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Damage structures in leaf epidermis and cuticle as an indicator of elevated atmospheric sulphur dioxide in early Mesozoic floras

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ABSTRACT

Volcanic episodes are considered to be potentially important drivers of many mass extinction events in Earth history, when a sharp decrease in the diversity and abundance of macroscopic organisms occurred. Such events are hypothesised to be associated with volcanic sulphur dioxide (SO_2) release. The effect of volcanism on atmospheric composition varies widely and is related to the magnitude and duration of the volcanic episode. Currently, there are limited methods for detecting the timing of SO_2 release in the geological past. In field conditions, the influence of SO_2 on plants can be difficult to separate from the effects of other volcanic emissions, which enter via stomata and can affect plant physiology, anatomy and morphology. In order to assess the direct effects of SO_2 associated with palaeo-volcanic episodes, we conducted a six month growth chamber experiment growing plants under control (zero parts per million (ppm)) and continuous elevated (0.2 ppm) sulphur dioxide atmospheres. Leaf morphological responses of ten species representative of Mesozoic gymnosperm and fern fossil floras were examined using cryo-scanning electron microscopy. Here we show that expanded, fully mature leaves record unambiguous damage structures associated with injury from SO_2 fumigation. In SO_2 treated plants, leaves were smaller and did not persist; distinct raised areas of cuticle surrounding stomata appeared; surface waxes altered; blistering of the cuticle occurred; and the stomatal complex became distorted. These results have clear implications for application to the fossil record as many of the observed damage structures have the potential to be preserved in fossil plant cuticle and thus allow precise pinpointing of elevated SO_2 episodes in the geological past.

1. Introduction

1.1. Sources of sulphur dioxide

Volcanic episodes are considered to be important drivers of mass extinction events in Earth history, when the diversity and abundance of macroscopic organisms declined sharply (Courtillot and Renne, 2003; Ganino and Arndt, 2009; van de Schootbrugge et al., 2009; Wignall, 2011). These events are believed to be associated with the volcanic release of sulphur dioxide (SO_2), for example, the Permian–Triassic extinction event of circa 252 Ma ago (Shen et al., 2011), considered to be the worst extinction event in Earth history (Benton and Twitchett, 2003), and the Triassic–Jurassic extinction event of circa 201 Ma ago (McElwain et al., 1999; Tanner et al., 2001; Hesselbo et al., 2002; van de Schootbrugge et al., 2009; Whiteside et al., 2010). Sulphur dioxide emitted by volcanic eruptions can be short-lived; the atmospheric impacts are dependent on ejection volume and height. Volcanoes may be forceful enough to project toxic gases including SO_2 into the stratosphere, leading to global cooling (Rampino et al., 1979; Rampino, 2002; Tanner et al., 2007), whereas effusive and fissure volcanoes more frequently result in volcanic gases remaining in the troposphere, leading to global warming (Gudmundsson, 1996). Volcanic eruptions also discharge many other gases, including hydrogen sulphide (H_2S), which rapidly oxidises in the atmosphere to form SO_2 (Brown, 1982; Kump et al., 2005), carbon dioxide (CO_2), hydrogen chloride (HCl), hydrogen fluoride (HF) and large amounts of water vapour (H_2O). Thus, volcanic gases, alone or in tandem, damage plants. Of all these gases, we chose to concentrate on one of the major components of volcanic gases, sulphur dioxide (Symonds et al., 1994). Yet currently, methods for detecting the timing and magnitude of release in the geological past of sulphur dioxide are limited.

