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Aldehyde Volatiles Emitted in Succession from Mechanically Damaged Leaves of Poplar Cuttings

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Plant aldehydes are volatiles necessary to defenses against environmental stress. To explore their emissions in response to wounding, we performed gas chromatography-mass spectrometry (GC-MS) on cuttings from poplar (*Populus simonii*×*P. pyramidalis* 'Opera 8277') that were mechanically damaged to mimic herbivore attack. We detected 16 aldehydes, including 11 linear saturated aldehydes, 3 linear unsaturated aldehydes, and 2 non-linear aldehydes. Emissions of these aldehydes were clearly enhanced by such treatment, and exhibited a similar pattern of change, i.e., increasing in the first 2 h, then sharply decreasing before rising again at about 12 h. Two release peaks for these aldehydes were observed. Therefore, we propose two pathways for the mediation of aldehyde emissions following damage. The first peak may represent emissions from plant storage pools, whereas the second release peak might result from greater formation *de novo* through an activated synthesis pathway.

Keywords: aldehyde emissions, emission mechanism, mechanical damage, poplar (*Populus simonii*×*P. pyramidalis* 'Opera 8277') cuttings

Unlike animals that can avoid injury by moving, plants utilize special defense systems against environmental stresses. In response to mechanical damage, herbivore wounding, or pathogen infections, plants can release a range of volatile organic compounds (VOCs) (Ping and Shen, 2001). Such VOCs can serve as a means of defense by directly repelling the herbivores or attracting predatory arthropods (Kessler and Baldwin, 2001, 2002). Likewise, some VOCs might act as interplant signals that are perceived by neighbouring plants to adjust their defensive phenotype according to the present risk of attack (Peñuelas and Llusià, 2004).

Plants emit substantial amounts of VOCs into the air, i.e., alkanes, alkenes, alcohols, aldehydes, ethers, esters, and carboxylic acids (Kesselmeier and Staudt, 1999; Katrien, 2002; Penuelas and Llusià, 2004). Some of these are constituent VOCs, while others are emitted only after damage occurs. The components of VOCs, released from various plant parts or at different times, fluctuate in their intensity or by species (Turlings et al., 1993). Moreover, specific damage treatment or insect wounding can lead to differences in VOCs (Hu et al., 2004).

Terpenes are found to be emitted from plants (Kesselmeier and Staudt, 1999). Two ester compounds, methyl jasmonic acid and methyl salicylic acid, are regarded as important molecular signals between plants (Ping and Shen, 2001). Aldehydes belong to an important class of volatiles that are indispensable to plants in response to environmental conditions. Nandi and Fries (1976) have found that pentanal, hexanal, and heptanal exhibit strong antifungal activities against several fungi, including two *Aspergillus* species in stored wheat seeds. Past studies of aldehydes have largely focused on aldehyde C6, a member of the green leafy volatiles (GLVs). These compounds, components of plant fragrance, can be emitted after damage and are considered to be direct or indirect defense signals (Kishimoto et al., 2005). Aldehydes C6-C10 are induced in six species -- sunflower, pine, corn, tomato, tobacco, and canola – when plants are exposed to ozone (Wildt et al., 2003), furfural is also released from Chinese white poplar and ashleaf maple following mechanical wounding (Ping et al., 2001b). However, the emissions of other aldehydes have not been well described, and the species and patterns of change in aldehyde levels, as stimulated by damage, are largely unknown in plants.

Here, we performed GC-MS to measure the emissions of aldehydes in cuttings from *Populus* plants after mechanical damage. Our objective was to investigate the potential physioecological functions of aldehyde volatiles.

MATERIALS AND METHODS

Plant Materials and Growing Conditions

One-year-old cuttings from poplar (*Populus simonii*×*P. pyramidalis* 'Opera 8277') were grown in pots (25.0 × 25.0 cm) containing nursery top soil. Greenhouse conditions at Beijing Forestry University included a 16-h photoperiod and day/night temperatures of 25/20°C. The cuttings were watered daily and supplied with a full Hoagland nutrient solution every two weeks (Hu et al., 2004). Their volatiles were collected in July of 2004.

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Damage Treatment

Mechanical damage was applied at 7:00 to 7:30 a.m. From the top of the cuttings, the third to seventh leaves were damaged by a punch (0.5 cm diameter). From each leaf, 20% was cut randomly so that this slow process began to mimic a degree of herbivore wounding. This treatment was repeated at least three times.

