Green Leaf Volatiles—The Forefront of Plant Responses Against Biotic Attack

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Green leaf volatiles (GLVs) are six-carbon volatile oxylipins ubiquitous in vascular plants. GLVs are produced from acyl groups in the biological membranes via oxygenation by a pathway-specific lipoygenase (LOX) and a subsequent cleavage reaction by hydroperoxide lyase. Because of the universal distribution and ability to form GLVs, they have been anticipated to play a common role in vascular plants. While resting levels in intact plant tissues are low, GLVs are immediately synthesized de novo in response to stresses, such as insect herbivory, that disrupt the cell structure. This rapid GLV burst is one of the fastest responses of plants to cell-damaging stresses; therefore, GLVs are the first plant-derived compounds encountered by organisms that interact with plants irrespective of whether the interaction is competitive or friendly. GLVs should therefore be considered important mediators between plants and organisms that interact with them. GLVs can have direct effects by deterring herbivores and pathogens as well as indirect effects by attracting predators of herbivores, while other plants can recruit them to prepare their defenses in a process called priming. While the beneficial effects provided to plants by GLVs are often less dramatic and even complementary, the buildup of these tiny effects due to the multiple functions of GLVs can amass to levels that become substantially beneficial to plants. This review summarizes the current understanding of the spatiotemporal resolution of GLV biosynthesis and GLV functions and outlines how GLVs support the basic health of plants.

Keywords: Green leaf volatiles • Herbivores • Direct and indirect defenses • Priming

Introduction

Green leaf volatiles (GLVs) is a generic name for volatile compounds consisting of six-carbon aldehydes, alcohols and their acyl esters that are derived from fatty acids. The history of GLVs dates back to 1881 when Reinke et al. at Gottingen University reported the presence of an aldehyde component in the steam distillate of plant leaves. In 1912, Curtius and Franzen at Heidelberg University isolated the aldehyde from European hornbeam (Carpinus betulus) and identified it as 2-hexenal (Curtius and Franzen 1912). At the same time, they detected the same component in the leaves of 19 plant species, including grapevine (Vitis vinifera) and eagle fern (Pteridium aquilinum), indicating that 2-hexenal is widely distributed in the green leaves of plants. Recently, the genome sequences of representative plant species and relevant microbes belonging to almost all plant taxa from cyanobacteria to angiosperms have been made available, and chemical analyses of leaves of the representative plant species and comprehensive searches of their genome sequence have revealed that while only a limited number of moss species possess GLVs, almost all green plants beginning with the emergence of lycophytes have the ability to form GLVs (Tanaka et al. 2021). Therefore, it can be inferred that GLVs are not specialized metabolites, but rather play a more general role in enhancing the survivability of vascular plants.

In the past two decades, it has become clear how GLVs affect plant fitness. One of the objectives of this review is therefore to provide an overview of these developments. GLVs are characterized by the fact that the biosynthetic pathway is latent and does not allow for the accumulation of large amounts in intact plant tissues. When plant tissues are damaged, the biosynthetic pathway is quickly activated and a massive amount of GLVs can be formed within seconds to a few minutes (D’Auria et al. 2007). This quick formation of GLVs is sometimes named the GLV burst (Mochizuki et al. 2016). This special feature of GLVs means that their production proceeds in a spatiotemporal manner, which is closely associated with the incidents that cause tissue damage. For organisms that associate with plants, GLVs are one of the first compounds of plant origin the organisms encounter after the onset of interaction. In most cases, GLVs bear real-time information about plants undergoing stress associated with tissue damage. In other words, GLVs are at the forefront of the interaction between plants and the organisms that are associated with plants.

A number of excellent reviews have been published on the biosynthetic mechanisms and physiological and ecological roles of GLVs (Matsui 2006, Scala et al. 2013a, Ameye et al. 2018, Engelberth 2020). With some overlap, we will first outline the biosynthetic mechanisms and discuss how immediate GLV...
production and release are achieved at the site of tissue damage. We will then discuss the role of GLVs in plant interactions with pathogens, herbivores and predators, and how and to what extent GLVs are involved in plant–plant communication, summarizing our current knowledge.

**Biosynthesis of GLVs**

For the formation of GLVs, a lipoxygenase (LOX) catalyzes a dioxygenase reaction on fatty acids either in their free or in their esterified form to yield fatty acid/lipid hydroperoxides (Nakashima et al. 2013) (Fig. 1). The hydroperoxides are subsequently cleaved by a noncanonical cytochrome P450 enzyme, hydroperoxide lyase (HPL), to yield the six-carbon (C6) volatile aldehydes concomitant with the 12-carbon (C12) oxo-acids/esters as the counterparts. A portion of C6 aldehydes is reduced to the corresponding C6 alcohols (Tanaka et al. 2018) and can be further converted into their acyl esters with the aid of acyl-coenzyme A (CoA) acyltransferase (D’Auria et al. 2007) or into their glycosides with the aid of UDP glycosyltransferase (Ohgami et al. 2015).

