Isomers of green leaf volatiles in Nicotiana attenuata and their role in plant-insect interactions
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Why do you whisper, green grass?

Why tell them all your secrets;
who kissed there long ago?

Don't you tell it to the breeze;
or she will tell the birds and bees
and everyone will know …
CHAPTER 6

GENERAL DISCUSSION

In this thesis I have introduced a novel biological process by which plant-derived green leaf volatiles (GLVs) can undergo modifications, prior to their release into the environment, as such that they contain an herbivore-specific signature. Previously the idea that GLVs serve as attractant for predators and parasitoids was generally accepted despite the fact that it was unclear how such natural enemies could differentiate between GLVs resulting from aspecific mechanical damage and those from the feeding activities of their actual prey. Hence, this thesis does not only show that the GLV bouquet of herbivore attacked plants indeed contain an herbivore-specific signature which attracts foraging natural enemies (Chapter 3) and repels conspecific gravid moths (Chapter 4), but also that this signature is due to the action of an enzyme present in the oral secretions of M. sexta caterpillars (Chapter 3 and 5). In this thesis I also report on one of the key upstream biosynthetic components of GLVs in the wild tobacco Nicotiana attenuata: a 13-lipoxygenase (NaLOX2), which specifically supplies substrate for the formation of GLVs (Chapter 2). The knowledge on this biosynthetic pathway has expanded rapidly and recent literature provides exciting additional insight into the role that NaLOX2 might play in the defense response of N. attenuata:

FACs are important plant elicitors present in M. sexta’s OS; they are responsible for the initiation of herbivore-specific defense responses in N. attenuata, including the transcriptional up-regulation of several defense-related genes (Halitschke et al., 2003), the accumulation of JAs and ET (Kahl et al., 2000; Wang et al., 2007) and related secondary metabolites (Zavala et al., 2004; Schuman et al., 2009; Heiling et al., 2010). The most abundant FAC in the OS of M. sexta caterpillars is N-linolenoyl-glutamate (18:3-Glu) (Halitschke et al., 2001). Recently it has been shown that 18:3-Glu is rapidly metabolized to 13-hydroperoxy-18:3-Glu when applied onto N. attenuata’s wounded leaf surface and subsequently converted to its derivatives 13-hydroxy-18:3-Glu and 13-oxo-13:2-Glu (VanDoorn et al., 2010). Interestingly, these modifications were largely dependent on the activity of NaLOX2; plants with reduced expression of NaLOX2 (ir-lox2) were less effective in oxidizing the fatty acid moiety of 18:3-Glu and had a much slower turnover rate of this FAC. Furthermore, these modifications resulted in the formation of an inactive compound, i.e. 13-hydroxy-18:3-Glu, and an active elicitor, 13-oxo-13:2-Glu, which did induce JA-accumulation as efficiently as 18:3-Glu (Fig. 6.1), but additionally induced higher emissions of two novel monoterpenes compared to 18:3-Glu elicitation (VanDoorn et al., 2010a; VanDoorn et al., 2010b).
Figure 6.1. NaLOX2 is involved in the modification of plant- and insect-derived fatty acid derivatives. When *M. sexta* feeds on *N. attenuata* leaves the FAC 18:3-Glu is introduced into leaf wounds and rapidly dioxygenated by NaLOX2 to 13-OOH-18:3-Glu. 13-OOH-18:3-Glu is further metabolized to the inactive 13-OH-18:3-Glu and the active 13-oxo-13:2-Glu (VanDoorn et al., 2010). Leaf wounding and herbivory also induces hydrolysis of α-linolenic acid and linoleic acid from plant membranes and these unsaturated fatty acids are dioxygenated as well by NaLOX2 as part of the GLV-pathway. The resulting 13-hydroperoxides (13-HPs) are cleaved between C11 and C12 by a hydroperoxide lyase (NaHPL) generating C6-aldehydes ((Z)-3-hexenal or n-hexanal) and 12-oxo-(9Z)-dodecenoic acid ((9Z)-traumatin). (9Z)-traumatin can undergo several enzymatic or non-enzymatic modifications with 9-hydroxy-12-oxo-(10E)-dodecenoic acid (9-OH-traumatin) as one of its main products which is in large parts generated by NaLOX2 activity. Other derivatives that are formed are, amongst others, 12-OH-(9Z)-dodecenoic acid, 12-oxo-(10E)-dodecenoic acid ((10E)-traumatin), (3Z)-dodecenedioic acid ((3Z)-traumatic acid) and (2E)-dodecenedioic acid ((2E)-traumatic acid). 9-OH-traumatin can be conjugated to reduced glutathione (GSH). This figure is adapted from Kallenbach *et al.* (2011); background image with permission of Anja Paschold.
The GLV-branch of the oxylipin pathway does not only lead to the formation of GLVs; the NaLOX2 derived 13-HPs are cleaved by NaHPL into a C6- and a C12-compound. Thus, with each molecule of (Z)-3-hexenal or n-hexanal also one molecule of (9Z)-traumatin is formed (Fig. 6.1). (9Z)-traumatin undergoes rapid enzymatic and non-enzymatic modifications like the isomerization of double bonds from (Z) to (E) and the oxidation of the aldehyde groups to carboxyl groups (Grechkin, 2002; Kallenbach et al., 2011; Mukhtarova et al., 2011). All (9Z)-traumatin derived compounds are referred to as traumatins hereafter.

