Research review

Emerging trade-offs – impact of photoprotectants (PsbS, xanthophylls, and vitamin E) on oxylipins as regulators of development and defense

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Received: *13 September 2012*
Accepted: *8 November 2012*

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New Phytologist (2013) **197:** 720–729
doi: 10.1111/nph.12100

Key words: defense, jasmonic acid (JA), lutein, oxylipin, PsbS, vitamin E, xanthophyll, zeaxanthin.

Summary

This review summarizes evidence for a mechanistic link between plant photoprotection and the synthesis of oxylipin hormones as regulators of development and defense. Knockout mutants of *Arabidopsis*, deficient in various key components of the chloroplast photoprotection system, consistently produced greater concentrations of the hormone jasmonic acid or its precursor 12-oxo-phytodienoic acid (OPDA), both members of the oxylipin messenger family. Characterized plants include several mutants deficient in PsbS (an intrinsic chlorophyll-binding protein of photosystem II) or pigments (zeaxanthin and/or lutein) required for photoprotective thermal dissipation of excess excitation energy in the chloroplast and a mutant deficient in reactive oxygen detoxification via the antioxidant vitamin E (tocopherol). Evidence is also presented that certain plant defenses against herbivores or pathogens are elevated for these mutants. This evidence furthermore indicates that wild-type *Arabidopsis* plants possess less than maximal defenses against herbivores or pathogens, and suggest that plant lines with superior defenses against abiotic stress may have lower biotic defenses. The implications of this apparent trade-off between abiotic and biotic plant defenses for plant ecology as well as for plant breeding/engineering are explored, and the need for research further addressing this important issue is highlighted.

Introduction

The present review examines connections between plant photoprotection and oxylipin hormone production. Previously published and new evidence is summarized to show that chloroplast photoprotection mechanisms, serving to prevent potentially damaging oxidative events (especially under abiotic stress), simultaneously suppress the formation of oxylipin hormones that function as regulators of development and defense (including defenses against herbivores and pathogens). These findings suggest a possible trade-off between abiotic and biotic stress tolerances. The present review also proposes that this interaction can represent a compromise between efficient carbon translocation throughout the plant and barricading these same transport routes for the purpose of limiting the spread of pathogens through the plant, and thereby achieving superior pathogen defense. Full recognition of possible trade-offs between abiotic and biotic defenses is essential to be able to anticipate side effects on biotic defenses of plants bred or engineered for augmented abiotic stress tolerance, and of plants naturally featuring superior abiotic stress tolerance.

A wide range of organisms employ redox signals as regulators of cellular metabolism (for reviews, see e.g. Foyer & Noctor, 2009; Ray *et al.*, 2012). Central agents in this regulation are reactive oxygen species (ROS) which modify oxidation/reduction-sensitive signaling proteins or polyunsaturated fatty acids involved in signal transduction cascades. In plants, the chloroplast (specifically photosynthetic light collection and electron transport) is a major site of ROS generation (Pitzchke *et al.*, 2006). Chloroplast

membranes furthermore contain a large amount of highly oxidation-sensitive polyunsaturated fatty acids, among which alpha-linolenic acid (ALA) is a major parent compound for an array of messenger compounds derived via oxidative modification by ROS (Fig. 1 and Schaller & Stintzi, 2009). Plant messengers derived from oxidatively modified polyunsaturated fatty acids are collectively termed oxylipins (see Howe, 2004), and include important plant stress hormones such as jasmonic acid (JA) as well as its precursor – and messenger in its own right – 12-oxo-phytodienoic acid (OPDA), and its derivate methyl jasmonate (MeJA) (Fig. 1). In nonphotosynthetic organisms (including humans), analogous signal transduction cascades are initiated by oxidative modification of polyunsaturated fatty acids to hormonal messengers (for reviews, see Wahle *et al.*, 2003; Fernandis & Wenk, 2007).

Gene regulation by lipid peroxidation-derived messengers is thus a key regulatory pathway in both plants and animals. These messengers modulate a broad range of key responses, including development and defense responses, such as the overall immune response in animals (e.g. Yaqoob, 2003) as well as defenses against pests and pathogens in plants (see the following paragraph). Specific responses regulated by these messengers include programmed cell death (see Danon *et al.*, 2005 for plants) and plant senescence (e.g. He *et al.*, 2001, 2002; Devoto & Turner, 2003; Ananieva *et al.*, 2004), as well as up-regulation of antioxidant defenses (Sasaki-Sekimoto *et al.*, 2005; Wolucka *et al.*, 2005 for up-regulation of the ascorbate pool in plants). In addition, plant oxylipins regulate key responses in development, carbon allocation, and reproduction (cf. Fig. 2).

