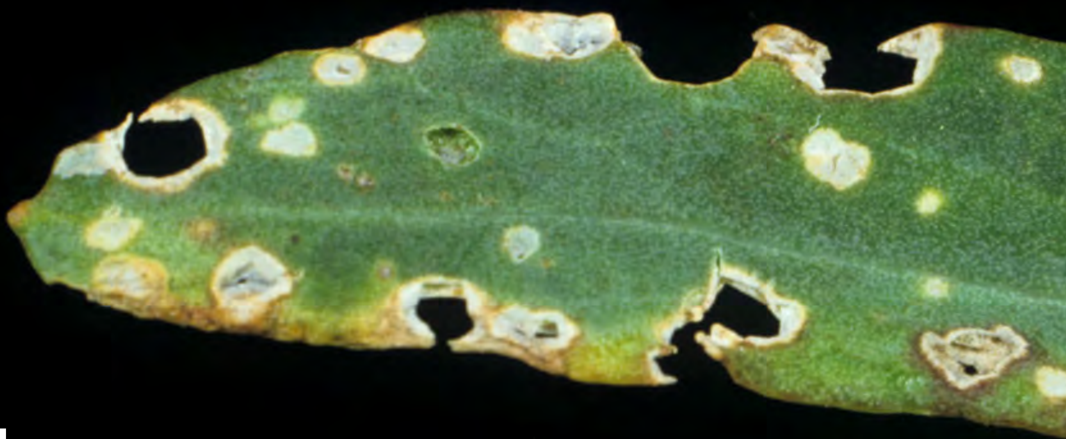


A Beetle
Overcomes a Plant's Defenses

Every cabbage plant conceals a bomb – a mustard oil bomb. For many insects, this makes the plant unpalatable. **Franziska Beran** from the **Max Planck Institute for Chemical Ecology** in Jena now knows, however, how insects can avert this danger: flea beetles, for example, outsmart the plants' defensive weapon and even commandeer it for their own protection.

Flea beetles are dreaded pests. When they are attracted in large numbers to rapeseed fields by pheromones released by other beetles of the same species, they can wreak havoc, leaving the foliage riddled with holes.

» To produce their mustard oil bombs, the beetles use components they produce themselves as well as substances they take up from plants on which they feed.

TEXT **KLAUS WILHELM**

Franziska Beran heard about the mustard oil bomb for the first time while studying biology at Humboldt-Universität zu Berlin. "It's a highly effective defense system that plants activate only when they are being eaten – a truly ingenious invention of nature," says Beran, who leads the Sequestration and Detoxification in Insects Research Group in Jena. Only plants in the crucifer family (Brassicaceae) are armed with this chemical defense system. The Brassicaceae include important sources of vegetables, spices and oils, such as white cabbage, broccoli, cauliflower, brussels sprouts, kohlrabi, mustard, oilseed rape, radishes and cress.

The mustard oil bomb is conceived as a two-component system. Component 1: mustard oil glycosides (glucosinolates), which are completely harmless. Component 2: the enzyme myrosinase. When a plant is attacked by, say, a beetle, the enzyme degrades the mustard oil glycosides to form spicy toxic substances. In the intact state, the plant stores the two components neatly separated so they don't come into contact with each other – so the bomb doesn't go off inadvertently. However, if the plant is injured, this separation breaks down and the mustard oil bomb is triggered.

The substances formed include mustard oils, or isothiocyanates, which give mustard its characteristic sharp taste. But isothiocyanates also interfere with the insects' digestion, so the mustard oil bomb can have a toxic effect. "This deters most natural enemies of cruciferous plants," Beran explains. This includes mainly insects, but isothiocyanates can also protect the plants against pathogenic fungi and bacteria.

Yet the mustard oil bomb doesn't work against all predators: some insects have, over the course of evolution, developed strategies to defuse the bomb. For instance, the voracious caterpillars of the cabbage white butterfly are unable to prevent the breakdown of mustard oil glycosides, but they accumulate a protein in their gut that interacts with the myrosinase to produce much less toxic nitriles instead of mustard oils.

