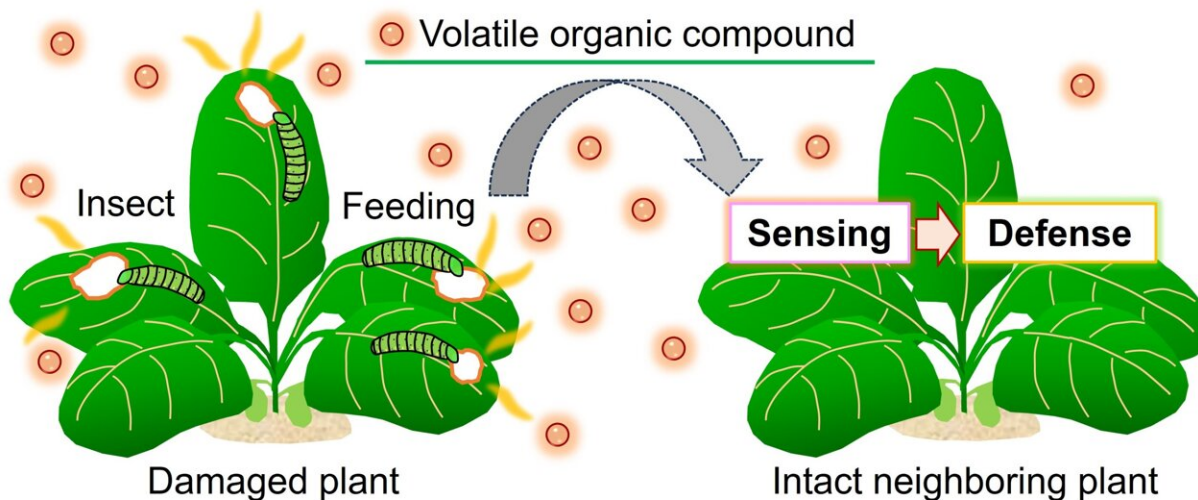


# Real-time visualization of plant-plant communications through airborne volatiles

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Plants release VOCs into the atmosphere when damaged by insects. Intact neighboring plants sense VOCs and activate pre-emptive defense responses against the insects. Credit: Masatsugu Toyota/Saitama University

Plants emit volatile organic compounds (VOCs) into the atmosphere upon mechanical damages or insect attacks. Undamaged neighboring plants sense the released VOCs as danger cues to activate defense responses against upcoming threats. This phenomenon of airborne communication among plants through VOCs was first documented in 1983 and has since been observed in more than 30 different plant species. However, the molecular mechanisms underlying VOC

perception to defense induction remain unclear.

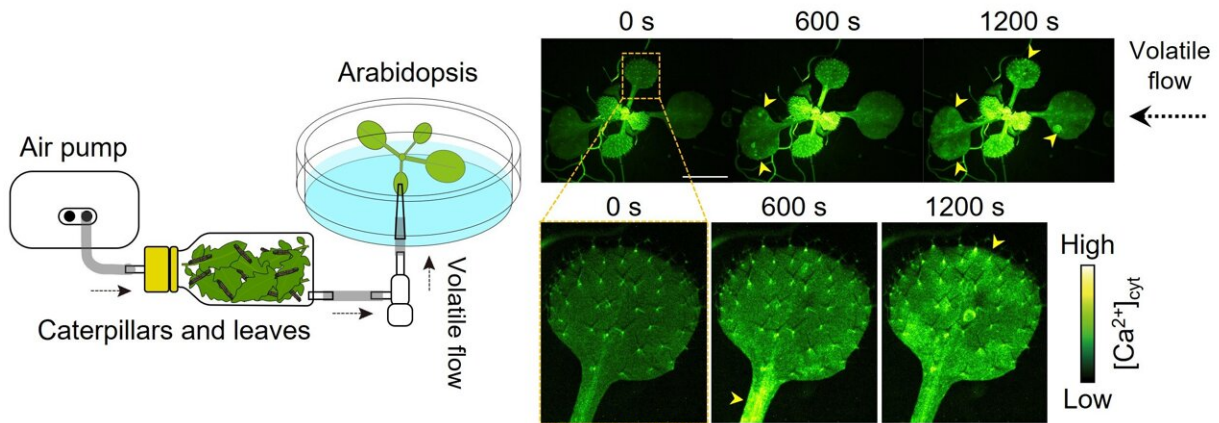
A research team, led by Professor Masatsugu Toyota (Saitama University, Japan), visualized plant-plant communications via VOCs in real time and revealed how VOCs are taken up by [plants](#), initiating  $\text{Ca}^{2+}$ -dependent defense responses against future threats.

This groundbreaking research is published in *Nature Communications*. Yuri Aratani and Takuya Uemura led the work as a Ph.D. student and a postdoctoral researcher, respectively, in Toyota's lab and collaborated with Professor Kenji Matsui at Yamaguchi University, Japan.

