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To cite this version:

Ferjani Ben Abdallah, Nada Elloumi, Imed Mezghani, Jean-Pierre J.-P. Garrec, Makki Boukhris. Industrial fluoride pollution of Jerbi grape leaves and the distribution of F,Ca, Mg, and P in them. Fluoride, 2006, 39 (1), pp.43-48. hal-02659605

INDUSTRIAL FLUORIDE POLLUTION OF JERBI GRAPE LEAVES AND THE DISTRIBUTION OF F, Ca, Mg, AND P IN THEM

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SUMMARY: Fluoride damaged leaves of the Jerbi grape vine tree (*Vitis vinifera* L) growing in the vicinity of a phosphate fertilizer manufacturing plant near Sfax, Tunisia, were used to study the distribution of the chemical elements F, Ca, Mg, and P in the leaves and stalks. Photosynthesis and the chlorophyll content of healthy leaf parts were also investigated to determine tolerance mechanisms of this species to fluoride. The subdivision of the necrotic zone into concentric necrotic halos evidently reflects a series of plant reactions to preserve a large portion of the leaf assimilatory surface. Photosynthesis still occurred with 30 to 40% necrosis of the leaves. Preferential accumulation of F was found in the leaf margins along with a parallel accumulation of Ca. Necrosis becomes evident when a fall in Ca occurs with an excess of F. After this happens, the plant tends to maintain high Mg and P concentrations in its healthy leaf parts. At 60% leaf necrosis, F seems to be effective in reducing photosynthesis and chlorophyll content.

Keywords: Calcium level; Fluoride tolerance; Fluorine trapping mechanism; Jerbi leaf bio-indicator; Leaf calcium; Leaf magnesium; Necrotic halos; Leaf phosphorus; Translocation.

INTRODUCTION

Sfax, the second city of Tunisia, has a considerable gene pool of indigenous grapevine varieties adapted to the local climatic conditions of arid regions. In spite of sensitivity of grapevines to air pollutants as reported in literature,[1-3] some local ecotypes of the Jerbi variety (*Vitis vinifera* L) still survive in the area surrounding the "Société Industrielle d'Acide Phosphorique et d'Engrais" (SIAPE) factory near Sfax. Analyses of the air surrounding the factory indicate that the fluoride (F) content of the air varies between 3 and 12 $\mu g/m^3$/day.[4] The F pollutants emitted into the air by SIAPE are mainly HF, SiF_4, and H_2SiF_6.[5-7]

The aim of this work is to show that, owing to its great surface, the grape leaf may constitute an excellent plant bio-indicator of morphological and physiological effects of fluoride pollution. Mineral analyses of necrotic and central leaf areas were performed in order to understand the role of certain elements in avoiding pollutant toxicity. In addition, our study allowed us to explore some adaptation strategies adopted by these vines surviving in such harsh conditions of pollution and aridity such as high temperatures ranging from 35 to 40ºC and low rainfall.[8]

Our weekly field observations of the evolution of leaf necroses showed that the margin subdivisions of the grape leaves into necrotic halos can be used as a useful indicator of morphological and physiological alterations.

MATERIALS AND METHODS

The Jerbi grapevine tree *Vitis vinifera* L studied here is indigenous to the Sfax area. The vine trees were marked in land plots close to the SIAPE factory. To study the distribution of leaf Ca and F as a function of time, sampling was done from

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April through October during the year 2004. Each sampling was repeated 3 times. Leaf samples from each local vine were taken from several branches in different parts of the vine tree side exposed to the factory fume. For F and mineral analyses of necrotic and healthy leaf areas, only grape leaves exhibiting marginal necroses and occupying the middle of the shoots were selected during July. Scissors were used to separate necrotic and central (healthy) leaf areas from each other and then analyzed within 24 hr. Control samples were gathered in non-polluted land plots situated at a distance of 30 km from the factory.

