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RESEARCH ARTICLE

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Effects of ethylene and (*Z*)-hexen-3-al on production of volatiles in *Populus simonii* × *P. pyramidalis* 'Opera 8277' cuttings

Abstract We studied the effects of ethylene, (*Z*)-hexen-3-al, combinations of ethylene and (*Z*)-hexen-3-al, methyl jasmonate on the release of terpenoids and green leaf volatiles (GLVs) of *Populus simonii*×*P. pyramidalis* 'Opera 8277' by fumigation. The results show that exposure to ethylene alone did not induce volatiles. However, it was induced by exposure to combinations of (*Z*)-hexen-3-al and ethylene, a large amount of hexenal and hexen-3-ol as compared to sole (*Z*)-hexen-3-al exposure, which indicated that the release of wounding signals of *P. simonii*×*P. pyramidalis* 'Opera 8277' could be synergized by ethylene and (*Z*)-hexen-3-al.

Keywords *Populus simonii*×*P. pyramidalis* 'Opera 8277', cuttings, ethylene, (*Z*)-hexen-3-al

1 Introduction

In response to mechanical damage and herbivore wounding, plants release a range of volatiles which can induce the defense responses in neighboring intact plants. This process is mediated by the interplant airborne transduction (Kong and Hu, 2003; Gao and Shen, 2006; Frost et al., 2007). Recent studies have shown that besides methyl jasmonate (MeJA) and methyl salicylate (MeSA), green leaf volatiles (GLVs), including C6 alcohols, aldehydes and esters, are immediately emitted from plants after wounding through the octadecanoid pathway and can act as important airborne signals between plants (Yan and Wang, 2006). The previous study has shown that (*Z*)-hexen-3-al led to emission of similar volatiles induced by herbivore wounding in maize plants. These volatiles not only serve as a measure of defense against herbivores by directly decreasing oviposition rate, but can also attract natural enemies of the herbivores (Ruther and Kleier, 2005). As one of the important phytohormones, ethylene plays a key role in plant defense response. Ethylene and jasmonate pathway co-regulate the plant defense against diseases and pests through synergic or antagonistic effects (Lu et al., 2006). This study aims to investigate the volatiles emitted from *Populus simonii*×*P. pyramidalis* 'Opera 8277' exposed to GLVs and ethylene.

2 Methods and materials

2.1 Plant materials

In this study, one-year-old poplar cuttings from a nine-year-old *P. simonii*×*P. pyramidalis* 'Opera 8277' in the nursery of the Beijing Forestry University were used. The cuttings were grown in pots (25 cm diameter, 25 cm height) containing nursery topsoil during March to July of 2006. The cuttings were watered daily and supplied with a 1/2 Hoagland nutrient solution every week (Arnon and Hoagland, 1940). The volatiles were collected in July in a greenhouse, and the cuttings were kept for three days in the greenhouse.

2.2 Treatments

The cuttings were placed in a glass chamber (60 cm×80 cm×150 cm) to be fumigated by exogenous volatiles. The purified air by activated carbon entered this chamber through an inlet at the bottom of the chamber. A mini fanner was put in the chamber to circulate air and accelerate the volatilization of exogenous volatiles. CH_2Cl_2 (Beijing Chemical Reagent Inc., China), (*Z*)-

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hexen-3-al (Sigma-Aldrich, USA), ethylene (Beijing Hua Yuan Gas Chemical Industry Co., Ltd, China) and MeJA (Sigma-Aldrich, USA) were used as exogenous volatiles in this study. (*Z*)-hexen-3-al and MeJA were dissolved in CH_2Cl_2 to 1 mol/L before use.

After exposure to 0.5 μmol/L (*Z*)-hexen-3-al, 50 μL/L ethylene, 0.5 μmol/L MeJA, and the mixture of 0.5 μmol/L (*Z*)-hexen-3-al and 50 μL/L ethylene, respectively, volatiles emitted from the poplar cuttings were collected. The concentration of ethylene referred to Ruther's study on maize (Ruther and Kleier, 2005). The poplar cuttings were placed together with absorbent cotton on evaporating dishes absorbing the chemicals in CH_2Cl_2 or CH_2Cl_2 alone as a control. The poplar cuttings were prevented from touching the chemicals. The ethylene was injected into the glass chamber with an injector from the inlet at the bottom. There were three replications for each treatment.

