# Plants Bite Back

## Insect-infested hosts starve out unwanted guests, and may even warn their neighbors

By INGFEI CHEN

Imagine yourself a beetle in culinary heaven, chomping on a tender tomato leaf. But wait: Your bites have tripped a biological security system, sending an alarm signal coursing through the plant. Your host starts to churn out noxious substances that permeate every leaf. If you finish the meal, you're asking for a serious case of indigestion that could eventually stunt your growth and lead to death by slow starvation.

And the entomological nightmare doesn't end when you flee to the nearest plant. Your wounded host has sent out a chemical alert, and every plant in the vicinity has started producing the same anti-beetle juice.

Your hopes of a decent meal anywhere in the neighborhood have been ruined.

Scientists have begun chewing on this scenario — which remains partly hypothetical — as a possible model for chemical signaling within and among plants. Over the past two decades, researchers have made steady progress in deciphering insect-triggered defenses within plants, but they have gathered only shaky evidence for airborne warning signals sent from one plant to another. New findings now place the concept of interplant "talk" on somewhat firmer ground.

In the October PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES (Vol.87, No.20), biochemists describe lab experiments showing that a volatile, plant-produced oil called methyl jasmonate — a common perfume ingredient — can spread through the air to induce nearby tomato, tobacco and alfalfa plants to manufacture insect-thwarting compounds called proteinase inhibitors.

Clarence A. Ryan and Edward E. Farmer, working at Washington State University's Institute of Biological Chemistry in Pullman, began one of their studies by applying various dilutions of methyl jasmonate, ranging from 0.5 to 100 nanoliters per sample, to cotton-tipped wooden dowels. They placed the dowels with tomato plants in airtight glass jars for 24 hours, then tested the leaves for the presence of proteinase inhibitors I and II. These two compounds, which inhibit the breakdown of proteins by major digestive enzymes in insects, are normally undetectable in healthy tomato leaves.

The researchers found that each tomato plant's production of proteinase inhibitors appeared to depend on the amount of methyl jasmonate in the incubation jar. In plants exposed to the highest oil concentrations, levels of proteinase inhibitors I and II soared to 300 and 260 micrograms per gram (μg/g) of leaf tissue, respectively.

These findings, supported by similar results in additional experiments with tobacco and alfalfa plants, demonstrate that methyl jasmonate is quite "powerful in turning on the proteinase inhibitor genes," Farmer says.

He and Ryan went on to show that sagebrush leaves — which naturally contain methyl jasmonate at concentrations comparable to those used in the dowel experiments — could elicit the same response. After incubating tomato plants with sagebrush branches, they detected significant levels of the anti-insect compounds in the tomato leaves, with production typically rising to about 70 μg/g of proteinase inhibitor I and 50 μg/g of proteinase inhibitor II. Tomato plants unexposed to sagebrush showed no trace of either compound, they report.

Overall, the results offer "a biochemical basis for a previously unrecognized form of defense gene regulation involving interplant communication," Ryan and Farmer propose.

Support for this theory also emerged in recent, unpublished genetic engineering work in which Ryan and his colleagues inserted a regulatory DNA sequence from potatoes into tobacco plants. They showed that methyl jasmonate can directly activate the potato DNA, which normally switches on a proteinase inhibitor gene found in potato leaves, Ryan says. He speculates that crop sprays laced with methyl jasmonate might someday prove valuable for enhancing plant resistance to insect pests or even disease-causing microbes.

"We don't yet know what significance this has biologically," says Farmer of the methyl jasmonate phenomenon. "But the fact that we can demonstrate it in a defined system will now allow us to ask the question: Does interplant communication by airborne signals occur in nature?"

For Ryan, the search for interplant defense signals represents a shift in focus. For almost 20 years, he has delved into anti-pest responses occurring *within* plants (SN: 5/25/85, p.327). In 1972, Ryan became the first to document such a reaction: the buildup of proteinase inhibitor I in damaged and undamaged leaves of a tomato plant hours after the plant was attacked by Colorado potato beetles. Since then, scientists have reported many instances of induced resistance in plants "inoculated" by the ravages of insects (SN: 2/14/87, p.101). Among their findings:

- Cotton plants infested by spider mites underwent protective chemical and physiological changes, causing poor development in mite populations that later attacked the same plants.
- Beet armyworm larvae suffered severely stunted growth when nurtured on

*Methyl jasmonate in sagebrush diffuses through the air and induces the production of anti-insect proteinase inhibitors in the leaves of a neighboring tomato plant.*

Farmer

leaves from tomato plants in which proteinase inhibitors I and II had accumulated after mechanical wounding.

- Physiological changes in sugar beets after attack by beetflies caused a mortality rise in beetfly larvae that later munched on the plants.
- Autumnal moth larvae showed abnormally slow development when fed undamaged leaves of birch branches from which researchers had torn a single leaf two days earlier.

A key question nags researchers who study such anti-pest responses within plants: What is the alarm signal that travels from a wound site through the plant's vascular system to activate the genes responsible for plant-wide defensive changes?