Volatile Collection

Reynolds oven bags (44.3 × 55.8 cm) were used for sampling because they release and absorb only a few volatiles. Cutting samples of similar leaf area were placed in each bag. A glass tube (15.0 × 0.3 cm; Chrompack, Middelburg, the Netherlands) containing Tenax-TA (60 to 80 mesh, Chrompack) was used as a volatile trap in each bag. We tried to avoid having the poplar cuttings touch the tube. A portable air sampler (QC-1; Beijing Municipal Institute of Labour Protection, China) was used as the pump. Volatiles were collected for 1 h, at a flow rate of 100 mL min^{-1} per cutting. The glass tube was then placed in a desiccator at 26±2°C. For the control, samples were collected from the cuttings before this damage treatment was begun.

Volatile Analysis

Using gas chromatography-mass spectrometry (GC-MS) (Trace 2000-Voyager, Finnigan; Thermo-Quest, Rodano, Milan, Italy), we de-sorbed the volatiles by heating them in a CP-4010 TCT thermal desorption device (Chrompack) at 250°C for 10 min, then cryo-focused them in a cold trap refrigerated by liquid N_2 at –100°C. This cold trap was then quickly heated to 260°C for 1 min to transport the volatiles into an analytical column (CP-Sil 5CB low bleed/MS 60 m × 0.32 mm i.d., with a 0.5 μm film thickness). The column was programmed from 40°C to 270°C, at 6°C min^{-1}, and held for 10 min. Helium at 20 kPa pressure was used as the propellant. The MS was operated in a 70 eV EI ionization mode, and scanning was done from *m/z* 10 to *m/z* 400, for 0.4 s per scan.

Volatile Identification and Quantification

Compounds were preliminarily identified by searching the NIST library in the data system of Xcalibur (Finnigan) and checking them against its retention index. To compare among the amounts of aldehydes released after different treatment periods, we used hexanal (C6) (Beijing Chemical Reagent Inc., China) as an external standard, as described previously (Ping et al., 2001a). After the hexanal was dissolved in ethanol, 1, 5, 10, or 50 μL of dilutions of 1 mmol mL^{-1} were applied to cotton-tipped wooden dowels. These were then placed in a collection bag without plants, thereby maintaining the same volume as when collecting volatiles from the cuttings. A characteristic ion intensity (E3) was used for further determining the amount of volatiles released (Ping et al., 2001b).

