Manduca sexta caterpillars' developed surprising detoxification mechanism against their host plant's sweet toxin

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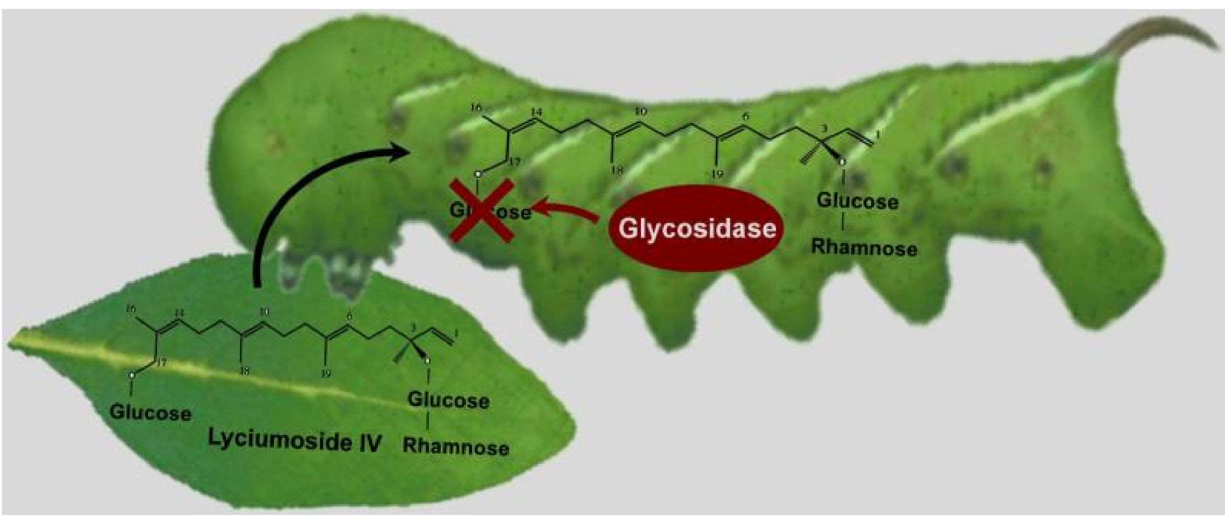
A Manduca sexta larva is feeding on a tobacco plant. Credit: Copyright: Anna Schroll

Plants produce a large arsenal of toxic compounds in order fend off herbivorous insects. To make sure that the toxicity of these defensive substances will not harm the plants themselves, many plants add a sugar molecule to some of their toxins. Digestive enzymes called glycosidases in the insect gut usually cleave off this sugar to release the toxin—with harmful effects on the insects. Scientists at the Max Planck Institute for Chemical Ecology in Jena, Germany, now found the opposite mechanism: a defensive compound of the wild tobacco species *Nicotiana attenuata* which is toxic with sugar molecules bound to it and a glycosidase in the gut of the tobacco hornworm *Manduca sexta* which removes one sugar from this toxin to convert it to a non-toxic form. This is the first time that the role of deglycosylation in detoxification as an insect counter-adaptation could be shown (*Nature Communications*, October 2015).

Plants add sugars to their defensive compounds in order to avoid self-intoxication and to facilitate their transport and storage. The process of adding sugar to a chemical compound is called glycosylation. When herbivorous insects ingest these glycosylated toxins while feeding on plants, sugar-cleaving gut enzymes called glycosidases cleave these sugars from the defensive substances and the toxins are released in the midgut. The toxins can then exert deleterious effects on the insects' growth and fitness.

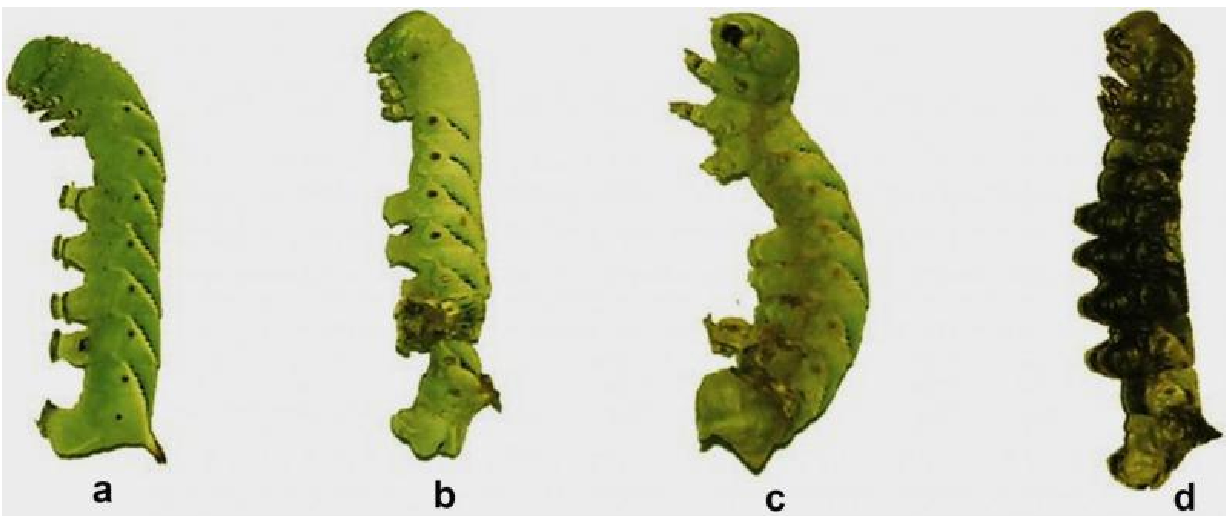
Scientists from the Max Planck Institute for Chemical Ecology examined defensive substances in the coyote tobacco *Nicotiana attenuata*. They were especially interested in a compound named lyciumoside IV which contains three sugar molecules and is toxic to larvae of the tobacco hornworm *Manduca sexta*. The ingestion of lyciumoside IV should usually cause severe body mass reduction in these caterpillars. It was thought that the sugar molecules were removed from lyciumoside IV in the *Manduca sexta* midgut and the released toxin caused these deleterious effects. However, what the researchers discovered contradicted the current understanding of the role of deglycosylation: lyciumoside IV is not completely deglycosylated, and what is more important, this compound itself and not its deglycosylated form is toxic. Glycosidases in the midgut of the tobacco hornworm larvae remove only one sugar molecule from lyciumoside IV which converts the toxin into a novel compound. Ingestion of this compound exerts no detrimental effects on the insects as those exerted by the ingestion of lyciumoside IV, suggesting that this novel compound is a detoxified form of lyciumoside IV. For the first time, scientists showed that removing a sugar molecule from a plant's defensive compound can also result in detoxification.



