Environmental and Experimental Botany 145 (2018) 21–38

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Research paper

Differential regulation of volatile emission from *Eucalyptus globulus* leaves upon single and combined ozone and wounding treatments through recovery and relationships with ozone uptake

Arooran Kanagendran[a,*], Leila Pazouki[a], Ülo Niinemets[a,b]

[a] Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, Tartu 51014, Estonia
[b] Estonian Academy of Sciences, Kohtu 6, 10130 Tallinn, Estonia

A R T I C L E I N F O

Keywords:
Acute ozone stress
Benzenoid emission
Isoprene emission
LOX products
Monoterpene emission
Sesquiterpene emission
Stomatal ozone uptake
Stress interaction
Synergistic effects

A B S T R A C T

Both ozone and wounding constitute two key abiotic stress factors, but their interactive effects on plant constitutive and stress-elicited volatile (VOC) emissions are poorly understood. Furthermore, the information on time-dependent modifications in VOC release during recovery from a combined stress is very limited. We studied the modifications in photosynthetic characteristics and constitutive and stress-induced volatile emissions in response to single and combined applications of acute ozone (4, 5, and 6 ppm) and wounding treatments through recovery (0.5–75 h) in a constitutive isoprene and mono- and sesquiterpene emitter *Eucalyptus globulus*. Overall, the photosynthetic characteristics were surprisingly resistant to all ozone and wounding treatments. Constitutive isoprene emissions were strongly upregulated by ozone and combined ozone and wounding treatments and remained high through recovery phase, but wounding applied alone reduced isoprene emission. All stress treatments enhanced emissions of lipoxygenase pathway volatiles (LOX), mono- and sesquiterpenes, saturated aldehydes (C7-C10), benzenoids, and geranylgeranyl diphosphate (GGDP) pathway volatiles. Once elicited, GGDP volatile, saturated aldehyde and benzenoid emissions remained high through the recovery period. In contrast, LOX emissions, and total mono- and sesquiterpene emissions decreased through recovery period. However, secondary rises in total sesquiterpene emissions at 75 h and in total monoterpenes at 25–50 h were observed. Overall, acute ozone and wounding treatments synergistically altered gas exchange characteristics and stress volatile emissions. Through the treatments and recovery period, stomatal ozone uptake rate and volatile emission rates were poorly correlated, reflecting possible ozone-scavenging effect of volatiles and thus, reduction of effective ozone dose and elicitation of induced defense by the acute ozone concentrations applied. These results underscore the important role of interactive stresses on both constitutive and induced volatile emission responses.

1. Introduction

Tropospheric ozone (O_3) is a major oxidative pollutant and a key plant stress elicitor (Karnosky et al., 2007). The bulk of tropospheric ozone is formed in the reactions involving nitrogen oxides NO and NO_2 (NO_X) and reactive volatile organic compounds (VOCs) in the presence of sunlight (Ryerson et al., 2003). Elevated ozone generates oxidative stress in plants that leads to biochemical adjustments and metabolic shifts as a result of ozone-induced gene expression changes and acclimation responses or hypersensitive or necrotic responses associated with foliar injury, impairment of shoot and root growth, and accelerated organ senescence (Calfapietra et al., 2008; Gerosa et al., 2009; Heath, 2008; Peñuelas and Staudt, 2010).

The current surface ozone level is around 40 ppb in most parts of the world (Sicard et al., 2017). Although it varies with the geographical location, the present tropospheric ozone levels are capable of causing physiological damage in plants (Ashworth et al., 2013; Proietti et al., 2016). Furthermore, it is anticipated that tropospheric ozone concentrations will increase by 2–4 folds in the next two decades, primarily due to industrialization and burning of fossil fuels, implying that ozone stress is expected to become more severe in the future (Vingarzan, 2004).