2.3 Volatile collection

After exposure to the exogenous chemicals for 12 h, the cuttings were taken out from the glass chamber and the volatiles were collected. Reynolds oven bags (44.3 cm ×55.8 cm) that release and adsorb few volatiles were used to collect the volatiles. A glass tube (15 cm×0.3 cm, Chrompack, Middelburg, the Netherlands) containing Tenax-TA (60–80 mesh, Chrompack) was used as the volatile trap, while avoiding touching the poplar cuttings. A portable air sampler (QC-1, Beijing Municipal Institute of Labor Protection, China) was used as collection pump. The air in the bag was quickly extracted with the pump, and then the clean air which was filtered through activated carbon and absorbent- GDX-101 was pumped into the bag. Then, a close air course was set up. The volatiles were collected for two hours at a flow rate of 100 mL/min for each cutting. The glass tubes with the adsorbing volatiles were placed in a desiccator. The leaves in the bag on the treated cuttings were gathered after collecting the volatiles, dried at 75°C and weighed.

2.4 Volatile analysis and identification

Using GC/MS (Trace 2000-Voyager, Finnigan, ThermoQuest, Rodano, Milan, Italy), the volatiles were desorbed by heating in a CP-4010 TCT thermal desorption device (Chrompack, Netherlands) at 250°C for 10 min, and then cryofocused in a cold trap refrigerated by liquid N_2 at −120°C. The cold trap was then quickly heated to 260°C in 1 min to transport the volatiles into the analytical column (CP-Sil 5CB low bleed/MS 60 m×0.32 mm ID. with a 0.5 μm film thickness). The column was programmed from 40 to 250°C at 6°C/min, and held for 3 min. Helium of 20 kPa was used as the carrier gas. The MS was operated in the 70 eV EI ionization. Scanning was done from m/z 10 to m/z 400 at 0.4 s per scan.

2.5 Volatile qualification and quantification

Preliminary identification of the compounds was made by searching the NIST library in the data system of Xcalibur (Finnigan) and checked according to its retention index. The intensity of mass spectrometry of the volatile per gram dry weight of leaves per hour was used to express the release rate of the volatile ($E3 \cdot g^{-1} DW \cdot 2h^{-1}$). The compounds detected in the volatiles were followed by the mass/charge ratio (*m*/*z*): *α*-pinene, 93; 6-methyl-5-hepten-2-one, 43; *β*-pinene, 93; trans-geranylacetone, 93; menthol, 81; hexanal, 44; 1-hexen-3-ol, 57.

3 Results

Previous studies show that terpenes and the products of cleaving fatty acid, linoleic acid and linolenic acid by LOX and HPL, GLVs were thought of the potential airborne signals between plants (Lou and Cheng, 2000; Weber, 2002). In this study, the emission of those compounds was measured.

Figure 1 shows the release rates of terpenes and GLVs after exposure to ethylene. Except *α*-pinene whose release rate was evidently higher than control, the enhanced release rates of *β*-pinene, menthol, hexanal and 1-hexen-3-ol was not induced by ethylene. Ethylene exposure clearly decreased the release rates of two monoterpenes, 6-methyl-5-hepten-2-one and trans-geranylacetone, showing ethylene inhibited their emission. Ethylene did not exhibit clear inducing effects on the emission of terpenes and GLVs in poplar cuttings.

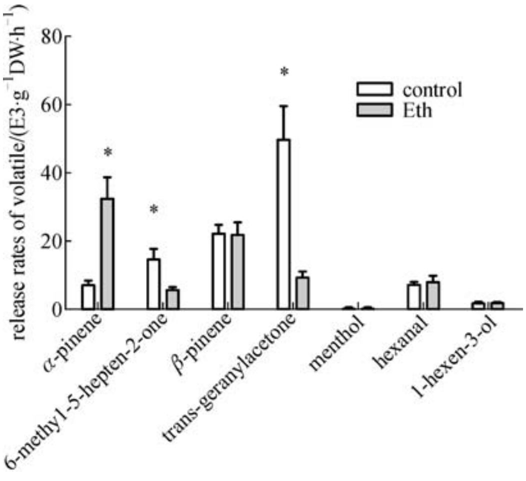

Fig. 1 Release rates of terpenoids and GLVs in ethylene treated poplar leaves (mean ± SD, *n* = 3). Control, health plants; Eth, ethylene treated plants; * indicates statistically significant difference between control and treatment (Student's *t*-test, $P < 0.05$)

As shown in Fig. 2, (*Z*)-3-hexenal, MeJA and mixture of ethylene and (*Z*)-3-hexenal all resulted in clearly higher total amount of volatiles than control, but the volatiles show different release rates exposed to different chemicals.