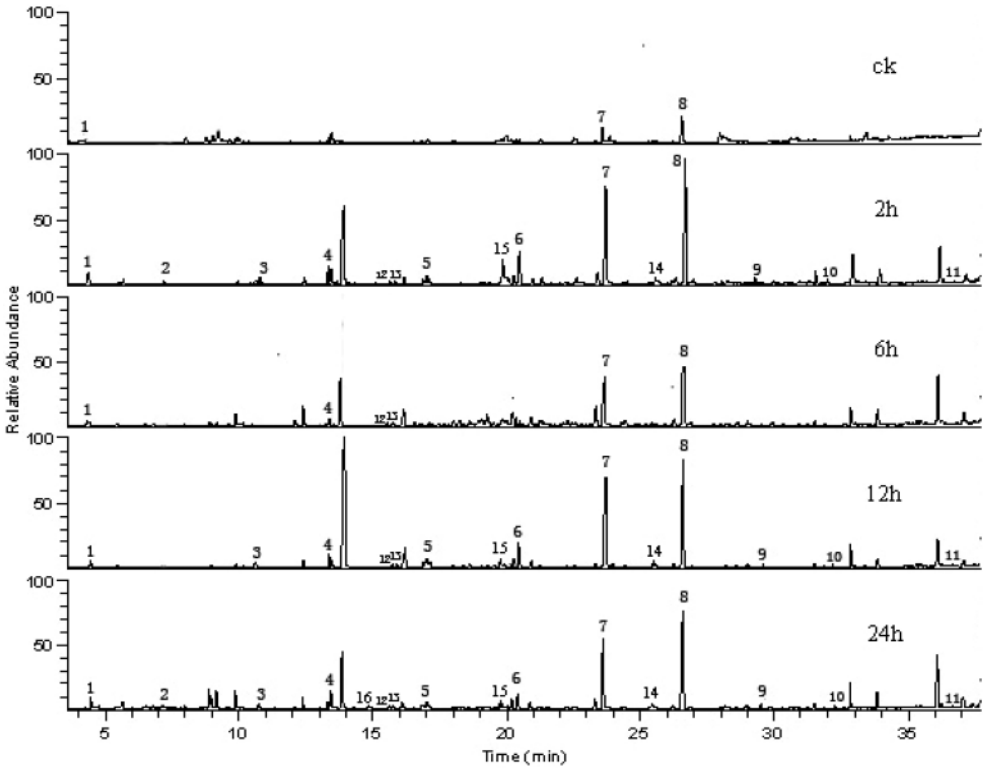

Figure 1. Chromatographic profiles of volatiles from poplar cuttings after mechanical damage. Peaks of 16 aldehydes show time-dependent pattern: 1, acetaldehyde; 2, butanal; 3, pentanal; 4, hexanal; 5, heptanal; 6, octanal; 7, nonanal; 8, decanal; 9, undecanal; 10, dodecanal; 11, tetradecanal; 12, (*Z*)-3-hexenal; 13, (*E*)-2-hexenal; 14, (*E*)-2-nonenal; 15, benzaldehyde; and 16, furfural. Volatiles were collected at 2, 6, 12, and 24 h after wounding occurred; samples collected from cuttings before treatment was applied served as control. Chromatograms were performed at mass/charge ratio (*m/z*) = 41 as qualifier ion.

RESULTS

Chromatographic profiles of volatiles from mechanically damaged poplar cuttings included 16 aldehydes: acetaldehyde, butanal, pentanal, hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal, tetradecanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, (*E*)-2-nonenal, benzaldehyde, and furfural (Fig. 1). All exhibited a time-dependent pattern of release after wounding.

In all, 11 linear saturated aldehydes -- acetaldehyde, butanal, pentanal, hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal, and tetradecanal -- were detected (Fig. 2). Their levels were clearly greater than from the undamaged control, and all exhibited the same pattern of change: a sharp increase 2 h after damage, followed by a sudden decrease, then a significant rise. However, the second release peak for each aldehyde was slightly different. For example, the amounts of acetaldehyde and butanal were induced to increase 5- to 9-fold at 2 h after damage before declining to a level similar to that of the control at 6 h and 12 h, before rising again at 24 h to the same level previously attained at 2 h after damage occurred. The released amount of undecanal showed a similar pattern except that its amount, released at 24 h, barely increased, to about 0.6 times that measured at 2 h. Releases of pentanal, hexanal, and tetradecanal returned to the same high levels seen at 12 h, after an earlier decline at 6 h, then continued to rise at 24 h. Heptanal, octanal, nonanal, decanal, and dodecanal showed a slightly different pattern of change: a decrease at 24 h that followed a clear increase at 12 h; however, those amounts released at 24 h were still far higher than those of the control. Overall, nonanal and decanal had values of almost 200 E3, while hexanal and octanal exceeded that level. The release of other aldehydes was relatively low, especially that of butanal and tetradecanal, where values were below 5 E3.

Three linear unsaturated aldehydes -- containing (*Z*)-3-hexenal, (*E*)-2-hexenal, and *(E)*-2-nonenal -- were also detected here (Fig. 3). The amounts of these volatiles were considerably stimulated by wounding, exhibited a pattern of

