MORPHOLOGICAL AND ANATOMICAL RESPONSES OF PEAR AND ALMOND TREES TO FLUORIDE AIR POLLUTION

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ABSTRACT: The effect of fluoride air pollution on the morphology and anatomy of the leaves of almond (*Prunus dulcis*) and pear (*Pyrus communis*) trees was studied by regular field observations of trees growing in the vicinity of a phosphate fertilizer producing factory. In almond leaves, the damage appeared from the beginning of the growing season and consisted of apical brick-yellow necroses that extended to the leaf margins. In pear leaves, the necroses were not as frequent and appeared later as apical burns. The lower pear leaf surfaces appeared to be covered with a layer of white dust which was difficult to remove. Chemical analysis of the whitish dust layer deposited on the lower surface of the pear leaves and of the necrotic, healthy, leaf stalk, and internodal areas of pear leaves, from both the polluted and non-polluted areas, indicated a mechanism of excluding excessive fluoride to the outside the leaf followed by its removal through leaching, thus allowing the plant to maintain the photosynthetic integrity of its remaining leaf area. Our results suggest the possibility of trapping fluoride ions by calcium, most likely in the form of CaF_2. The damage seemed to appear when calcium was in deficit. Anatomical sections of polluted almond and pear leaves revealed several structural changes induced in response to the fluoride air pollution involving: (i) an elongation in the mesophyll palisade cells, (ii) a disruption in the mesophyll spongy cells, with this being more pronounced in pear leaves, and (iii) a decrease in epidermal cell size associated with thickening of the cuticle. These changes demonstrate the tendency of these species to develop survival strategies in the restrictive condition imposed by the pollution. The presence of morphologically recognizable damage on almond leaves due to fluoride air pollution suggests that almond leaves may be a suitable bio-indicator of fluoride air pollution.

Key words: Air fluoride pollution; Almond; Bio-indicator; Leaves; Morphology; Pear.

INTRODUCTION

SIAPE is a phosphate fertilizer producing factory, located in the southern suburb of Sfax, that converts crude phosphate with a high fluoroapatite [$Ca_5(PO_4)_3F$] content into a granular phosphate fertilizer easily assimilated by plants. During the phosphate digestion by sulphuric and phosphoric acids, fluoride compounds such as HF, H_2SiF_6, and CaF_2 are given off by the factory chimney.[1,2,3] Analysis of the air surrounding the factory showed that fluoride content of the air oscillated between 3 and 12 µg /dm^3/day.[1,4]

Since the installation of the SIAPE factory, the agrosystems in its surroundings have undergone increasing degradation. In the vicinity of the factory, some local fruit species, such as pear and almond trees, grow in a naked landscape and are subjected daily to fluoride air pollution. For the almond tree, which has often been considered as sensitive to fluoride pollution,[5,6] we did not observe any flowers or fruit within a radius of 1.5 km from the source of the pollution. However, for the

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pear tree, classified among the fruit species which are tolerant to fluoride pollution,[6] some ecotypes thrived and fructified in the most polluted area. In an attempt to explore some of the adaptation strategies adopted by both the resistant and the sensitive fruit species for survival in such a fluoride polluted area, and to explain the different plant responses to air pollutants, we studied the distribution of calcium and fluoride in healthy and necrotic leaf areas.

In addition, anatomical sections were examined to (i) identify plant tissues affected by air pollution and (ii) to explore some of the morphological reactions adopted by the species which were still surviving in such restrictive environmental conditions of aridity and temperatures ranging from 35 to 40ºC.[7]

MATERIALS AND METHODS

The plant material for the present study consisted of 2 fruit tree species, indigenous to the Sfax area: almond (*Prunus dulcis*) and pear (*Pyrus communs*) trees.

