The effect of methyl salicylate on the induction of direct and indirect plant defense mechanisms in poplar (*Populus × euramericana* 'Nanlin 895')

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RESEARCH ARTICLE

The effect of methyl salicylate on the induction of direct and indirect plant defense mechanisms in poplar (*Populus × euramericana* 'Nanlin 895')

Fang Tang*, Ying-Ying Fu and Jian-Ren Ye

Department of Forest Protection, College of Forest, Nanjing Forestry University, Nanjing 210037, China

The objective of this study was to investigate the effect of different concentrations of methyl salicylate (MeSA) on direct defense and indirect defense in poplar cuttings (*Populus × euramericana* 'Nanlin 895'). Four defense-related enzyme activities, such as superoxide dismutase (SOD, EC1.15.1.1), oxydoreductases peroxidase (POD, EC1.11.1.7), catalase (CAT, EC1.11.1.6), and polyphenol oxidase (PPO, EC 1.14.18.1), were measured. The results showed that the SOD activities were induced by 1.0 and 10.0 mM MeSA and the POD activities were induced by MeSA. The CAT activity was induced at low concentrations of MeSA but was inhibited by high concentrations, and the PPO activity was affected by 0.1, 1.0, and 10.0 mM MeSA. Furthermore, the volatiles were detected using gas chromatography–mass spectrometry and gas chromatography. Twenty-one volatile compounds were tentatively identified in the emissions from plant leaves. Of these volatile compounds, (*Z*)-3-hexen-1-ol and *cis*-3-hexenyl acetate emissions were the highest. (*Z*)-3-Nonen-1-ol, (*E*)-2-hexen-1-ol, 1-octanol, (*E*,*Z*)-3,6-nonadien-1-ol, *β*-ionone, and hexadecanamide were six compounds that were only produced after treatment by 10.0 mM MeSA. These results showed that direct and indirect defense mechanisms in poplars were induced by MeSA.

Keywords: methyl salicylate; poplar; volatile compounds; direct defense; indirect defense

Abbreviations: MeSA, methyl salicylate; SOD, superoxide dismutase; POD, oxydoreductases peroxidase; CAT, catalase; PPO, polyphenol oxidase; PVPP, polyvinylpolypyrrolidone; GC–MS, gas chromatography–mass spectrometry; SA, salicylic acid; HIPV, herbivore-induced plant volatile

Introduction

Plants have evolved protective mechanisms to keep deleterious reactions to a minimum, such as antioxidative enzymatic defenses, including oxydoreductases peroxidase (POD), polyphenol oxidase (PPO), and catalase (CAT), or nonenzymatic defenses (Qin et al. 2005; Mahdavian et al. 2007). Methyl salicylate (MeSA) is a volatile organic compound that is widespread in the plant world. It is synthesized from salicylic acid (SA), a phytohormone that contributes to plant systemic resistance defenses. MeSA is synthesized by members of a family of *O*-methyltransferases (Tieman et al. 2010). MeSA and SA are key compounds in the shikimic acid pathway and are involved in the induction of direct and indirect plant defenses (Dicke & Hilker 2003). MeSA is an important component of the signal transduction cascades, which activate the plant defense response, and it induces an oxidative burst in sunflower seedling roots (Garrido et al. 2009).

MeSA is naturally produced by plants in response to herbivore damage (Van Den Boom et al. 2004). It is a herbivore-induced plant volatile (HIPV; Gadino et al. 2012) and has been shown to attract natural enemies and affect herbivore behavior (Mallinger et al. 2011). HIPVs attract the arthropod natural enemies of herbivores and therefore may potentially be able to enhance biological control. However, HIPVs can also affect the behavior of arthropod herbivores and possibly higher-order natural enemies, which may complicate the use of HIPVs in biological control (Orre et al. 2010). The positive response of beneficial arthropods toward MeSA and other herbivore-induced volatiles has been demonstrated in a number of laboratory assays (De Boer & Dicke 2004; Ishiwari et al. 2007; Shimoda 2010). For example, *Coccinella septempunctata* may use MeSA as the olfactory cue for prey location (Zhu & Park 2005).

