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Green leaf volatiles: airborne signals that protect against biotic and abiotic stresses

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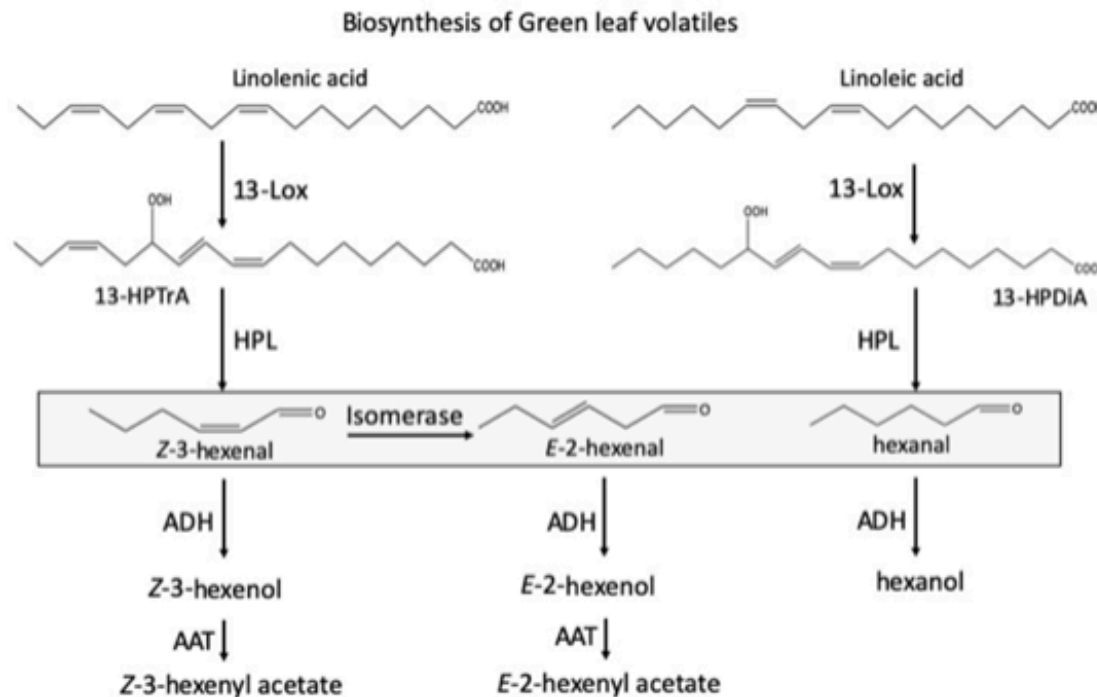
Abstract: Green leaf volatiles (GLVs) are rapidly released by plant leaves upon damage. This makes them ideal signals to convey the presence of a damaging threat to other parts of the same plant, but also to plants nearby. There, GLVs were first found to activate defense responses against insect herbivores and necrotrophic pathogens. Aside from providing direct protection, GLVs also prime those responses resulting in an enhanced and/or accelerated response to these biotic stressors. Recently, it was shown that GLVs also protect against cold stress in plants, resulting in stress-specific transcript accumulation and subsequent reduced damage. This response was further associated with a stimulation of growth after the stress subsided. Common to all those stresses is that they can also cause the release of these compounds. However, the quantities and qualities of the emitted GLVs can vary significantly even in closely related species, suggesting that eco-physiological factors related to biotic and abiotic stresses rather than systematic relationships may have been the driving force for the highly variable emission of these compounds. Still, too little is known about the regulation of GLV emissions, signaling, and responses to support this hypothesis. In this presentation we will provide an overview of current knowledge regarding biosynthesis and signaling of GLVs in plants and will give an outlook into future areas of research that may provide essential information about the complex biological activities of these compounds.

