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Influence of volcanic gases on the epidermis of *Pinus halepensis* Mill. in Campi Flegrei, Southern Italy: A possible tool for detecting volcanism in present and past floras

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ABSTRACT

Cuticle micromorphology together with epidermal and epistomatal wax, in both current- and first-year-old needles of conifer *Pinus halepensis* (Aleppo pine) trees growing under volcanic gas fumigation was analysed in Pisciarelli area, Campi Flegrei, Southern Italy. As a control, current- and first-year-old needles growing far from volcanic gas emission were also sampled. Using a multidisciplinary approach with SEM, TEM and X-ray, volcanic gases were shown to cause degradation on epicuticular and epistomatal waxes. Significant statistical variations of ultrastructural components of the cuticle, with 30 measurements, including total thickness of the cuticle, and details and proportions of all different layers, and use of confidence interval, revealed a high degree of sensitivity of Aleppo pine to this extreme environment. In the present study, non-significant thickness variations of the cell wall plus cuticle among current- and first-year-old needles of both fumigated and non fumigated trees have been found. However, at the ultrastructural level, significant variations in cell wall and total cuticle thickness, especially within the three zones of B1 fibrillar layer, revealed different equilibria for each of the four types of material. Using energy dispersive X-ray microanalysis, no sulphur was found in either cuticle or epidermal cells, but the presence of H_2S in the fumarole gas is suspected to cause indirect and/or direct cuticle alterations of wax structure. Ultrastructural characters of plant cuticles related to emission of volcanic gases during the geological past are also discussed. Among these considerations, an identification key enabling distinction between non fumigated and fumigated materials with 9 characters, provides a good tool detecting the influence of volcanism for extant and fossil plants.

1. Introduction

Over the geological history of the planet, among chronic environmental stress factors advocated as killing agents (Visscher et al., 2004), changes in atmospheric chemistry have had world-wide dramatic effects on terrestrial plant life (e.g. Visscher et al., 1996; Meyer and Kump, 2008). For instance, among chemical contaminants that could have disrupted the end-Permian biota, volcanogenic SO_2 (Visscher et al., 2004) and biological H_2S (euxinia mechanism: Kump et al., 2005; Berner and Ward, 2006) gases are favoured to explain extinction. In particular, volcanism subsequently played a role in both maintaining and perturbing the atmospheric chemistry and physics, with important implications in terms of the evolution of life (Mather, 2008). The development of large igneous provinces (LIP) and continental flood basalt provinces (CFBP) (Courtillot and Renne, 2003; Jerram and Widdowson, 2005; Keller, 2008; Bryan et al., 2010) commonly coincides with mass extinction events (Wignall, 2001, 2005; Rampino, 2010; Whiteside et al., 2010) and results in the release of significant volumes of gases, such as CO_2, H_2S and SO_2 into the atmosphere (Beerling and Berner, 2002; Berner and Beerling, 2007; Hori et al., 2007). It is widely recognized that volcanic sulphur dioxide (SO_2) and hydrogen sulphide (H_2S) emissions are significant sources of sulphur release to the atmosphere (Bates et al., 1992; Berner and Berner, 1996).

Gases emitted by volcanoes represent a factor inhibiting vegetation development (Whittaker et al., 1989) and could have been responsible of the decline of vegetation during periods of global-scale volcanism (Visscher et al., 2004; McElwain and Punyasena, 2007; Bond et al., 2010; Whiteside et al., 2010). In particular, H_2S is often thought to be a phytotoxin, being harmful to the growth and development of plants (Lisjak et al., 2010) especially when the quantities are higher than plant necessity (Thompson and Kats, 1978; Lorenzini and Nali, 2005).

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Moreover, atmospheric pollutants produced by volcanic activity and OAEs (Oceanic Anoxic Events), such as SO_2 and H_2S, are said to be absorbed via the cuticle as well as the stomata (Haworth and McElwain, 2008).