To study the distribution of leaf calcium and fluoride, sampling was done during the growing season. Each sampling was repeated 3 times. Leaf samples from each species were taken from several branches of the side of the tree exposed to the factory fumes. Almond leaves exhibiting marginal necroses in the middle of the shoots were selected during July for fluoride ion (F) and mineral analyses.The necrotic and central (healthy) leaf areas were separated from each other using scissors and then analyzed within 24 hr. Control samples were gathered in non-polluted land plots situated at a distance of 45 km from the factory. The dust and particles, deposited on the pear leaves, were removed by gentle hand shaking. The leaves were then washed with tap water, dipped into 0.01 M HCl for 5 min, and then thoroughly washed with deionised water.

The damaged leaf areas were carefully cut off so that the central (healthy) leaf surfaces remained attached on mother plant shoots for photosynthesis measurements. Leaf photosynthesis was measured using a portable infrared gas analyzer (CID 301 PS, Vancouver, USA) on attached leaves in the field, between 9:30 and 10:00 am. Only the central leaf area was introduced into the leaf chamber. The leaf surface area as well as the leaf percentage necroses were estimated as reported by the method of Mabrouk and Carbonneau.[8] In order to avoid the effect of light intensity variation, all measurements were taken on sunny days, with the Photosynthetic Active Radiation (PAR) being higher than 1600 μmol/m$^{2/}$sec, by orienting the leaf chamber to obtain the maximum light absorption. The PAR was measured directly by the infrared gas exchange analyser system. The average leaf temperature was 34±2.55ºC.

For the analysis of F and Ca, different plant tissues (leaf blade, leaf stalk, and internodes) were dried at 80ºC for 48 hr and then ground to pass through a 40-mesh sieve in a Willey hammer mill. The leaf powder was re-dried for 1 to 2 hr prior to weighing the sub-samples for analysis at either 80ºC for F or 105ºC for Ca. Fluoride concentrations were determined using the potentiometric technique described by the Association of Official Analytical Chemists AOAC.[9] After digesting the plant powder with nitric and perchloric acids (2 volumes/1 volume), Ca was determined by the atomic absorption spectrophotometry technique with a polarized Zeeman atomic absorption spectrometer (HITACHI, Z-6100).

For the anatomical sections, fresh polluted and non-polluted leaves were gathered and fixed for 24 hr in a Formalin-Acetic-Alcohol mixture (FAA) according to the Sas fixation procedure.[10] The fixed material was rinsed with water and then divided into two lots. The first one was dehydrated in alcohol, embedded in paraffin, cut, and stained with triple stain (Safranin, Heidenhain's and blue aniline).[10] The second lot was cut with a freezing microtome and stained with aceto-carmin.[11] The frozen and serial cross sections were observed under a light Reichert LKB microscope equipped with a camera.

Statistical analyses were performed with the SAS package (Statistical Analysis System, version 6.12, Cary, NC, USA) using both the Duncan multiple range and Student Tests at the 5% significance level.

RESULTS AND DISCUSSION

Morphological responses of the polluted plants: The regular monitoring in the polluted area made it possible to recognize the various expressions of damage caused by fluoride to the almond and pear trees. In the almond trees, the damage appeared on the leaf tips and margins from the beginning of the growing season. and consisted of apical brick-yellow necroses that extended to the leaf margins (Figure 1).