**Lipoxygenase**

The GLV-biosynthesis pathway illustrated in most review articles starts with free linolenic acid. This is mostly because most plant LOXs prefer free linolenic acid when assayed in vitro in an aqueous solution. Also, an enzyme catalyzing the hydrolysis of glycerolipids to release free fatty acids has been believed to be essential for GLV production even without any direct evidence. One large group of acyl-hydrolyzing enzymes are lipolytic acyl hydrolases that are structurally related to patatins, storage proteins in potato tubers, and the other group consists of GDSL lipases (Wang et al. 2019). The involvement of these acyl hydrolases in oxylipin formation has been occasionally reported, e.g. involvement of patatin-like protein 2 in the generation of a product of the oxylipin in the α-dioxygenase pathway (La Camera et al. 2009) or DEFECTIVE IN ANther DEhiscence1 (DAD1) belonging to the family of GDSL lipase.
in jasmonates (JAs) production in Arabidopsis inflorescence (Ishiguro et al. 2001); however, direct evidence of the involvement of these acyl hydrolases in GLV formation has never been provided, with only one exception in tomato fruits. One of the GDSL lipases, SI-LIP8, is essential for GLV formation in tomato fruits, while a loss-of-function mutant showed no effect in wound-associated GLV formation in leaves (Li et al. 2020). Taken together, no direct evidence for the involvement of acyl hydrolase in GLV production at least in leaf tissues has been provided, and therefore, the old idea that an acyl hydrolase is essential for GLV formation needs to be reconsidered.

When Arabidopsis leaves are damaged, (Z)-3-hexenal is formed concomitant with a substantial amount of C12 oxo-acids esterified to galactolipids (Nakashima et al. 2013). This suggests that the conversion of galactolipids to galactolipid hydroperoxide by a LOX and the subsequent cleavage of acyl groups by HPL proceeds without the hydrolysis of galactolipids. The galactolipids harboring C12 oxo-acids as their acyl groups are found in larger quantities than free C12 oxo-acids not only in Arabidopsis but also in the leaves of Brassica oleracea, Nicotiana tabacum, Solanum lycopersicum and Phaseolus vulgaris (Nakashima et al. 2013). These results indicate that free fatty acid supply by acyl hydrolysis is not necessarily required for GLV production in the leaves and that LOX acts directly on membrane lipids to produce lipid hydroperoxides as the initial step. If this would be the case, then lipid peroxidation catalyzed by a LOX is the step governing GLV burst after leaf damage. Arabidopsis ecotype Col-0 is deficient in GLV burst because of the deletion of the HPL gene (Duan et al. 2005); thus, it was expected that the substrates for HPL (i.e. the products of LOX) would accumulate in wounded leaf tissues. This is the case, and the accumulation of galactolipid hydroperoxides was detected in Col-0 leaves after damage (Mochizuki and Matsui 2018). This also supports the direct oxygenation of galactolipids by a LOX in the course of a GLV burst. With most plant LOX studied so far, acyl lipids are not good substrates in vitro; however, the appropriate preparation of the aqueous suspension of acyl lipids, for example, by using a detergent adequately, has been shown to make them suitable for the LOX reaction (Nakashima et al. 2011). The kinetics of lipid hydroperoxide formation by LOX in the course of a GLV burst needs to be reevaluated, by simulating the physicochemical state of lipid substrates in cell membranes in damaged cells.

If LOX is the primary enzyme causing the GLV burst, then LOX activation is the primary factor in regulating a cell-damage-associated GLV burst. However, any attempt to examine the LOX activation mechanism failed because in most cases no LOX activity was detected in vitro in leaf homogenates even though it was apparent that LOX had been active, at least for a while, to support the GLV burst. It is likely that upon leaf homogenization, LOX oxidizes glycolipids only for a short period, but is quickly inactivated, for example by a suicide inactivation during its catalysis (Kishimoto et al. 1996). Accordingly, the mechanism of how LOX is activated after cell damage is still an open question. Indirect evidence for LOX activation was obtained with the addition of Ca$^{2+}$ chelating agents, such as 1,2-bis(2-aminophenoxy)ethane-N,N,N’,N’-tetraacetic acid (BAPTA) and EGTA. The addition of BAPTA or EGTA effectively suppressed the GLV burst in Arabidopsis Ws-0 and galactolipid hydroperoxides formation in HPL-less Col-0 (Mochizuki and Matsui 2018). Since the formation of galactolipid hydroperoxides was suppressed, it must be the LOX reaction where the Ca$^{2+}$ chelating agents act. It has been reported that Ca$^{2+}$ binds to the N-terminal PLAT domain of animal LOXs, which causes a conformational change in the domain and subsequently promotes the transport of the proteins to the membrane (Newcomer and Brash 2014). AtLOX2 is the isoform exclusively involved in GLV burst in Arabidopsis (Mochizuki et al. 2016) and is a soluble protein in the chloroplast stroma. It also consists of an N-terminal PLAT domain and a C-terminal catalytic domain. The increase in Ca$^{2+}$ concentration in the stroma due to cell disruption may induce a conformational change in the PLAT domain of AtLOX2 and its recruitment to the membrane, which may lead to the peroxidation of galactolipids. This is one possible scenario for the mechanism of GLV burst activation but needs further confirmation. With maize seedlings, light touching alone causes GLV burst, even though cell disruption by light touching was unlikely (Markovic et al. 2019). GLV burst from intact tissues is also reported in gray poplar leaves during light–dark transitions (Graus et al. 2004). In either case, cytoplasmic Ca$^{2+}$ level seems to increase (Nomura and Shiina 2014), which supports the hypothesis of the GLV burst being elicited by LOX activation through Ca$^{2+}$. As described above, the physicochemical condition of the lipid substrate in an aqueous solution has a significant impact on LOX activity, and the effect of Ca$^{2+}$ on the physicochemical state of lipid substrates should also be considered.

