

USING A TREE'S NATURAL DEFENSES

TEXT: CATARINA PIETSCHMANN

56 Trees stand for strength and steadfastness. However, the latter can also be a handicap. This is because trees can neither run nor hide from enemies. Nevertheless, trees are by no means defenseless. Sybille Unsicker from the Max Planck Institute for Chemical Ecology in Jena is investigating how black poplars defend themselves against voracious insects.

Not only the ravages of time but also a multitude of large and small herbivores, bacteria, fungi, and viruses eat away at a tree throughout its life. Which pests infest which parts of a tree depends, among other things, on its age (and that of its leaves), the season, and the weather. Different conditions also prevail within the tree crown. Leaves can have different micro-climates, depending on how high they are off the ground, the direction they face, and their position on the tree. “You have to think of a tree as more like a meadow in which there are various ecological niches,” explains Unsicker. The tree as an ecosystem, so to speak.

Predators also have to cope with these constantly changing and locally varying conditions. In the plant kingdom, a common way to spoil the appetite of attackers is to produce very indigestible or even toxic substances. However, there are hardly any toxins that keep all predators equally at bay. What is toxic to one species may be attractive to another. For example, the nicotine in tobacco plants, the caffeine in coffee plants, or the piperine in pepper plants are produced to make the leaves or fruits of their respective plants inedible. And this works against most insects. But for humans, these substances are popular stimulants. True to the motto: “The enemy of my enemy is my friend,” other plants get external help for defense. And Unsicker aims to find out exactly how these alliances work.

For her diploma thesis, she examined tree crowns on the Ivory Coast. She is currently conducting research in a riparian forest on Küstrin-Kietz, an island in the Oder River, which is a nature reserve directly on the German–Polish border. The more than 300 black poplars (*Populus nigra*) growing along the banks of the Oder are among the last naturally occurring black poplars in Germany. While elsewhere more competitive species make life difficult for them, the battles at the end of the Second World War destroyed almost all the vegetation here. This was a stroke of luck for these trees because they are weak competitors. As seedlings, they can rarely prevail against other plants.

“I was eager to study a native tree species that occurs in an ecosystem with great

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Using professional climbing equipment, Sybille Unsicker can examine the treetops even at these heights.

Maybe not pure herbivores after all? In addition to leaves, the caterpillars of the gypsy moth (*Lymantria dispar*) like to eat fungi. The orange-colored spore deposits of poplar leaf rust alter the mixture of odorous substances that the poplars release from their leaves.



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biodiversity. Riparian forests are hot spots of biodiversity. The fact that black poplars have become rare in Europe has given me additional motivation. Our results might even contribute to the preservation of these trees,” says Unsicker. Since 2009, she and her team have been studying how poplars react to insect attack – both in the field and in the laboratory. For this purpose, Unsicker has equipped several trees in the riparian forest with ropes. Researchers can use these to climb into the tree crowns and take leaf samples from different heights. They regularly encounter caterpillars of the gypsy moth, a moth that is about three centimeters in size. The investigations have shown that the insects prefer to feed on older, already hardened leaves.

To avoid having to climb into the tree-tops every time they want to observe the interplay between caterpillars and poplars, Unsicker and her team have planted poplar cuttings from the floodplain forest in their greenhouse in Jena. For their analyses, the researchers wrapped the branches in fine nets and placed gypsy moth caterpillars inside. “You can easily smell that the insects change the smell of the leaves,” says Unsicker. Once the caterpillars have begun to destroy the leaves, the researchers replace the nets with gas-impermeable bags. These collect the volatile substances of the trees that are being attacked. The researchers then capture the volatiles with a filter. The filter content is rinsed with solvent and analyzed using gas chromatography and mass spectrometry.

The difference between the damaged and the undamaged leaves is enormous. “Infested leaves produce large quantities of volatile compounds, which give crushed leaves their characteristic odor. We can also detect sesqui- and monoterpenes, aromatic hydrocarbons, and nitrogen-containing volatiles, including benzyl cyanide,” says Unsicker.