Keywords: green leaf volatiles; biotic stress; abiotic stress, protection

The Biological Activity of Green Leaf Volatiles

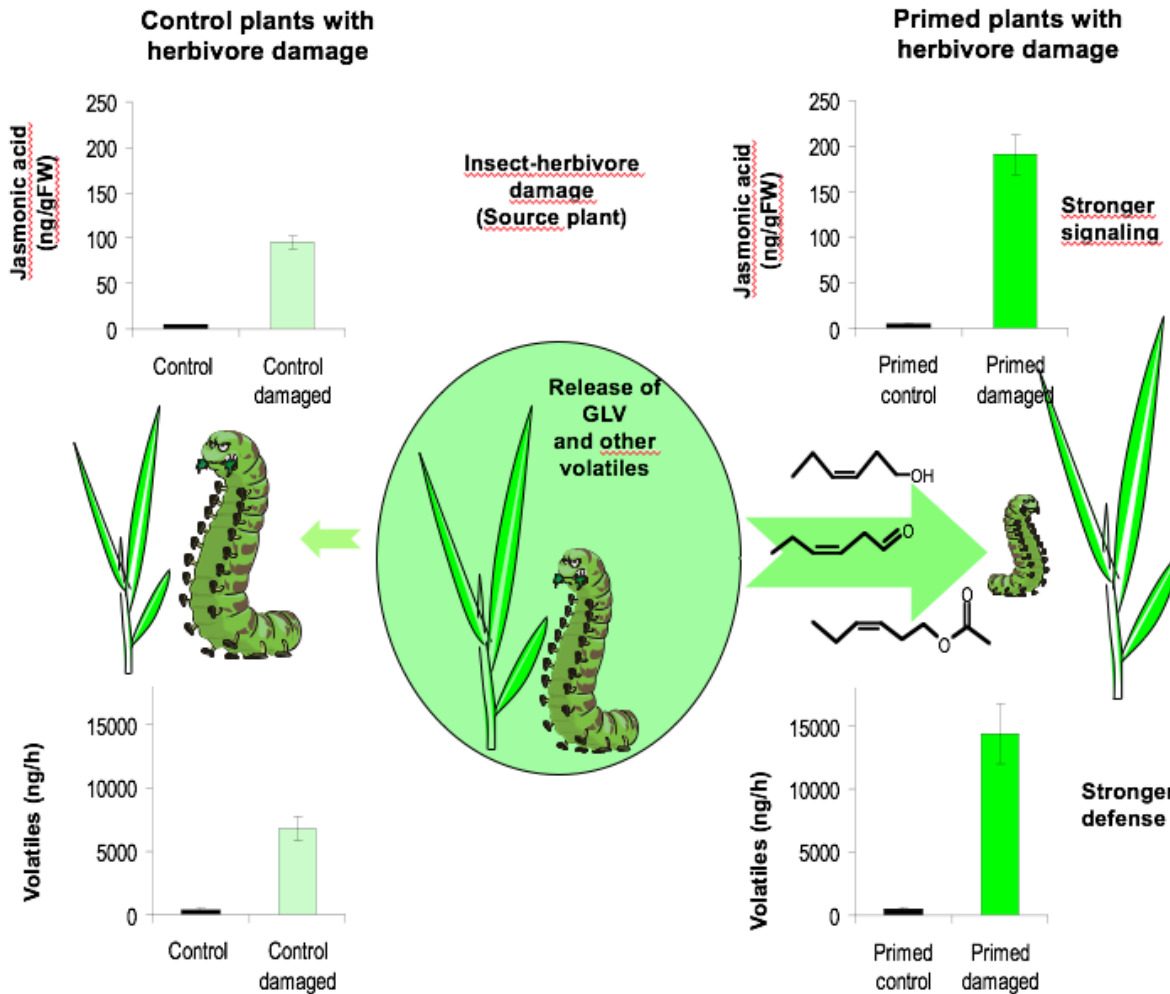
Biosynthesis

Figure 1. Biosynthetic pathways leading to green leaf volatile production in plants. A 13-lipoxygenase (13-LOX) catalyzes the addition of molecular oxygen at position 13 in linolenic acid and linoleic acid to form 13-hydroperoxy octadecatrienoic and 13-hydroperoxy octadecadienoic acid (13-HPTrA and 13-HPDiA). These oxygenated fatty acids are then converted to (*Z*)-3-hexenal and hexanal, as well as 9-(*Z*)-traumatol, by hydroperoxide lyase (HPL). An isomerase converts (*Z*)-3-hexenal into (*E*)-2-hexenal. (*Z*)-3-Hexenal and (*E*)-2-hexenal can be reduced to (*Z*)-3-hexenol and (*E*)-2-hexenol, respectively, by alcohol dehydrogenase(s) (ADH). (*Z*)-3-Hexenol and (*E*)-2-hexenyl acetate can further be converted to (*Z*)-3-hexenyl acetate and (*E*)-2-hexenyl acetate by alcohol acyltransferase(s) (AAT). Boxed compounds are those produced mainly by damaged plant tissue.



The Biological Activity of Green Leaf Volatiles

Insect Herbivory



Corn seedlings that were previously exposed to green leaf volatiles (GLV, right) responded stronger and faster to subsequent treatment with insect elicitors when compared to controls (left).

Airborne signals prime plants against insect herbivore attack

Juergen Engelberth*, Hans T. Alborn, Eric A. Schmelz, and James H. Tumlinson**

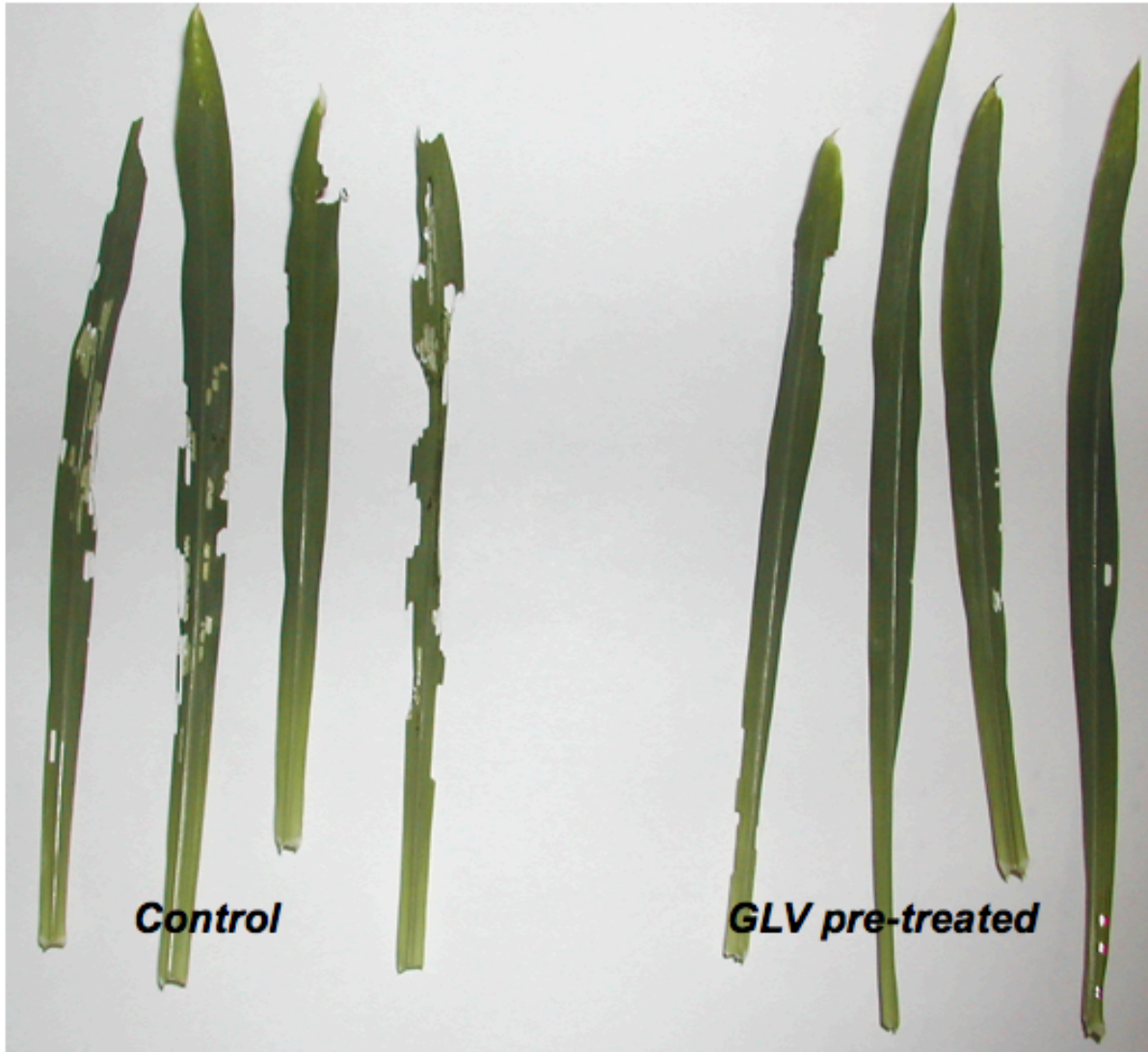
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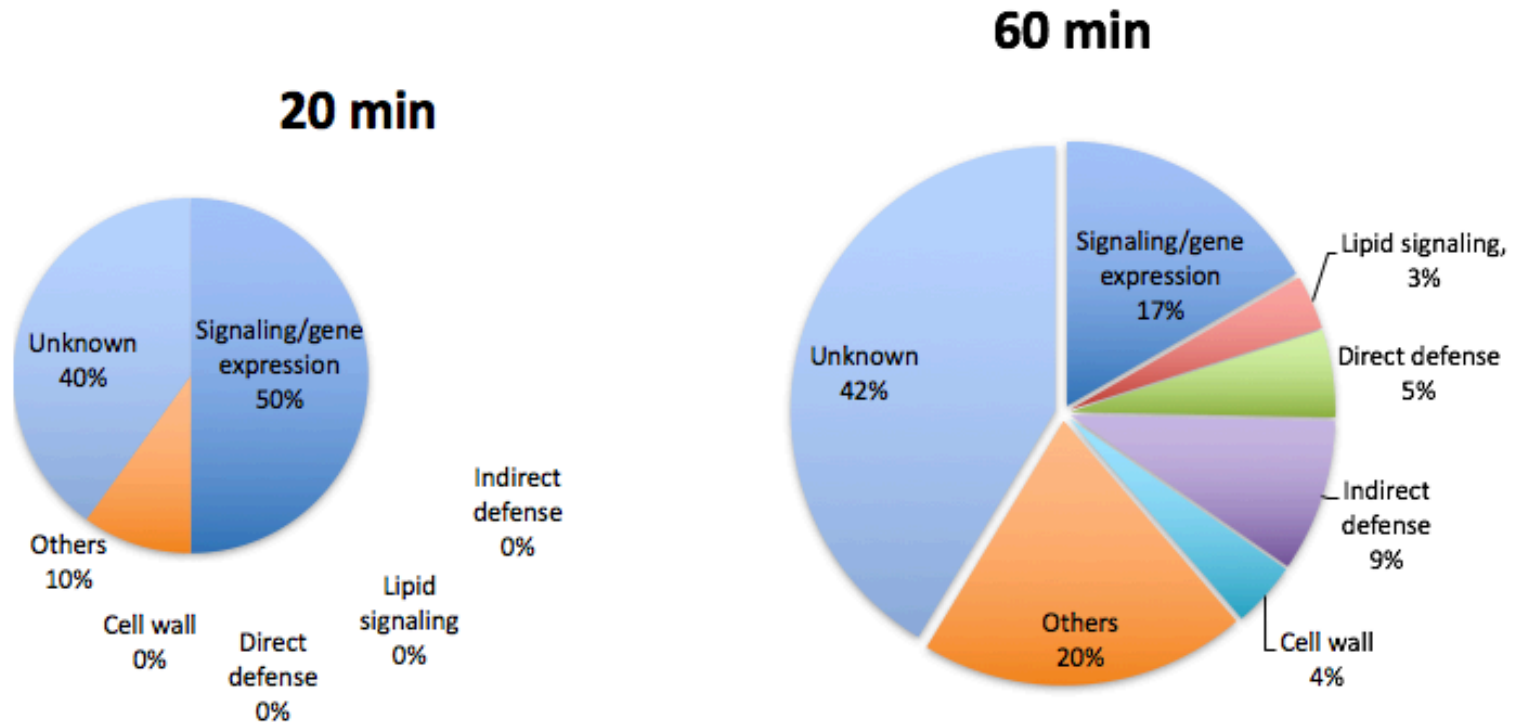
Insect Herbivory