Proposed candidates include plant enzymes, hormones, lipids, electrical signals and fragments of the leaves' damaged cell walls. So far, however, attempts to identify a leading contender have yielded conflicting results. Indeed, many investigators suspect that different alarm signals trigger the different defensive responses, which can occur hours, days or weeks after wounding and are likely to arise from diverse mechanisms.

In the case of proteinase inhibitors, scientists have long speculated that cell-wall fragments from wounded leaves might serve as the call to action. This notion arose from experiments in the early 1980s showing that the fragments, which consist of small carbohydrate molecules called oligosaccharides, can activate several defensive plant genes, including those coding for proteinase inhibitors. In a 1985 study, however, researchers applied radioactively labeled plant oligosaccharides to leaf wounds and observed no movement of these purported signals out of the damaged leaves and into the rest of the plant.

Several investigators now suggest that the cellular fragments initiate some other signal that then spreads throughout the plant. Last July, biochemists at the University of Leeds in England reported in Plant, Cell and Environment that oligosaccharides trigger immediate depolarization of cell membranes in leaves, causing a decrease in the membrane potential (the difference between the electrical charges on either side of the membrane). Study coauthor Dianna J. Bowles speculates that the release of oligosaccharides during wounding could lead to electrical changes that "may be causative in setting up long-range systemic signaling."

German scientists suggested last year that a plant hormone called abscisic acid (ABA) may act as the plant-wide signal to increase proteinase inhibitors. The team, led by Lothar Willmitzer of the Institute for Biogenetic Research in Berlin, showed that ABA levels usually rise in both damaged and undamaged leaves of mechanically wounded potato and tomato plants. In contrast, mutant, ABA-deficient plants showed "drastically reduced" activation of proteinase inhibitor genes when wounded, they found. However, spraying ABA on intact mutant plants appeared to trigger the production of large amounts of proteinase inhibitor II. These findings "are compatible with a model assuming this hormone [ABA] to be the actual mediator of the systemic wound response," the researchers conclude in the December 1989 Proceedings of the National Academy of Sciences (Vol.86, No.24).

Other reports seem to contradict that hypothesis. A 1974 study by Ryan, for instance, indicated that ABA had no effect on proteinase inhibitor levels in tomato plants. The hormone probably does not serve as the primary signal, he says, because too little of it occurs naturally in plants.

Ryan and Farmer are now looking at how methyl jasmonate and a related compound, jasmonic acid, might fit into the signaling cascade. They hypothesize that wounded cell membranes release a fatty acid, called linolenic acid, which the plant converts to jasmonic acid. The two researchers have observed that jasmonic acid induces proteinase inhibitors in tomato plants, although at levels lower than those elicited by methyl jasmonate.

Jasmonic acid "might be a common component of signaling pathways leading to proteinase-inhibitor gene expression," says Farmer, noting that the chemical is ubiquitous among higher plants, though in varying amounts. He now seeks to determine whether the acid can migrate through the plant vascular system to produce a systemic defense response.

Farmer further speculates that a plant under attack might convert jasmonic acid to the more volatile methyl jasmonate, which could diffuse into the surrounding air. If so, this may represent the sought-after mechanism linking a plant's self-protective response with an airborne warning system that mobilizes neighboring plants.


Farmer

*Sagebrush, the most abundant shrub in the United States, is naturally rich in methyl jasmonate.*


Ryan

*When a Colorado potato beetle munches on a tomato leaf, an alarm signal courses through the plant, triggering the synthesis of proteinase inhibitor I in both damaged and undamaged leaves.*

The sagebrush-tomato findings may help revive the notion of "talking trees," which has won little scientific support since two research teams proposed it in the early 1980s. In one of those studies, David F. Rhoades of the University of Washington in Seattle used young western tent caterpillars to assess leaf quality in 10 Sitka willows previously infested by the same insect species, in 10 unattacked neighboring trees, and in 20 unattacked trees that were farther away. In the lab, he found that caterpillars raised on leaves from the damaged trees and those eating leaves from the nearby uninjured willows grew at a slower rate than caterpillars reared on leaves from the more distant trees.

Rhoades, who described the work at a 1982 American Chemical Society meeting, says the results pointed to an altered leaf chemistry not only in the attacked willows but also in the nearby trees, suggesting that "the trees were talking to each other." Noting that he found no sign of root connections between the infested and control trees, he maintains that the damaged willows released an airborne substance that warned uninjured neigh-

### Pest-eating allies: Calling up the reserves

As if indigestion-inducing chemical warfare weren't enough, insect-injured plants may also broadcast SOS signals that call in pest-killing parasites. New research suggests that some plants, when attacked by caterpillars, release volatile chemicals that attract certain female wasps. The wasps sting the caterpillars, temporarily paralyzing them, and lay their eggs in the pests; when the wasp larvae hatch, they devour their insect hosts.

Douglas W. Whitman of Illinois State University in Normal and Fred J. Eller of the U.S. Department of Agriculture in Peoria, Ill., analyzed cowpea leaves that were either chewed by caterpillars or cut with scissors. Both types of injured leaves produced higher levels of "green leaf odors" – which attract wasps – than did undamaged leaves, the researchers report in the fall issue of CHEMOECOLOGY (Vol.1, No.2).