In this study, we investigated both direct and indirect plant defenses in poplar cuttings (*Populus × euramericana* 'Nanlin 895'). We focused on the change in enzymes related to direct defense and in the volatile compounds related to indirect defense. The aim of this study was to determine if there were any differences in poplar chemical defenses when treated with different concentrations of MeSA, compared to the control plants, in order to see whether these treatments had an effect on the antioxidant defense system and indirect volatiles defense systems, and thereby ascertain the changes in the direct and indirect defense mechanisms of poplar when treated with exogenous MeSA.

Materials and methods

Plant materials and growth conditions

Poplar cuttings (*P. × euramericana* 'Nanlin 895') were provided from the forest cultivation seedling base at

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*Corresponding author. Email: tangfang76@sohu.com

Nanjing Forestry University. The seedlings were grown in a growth chamber at $25 \pm 1°C$ and with a L16:D8 photoperiod. They were watered daily and supplied with Hoagland nutrient solution (Afrousheh et al. 2010) every 2 weeks in order to avoid water and nutrient stress. They were used in experiments after they had grown to a height of 60–80 cm.

Plant treatment

Similar plants were chosen for the MeSA experiment in order to minimize the effects of differences between plants. The leaves were sprayed with 0.001, 0.01, 0.1, 1.0, and 10 mM MeSA (purity >99.5%; Sigma-Aldrich, St Louis, MO, USA) solution (with 50% alcohol as a solvent) at about 25 μl solution cm^{-2}. Both sides of the leaves were sprayed. They were then used to assay for enzyme activities. For the volatile compounds experiment, the leaves were sprayed with 10 mM MeSA solution then all the volatile compounds were determined. The control plants were sprayed with 50% alcohol solution only. The leaves were harvested with their petiole after 24, 48, and 72 h. All the experiments were repeated three times and three replications were run at each time point in each treatment. New plants were sampled at each time point.

Assay for enzyme activities

Superoxide dismutase (SOD) activity was determined according to Oncel et al. (2004) with slight modification. Exactly 200 mg of fresh leaf with 20 mg polyvinylpolypyrrolidone (PVPP) was ground and extracted in 3 ml 0.05 M phosphate buffer (pH 7.8). The homogenate was centrifuged at 1737 g for 10 min at 4°C. The reaction mixture (3 ml) contained 1.5 ml potassium phosphate buffer (pH 7.8), 50 μl enzyme extract, and 250 μl distilled water, 300 μl 130 mM L-methionine, 300 μl 750 μM nitroblue tetrazolium chloride (NBT), 300 μl 20 μM riboflavin, and 300 μl 100 μM ethylenediamine tetra acetic acid disodium salt. The reaction mixture was kept under 4000-l × fluorescent light for 30 min at 25°C. NBT reduction ratios were measured with a spectrophotometer adjusted to 560 nm (Cary 50). One SOD unit was described as the amount of enzyme where the NBT reduction ratio was 50%.

POD activity was assayed according to Yingsanga et al. (2008) with slight modification. Exactly 100 mg of fresh leaf was homogenized in 2 ml 0.1 M ice-cold sodium phosphate buffer (pH 7.0). Then the homogenate was centrifuged at 1646 g for 10 min at 4°C and the supernatant was stored at −20°C until it was needed for the POD spectrophotometric assays. The substrate used in the POD activities analysis consisted of 50 μM guaiacol (Sigma) with 0.04% H_2O_2 (wt/wt) added as a cofactor. An aliquot of 500 μl of leaf enzyme extract was added to 1.5 ml of substrate in phosphoric acid buffer (0.1 M; pH 6.0) and the change in absorbance of the mixture at 470 nm was measured for 1 min (Cary 50). One unit of enzyme activity was defined as a change of 0.01 in absorbance per min.

CAT activity was assayed according to Tejera García et al. (2007) with slight modification. Exactly 100 mg of fresh leaf was homogenized in 2 ml 0.2 M ice-cold sodium phosphate buffer (pH 7.8) containing 10 mg PVPP (Sigma). Then the homogenate was left for 10 min at 4°C and the supernatant was centrifuged at 1737 g for 15 min at 4°C. The supernatant was stored at −20°C until it was needed for the CAT spectrophotometric assays. An aliquot of 50 μl of leaf enzyme extract was added to 2.95 ml of 3.39 mM H_2O_2 in phosphoric acid buffer (0.05 M; pH 7.8) and the change in absorbance of the mixture at 240 nm was measured for 1 min (Cary 50). One unit of CAT activity was defined as the amount of protein decomposing 1 μmol of H_2O_2 per min, when compared to a control reaction without substrate.