Contemporary anthropogenic sources of SO_2 result from the processing and combustion of fossil fuels containing sulphur. Acid rain occurs when SO_2 reacts in the atmosphere with water, oxygen and other chemicals to form a mild solution of sulphuric acid (H_2SO_4) (Grattan, 2005), which is then deposited on plants by wet or dry deposition depending on weather conditions (Kim et al., 1997). A wealth of research in the 1980s and 1990s into the effects of simulated acid rain on leaves confirms that acid rain alters the physical and chemical properties of cuticle and epicuticular waxes, in some cases leading to deformed stomatal complexes during development of the cuticle (Haines et al., 1985;

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Percy and Baker, 1987, 1990; Turunen and Huttunen, 1990; Tuomisto and Neuvonen, 1993; Turunen et al., 1995). Early greenhouse and plant growth chamber studies confirmed that, in addition to epicuticular damage, simulated acid rain composed of SO_2, HCl and HF, either alone or in combination, resulted in the following effects: permanent chromosomal and phenotypic changes in the offspring of HF-fumigated tomato seeds (Mohamed, 1968); alterations in leaf area and internode length in *Citrus sinensis* exposed to a mixture of SO_2 and HF (Matsushima and Brewer, 1972); leaf chlorosis with increased leaf fall rate in *Citrus unshu* subjected to HF alone (Matsushima and Brewer, 1972); and stomatal closure leading to reduced transpiration and raised leaf temperature in soybean (*Glycine max*) under HF fumigation (Poovaiah and Wiebe, 1973). Phytotoxic gases (atmospheric gases that inhibit plant growth or poison plants) can have major deleterious effects on plants growing near the source of a pollutant. Plants that grow close to volcanic vents and are regularly subjected to phytotoxic gases, such as SO_2, may develop resistance to the pollutant (Winner and Mooney, 1985; Haworth et al., 2010), whereas those exposed to occasional and catastrophic amounts of toxic gases may succumb to foliar injury and die (Haworth et al., 2012). In a study on the influence of volcanic gases on *Pinus halepensis*, Bartiromo et al. (2012) showed degradation of epicuticular and epistomatal waxes as fusion of the wax structures; the presence of H_2S in the fumarole gas likely caused the damage. In a further study, Bartiromo et al. (2013) undertook comprehensive research into the effects of long-term exposure to volcanic gases on an angiosperm, *Erica arborea*; however, it was unclear whether the alterations in cuticle that occurred resulted from elevated CO_2 or SO_2 or both. Regular exposure to volcanic gases has also been shown to modify leaf physiognomy (Bacon et al., 2013) and change stomatal density and its ratio to the stomatal index (Haworth et al., 2012). Therefore, development of leaf damage and changes in leaf structure and shape can provide a record of exposure to toxic gases. If such plants were subsequently preserved through fossilisation, it may be possible to detect signs of SO_2 damage in the preserved cuticles and use this record of leaf damage as a proxy for toxic gases in the geological past. However, there are currently no studies where leaf-level SO_2 damage has been investigated and categorised in rigorously controlled experimental conditions in plant growth chambers with a view to developing a palaeo-SO_2 proxy.

1.2. Effects of sulphur dioxide on plants

SO_2 enters plants through minute epidermal pores called stomata. When stomata open to allow exchange of carbon dioxide, oxygen and water vapour with the atmosphere, sulphur dioxide enters by diffusion due to the lower concentration gradient of SO_2 inside the plant. Stomata close in response to low concentrations of atmospheric SO_2, whereas high concentrations may cause continuous stomatal opening as the epidermal cells surrounding the guard cells are damaged by SO_2 and no longer provide the structural support required for effective stomatal function (Black and Black, 1979; Neighbour et al., 1988; Robinson et al., 1998; McAinsh et al., 2002). In addition, SO_2 can affect stomata by slowing their ability to close, thus impairing stomatal control (McAinsh et al., 2002). Once inside the aqueous environment of the cell, SO_2 is converted to sulphite (SO_3^{2-}) and hydrogen sulphite (HSO_3^-); sulphite is then partly oxidised to sulphate (SO_4^{2-}) (Zeigler, 1972). A certain amount of sulphur can be metabolised by plants but beyond a critical threshold level, determined by the concentration of gas (Black and Black, 1979) and duration of exposure (Ashenden, 1979), damage occurs. Sulphur oxides are acidic and, at high concentrations, acids denature membrane-associated proteins embedded in the phospholipid bilayer of the plasma membrane that are essential for osmotic regulation (Heath, 1980). Membrane-associated calcium (mCa) ions are important second messengers in signal transduction responses to environmental stimuli. The calcium ions influence permeability by stabilising membrane structure, especially in conifers (DeHayes et al., 1999). Hydrogen ions in acids, such as sulphuric acid, can displace calcium ions in the plasma membrane, thus impairing physiological responses to environmental stresses.