Another group of insects that are not impressed by the mustard oil bomb are the flea beetles – beetles that jump like fleas. It is this trait that gave them their name, which is as apt as it is misleading, as they are not related to fleas. In some parts of the world, flea beetles are dreaded pests that result in substantial crop losses. In Canada, for example, they attack rapeseed fields in hordes, leaving the plant leaves riddled with holes.

A PEST IN ASIA

The infestation of crops by these pests has increased considerably in recent years, especially in Southeast Asia. During an internship at the World Vegetable Center in Taiwan, the Max Planck researcher observed first-hand how flea beetles can wreak havoc on valuable cruciferous crops. The biggest culprit is the striped flea beetle, *Phyllotreta striolata*. "When we planted bok choy or radish seedlings on Friday, the plants were already devoured by Monday."

On closer inspection, the rearmost of the three pairs of legs of flea beetles appear to be much thicker than the others. Inside is a kind of spring that

Theresa Sporer collects the beetles once a week from mustard plants and provides them with fresh plants (top). Because the beetles are powerful jumpers and are thus difficult to catch, Sporer uses a small handheld, battery-operated vacuum apparatus to capture them (bottom).

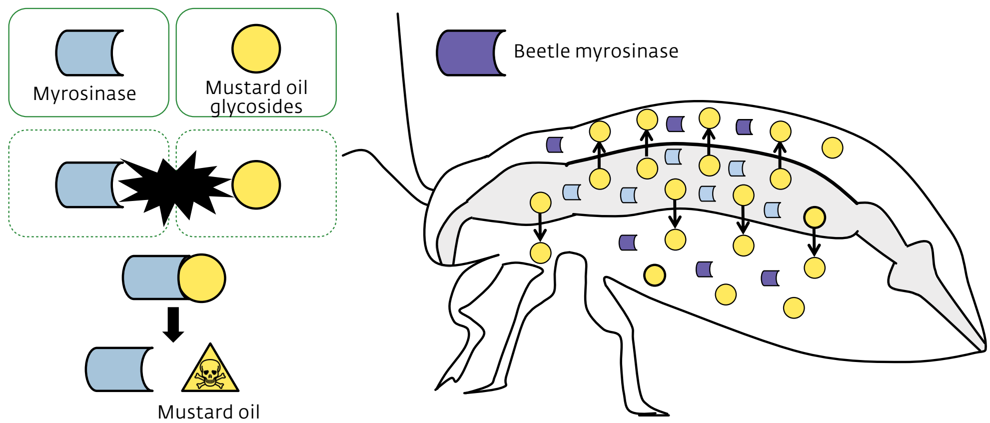
Photos: Anna Schroll (2)

the beetles can use to catapult themselves into the air to evade enemies. Beran's colleague Theresa Sporer, who cultivates the native horseradish flea beetle (*Phyllotreta armoraciae*) in the basement of the Max Planck Institute, is often treated to stunning athletic feats: the tiny insects, which measure only about three millimeters in length, achieve leaps of half a meter with ease. Once, a beetle escaped from the breeding room despite extensive security measures, and sated itself on the experimental plants of other colleagues in the Institute's greenhouse. "These colleagues were understandably not very pleased about this," says Sporer. Measures to prevent such breakouts have since been tightened.

A COMFORTABLE LIFE IN THE LAB

But why on earth would the little beetles want to escape? After all, they lead a very good life in their cozy basement. They have warmth and light and can chomp on mustard leaves to their heart's content. A few weeks after hatching, they lay eggs from which larvae develop. The larvae feed inside leaf stalks and finally pupate in the soil. Thanks to the lab's breeding program, "fresh" beetles are available daily for research. In the wild, in contrast, the horseradish flea beetle breeds just once a year. The striped flea beetle manages to do so up to nine times a year, thanks to the tropical climate in Asia.

