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Ozone disrupts adsorption of *Rhododendron tomentosum* volatiles to neighbouring plant surfaces, but does not disturb herbivore repellency

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ABSTRACT

The perennial evergreen woody shrub, *Rhododendron tomentosum*, confers associational resistance against herbivory and oviposition on neighbouring plants through passive adsorption of some of its constitutively emitted volatile organic compounds (VOCs). The adsorption process is dependent on transport of VOCs in the air. In polluted atmospheres, the VOCs may be degraded and adsorption impeded. We studied the effect of elevated ozone regimes on the adsorption of *R. tomentosum* volatiles to white cabbage, *Brassica oleracea*, and the oviposition of the specialist herbivore *Plutella xylostella* on the exposed plants. We found evidence for adsorption and re-emission of *R. tomentosum* volatiles by *B. oleracea* plants. Ozone changed the blend of *R. tomentosum* volatiles and reduced the amount of *R. tomentosum* volatiles recovered from *B. oleracea* plants. However, plants exposed to *R. tomentosum* volatiles received fewer *P. xylostella* eggs than control plants exposed to filtered air irrespective of whether *R. tomentosum* volatiles mixed with ozone. Ozone disrupts a volatile mediated passive plant-to-plant interaction by degrading some compounds and reducing the quantity available for adsorption by neighbouring plants. The change, however, did not affect the deterrence of oviposition by *P. xylostella*, suggesting that aromatic companion plants of *Brassica* crops may confer pest-deterring properties even in ozone-polluted environments.

1. Introduction

Biogenic volatile organic compounds (BVOCs) are secondary organic metabolites that mediate plant interactions within and across trophic levels (Dicke and Baldwin, 2010). These interactions include plant-to-plant communication, herbivore foraging, oviposition and pollination (Dicke and Baldwin, 2010; Holopainen and Blande, 2013). Some of these interactions are dependent on transport of BVOCs in the air (Karban et al., 2006), where they are subject to atmospheric reactions that may hinder the ecological processes they mediate, especially in polluted atmospheres (Blande et al., 2010). Tropospheric ozone is one of the most important atmospheric pollutants with levels expected to increase in many parts of the world in the future due to global warming and land cover changes (Martin et al., 2015; Prather et al., 2013). Currently, background ozone levels in the northern hemisphere range between 35 and 45 ppb, with common occurrences of peak emissions of above 100 ppb (Cionni et al., 2011; Fowler et al., 2008). Although the global average ozone concentrations are not expected to increase significantly over the coming years, average 8-h daily averages of up to 80 ppb are still experienced in some areas (Cionni et al., 2011; Oksanen et al., 2013).

At higher ozone levels, some volatile compounds are degraded, resulting in the disruption of their ecological roles. Volatile mediated-herbivore foraging (Li et al., 2016), pollinator attraction (Farré-Armengol et al., 2016) and plant-to-plant interactions (Girón-Calva et al., 2016; Li and Blande, 2015) have all been shown to be disrupted under elevated ozone regimes. Volatile-mediated plant-to-plant interactions may be active, whereby volatiles trigger a physiological response in the receiver plant (Frost et al., 2008; Heil and Kost, 2006; Kost and Heil, 2006) or passive, whereby volatiles stick to the surfaces of neighbouring plants (Li and Blande, 2015). Both active and passive processes may confer herbivore resistance on receiving plants in a process known as associational resistance (Kost and Heil, 2006; Karban et al., 2006; Himanen et al., 2010; Himanen et al., 2015). Elevated ozone regimes have been shown to disrupt active plant-to-plant interactions by degrading the volatiles and reducing the effective interaction distance between emitter and receiver plants (Blande

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et al., 2010). The effects of elevated ozone on passive plant-to-plant interactions remain largely unstudied.

Here, we studied the effect of ozone on interactions between *Rhododendron tomentosum* and *Brassica oleracea*, a model system for passive plant-to-plant interactions. *Rhododendron tomentosum* is a small woody perennial evergreen shrub distributed throughout boreal ecosystems (Butkienė et al., 2008). The species has a high volatile terpenoid content stored in glandular trichomes distributed throughout its leaves and stem that gives it a characteristic smell. Some of these terpenoid compounds are myrcene ($C_{10}H_{16}$), which is the main monoterpene compound, as well as species-specific C15 semi-volatile compounds, ledene ($C_{15}H_{24}$), ledol ($C_{15}H_{26}O$), and palustrol ($C_{15}H_{26}O$). (Dampc and Luczkiewicz, 2013; Himanen et al., 2010; Himanen et al., 2015). *R. tomentosum* extracts and essential oils have been shown to have herbivore repellent properties (Egigu et al., 2011; Jaenson et al., 2005).

Passive adsorption of *R. tomentosum* volatiles on neighbouring plant surfaces was first reported by Himanen et al. (2010) when the species-specific volatile sesquiterpenes – palustrol, ledene and ledol were recovered from the surfaces of neighbouring silver birch branches. *Betula pendula* and *Brassica oleracea* plants exposed to volatiles from neighbouring *R. tomentosum* showed increased resistance to herbivore-feeding and *Plutella xylostella* oviposition (Himanen et al., 2010; Himanen et al., 2015). Plant volatiles are used as cues in host finding and selection by *P. xylostella*, once a plant is selected, the leaf surface physical and chemical characteristics are used to determine its suitability for oviposition (Renwick and Chew, 1994; Badenes-Perez et al., 2004).