Corn seedlings that were exposed to GLV show faster and stronger response to insect herbivore attack. Consequently GLV exposed pretreated plants show sustain less damage compared to control plants when challenged with an insect herbivore.



The Biological Activity of Green Leaf Volatiles Insect Herbivory



Early transcriptional analysis revealed distinct transcriptional networks in corn after exposure to GLV.

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The Biological Activity of Green Leaf Volatiles

Cold Stress

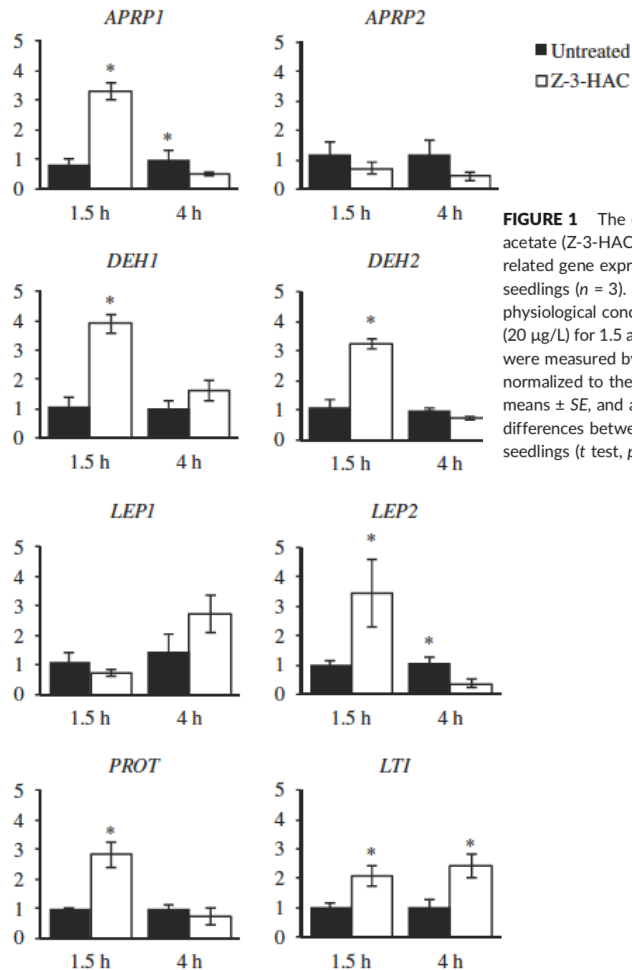


FIGURE 1 The effect of (Z)-3-hexen-1-yl acetate (Z-3-HAC) treatment on cold stress-related gene expression in maize (*Zea mays*) seedlings ($n = 3$). Seedlings were treated with physiological concentrations of Z-3-HAC (20 $\mu\text{g/L}$) for 1.5 and 4 hr, and transcript levels were measured by RT-qPCR. Data are normalized to the controls. Values are means \pm SE, and asterisks indicate significant differences between treated and untreated seedlings (t test, $p < .05$)