In a recent study from Kallenbach et al. (2011) it was shown that almost 98% of the (9Z)-traumatin was converted to 9-OH-traumatin and that two thirds of this conversion were dependent on NaLOX2 activity. When a 1:1 mixture of (9Z)-traumatin and 9-OH-traumatin was applied onto wounded leaves of plants with reduced expression of NaLOX2 and NaLOX3 (ir-lox2/ir-lox3) this caused an up-regulation of several oxidative stress and defense related genes suggesting that the C12-cleavage products of NaHPL might also play important roles in mediating plant responses to wounding and herbivory in *N. attenuata*.

Traumatins have been shown to promote cell division after wounding (English and Bonner, 1937; Zimmerman and Coudron, 1979; Farmer, 1994; Ivanova et al., 2001) and because of this characteristic they have been referred to as ‘wound hormone’. This potentially very important function has been studied already in the early 20th century (English and Bonner, 1937), but in more recent years the research relayed its focus to the functional characterization of GLVs i.e. the C6-portion of HPL cleaved 13-HPs. This is not surprising given the diverse functions that have been reported for GLVs and that have been summarized in the general introduction. Shortly, some GLVs have antimicrobial properties against several bacteria and fungi; they can serve as an anti-herbivore compound by repelling or poisoning herbivores or as feeding stimulant; they mediate indirect defenses by attracting predators and parasitoids; but they can also attract herbivores; and finally they also induce and prime defense responses in neighboring plants or in adjacent leaves or branches (for review see Matsui, 2006).

Our discovery of a (3Z):(2E)-enal isomerase in the oral secretions of *M. sexta* caterpillars raises many questions. For example, whether this isomerase is specific and unique to *M. sexta* caterpillars or whether it is also present in other (lepidopteran) species. First of all, we recently detected isomerase activity in the OS of *Spodoptera littoralis* (Fig. 6.2) and secondly, also Turlings et al. (2004) reported that maize, damaged by *S. frugiperda*, emitted far more (E)-2-hexenal than maize damaged by *S. exigua*, suggesting that isomerase activity exists also in other lepidopteran species. Future work will reveal how widespread this enzyme
is and whether homologs are also present in plants (Takamura and Gardner, 1996; Noordermeer et al., 1999).