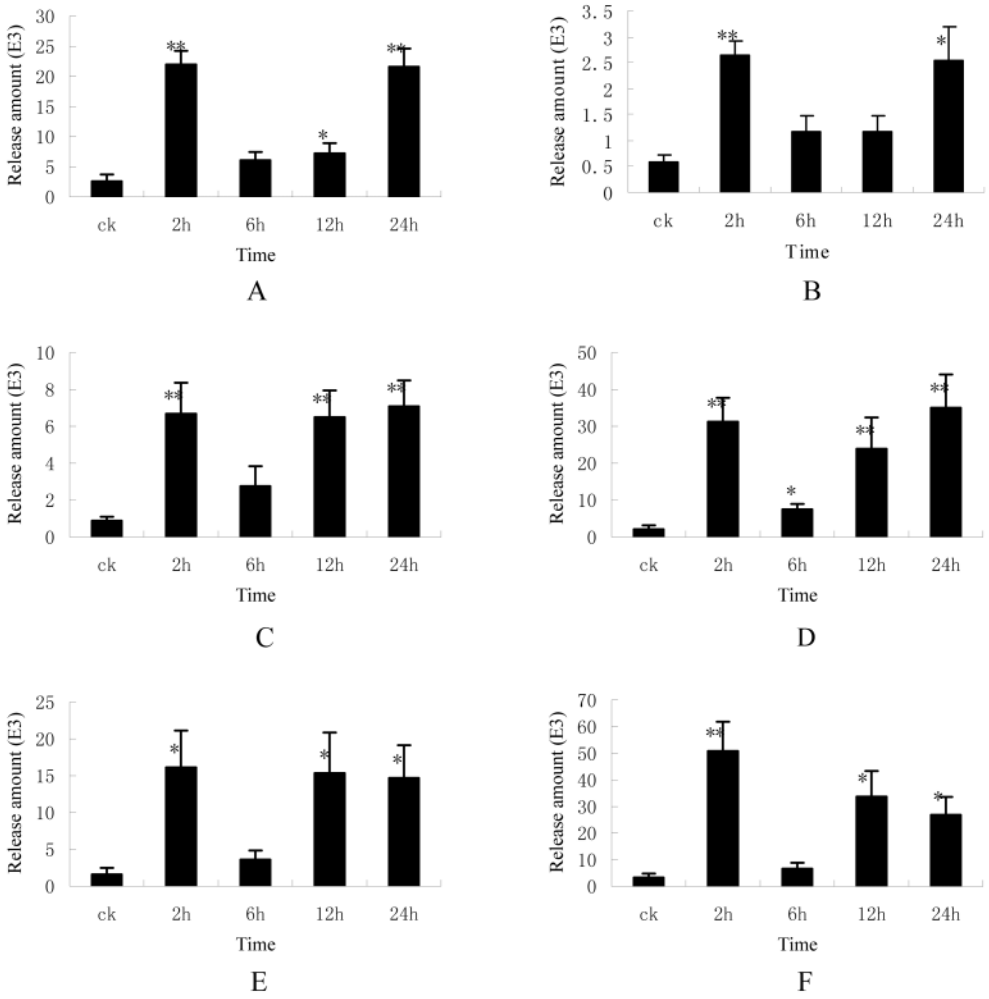

Figure 2. Released amounts of acetaldehyde (**A**), butanal (**B**), pentanal(**C**), hexanal (**D**), heptanal (**E**), octanal (**F**), nonanal (**G**), decanal (**H**), undecanal (**I**), dodecanal (**J**), and tetradecanal (**K**) from poplar leaves after mechanical damage. Aldehyde emissions clearly increased after treatment, exhibiting regular pattern. Two emission peaks are shown for aldehydes. Each point is average of at least three independent repeats. Statistical significance [least significant difference (LSD) test] of differences between before- and after-damage treatments is indicated by '*' ($p < 0.05$) and '**' ($p < 0.01$). Standard errors are shown.

