PHOTO: MPI FOR CHEMICAL ECOLOGY

The discovery of insect pheromones revolutionized the battle against harmful insects – a highlight of chemical ecology. At the 23rd Annual Meeting of the International Society of Chemical Ecology (ISCE) held in Jena in July and co-organized by the **MAX PLANCK INSTITUTE OF CHEMICAL ECOLOGY**, *some 300 scientists from 30 countries discussed the latest findings on the complex relationships between plants, animals and microbes.*

The Silent Scream of the **Lima Bean**

Relaxing in a summer meadow, listening to the buzz of bees, watching a caterpillar leisurely inch its way up a stalk, aphids obligingly allowing ants to rub them with their antennae and milk them – a peaceful scene on a gentle slope above Jena in the German state of Thuringia. But what an illusion! These are dramas playing out in meadows, forests and hedges – dramas of death and downfall, hunting and enticement, attack and defense. Just because humans lack the appropriate senses, we fail to notice the daily fight for survival that continuously goes on between plants and their adversaries: insects, fungi, bacteria and viruses.

Alluring calls, warning calls, calls for help – we rarely perceive them because they are not acoustically or optically encoded. We know them only as the seductive fragrance of a spray of flowers, or as the heavy bouquet of a freshly mown field. Fragrant chemicals, emitted in extremely low concentrations, are what plants use to communicate. If we could see them, a meadow or a section of forest would be awash in trails of molecules and softly billowing clouds of fragrance. If we could hear them, the screams of battle would destroy any summer idyll. But people have no natural sense or comprehension of the molecular language of plants.

Down below, in the city on the Saale, a group of scientists gathered who do understand this language, who decode it, molecule for molecule. In their research life, chemical ecologists collect substances like taxonomists collect beetles in their boxes. Every new substance that they successfully decode and synthesize is one more letter in the alphabet of chemical communication. They presented and discussed their latest findings with their colleagues at the Annual Meeting of the International Society of Chemical Ecology (ISCE). It was no coincidence that the 23rd meeting was held in Jena, the city of Ernst Haeckel, the "German Darwin," who introduced the term ecology. "The Max Planck Institute of Chemical Ecology is celebrating its 10-year anniversary this year," says conference organizer Wilhelm Boland, Director of the Department of Bioorganic Chemistry at the institute in Jena.

Furthermore, Jena is even considered one of the birthplaces of chemical ecology and of the study of the defense chemistry of plants: this was where, 120 years ago, Ernst Stahl investigated how plants react to slug damage. However, as has so often happened in the history of science, his approach was lost. It wasn't until the 1950s and 1960s that insect researchers turned the attention of chemists to plants' reactions to insect attacks. Since then, they have deciphered large portions of the communication puzzle. "For example, we have learned that secondary metabolic products that the plant organism does not need directly for survival certainly do have a function," says Boland. The scientists now know of more than 200,000 such secondary metabolites.

THE VARIETY OF SECONDARY METABOLITES

While primary metabolites, such as amino acids and sugars, perform fundamental survival functions, secondary metabolites are rather an indirect benefit to survival: Plants use them, for instance, as dyes and scents in flowers and fruits to attract pollinators or animals that disperse the seeds. But they also make up a large part of the plants' defense forces, either as messenger substances, like the plant hormones jasmonic acid and salicylic acid, or as defense substances, like the mustard oil glycosides, the terpenes or the tannins.

It takes some time before a plant activates its defenses: genes for their production are adjusted up and down, enzymes are produced, and defensive compounds are synthesized. Scientists now have a very good understanding of all of this. It was only what happens in the first seconds to minutes when a caterpillar takes its first bite from a leaf that long remained in the dark. Massimo Maffei from the University of Turin and Wilhelm Boland have shed light on this early time period. Their first key discovery: the feeding attack of a caterpillar changes the electrical potential of the affected cells and their neighbors in such a way that the resulting depolarization wave propagates alarmingly, within just minutes, across the entire leaf. "Now we have to find the connection with the subsequent signal cascades," says Maffei.

To undertake controlled studies of these and other reactions of the plants to attacks by herbivores, Boland and his colleagues are counting on a mechanical assistant – MecWorm, a kind of stamping robot:

PHOTO: NORBERT MICHALKE

Around 100 lectures were held at the 23rd Annual Meeting of the International Society of Chemical Ecology in Jena. The topics ranged from plant defense and interaction with microbes to evolution and adaptation.

Photo: Norbert Michalke

Meetings among the posters offered many occasions for animated discussions.

"This allows us for the first time to imitate, continuously and over a long period, the wounding by herbivores and, above all, to study separately what impact the saliva of herbivores has and what that of pure mechanical damage is," says Boland.