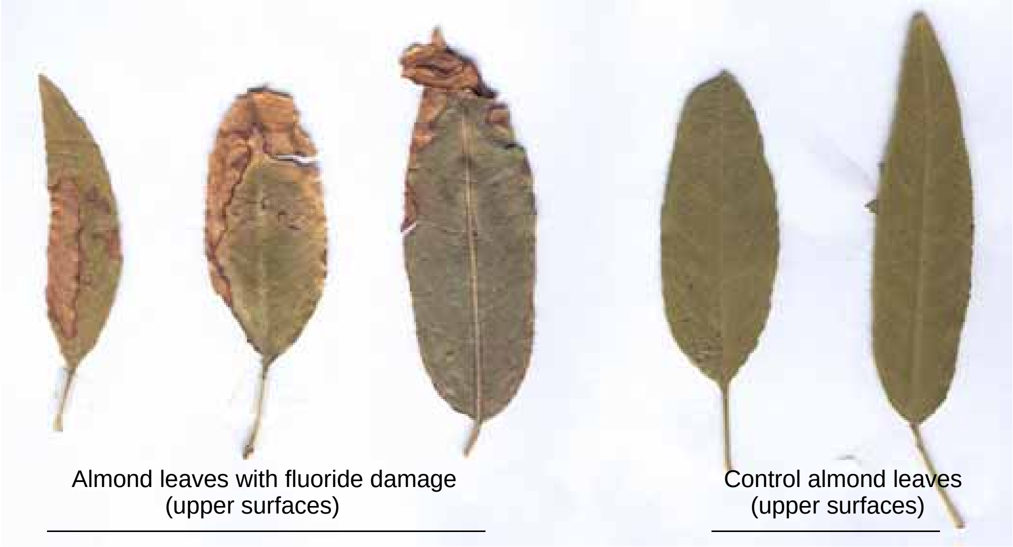

Figure 1. Almond leaves with fluoride damage (left) and control almond leaves (right).

Miller[12] and Fornasiero,[13] reported that gaseous fluorides enter the leaf by diffusion through the stomata, and dissolve in the humid space of the substomatal cavity. The ions are then carried with the transpiration stream to the sites of greatest evaporation, which are usually the leaf margins and tips where the concentrated amounts of F cause the first signs of damage.

As shown in Figure 1, the necrotic tissues were limited by a dark violet borderline consisting of anthocyanin pigments which are secreted by the plant leaf cells when subjected to any stress.[14] The secretion of anthocyanins along a wavy line appears to be a primary defense reaction developed by the plant in order to limit damage at the leaf extremities.

The follow-up of the status of some ecotypes of pear trees exposed to the fumes given off by the polluting factory allowed us to identify the damage caused to this species. Compared to the necroses exhibited on the almond leaves, those on pear leaves were infrequent and appeared later as burns occupying the leaf margins (Figure 2).

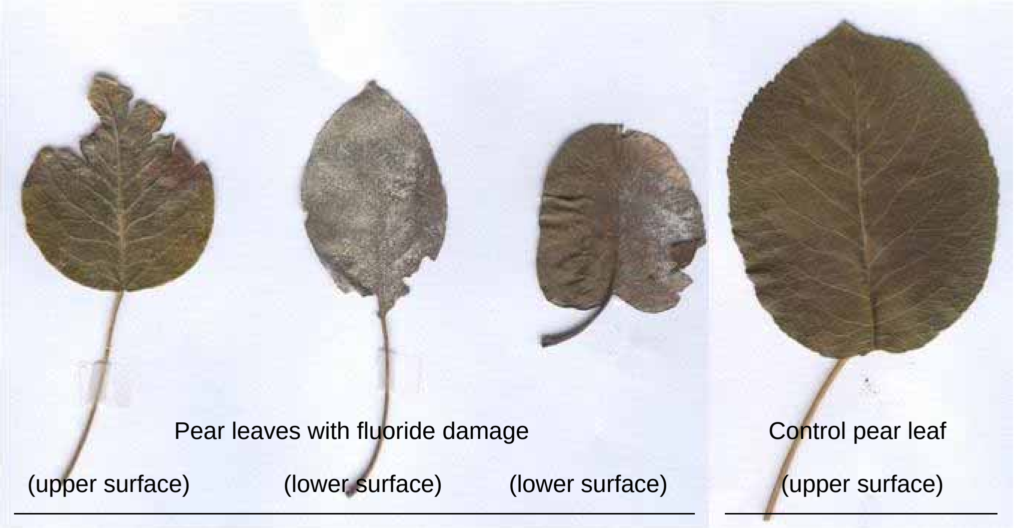

Figure 2. Pear leaves with fluoride damage (left) and control pear leaf (right).

Our results are in agreement with those of Ben Abdallah et al.[15] who considered that this species was a resistant fruit species and tolerant to fluoride air pollution as it accumulated fluoride without showing symptoms either of F toxicity or growth restriction.