Is this the “cry for help” we are looking for – the message to the enemy of the enemy? The team had colleagues from Austria send one of the caterpillars’ most formidable enemies: parasitoid (i.e., Braconid) wasps. These insects deposit several eggs inside the body of the caterpillars. The hatched braconid wasp larvae feed on the tissue of their hosts but spare the vital organs. The caterpillar thus continues to live until the wasp larvae bore

through its skin to the outside and pupate. At this stage, the caterpillar behaves as if it were under the control of an external force and even defends its deadly tenants against attackers. Its strength is eventually exhausted, and it dies. The volatile analysis shows that the parasitoid wasps can detect the odorant molecules from the injured leaves extremely well. Faced with the choice between the odor of intact or damaged leaves, they clearly prefer the damaged leaves. Only a few odorant molecules are required for the highly sensitive antennae of the parasitoid wasps to locate their prey. But is this also the situation in the wild, where – in addition to poplars – other plants also release their volatiles and the wind swirls all the scents together? To find out, the researchers placed the presumed attractants on glue traps in the riparian forest. “Af-



PHOTO: FRANZISKA EBEL

ter only a short time, we had braconid wasps and other parasitic insects such as ichneumonid wasps in the traps,” says Unsicker.

Examinations of the cuttings in the greenhouse confirmed the findings. As soon as the researchers removed the caterpillars from the leaves, the concentration of the nitrogen-containing volatiles dropped to zero. Because the wasps are attracted to these very odors, it was clear: “We had found the distress call molecules produced by the trees,” says Unsicker. Poplars produce volatiles mainly during the day when they also carry out photosynthesis. At night, however, they largely stop production. The kinds of volatiles released by the leaves depend on the type of injury they sustain. If they are cut with scissors, for example, they release diffe-

rent substances than when they are infested by caterpillars. But how does a leaf know what is causing it harm? “Leaves can recognize insects by their saliva, which penetrates the leaf tissue. They also perceive the way an animal eats and how much leaf area is lost in the process,” explains Unsicker. “Sucking aphids trigger a different reaction than caterpillars that feed on large areas, or leaf beetles that punch small holes in the leaves.”

The trees can thus not only call for outside help but also warn parts of the crown that may not have been infested yet. After all, branches of large trees are often many meters apart. “Communication within the tree crowns via volatiles would thus be comparatively quick. It would take much longer to send information through the tree’s supply lines, from the leaf to the branch to the trunk, and from there back to another branch and so on,” says Unsicker. In the coming years, Unsicker hopes to find out exactly how one leaf perceives the odor of another as well as which receptors and transport molecules carry the volatiles through the cell membranes.

Caterpillar toxins in the leaves

But why don’t the attacked poplar leaves produce toxins that would repel the caterpillars like tobacco, coffee, and pepper plants do? “The leaves of the black poplars also contain substances that are toxic in high concentrations. For example, salicinoids can be toxic, especially for generalists (i.e. caterpillars that feed on various tree species) like gypsy moth caterpillars. But there is a considerable danger that such a toxin would also affect all the many beneficial insects that live on and in a tree.” Unsicker suspects that the arsenal of defense molecules that

can be found in plants is highly complex and used in a variety of ways. Only in this way is it possible to conceive that a poplar tree – which reaches sexual maturity no earlier than the age of 10 years and then lives on for another 90 years – can survive the attacks of its extremely diverse predators.

This is supported by the fact that the volatiles that summon the parasitoid wasps can be produced very easily. The starting point is the amino acid phenylalanine, which is converted into benzyl cyanide in only two steps. The intermediate stage, phenylacetal oxime, can accumulate in the leaf and is toxic. The higher the concentration

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SUMMARY

Black poplars emit odorous substances from their leaves; these attract parasitoid wasps. The insects parasitize caterpillars and thus help the trees keep the voracious larvae at bay.

Odorless substances are also suitable media for transporting information from tree to tree as well as within the tree crown. They reach their destination relatively quickly and are easy to produce.