Hydroperoxide lyase

HPL is the cytochrome P450 enzyme (CYP74B) that catalyzes the rearrangement reaction of lipid hydroperoxide to cleave its C–C bond and form hexenal and oxo-acid. Unlike a canonical P450 enzyme, HPL requires neither the reducing power usually provided by an NADPH cytochrome P450 reductase nor molecular oxygen. HPL completes the reduction and oxidation of its heme iron through homolysis of the O–O bond of the fatty acid hydroperoxide and the subsequent rearrangement of the radical species (Grechkin and Hamberg 2004), which is similar to mammalian P450 enzymes that exclusively catalyze the so-called peroxide shunt pathway (Hrycay and Bandiera 2012). HPL and allene oxide synthase (AOS) (CYP74A) are homologous P450 enzymes located at the branching point leading to either GLVs or JAs, the two major oxylipins formed from linolenic acid hydroperoxides. HPL and AOS compete for the same substrate; however, they seem to be well regulated to avoid competition. In tomato, potato, wild tobacco (Nicotiana attenuata), maize and rice, there are respective LOXs for either the HPL pathway or the AOS pathway (Mwenda et al. 2017). One way to avoid
Hexenal reductase and acetyl transferase

Because some of the nicotinamide adenine dinucleotide, reduced (NADH)-dependent alcohol dehydrogenases (EC 1.1.1.1) show wide substrate specificity and can catalyze the NADH-dependent reduction of (Z)-3-hexenal, it has been assumed that alcohol dehydrogenases are responsible for the formation of (Z)-3-hexenal from (Z)-3-hexenal in plants. This seems to be the case with tomato fruits, and the genetic modification of S. lycopersicum alcohol dehydrogenase 2 (SiADH2) led to higher levels of C6-alcohols in the fruits with increased ADH activity and reduced levels of them in those with low ADH activity in homogenates prepared from tomato fruits (Speirs et al. 1998). In contrast, the addition of nicotinamide adenine dinucleotide phosphate, reduced (NADPH), but not NADH, to crude homogenates prepared from Arabidopsis leaves efficiently enhanced the formation of hexenol (Matsui et al. 2012), suggesting that another reductase was involved in the conversion of hexenal to hexenol. Purification of the enzyme identified a protein formerly designated as cinnamyl alcohol dehydrogenase 2 as the one responsible for the formation of (Z)-3-hexenal from (Z)-3-hexenal; accordingly, the protein was renamed as cinnamaldehyde and hexenal reductase (CHR) (Tanaka et al. 2018). Phylogenetic analysis indicated that Arabidopsis CHR was related to oxidoreductases involved in plant-specialized metabolisms, such as tetrahydroalstonin synthase and 8-hydroxygeraniol dehydrogenase in Catharanthus roseus. As the disruption of AtCHR resulted in higher sensitivity to deleterious effects caused by (Z)-3-hexenal vapor, it was assumed that from an evolutionary perspective one of the oxidoreductase genes involved in specialized metabolism was diverted to become specialized for the detoxification of hexenal formed at the site of tissue disruption.

The amount and composition of GLVs formed after wounding vary spatiotemporally. The GLV burst starts with the rapid formation of hexenal in the damaged cells. The reduction of hexenal to hexenol in the damaged cells is inefficient due to the limited supply of NADPH. Because of the low polarity and small size of hexenal, some of the hexenal produced in the damaged cells diffuse into the surrounding intact tissues, where it is reduced to hexenol by NADPH-dependent CHR. Therefore, microscopically speaking, the sites where hexenal is mainly found and those where hexenol is found are different, with hexenal being abundant in the injured/damaged tissue and hexenol being abundant in the surrounding/intact tissue (Fig. 2). In terms of the time course of formation of each metabolite, hexenal is produced first, reaching maximum emissions after approximately 30–45 s, while hexenol production reaches its maximum at 2.5 min (D’Auria et al. 2007). Hexenyl acetate formation requires a trans-esterification reaction with acetyl-CoA by acetyl transferase (D’Auria et al. 2007), which may also acquire intact cells to provide the sufficient supply of acetyl-CoA. The peak of hexenyl acetate formation is further delayed, peaking 4.5–5.5 min after injury. In conclusion, these sequential changes in quantity and quality reflect when and where the tissue damage occurs and thus provide spatiotemporal information about the damage, which can also be utilized by other organisms including herbivores and predators. In fact, some herbivores are known to respond more strongly to a mixture of GLV compounds than to a single GLV compound (Bruce and Pickett 2011). It should be noted that the artificial mechanical wounding is mostly instantaneous but feeding damage by herbivores is usually continuous, so these spatiotemporal changes are thought to be continuously repeated while changing locations.