![Bar graph showing percent conversion to (E)-2-hexenal by OS from different lepidopteran species. OS from M. sexta, S. exigua, and S. littoralis were diluted 1:20 with 0.02% Tween-20 and the percent conversion to (E)-2-hexenal was determined (M. sexta n=6; S. exigua, S. littoralis n=3) using the in vitro assay as described in Chapter 3. The percent conversion to (E)-2-hexenal in control samples (only 0.02% Tween without OS) has been subtracted from these values (bars 1-3 have already been presented in Chapter 3, Fig. S3.6).](image)

**Figure 6.2. (3Z):(2E)-enal isomerase activity is also present in the OS of Spodoptera littoralis.** Percent conversion to (E)-2-hexenal by OS from different lepidopteran species. OS from *M. sexta*, *S. exigua* and *S. littoralis* were diluted 1:20 with 0.02% Tween-20 and the percent conversion to (E)-2-hexenal was determined (*M. sexta* n=6; *S. exigua*, *S. littoralis* n=3) using the in vitro assay as described in Chapter 3. The percent conversion to (E)-2-hexenal in control samples (only 0.02% Tween without OS) has been subtracted from these values (bars 1-3 have already been presented in Chapter 3, Fig. S3.6).

The most puzzling question however is: why does *Manduca* produce an enzyme that generates volatiles which betray the insect to its enemies and so why did evolution not select against this isomerase? Possibly the enzyme really is maladaptive and is, or will be, under negative selection. However, the occurrence of this specific isomerase activity in at least two lepidopteran species (Fig 6.2) suggests that it may have either (a) vital or non-vital but beneficial function(s) that outweigh the caterpillar’s net costs of maintaining such an enzyme. If the enzyme has a vital primary function for the caterpillar’s physiology this would prevent rapid selection against it.

The fact that pathogen and herbivore-derived elicitor compounds are generally present across species indicates that these have beneficial functions which soften the selection on them. For example, flagellin and chitin both act as pathogen-associated molecular patterns (PAMPs), but they also have very obvious beneficial functions for motion and structural integrity in bacteria and fungi, respectively (Zipfel, 2009). However, for plant-herbivore interactions, data, as well as our knowledge, on occurrence and maintenance of elicitors is more limited as, for example, the persistence of FAC elicitors for long did not have an obvious explanation. However, recently Yoshinaga *et al.* (2008) reported that FACs are used by the insect for nitrogen assimilation and glutamine storage and are thus vital for the insect’s
survival. Hence revealing the spectrum of physiological properties of elicitors will be essential for understanding why they emerge, persist, disappear and possibly re-appear through time and influence species interactions.

To provide a frame work for understanding the forces that could influence the persistence in isomerase production in *M. sexta*, and probably also in other species, I will discuss possible costs and benefits for *M. sexta* in the following paragraphs (summarized in Fig. 6.3).

*Possible benefits of a (3Z):(2E)-enal isomerase for M. sexta caterpillars:*

1) Direct positive effects: Cell survival/antimicrobial effects in *M. sexta* caterpillars:

From greenhouse experiments we know that *M. sexta* caterpillars grew smaller on transgenic *N. attenuata* plants that produced less GLVs and that larval performance was restored to WT levels when synthetic GLVs were added to the transgenic plants (Halitschke et al., 2004; Meldau et al., 2009). Interestingly, the reduced caterpillar performance on GLV-depleted plants was not caused by an increase in plant defense responses but by a reduced consumption of leaf material (Halitschke et al., 2004). This clearly suggests that outside of a natural environment and thus without having to deal with natural enemies and competitors, the presence of GLVs has a positive and probably direct effect on *Manduca*'s health/feeding behavior. While previous papers suggested that these volatiles act as feeding stimulants (Halitschke et al., 2004; Meldau et al., 2009) there is also plenty of room for alternative, not mutually exclusive, explanations. Firstly, *M. sexta* caterpillars may actively convert (Z)-3-hexenal to (E)-2-hexenal to protect themselves from endogenous and exogenous stresses: (E)-2-hexenal, but not (Z)-3-hexenal is a reactive electrophile species (RES; Alméras et al., 2003) as it possesses an α,β-unsaturated carbonyl group which shows high reactivity with nucleophilic atoms and can cause protein carbonylation (Farmer and Davoine, 2007; Mueller and Berger, 2009). RES induced carbonylation often leads to the inactivation of proteins (Liebler, 2008). However, it can also result in the activation of Cap’n’collar (Cnc) proteins which play an important role in regulating cellular defenses against oxidative and electrophilic stress (Nguyen et al., 2009). Cnc proteins are a family of basic leucine zipper transcription factors (TFs) that are conserved in insects, fishes, birds, and mammals (Sykiotis and Bohmann, 2010). The most studied Cnc TF is the vertebrate homolog Nrf2 (Dinkova-Kostova and Talalay, 2008; Sykiotis and Bohmann, 2010). In non-stressed cells Nrf2 is
targeted for degradation by the Keap1 protein. Keap1 associates with a Cullin3-dependent ubiquitin ligase (E3) complex that ubiquitinates Nrf2 leading to its degradation by the proteasome. Keap1 functions as a sensor of free RES: RES can modify the critical Cys residues in Keap1 (Dinkova-Kostova and Talalay, 2008), which results in a conformational change in Keap1 disabling E3 to ubiquitinate Nrf2 (Surh et al., 2008; Nguyen et al., 2009). The released Nrf2 escapes proteasomal degradation and transactivates the expression of several cytoprotective genes which enhance the cell survival (Kensler et al., 2007). So far Cnc TFs have not been reported for *M. sexta* and it thus needs to be tested whether Manduca caterpillars possess such Keap1/Nrf2- or similar RES activated pathways with cytoprotective functions; whether (E)-2-hexenal plays a role in its activation and whether this activation indeed helps the caterpillar to improve their resistance to oxidative stress.

Secondly, *M. sexta* caterpillars may actively produce and ingest (E)-2-hexenal to protect themselves from pathogen attack: GLVs and especially C6-aldehydes have antimicrobial activities against gram-positive and -negative bacteria (Nakamura and Hatanaka, 2002) and against fungi (Hamiltonkemp et al., 1992; Vaughn and Gardner, 1993). Only few studies exist that directly compared the antimicrobial properties of (Z)-3- and (E)-2-hexenal. While Nakamura and Hatanaka (2002) found no obvious differences between the two alkenals, others reported higher antimicrobial activities of (E)-2-hexenal compared to (Z)-3-hexenal (Kishimoto et al., 2005; Prost et al., 2005). These enhanced antimicrobial properties of (E)-2-hexenal are probably due to the α,β-unsaturated carbonyl moiety and the resulting high reactivity with nucleophilic atoms (Alméras et al., 2003). Because of its highly effective antimicrobial properties (E)-2-hexenal has recently been put forward as a natural alternative to synthetic antibiotics against American Foulbrood Disease of honey bees (Flesar et al., 2010). In addition it was shown that ingested antibiotics can inhibit the reproduction and colonization of *M. sexta*’s entomopathogenic nematode *Steinernema carpocapsae* and its bacterial symbiont *Xenorhabdus nematophila* in the gut while significantly promoting insect growth rates (Van Der Hoeven et al., 2008). This justifies the hypothesis that (3Z):(2E)-enal isomerase activity may contribute to the insect’s immunity repertoire by converting the probably less active (Z)-3-hexenal into the potent antimicrobial substance (E)-2-hexenal.

