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# Conversion of volatile alcohols into their glucosides in Arabidopsis

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Exposure of tomato plants to volatile chemicals emitted from common cutworm (*Spodoptera litura*)-infested conspecifics led to accumulation of the glycoside, (*Z*)-3-hexenyl vicianoside. Accumulation of (*Z*)-3-hexenyl vicianoside in the exposed plants has adverse impacts on the performance of the common cutworms. The aglycon of (*Z*)-3-hexenyl vicianoside is derived from airborne (*Z*)-3-hexenol emitted from infested plants. The ability to incorporate and convert (*Z*)-3-hexenol to its corresponding glycoside is widely conserved in an array of plant species. However, the specificity of this ability to discriminate between the chemical structures of different volatile alcohols remains unknown. In this study, we investigated glycosylation of several volatile alcohols in Arabidopsis (*Arabidopsis thaliana*). The exposure of Arabidopsis to a variety of volatile alcohols, (*Z*)-2-pentenol, (*Z*)-3-hexenol, (*Z*)-3-heptenol, (*Z*)-3-octenol, (*Z*)-3-nonenol, cyclohexanol, benzyl alcohol, verbenol, perillyl alcohol, myrtenol, geraniol, or linalool led to the detection of the putative corresponding glucosides. These results suggest that Arabidopsis might convert a broad range of volatile alcohols into the corresponding glucosides.

## Introduction

Plants emit a variety of volatile compounds in response to being attacked by herbivores, and the results of numerous studies have shown that these compounds can work as direct and/or indirect defense weapons against herbivores.[1] For example, the suppression of hydroperoxide lyase, which is responsible for the biosynthesis of green leaf volatiles consisting of 6-carbon-based compounds, led to an increase in the performance of aphids on potato leaves.[2] This result suggested that the products resulting from the activity of this enzyme, such as green leaf volatiles, made a direct contribution to the defense of the plant against aphids. Regarding indirect defense, the emission of herbivore-induced plant volatiles resulted in the recruitment of carnivorous arthropods that preyed upon the herbivores. When the recruitment results in reduction of the damage caused by herbivores, the carnivores are called "bodyguards" of the plants. This type of indirect defense has been reported in a broad range of plant taxa.[1]

A growing number of studies have shown that the volatile compounds emitted from infested plants can also mediate plant-plant communication, with the neighboring uninfested plants becoming more defensive against subsequently coming herbivores.[1] For example, the exposure of intact lima bean plants to the volatiles emitted from neighboring conspecific plants infested with two-spotted spider mites (*Tetranychus urticae*) made these plants become more defensive toward the spider mites.[3] Such responses in exposed plants have also been reported for many plant species, including maize.[4] Among the mechanism of defensive trait in exposed plants, transcriptional changes in the defensive genes of maize and Arabidopsis plants have been observed when they are exposed to an authentic volatile compounds.[4-6] By contrast, there have been no reports concerning the mechanisms by which plants receive and recognize volatile compounds from other plants.

We recently reported that the exposure of uninfested tomato plants to the volatiles emitted from conspecific plants infested with common cutworms (*Spodoptera litura*) led to the accumulation of (*Z*)-3-hexenyl vicianoside, which works as a defense chemical against common cutworms.[7] The aglycon of (*Z*)-3-hexenyl vicianoside, (*Z*)-3-hexenol, is originated not from volatile-exposed tomato plants but from infested ones, and the glycosylation of airborne (*Z*)-3-hexenol have been observed in various plant species belonging to a range of different genera.[7] This result indicated that the conjugation of the hydroxyl group of (*Z*)-3-hexenol with a sugar unit represents one of the key reception mechanisms for the recognition of volatile compounds in plants.

The blend of volatile chemicals derived from common cutworm-infested tomato plants contains only one volatile alcohol, namely (*Z*)-3-hexenol, and this would explain why (*Z*)-3-hexenyl vicianoside was the only glycosylated compound detected in the plants exposed to this particular blend of volatiles.[7] Plant volatiles, however, may contain a variety of volatile alcohols, including aliphatic alcohols, terpene alcohols and aromatic alcohols, and it is not yet known whether plants can receive and convert a

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variety of volatile alcohols to the corresponding glycosides. To clarify this issue, we exposed Arabidopsis to a variety of different volatile alcohols and then analyzed whether the corresponding glycosides were produced.