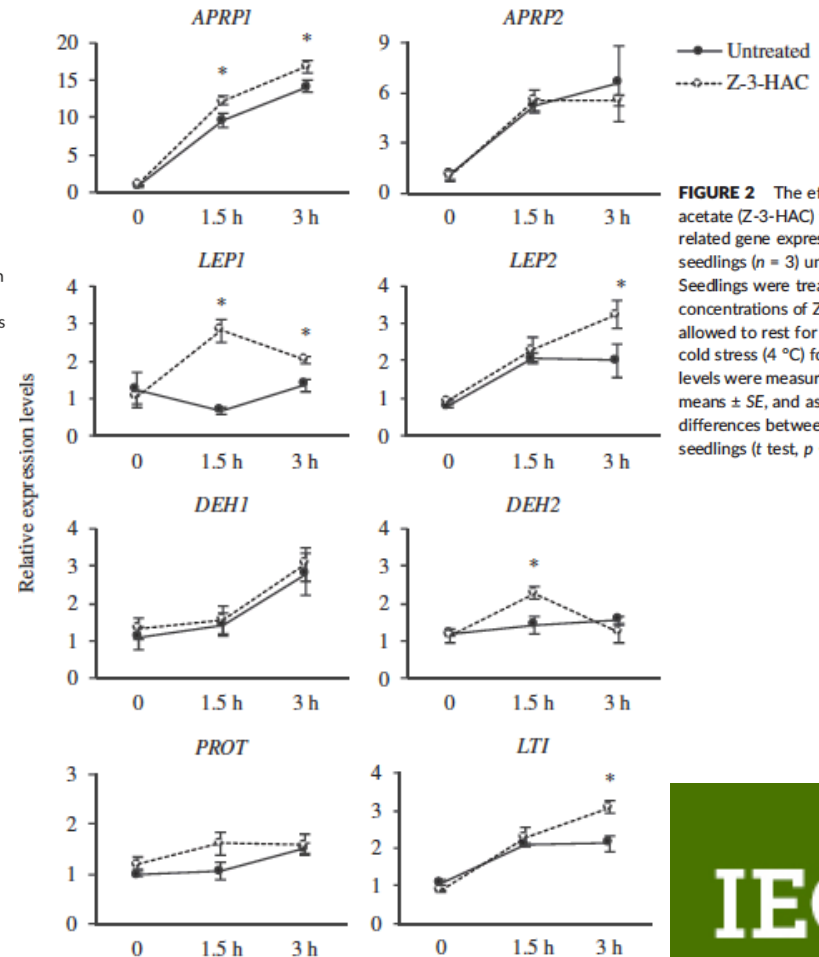


FIGURE 2 The effect of (Z)-3-hexen-1-yl acetate (Z-3-HAC) treatment on cold stress-related gene expression in maize (*Zea mays*) seedlings ($n = 3$) under cold stress (4 °C). Seedlings were treated with physiological concentrations of Z-3-HAC (20 $\mu\text{g/L}$) for 1 hr, allowed to rest for 3 hr, and then exposed to cold stress (4 °C) for 1.5 and 3 hr. Transcript levels were measured by RT-qPCR. Values are means \pm SE, and asterisks indicate significant differences between treated and untreated seedlings (t test, $p < .05$)

Relative expression levels

Relative expression levels

The Biological Activity of Green Leaf Volatiles

Cold Stress

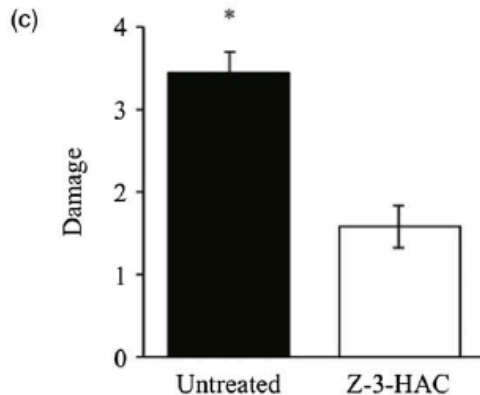
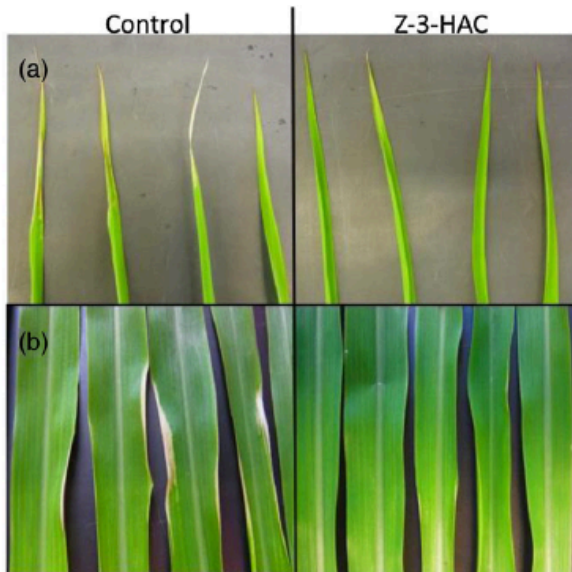


FIGURE 3 The effect of (Z)-3-hexen-1-yl acetate (Z-3-HAC) treatment on leaf damage in maize (*Zea mays*) seedlings after 16 hr at 4 °C. Typical (a) leaf tip and (b) leaf blade damage after 15 hr at 4 °C was strongly reduced in Z-3-HAC treated plants relative to untreated controls. (c) Assessment of damage on a scale from 1 (no damage) to 4 (heavy damage) by visual inspection of at least eight individual seedlings per treatment. Values are means ± SE, and asterisks indicate significant differences between treated and untreated seedlings (Wilcoxon rank sum test, $p < .05$)

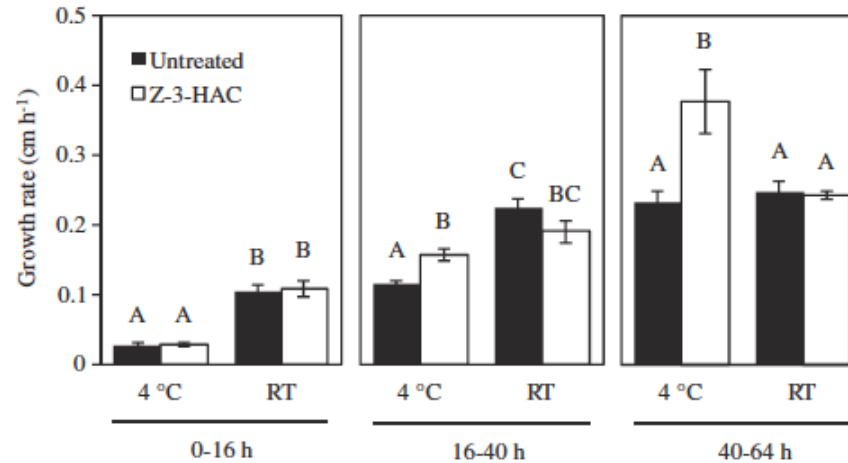


FIGURE 4 The growth response of (Z)-3-hexen-1-yl acetate treated (Z-3-HAC) and untreated maize (*Zea mays*) seedlings ($n = 4$) after 16 hr at 4 °C and at room temperature (RT). Growth rate is shown for the indicated time unit and is calculate as cm/hr. Values are means ± SE, and different letters indicate significant differences (Kruskal-Wallis, Steel-Dwass test, $p < .05$)

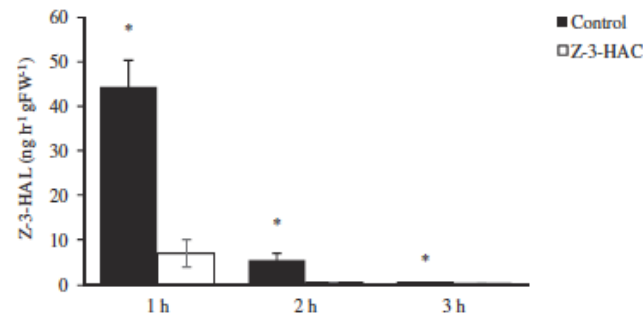


FIGURE 5 Damage assessment through quantification of (Z)-3-hexen-1-yl (Z-3-HAL) emissions from (Z)-3-hexen-1-yl acetate (Z-3-HAC) treated and untreated maize (*Zea mays*) seedlings ($n = 4$) after 3 min at -15 °C. Values are means ± SE, and asterisks indicate significant differences between treated and untreated seedlings (t test, $p < .05$)

The Biological Activity of Green Leaf Volatiles

Cold Stress

Figure 1. Release of green leaf volatiles from cold stress damaged maize (*Zea mays*) seedlings. Maize seedlings were either placed at $-5\text{ }^{\circ}\text{C}$ or at room temperature (RT), and volatiles were collected for 1 h ($n = 4$). Values are means \pm standard deviation (SD), and asterisks indicate significant differences between treated and untreated seedlings (t -test, $p < 0.05$; n.d., not detectable).

