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Volatile organic compound emissions from *Alnus glutinosa* under interacting drought and herbivory stresses

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ABSTRACT

Plant volatile organic compounds (VOCs) elicited in response to herbivory can serve as cues for parasitic and predatory insects, but the modification of VOC elicitation responses under interacting abiotic stresses is poorly known. We studied foliage VOC emissions in the deciduous tree *Alnus glutinosa* induced by *feeding* by the larvae of green alder sawfly (*Monsoma pulveratum*) under well-watered and drought-stressed conditions. Drought strongly curbed photosynthesis rate and stomatal conductance, but there were no effects of insect feeding on photosynthetic characteristics. Feeding induced emissions of volatile products of lipoxygenase pathway and monoterpenes, and emissions of stress marker compounds (*E*)-β-ocimene and homoterpene DMNT. The emissions were more strongly elicited and reached a maximum value earlier in drought-stressed plants. In addition, methyl salicylate emissions were elicited in herbivory-fed drought-stressed plants. Herbivores were more strongly attracted to well-watered plants and consumed more than a four-fold greater fraction of leaf area than they consumed from drought-treated plants. Overall, this study demonstrates an important priming effect of drought, suggesting that plants under combined drought/herbivory stress are more resistant to herbivores.

1. Introduction

Under natural conditions, plants are often exposed to a combination of two or more simultaneous or sequential stress factors (Holopainen and Gershenzon, 2010; Mittler, 2006; Niinemets, 2010a,b). In the case of simultaneously occurring stresses, one type of stress could weaken or enhance the effects of another simultaneous stress factor by direct physiological cumulative or interactive effects (Ibrahim et al., 2008; Niinemets, 2010a). Sequential or superimposed stress effects can be further complicated by stress priming (Frost et al., 2008; Heil and Kost, 2006; Kessler et al., 2006; Niinemets, 2010b) that can induce partial acclimation responses to similar type of stress or result in metabolic changes that protect directly or indirectly against a different type of stress. For instance, abiotic stress-driven metabolic changes can affect biotic stress response, or priming in responses to one type of biotic stress can affect responses to a different type of stress (Cardoza et al., 2002; Dicke, 2009; Holopainen and Gershenzon, 2010; Peng et al., 2011; Thaler et al., 2002).

Plant volatile emission patterns change quantitatively and qualitatively in response to damage by herbivores (Arimura et al., 2005; Brilli et al., 2009; Dicke, 2009; Kant et al., 2009; Niinemets et al., 2013; Trowbridge and Stoy, 2013). Most plants have evolved effective defense strategies in order to reduce insect attacks (reviews in Dicke, 2009; Dicke and Baldwin, 2010; Dicke and Loreto, 2010; Holopainen and Gershenzon, 2010). Presence of multiple stresses can significantly affect the volatile emissions, but so far, the interactive effects are still poorly understood. For example, simultaneous fungal and lepidopteran herbivory treatment resulted in similar emissions of herbivory-induced volatiles in *Arachis hypogea* (Cardoza et al., 2002) and ca. 50% reduced emissions in maize (*Zea mays*) (Rostás et al., 2006), but the herbivore performance was not affected (Rostás et al., 2006) or was even enhanced (Cardoza et al., 2002) in fungal-infected plants, demonstrating complex response to multiple biotic stresses. On the other hand, in *Z. mays*, herbivore-induced VOCs were reduced by 75% in the case of nutrient deficiency (Gouinguene and Turlings, 2002), indicating reduced capacity for indirect defense.

The typical fast response of the plants to herbivore attacks is the emission of volatile products of lipoxygenase pathway – LOX products (also called green leaf volatiles) consisting of various

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C6 aldehydes and alcohols (Copolovici et al., 2011; Gosset et al., 2009; Pinto et al., 2007; Toome et al., 2010). LOX product emission is a ubiquitous stress response that has been observed in response to other biotic stresses such as fungal attacks (Steindel et al., 2005; Toome et al., 2010) and many abiotic stresses including heat and frost (Copolovici et al., 2012), flooding (Copolovici and Niinemets, 2010), ozone (Beauchamp et al., 2005; Heiden et al., 2003), high light (Loreto et al., 2006) and mechanical wounding (Loreto et al., 2006). This initial rapid response is followed by emissions of induced volatile isoprenoids, in particular, mono- and sesquiterpenes (Beauchamp et al., 2005; Copolovici et al., 2012; Steindel et al., 2005; Toome et al., 2010) and in some cases by emissions of benzenoid methyl salicylate (Beauchamp et al., 2005; Cardoza et al., 2002; Zhao et al., 2010). These compounds can play a role in host detection of herbivores as well as in eliciting priming responses in plants (Choudhary et al., 2008; Peñuelas et al., 2007; Ton et al., 2007), thereby playing a major role in determining the integrated response of previously imposed biotic or abiotic stress and subsequent herbivory stress.