2) Indirect positive effects by decreasing the absolute amounts of GLVs or their activity:

Besides its possible direct effects on *M. sexta* caterpillars, the catalyzed conversion from (Z)-3- to (E)-2-hexenal might also cause a less durable, i.e. less chemically stable, plant-derived volatile signal reducing the efficiency of the volatile blend in attracting natural
enemies or in inducing plant defense responses: GLVs are relatively unstable in the atmosphere and can easily break down when reacting with photons (O'Connor et al., 2006). While \((E)-2\text{-hexenal}\) has an atmospheric photolytic lifetime of approx. three hours, \((Z)-3\text{-hexenal}\) is less sensitive to photolysis and has an average lifetime of 13 hours (O'Connor et al., 2006). Thus, in theory conversion of \((Z)-3\text{-hexenal}\) to \((E)-2\text{-hexenal}\) by \textit{M. sexta}'s OS could lead to reduced GLV concentrations in the air due to increased photolysis of the \((E)-2\text{-aldehydes}\) and subsequent degradation. However, GLVs are not only prone to photolysis but also to oxidation by OH, NO\textsubscript{3} or O\textsubscript{3} and while \((Z)-3\text{-hexenal}\) is already very unstable by itself it also undergoes rapid oxidation (Arimura et al., 2009). Additionally, since we were able to detect \((E)-2\text{-hexenal}\) but not \((Z)-3\text{-hexenal}\) in our field experiments (\textit{Chapter 3} and \textit{4}) it is rather likely that the net atmospheric lifetime of \((Z)-3\text{-hexenal}\) is shorter than that of \((E)-2\text{-hexenal}\) which would falsify the above mentioned ‘weakening’ hypothesis. Furthermore, GLVs are immediately produced and released from the freshly wounded site of the leaf and almost as fast as the burst occurs it also ceases. The GLV bouquet thus offers immediate but transient information about the location of the herbivore which, while feeding, continuously creates fresh wounds. Long-lasting stability of GLVs is thus not necessary to serve predators and plants as a reliable signal.

While \((E)-2\text{-hexenal}\) is probably the more stable signal compared to \((Z)-3\text{-hexenal}\) this does not imply that this compound is also more active in inducing plant defense responses; exposure of tomato plants to \((E)-2\text{-hexenal}\) elicited three times higher monoterpene emissions than exposure to \((Z)-3\text{-hexenal}\) (Farag and Paré, 2002) and this fits with the hypothesis that \(\alpha,\beta\)-unsaturated carbonyls like \((E)-2\text{-hexenal}\) may play an important role in the induction of plant defense responses (Farmer, 2001). However, this was not supported by Ruther and Fürstenau (2005) who showed that exposure to \((Z)-3\text{-GLVs}\) (aldehyde and alcohol) generally induced slightly stronger volatile emissions in maize plants than exposure to \((E)-2\text{-GLVs}\).

3) Plant volatiles as ‘megaphone’ for insects to ‘communicate’ with each other:

It is well known that plants can exchange information above ground by releasing volatiles into the air (Baldwin, 2010), or below ground via exudation of allelochemicals through the roots (Lovett et al., 1989). Also insects communicate with each other through tactile, chemical, acoustic and visual signals (Billen, 2006). Insects can use plant derived volatiles as ‘megaphone’ by giving the herbivore induced volatile blend a ‘personal’ note - in our case, by converting \((Z)\text{-GLVs}\) to \((E)\text{-GLVs}\) and by changing the \((Z)/(E)\) ratio. What could be the message that \textit{M. sexta} caterpillars are bringing across? Firstly, their message may serve
to reduce the number of competitors on their host plant by informing conspecific gravid moths that this plant is already occupied and, possibly, that egg predators could be around. In Chapter 4 we have shown that female moths are indeed able to distinguish between (Z)- and (E)-GLVs, and in field experiments gravid moths preferred to oviposit on (Z)- than on (E)-baited plants.