To assay for PPO activity, 100 mg of fresh leaf was homogenized in 1.5 ml 0.1 M ice-cold sodium phosphate buffer (pH 7.5) that contained 10 mg PVPP (Sigma). Then the homogenate was centrifuged at 27,664 g for 20 min at 4°C and the supernatant was stored at −20°C until it was needed for the PPO spectrophotometric assays. PPO activity was assayed according to Vanitha and Umesha (2011) with slight modification. An aliquot of 50 μl of leaf enzyme extract was added to 2.95 ml of 13.56 mM catechol (Sigma) in phosphoric acid buffer (0.2 M; pH 4.0) and the change in absorbance of the mixture at 420 nm was measured for 1 min (Cary 50). One unit of enzyme activity was defined as a change of 0.01 in absorbance per min.

Collection of volatile compounds

Headspace solid-phase micro extraction (SPME) was used to analyze the volatiles coming from the treated leaves (Zhang & Pawliszyn 1993). The fiber coating was PDMS-100 (100 μm polydimethylsiloxane; Supelco, Bellefonte, PA, USA). The leaf sample (2 g, shredded) was placed in a 15 ml glass vial that had been closed with a septum cap. The septum was punctured and the SPME needle inserted into the headspace. Samples were extracted at 45°C and the fiber was exposed for 30 min. After this, the fiber was inserted into the injector of a gas chromatography (GC) or a gas chromatography–mass spectrometry (GC–MS) system for thermal desorption of the volatiles (Abel et al. 2009). All the experiments were repeated three times, and three replications were run at each time point for each treatment. New plants were used at each time point.

Chemical analyses

Gas chromatography–mass spectrometry (GC–MS)

GC–MS analyses were undertaken using an Agilent HP7890A GC coupled to an Agilent 5975C system (Agilent Technologies, USA) with the injector set at 250°C in the splitless mode. Analyses were performed using a DB-WAX column (30 m × 0.32 mm, 0.25 μm,

Agilent J&W; 42 cm s^{-1} He was used as the carrier gas, temperature increased from 70°C [2 min] to 230°C at 6°C min^{-1} with a 6 min hold). The temperatures of the transfer line, the ion source, and the quadrupole were 250°C, 230°C, and 150°C, respectively. Electron ionization mass spectra were recorded (with a 4 min solvent delay) at 70 eV over the *m/z* range of 25–350 with a threshold abundance of 10.

The compounds were identified using the mass spectral libraries: NIST08.L (Agilent Technologies, Palo Alto, CA, USA) and Wiley275, and confirmed with authentic standards where available.

Gas chromatography (GC)

GC analyses were carried out using a HP6890 GC system (Agilent Technologies, USA). The injection port (250°C) was in the splitless mode and the flame ionization detector was heated to 250°C. A capillary column was used with N$_2$ (37 cm s^{-1}) as the carrier gas. A polyethylene glycol DB-WAX column (30 m× 0.32 mm, 0.25 µm, Agilent J&W) was maintained at 70°C for 2 min, then increased at 6°C min^{-1} to 230°C, which was maintained for 5 min (Tooker et al. 2008). The total ion peaks of each compound were integrated and the amount of each compound was calculated based on external calibration curves constructed from 1 µl injections of known *n*-heptanol, which was generated using authentic standards (Schmelz et al. 2003). All statistical analyses were performed using GraphPad InStat version 3.00.

Results

Induction of defense enzymes

The SOD, POD, CAT, and PPO activities induced by MeSA treatment over 72 h are shown in Figure 1.

Treatment with the different concentrations of MeSA led to different effects on SOD activity in poplar leaves. At 24 and 48 h, the results for the 0.001, 0.01, and 0.1 mM concentrations were not significantly different ($P > 0.05$) from the controls, but the 1.0 and 10.0 mM treatments were significantly higher ($P < 0.05$) than the controls. After 72 h, none of the treatments were significantly different from the controls (Figure 1A).