Abbreviations: BVOC, biogenic volatile organic compound; GGDP, geranylgeranyl diphosphate; GLM, generalized linear model; GLV, green leaf volatiles; LOX, lipoxygenase pathway; MEP/DOXP pathway, 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway; MVA pathway, mevalonate pathway; PSII, photosystem II; ROS, reactive oxygen species

* Corresponding author.
E-mail address: kanagendran.arooran@emu.ee (A. Kanagendran).

http://dx.doi.org/10.1016/j.envexpbot.2017.10.012
Received 11 June 2017; Received in revised form 16 October 2017; Accepted 17 October 2017
Available online 18 October 2017
0098-8472/

Plants are a significant sink for atmospheric ozone both due to stomatal ozone uptake and non-stomatal ozone deposition (Fares et al., 2008). Stomatal uptake is the primary passage through which ozone enters the leaf intercellular spaces and generates physiological and oxidative damage in plant cells (Beauchamp et al., 2005; Gerosa et al., 2007). Apart from alteration of basic metabolic processes such as photosynthesis and plant growth, ozone exposure leads to major changes in plant volatile emission rates and emission profiles during the initial stress impact and through recovery. Upon acute ozone exposures, damaged plant cells release free fatty acids from their membranes, leading to an activation of lipoxygenase pathway (LOX) and rapid emission burst of LOX volatiles (also called green leaf volatiles, GLV) (Beauchamp et al., 2005; Copolovici et al., 2014; Portillo-Estrada et al., 2015). These early stress responses ultimately trigger the signal transduction pathways that activate defense reactions primarily through jasmonate (JA) and ethylene regulated transcription factors (Bailey et al., 2005). Typically, ozone-caused longer-term responses include elicitation of terpenoid and benzenoid emissions for hours to days following the initial stress impact (Beauchamp et al., 2005).

In nature, plants often encounter multiple stress factors simultaneously or in sequence. Interacting stresses can strengthen or weaken the effect of individual stress factors due to modification of overall stress severity or complex stress-priming responses (Copolovici et al., 2014; Ibrahim et al., 2008; Niinemets, 2010a,b). Leaf wounding is a key mechanical stress in the field that primarily results from herbivore feeding, but also from mechanical damage due to wind, falling debris and heavy precipitation (Benikhlef et al., 2013; Portillo-Estrada et al., 2015). Similarly to ozone, wounding leads to emissions of LOX volatiles that are quantitatively related to the degree of damage (Copolovici and Niinemets, 2015; Copolovici et al., 2017; Portillo-Estrada et al., 2015).

In fact, LOX emission is a very characteristic early stress response, followed by volatile isoprenoids, emissions of which have been detected upon many abiotic stresses such as heat shock in *Solanum lycopersicum* (Pazouki et al., 2016), drought and herbivory in *Alnus glutinosa* (Copolovici et al., 2014) and ozone in *Nicotiana tabacum* (Beauchamp et al., 2005), and biotic stresses such as feeding by larvae of common white wave (*Cabera pusaria*) in leaves of *Alnus glutinosa* (Copolovici et al., 2011) and leaf rust infection in *Salix* spp. (Toome et al., 2010) and *Populus* (Jiang et al., 2016).