## Results and Discussion

The exposure of Arabidopsis plants (i.e., ecotype Col-0) to authentic (*Z*)-3-hexenol, which is normally emitted from herbivore-infested plants,[8] led to the accumulation of (*Z*)-3-hexenyl glucoside (**Table 1**). Col-0 does not possess a functional genes for hydroperoxide lyase, which is required for (*Z*)-3-hexenol synthesis,[9] suggesting that the aglycon of (*Z*)-3-hexenyl glucoside was therefore synthesized from airborne (*Z*)-3-hexenol. This conversion of (*Z*)-3-hexenol to the corresponding glucoside is also consistent with our previous report involving another ecotype of Arabidopsis.[7]

We also investigated whether Arabidopsis could glucosylate volatile alcohols of various carbon chain lengths, such as (*Z*)-2-pentenol, (*Z*)-3-heptenol, (*Z*)-3-octenol, and (*Z*)-3-nonenol, following the exposure of the plants to these compounds. Compared to the control plants, the exposed plants accumulated significantly higher levels of compounds with molecular weights corresponding to the glucosides of these volatile alcohols (**Table 1** and **Fig. 1A-E**). This result indicated that Arabidopsis could be used to convert volatile alcohols of different carbon chain lengths to the corresponding glucosides.

Next we investigated the exposure of Arabidopsis to several other volatile alcohols, including the secondary alcohol cyclohexanol. Following its exposure to cyclohexanol, Arabidopsis accumulated a compound with a nominal mass that was consistent with the corresponding glucoside (**Table 1** and **Fig. 1F**). This result therefore suggested that Arabidopsis could glycosylate cyclic alcohols as well as straight chain alcohols. Furthermore, this result indicated that Arabidopsis could convert secondary alcohols to the corresponding glucosylated species. The exposure of Arabidopsis to aromatic and monoterpene alcohols was also investigated, and the plants exposed to benzyl alcohol accumulated significant levels of the putative benzyl glucoside (**Table 1**). Additionally the plants exposed to the monoterpene alcohols accumulated significant levels of the corresponding monoterpene glucosides (**Table 1**). It is noteworthy that the retention times of the compounds accumulated in the plants exposed to 5 different monoterpenes were different from each other (**Fig. 1H–L**). The accumulation of a putative linalyl glucoside suggested that Arabidopsis possesses the ability to convert tertiary alcohols to the corresponding glucoside.

Taken together, the results of this study suggest that Arabidopsis converts a wide variety of airborne volatile alcohols into the corresponding glucosides. Exposure to (*Z*)-3-hexenol induced defensive responses toward gray mold disease, probably via the induction of defense-related genes in Arabidopsis.[6] There is, however, a possibility that the glucosides of (*Z*)-3-hexenol worked as direct defensive compounds against the pathogen. Recently, increasing number of reports have identified the genes for glycosyltransferases acting on volatile compounds.[10–12] The enzymes converted the volatile compounds into glycosides that were thought to be accumulated as precursors of volatiles.[12–14] The function of glycosides as volatile precursors in tomato or kiwi fruits[12,14] implies that the glycoside in leaves of volatile-exposed plants might have a role not only as direct defensive compounds but also as a source of volatiles emitted immediately after herbivory to attract natural enemies of herbivores as an