In MeSA-treated plants, the POD specific activities increased ($P < 0.05$) for all treatments at 24 and 48 h compared to the control leaves. At 72 h, the 0.001 mM treated leaves were not significantly different ($P > 0.05$) from the controls, but the other treatments were all significantly higher than the controls. At 48 h, all treatments reached their peak rates ($P < 0.001$) for POD (Figure 1B).

At 24 h, the CAT activity in leaves treated with 0.001 and 0.01 mM of MeSA was higher ($P < 0.05$) than the controls. The 0.1 mM treatment did not differ ($P > 0.05$) from the control, but the CAT activity was lower ($P < 0.05$) in the 1.0 and 10.0 mM MeSA-treated leaves, compared to the control leaves. At 48 h, the CAT activity in the leaves treated with 0.001 and 0.01 mM MeSA was still higher ($P < 0.05$) than in the controls. The 0.1 and 1.0 mM treatments were not significantly different ($P > 0.05$) from the controls, but the 10.0 mM treatment was significantly lower than the controls. At 72 h, the CAT activity in the leaves treated with 0.001 mM was
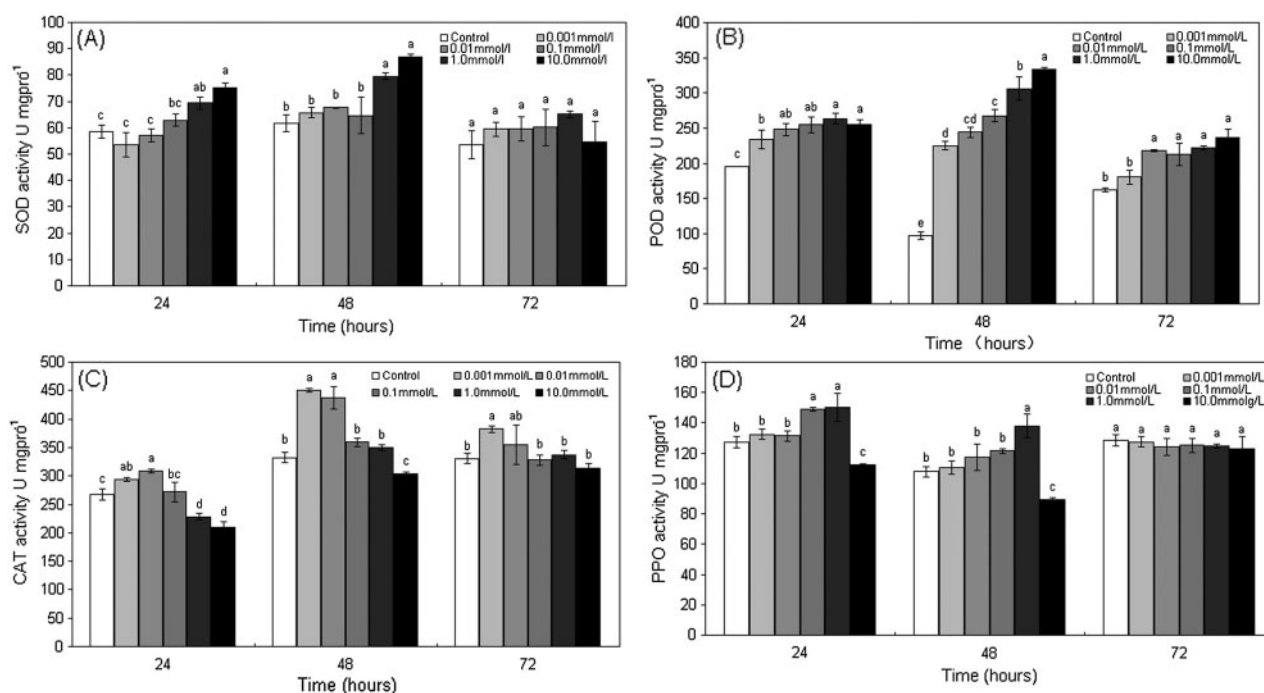

Figure 1. The activity change in SOD (A), POD (B), CAT (C), and PPO (D) in poplar leaves after treatment with MeSA for different lengths of time.
Note: All values are mean ± standard deviation of the mean (SD). Different letters indicate significant differences between the treatment and the control ($P < 0.05$; *t* test).

higher ($P < 0.05$) than the controls, but the other treatments were not significantly different (Figure 1C).