The biologists then studied flight patterns of female *Microplitis croceipes* and *Netelia heroica* wasps, using a wind tunnel to provide an even, uncontaminated airflow. The wasps homed in on scissor-snipped leaves from cowpea, cotton and hyacinth bean plants placed in the tunnel, the researchers found. Similar "orienting" occurred when they replaced the leaves with thin glass tubes filled with various lab-made, wasp-luring substances – including synthetic versions of the green leaf odors assayed in the injured leaves – but not when they used intact leaves or empty tubes.

The results imply that plants "talk" with insects that can help protect them, Whitman and Eller conclude. An understanding of such chemical communication could prove valuable in agricultural pest control, Whitman told SCIENCE NEWS. He suggests that scientists may someday develop genetically engineered crops that lure helpful parasites when attacked.

Some researchers, however, view the findings as inconclusive, noting that the experiments did not establish that the wasp behavior was a direct response to caterpillar-damaged leaves. Others reject the conclusion that the results point to purposeful chemical signaling. Entomologist Elizabeth A. Bernays of the University of Arizona in Tucson argues that it's no surprise that damaged plants release odors or that wasps detect those odors, but "it's not a defense against anything – that just happens." For instance, she says, when an half-eaten apple turns brown, discouraging a person from finishing it later, that doesn't mean the apple is defending itself.

Echoing scientists' widespread skepticism of the notion of "talking" plants, Bernays says: "It's . . . totally exaggerated to talk about communication [between plants and pest-eating parasites]." *– I. Chen*

Whitman

*Female* Netelia heroica *wasps sting, paralyze and lay their eggs in leaf-devouring caterpillars. The eggs later hatch into larvae that kill the caterpillars by consuming them from within.*

bors of an impending attack.

A team at Dartmouth College in Hanover, N.H., reported further hints of interplant communication in the July 15, 1983 SCIENCE. In lab experiments, biologists Ian T. Baldwin and Jack C. Schultz detected increased quantities of phenolics and tannins – compounds that make certain plants indigestible for insects – in potted poplars whose leaves were torn and in undamaged poplars sharing the same airtight enclosure. Within hours, these boosted concentrations reached levels significantly higher than those seen in untouched, control poplars housed in a separate chamber. Baldwin and Schultz observed a similar response in experiments with damaged sugar maple seedlings.

Researchers have criticized these studies for lacking adequate scientific controls. "The experiments were suggestive but not convincing," says Richard Karban of the University of California in Davis. However, he adds, "I personally believe they're onto something."

For years after the "tree talk" hypothesis emerged, its supporters speculated that the plant hormone ethylene served as the airborne communicator. This gas, known to induce ripening when it diffuses from one piece of fruit to another, seemed the perfect candidate. Indeed, scientists have demonstrated that ethylene can turn on some defensive genes in plants. But Ryan says no one has proved that plants naturally emit enough ethylene to induce anti-insect responses in their neighbors.

Ryan asserts that his recent tomato studies establish methyl jasmonate as the first compound shown to carry a defense-activating message through the air from one plant to another. Two authors of the earlier controversial reports today voice strong support for the new work. Baldwin, now at the State University of New York at Buffalo, says the evidence for methyl jasmonate's specific defense-inducing abilities makes the compound "one of the best-established molecular cues [for proteinase-inhibitor gene activation]." And Rhoades calls the research "a major advance" in the field of plant communication because it clearly demonstrates a biochemical mechanism responsible for the observed sagebrush-tomato interactions.

However, many ecologists question whether methyl jasmonate or jasmonic acid has significant interplant effects in nature. They wonder, for instance, whether sagebrush releases either compound in concentrations intense enough to diffuse through open air and trigger a defense response in nearby plants. Some point out that sagebrush and tomatoes seldom grow near each other.

"It's no great surprise that there are chemicals that can communicate among plants," says insect ecologist Judith H. Myers of the University of British Columbia in Vancouver. But she remains unconvinced that the methyl jasmonate effects offer proof of an interplant warning system. From an evolutionary standpoint, Myers says, it would not make sense for a plant to release chemicals that activate defenses in a neighboring plant of a different species, because the damaged plant "would not get any benefit from warning the other."

On the other hand, a plant insensitive to such signals "may be more vulnerable to attack," notes C. Ronald Carroll of the Institute of Ecology at the University of Georgia in Athens. Many plants growing in the same area face the same sources of stress, he says, so "it makes sense" for a plant to respond to chemicals released from damaged plants close by. And even if sagebrush and tomatoes are not natural neighbors, Carroll asserts that the point of the new finding is that plants may share universal alarm signals.

Clearly, Ryan and Farmer have much work ahead. Among other things, they hope to determine whether the methyl jasmonate released by insect-injured plants in nature remains potent enough to induce proteinase inhibitor synthesis in other plants after traveling through the air. Says Farmer, who plans to get out into the field next summer to study interactions between sagebrush and neighboring plants: "We're very excited about the interplant communication aspect. But we're also very cautious." □