Plants exposed to poisonous volcanic gases may show signs of damage including total defoliation and death (e.g. Dickson, 1965; Clarkson and Clarkson, 1994; Delmelle et al., 2002). However, plant damage is related to gas concentration (Delmelle, 2003), gas persistence (Grattan et al., 1998) in the atmosphere, identity of the gases (Thomas, 1951) and plant resilience (Abe and Hasegawa, 2008). Under severe pollution conditions, the direct phytotoxic effects of gaseous pollutants as well as long-term effects of acid washout (Grattan and Pyat, 1994) can even be considered as potential environmental mutagens disturbing plant growth and community structure (Visscher et al., 1996). As a matter of fact, as Visscher et al. (2004) pointed out, variation in structure and composition of leaf cuticles is a potential source of botanical evidence of mutational effects of environmental stress factors.

Therefore, leaves in natural environments are subjected to a range of physical processes which may damage their surfaces, leading to alterations in the structure and integrity of the cuticle, and consequently changes in the physical properties of the leaf surfaces (van Gardingen et al., 1991).

To this end, numerous articles have been published in relation to the effects and interactions of volcanic products (e.g. tephra or ash fall) on both fossil (e.g. Kovar-Eder et al., 2001; García Massini and Jacobs, 2011) and extant plants (Eggler, 1948; Winner and Mooney, 1980b; Cook et al., 1981; Seymour et al., 1983; Dale et al., 2005). Moreover, in extant plants concentration of chemical elements in the leaves (Notcutt and Davies, 1989; Bellomo et al., 2007; Martin et al., 2009a, 2009b) and analysis of the trunks (Baillie and Munro, 1988; Battipaglia et al., 2007) together with field studies led to significant advances in understanding the composition and dispersion of volcanic emissions at source (e.g. Kempter et al., 1996; Delmelle et al., 2002), including major "gas species" (Costa et al., 2005; Chiodini, 2008; Chiodini et al., 2010a).

Leaves of plants act as passive and active collectors for natural (e.g. Martin et al., 2009a) and anthropogenic (e.g. Bačić et al., 1999) airborne pollutants (e.g. gas, aerosols and dusts) and are more sensitive to air quality than other plant organs (e.g. roots) (Landolt et al., 1989; Casseles, 1998; Kabata-Pendias, 2001). Gas exchange between plants and surrounding atmosphere is mediated by the cuticle; a non-living (Riederer, 2006) thin (<0.1–10 μm thick in extant plants) and heterogeneous membrane (van Gardingen et al., 1991) that covers the epidermis of aerial parts of many tracheophytes (Guignard et al., 2004). Cuticle consists of a polymer matrix (cutin), polysaccharides and associated solvent-soluble lipids which are synthesised by the epidermal cells and deposited on their outer wall (Kirkwood, 1999; Riederer and Schreiber, 2001). The outer surface of the cuticle is coated with epicuticular waxes, a general term (Jeffree, 2006) designating very long chain hydrocarbons found embedded within the cuticle and also in the crystalline epicuticular wax layer (Bird and Gray, 2003). The main function ascribed to waxes is to limit the diffusional flow of water and solutes across the cuticle (Heredia and Dominguez, 2009), providing protection for the leaf cells (Turunen and Huttunen, 1990) and acting as the main barrier to air pollutants (e.g. Jeffree, 1986). Composition and amount of waxes in cuticle have been shown to vary depending on environmental conditions of the plant (Baker, 1982; Bird and Gray, 2003). According to many authors air pollution seems to increase the rate of wax tubule degradation (e.g. Huttunen and Laine, 1983; Riding and Percy, 1985; Berg, 1987; Turunen and Huttunen, 1990, 1991; Huttunen, 1994). In particular, wax load and structure can be used as an indicator of pollution level (Holroyd et al., 2002; Hansell and Oppenheimer, 2006). Epicuticular wax of pine needles undergoes an ageing procedure during needle lifetime (Turunen and Huttunen, 1996; Bačić et al., 1999) and is disturbed by polluted air (Huttunen, 1994). The literature is replete with references to structural changes in epicuticular waxes following exposure to air pollutants (see Turunen and Huttunen, 1990), and erosion of epicuticular wax is a relevant factor of multiple forest decline syndrome (Turunen and Huttunen, 1990).