Evidence of plant survival mechanisms in the polluted area: The high levels of fluoride in unwashed pear tree leaves (Table 1) and the presence of a whitish layer of dust covering the lower leaf surface (Figure 2) confirm the existence of a particulate pollution[3]. The chemical analyses of the dust deposited on the pear leaves showed high levels of both Ca and F (Table 1).

This suggests the existence of a possible fluoride ion regulatory mechanism by which excess fluoride may be excreted onto the outside of the leaf, followed, probably, by its removal through leaching. Elloumi et al.[7] reported that the particle cover, which was difficult to be remove after washing, seemed to be related to both plant specificity and to the direction of the prevailing winds.

Table 1. Fluoride and calcium contents in the dust covering polluted pear leaves, in non-washed and washed polluted pear leaves, and in non-polluted pear leaves
(Values are the mean±SD of 10 replicates)

Specimen	Fluoride (µg/g dry weight)	Calcium (mg/g dry weight)
Dust	435.5±31.1	140.3±12.2
Non-washed leaves	270.5±14.3	92.0±8.1
Washed leaves	86.0±6.0	73.2±7.1
Non-polluted leaves	15.0±1.1	22.0±1.3

In the absence of rain, particles deposited on the surface of the leaves may obstruct the stomata and cover the assimilatory surfaces. Such particles are not phytotoxic: it is a latent pollution. However, in the rainy season, if a portion of fluoride particulate is leached, another part may be dissolved and then enter the leaf tissue.[16]

The data acquired from our chemical analyses revealed (i) a high fluoride and calcium content in the necrotic almond leaf tips and margins relative to the central part of the leaf, (ii) a low fluoride and calcium content in the healthy leaf areas, in the leaves in the polluted area, in comparison with those of non-polluted leaves from the non-polluted area, and (iii) a non-significant fluoride content in the internodes and leaf stalks adjacent to the polluted leaf blades (Figure 3).

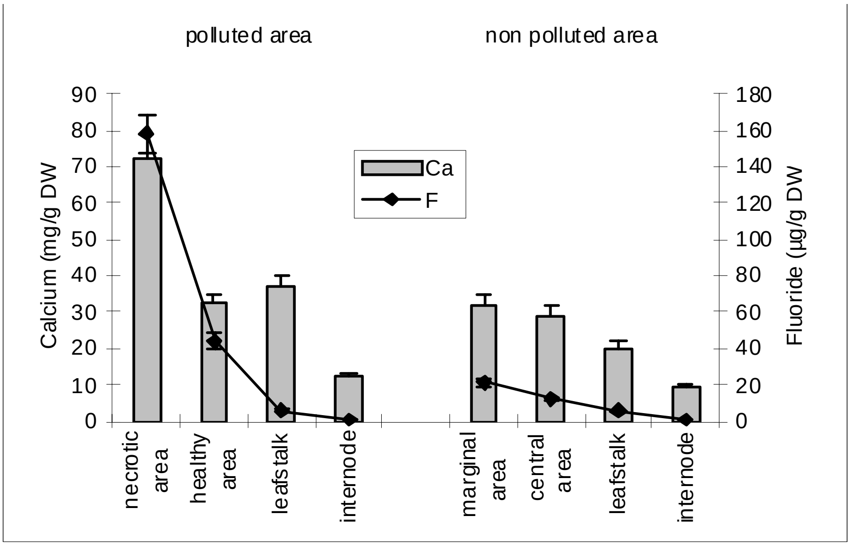

Figure 3. Distribution of calcium and fluoride contents in some organs of polluted and non-polluted almond trees. Values are the means of 10 replicates.