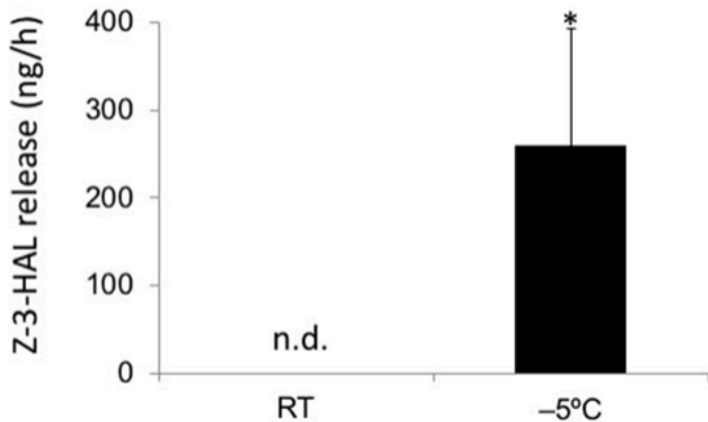
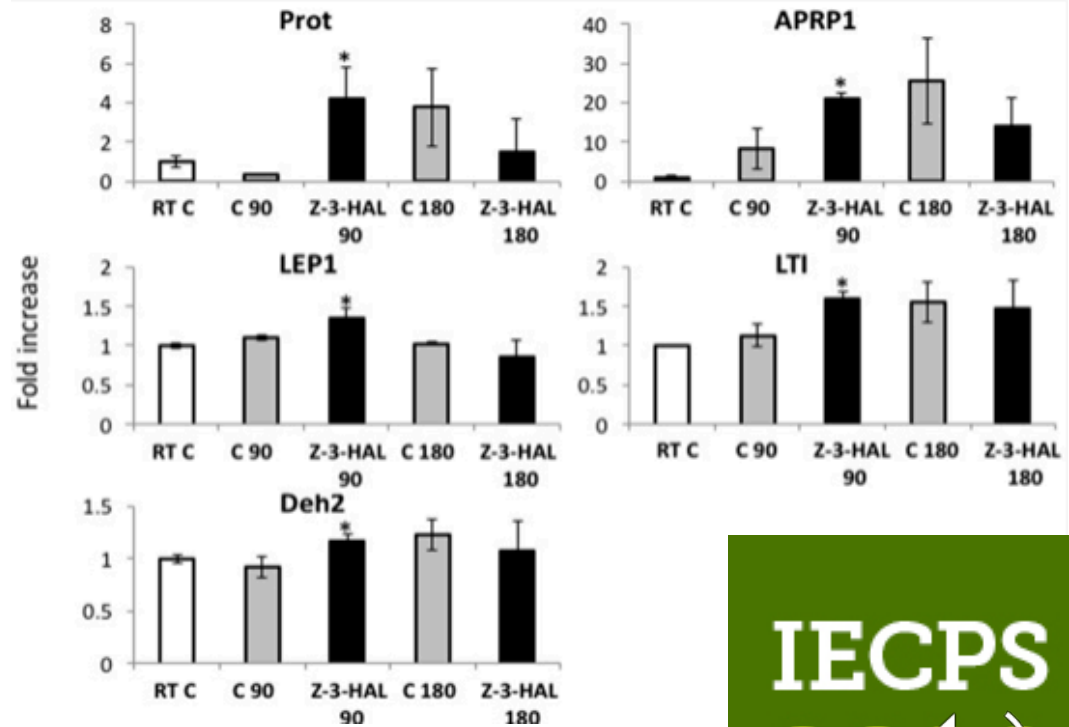


Figure 2. The effect of in-cold (Z)-3-hexen-1-al (Z-3-HAL) treatment on cold stress related gene expression in maize (*Zea mays*) seedlings ($n = 3$) under cold stress ($5\text{ }^{\circ}\text{C}$). Seedlings were treated with physiological concentrations of Z-3-HAL ($20\text{ }\mu\text{g L}^{-1}$) 30 min after being placed at $5\text{ }^{\circ}\text{C}$ for 90 min and 180 min. Transcript levels were measured by RT-qPCR. The values are means \pm standard error of the mean (SEM), and the asterisks indicate significant differences between the treated and untreated seedlings (t -test, $p < 0.05$).



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The Biological Activity of Green Leaf Volatiles Cold Stress

Figure 3. The effect of in-cold (Z)-3-hexen-1-al (Z-3-HAL) treatment on cold stress related ion leakage in maize (*Zea mays*) seedlings ($n = 6$). The relative conductivity is expressed as the ratio between ion leakage after short cold stress, divided by the total ion leakage after overnight freezing and thawing. The values are means \pm SD, and different letters indicate significant differences between the treated and untreated seedlings (ANOVA, $p < 0.05$).

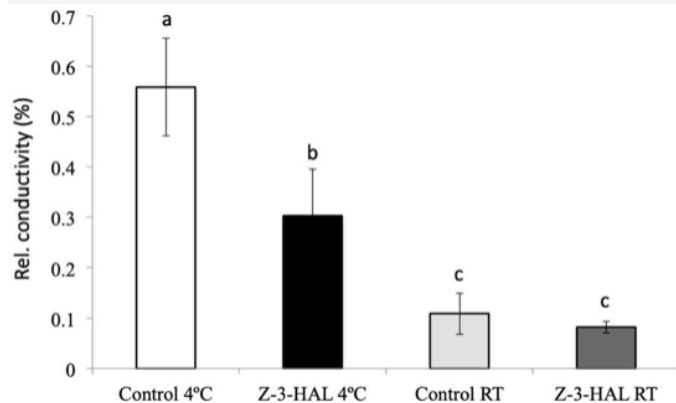


Figure 4. The effect of in-cold (Z)-3-hexen-1-al (Z-3-HAL) treatment on damage in maize (*Zea mays*) seedlings. Assessment of damage on a scale from 0 (no damage) to 4 (dead). The values are means \pm SD, and the asterisks indicate significant differences between the treated and untreated seedlings (Students t -test, $p < 0.05$).