Leaves infested with fungi also release characteristic volatiles. Their smell attracts butterfly caterpillars, which apparently do not live a purely vegetarian life but also eat the fungi as a source of nitrogen.

of this substance in the leaves eaten by gypsy moth caterpillars, the higher the mortality of the caterpillars. “The production of these toxic substances thus serves not only the indirect but also the direct defense. The tree essentially has a double defense strategy,” explains Unsicker.

The poplars communicate not only with the parasitoid wasps but also exchange information with each other – a phenomenon that has been communicated to the public as well, thanks in part to the books by Peter Wohlleben. It has been known since the 1980s that trees can pass on information about pests with the help of volatiles. Trees are also connected to each other via fungal networks at the roots, the mycorrhizae. However, critics accuse Wohlleben of humanizing trees. “Even for my taste, he sometimes embellishes the facts too much. But I

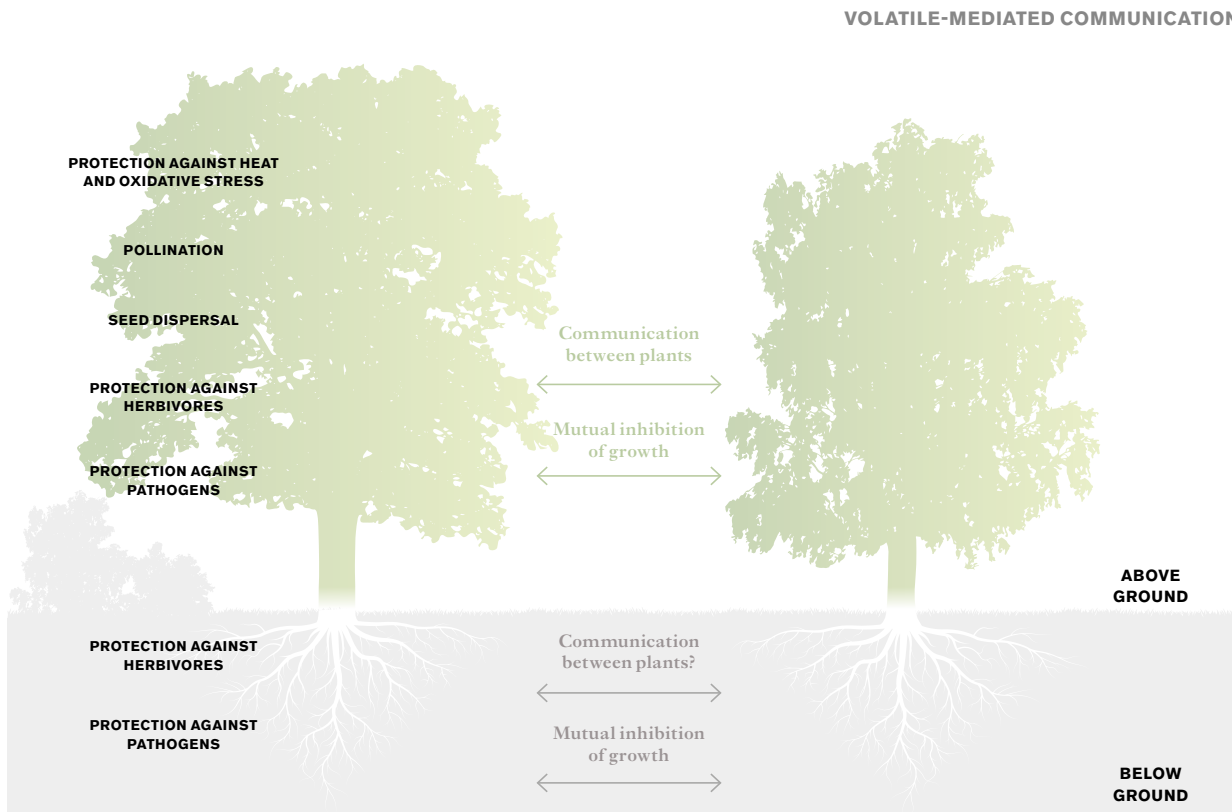
think it’s great that someone has managed to draw attention to the forest ecosystem,” says Unsicker.