These findings confirm the tendency of polluted species to balance the fluoride accumulation by a parallel calcium accumulation in the leaf margins thus suggesting the possibility of trapping fluoride ions by calcium, most likely in the form of CaF_2. Therefore, tolerant varieties accumulating high levels of fluoride in their leaves are able to sequester it as CaF_2. When trapped in this form, fluoride cannot disturb the plant metabolism.[17] Our findings are in favor of the non-translocation of fluoride, through phloem towards lower plant organs as demonstrated in previous work.[18] The interaction between fluoride and calcium has also been reported with other cations such as silicon and aluminium.[19,20] Therefore, the pear tree, through assimilating calcium ions, would have the ability, not only to fight fluoride ion toxicity through trapping the ions as an insoluble complex of CaF_2, but also to delay the appearance of necroses as late as possible. Such behavior would to explain the relative thriving and fructifying of the pear trees despite their existence in the most polluted area. Ben Abdallah[16] attributed the appearance of necroses to the quantity of calcium available in the leaf tips and/ or margins.

The separation between the healthy and the damaged leaf areas by anthocyanins secreted as a dark wavy borderline would suggest the existence of a possible isolation mechanism developed by the plant in order to avoid the harmful effects of fluoride on its metabolism. By limiting the damage to the margins and leaf tips by this mechanism, the plant is able to maintain the photosynthetic integrity of its remaining leaf area. A significant proportion of the leaf assimilatory surfaces is thereby preserved, thus explaining the ability of the plant to photosynthesize, even when 30 to 40% of its leaf surface is damaged (Table 2). In addition, the evidence at the study site of morphologically recognizable damages on almond leaves due to fluoride air pollution suggests the suitability of almond leaves as a bio-indicator of fluoride air pollution. [21]

Table 2. Net photosynthesis of the healthy areas of almond leaves as a function of the leaf necrosis percentage (n=19)

Parameter	Leaf groups with % of necrosis				
	Control	Leaves with necroses			
	0	10–20	30–40	50–60	>60
Net photosynthesis (μmol $CO_2/m^2/sec$)	18.1±1.1	16.9±1.2	15.16±1.34*	12.24±1.17*	8.0±1.0*

Compared to the control group: *$p \leq 0.001$.

Plant anatomical responses: The anatomical sections of the polluted almond and pear leaves showed a decrease in epidermal cell size, in comparison with the non-polluted controls (Figure 4).