Potential changes in host quality and both constitutive and induced resistance induced by abiotic stresses could influence herbivores' development and health, and thereby determine the degree of herbivory damage (Lerdau et al., 1994). The share of the constitutive vs. induced response is expected to depend on plant characteristics (growth rate, defense strategy) and the specific type of stress. A key abiotic stress that strongly curtails plant carbon gain and growth is drought, but the influence of water deficiency on the strategies of plants to cope with different biotic attacks has received limited attention, and the studies have mainly focused on non-volatile primary or secondary metabolites (e.g., Gutbrodt et al., 2012; Khan et al., 2011) or plants' growth characteristics (Eranen et al., 2009; Halpern et al., 2010). For deciduous trees in general, there are very few studies investigating the impact of multiple stresses on plant performance and, especially limited are studies combining abiotic and biotic stresses (Dicke and Loreto, 2010; Holopainen and Gershenzon, 2010; Niinemets, 2010a; Niinemets and Monson, 2013). This is an important omission because in trees with longer-living foliage and slower leaf turnover (Wright et al., 2004), the constitutive stress response is expected to be of greater significance than in herbaceous species with faster leaf turnover and more dynamic stress responses.

Here we investigated the kinetics of VOC emission in black alder (*Alnus glutinosa*) leaves in response to combined drought and herbivory by green alder sawfly (*Monsoma pulveratum*) larvae, and studied the degree of herbivory damage and plant attractiveness to herbivores in drought-stressed and well-watered plants. *A. glutinosa* is a relatively short-living (ca. 40 yrs) wide-spread fast-growing tree species colonizing habitats along stream banks and wet forests. It has a very low drought tolerance (Niinemets and Valladares, 2006). Herbivores and pathogens have a strong impact on *Alnus* spp. altering their survival, growth and even reproduction, thereby influencing the composition of early successional alder dominated ecosystems. So far, only a few studies have investigated stress induced by herbivore attack (e.g., Blande et al., 2010; Dolch and Tscharntke, 2000; Giertych et al., 2006; Tscharntke et al., 2001) or by drought (Arbellay et al., 2010; Francis et al., 2005; Hacke and Sauter, 1996; Sundstrom and Hussdanell, 1995) in *Alnus* spp., but the combined stress has not been studied in alder to our knowledge.

We tested the hypotheses that priming by drought stress results in altered kinetics of VOC emissions, with drought-stressed plants responding earlier to herbivory stress than well-watered plants and we also expected the drought-stressed plants to be less attractive and less palatable to the herbivores due to priming of defense responses by water stress.

2. Materials and methods

2.1. Plant material

A. glutinosa seedlings of local (Estonian) origin were grown in 5 L clay pots filled with a 1:1 mixture of commercial potting soil (Biolan Oy, Finland) and quartz sand under light intensity of 200 μmol m^{-2} s^{-1} (HPI-T Plus 400 W metal halide lamps, Philips) with day/night temperatures of 24/18 °C for a 12 h light period. The plants were watered daily and fertilized once per month with a slow release NPK (3-1-2 ratio) fertilizer containing microelements. In all experiments, we used similar-sized 2-year-old seedlings with 20–25 leaves. The experiment was conducted with plants having fully mature leaves, and no leaf area expansion was observed throughout the experiment in both the control and insect-treated plants.

2.2. Insects

Larvae of green alder sawfly *M. pulveratum* (Hymenoptera: Tenthredinidae) at final instar were used as herbivores. *M. pulveratum* is a common Palearctic species (recently also found in North America) whose folivorous larvae feed on *Alnus* species, occasionally causing total defoliation of the trees. The larvae were collected from the vicinity of Tartu, Estonia (58°22′ N 26°43′ E) a few days prior to the experiment and kept in plastic containers with access to fresh *Alnus* leaves. Larvae of similar size, approximately 1.2 cm in length, were selected for the experiment.

2.3. Experimental setup

Whole plants were placed in a dynamic headspace sampling cuvette system consisting of eight 3 L glass chambers similar to the system described in detail in Toome et al. (2010) and Copolovici et al. (2011). In this system, roots with the potting medium stayed outside the chambers, and above-ground plant parts were hermetically sealed in individual cuvettes. The air flow through each chamber was 0.25 L min^{-1} and a fan (Sunon Group, Beijing, China) was installed in the chamber resulting in high turbulence. During the experiment, light was provided by Philips HPI-T Plus 400 W metal halide lamps for 12 h photoperiod at a level of light intensity of 200 μmol m^{-2} s^{-1}. After hermetic installation of the plants (day −1), all plants were watered to field capacity. Three plants were randomly chosen for drought treatment (no water provided for 6 days), while the remaining five were watered daily. After 48 h (day 1), the larvae of *M. pulveratum* were placed on three drought-stressed and on three well-watered plants to initiate the herbivory stress, while two well-watered plants were left untreated as controls. Five larvae were placed on each herbivory-treated plant. After the introduction of the larvae, the plants were stabilized in the system with dynamic air flow for two more hours before VOC sampling. The larvae were left to feed on the leaves for 7 days. After 5 days of feeding, the plants under drought stress were watered to field capacity and their recovery was followed for another 2 days with the larvae still feeding. A similar experiment was performed using the same set-up with 4 plants as controls and 4 plants drought-stressed without the herbivory treatment. Both experiments were replicated twice.

2.4. Leaf structural measurements

After the last collection of VOCs, all leaves were harvested and scanned at 300 dpi. Projected leaf area was determined from the scanned images by a custom made software. Both the total leaf area after the experiment and before the feeding treatment (non-damaged area, i.e., the holes and fed margins added) were