Auxiliary metabolism of GLVs

In addition to the major metabolic pathways described above, there are also branching pathways where each component of GLV is converted to other compounds that are also involved in regulating the interactions of plants with the organisms associated with them. (Z)-3-Hexenal is prone to be isomerized to (E)-2-hexenal, which harbors reactive α,β-unsaturated aldehyde moiety, spontaneously, for example under mild basic conditions, or enzymatically as found in some (but not all) plants such as alfalfa and cucumber (Noordermeer et al. 1999, Kunishima et al. 2016). The α,β-unsaturated aldehyde readily
Fig. 2 A schematic diagram of ‘GLV-burst’ from plant leaves caused by insect herbivory. (Z)-3-Hexenal is formed in the damaged tissues from disorganized membranes in collapsed cells through rapid activation of lipoxygenase and subsequently diffuses out to the neighboring undamaged tissues. (Z)-3-Hexenal reaches the undamaged tissues is reduced to form (Z)-3-hexenol, and a portion of (Z)-3-hexenol is further converted into (Z)-3-hexenyl acetate. Because each biochemical and biophysical step progresses in succession with slightly different phases, the formation of the aldehydes, alcohols and acetates is spatiotemporally varied. As insect herbivory proceeds almost constantly for a while until it moves on, GLV bursts occur many times in short intervals, resulting in an apparently constant volatile production. Accordingly, the GLV composition varied depending on the degree of damage to the tissues.

(Z)-3-Hexenal reacts with nucleophilic biomolecules, such as amines and thiols, and was found to inactivate several enzymes in the Calvin cycle.

(Z)-3-Hexenal has bisallylic acidic hydrogens; therefore, it is also prone to be oxygenated spontaneously or by a LOX action to give 4-hydroperoxy-(E)-2-hexenal (HPHE) (Takamura and Gardner 1996). HPHE appears to be unstable and converted to 4-hydroxy-(E)-2-hexenal (HHE). HHE is one of the most reactive α,β-unsaturated carbonyl species and can form covalent adducts with nucleophilic biomolecules enzymatically or nonenzymatically (Pillon et al. 2010). HHE efficiently inhibits the growth of plant pathogenic fungi (Vaughn and Gardner 1993) and is found in large amounts in the plant–pathogen interface of Botrytis cinerea-infected bell pepper fruits (Delighton et al. 1999). The high antimicrobial activity found with (Z)-3-hexenal, contributing to the direct defense, can be partly explained by the fact that (Z)-3-hexenal is readily converted into highly reactive HHE. Quite recently, an unique metabolic pathway where (Z)-3-hexenal is employed as a Michael reaction donor to form a conjugate with a quinone was reported (Bai et al. 2022). The metabolite formed through the pathway exerted nonhost resistance against a herbivore.

Glutathione reacts with C6-monounsaturated aldehydes spontaneously or with the aid of glutathione S-transferase (Davoine et al. 2006, Yalcinkaya et al. 2019). One of the GSH adducts, 1-hexanol-3-GSH, accumulated during the hypersensitive response of tobacco leaves induced by cryptogein (Davoine et al. 2006), but it has not been known whether the GSH adduct is involved in the plant defense response or it is merely a product formed during the response. Interestingly, 1-hexanol-3-GSH is further metabolized into 3-mercaptop-1-hexanol, a key aroma of Sauvignon blanc wine in grapevines (Clark and Deed 2018).

Some volatile alcohols are converted into their glycosides. Tea plants (Camellia sinensis) convert endogenously formed (Z)-3-hexenol as a glucoside or primeveroside (glucose–xylose) with UDP glycosyltransferases (Ohgami et al. 2015). Water-soluble hexenol glycosides are stored in intact leaves and hydrolyzed by glucosidase or primeverosidase to yield volatile (Z)-3-hexenol when plant tissues are damaged. Therefore, damage-induced hydrolysis of hexenyl glycosides is another way to form the volatiles quickly after tissue damage. In tea and tomato, hexenol glycosides are formed not only from endogenous hexenol but also from that formed and emitted by neighboring conspecifics (Sugimoto et al. 2014, Jing et al. 2019). Treating tomato plants with the vapor of (Z)-3-hexenyl acetate also resulted in the accumulation of (Z)-3-hexenyl vicianoside, suggesting that the (Z)-3-hexenyl acetate taken up by tomato leaf tissue is hydrolyzed to yield (Z)-3-hexenol, which is subsequently converted into the vicianoside (Sugimoto et al. 2021). In tomato, (Z)-3-hexenyl vicianoside thus formed in the receiver plants has a feeding inhibitory activity against the common cutworm (Spodoptera litura), indicating that the release of hexenol and its glycosylation followed by absorption by neighboring conspecific is one of the entities of plant–plant communication (Sugimoto et al. 2014).