The 0.1 and 1.0 mM MeSA treatments increased the PPO activity in poplar leaves at 24 h, but 10.0 mM decreased activity. At 48 h, the PPO activity in poplar leaves treated with 1.0 mM of MeSA was higher ($P < 0.05$) than the controls but was lower in the leaves treated with 10.0 mM MeSA. At 72 h, the PPO activity in all treatments was not significantly different to the controls (Figure 1D).

Volatile compounds

In total, 21 volatile compounds were tentatively identified in the emissions from the treated and control plant leaves by comparing their retention indices and mass spectra with those of synthetic standards (Figure 2A and 2B).

Most volatiles released from the poplar plants were common plant volatiles. However, further quantitative GC and GC–MS analyses revealed that there were differences in emissions between the treated and control leaves at the different time periods for the 21 compounds identified. The contents of the first 19 compounds were lower than the last two and they changed over time (Figure 2C, 2D, and 2E). The content of hexyl formate, 2-ethyl-1-hexanol, and methoxy-phenyl-oxime- increased significantly at 72 h after treatment with MeSA. There were no significant differences in the contents of (Z)-butanoic acid-3-hexenyl ester between the treated and
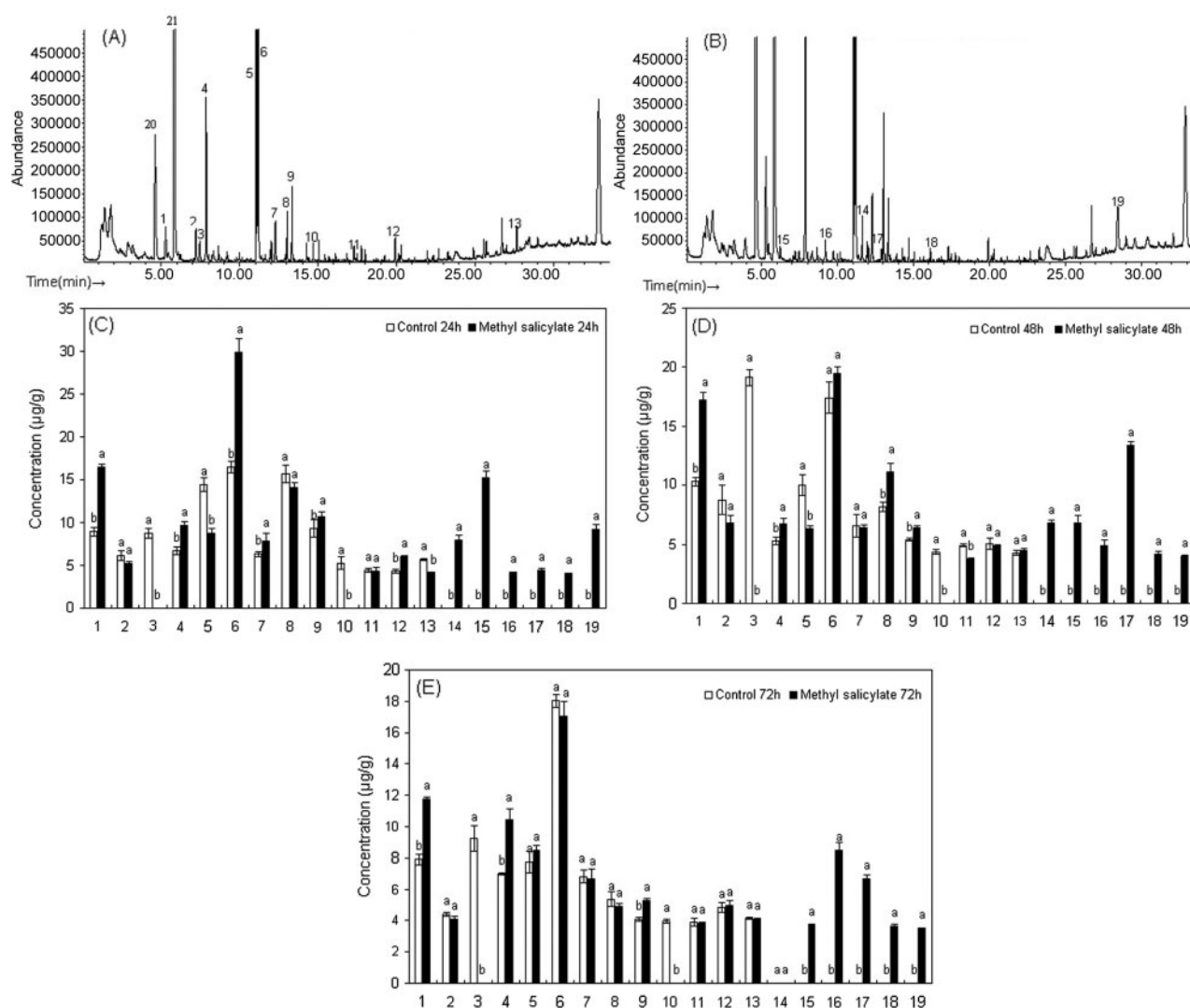