**Table 1.** Relative amount of compounds accumulated by exposure of indicated volatile compounds

| Exposed volatiles | Molecular formula | Formula weight | Calculated nominal mass of its glucoside | Selected ions for glucoside analysis ([M+HCOO$^-$]) | Control (mean ± SE) | Exposed (mean ± SE) |
|---|---|---|---|---|---|---|
| | | | *Straight chain alcohols* | | | |
| (*Z*)-2-pentenol | $C_5H_{10}O$ | 86.13 | 248 | 293 | 0.004 ± 0.000 | 1.179 ± 0.050* |
| (*Z*)-3-hexenol | $C_6H_{12}O$ | 100.16 | 262 | 307 | 0.021 ± 0.004 | 0.673 ± 0.151* |
| (*Z*)-3-heptenol | $C_7H_{14}O$ | 114.10 | 276 | 321 | 0.007 ± 0.003 | 0.138 ± 0.023* |
| (*Z*)-3-octenol | $C_8H_{16}O$ | 128.21 | 290 | 335 | 0.007 ± 0.002 | 0.124 ± 0.018* |
| (*Z*)-3-nonenol | $C_9H_{18}O$ | 142.24 | 304 | 349 | 0.004 ± 0.001 | 0.235 ± 0.047* |
| | | | *Cyclic alcohol* | | | |
| cyclohexanol | $C_6H_{12}O$ | 100.16 | 262 | 307 | 0.005 ± 0.002 | 0.735 ± 0.139* |
| | | | *Aromatic alcohol* | | | |
| benzyl alcohol | $C_7H_7O$ | 108.19 | 270 | 315 | 0.007 ± 0.001 | 0.772 ± 0.161* |
| | | | *Straight chain monoterpene alcohols* | | | |
| linalool | $C_{10}H_{18}O$ | 154.25 | 316 | 361 | 0.007 ± 0.004 | 0.113 ± 0.020* |
| geraniol | $C_{10}H_{18}O$ | 154.25 | 316 | 361 | 0.001 ± 0.000 | 0.080 ± 0.019* |
| | | | *Cyclic monoterpene alcohols* | | | |
| verbenol | $C_{10}H_{16}O$ | 152.23 | 314 | 359 | 0.001 ± 0.000 | 0.834 ± 0.039* |
| perillyl alcohol | $C_{10}H_{16}O$ | 152.23 | 314 | 359 | 0.001 ± 0.000 | 0.480 ± 0.095* |
| myrtenol | $C_{10}H_{16}O$ | 152.23 | 314 | 359 | ND | 0.652 ± 0.111 |

Values are area ratios of target compounds to internal standard.
*: $P < 0.05$ (t-test)
ND: not detected