Some SO_2 permeates the cuticle but most enters via the stomata due to the lower resistance pathway. The physical and chemical properties of some plant cuticles facilitate repellence of water and particles that could otherwise have negative effects on the plant (Neinhuis and Barthlott, 1997; Haworth and McElwain, 2008). Additionally, wax structures on the cuticle reflect and scatter photosynthetically active and UV radiation; therefore, any phytotoxic gas that degrades plant cuticle induces increased absorbance of light and possible photo-oxidative damage in leaves (Shepherd and Wynne Griffiths, 2006). The composition of cuticular waxes is known to be affected by many environmental factors (Holroyd et al., 2002). Water stress or low nitrogen levels lead to increases in epidermal wax in *Pinus palustris* (Prior et al., 1997), ozone pollution leads to a reduction in waxes in *Populus tremuloides* (Mankovska et al., 1998), whilst nitrogen oxides (NO_x) and aerosol black carbon from traffic pollution cause degradation of wax crystal structure in *Picea abies* (Viskari et al., 2000).

Once inside the plant, atmospheric pollutants cause different types of injury. Prolonged exposure may cause chronic damage, whilst substantial episodic exposure can cause acute injury. Physiological damage by SO_2 includes down-regulation of photosynthesis (Noyes, 1980; Hallgren and Gezelius, 1982; Haworth et al., 2012). Anatomical damage leads to disruption of water regulation as the epidermal and neighbour cells that provide structural support to the guard cells collapse, leaving the stomata permanently open (Neighbour et al., 1988; Mansfield, 1998; Robinson et al., 1998). Morphological damage to the plant cuticle negatively impacts cuticular waxes, breaching the protective barrier between the plant interior and exterior (Thompson and Kats, 1978; Bartiromo et al., 2012). Kim et al. (1997) found that differences in the uptake of SO_2 also depend on plant type. They showed that low uptake of SO_2 by gymnosperms (ca. <0.4% leaf sulphur content) led to damaged cuticle, whilst higher SO_2 uptake damaged angiosperm cuticle (ca. 0.4–0.6% leaf sulphur content) and very high levels of uptake were required to damage the cuticle of *Ginkgo biloba* (ca. >0.7% leaf sulphur content).

The purpose of this work was to analyse and categorise the impact of continuous SO_2 fumigation on plant epidermal and cuticle morphology to determine if elevated SO_2 resulted in universal damage type(s) that would be capable of detection in the fossil plant record.

2. Methods and materials

2.1. Controlled environment conditions

Ten vascular plant species (three deciduous and seven evergreens) representative of Mesozoic gymnosperm and fern fossil floras (Table 1) were analysed for changes in epidermal and cuticle morphology when grown under continuous SO_2 fumigation compared to a control treatment. Future work will include analysis of angiosperms. For plant growth methods see Haworth et al. (2012). Plant species included: *Lepidozamia hopei* and *Lepidozamia peroffskyana* cycads grown from seed, six month-old specimens of the fern *Osmunda regalis* and two year-old specimens of gymnosperms *Ginkgo biloba*, *Nageia nagi*, *Podocarpus macrophyllus*, *Araucaria bidwillii*, *Agathis australis*, *Taxodium distichum* and *Wollemia nobilis*. All species were grown for a minimum of six months in CONVIRON BDW40 (Winnipeg, Canada) walk-in growth rooms at the Programme for Experimental Atmospheres and Climate (PÉAC) facility in University College Dublin. Six months was deemed an appropriate duration for the experiment as this allowed adequate time for each species to grow new leaves under experimental conditions and for stabilisation of the plants' physiological response to SO_2, allowing us to measure long-term responses of plants to chronic SO_2 fumigation in a simulated volcanic episode. Plants were grown under control atmospheric conditions (zero ppm SO_2; 20.9% O_2; 380 ppm CO_2) or continuous fumigation with SO_2 (0.2 ppm SO_2;