Interaction with Pathogens

Because some GLVs, especially C6-aldehydes, have substantial reactivities with other biomolecules as described above, they have also been thought to play a direct defense function
against pathogens. However, there are only a few reports confirming the defensive role of GLVs in vivo against pathogens. This is very small compared to the number of studies on GLVs associated with defense against herbivores. The review by Hammerbacher et al. (2019) comprehensively summarizes the activities of GLVs against pathogens, but some studies cited in the review only looked at the correlation between GLV amounts and pathogen susceptibility, and some looked at the effect of enhancing GLV production through gene overexpression. However, direct evidence indicating an involvement of GLVs in the direct defense against pathogens is limited. It is important to examine pathogen susceptibility with a plant deficient in GLV formation by knocking down/out the GLV-biosynthetic pathway. In vivo assays of Arabidopsis plants with a lowered ability to form GLVs by antisense suppression of the HPL gene with a necrotic fungal pathogen, B. cinerea, indicated that GLVs are involved in the defense of Arabidopsis against B. cinerea, most possibly through direct defense effects of the C6-aldehydes (Shiojiri et al. 2006, Kishimoto et al. 2008). As far as we know, this is the only report showing the more direct significance of the ability to form GLVs in plants. However, a similar experiment was conducted later, and it was reported that the presence or absence of HPL had little effect on the resistance of Arabidopsis was conducted later, and it was reported that the presence or absence of HPL had little effect on the resistance of Arabidopsis to two necrotrophic fungal pathogens, B. cinerea and Alternaria brassicicola, in their experimental system (Chehab et al. 2008); therefore, a more detailed study is required to confirm the direct defense effects of GLVs against pathogens. Another study indicated that GLVs function as signaling molecules to induce defenses, but not as direct defense substances. The antisense suppression of hexenal acyltransferase in tomato fruits resulted in lower amounts of acylated hexenol including (Z)-3-hexenyl and n-hexyl acetate, concomitant with higher susceptibility to a biotrophic bacterial pathogen Pseudomonas syringae. Acylated hexenols promote stomata closure, and as a result, suppressed P. syringae invasion into the leaf tissues (López-Gresa et al. 2018).

On the other hand, several studies showed that the suppression of the ability to form GLVs resulted in higher resistance to pathogens. In Arabidopsis, an HPL-deficient mutant was more resistant to P. syringae DC3000 (Scala et al. 2013b); the change in resistance to P. syringae was attributed to lower JA levels and higher SA levels in the mutant. Similar antagonistic crosstalk was reported in maize, where GLV production was suppressed by a disrupted ZmLOX10 gene, resulting in increased resistance to the hemibiotrophic fungus Colletotrichum graminicola (Gorman et al. 2020). Since the amount of SA was also higher in the maize mutant, it is clear that GLVs affect SA as well as JA signaling. (Z)-3-Hexenol treatment primed tomato plants for enhanced defense against Tomato yellow leaf curl virus transmission by the whitefly Bemisia tabaci (Su et al. 2020). In the (Z)-3-hexenol-primed tomato plants transcripts of JA biosynthetic genes, as well as whitefly-induced transcripts of SA biosynthetic genes, were increased. As such, the defense signaling pathway mediated by GLVs interacts differently with JA and/or SA pathways depending on the plant species examined. Detailed molecular analyses of the signaling pathways activated by GLVs need to be carried out to dissect the agonistic and antagonistic crosstalk among the involved signaling pathway.

On the other hand, there are many reports that exogenous GLV treatment of plants increases resistance to pathogens. To give a few examples, exposing potato plants to the vapor of (Z)-3-hexenyl acetate decreased the severity of late blight disease caused by Phytophthora infestans (Najdabbasi et al. 2021). Postharvest fumigation of (E)-2-hexenal on kiwifruit enhanced resistance to B. cinerea (Hyun et al. 2022). Most of such reports are based on the expectation that GLVs act as a biostimulant and not as a signal in plant–plant communication in nature, so the concentrations and treatment methods employed are usually eco-physiologically irrelevant. Nevertheless, the possibility of GLVs for applications in agriculture should be acknowledged.

**GLVs and Plant Defenses against Insect Herbivores**

Communication between plants by volatile signals has been described first by Baldwin and Schultz (1983) and Rhoades (1983) independently. Both found that plants exposed to volatiles released by other mechanically damaged plants were less attractive to insect herbivores. Taking into consideration that damaged green plant tissues mainly release GLVs, the studies provided the first evidence that these compounds might have been the bioactive signal. However, no specific volatile compound was described as being responsible for this response. Arimura et al. (2000) convincingly described specific volatiles emitted from herbivore-infested plants that can signal the activation of defense-related gene expression in nearby uninfested plants. Among the volatiles identified were several terpenes including ocimene, but also GLVs. There has been evidence from earlier studies, which showed that GLVs can significantly affect plant defense responses, in particular those against insect herbivores. Zeringue (1992) showed that GLVs induced certain phytoalexins. Hildebrand et al. (1993) found that GLV had a negative effect on aphid fecundity by modifying leaf chemistry. Vancanneyt et al. (2001) confirmed these results by also finding a negative effect of GLVs on aphid performance. While these results demonstrated that GLV had the potential to serve as signals between plants by inducing defense-related processes and consequently had a negative effect on the performance of the attacking insect herbivore, their role in signaling potentially damaging events between plants still needed to be discovered.