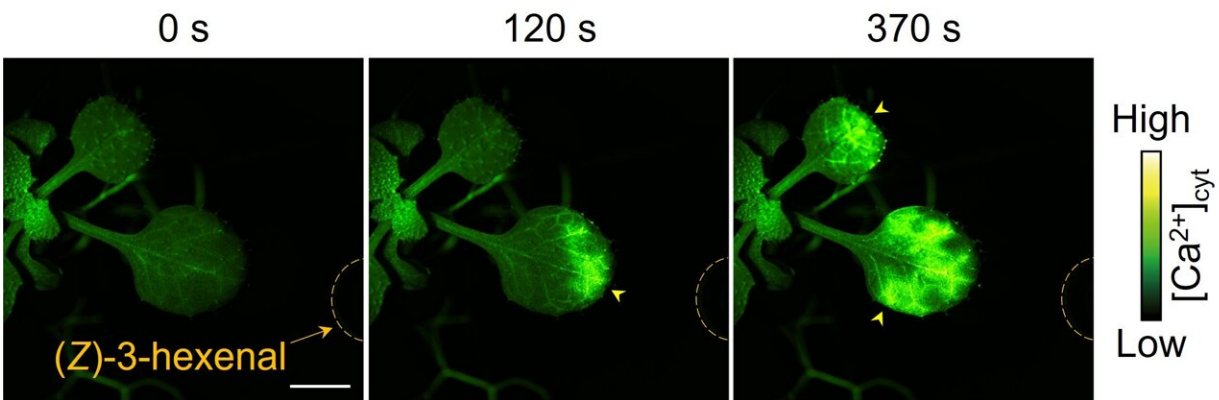
"We constructed equipment to pump VOCs emitted from plants fed by caterpillars onto undamaged neighboring plants and combined it with a wild-field, real-time fluorescent imaging system," says Toyota.

This innovative setup visualized bursts of fluorescence spreading in a mustard plant *Arabidopsis thaliana* after exposure to VOCs emitted from the insect-damaged plants. The plants create fluorescent protein sensors for intracellular  $\text{Ca}^{2+}$  and therefore, changes in intracellular  $\text{Ca}^{2+}$  concentration can be monitored by observing changes in fluorescence.

"In addition to insect attacks, VOCs released from manually smashed leaves induced  $\text{Ca}^{2+}$  signals in undamaged neighboring plants," says Toyota.



Left panel: Equipment for exposing intact *Arabidopsis* to VOCs emitted by insect-damaged plants (dashed arrow). Right panel:  $\text{Ca}^{2+}$  signals (yellow arrowheads, 600 and 1200 s) were induced by VOCs released from insect-damaged plants (dashed arrow). Credit: Masatsugu Toyota/Saitama University



Airborne Z-3-HAL (orange broken line) induced  $\text{Ca}^{2+}$  signals (yellow arrowheads, 120 and 370 s) in *Arabidopsis* leaves. Credit: Masatsugu Toyota/Saitama University

To identify what type of VOCs induced  $\text{Ca}^{2+}$  signals in plants, Toyota's team of scientists investigated various VOCs known to induce defense responses in plants. They found that two VOCs, (Z)-3-hexenal (Z-3-HAL) and (E)-2-hexenal (E-2-HAL), both six-carbon aldehydes, induce  $\text{Ca}^{2+}$  signals in Arabidopsis. Z-3-HAL and E-2-HAL are airborne chemicals with grassy smells and are known as green leaf volatiles (GLVs) emitted from mechanically- and herbivore-damaged plants.

Exposing Arabidopsis to Z-3-HAL and E-2-HAL resulted in the upregulation of defense-related genes. To understand the relationship between the  $\text{Ca}^{2+}$  signals and the defense responses, they treated Arabidopsis with the  $\text{Ca}^{2+}$  channel inhibitor,  $\text{LaCl}_3$  and the  $\text{Ca}^{2+}$  chelating agent, EGTA.

These chemicals suppressed both the  $\text{Ca}^{2+}$  signals and the induction of defense-related genes, providing evidence that Arabidopsis perceives GLVs and activates defense responses in a  $\text{Ca}^{2+}$ -dependent manner.

### **Guard cells: The gateway to awareness**

They also identified which specific cells exhibited the  $\text{Ca}^{2+}$  signals in response to GLVs by engineering transgenic plants expressing the fluorescent protein sensors exclusively in guard, mesophyll, or [epidermal cells](#).

Upon Z-3-HAL exposure,  $\text{Ca}^{2+}$  signals were generated in [guard cells](#) within approximately 1 minute and then in mesophyll cells, whereas epidermal cells generated  $\text{Ca}^{2+}$  signals more slowly. Guard cells are bean-shaped cells on plant surfaces and form stomata, small pores that connect inner tissues and the atmosphere.

"Plants do not possess a 'nose,' but stomata serve as a plant gateway mediating rapid GLV entry into interspaces in leaf tissues," says Toyota.

In fact, the team found that pretreating with [abscisic acid](#) (ABA), one of the phytohormones known for its ability to close stomata, reduced  $\text{Ca}^{2+}$  responses in wild-type leaves. On the other hand, mutants with impaired ABA-induced stomatal closures maintained normal  $\text{Ca}^{2+}$  signals in leaves even when treated with ABA.

"We have finally unveiled the intricate story of when, where, and how plants respond to airborne 'warning messages' from their threatened neighbors," he says. "This ethereal communication network, hidden from our view, plays a pivotal role in safeguarding neighboring plants from imminent threats in a timely manner."

This pioneering research not only deepens our appreciation for the astonishing world of plants but also underscores the remarkable ways in which nature has equipped them to thrive and adapt in the face of adversity. The profound implications of these findings resonate far beyond the boundaries of plant science, offering a glimpse into the intricate tapestry of life on Earth.

**More information:** Green leaf volatile sensory calcium transduction in *Arabidopsis*, *Nature Communications* (2023). [DOI: 10.1038/s41467-023-41589-9](#)

Provided by Saitama University

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