Figure 2. Volatile compound content changes in poplar treated by MeSA. Typical gas chromatograms (on a DB-WAX column) of volatile compounds emitted from control (non-MeSA treated plants) (A) and MeSA treated plants (B) after 24 h and the content changes of 19 low-quantity volatile compounds in poplar treated for 24 h (C), 48 h (D), and 72 h (E).

Notes: The bars represent hexyl formate (1), (Z)-butanoic acid-3-hexenyl ester (2), *cis*-3-hexenyl-2-methylbutyrate (3), 2-ethyl-1-hexanol (4), unknown (5), 2-hydroxy-benzaldehyde (6), 2,6-dimethyl-5-heptenal (7), methylsalicylate (8), methoxy-phenyl-oxime- (9), geraniol (10), phenol (11), eugenol (12), dibutylphthalate (13), (Z)-3-nonen-1-ol (14), (E)-2-hexen-1-ol (15), 1-octanol (16), (E,Z)-3,6-nonadien-1-ol (17), *β*-ionone (18), and hexadecanamide (19). Each compound was more than 80% matched. For each number, different small letters indicate statistically significant differences in volatile release concentrations between the treated and the control plants (GraphPad InStat version 3.00; $N = 3$; $P < 0.05$).

control leaves over the three time periods. *cis*-3-Hexenyl-2-methylbutyrate and (*E,Z*)-3,6-nonadien-1-ol occurred only in the control plants and were not detected in the treated plants. The 2-hydroxy-benzaldehyde and 2,6-dimethyl-5-heptenal contents in the treated leaves were higher than in the control leaves at 24 h, but there were no significant differences at 48 and 72 h. The MeSA content was higher in the treated leaves than that in the control leaves at 48 h, but there were no significant differences at 24 and 72 h. The phenol contents of the treated leaves were not significantly different from the controls at 24 and 72 h but significantly lower than the controls at 48 h. The dibutylphthalate content was lower in the treated plants than that in the controls at 24 h, but there were no significant differences at 48 and 72 h. (*Z*)-3-Nonen-1-ol was only present in the treated plants and occurred at 24 and 48 h. (*E*)-2-Hexen-1-ol, 1-octanoloctanol, (*E,Z*)-3,6-nonadien-1-ol, *β*-ionone, and hexadecanamide were also only detected in the treated plants and occurred at 24, 48, and 72 h.

Table 1 shows the content changes for the six compounds only found in the treated leaves at the different time periods. (*Z*)-3-Nonen-1-ol was detected at 24 and 48 h and its content at 24 h was significantly higher than at 48 h. The content of (*E*)-2-hexen-1-ol fell over time and each time period was significantly different. Its concentration reached a maximum (15.19 µg g^{-1}) at 24 h. The content of 1-octanol increased over time and reached a maximum concentration (8.52 µg g^{-1}) at 72 h. There was also a significant difference in concentration of (*E,Z*)-3,6-nonadien-1-ol between the 24 and 48 h time periods. At 24 and 48 h, the *β*-ionone concentrations were not significantly different, but they were significantly higher than at 72 h. The hexadecanamide concentration reached a maximum (9.16 µg g^{-1}) at 24 h and then declined.