Oxylipins are derived from peroxidation products of polyunsaturated fatty acids (Fig. 1). These fatty acids are subject to both nonenzymatic and, mainly via the lipoxygenase (LOX) pathway, enzymatic peroxidation (Berger *et al.*, 2001). In response to a host of environmental (abiotic and biotic) as well as developmental cues (Bell *et al.*, 1995), fatty acids such as ALA are excised from chloroplast lipids by phospholipase, oxidized by LOX, and processed by a series of additional steps taking place in the chloroplast envelope and then in peroxisomes (Chrispeels *et al.*, 1999), leading to the formation of messengers such as OPDA, JA, and MeJA (Fig. 1; for reviews, see Creelman & Mullet, 1997; Howe & Schilmiller, 2002; Turner *et al.*, 2002; Wasternack & Hause, 2002; Devoto & Turner, 2003, 2005). JA is an important plant messenger that regulates, among other key responses, the expression of genes involved in plant defense (Fig. 1; Berger, 2002; Halitschke & Baldwin, 2003; Thaler *et al.*, 2004; Devoto & Turner, 2005). Jasmonates play an important role in defense against insect attack and wounding in general (Berger, 2002; Ellis *et al.*, 2002; Bostock, 2005). In addition to JA, OPDA also up-regulates defenses in response to pathogen and insect attack (Fig. 1; Stintzi *et al.*, 2001) and, in general, acts as a messenger independently of JA (Landgraf *et al.*, 2002; Danon *et al.*, 2005; Taki *et al.*, 2005).

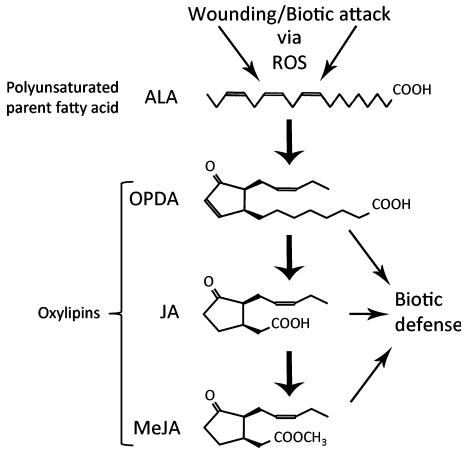

Fig. 1 Biosynthesis of oxylipins in *Arabidopsis* (schematic depiction abbreviated after Devoto & Turner, 2005). ROS, reactive oxygen species; ALA, alpha-linolenic acid; OPDA, 12-oxo-phytodienoic acid; JA, jasmonic acid; MeJA, methyl jasmonate; ROS, reactive oxygen species.

Specific plant oxylipins have thus been characterized as messengers initiating and coordinating plant defenses against biotic stress from pest or pathogen attack (Fig. 1). Formation of ROS is triggered by wounding and/or biotic attack (Torres, 2010), developmental cues (Bell *et al.*, 1995), and abiotic stresses (Suzuki *et al.*, 2012). Oxylipin formation would thus appear to be a strong candidate for interactive 'crosstalk' between biotic and abiotic stresses. A wide range of abiotic stresses – such as drought, unfavorable temperatures, and many others that can impede plant growth – have been shown to slow photosynthetic electron transport and thereby cause excitation energy to accumulate, which increases the potential for ROS formation in light-collecting pigment complexes (light absorption; Fig. 2) and via components of the electron transport chain (charge separation and electron transport; Fig. 2). Intense sunlight has the potential to do the same even in the absence of additional abiotic stress factors (Amiard *et al.*, 2007). All abiotic stresses increase plant photoprotection, including antioxidant production.

The chloroplast features a multilayered cascade of photoprotective processes (Fig. 2), all of which act to lower ROS production. High light and/or abiotic stresses trigger up-regulation of these photoprotective processes (for a review, see Niyogi, 1999). It would seem that the potency of these photoprotective processes – augmented in response to abiotic stress – should affect the level of oxidatively formed messengers that, in turn, trigger plant biotic defenses. If so, future research should take a comprehensive view of plant abiotic and biotic stress and the resulting stress responses in order to identify likely synergisms and trade-offs between abiotic and biotic stresses. This review summarizes predictions based on known features of chloroplast-based photoprotection and oxylipin production, and presents the evidence currently available to evaluate these predictions.