While Wohlleben promotes greater appreciation of trees and the forest, Unsicker and her team continue to collect scientific data. In addition to the gypsy moth, trees usually have to deal with a host of other enemies. For example, black poplars are often attacked by rust fungi, which can spread to most of the tree crown in late summer. The fungal spores are blown onto the leaves by the wind or washed by rain onto lower parts of the crown. They then penetrate the leaves through the stomata. After a few days, they form spore deposits that penetrate the leaf surface as orange pustules from which new spores are released. In a pilot study, Unsicker’s co-worker Franziska Eberl sprayed some of the small poplars in

the greenhouse with rust spores and also put gypsy moth caterpillars on the leaves. An analysis of the leaf scents showed that leaves exposed to such a double attack gave off a completely different odor profile than leaves on which only caterpillars had chewed. “The leaves smell a bit like mushrooms. The proportion of terpenes is considerably lower, but the samples contain hydrocarbons that are typical for fungi,” explains Unsicker. The nitrogen-containing substances that predominate in the distress call to the parasitoid wasps are not affected by the rust.

Caterpillars love fungi

In particular, fungi can change the phenol content of leaves. For example,



Volatiles released from plants perform various tasks – both above and below ground. Trees thus attract beneficial insects such as pollinators, seed dispersers, and enemies of herbivorous insects. They are also in contact with each other – some volatiles can inhibit the growth of their neighbors or warn them of heat and pests.

GLOSSARY

GYPSY MOTH (*LYMANTRIA DISPAR*)

Moth belonging to the family of owlet moths. The name refers to the behavior of its larger caterpillars, which generally migrate each day from the leaves to rest in shaded spots. The eggs are laid on the trunks of the trees and survive the winter. The caterpillars hatch in the spring; they prefer to eat oak leaves but do not reject the foliage of other deciduous and coniferous trees.

Gypsy moths are among the most feared pests in oak forests. A single caterpillar can destroy one square meter of leaf surface from the time it hatches to the time it pupates. Their mass propagation can defoliate entire oak forests and cause permanent damage to the trees.

PHOTO: SYBILLE UNSICKER / MPI FOR CHEMICAL ECOLOGY



The researchers capture the gaseous odor signals of the leaves in plastic bags and pass them through a filter. They then determine the chemical structure of these volatile substances in the laboratory.

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they trigger the formation of catechins, the basic building blocks of various tanning agents, in the leaves. These are harmful to the fungus. Because rust can pervade the treetops in late summer, it could also influence the prevalence of other natural enemies of the poplars. But the real surprise for the researchers was that the fungal odor apparently does not bother the caterpillars! On the contrary: faced with the choice between healthy and fungus-infested leaves, the caterpillars clearly prefer the latter. Long-term observations even show that especially very young caterpillars are attracted by the odor. They first nibble the rust from the leaves before they attack the leaves themselves days later.

The researchers initially considered their findings to be a measurement error – the gypsy moth caterpillars

were thought to be pure herbivores. “We therefore conducted the same experiment with the closely related *Orygia antiqua* – with the same result: it is also crazy about the rust.”

High protein diet

At first, Unsicker suspected that the fungus-infested leaves might produce fewer toxins because the caterpillars develop faster on them and pupate earlier. But that is not the case. It is rather the components of the fungi that are the cause: the spores are rich in nitrogen and contain many amino acids and B vitamins essential for the development of the caterpillars. “Fungi and other microorganisms might therefore have had a stronger influence on the communal evolution of plants and insects than previously assumed,” says Unsicker.

The extent to which the findings can be transferred from poplars to other tree species is not yet fully understood. For example, during some years, oaks can suffer severe damage from massive infestations of gypsy moths. Forest rangers and forest owners currently spray forests with pesticides in order to limit the economic damage. “From an ecological point of view, it is quite natural that species like the gypsy moth repeatedly appear in great numbers. Nature would normally regulate this itself in each case because the natural enemies respond to the increased food supply and decimate the gypsy moth population again. Pesticides should therefore be used only in such a way – if at all – that enough predators will be left to keep future moth populations in check.”

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