**GLVs are rapid inducers of protective gene expression**

Bate and Rothstein (1998) were the first to describe the activation of certain defense genes upon exposure to GLVs in Arabidopsis. However, this analysis was very limited in its approach and not all genes tested were affected by (E)-2-hexenal.
GLVs tested to date have shown almost identical activities in maize seedlings as the major defense hormone. While in maize seedlings all those that regulate protection including the corresponding alcohols to remain fully functional so that they can thus assist in the immediate vicinity of the damage site and allows them to respond within minutes. Interestingly, while each compound did not give the full response in pyrethrum plants, a blend of these five compounds did. In nature, plants do mainly emit a blend of compounds upon damaging stresses. Therefore, all living organisms must be surrounded by a blend consisting of various volatile compounds. Accordingly, there might be a system to distinguish a blend; however, nothing is known about a system for ‘sniffing out’ blends. Its detail is a big open question.

Most studies on the direct or immediate effects of GLVs on defense gene expression are limited to a few selected genes and it is therefore impossible at the current time to determine which of those differential effects are common among plants. Nonetheless, GLVs in general can provide rapid, direct protection against many biotic and abiotic stresses in addition to serving as long-distance signal.

GLVs can serve as priming agents against biotic stressors

The rather limited activation of typical insect herbivore defense responses by GLVs was always considered to be the weak spot in the discussions about their effectiveness. Added to this were doubts on whether or not the quantities of GLVs that are released by damaged plants were sufficient to activate effective responses in neighboring plants. However, it has been shown that GLVs are often produced in large quantities within seconds after damage and that this release is often sustained over prolonged periods, often by changing the profile from being mainly aldehydes to the more reduced forms, e.g. hexenol and hexenyl acetate (D’Auria et al. 2007). This makes them rather sensible signals that can report the status of a plant to distal, yet unaffected parts of the same plant, but also to other plants nearby. Caterpillars often eat on one plant until it becomes either indigestible or even toxic and then move on to other plants nearby. This may take hours and therefore any signals emitted by the previously damaged plant should ideally provide protection for nearby plants that lasts sufficiently long. To further characterize this long-term defensive effect of GLVs on plants, Engelberth et al. (2004) designed a series of experiments that helped to better understand how GLVs affected defense responses hours after perceiving the signal. While maize seedlings exposed to a variety of GLVs quickly responded by accumulating small but significant amounts of JA, a more pronounced effect was found much later. When treated first overnight with GLVs, either by using a physiological concentration of pure chemicals or by using herbivore-damaged plants as a source for GLVs and then challenged with an IE the next morning, maize seedlings responded by producing significantly more JA and typical herbivore-induced plant volatiles than their respective controls. This effect was not observed when GLV-treated plants were only mechanically damaged, suggesting a specific link between the initial treatment with GLVs and the actual insect herbivory. This was the first demonstration of a priming effect of GLVs on plant defense responses against insect herbivores by stimulating the enhanced production of typical defensive measures many hours after perceiving the first volatile signal.
This priming effect of GLVs has since been demonstrated for many other plant species. Kessler et al. (2006) used a microarray approach to study the effect of GLVs on defense-related genes in wild tobacco that was exposed to volatiles from clipped sagebrush, which is rich in GLVs. These plants also responded to herbivory by Manduca sexta by an accelerated production of a trypsin proteinase inhibitor and consequently, less herbivore damage and a higher mortality rate among young Manduca caterpillars. In a study by Ton et al. (2007) a priming effect on defense-related gene expression was shown, and Frost et al. (2008) found that (Z)-3-hexenyl acetate can also prime poplar trees against insect herbivory. Overall, these studies established GLVs as priming signals and explained why the full activation of defenses is not always necessary to mount an effective defense. Here, plants perceive signals that report the status of plants nearby without inducing the full activation of defenses. As such priming appears to be less costly by not investing massive resources into defense. This was supported by growth studies performed on maize (Engelberth and Engelberth 2019, Engelberth 2020) and lima beans (Phaseolus lunatus) (Freundlich et al. 2021). In maize, the initial exposure to GLVs caused a significant reduction in growth, but plants recovered quickly and assumed normal or even enhanced growth rates again. Even after elicitation with IE or mechanical damage alone, GLV-primed plants showed an enhanced growth response not only in the affected leaves but also in systemic leaves, where mechanically damaged plants responded similar or even stronger than those treated with IE (Engelberth and Engelberth 2019). For lima beans, it was also found that exposure to GLVs also increased growth rates and seed production (Freundlich et al. 2021). However, this does not appear to be a general response. In pepper (Capsicum annuum), the same authors found a reduced growth rate as well as reduced seed production after continuous exposure to (Z)-3-hexenyl acetate. It still raises the question of whether these effects of GLVs on growth and general fitness are common features among different plant species.