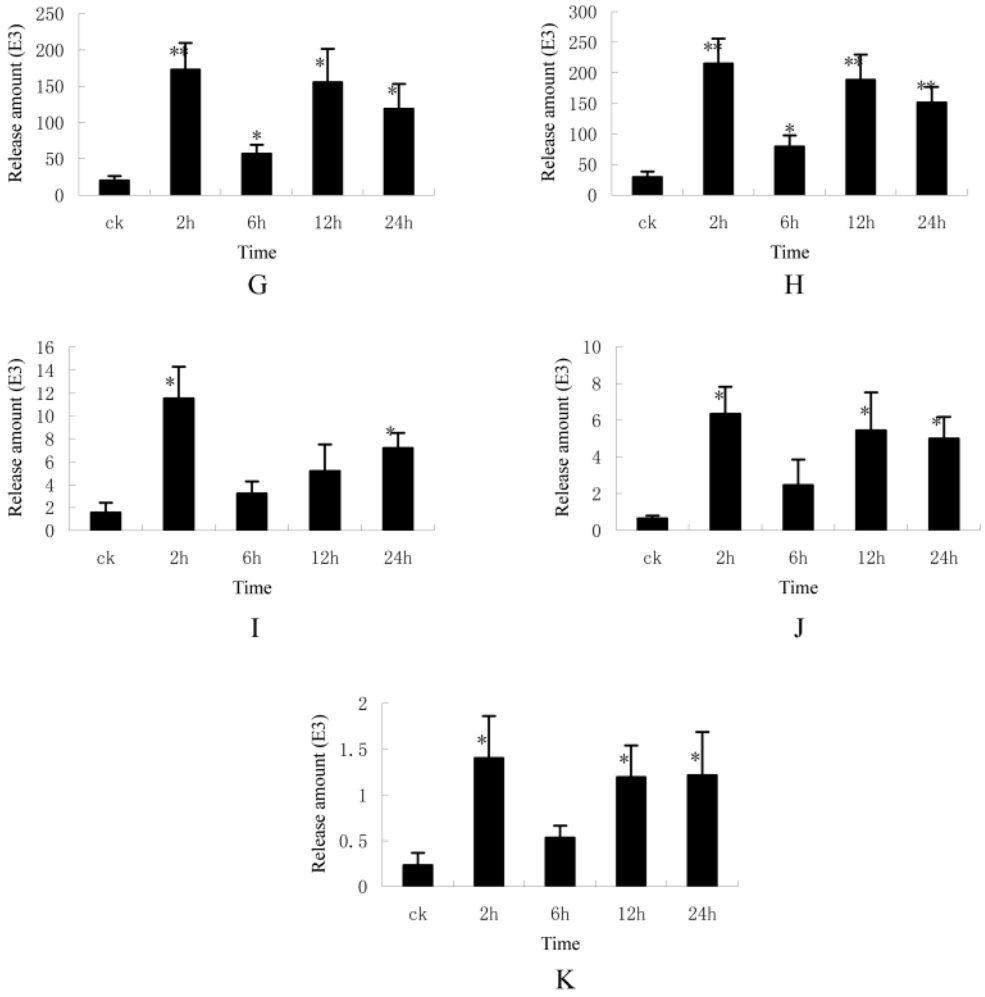

Figure 2. Continued.

change resembling that of the linear saturated aldehydes (see Fig. 2). Except for (*Z*)-3-hexenal, whose highest peak of release occurred at 24 h, the released amounts of (*E*)-2-hexenal and (*E*)-2-nonenal reached their maximums at 12 h. At 24 h, the released amount of (*Z*)-3-hexenal was about 20-fold higher than the control, while the level of (*E*)-2-nonenal at 24 h decreased by only 0.3 times the amount at 12 h. Only a slight drop in (*E*)-2-hexenal was observed at 24 h. All values were under 10 E3. Comparatively, the released amount of (*E*)-2-nonenal was higher than that of the other two aldehydes.

We also detected two nonlinear aldehydes -- benzaldehyde and furfural – whose patterns of change in emissions varied from those of the linear aldehydes (Fig. 4). For example, released amounts of benzaldehyde increased considerably at 2 h after treatment, then rapidly decreased at 6 h. Although these amounts rose again at 12 h and 24 h, they did not differ significantly from the control. The increase in the amount of released furfural was 8-fold greater than that of the control at 2 h, but did not reach a peak. Following a reduction by half at 6 h and 12 h, a sudden 20-fold increase occurred at 24 h. The amount of benzaldehyde was far higher than that of furfural, with a peak value for the former exceeding 40 E3 versus 6 E3 for the latter.

DISCUSSION

When plants undergo various stresses, e.g., herbivore wounding, pathogen infection, or mechanical damage, a range of defense responses are activated, including the emissions of VOCs (Baldwin and Schultz, 1983; Kesselmeier and Staudt, 1999; Dicke and Bruin, 2001; Kessler and Baldwin, 2001, 2002). These volatiles play a central role in the relationship between plants and herbivorous insects, as well as between insects and their natural enemies (Kesselmeier and Staudt, 1999; Karban et al., 2003; Engelberth et al., 2004). For example, these volatiles function as reliable and precise signals to distinguish the diverse insects attacking plants from parasites and natural enemies (Kessler and Baldwin, 2001, 2002). Likewise, these volatiles can be utilized as cues by insects to choose host plants. Wounding-induced volatiles can serve as signalling molecules between plants, sending alarms to neighbouring intact plants in order to prime them against herbivore attacks (Farmer, 2001). Moreover, such volatiles are crucial airborne signals in ecological systems. Aldehydes are important components of plant volatiles, especially GLVs such as C6-aldehydes containing (*E*)-2-hexenal, (*Z*)-3-hexenal and n-hexanal. These can trigger the expression of defense genes, including CHS and AOS in

