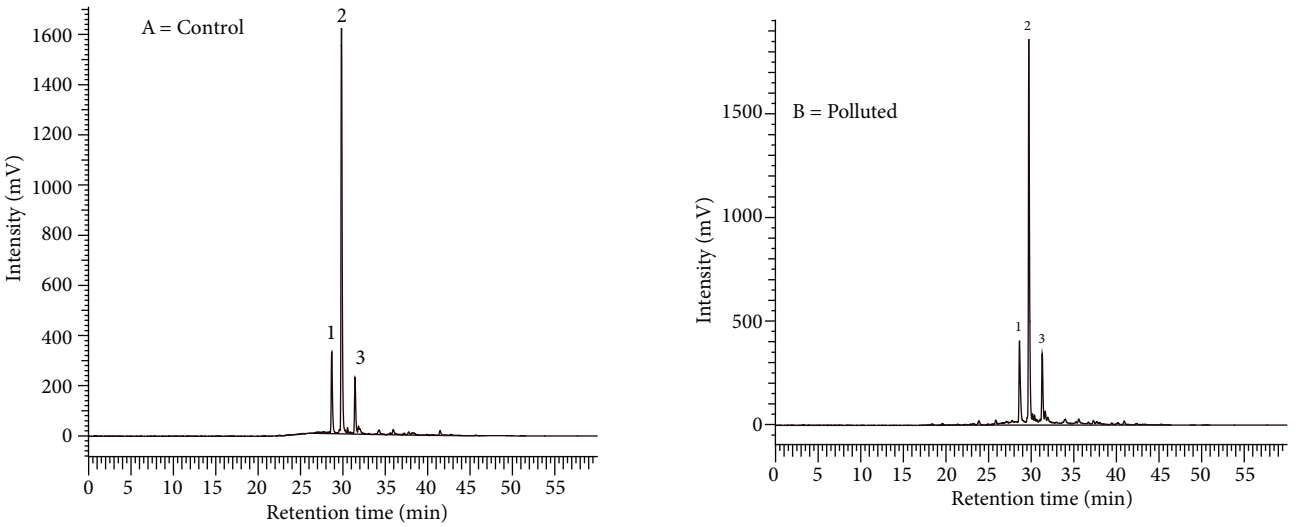

Figure 4. HPLC chromotograms of flavonoids from pollen grains in *Petunia hybrida* grown in control and polluted areas, respectively. Key components are designated by their peak retention times (min).

our results showed an increase in flavonoids with air pollutants together in a natural environment. It seems all air pollutants together, especially APMs, have synergic effects and induce defence mechanisms such as flavonoids biosynthesis. Our search of the literature showed no research about the effect of APMs on pollen flavonoids. It is noteworthy that the concentration of these materials in polluted areas was 5- to 6-fold higher than in control areas. Angiosperm pollen is metabolically very active, and successful fertilisation is highly dependent on 2 factors: rapid germination and pollen tube growth. Our results demonstrated increased flavonoid levels in the presence of air pollutants. Flavonoids are effective in pollen regularity, pollen germination, and pollen tube growth; therefore, this increase is a defence mechanism because air pollutants affect the above-mentioned characters in pollen (Emberlin, 2000; Rezanejad et al., 2003; Rezanejad, 2007). Several lines of inquiry indicate that most air pollutants enter plant tissues and act primarily through the production of reactive oxygen species (ROS)—also called oxidative-free radicals—as is the case with most other abiotic stresses (Alscher et al., 1997). There are 3 important ROS that are highly toxic and cause changes in DNA, proteins and lipids, and membrane organisation: superoxide anion (O_2^-), hydroxy-free radical (OH^-), and H_2O_2. Several in vitro studies have demonstrated that flavonoids can directly scavenge ROS (Bors et al., 1994). This is apparently through the participation of flavonoids in peroxidase-mediated catabolism of H_2O_2, where flavonoids may act as electron donors (Takahama, 1989). There are 2 major flavonoids—quercetin and kaempferol and their glycosides—which can be oxidised by H_2O_2 in the presence of horseradish peroxidase or a cell-free extract of the leaves (Yamasaki, 1997). The leaf extract apparently contains a peroxidase which may use flavonols as a reducing agent for the oxidation of H_2O_2. Thus, flavonoid content increases in response to air pollution, since these compounds can scavenge ROS and induce pollen development, pollen germination, and pollen tube growth, thereby enhancing plant fecundity.

The difference between the 2 profiles in pollen grains of *Petunia hybrida* was not very great, although in this genus the height of peak 2 (RT 30) was higher in polluted than in control groups as well. It is estimated that daily irrigation by fountain removes some of these pollutants from the plant surface. In addition, it is possible that the type of plant species causes these differences.

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