Figure 3 shows the concentrations of *cis*-3-hexenyl acetate and (*Z*)-3-hexen-1-ol in the poplar leaves at the 24, 48, and 72 h time periods. The results indicated that the amount of *cis*-3-hexenyl acetate released from the treated leaves was higher (*P* < 0.05) than that from the control leaves for the entire length of the experiment. The amount of (*Z*)-3-hexen-1-ol released from the treatment leaves was not significantly different from the control leaves (*P* > 0.05) at 24 h, but then increased
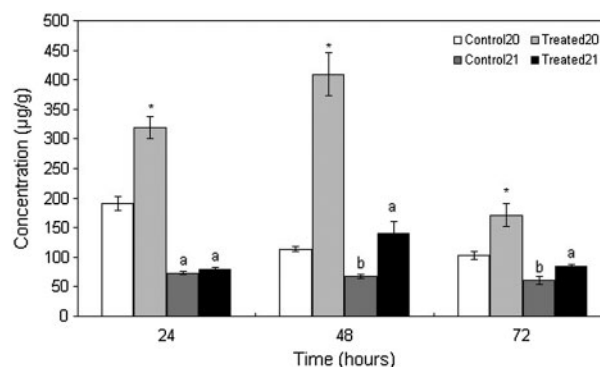

Figure 3. Concentrations of high-volume volatile compounds: *cis*-3-hexenyl acetate (20) and (*Z*)-3-hexen-1-ol (21), in poplar leaves at the 24, 48, and 72 h time periods.
Note: Columns with an asterisk or with different small letters have significantly different volatile release concentrations between the treated and control leaves (GraphPad InStat version 3.00; *N* = 3; *P* < 0.05).

and became higher than the controls at 48 and 72 h. The peak release quantities of these two compounds were 319.50 and 139.90 µg g^{-1}, respectively, and occurred at 48 h.

Discussion

Diverse environmental stresses affect plant processes in various different ways and can lead to the loss of cellular homeostasis and the formation of reactive oxygen species (ROS; Almeselmani et al. 2006), which induce the production of superoxide radicals (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-; Foyer et al. 1994). They cause oxidative damage to membranes, lipids, proteins, and nucleic acids (Srivalli et al. 2003). Antioxidant enzymes play important roles in adaptation to stress conditions. SOD, POD, and CAT are protective enzymes in plants and they can be affected by light and cold stress (Gechev et al. 2003), salinity (Rios-Gonzalez et al. 2002; Misra & Gupta 2006), high temperature stress (Ali et al. 2005; Almeselmani et al. 2006), and drought stress (Sun et al. 2010). The coordinated functions of antioxidant enzymes, such as SOD, CAT, and POD, facilitate ROS processing and

Table 1. The content changes of six volatile compounds only produced in the treated leaves (among 19 low-quantity volatile compounds) at the different time periods.

Compound	Concentration (µg g^{-1}) ± SD		
	24 h	48 h	72 h
(*Z*)-3-Nonen-1-ol	7.91 ± 0.56a	6.83 ± 0.25b	NDc
(*E*)-2-Hexen-1-ol	15.19 ± 0.85a	6.84 ± 0.58b	3.73 ± 0.07c
1-Octanol	4.15 ± 0.02b	4.85 ± 0.52b	8.52 ± 0.44a
(*E,Z*)-3,6-Nonadien-1-ol	4.56 ± 0.18c	13.37 ± 0.32a	6.66 ± 0.25b
β-Ionone	4.07 ± 0.03a	4.14 ± 0.25a	3.63 ± 0.13b
Hexadecanamide	9.16 ± 0.64a	3.97 ± 0.13b	3.50 ± 0.03b

Notes: ND means that the compound was not detected. The same compounds at the various time periods with different small letters have significantly different volatile release concentrations (GraphPad InStat version 3.00; *N* = 3; *P* < 0.05).

the regeneration of redox ascorbate and glutathione metabolites (Foyer & Nector 2000).