Schematic representation of lyciumoside IV deglycosylation by a glycosidase in the gut of *Manduca sexta* caterpillar. Credit: Sagar Pandit, Max Planck Institute for Chemical Ecology

"That glucosidases which play an important role in digestion and toxin activation also function the opposite way and detoxify plant defensive substances, opens a new dimension of the plant-herbivore arms race.

This discovery of an unusual detoxification mechanism can be mainly credited to our unbiased, reverse genetics-based 'ask the herbivore' and 'ask the ecosystem' approaches. The results obtained by using these approaches enabled us to find, understand and explain the mechanism which functions exactly contrary to the current understanding of glycosidases and detoxification mechanisms. Thus, this unbiased approach is one of our major contributions to the understanding of the complexity of plant defenses and insect adaptations," explains Sagar Pandit, one of the senior authors of the study.



Molting failure was caused by lyciumoside IV ingestion in glycosidase-silenced *Manduca sexta* caterpillars (b, c, d) in comparison to the control (a). Credit: Sagar Pandit, Max Planck Institute for Chemical Ecology

The study revealed the significance of this unusual detoxification mechanism to the caterpillars in nature. The scientists first found the glycosidase responsible for removing the sugar from lyciumoside IV. Then they generated larvae with a suppressed glycosidase activity by silencing the respective larval glycosidase gene to study the effect of lyciumoside IV ingestion on the disabled larvae. The larval gene was silenced using a modern method called "plant-mediated RNA interference" in which transgenic tobacco plants were generated to produce the specific gene-silencing signal, which was transferred to larvae feeding on these plants. Interestingly, when the glycosidase-silenced larvae ingested lyciumoside IV, they failed to molt and eventually died. Glycosidase-silenced larvae which ingested the deglycosylated product did not show such molting failure and mortality. This suggested that the detoxification of lyciumoside IV is necessary to avoid these deleterious effects.

Many herbivores are known to store their host plant's toxic compounds in specialized compartments of their body, a process which is called sequestration. Usually, herbivores use sequestered compounds as their own defense against predators and parasites. The scientists therefore examined whether lyciumoside IV or its detoxified product protect *Manduca sexta* caterpillars against their natural enemies. Field experiments with glycosidase-silenced caterpillars revealed that the wolf spider *Camptocosa parallela*, a predator of tobacco hornworm larvae, captured and killed about the same number of glucosidase-silenced larvae and controls, but ingested significantly fewer glucosidase-silenced larvae. Larvae that had been coated with lyciumoside IV deterred the spiders; such deterrence was not observed when they were coated with the detoxification product. This clearly demonstrates that lyciumoside IV would have a protective effect if it was sequestered by the larvae. However, as the researchers showed, *Manduca sexta* larvae prefer to detoxify this plant defensive compound rather than sequestering it. The scientists infer that these larvae have not yet evolved the mechanism to co-opt lyciumocide IV and so they have to detoxify it in order to avoid the molting failure and mortality caused by its ingestion.

Now, the scientists want to find out whether there are natural *Manduca* *sexta* variants or related *Manduca* species that have already evolved a mechanism to co-opt lyciumoside IV and simultaneously avoid mortality and impairments. They are also interested in the question why the deglycosylation is restricted to the removal of only one sugar. By studying the responses to lyciumoside IV in *Nicotiana attenuata*'s other specialist and generalist herbivores, they want to shed more light into this particular plant defense and the insects' counter-adaptations. [AO]

More information: Spoorthi Poreddy et al. Detoxification of hostplant's chemical defence rather than its anti-predator co-option drives β-glucosidase-mediated lepidopteran counteradaptation, *Nature Communications* (2015). DOI: 10.1038/ncomms9525

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