Another way to get rid of competitors would be to betray them to their own enemies as shown in Chapter 3. This is of course a risky strategy. The known predators which detect and respond to herbivore-specific volatiles are generally small which means that late instars which can reach a size of approx. 8-10 cm have outgrown this predation risk. Bigger caterpillars create bigger wounds and produce more OS. The increased OS production and application on wound leaves could in principle cause a greater release of (E)-GLVs compared to smaller caterpillars and attract greater numbers of predators, assuming that greater quantities of (E)-GLVs also increase the foraging efficiency of predators (Shiojiri et al., 2006). Late instars could thus riskless call for predators, which may remove small caterpillars before they can become serious competitors. Here it would be interesting to test whether isomerase activity changes during development with e.g. low levels during the early and high levels during the late instars. So far we can only say that OS from late instars posses isomerase activity, because *M. sexta*’s OS are normally collected from 3rd to 5th instar caterpillars. However, it is a dangerous game that caterpillars would play by calling their own enemies, because also bigger predators can respond to volatiles: recently it has been shown that branched chain aliphatic acids, derived from the caterpillar’s digestion of plant *O*-acyl sugars which are present in trichomes of *N. attenuata* (Weinhold and Baldwin, 2011), may betray *Manduca* larvae to lizard predators during late developmental stages as well (Stork et al., 2011).

As a fourth option it is of course also possible that the conversion of (Z)-3-hexenal to (E)-2-hexenal is only a side effect and that the enzyme mainly catalyzes a different, for the caterpillar beneficial or even vital function. However, we can only start to verify this hypothesis after we have identified the enzyme.

**Possible costs of (3Z):(2E)-enal isomerase for *M. sexta* caterpillars:**

1) Natural enemy attraction by (Z)- to (E)-conversion of GLVs:

The existence of a (3Z):(2E)-enal isomerase in the OS of *M. sexta* has also very obvious disadvantages for the caterpillars which have been described in Chapter 3: by converting (Z)-3-hexenal to (E)-2-hexenal *M. sexta* enables natural enemies to discriminate
between a general wound-elicited and an herbivore-specific volatile bouquet, and natural enemies might learn to respond to these herbivore-specific changes in the volatile profile. Our field experiments showed that *Geocoris* spp. were able to distinguish between (Z)- and (E)-GLV mixtures that consisted of each 4 compounds (aldehyde, alcohol, hexenyl acetate and hexenyl butyrate). They were also able to distinguish GLV mixtures that contained both (Z)- and (E)-GLVs but in different ratios (Allmann and Baldwin, 2010 and Chapter 3). *Geocoris* spp. are polyphagous predators, also called big-eyed bugs, that feed on aphids, whiteflies, beetle larvae, and the eggs and early instars of lepidopteran species (Crocker and Whitcomb, 1980). Already earlier experiments showed that (E)-2-hexenal is attractive to *Geocoris*. In a field experiment fifteen HIPVs were tested for their attractiveness to beneficial insects by using sticky traps baited with single volatile compounds. The most attractive volatile for *Geocoris* turned out to be (E)-2-hexenal and interestingly, *Geocoris* was also the only insect in this study that was attracted to this compound. Sticky traps baited with (Z)-3-hexenol or (Z)-3-hexenyl acetate did not catch significantly more big-eyed bugs than control traps (James, 2005). Similar results have been documented in field experiments in the Utah desert; eggs attached to artificial leaves that were treated with (E)-2-hexenal had 3.3-times higher predation rates than eggs on control leaves while the application of (Z)-3-hexenol and (Z)-3-hexenyl acetate did not significantly increase predation (Halitschke et al., 2008). Interestingly, (E)-2-hexenal was also found in extracts of homogenized adults of *Geocoris punctipes* (Marques et al., 2000). This could explain as well why this compound is so attractive to these predators. While (E)-2-hexenal is clearly used by *Geocoris* spp. when foraging, it is not essential for predator attraction; mixes without the aldehydes resulted in similar predator responses than mixes with the aldehydes (Allmann and Baldwin, 2010).