Before turning her attention to the mustard oil bomb, Franziska Beran studied how striped flea beetles suddenly congregate on cabbages in hordes for



The principle of the mustard oil bomb: As long as the enzyme myrosinase and mustard oil glycosides are stored in separate cells, the substances are harmless. Only when they encounter each other, for example when the cells are injured, are toxic mustard oils formed. Flea beetles use components of various origins to produce their mustard oil bombs: the mustard oil glycosides are derived from the plants they feed on. They are absorbed through the gut and accumulate in the insects' body. The myrosinase contained in the plant, in contrast, remains unused and is synthesized by the beetles themselves.

a shared meal – despite only a few beetles having initially discovered the food source. The invasion is simply too fast and overwhelming for the plants' protection mechanism to be effective: the plants are consumed before they can mount a defense.

Beran, who is a native of Berlin, discovered that flea beetles are drawn to the feast by a pheromone. When a beetle finds a new food source, it emits a pheromone to attract fellow beetles, which can detect the scent over great distances. "Aggregation pheromones act in a similar way to sex pheromones, but in this case, they are released by the males and attract both sexes," Beran explains.

At first glance, it would appear to be counterproductive for a flea beetle to inform its fellow beetles when it has discovered food. After all, this seemingly selfless act attracts competitors to the scene. Nevertheless, it is beneficial for the beetles to beckon others, because together they can penetrate the hard outer layer of a leaf more quickly. The leaf layers that the beetles relish are only found under the waxy surface of many cruciferous plants. Studies have shown that each beetle can consume larger amounts of plant food when many animals eat together.

INVITATION TO THE FEAST

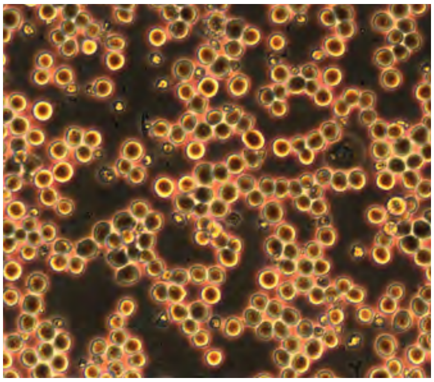
The male beetles don't release the pheromone signal until they have begun to munch on the plant. With their antennas, their fellow beetles can sense one millionth of a gram of pheromone and then follow the scent trail to its source.

Chemically, these pheromones are so-called terpenes, or more precisely, sesquiterpenes – a widely distributed group of secondary metabolic products that occur in fungi, bacteria and plants. These substances are responsible, for example, for the typical resinous odor of conifers. The biologist has shown that male *Phyllotreta striolata* beetles produce a terpene blend consisting of eight structurally related bicyclic sesquiterpenes. Franziska Beran has not yet been able to test the attractant power of the complete mixture, since not all the components are available. "Actually, it wasn't even necessary, because a mixture of just two terpenes reliably attracts the beetles."

Most insects don't produce terpenes. *Phyllotreta striolata* is an exception: according to studies Beran has carried out, the beetle possesses specific enzymes called terpene synthases. The researcher analyzed the genes that code for the enzymes and discovered that the beetles' enzymes differ from those found in bacteria and plants. This means that beetles have, so to speak, reinvented the production of terpenes: instead of using the terpenes of the plants they feed on or exploiting symbiotic bacteria that produce the substances, they synthesize the terpenes themselves.

Of course, these results raise the question of whether the terpenes can be used to the detriment of flea beetles, for example in the form of pheromone traps that selectively attract them. For Beran, however, this is a long way off. "We still know too little about how the beetles use their messenger substances to communicate," she says. She tested the effectiveness of pheromones in a field experiment and found that, although the artificial scents attract the beetles, they

Graphic: Franziska Beran/MPI for Chemical Ecology



Franziska Beran observes cultured insect cells under the microscope. The cells are genetically modified to produce the myrosinase of the striped flea beetle *Phyllotreta striolata* (bottom). Beran isolates the enzyme from the cells to investigate its properties.

