Isomers of green leaf volatiles in Nicotiana attenuata and their role in plant-insect interactions

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SUMMARY

Plants use volatiles like we use our voice – for communication: we can speak softly or loud, in a pleasant or aggressive tone, and we use different phonemes to form different words and to transfer different massages. Likewise, plants can tune their volatile bouquet by releasing different volatile compounds in variable amounts thereby providing their surroundings with information about their physiological state or the type of attacker. Since plants are frequently attacked by herbivores, they have developed sophisticated defense strategies. Some defenses are direct and constitute accumulation of feeding deterrents and toxins. Others are indirect and constitute the attraction of natural enemies of herbivores via the release of specific volatile signals. Fatty-acid-derived green leaf volatiles (GLVs) are an important group of herbivore induced plant volatiles which can function as indirect defense response. They are rapidly released after wounding or herbivore attack and can thus provide an immediate signal to the environment. Many herbivores, especially the ones that chew leaf material, produce oral secretions (OS) while eating, and these OS play an important role in the fine tuning of a plant's volatile bouquet; OS contain small molecules and enzymes which are recognized by the plant upon contact with the wounded leaf surface and which elicit specific defense responses including the release of herbivore-induced plant volatiles.

In this thesis I described the biosynthesis of GLVs in the wild tobacco *Nicotiana attenuata*; how herbivory by the tobacco hornworm *Manduca sexta* affects the plant's GLV composition and what the consequences of these herbivore-induced changes are for herbivores and predators.

GLVs are derived from the oxylipin pathway, and this pathway consists of several branches. While one of these branches leads to the formation of GLVs another results in the formation of the plant hormone jasmonic acid (JA). Both branches use precursors derived from the action of the enzyme lipoxygenase (LOX), which incorporates molecular oxygen into fatty acids. Plants can possess more than one LOX and often these LOXs provide substrate only to a specific branch of the oxylipin pathway. We showed that two LOXs of the wild tobacco *N. attenuata* (NaLOX2 and NaLOX3) supply only specific branches: while NaLOX3 activity is necessary for the formation of JA, NaLOX2 activity is needed to produce GLVs. This we concluded by generating transgenic tobacco plants with reduced expression of either *NaLOX2* (ir-*lox2*) or *NaLOX3* (ir-*lox3*) (**Chapter 2**).

GLVs comprise a group of volatile compounds with a C6 chain as backbone. The most abundant GLV released from tobacco plants is (*Z*)-3-hexenal. This aldehyde can be further metabolized to its corresponding alcohol or ester, but it can also be converted to its (*E*)-isomer, (*E*)-2-hexenal. We showed that mechanically damaged leaves of the wild tobacco *N. attenuata* release large amounts of (*Z*)-GLVs and small amounts of (*E*)-GLVs, but that feeding by the specialist herbivore *M. sexta*, or the application of their OS onto leaf wounds, dramatically shifts the isomer ratio from (*Z*)- to (*E*)-GLVs. Although (*Z*)- and (*E*)-isomers have the same molecular formula their fragrances can differ tremendously. For example, while (*Z*)-3-hexenal mainly accounts for the characteristic smell of freshly cut grass, (*E*)-2-hexenal has a fruity, pungent smell. In field experiments we showed that the big-eyed bug *Geocoris*, a natural enemy of tobacco hornworms, which feeds on *Manduca* eggs and early larval instars, can distinguish between (*Z*)- and (*E*)-GLVs and between different (Z)/(E)-ratios and thus uses the herbivore-induced changes in the GLV profile to find plants with prey. Surprisingly, the re-arrangement from (*Z*)-GLVs to (*E*)-GLVs was catalyzed by an enzyme present in the OS of *M. sexta* caterpillars indicating that the insect is responsible for its own betrayal (**Chapter 3**).

Not only *Geocoris*, but also female *Manduca* moths are able to distinguish between (*Z*)- and (*E*)-GLVs and to use this information for choosing appropriate host plants for their offspring. We showed that different regions in the brain (the antennal lobe) of *M. sexta* females are activated upon stimulation with (*Z*)- or (*E*)-isomers. When tested in the field female moths laid more eggs on those plants that emitted only or more (*Z*)-GLVs and no or less (*E*)-GLVs (**Chapter 4**). In this way female moths could avoid those plants that are already occupied by competitors and which have likely already induced their defenses.

We have made the first attempts in identifying the enzyme from *M. sexta's* OS that converts (*Z*)-3- to (*E*)-2-hexenal. We collected large amounts of OS from *M. sexta* caterpillars and we purified with several biochemical fractionation techniques an active compound, which was larger than 50kDa and active over a wide pH range (**Chapter 5**). With the forthcoming genomic and transcriptomic databases of *Manduca sexta* we are confident to succeed in identifying this isomerase.