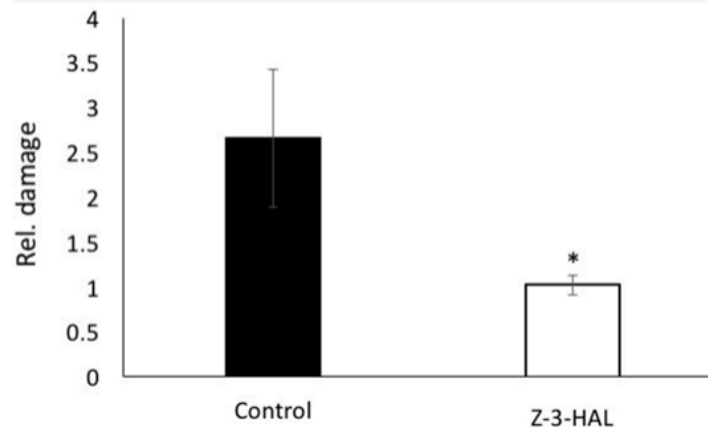
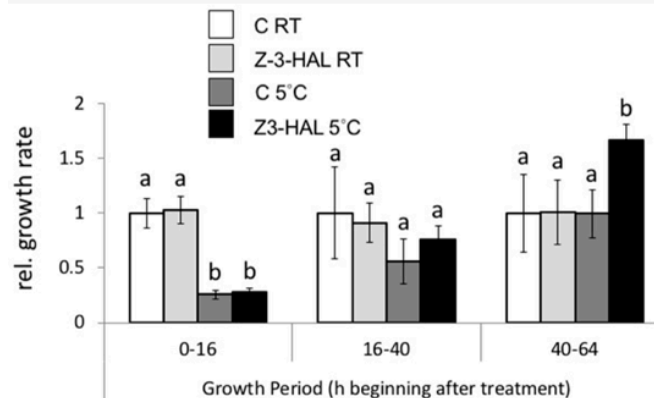


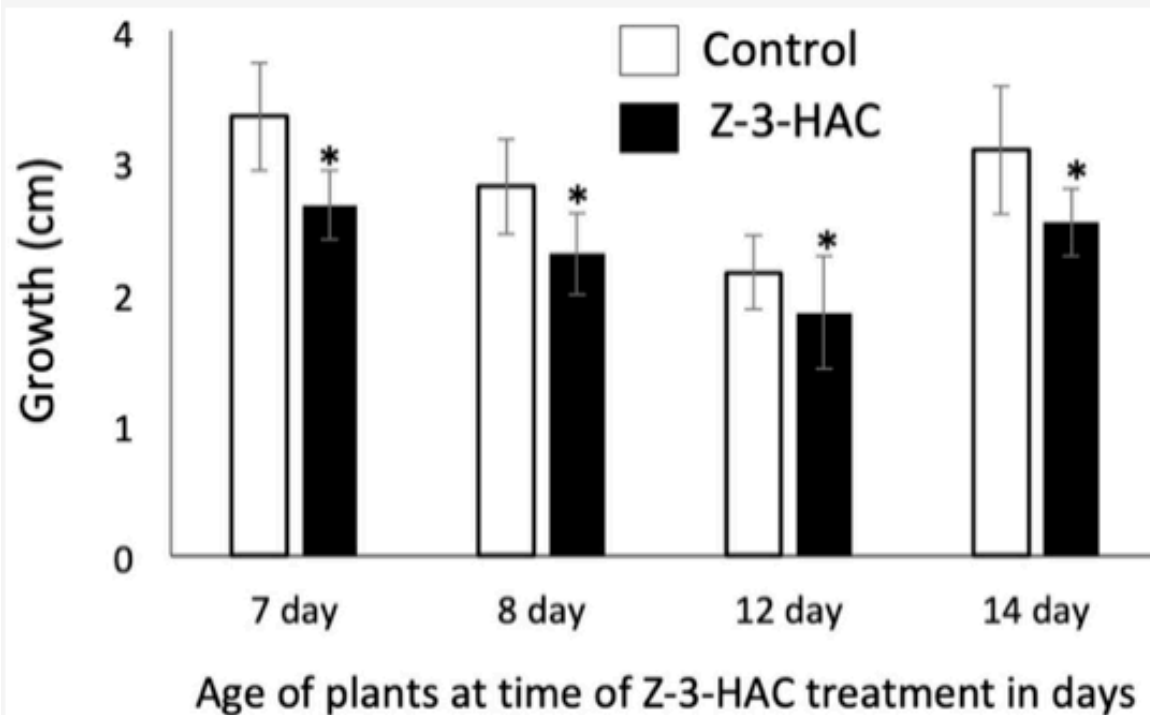
Figure 5. The growth response of in-cold (Z)-3-hexen-1-al (Z-3-HAL) and untreated maize (*Zea mays*) seedlings ($n = 6$) after 16 h at 5 °C and at room temperature (RT). Growth is expressed in relative units, with the RT control plants set at 100% for the observed growth period. The values are means \pm SD, and the different letters indicate significant differences (ANOVA, $p < 0.05$).



The Biological Activity of Green Leaf Volatiles

The Costs of Priming/Protection

Figure 1. The effect of Z-3-hexenyl acetate (Z-3-HAC) exposure on growth in maize (*Zea mays*) seedlings ($n \geq 15$) at different ages/developmental stages. Seedlings were 7-, 8-, 12- and 14-days old when treated with physiological concentrations of Z-3-HAC ($20 \mu\text{g L}^{-1}$) overnight (15 h). The growth of the actively growing 2nd, 3rd, 4th and 5th leaf was measured. The values are means \pm SE and the asterisks indicate significant differences between control- and Z-3-HAC-treated seedlings (t -test, $P < 0.05$).



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The Biological Activity of Green Leaf Volatiles

The Costs of Priming/Protection

Figure 2. The effects of priming by (Z)-3-hexen-1-yl acetate (Z-3-HAC) on mechanical wounding (MW) and insect elicitor (IE) induced growth responses in maize (*Zea mays*) seedlings (expressed as growth rate in $\text{cm} \cdot \text{h}^{-1}$, $n \geq 7$). Responses in control maize plants at different ages are shown in the left column and responses of Z-3-HAC-treated plants are shown in the right column. Maize seedlings were first exposed to physiological concentrations of Z-3-HAC ($2 \mu\text{g L}^{-1}$ air volume) for 15 h before being an actively growing leaf was treated with MW or IE (in-leaf treatment). The growth of the treated leaf was measured for 3 days and is expressed as growth rate ($\text{cm} \cdot \text{h}^{-1}$). Values are means \pm SE and different letters indicate significant differences between the different treatments (ANOVA, $P < 0.05$). Circled time points indicate no significant differences between treatments on day 2. Day 1 and Day 3 growth rates show no significant differences between treatments, with only 13-day-old Z-3-HAC-treated seedlings showing different growth rates on day 3 (circled).