Damaged leaf areas were cut off carefully in such a way that central (healthy) leaf surfaces remain attached on mother plant shoots for photosynthesis measurements. Leaf photosynthesis was measured using a portable infrared gas analyzer (CID 301 PS, USA) on attached leaves in the field, between 9:30 and 10:00 am. Only the central leaf area was introduced in the leaf chamber. Leaf surface as well as leaf percentage necroses were estimated as reported by the method of Mabrouk and Carbonneau.[9] In order to avoid the effect of light intensity variation, all measurements were taken on sunny days, with Photosynthetic Active Radiation being higher than 1600 μmol/m^2/s, by orienting the leaf chamber to obtain maximum light absorption. The average leaf temperature was 34ºC ± 2.55ºC. After photosynthesis measurements, attached central leaf areas were picked to determine their chlorophyll content. The latter was estimated by absorbance at 645 and 663 nm of an 80% acetone extract of ten leaf discs obtained from each sample used for photosynthesis measurements as reported by Arnon.[10]

For analysis of F and nutrient elements Ca, Mg, and P, different plant tissues (leaf blade, leaf stalk, and internodes) were dried at 80ºC for 48 hr and ground to pass through a 40-mesh sieve in a Willey hammer mill. Leaf powder was re-dried for 1 to 2 hr prior to weighing sub-samples for analysis at either 80ºC for F or 105ºC for Ca, Mg, and P.

Fluoride concentrations were determined using the potentiometric technique described by the Association of Official Analytical Chemists.[11] After digesting plant powder with nitric and perchloric acids (2v/1v), Ca and Mg were determined by the atomic absorption spectrophotometry technique, while P (as phosphate) was determined by the vanado-molybdate phosphoric yellow colour method. [12]

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

RESULTS

1. Morphological responses of the grape leaf: In the polluted area, grape leaves exhibited marginal necroses of brick red color, occupying lobe extremities (Figure 1). Necrotic areas are generally separated from healthy ones by a dark violet borderline. As time advances, new necrotic tissues are limited by a new borderline, thus showing concentric halos of necrotic zones giving the leaf margin a mosaic appearance (Figure 1).

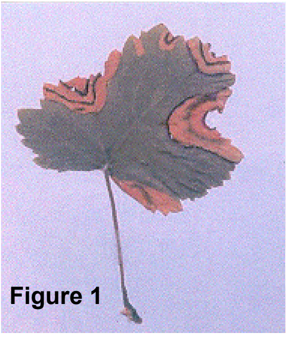

Figure 1. Necrotic halos on grape leaf margins in polluted area.

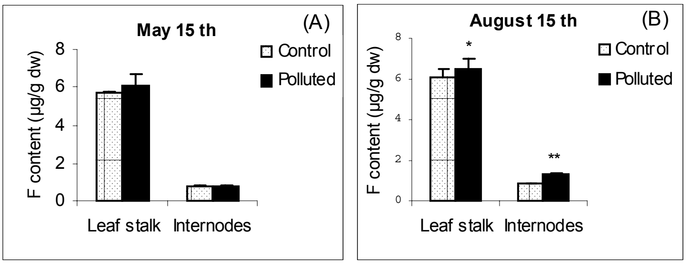

Figure 2. Fluoride content (µg/g/dw) of leaf stalk and internodes of Jerbi variety, during the year 2004, at two different periods (A) and (B) in polluted and non-polluted areas. Values are means SE (n = 10). *p< 0.05; **p < 0.001.

2. Leaf fluoride accumulation sites: As seen in Figures 2A and 2B, low fluoride concentrations in leaf stalks and internodes were recorded at the beginning as well as at the end of the growing season. Analyses of necrotic and central leaf slices show a significant F accumulation in the necrotic leaf margins areas.

On the other hand, as indicated in Figure 3, the F content of the central leaf parts including the main veins was in the range of the controls.