Previous studies have revealed that the impacts of elevated ozone (Beauchamp et al., 2005; Llusià et al., 2002; Loreto et al., 2001, 2004; Peñuelas et al., 1999; Velikova et al., 2005), and wounding (Brilli et al., 2011; Loreto and Sharkey, 1993; Portillo-Estrada et al., 2015) alter primary and secondary metabolic process of plants and especially, LOX, MEP/DOXP (2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate) and MVA (mevalonate) pathways responsible for volatile emission. In all these studies, volatile emissions were quantitatively associated with the stress severity, characterized as the spread of damage or degree of biological infection, but it is unclear how combined application of two different stresses capable of causing cell-level injury can simultaneously affect plant volatile emission. Although both ozone and wounding stresses are known to induce LOX emissions, and ozone exposure further elicits isoprenoid emissions during recovery, it is also unclear whether wounding alone can also elicit volatile isoprenoid emission responses and whether wounding following ozone exposure amplifies the volatile emission responses during recovery. Furthermore, not only do the past studies lack the interaction effects, but they have typically analyzed immediate plant responses to stress rather than plant responses through recovery phase. Interaction studies are pertinent because in natural environments, occurrence of multiple stress factors with varying strength and duration is common and multiple stresses can significantly influence tree physiological responses through additive, synergistic, and antagonistic effects. In old-growth forest ecosystems, for example, low understory light levels combined with low soil nutrient and water availabilities can frequently limit understory regeneration (Bergh et al., 1999; Niinemets, 2010b). In addition to interacting stresses, an already existing stress can be superimposed by another stress factor, further complicating the stress situation. Due to stress priming and acclimation responses, the effects of superimposed and successive environmental stresses are often not additive, but different stresses can interact, leading to synergistic or antagonistic responses. The interactive effects on plant performance can either be negative, indicating an enhanced plant response to the given stressor due to an additional stress, or positive, implying a reduced plant response due to an additional stress (Niinemets, 2010b). Thus, the plant responsiveness to the given stress varies with the type and duration of the combined stresses imposed. In this study, wounding is a disturbance stress defined as a single episodic event or chronic impact that is associated with full or partial destruction of plant biomass, impairing plant physiological and functional activities such as photosynthesis and growth, whereas ozone is an oxidative stress that leads to a sustained deviation from optimal environmental conditions, causing reduced plant productivity and growth rates (Bansal et al., 2013). Therefore, ozone and wounding treatments are expected to lead to synergistic effects on plant functional activity. Especially for ozone, this is relevant as early and late stress responses can be qualitatively different (Calfapietra et al., 2013; Niinemets, 2010a).

Eucalyptus spp. are the dominant plant species in Australian forests and woodlands. Due to their high growth rates, they are considered economically highly valuable hardwoods for pulp industry (Külheim et al., 2015), and are therefore, widely grown in forest plantations around the world especially in tropical, sub-tropical and warm temperate regions (Loreto et al., 2000). Eucalypts are significant isoprene and monoterpene emitters under non-stressed conditions (Funk et al., 2006; Guenther et al., 1991; He et al., 2000; Loreto et al., 2000; Street et al., 1997; Winters et al., 2009). In this study, we used Tasmanian bluegum (*Eucalyptus globulus* Labill.) that is a classic species used in constitutive isoprenoid emission studies (Guenther et al., 1991; Loreto et al., 2000). However, much less is known of stress responses of volatile emissions in eucalypt species. We investigated how acute ozone and wounding stresses alone and in interaction change foliage photosynthetic characteristics, and volatile emissions through different recovery times in *E. globulus* leaves. Since plant-produced volatiles can quench ozone in ambient air and in leaf intercellular air spaces due to direct reaction with ozone (Fares et al., 2010), eucalypts are expected to be highly resistant to ozone stress, and we used acute ozone exposures in this study.

We addressed the following questions: (1) how do individual and interactive effects of acute ozone and wounding influence foliage photosynthetic characteristics, overall emission amounts and blend of emissions through recovery? (2) how are the quantitative emission responses through recovery phase associated with stress severity, including ozone dose and stomatal ozone uptake rate? Both the plant responses to immediate ozone and wounding application, and recovery of those responses upon returning the plants to ambient environment after stress were analyzed to gain conclusive insight into the correspondence among photosynthetic characteristics, ozone and wounding treatments, stomatal ozone uptake rates and volatile emissions. We hypothesized that (1) single and combined applications of acute ozone and wounding stresses would result in a major reduction in foliage photosynthesis rate, stomatal conductance to water vapor, and reduction in maximum quantum yield of photosystem II (PSII, F_v/F_m); (2) application of ozone and wounding treatments alone would lead to a strong emission response of LOX and volatile isoprenoids, saturated aldehydes, benzenoids, and GGDP pathway volatiles and that these responses scale with ozone dose and are greater than the responses due to wounding; (3) the combined application of ozone and wounding leads to synergistic effects on gas exchange and VOC emission responses; and (4) as *E. globulus* is a strong isoprenoid emitter, a major proportion of volatile isoprenoids and antioxidants scavenges ozone in leaf intercellular airspaces and ROS in plant cells, eventually resulting in a poor relationships between stomatal ozone uptake vs. volatile