Within a cell, the SODs constitute the first line of defense against ROS (Alscher et al. 2002). SODs are enzymes that play a pivotal role in metabolizing O_2^-. They preempt oxidizing chain reactions that cause extensive damage and stop the formation of a number of deleterious ROS including: H_2O_2, hypochlorite (OCl^-), peroxynitrate (ONO_2^-), and OH^- (Miller 2012). SOD converts one form of ROS (O_2^-) to another equally toxic one (H_2O_2; Almeselmani et al. 2006). The increased SOD activity could be a consequence of excess O_2^- generation when leaves have been treated with high MeSA concentrations. MeSA induces antioxidant enzyme production, which might have resulted in the increased production of SOD seen at the 24 and 48 h time periods. The rise in SOD coincided with an activity increase in the H_2O_2 scavenging enzymes. SOD generates H_2O_2, which is then eliminated by POD (Rios-Gonzalez et al. 2002). PODs are polyfunctional enzymes that undergo two reaction cycles: the peroxidase–oxidase cycle (with NADH as substrate, also called NADH peroxidases) resulting in H_2O_2 production (Halliwell 1978) and the peroxidase cycle (with guaiacol as phenol substrate, also called guaiacol peroxidase) leading to H_2O_2 consumption (Fecht-Christoffers et al. 2003). PODs are believed to act as induced and constitutive defenses against leaf feeding insects (Barbehenn et al. 2010). The POD activity increased between 24 and 72 h in this study. CAT was the first antioxidant enzyme to be discovered and characterized. The typical CAT reaction is the dismutation of two molecules of H_2O_2 to water and O_2 (Mhamdi et al. 2010). The CAT activity increased between 24 and 72 h when leaves were treated with low concentrations of MeSA, whereas high concentrations of MeSA caused CAT to decline over the same time frame. In these experiments, the H_2O_2 was probably of enzymatic origin, considering the oxygen concentration in the system and the positive effects of the various inhibitors of H_2O_2-producing enzymes (Blokhina et al. 2003). PPOs are metalloenzymes that catalyze the oxygen-dependent oxidation of phenols to quinones. Eolini et al. (2004) suggested that the physiological regulation of PPO expressions was part of the plant defense system and was related to other metabolic pathways. Moreover, they also suggested that these enzymes were involved in plant responses to different stress conditions. Highly reactive intermediates and their secondary reactions are responsible for much of the oxidative browning that accompanies plant senescence, wounding, and responses to pathogens and herbivore attack. In addition, the role of PPO in the generation of ROS is evident from the large contribution PPO-based phenolic oxidation makes to H_2O_2 production in plant extracts (Thipyapong et al. 2004). Compared with the controls, PPO increased after leaves were treated with 0.1 and 1.0 mM MeSA at the 24 h time period but declined at the 24 and 48 h time periods after the plant leaves were treated with 10.0 mM MeSA.

Plant odors are the most ubiquitous volatiles in nature. Any type of surface damage commonly causes plant leaves to release so-called green leaf volatiles (Hoballah & Turlings 2005). Han and Chen (2002) showed that tea can produce different compounds after being mechanically pierced or attacked by the tea aphid. This study investigated the effect of MeSA on the production of volatile blends in poplar. The amount of MeSA seen in poplar treated with 10 mM MeSA was significantly higher than in the control leaves (Figure 2) and MeSA has been shown to trigger the plant's own production of HIPVs (Khan et al. 2008). MeSA also acts as a transmission signal and recent evidence supports this conclusion (Schulaev et al. 1997; Chen et al. 2003; Koo et al. 2007; Park et al. 2007). It is also known to be a HIPV that attracts natural enemies of the herbivores feeding on the plants (Girling et al. 2006). After the leaves had been treated with MeSA, (Z)-3-hexen-1-ol, and (Z)-3-hexenyl acetate levels increased. This was consistent with others' results (Ruther et al. 2002; Fraser et al. 2003). Furthermore, Ruther's experiment showed that (Z)-3-hexen-1-ol was highly attractive to male *Melolontha hippocastani* antennae (Ruther et al. 2002). Fraser et al. (2003) confirmed that male *Manduca sexta* can detect (Z)-3-hexenyl acetate and that MeSA elicited large electroantennogram responses, even when it was present in trace amounts. To the contrary, Snoeren et al. (2010) demonstrated that MeSA acts as a repellent to naïve *Diadegma semiclausum* and that the repellent effect of MeSA was dose-dependent. Therefore, the effect of these plant volatile organic compounds on natural enemies of poplar pests needs further studies.

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