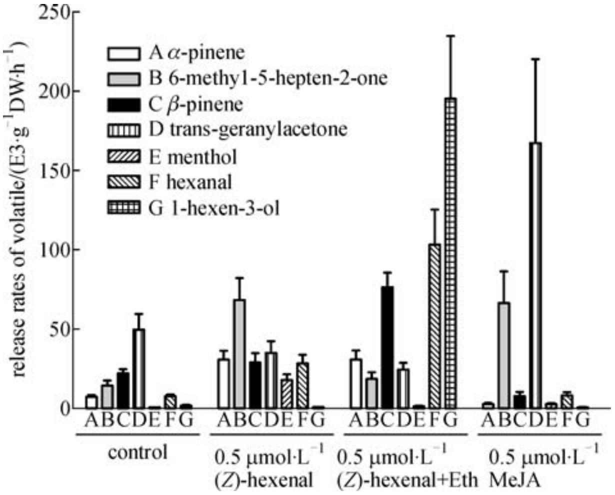

Fig. 2 Release rates of terpenoids and GLVs in different treated poplar leaves (mean ± SD, $n = 3$). Control, health plants; 0.5 μmol/L (*Z*)-hexenal, 0.5 μmol/L (*Z*)-hexen-3-al treated plants; 0.5 μmol/L (*Z*)-hexenal + Eth, 0.5 μmol/L (*Z*)-hexen-3-al + 40 μL/L Eth treated plants; MeJA, 0.5 μmol/L MeJA treated plants.

(*Z*)-hexe-3-nal induced a high emission of 6-methyl-5-hepten-2-one and hexenal from poplar cuttings, and their release rates were 4.5-fold and 3.7-fold as much as control respectively. (*Z*)-3-hexenal did not cause the emission of 1-hexen-3-ol from the poplar cuttings, but the mixture of (*Z*)-3-hexenal and ethylene resulted in a high emission of hexanal and 1-hexen-3-ol, and their release rates were 13-fold and 109-fold higher than control respectively. MeJA exposure induced the high emission of two monoterpenes, 6-methyl-5-hepten-2-one and trans-geranylacetone, but did not induce the emission of hexanal and 1-hexen-3-ol.

The results show that MeJA treatment resulted in a high emission of 6-methyl-5-hepten-2-one and trans-geranylacetone possibly mediated by the activation of terpenes synthesis genes, and the mixture of ethylene and (*Z*)-3-hexenal exhibited significant inducing effects on the emission of hexanal and 1-hexen-3-ol. Contrarily, little 1-hexen-3-ol was emitted from control, but its eco-physiological function in interplant signaling communication and indirect defense in *P. simonii*× *P. pyramidalis*'Opera 8277' plants need to be investigated in future studies.

4 Discussion

Jasmonate pathway plays an important role in the generation of wounding signals in plants, whereas it cannot induce whole defense response in plants after mechanical damage and herbivore wounding. In the transgenic potato plants superexpressing allene oxide synthase (AOS) that is a key enzyme in jasmonate pathway. The content of jasmonic acid is 6–12 fold higher than wild plants, but the proteinase inhibitor genes is not been activated (Harms et al., 1995). Thus, besides the jasmonate pathway, some other pathways may regulate the emission of volatiles in plants after mechanical damage and herbivore wounding.

A large number of GLVs are emitted from plants at once after mechanical damage and herbivore wounding. The latest study shows that (*Z*)-3-hexenol and (*E*)-2-hexenal emitted from lima beans induced by two spotted spider mites trigger the expressions of many defense genes in intact individuals (Arimura et al., 2004). These two volatiles can act as the early airborne signals to induce the defense response rapidly in the neighbouring intact plants, weakening the lag of induced resistance.

The results indicated that ethylene play an important role in the volatiles production of *P. simonii*×*P. pyramidalis* 'Opera 8277' cuttings, but ethylene alone could not promote the emission of volatiles. The mixture of ethylene and (*Z*)-3-hexenal significantly accelerated the emission of volatiles, showing that many signaling pathway co-regulated the volatiles synthesis in poplar cuttings.

In the following study, 0.5 μmol/L (*E*)-3-hexenal did not induce the emission of volatiles in poplar cuttings (data not shown), indicating that the poplar cuttings could recognize different structural hexenals. This might result from the different corresponding signal receptors. It is a problem in the field of phytochemical communication to explore that the recognition of plants to the airborne signals. Only the receptor of ethylene has been found in the plants among the interplant signals so far. MeJA may diffuse into plants through stoma, and it would be hydrolyzed to jasmonic acid by esterases, accomplishing the conversion from airborne signal to intracellular signal (Farmer, 2001). The understanding of mechanism in signaling communication between plants depends on the research of the receptors of airborne signals.

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