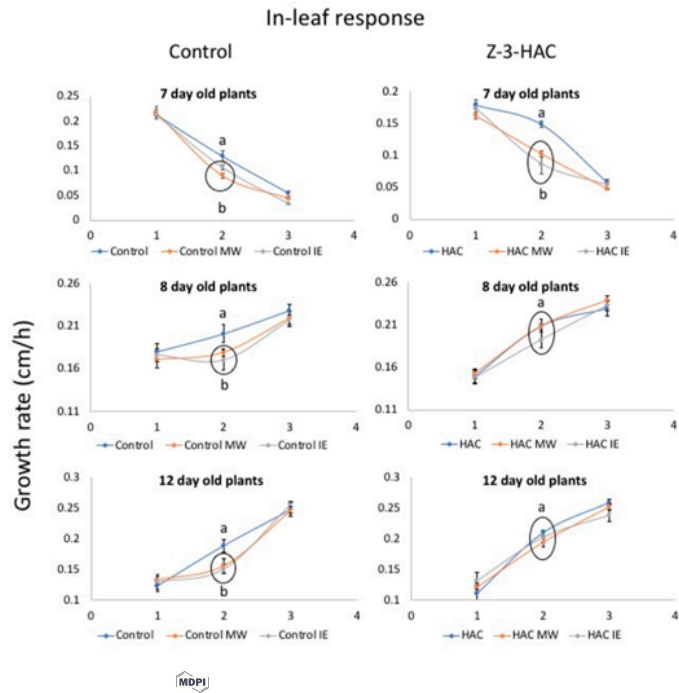
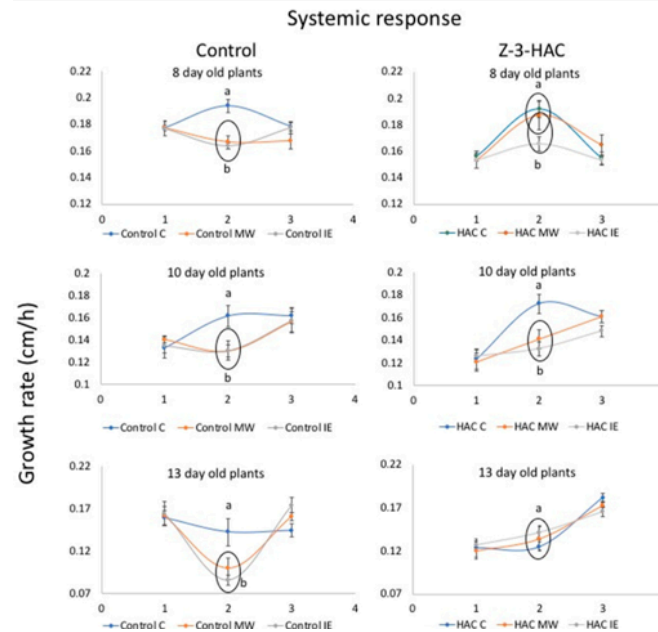


Figure 3. The effects of priming by (Z)-3-hexen-1-yl acetate (Z-3-HAC) on mechanical wounding (MW) and insect elicitor (IE) induced growth responses in systemic leaves of maize (*Zea mays*) seedlings (expressed as growth rate in $\text{cm} \cdot \text{h}^{-1}$, $n \geq 7$). Responses in control maize plants at different ages are shown in the left column and responses of Z-3-HAC-treated plants are shown in the right column. Maize seedlings were first exposed to physiological concentrations of Z-3-HAC ($2 \mu\text{g L}^{-1}$ air volume) for 15 h before the MW or IE treatment was applied to an actively growing leaf (7- and 12-day-old seedlings) or a fully developed leaf (8- and 14-day-old seedlings). The growth of the next (systemic) leaf was measured for 3 days and is expressed as growth rate ($\text{cm} \cdot \text{h}^{-1}$). Values are means \pm SE and different letters indicate significant differences between the different treatments (ANOVA, $P < 0.05$). Circled time points indicate no significant differences between treatments. Day 1 and Day 3 growth rates show no significant differences between treatments, with only 13-day-old Z-3-HAC-treated seedlings showing different growth rates on day 3.



The Biological Activity of Green Leaf Volatiles

The ecological relevance



Article
Variability in the Capacity to Produce Damage-Induced Aldehyde Green Leaf Volatiles among Different Plant Species Provides Novel Insights into Biosynthetic Diversity

Jürgen Engelberth and Marie Engelberth

Figure 2. Damage-induced biosynthesis of green leaf volatiles (GLVs) from different plant species. Shown are amounts for (Z)-3-hexenal, hexanal, and (E)-2-hexenal in ng/g fresh weight (FW). Results for each plant are averages from at least three biological replicates. Data including standard deviation are shown in [Table S1 \(Supplementary Materials\)](#).

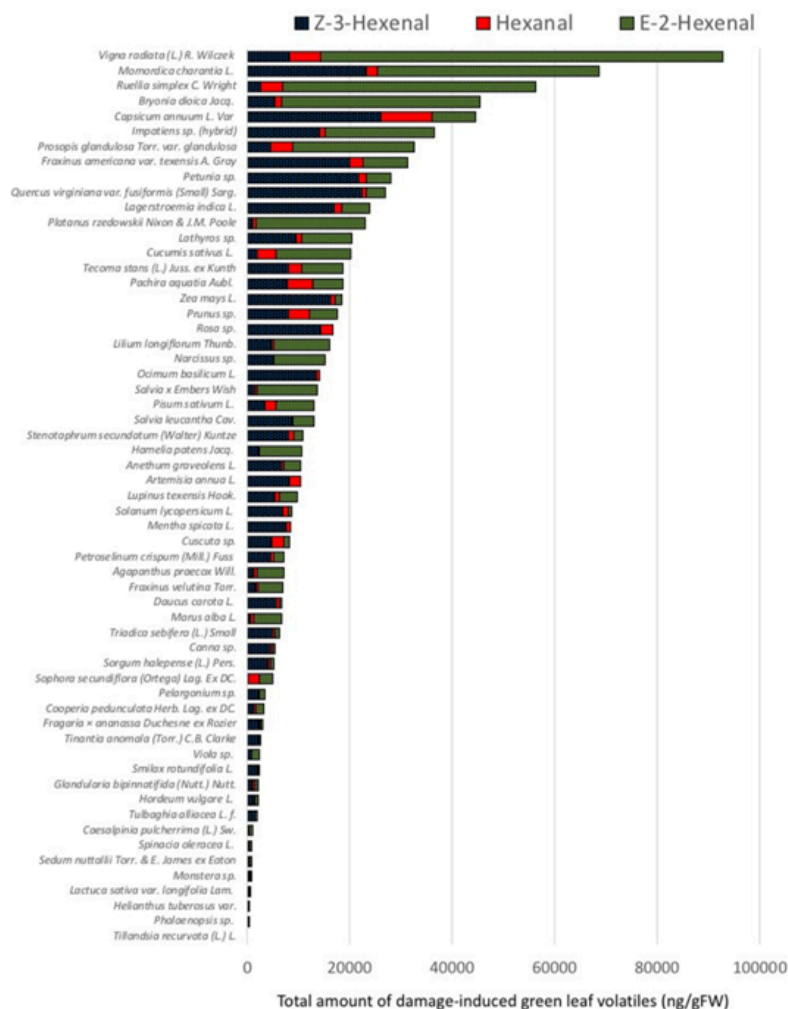
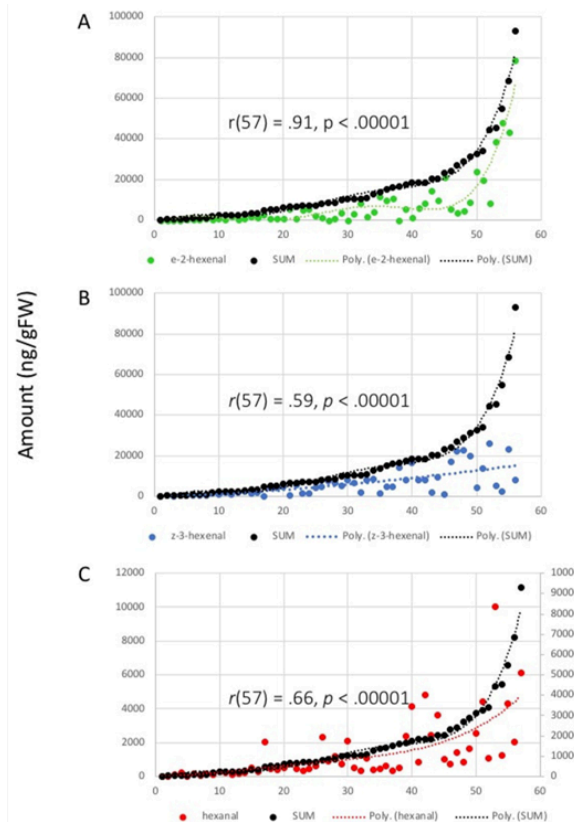