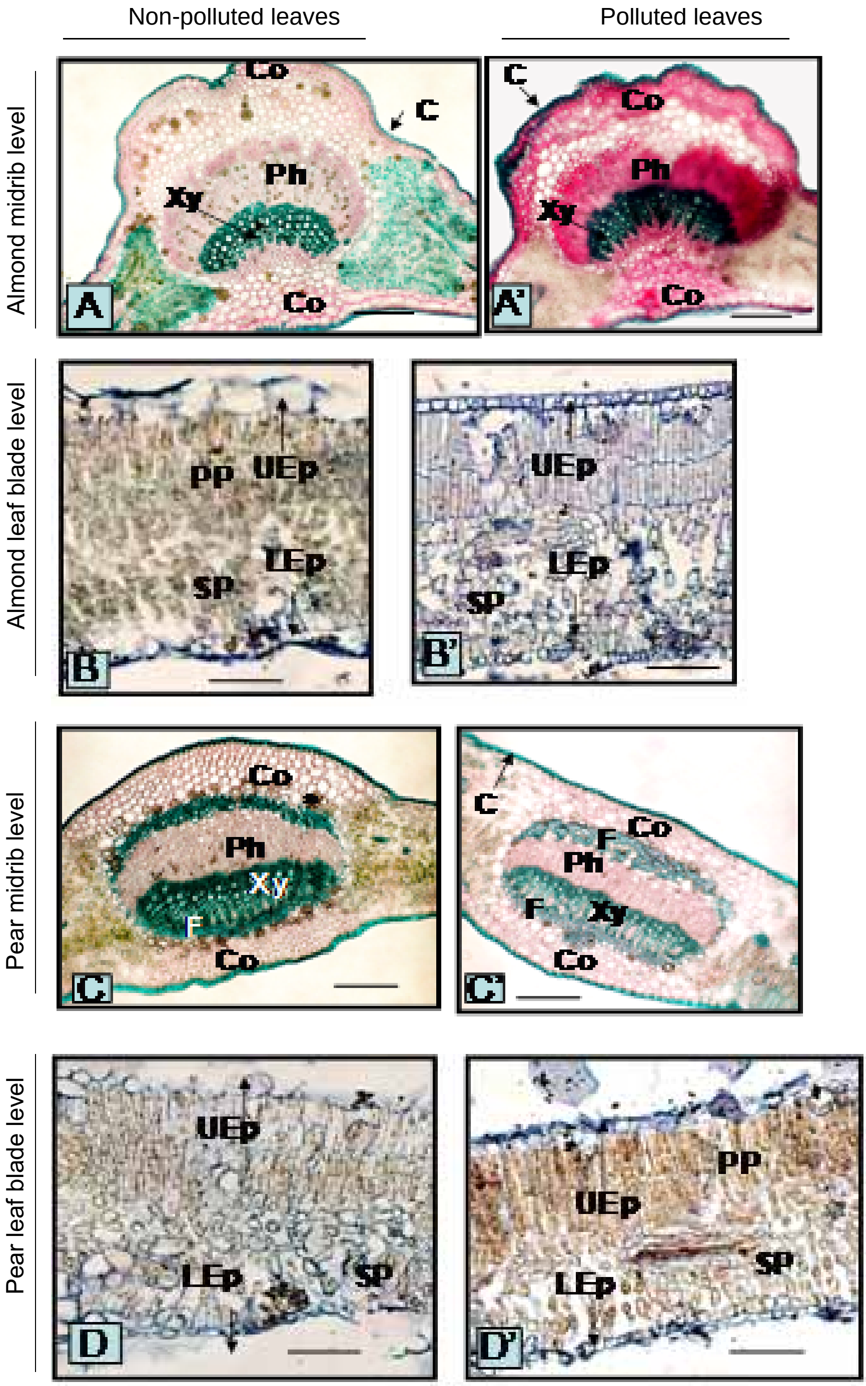

Figure 4. Transverse sections in almond and pear leaves at the midrib and leaf blade levels: (i) At the midrib level (scale bar = 10 µm): A: non-polluted almond leaf, A': polluted almond leaf, C: non-polluted pear leaf, C': polluted pear leaf;
(ii) At the leaf blade level (scale bar = 100 µm): B: non-polluted almond leaf, B': polluted almond leaf, D: non-polluted pear leaf, D': polluted pear leaf.
Abbreviations: C = cuticle; Co = collenchyma; LEp = lower epidermis; Ph = phloem; PP = palisade parenchyma; SP = spongy parenchyma; UEp = upper epidermis; Xy = xylem.
F= Fiber.

Furthermore, with fluoride pollution, the cuticle became thicker in the both the lower and upper epidermis. The thickening of the cutin layers is thought to be the usual response of plants subjected to abiotic stress.[22,23] This thickening, already reported by Stevens et al.,[24] working on *Avena sativa* and *Lycopersicon esculentum*, seems to be more important at the midrib, the lower epidermis of which is folded by the collapse of some of its cells. This folding which is less important in the case of the pear leaf, becomes more important for the almond leaf at the end of the growing season.

In summary, the structural changes found were: (i) in the pear, an increase in the number of fibers at the midrib in such a way as to form a multilayered ring on the side of the phloem, and a remarkable and progressive thickening of the collenchyma cell walls, and (ii) in both species, an elongation in the mesophyll palisade cells, a disruption in the mesophyll spongy cells, which was more pronounced in pear leaves, and a decrease in the epidermal cell size associated with a thickening of the cuticle.

Our results corroborate those of Millar[12] and Zuiazek and Shay[25] who reported that leaf damage occurs first in the spongy mesophyll and lower epidermis, and later a total collapse occurs with an alteration of the leaf architecture.[12,25]

CONCLUSIONS

The morphological and structural changes occurring in the leaves of almond and pear trees in response to fluoride air pollution demonstrate the tendency of these species to develop survival strategies in the restrictive condition imposed by the pollution. The presence of morphologically recognizable damage on almond leaves due to fluoride air pollution suggests that almond leaves may be a suitable bio-indicator of fluoride air pollution

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