3. Leaf calcium, magnesium, and phosphorus distribution: As also seen in Figure 3, the mineral analyses of the central and necrotic grape leaf areas reveal high Ca and F concentrations in the leaf margins. The polluted grapevine seems to retain more Mg and P in the central leaf areas than in the leaf margins.

4. Leaf calcium and fluoride distribution as a function of time: In the polluted area, the distribution of leaf F and Ca showed a considerable increase with time until the end of August, when maximum leaf F coincided with a drop in Ca content (Figure 4). Our weekly field observations showed that typical necroses from F generally appeared after Ca had fallen (indicated by arrow on the curve). Such variations were not observed in controls from the non-polluted area (Figure 5).

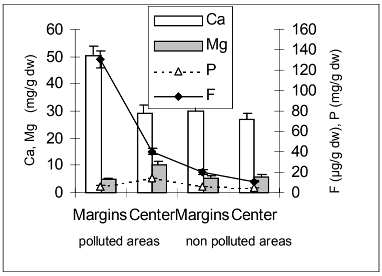

Figure 3. Fluoride, calcium, magnesium, and phosphorus concentrations in central and necrotic leaf areas of Jerbi variety during the year 2004 in polluted and non-polluted areas. Values are means SE (n=10).

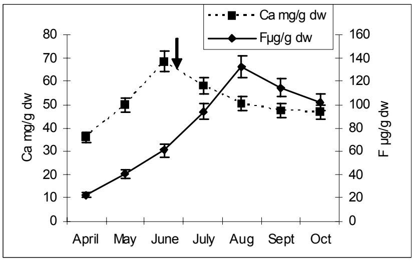

Figure 4. Temporal distribution of Jerbi leaf fluoride and calcium contents, during the year 2004, in polluted area. Values are means SE (n=10). The arrow indicates appearance of necroses.

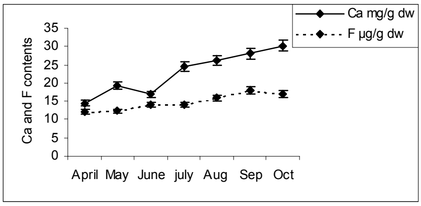

Figure 5. Temporal distribution of Jerbi leaf calcium (mg/g dw) and fluoride (µg/g dw) contents, during the year 2004, in non-polluted area. Values are means SE (n=10).

5. Photosynthesis and chlorophyll content of healthy leaf areas: Our results show that photosynthesis and chlorophyll contents of central (healthy) leaf areas decreased as the percentage of leaf necroses increased (Figures 6 and 7) and that the plant could still photosynthesize while 30 to 40% of its leaf surface was necrotic. However, when 60% or more of the leaf surface was damaged, and there was less than 40% of healthy leaf area, low chlorophyll content and low net photosynthesis of the central leaf area was observed.

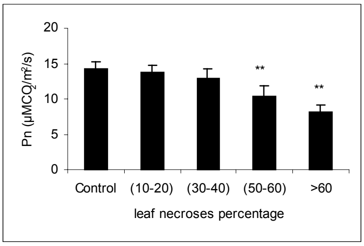

Figure 6. Net photosynthesis in central leaf areas of Jerbi variety in relation to percentage of leaf necroses. Values are means SE (n=19). **p < 0.001.

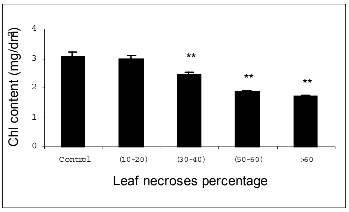

Figure 7. Chlorophyll content (mg/dm^2) of central leaf areas of Jerbi variety in relation to percentage of leaf necroses.