The volatile constituents of *R. tomentosum* are mostly terpenoids, which are prone to oxidation reactions with ozone in the atmosphere (Atkinson and Arey, 2003). Elevated ozone may change the blend of *R. tomentosum* volatiles in the air by degrading some of its volatile constituents and subsequently reducing the availability for adsorption to neighbouring plants. Degradation reactions may also produce compounds whose ecological significance remains unknown. We tested the effects of an elevated ozone regime on the volatile blend of *R. tomentosum* after emission and the adsorption of these compounds to *B. oleracea* plants. We also tested the effects of adsorption of volatile compounds emitted by *R. tomentosum* on oviposition on *B. oleracea* by *P. xylostella*.

2. Materials and methods

2.1. Plant material

We collected *Rhododendron tomentosum* (henceforth referred to as RT) plants in August 2016 from a ditched pine forest site in Suonenjoki, Finland (62.6456° N, 27.0649° E) and stored them at 4 °C for 2 weeks before the start of experiments. White cabbage, *Brassica oleracea convar. capitata var. alba* seeds were sown in a mixture of peat:mull:sand (3:1:1) in 1 L pots and grown in a plant growth chamber (Weiss Bio 1300, Germany) [Day 16 h (photosynthetically active radiation 300 μmol^{-2} s^{-1}), 23 °C, 60% humidity: Night 8 h dark, 18 °C, 80% humidity].

2.2. Exposure system

In the exposure system (Fig. 1), 30 g of RT shoots were arranged in an Erlenmeyer flask filled with water and enclosed in a pre-cleaned (+120 °C for 1 h) polyethylene terephthalate (PET) bag (45 × 55 cm). Activated carbon-filtered air was passed through Teflon tubes into the RT enclosure at 2 L min^{-1} and the outlet air from the RT enclosure was split evenly into two 22.4 L glass desiccators (mixing chambers). One of the mixing chambers was supplemented with ozone-enriched air up to a level of 100 ppb and

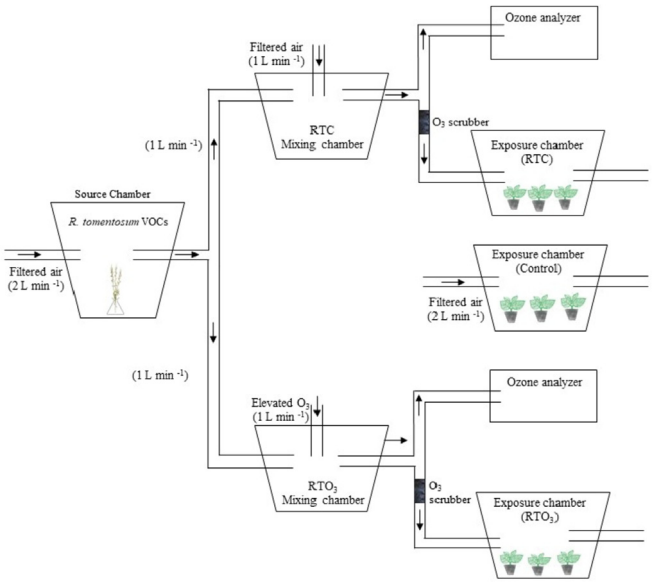

Fig. 1. Schematic illustration of exposure to RT volatiles, RT volatiles +100 ppb ozone and filtered air (control). Exposure lasted for 24 h in each experiment and was repeated five times.

ozone-free air was added to the other mixing chamber at a rate of 1 L min^{-1}. Ozone (O_3) was produced from filtered air with an ozone generator (Dasibi 1008-RS; Dasibi Environmental Corp., Glendale, CA, USA) and ozone analysers (Environnement S.A O342M, Environnement S.A, Poissy, France) were used to monitor ozone levels. Outgoing air from each mixing chamber was split into two; one stream passed into an ozone analyser and the other was passed through O_3 scrubbers [potassium iodide (KI) coated copper tube] to remove ozone before passing into 22.4 L chambers (exposure chambers) each containing three 4-week-old cabbage plants. The setup also included a control exposure chamber through which activated carbon-filtered air was passed at a rate of 2 L min^{-1}. Thus, there were three treatments: control plants exposed to filtered air, plants exposed to RT emissions (RTC) and plants exposed to RT emissions after they had been mixed with ozone (RTO_3). Outgoing air from the exposure chambers was released into a fume hood. Each exposure treatment period lasted 24 h (16 h light, 8 h dark) and was repeated five times.

2.3. Volatile analysis

All volatiles were collected in stainless steel tubes filled with 200 mg Tenax TA 35/60 adsorbent (Markes International, UK). Volatiles were collected from the air space of mixing chambers 2 h after setup for 10 min at ~0.2 L min^{-1} with a suction pump (KNF, Neuberger D-79112, Germany) in order to measure the concentrations of *R. tomentosum* volatiles in the mixing chamber. The mixing chamber concentrations were expressed in ng L^{-1}. The exposure system was established five times with two *B. oleracea* plants from each exposure chamber selected for plant volatile analysis and leaves detached from the 3rd plant and used for oviposition tests. In a 5th exposure cycle, one plant was used for volatile analysis and two for oviposition tests. In total, we collected volatiles from nine plants and used thirty-six detached leaves for oviposition tests per treatment. Dynamic headspace analysis was used for plant volatile collection; plant shoots were enclosed in a pre-cleaned PET bag (25 × 55 cm) and filtered air was passed through one end of the bag at ~ 0.3 L min^{-1}. A Tenax TA adsorbent-filled tube was attached to the other end of the bag with a suction