Photo: Anna Schroll (top), Yannick Pauchet/MPI for Chemical Ecology (bottom)

are far less potent than the originals and are too weak to prevent the insects from feeding on crops. “Either all eight terpenes are needed, or other as-yet unknown substances play a role in the mass gatherings of the insects.”

But back to the mustard oil bomb. Thanks to the findings of the researchers in Jena, we now know that flea beetles take up certain mustard oil glycosides from their food plants and accumulate them in their body. In fact, mustard oil glycosides make up nearly 2 percent of the beetles’ body weight. The scientists now want to find out how the beetles prevent the mustard oil bomb from going off while they are feeding on the plants. After all, injury to the plant is supposed to cause myrosinase to break down the mustard oil glycosides into toxic mustard oils.

In fact, the beetles don’t appear to be completely immune to the mustard oil bombs of cruciferae. Plants with very high myrosinase activity and a correspondingly high isothiocyanate content are better protected against the pests. “This means that the beetles are unable to completely disarm the plants’ defense system,” says Beran.

But what do the beetles do with the stored mustard oil glycosides? Is it possible that they have their own mustard oil bomb? In that case, the necessary enzyme would also have to be present. In fact, an analysis of the genome of flea beetles has revealed that they have the blueprint for producing myrosinase in their genes. “The beetles developed, independently of the plants, their own enzyme that breaks down mustard oil glycosides to form isothiocyanates. The

Below Sticky trap in a cabbage field in Taiwan. The trap is baited with an artificially produced beetle pheromone. The wind carries the scent into the surroundings and attracts the pests so that they become stuck in the trap.

Right During an internship, Franziska Beran observed first-hand the damage flea beetles can wreak on economically important cabbage varieties in Asia. Her research has since revealed a few secrets of the tiny insects. She hopes that her findings will help farmers combat the pests both selectively and in an environmentally friendly manner.

mustard oil bomb of the cabbage flea beetle is therefore based on myrosinase that it produces itself and mustard oil glycosides obtained from food plants," Beran explains.

It is conceivable that the beetles' real enemies aren't other animals – after all, their powerful legs enable them to deftly leap away – but rather pathogens such as bacteria and fungi. Or the mustard oil bomb may protect the beetle larvae, which feed on roots in the soil, where they are exposed to numerous enemies, including bacteria.

Flea beetles have therefore not only learned to render the defenses of their feed plants harmless; they also appropriate them for their own protection. Scientists can thus learn a great deal about the interrelationships between plants and insects from the tiny long-jumpers – knowledge that may one day be used to protect agricultural crops. ◂

TO THE POINT

- **Cruciferous plants convert mustard oil glycosides into toxic isothiocyanates with the help of the enzyme myrosinase. Only when the plants are being eaten does this enzyme come into contact with the glycosides to form toxins.**
- **Flea beetles can at least partially prevent the breakdown of mustard oil glycosides by myrosinase, thus circumventing the plants' defense system. They store some of the mustard oil glycosides in their body and use them together with self-produced myrosinase for their own protection.**
- **Flea beetles release a mixture of terpenes to attract fellow beetles to food plants. Together, the beetles are better able to penetrate the hard leaves of the plants.**

GLOSSARY

Flea beetles: These beetles measure about one and a half to three millimeters in size and are dark blue to black or yellow striped. They overwinter in the soil, and the females lay their eggs in young plants in the spring. The inconspicuous white larvae of flea beetles feed on plant roots or burrow into leaf stalks without causing significant economic damage. The young beetles appear in summer. They feed on leaves, boring round holes in them. Flea beetles can transmit plant pathogens such as radish mosaic virus. Fine-meshed insect netting can prevent the beetles from reaching the plants on which they feed.

Photos: Franziska Beran (left), Anna Schroll (right)