DISCUSSION

In our study, we observed that necrotic and healthy portions of leaf tissues were separated by a dark violet borderline. The latter consists of anthocyanin pigments secreted by grapevine leaf cells when under stress.[13] The subdivision of necrotic leaf margins into concentric halos, the number of which increases with time, seems to reflect a series of primary defense reactions developed by the plant in order to limit damage to the leaf blade extremities. A significant proportion of the leaf assimilatory surfaces is thereby preserved, thus explaining the ability of the plant to photosynthesize while 30 to 40 % of its leaf surface is damaged. In addition, the evidence of fluoride air pollution in the study site [4] suggests that damaged grape leaf can be used as a bio-indicator of fluoride pollution.[14] Our observations indicate preferential accumulation of fluoride in leaf tissues extremities, where necroses appear, with relatively little fluoride being retained by internodes, leaf stalks, and central leaf parts including the main veins, thus confirming that apical and marginal necroses can be considered as typical symptoms of fluoride compounds as previously described.[15-19] Furthermore, by still surviving in the most polluted area, without showing growth restrictions, the Jerbi variety of grapes can be considered to be fairly tolerant to air fluoride pollution. The tendency of this local variety to balance the fluoride accumulation by a parallel calcium accumulation in its leaf margins suggests trapping of fluoride in the form of CaF_2. Therefore, tolerant varieties accumulating a high leaf fluoride content are able to sequester it, as CaF_2. When trapped in this form, fluoride cannot disturb the plant metabolism.[20] Our findings favour the non-translocation of fluoride, through phloem towards lower plant organs as demonstrated in previous work.[21] The interaction between fluoride and calcium has also been reported with other cations such as silicon[22] and aluminium.[23] Since magnesium is a central component of the chlorophyll molecule as well as a compound of the cell wall pectin, and in view of its important role for enzyme balance and protein synthesis,[24] the tendency to considerably increase the Ca content where F is present and to keep more Mg in the central leaf parts also implicates Mg in a detoxification mechanism that consists in trapping fluoride in the form of MgF_2. Both mechanisms allow the plant to maintain its Ca and Mg concentrations at an adequate level compatible with its survival under such challenging conditions.

Since fluoride exclusion from protoplasm and cellular fluids is most likely to be as insoluble CaF_2,[20] the coincidence of the decline in Ca content with the appearance of the first leaf necroses points to the role played by Ca in detoxifying F. When no more Ca is available, probably corresponding to its fall, damage seems to appear in the form of necroses. Therefore, Mg might be taken from the chlorophyll molecule as MgF_2, thus possibly explaining the decrease of chlorophyll concentrations and net photosynthesis as the percentage of leaf necroses increases with time. This reduction in chlorophyll as leaf necrosis increases may also reflect the breakdown of membrane structure within the chloroplasts or the direct effects of F on chlorophyll biosynthesis.[17]

On the other hand, given the abnormal increase of phosphorus (P) in the central parts of the leaf as the outer areas of leaf necrosis increases (unpublished data of our laboratory), we consider this increase in P and Mg concentrations recorded in the central parts of the leaf to be a strategy adopted by the Jerbi grapevine in an arid and polluted area to minimize damage by F as much as possible. Moreover, it is known that Mg is a key component for the activity of enzymes involved in the transfer of phosphate.[25] Similarly, a correlation has been observed between symptoms of grapevine saline toxicity injury and a low supply of P.[26] On the other hand, investigations on the algae *Chlorella vulgaris* showed that entry of aluminium into the cell was restricted by phosphate but promoted by fluoride.[27] We therefore propose that dust and particles in the form of crushed crude phosphate drifted daily by winds in the area[7] may contribute as an adaptation factor to fluoride pollution. In addition, the adaptation of the Jerbi variety to the harsh climatic conditions of the Tunisian Sahara with drought, sandy soils, low rainfall, etc.[28] seems to go hand in hand with its tolerance to pollution.

ACKNOWLEDGMENTS

Support for this research from the Regional Laboratory of Environmental Sciences (LARSEN) at ENIS-Sfax and from the Olive Institute is greatly appreciated.

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