Aside from serving as a potential defense signal for neighboring plants, GLVs may further play a role in signaling damage to distal or systemic parts of the same plant. While insect herbivory can cause the generation of an electric signal that moves rapidly through the plant (Zimmermann et al. 2009, 2016, Farmer et al. 2020) or activates the transfer of chemicals including jasmonates from the damage site to systemic parts of the attacked plant (Li et al. 2002), highly sectorial plants may use GLVs as their long-distance signal. Karban et al. (2006) investigated the role of volatiles as signals between different branches of sagebrush (Artemisia tridentata) and found that airflow was essential for the induced resistance against insect herbivory in systemic parts of the same plant. Sagebrush, like many other desert plants, is highly sectorial, which allows it to better protect itself against drying out. However, this also restricts the exchange of signaling molecules through vascular connections. Here, volatiles are used to overcome these restrictions by serving as a long-distance signal. A similar effect was described for lima beans (P. lunatus), where it was also shown that GLVs can serve as a systemic signal (Heil and Bueno 2007, Heil and Ton 2008). Indeed, when considering the evolutionary development of GLV-induced priming, it may very well be that this is in fact the original function of GLVs within the overall defensive response to damage-causing stresses.

It must be noted here that GLVs, when released by damaged plants, do not follow an isotropic diffusion in nature, but rather move like a plume of smoke (Beyaert and Hilker 2014). This physical behavior may cause local GLV concentrations to be much higher and therefore may increase their biological activity in natural settings. Also, the repeated perception of even small quantities of GLVs over a prolonged period is sufficient to activate significant defenses (Shiojiri et al. 2012). The capacity of plants to produce these compounds must also be taken into consideration when investigating their biological activity. Significant differences in quantities and qualities are produced among different plant species (Engelberth and Engelberth 2020), but also within a species depending on the developmental stage of the plant (Engelberth and Engelberth 2022).

Little is known about the specific signaling events that regulate GLV-induced priming against insect herbivory. While many postulate receptors for volatile compounds in plants, to date, no such plant volatile receptor has been identified except for a transcription corepressor TOPLESS in tobacco as a candidate for being a caryophyllene receptor (Nagashima et al. 2019). Studies by Asai et al. (2009) and Zebelo et al. (2012) showed that rapid depolarization of membrane potentials and increases in cytosolic Ca\(^{2+}\) are among the fastest responses upon the perception of these volatile signals. This implies the involvement of a ligand-gated ion channel in plant responses against GLVs, but confirmation is needed. Based on most results published to date, a close correlation between GLVs and the JA pathway can be found. GLV exposure of maize seedlings directly and quickly caused the accumulation of JA with a maximum already found after 15 min (Engelberth et al. 2004, 2007). Priming by GLV increased subsequent IE-induced JA production by almost 100% in maize (Engelberth et al. 2004, 2007). JA signaling mutants were less responsive to GLV (Kishimoto et al. 2006). Accordingly, it is assumed that priming by GLVs depends on a functioning JA pathway.

Further insights into the biological activities of GLVs came from studies with mutant lines that were incapable of producing GLVs. Christensen et al. (2013) identified several distinctive effects in maize mutants that were defective in a GLV-pathway-specific LOX (ZmLOX10). These mutants produced significantly less JA, released less herbivore-induced plant volatiles, attracted fewer parasitoids and were generally much more susceptible to herbivore attack than their respective wild types. While similar pathway-specific LOXs were found for other plant species including tobacco and tomato (Allmann and Baldwin 2010, Shen et al. 2014), their involvement in defense activation varies...
Fig. 3 The interaction of stress-induced GLVs with other plants. Various stressors can induce the production of GLVs in plants, mainly through tissue damage. Aside from serving as signals for systemic parts of the damaged plant, GLVs can also be perceived by other plants nearby and rapidly activate direct defenses as well as genes involved in cellular structural integrity. GLVs can also prime those plants against future attacks, resulting in better preparedness against herbivore attacks at later time points. Additionally, in some plants the stimulation of growth was found after exposure to GLVs.

between different plant species. This may be caused by the fact that in some plants the HPL and the jasmonate pathway compete for the same substrate (Rustgi et al. 2019), resulting in ambiguous consequences for attacking insect herbivores. However, HPL-depleted plants including potato, tobacco and rice were also found to be more susceptible to insect herbivory than their wild-type control plants (Vancanneyt et al. 2001, Halitschke et al. 2004, Tong et al. 2012). These and other studies strongly suggest that GLVs are important for the regulation of insect herbivore-directed defense responses through the activation of direct defense responses, priming for delayed responses, and preparation for increased cellular structural integrity (Fig. 3). However, it must also be stated that for example Arabidopsis thaliana ecotype Col-0, which is incapable of producing GLV because of a natural mutation in its HPL gene, has not shown any differences compared with an HPL-active ecotype in its capacity to fend off insect herbivores. This can be attributed to alternative defense systems including jasmonates for signaling and glucosinolates for defense.