Attraction of natural enemies to OS-induced changes in the (Z)/(E) profile of GLVs have so far only been recorded for the generalist predator *Geocoris* spp. and the question arises whether predation by this natural enemy would be a sufficient source of mortality on *M. sexta* to select against this enzyme when alternative versions of the enzyme, i.e. that do not elicit the GLV-isomerization, have emerged somewhere in a population? While only future can tell this, we do know that *Geocoris* is a very abundant predator in the Utah desert; Kessler and Baldwin (2001) reported that *G. pallens* was responsible for 95% of the *M. quinquemaculata* and *M. sexta* larvae mortality in the Utah field experiments. Further field work will reveal whether also other natural enemies are attracted to (E)-GLVs or decreased (Z)/(E)-ratios, and how they influence the mortality rate of *M. sexta*. 

160
Figure 6.3. Summary of potential costs and benefits for *M. sexta* caterpillars to possess a (3Z):(2E)-enal isomerase. Potential benefits: cytoprotective / antimicrobial, (E)-2-hexenal might enhance cell survival or might have antimicrobial properties; communication, caterpillars might “communicate” via isomerase-modified GLV-emissions of plants with conspecific gravid moths (“this plant is occupied”) or with predators (“take the small one”); signal weakener, conversion to (E)-GLVs might weaken the efficiency of GLVs to induce or prime neighboring plant defenses. Potential costs: predator attraction, natural enemies of *Manduca* caterpillars can be attracted to (E)-GLVs or low (Z)/(E)-ratios; induces plant defenses, (E)-2-hexenal, its derivatives or low (Z)/(E) ratios could serve as herbivore specific signal for plants to induce or prime defenses. If the costs outweigh the benefits this trait will be under negative selection pressure, and if the benefits outweigh the costs then the trait will be under positive selection. However, if this trait has a primary essential function for the caterpillar, costs will never outweigh the benefits (anchor).

2) Induction or priming of plant defenses:

GLVs can be used by neighboring plants or by adjacent leaves of the same plant to induce or prime their defenses, and this can help the plant to prepare itself for the upcoming herbivore attack. However, as already mentioned earlier, only few studies directly compared the efficiency of (Z)- and (E)-GLVs in inducing plant defense responses (Farag and Paré, 2002; Ruther and Furstenau, 2005). It is therefore not clear whether one group of isomers is
more active than the other, or in other words, whether OS-induced conversion from \((Z)\) to \((E)\) causes a weakening or an amplification of the signal or whether conversion has no impact on the strength of the signal at all. In any case \((E)\)-2-hexenal has been shown in several cases to serve as a potent inducer of plant defenses:

\((E)\)-2-hexenal exposure induced phytoalexin accumulation in cotton balls (Zeringue Jr, 1992). In *Arabidopsis thaliana* it induced several defense-related genes (Bate et al., 1998) that closely mimicked methyl jasmonate induction (Arimura et al., 2001). \((E)\)-2-hexenal exposure furthermore triggered a local and systemic release of several mono- and sesquiterpenes in tomato plants (Farag and Paré, 2002) which was greater than that of \((Z)\)-3-hexenal exposed plants. Additionally, Kessler *et al.* (2006) showed that *N. attenuata*, when exposed to \((E)\)-2-hexenal prior herbivore attack by *Manduca* caterpillars, increased their trypsin proteinase inhibitor production more rapidly than did unexposed control plants.

**CONCLUSION**

In the case of the insect isomerase it remains an open question whether the enzyme activity is simply maladaptive and hence could be under negative selection; if there is a life-stage dependent or ecological trade-off with respect to the effects of enzyme expression or whether it is the plant that has adapted to make isomerase-expression backfire by synthesizing \((Z)\)-3-hexenal i.e. as a “green booby-trap”. Thus, taking the evolutionary time scale into account, it is still open to debate whether it is the herbivore betraying itself or the plant betraying the herbivore. By identifying the isomerase from the oral secretions of *M. sexta* caterpillars and by silencing the gene in the caterpillar via plant-mediated RNAi we will shed more light on the role \((Z)/(E)\)-isomerisation of GLVs plays for *M. sexta* caterpillars.

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