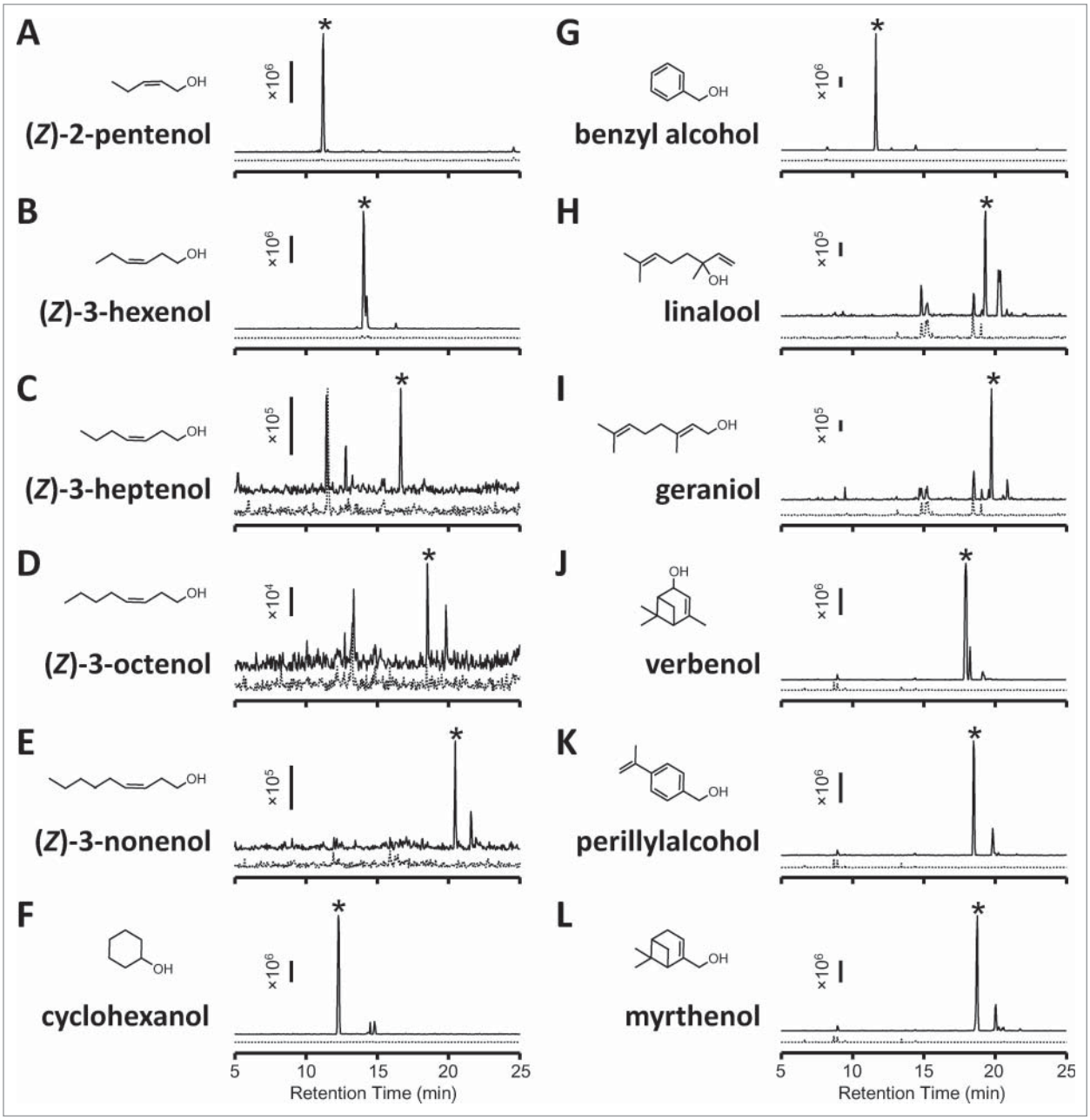


**Figure 1.** Chromatograms of the leaf extracts exposed to several volatile compounds. Extracts of Arabidopsis leaf tissues exposed to volatile compounds for 6 h were analyzed by LC-MS. The solid and dotted lines represent the volatile-exposed and mock-treated samples, respectively. Vertical bars indicate the signal intensity of mass spectrometry. Selected ions from each chromatogram are shown in **Table 1**.

indirect defense.[1] In future studies, the direct and indirect defensive functions of (*Z*)-3-hexenyl glucoside and other candidate glucosides detected in this study should be examined against various herbivores and pathogens.

## Materials and Methods

### Plant materials

*Arabidopsis thaliana* ecotype Col-0 seeds were sown on the surface of soil (Metro-Mix, Sun Gro Horticulture, Agawam, MA, USA), which had been premixed with an equal volume of vermiculite and placed in plastic pots 6 cm in diameter. After 2 d of cold acclimation at 4°C, the plants were transferred to a growth room and grown at 22°C under a photoperiod of 14 h light (60 μmol photons $m^{-2}$ $s^{-1}$) and 10 h darkness. At 5 weeks old, the plants were used for the volatile exposure and metabolite extraction experiments.

### Volatile exposure

The volatiles used in this study are listed in **Table 1**. All of the volatile compounds were dissolved in dichloromethane at a

concentration of 100 mM, and 10 μL of the volatile solutions were adsorbed on cotton swabs and individually enclosed in a large glass vessel (1000 $cm^3$) containing an Arabidopsis plant. Dichloromethane containing no volatile alcohols was used in the control experiments. The vessels were kept under growth conditions described above for 6 h to allow for effective exposure of the plant to the volatile compounds. (*Z*)-2-Pentenol and (*Z*)-3-heptenol were purchased from Alfa Aesar (Tokyo, Japan). (*Z*)-3-Hexenol, cyclohexane, benzyl alcohol, and linalool were purchased from Wako Pure Chemical (Osaka, Japan). (*Z*)-3-Octenol, (*Z*)-3-nonenol, and geraniol were purchased from TCI (Tokyo, Japan). Verbenol, perillyl alcohol, and myrtenol were kindly provided by Nippon Terpene Chemicals (Kobe, Japan).

### Analysis of the glucosides

The leaf tissues were ground with liquid nitrogen to a fine powder, and a small portion of this powder (ca. 100 mg) was extracted with methanol containing 1 μg $mL^{-1}$ of formononetin as an internal standard. Three microliters of the resulting extract were analyzed by UFLC-MS using a Shimadzu UFLC system (Shimadzu, Kyoto, Japan) equipped with a 3200 Q TRAP mass spectrometer (AB SCIEX, Foster City, CA, USA). The metabolites were separated on a Mightysil RP-18 column (5 μm, 2 × 150 mm, Kanto Chemical, Tokyo, Japan) at 40°C using water (A) and acetonitrile (B) containing 0.1% formic acid as the mobile phases. The LC conditions were as follows: 20% B for 10 min, 20–50% B from 10 to 20 min, 50–95% B from 20 to 40 min, and 95% B from 40 to 45 min, with a flow rate of 0.2 mL $min^{-1}$. The separated compounds were directly analyzed by electrospray ionization on the mass spectrometer in the negative ion mode with scanning in the *m/z* range of 150–750. The relative contents of the target compounds were calculated using the integrated peak areas of the formic acid adducts listed in **Table 1**, which were normalized relative to the peak area of the internal standard.

### Statistics

Three to 9 independent experiments were performed for the volatile exposure studies. All of the data were statistically analyzed by the t-test using R-software (version 3.0.1).


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