Initial genetic analyses using *Arabidopsis thaliana* show that the saliva does, in fact, cause numerous completely different genes in the plant to be adjusted up or down differently than with pure wounding. "And not only in the wounded leaf, but also in far distant regions of the plant," says Boland. This is evidence that there are signal substances in the saliva that are responsible for transmitting the stimuli from the damaged location to other parts of the plant.

"We now know that there are substances in the saliva that are channel-forming in cell membranes," explains the Max Planck researcher. Channels in cell membranes are the gateway for calcium and chloride ions, for example, which contribute to building up the electrical potential at the membranes and then travel from cell to cell. Massimo Maffei's studies of the electrochemical potentials in the first seconds following an attack also indicate that there are important substances in the saliva of the herbivores: "With the purely mechanical damage by MecWorm, the depolarization is much weaker than in the case of a true herbivore," says Maffei.

It almost looks as if, with substances in the saliva, the attacker assists the plant in sounding the alarm. Would that do an insect or a caterpillar any good? "We can never completely rule that out, as we mustn't forget that plants and herbivores are in a constant arms race on every level. And we should expect that the insect manipulates the plant in its favor in any way it can," explains Boland. Studies have shown again and again that the enemies of the plants have also developed counterstrategies to sideline the plants' defense. For example, together with their saliva, they send enzyme inhibitors into the wound to neutralize the defense substances.

However, some plants – such as peas, beans and elms – elegantly avoid the arms race. They try to defeat their opponents at the earliest possible stage, rather than waiting until an insect flies in for a visit, or a caterpillar comes crawling up the stem to take its first bite. Their strategy is to obliterate the offspring as early as possible. And that can be taken literally. "These plants try to combat the eggs so that no herbivores develop in the first place," says Monika Hilker, professor at the Institute of Biology at the Free University of Berlin.

The plants have two options for this: bean, rice and potato plants, for example, form new tissue or produce toxic substances to effectively repel the pests' eggs. Elms, pines and even brussels sprouts use fragrances to call parasitoid wasps to their aid. These destroy the leaf parasite by using its eggs as living incubators for their offspring.

Leaving the work to others is a type of defense that is also used by plants that are not put on alarm until they have been attacked by adult herbivores. Such plants include the lima bean, a creeper from Latin America, on which Wilhelm Boland's working group also focuses. The beanstalk uses a "sweet call for help" to attract virtual defense troops when attacked by aphids or beetles. Nibbling on their leaves triggers nectar production in the plant: Tiny shimmering beads of nectar are then secreted by small extrafloral nectaries. Their sweet fragrance attracts ants that take on the attackers and suck up the high-calorie treat as their reward.

Vital Information by Air

But the lima bean not only attracts foreign aid, it also uses fragrances to warn neighboring plants that can thus prevent an attack by likewise producing drops of nectar. However, this observation presents biologists with a general problem: "Why does a plant warn its neighbors, with which it competes for resources?" asks Martin Heil, professor at the University of Duisburg-Essen, and who researched the lima bean in a joint project with Wilhelm Boland. The solution: the actual recipient isn't the neighboring plant at all. Rather, with the scents, a lima bean that has been nibbled on is preparing further leaves on the same tendril for the attack. "Informing neighboring tendrils about the attack in this way is much quicker than sending a signal through the plant," explains Heil.

The warning signal would have to run from the attacked leaf to the tendril down to the main vine and from there to the neighboring tendril: "That can easily add up to a couple of meters," says the ecologist. As the adjacent leaf is just a few centimeters away, the linear distance is the shortest path for the lima bean, too. Evolution couldn't pass up such a shortcut. The neighboring plants just happen to be unintended beneficiaries of this aromatic warning.

In the fight for survival, volatile organic compounds play a role not only above ground, but also below the Earth's surface. They benefit the plants there, too, but they stem from a different creature, as Birgit Piechulla, a biochemist at the University of Rostock, was one of the first to discover. Single-celled organisms emit an as yet largely unknown scent cocktail in the soil and thus improve the growing conditions for the plant. "The volatile organic compounds act as antibiotics," says Piechulla. "Since there is enough space between the soil particles, they can disperse better there than, for instance, in a liquid."

In several experimental series, the scientists tested molecular cocktails of various soil-inhabiting bacteria, such as *Stenotrophomonas maltophilia* and *Bacillus subtilis*, on various fungi.

In some fungi, the fragrances nearly completely prevented growth, although other fungi were hardly affected or were not affected at all. "We now know that the bacteria can have a negative impact on the fungi occurring in the soil," says Birgit Piechulla. But some substances in the bacterial scent cocktail may even have a growth-promoting effect on the plants. When Piechulla used the bacterial fragrance to fumigate the roots of *Arabidopsis* under sterile conditions and with no fungus growth, they grew better than they had without the scent treatment.