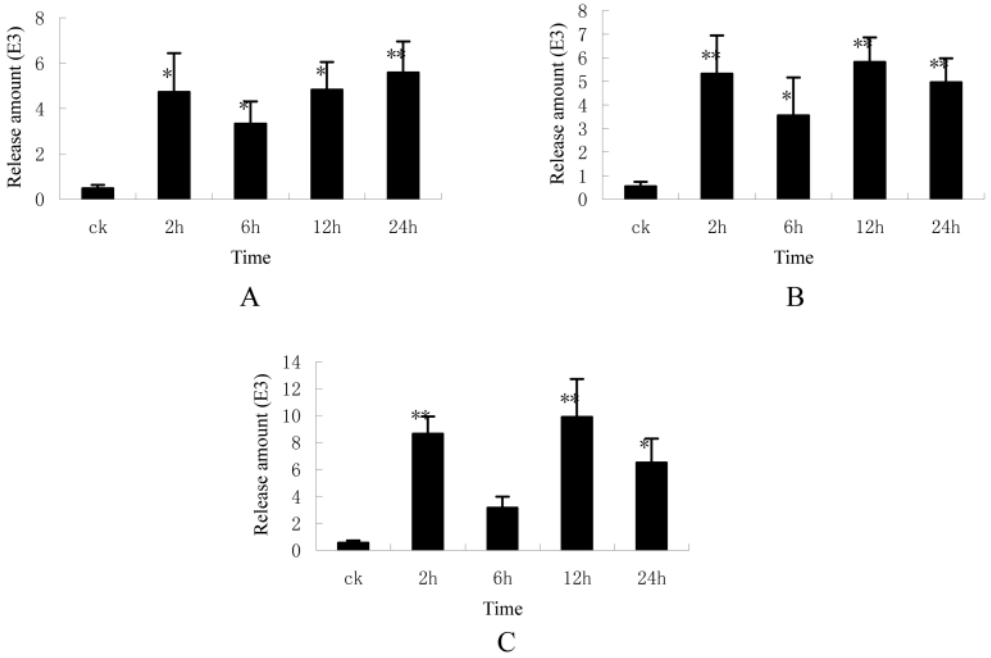

Figure 3. Amounts of (*Z*)-3-hexenal (**A**), (*E*)-2-hexenal (**B**), and (*E*)-2-nonenal (**C**) released from poplar leaves after mechanical damage. Aldehyde emissions clearly increased after treatment, exhibiting regular pattern. Two emission peaks are shown for aldehydes. Each point is average of at least three independent repeats. Statistical significance [least significant difference (LSD) test] of differences between before- and after-damage treatments is indicated by '*' ($p < 0.05$) and '**' ($p < 0.01$). Standard errors are shown.

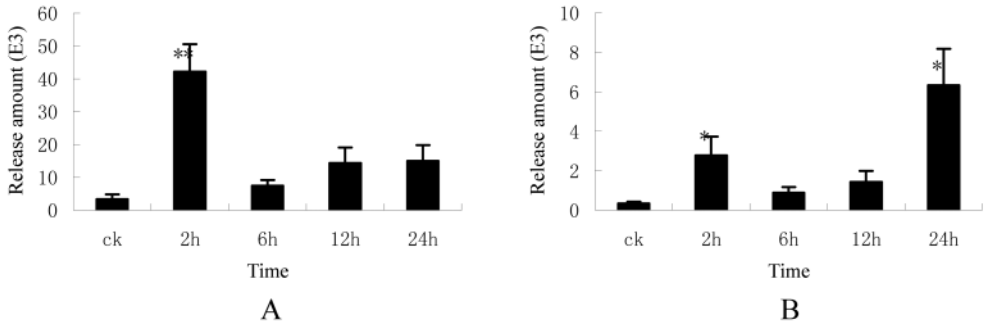

Figure 4. Amounts of benzaldehyde (**A**) and furfural (**B**) released from poplar leaves after mechanical damage. Aldehyde emissions clearly increased after treatment, exhibiting regular pattern. Two emission peaks are shown for aldehydes. Each point is average of at lease three independent repeats. Statistical significance [least significant difference (LSD) test] of differences between before- and after-damage treatments is indicated by '*' ($p < 0.05$) and '**' ($p < 0.01$). Standard errors are shown.

