# Press Information

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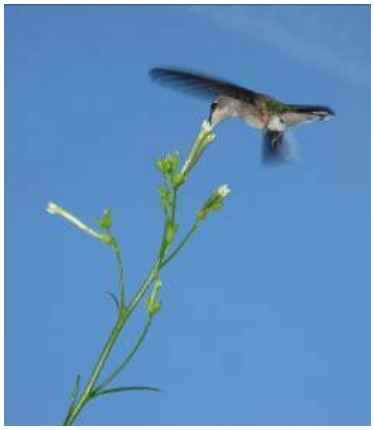
Max Planck Institute for Chemical Ecology

## Changing flowering times protect tobacco plants against insect herbivory

### Messenger molecule in oral secretions of herbivorous insects changes flower opening time of their host plants: Hummingbirds take over role as pollinators from moths

Butterflies and moths are welcome visitors to many plant species. Plants attract insect pollinators with the colors, forms, nectars and scents of their flowers to ensure fertilization and reproduction. However, female moths are also threatening to the plant: Once attracted by the flower's scent, they lay their eggs on the green leaves, and shortly voracious young caterpillars hatch. Scientists from the Max Planck Institute for Chemical Ecology have now discovered how tobacco plants successfully solve this dilemma. The researchers found that herbivory changed the opening time of the flower buds from dusk to dawn. In addition the emission of flower scents was dramatically reduced. This change in flower timing was elicited by specific molecules in the oral secretions of the larvae, and required the jasmonate signaling cascade, which is known to elicit a host of other defense responses in plants. Instead of night-active moths, these morning-opening flowers attract day-active hummingbirds which are also able to transfer pollen - without threatening the plant's life. (Current Biology, online first, January 21, 2010)



*Picture: Wild tobacco (Nicotiana attenuata) native in North America is flowering during the nighttime and attracts night-active moths as pollinators by emitting the attractant benzyl acetone. However, as soon as female moths start laying their eggs on the plant and the young caterpillars become a serious danger, the plant postpones the opening time of the flowers by 12 hours to dawn and additionally stops producing benzyl acetone. Moths stay away and hummingbirds take over pollination.*

*Photos: Danny Kessler, Max Planck Institute for Chemical Ecology, Jena, Germany*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MAX-PLANCK-GESELLSCHAFT

**Outbreak of tomato hornworms**

During field experiments performed by PhD students of the Department of Molecular Ecology headed by Prof. Ian T. Baldwin in the Great Basin Desert of Utah (USA) in summer 2007, a massive outbreak of tomato hornworms (*Manduca quinquemaculata*) occurred. Almost every tobacco plant of the native species *Nicotiana attenuata* on the field site was attacked by these herbivores which prefer plants of the nightshade family. Danny Kessler intensively studied the infested plants and noticed that these plants had many flowers that opened after sunrise – although tobacco is typically a night-flowering plant and usually opens its flower buds after sunset. This finding resulted in experiments conducted in the following two years that showed that the flowering time postponed by 12 hours was directly related to herbivory.

**Pollination wanted, but no oviposition**

Ecologists had already noticed that female moths attracted for pollination laid their eggs, and shortly leaf-eating larvae hatched to feed on the same plant. The scientists considered whether plants would actually submit without reserve to this life-threatening disadvantage - just for pollination. They intensively studied the remarkable morning-opening flowers (MoF) which were only produced by plants that had been attacked by insect larvae and compared them to the usually occurring night-opening flowers (NoF). The first experiment already revealed an astounding result: MoF did not emit the attractant benzyl acetone anymore (see also Kessler et al., Science 321, 2008) and also the sugar concentration in the floral nectar was considerably reduced. Furthermore, it was striking that the petals of MoF only opened to a third of the size of NoF. All in all, the MoF were rendered literally unnoticeable by the moths – however, they may become interesting for different pollinators living nearby the field station: hummingbirds.

**Hummingbirds visit the morning-opening flowers and serve as pollinators**

To find out whether moths or birds successfully transferred pollen from flower to flower, the scientists determined the outcrossing rate of plants visited by moths or hummingbirds in field experiments. They removed the anthers from young flower buds to rule out self-pollination. Then an unattacked and an insect-attacked tobacco plant were covered with a mesh-covered wire cage until the morning of the next day to exclude night-active pollinators. A second pair of plants remained uncovered and thereby accessible to night-active pollinators. Before dawn the cages were exchanged, so that the plants that had been uncovered during the night were now covered and the plants that had been covered at night became accessible to pollinators during the day. In the evening all experimental plants were covered and the plants remained so until seed capsules were produced. Counting of the capsules revealed that a significant majority of capsules on plants that had not been attacked by caterpillars originated from flowers that were pollinated during the night between 8:00 p.m. and 6:00 a.m., whereas in caterpillar-infested plants successful pollination had occurred in majority during the day between 6:00 a.m. and 8:00 p.m., therefore by hummingbirds.

The scientists verified the assumption that actually hummingbirds visit the MoFs and drink their nectar by directly observing and counting out more than 1000 flowering wild tobacco plants. 18 humming bird visitations were intensively studied which showed hummingbirds visiting larvae-infested plants. As a matter of fact, more than 90% of the birds preferred the MoF compared to NoF, even if only a few MoF were on a plant. “It is likely that the hummingbirds can recognize the special shape of the partially open corollas of the MoF in the morning and associate these characteristics with the reliable quality and quantity of the nectar in these flowers,” says Celia Diezel, co-author of the study.

**Experiments using larval oral secretions and transgenic tobacco plants**

In further experiments the scientists studied how attacked plants recognize herbivory and subsequently change the developmental program of the flowers to favor hummingbirds. Instead of infesting the plant by putting caterpillars on the leaves, the researchers mechanically wounded a leaf with a pattern wheel and applied oral secretions from hornworm larvae on the wounds. The plant reacted as after direct insect attack: After approximately 3 days more morning-opening flowers compared to non-induced plants were produced. “Maybe the fatty acid amino acid conjugates present in the oral secretions of the larvae elicit this reaction. We already know that they switch on the plant’s defense against herbivory, for instance by producing toxic substances to fend off the attacker,” Danny Kessler, PhD student at the institute, explains. In an additional

experiment he used genetically modified tobacco, in which the signaling pathway between the messenger molecule in the oral secretion and the defense reaction was interrupted; these plants were unable to produce jasmonate, a plant hormone initiating plant defense responses. In fact, the transgenic jasmonate-deficient plants used in the field experiment did not produce MoF after spit induction, but could if the plants were sprayed with jasmonate, which showed that the reprogramming of the flower production is actually related to the pathway that switches on defense mechanisms.

Why do plants risk attracting tomato hornworm moths as pollinators, although the insects' larvae feed on the plants? "We cannot answer this question from the perspective of one single plant, but, if at all, from an evolutionary and ecological background," says Ian Baldwin. Wild tobacco populations grow on vast areas after fires, comparable to synchronized monocultures with thousands of widespread plants. Hummingbirds may not be the most reliable pollination service the plant species needs for outcrossing and reproduction. Using volatiles, the plants can attract moths from large distances, whereas hummingbirds are only available, if their nests are accidentally in the vicinity of the tobacco populations. Moreover, looking at the special mode of hummingbird pollination, it is more likely that flowers of one single plant are pollinated with pollen from the same plant than from flowers of different plants. This can decrease the genetic variability of the seeds produced. Moths may move more frequently among plants and this behavior may results in greater genetic variability for the seed produced from their pollination services. [JWK/AO]

**Original Publication**

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