As with the lima bean, here, too, the question arises: How do bacteria benefit when they produce scents that are so advantageous for the plant? In an ecological system, there are many different organisms that not only communicate with each other, but that must also keep each other in check. Perhaps the scents inhibit competitors. Conceivable opponents could be organisms such as viruses, bacteria, nematodes and fungi – but of course also plants.

Bacteria Exposed as Toxin Producers

But bacteria can wipe out vegetation, even in alliance with fungi. They do this in such a clever way that they aren't even recognized as the real killer. One example is the microscopically small fungus *Rhizopus microsporus*. It attacks the roots of young rice plants, making it a predator that strikes fear in the hearts of farmers worldwide. The fungus secretes a toxin called rhizoxin that causes rice seedling blight. The toxin's attack mechanism is well known: "The toxin inhibits cell division by specifically binding to the protein beta-tubulin, thus preventing mitosis," explains Christian Hertweck from the Hans Knöll Institute in Jena, who tracked down the actual toxin producer. It isn't the fungus at all, but rather a bacterium of the genus *Burkholderia* that inhabits the fungus and, under its protection, produces the rhizoxin, as Hertweck and his colleague Laila Partida-Martinez were able to prove beyond a doubt.

The researchers knew that a polyketide synthase, an enzyme for producing a polyketide, is needed to produce the toxin (polyketides are one of the largest classes of natural compounds). When they were examining the fungus genome for the corresponding genes, to their own surprise, they found none. Instead, the old speculation that bacteria could be the real producers of many mycotoxins put them on the right track. "We were actually the first to succeed in isolating the bacterium, cultivating it and producing the toxin," says Hertweck.

Photo: Norbert Michalke

Gaining their bearings in the lecture marathon.

As is typical for a symbiosis, both the fungus and the bacterium benefit from the fatal alliance because they draw nutrients from the dying rice plant. The fungus is so dependent on the bacterium that it can no longer even breed without it. "When we remove the bacterium, the fungus no longer produces spores," says Hertweck. However, it still remains to be seen whether this means that rice seedling blight can now be controlled better: "We can't spray the fields with antibiotics against the bacterium."

But since the scientists already know what the true toxin factory is, the bacterium, it may be possible to use this knowledge to develop more effective variants of rhizoxin as a tumor toxin, because it has been known for some time that the toxin also prevents cell division in cancer tumors, and has repeatedly sparked the interest of cancer researchers. However, there has not yet been any resounding success. That could be a new highlight in chemical ecology.

Marcus Anhäuser

"Plants were mostly underestimated"

Researchers have known for years that, in plants, attacks by chewing insects trigger defense reactions that are communicated within the organism by chemical signal cascades. However, early events triggered in the plant by such an attack long remained in the dark. **Massimo Maffei** *from the University of Turin and* **Wilhelm Boland** *from the* **Max Planck Institute of Chemical Ecology** *in Jena are pioneers in the study of these initial moments of insect-plant interaction.* MaxPlanckResearch *interviewed the two scientists at the Annual Meeting of the International Society of Chemical Ecology.*

MaxPlanckResearch: *Scientists now understand quite well what occurs in a plant when an insect attacks. Only right at the beginning, in the first seconds and minutes, was it long uncertain what happens. Why was that previously overlooked?*

Wilhelm Boland: No one was working on these questions. Until five or six years ago, everyone was focused on plant hormones, which are produced only much later after an attack – how they interact with other substances and how they control the defense. And of course a key focus was measuring secondary metabolites, about which it was previously thought that they had no function whatsoever. Today, we know how important they are for the defense of the plant. This was studied with established biology and chemistry methods, such as microarrays and other molecular biology techniques. Anyone can work with these. However, in the case of the "early events," in other words the reactions of the plant in the first seconds up to about 20 or 30 minutes into an insect attack, the situation is completely different. This requires a great understanding of chemistry and physics, especially of electrochemical methods – but there are only a very few specialized labs in the world that can conduct these studies – including ours here in Jena and the one in Turin.

MPR: *So what happens in this early phase when, for example, a caterpillar tears its first pieces from a leaf?*

Massimo Maffei: There are various substances that act in different time periods, and each substance has its own timing. First, there is the interaction of the insect with the cell membrane, which upsets the balance of the ions located on both sides of the membrane. A membrane uses ion pumps to actively maintain a difference between the ions on the inside of a cell and those on the outside. One side is more negatively charged than the other – we call this the membrane potential. This difference between the outside and the inside must remain constant because it constitutes the balance of the living cell. Influences from outside can shift this balance or completely destroy it. Those are the first early signals with which the cell perceives that something is happening outside.