Figure 4. Correlation between total amount of green leaf volatiles and individual compounds after damage. (A) correlation of (E)-2-hexenal with total amount; (B) correlation of (Z)-3-hexenal with total amount; (C) correlation between hexanal and total amount. Note that, for hexanal (C), a separate scale was used due to the large difference in produced amounts. Correlation coefficients (r) and p -values are shown on the graphs. Trendlines are shown in corresponding colors (dotted lines). Results for each plant are averages from at least three biological replicates.



The Biological Activity of Green Leaf Volatiles

The ecological relevance

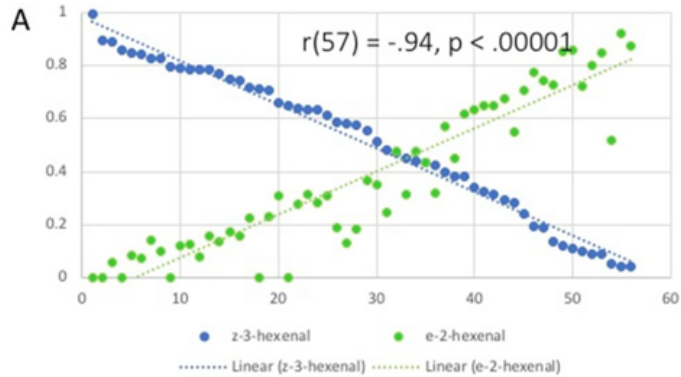
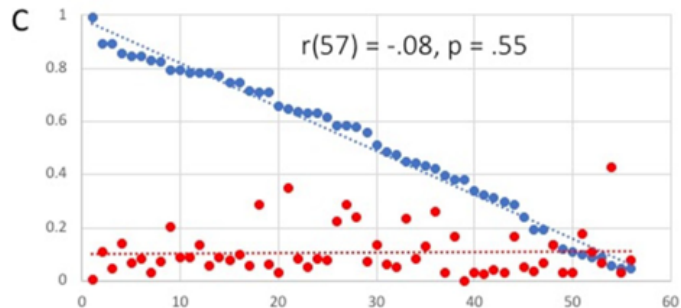
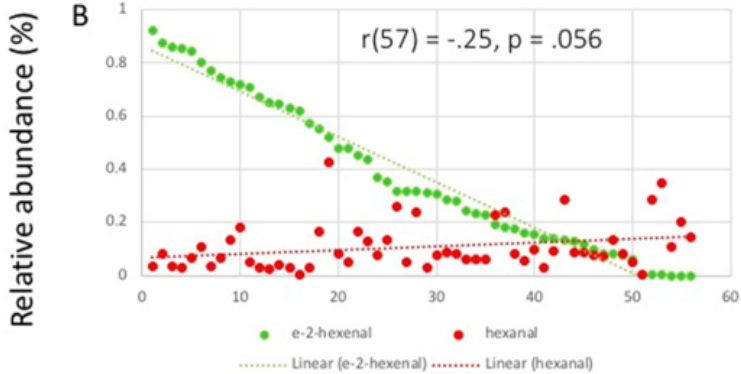
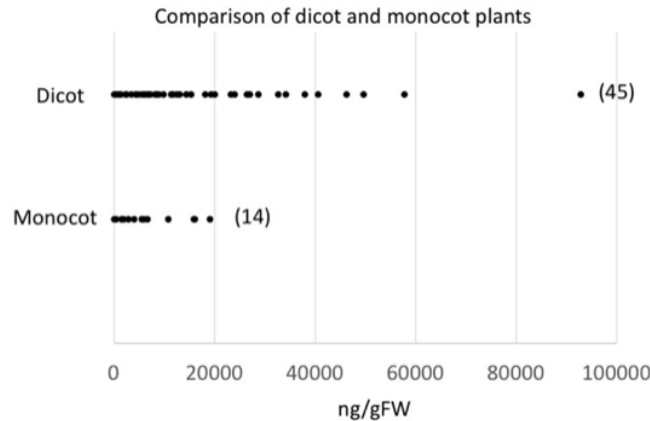


Figure 5. Correlation between relative proportions of individual compounds after damage. **(A)** correlation of (Z)-3-hexenal with (E)-2-hexenal; **(B)** correlation of (E)-2-hexenal with hexanal; **(C)** correlation between (Z)-3-hexenal and hexanal. Correlation coefficients (r) and p -values are shown on the graphs. Trendlines are shown in corresponding colors (dotted lines). Results for each plant are averages from at least three biological replicates.

Figure 6. Comparison of green leaf volatile (GLV) release (total amount in ng/g FW) from dicot and monocot plant species. Numbers in brackets indicate the number of plant species tested in each group. Results for each plant are averages from at least three biological replicates.



Article
Variability in the Capacity to Produce Damage-Induced Aldehyde Green Leaf Volatiles among Different Plant Species Provides Novel Insights into Biosynthetic Diversity

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Summary

GLV provide significant protection against biotic stresses including insect herbivory and necrotrophic pathogen infection.

GLV provide significant protection against abiotic stresses including cold, drought, and heat.

GLV are active even under cold stress conditions

Ecophysiological pressure rather than systematic relationships seem to regulate GLV production capacities

The ability to synthesize E-2-hexenal is essential for maximum GLV production

The Biological Activity of Green Leaf Volatiles

Outlook

GLV provide broad protection against a variety of biotic and abiotic stresses:

- Insect herbivory constitutes major threat to crops world-wide
- So far, all abiotic stressors that GLVs protect against are related to water stress

How can we use this to improve crop protection?

- Alter the biosynthesis of GLVs in plants.
- Treat crops with GLVs at regular intervals
- Improve perception of GLVs in plants

However, non of these aspects have so far been addressed!

Adaptation is greatest problem for genetically manipulated plants

Acknowledgments



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