Caterpillars can suppress and alter the production of specific GLVs by interfering with the biosynthesis

GLVs interact with herbivores on multiple levels. They can serve as attractants and as repellents. They can provide protection directly in the damaged plant and can even do so for other plants. Altogether, GLVs have been coined as the plant’s multifunctional weapon (Scala et al. 2013a). However, during evolution insect herbivores developed countermeasures throughout their larval stadiums that appear to either eliminate or at least modify the production of GLVs, and in recent years increasing evidence has been provided the widespread distribution of these countermeasures among different clades of insect herbivores. Allmann and Baldwin (2010) found that the saliva of the tobacco hornworm (M. sexta) contained an isomerase that quickly converted (Z)-3-hexenal into its (E)-2 form. While this eliminated the emission of (Z)-3-hexenal and its corresponding alcohol and esters, this conversion was found to be even more detrimental for the caterpillar since it made it more attractive for the generalist hemipteran predator Geocoris ssp. by tripling its foraging efficiency. In contrast, mechanisms effective for the elimination of GLV emissions by oral secretions from a caterpillar were first described by Savchenko et al. (2013) and Savchenko and Dehesh (2013). They demonstrated that oral secretions from beet armyworm (Spodoptera exigua) and cabbage looper (Trichoplusia ni) larvae significantly reduced the emissions of GLVs from damaged plants. This was surprising since many studies before had shown that GLVs are among the typical HIPV emitted from plants under herbivore attack. Further evidence came from a study by Takai et al. (2018), who identified a fatty acid hydroperoxide dehydratase in the saliva of silkworms (Bombyx mori) that converts 13(S)-hydroperoxy-linolenic acid, the substrate for HPL, into its keto-derivative in a stereospecific manner, thereby removing it from the biosynthetic pathway for GLVs. Jones et al. (2019) characterized another mechanism by which caterpillars can reduce GLV emissions from plants. A relatively small molecule of less than 1000 Da was found to bind (Z)-3-hexenal directly and was named HALT (hexenal trapping molecule).

In an extended study on the distribution of these GLV-suppressing mechanisms, it was found that among the 10 caterpillars analyzed all employed at least one of these mechanisms with high efficiency (Jones et al. 2022). For example, T. ni possessed HALT and dehydratase activities, while M. sexta oral...
GLVs are almost universally produced by plants. This general widespread ability to produce them, often in quite large quantities, must be of significance and here we have presented some of the explanations or hypotheses for these, most of which have been investigated over the last several decades. Among the communities, it was also found that the capacity to produce GLVs varies significantly among different plant species and at different developmental stages of the plant. This further complicates an assessment of the biological role these compounds play in natural as well as agricultural settings. Adding to the complexity of GLV production in nature is the ability of different insect herbivores to actively suppress the production and the release of aldehyde GLVs by damaged plants, suggesting a prominent role of GLVs in the defense arsenal of plants, either by serving as a signal or by having a direct defensive effect on the attacking herbivore. Avoiding or altering these functions may therefore represent an essential element in the ever-evolving arms race between plants and their insect herbivores.

**Summary and Outlook**

GLVs are almost universal among plants. This general widespread ability to produce them, often in quite large quantities, must be of significance and here we have presented some of the explanations or hypotheses for these, most of which have been investigated over the last several decades. Among the communities, it was also found that the capacity to produce GLVs varies significantly among different plant species and at different developmental stages of the plant. This further complicates an assessment of the biological role these compounds play in natural as well as agricultural settings. Adding to the complexity of GLV production in nature is the ability of different insect herbivores to actively suppress the production and the release of these compounds, which strongly supports the active role that GLVs play in regulating the interactions of plants and their insect herbivores. However, to date very little is still known about how exactly this is managed, either by the plant or by the insect herbivore. How do caterpillars benefit from either the effective suppression or the isomerization of these compounds? How do plants benefit from producing often large quantities of GLVs despite the efforts of the insect herbivores to suppress them? We have just started to explore this exciting new area of research, but it is already obvious from the first published results that the interaction of plants and their insect herbivores is much more complex than hitherto thought and what the precise role of GLVs is in this interaction. GLVs play a role as a feeding deterrent, can serve as an attractant, function as a long-distance signal in certain plants, act as a local mediator upon damage, are antibacterial and can signal damage to other plants nearby, allowing them to prepare or prime their defenses, just to name some of the roles GLVs can play in a natural habitat. Furthermore, GLVs can function as potent signals in regulating abiotic stresses, mostly those that can lead to severe cellular damage like low temperatures and drought. All of these make them a worthy target for biotechnological modifications with the goal to improve the viability of plants under a plethora of biotic and abiotic stresses in agricultural and horticultural settings. However, there are as always also negative aspects to these activities, most prominently the positive effects these compounds have on certain biotrophic pathogens. Therefore, new approaches for targeted modifications of GLV activities need to be established first, and a better understanding of their eco-physiological role in nature is essential in developing these.

**Data Availability**

No new datasets were generated or analyzed in this study.

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**Disclosures**

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