Boland: *And it is the first effect that we can measure with our methods: when a tissue is destroyed, depolarization of the membrane occurs in the first seconds.*

Maffei: Precisely. But initially only at the location of the bite. The depolarization is so strong that it triggers action potentials in the cells. An electrical wave then propagates outward from the bite site across the entire leaf at a speed of about one centimeter per minute. Seconds after depolarization, massive quantities of calcium ions flow into the cell. A little bit later, hydrogen peroxide production is increased, which is a known reaction when plants are attacked by herbivores. Only some 10 to 20 minutes later do we register a change in the concentration of such plant hormones as jasmonic and salicylic acid. And after about an hour, we see the first changes in gene regulation and the concentration of secondary metabolites that are then used as defense compounds.

Massimo Maffei

MPR: *What role do substances such as calcium play in an insect attack?*

Maffei: These substances are involved in signal transduction. This is comparable with what is happening here in our interview: there is a kind of microphone that receives the signal, and something that then amplifies it. Here, we have the recorder that takes care of amplification. In plants, it is the cell. Something is needed to boost the signal and then transmit it. Calcium is involved in transmitting the signal.

Photo: Norbert Michalke

Wilhelm Boland

MPR: *So what is the connection between these early events and the later signal cascade that ultimately leads to the defense reaction?*

Boland: That is one of the key questions to which we don't yet have an answer. There are assumptions, especially regarding the role of the calcium, but no final proof. However, we know that many of the enzymes that are related to the production of plant hormones, such as phospholipases, are activated by calcium ions. This means that, when there is a massive increase of calcium ions in the cell, this could activate the phospholipases – and ultimately the entire cascade, right down to the phytohormone jasmonic acid. This has not yet been shown, but it is a logical sequence that makes sense. To put it simply, the key question is: How does an electrical wave become a meaningful physiological signal?

MPR: *Are their electrophysiological measurements comparable with those from animal cells?*

Maffei: From a technical standpoint, there is no difference. In plants, however, the cell wall makes the task more difficult. The goal is the same, too: we want to measure the charge distribution on both sides of the cell membrane. When positive ions, such as calcium, flow into the cell, depolarization occurs; when they flow out in large numbers, hyperpolarization occurs. Accordingly, negative ions, such as chloride, cause the opposite reaction. In plants and animals, the membrane potential is actively maintained, but in different ways. It is very difficult to say how far the similarities go. We shouldn't overtax the comparison. After all, plant and animal evolution have been separate for a couple hundred million years now.

MPR: *But there are researchers who believe that the differences in signal processing and perception between animals and plants aren't nearly as great as we always think. After all, the term plant neurobiology has since spread through all the newspapers. What do you think of that?*

Boland: As is so often the case, the truth is somewhere in between. The term plant neurobiology accomplished exactly what it was intended to: bringing people's attention to a special kind of interaction between plants and their environment. Quite simply, the term includes the entire processing system, from signal perception to reaction. It was this analogy that led those involved to call it "plant neurobiology". It was good that they chose this term – it fulfilled its purpose of gaining attention. But now, three or four years later, we should create and use a more precise term.

MPR: *And what might that be?*

Boland: That's the problem! We can really only describe what it means in a couple of sentences. That is also what makes the term "plant neurobiology" so appealing. Everyone knows what is meant. What we mean is nothing other than a very comprehensive description of "picking up a signal, processing the signal, and responding to the signal."

Maffei: Interestingly enough, the same debate took place over plant physiology at the end of the 19th century. Then, too, people claimed: there is no such thing as a physiology of plants, that it applies only to animals. But plant physiology is now a completely established field.

Boland: The debate arose over plant hormones, too. Today, we call them phytohormones. And no one gets upset about it anymore.

MPR: *But aren't some proponents taking it a bit too far when they use such terms as intelligence or brain in connection with plants?*

Maffei: In my view, they do this to dispel the preconception that only humans and animals are capable of interpreting signals and stimuli and reacting to them, while plants are inactive and still. And one method of breaking down preconceptions is by choosing specific words: speaking, for instance, of a kind of self-awareness in plants because the roots can distinguish themselves from those of another plant. Of course this is not the same as human self-awareness, but the cells do recognize: those are roots of another plant, not their own.

Boland: It is also a means of showing that plants were largely underestimated. A plant can be viewed as a complex processing system, just like an animal, with many sensors and many response elements. The plant always notices what is happening in the distant roots or in the leaves. When one part is destroyed, this is somehow perceived, and that part then interacts with the other parts. For example, damage to the roots can cause the leaves to emit fragrances that are then noticed by parasites or whoever. The plant lives in a vast interactive sphere, both above and below the Earth, filled with chemical compounds that supply countless tidbits of information. It is exposed to numerous interactions, and that is why it must also be constantly aware of what is going on around it.

Interview: Marcus Anhäuser