Arabidopsis (Bate and Rothstein, 1998), and LOX and PAL in lima beans (Arimura et al., 2001).

The 16 aldehydes detected here included 11 saturated linear aldehydes (e.g., acetaldehyde and butanal), 3 unsaturated linear aldehydes [e.g., (*E*)-2-hexenal and (*E*)-2-nonenal], and two nonlinear aldehydes, benzaldehyde and furfural. Although the amounts of these aldehydes released by intact poplar cuttings were low, tissues that were mechanically wounded showed greatly enhanced emissions of volatiles that followed a regular pattern of release over time. Arimura et al. (2000) also have reported that emissions of GLVs, including hexenal and hexanal, are very low in healthy plants, but mechanical wounding, pathogen infections, and herbivore damage can induce their rapid synthesis. Moreover, the emissions of aldehydes C6 through C10, which are barely detectable from intact plants, are stimulated in those tissues exposed to ozone (Wildt et al., 2003).

These aldehydes in our study exhibited similar patterns after mechanical damage: i.e., a strong increase at 2 h, then a sudden drop in levels before another rise. For linear saturated aldehydes with fewer than 10 carbon atoms, the addition of other carbon atoms was associated with a gradual increase in amounts of all aldehydes except acetaldehyde and heptanal. In contrast, levels were extremely low for aldehydes with more than 10 carbon atoms. Three linear unsaturated aldehydes -- (*E*)-2-hexenal, (*Z*)-3-hexenal, and (*E*)-2-nonenal -- also released only small amounts.

We observed two peaks, at 12 h and 24 h, suggesting that two pathways might mediate these aldehyde emissions. The first might be the result of diffusion from special storage pools or cell vacuoles in plants. Terpenes are the main components of volatiles emitted by coniferous plants; monoterpenes, released from storage pools, exit the leaf together with the transpiration stream, which is purely physical in ori-

gin (Tingey et al., 1991). In our study, when poplar leaves were damaged, their cell vacuoles were broken and the stored aldehydes emitted. In contrast, plants might actively regulate storage pools to enhance aldehyde emissions. Given that gene expression does not need to be synthesized *de novo*, these aldehydes may have been emitted quite early from the damaged leaves, so that a significant increase in the amount released occurred after 2 h. However, because the amounts stored would be limited, release rates would gradually have declined following maximum emissions after 2 h.

The second release peak might have resulted from the synthesis pathway for aldehyde activation. After mechanical damage to the tissue, that pathway could have been elicited via signal transduction to produce aldehydes. In the initial stage after wounding, only a small amount of aldehydes was synthesized and emitted due to incomplete expression of the aldehyde-synthesis pathways. Concurrently, the release pattern of aldehydes would have been attributed to emissions from the storage pools. Gradually, the synthesis pathway was completely activated and the genes fully expressed, resulting in maximum synthesized amounts, such that another release peak appeared at 12 h and 24 h. For linear aldehydes, the amounts produced *de novo* were limited to the amounts in storage pools and, except for acetaldehyde and butanal (whose second release peak occurred at 24 h), other aldehydes already exhibited significant released amounts at 12 h. The level of a wound-induced nonlinear aldehyde or benzaldehyde only increased appreciably again at 12 h and 24 h, indicating that the synthesis pathway had not been completely activated. This, in turn, suggests that emission from storage pools was the main pathway within 24 h after treatment. In contrast, the amount of furfural synthesized *de novo* by a synthesis pathway was more than the emission from storage pools. This implies that diverse pathways mediate the synthesis of different aldehydes.

Aldehydes C6 and C9, including (*E*)-2-hexenal, (*Z*)-3-hexenal and hexanal, can be synthesized through a LOX/HPL pathway (Matsui et al., 2000). When plants are exposed to herbivore wounding, many aldehydes, as the byproducts of membrane lipid peroxidation, can be synthesized through this LOX pathway or by reactive oxygen species and, thus, possess potent electrophilic activity (Thoma et al., 2003). Percentage wise, aldehydes C6 through C9 may be relatively abundant in the volatiles emitted from cotton leaves, being decay products of unsaturated fatty acids (Zeringue and McCormick, 1990). Aldehyde synthesis is thought to be related to the oxidation process caused by ROS (e.g., O_2^- and H_2O_2) and O_3 (Wildt et al., 2003). However, the mechanisms for synthesizing other aldehydes are largely unknown. Thus, they require further study.

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