Few palaeobotanical works have been published on cuticular characters related to volcanic stress. Archangelsky et al. (1995) and Villar de Seoane (2001) studied Early Cretaceous plants from Patagonia (recovered in Baqueró and Springhill Formations, respectively) demonstrating that volcanic ash fall played an important role in the formation of xeromorphic structures. Haworth and McElwain (2008) claimed that the effect of toxic atmospheric gases and volcanic dust would explain xeromorphic features observed in *Pseudofrenelopsis parceramosa* (Fontaine) Watson from the Early Cretaceous of England. Moreover, the relationship between ultrastructural characteristics of cuticle and the environment is still poorly understood for extinct as well as extant plants (Guignard et al., 2001). Cuticular ultrastructure data are not numerous for fossil conifers (e.g. Guignard et al., 1998; Villar de Seoane, 1998; Yang et al., 2009) and seem to be still lacking for some species belonging to the genus *Pinus* (Jeffree, 2006).

However, to date, no studies have been carried out relative to the response of the ultrastructural features of plant cuticle exposed to the persistent volcanic gases. Conifers are well suited for studies of pollutant levels because they are evergreen and often have long-lived foliage. Usually the needles have a life cycle of several years (Hellström, 2003). Therefore, the protective role of the epicuticular waxes is particularly important for conifers that have to ensure their investment in leaf tissue for several years (Chabot and Chabot, 1977). In the volcanic area of Pisciarelli (Campi Flegrei, Southern Italy) the gymnosperm *Pinus halepensis* Mill. (Aleppo pine) is the only conifer growing adjacent to the fumaroles, and much of the surrounding vegetation (under study) displays indications of damage caused by toxic gases. *Pinus halepensis* is the most abundant pine species in the western Mediterranean Basin, where it occupies 2.5 million ha (Quézel, 2000) and it is considered as an opportunistic species (Nathan and Ne'eman, 2000) which is able to regenerate either in the absence or as a result of fire. In addition, *P. halepensis* has an elevated resistance to drought (Boddi et al., 2002), so much so that Emberger (1930) identifies it as being semiarid, and Oppenheimer (1968) considers it as the most arid-tolerant of all the *Pinus* species. The present study aims to assess the cuticular response of this conifer to a prolonged exposure to volcanic gases using both SEM and TEM approaches. Moreover, to our knowledge, this is the first study that tests cuticle ultrastructure behaviour during two subsequent years (current- and first-year-old needles) in response to the fumigation by volcanic gases containing H_2S.

The purpose of this research was to investigate: (1) response of plants to volcanic gases through different aspects: epicuticular and epistomatal waxes and ultrastructural features of the cuticle; (2) potential implications of the conifer cuticle response across environmental stress periods during the geological past; and (3) a new method detecting the influence of volcanism for extant and fossil plants.

2. Plants studied and methods

Material was collected from two localities in the Phlegrean Fields (Campi Flegrei, Campania Region), an active caldera which spans the last 50,000 years (Scandone et al., 2010), characterised by significant recent ground deformation (Morhange et al., 2006) and considered as one of the most dangerous volcanic areas in the world due to future eruptions (Chiodini et al., 2010a). In particular, pine needles were recovered from the famous fumarole field in Pisciarelli locality (40° 49′ 48.88″ N, 14° 08′ 46.95″ E) about 1 km SE of the Solfatara volcano, both sites characterised by volcanic degassing (Fig. 1A,B). Control samples of needles were collected from an area (Cigliano: 40° 50′ 46.46″ N, 14° 07′ 36.31″ E) about 2.5 km from Pisciarelli and characterised by the absence of volcanic gas emissions and the presence of clean air. Both localities are characterised by the same soil features (Di Gennaro and Terribile, 1999; Di Gennaro, 2002) and sun exposure, each is far away from traffic and industries.