Chloroplast photoprotection and oxylipin production

When more excitation energy is absorbed by chloroplast pigments than can be utilized in photosynthetic electron transport, the (singlet) excited state of chlorophyll (^{1}Chl*) may temporarily accumulate (Fig. 2). If there is insufficient de-excitation of this accumulating singlet excited state of chlorophyll via PsbS (an intrinsic chlorophyll-binding protein of photosystem II) and xanthophyll pigments such as zeaxanthin, excited triplet chlorophyll (^{3}Chl*) can be formed and pass excitation energy on to

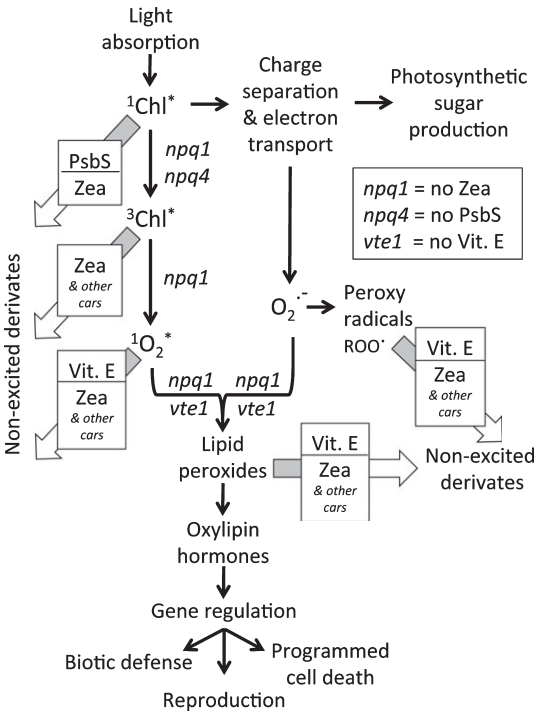

Fig. 2 Schematic depiction of the formation of various excited molecular species during light absorption and photosynthetic electron transport in the chloroplast. The initial excited species formed during light absorption is singlet excited chlorophyll a (^{1}Chl*), and the excited species potentially formed during charge separation and electron transport is singly reduced oxygen (superoxide $O_2^{\cdot-}$). The straight downward-pointing black arrows depict the successive conversion of each of the initially formed excited species to their products; abbreviations in italics next to the arrows depict mutants deficient in the respective steps; broad, angled, downward-pointing open arrows and rectangular boxes superimposed upon these arrows depict harmless de-excitation pathways (leading to nonexcited derivatives) and the protein/metabolite(s) catalyzing these reactions, respectively. Zea, zeaxanthin; Vit. E, vitamin E; cars, carotenoids; *npq1*, mutant deficient in violaxanthin de-epoxidase (and thus deficient in zeaxanthin formation); *npq4*, mutant deficient in PsbS (an intrinsic chlorophyll-binding protein of photosystem II) (and thus the rapid, pH-dependent engagement of thermal energy dissipation); *vte1*, mutant deficient in tocopherol cyclase (and thus deficient in vitamin E).

oxygen, thereby forming highly reactive singlet excited oxygen ($^1O_2^*$) (Fig. 2). Singlet oxygen, in turn, readily oxidizes polyunsaturated fatty acids, leading to lipid peroxide formation (Fig. 2). In addition, excess excitation can lead to the transfer – during charge separation and/or electron transport reactions – of a single electron to oxygen, leading to the formation of the highly reactive radical anion superoxide ($O_2^{\cdot-}$) that can also cause lipid peroxidation (Fig. 2). Lipid peroxides furthermore lead to the formation of oxylipin hormones (Fig. 2) with a wide range of gene regulatory functions in plant development and defenses.

Chloroplast defenses against oxidative stress are integrated with each other as well as with other components of the cellular antioxidant network (Noctor *et al.*, 2000; Pfannschmidt *et al.*, 2003; Baier & Dietz, 2005; Beck, 2005; Mullineaux & Rausch, 2005). In addition to protecting chloroplast integrity, antioxidants have a crucial role in redox sensing and signaling (Foyer & Noctor, 2003, 2005; Ledford & Niyogi, 2005). Cellular redox balance, in turn, plays a key role in the modulation of growth and development, via, for example, regulation of the cell cycle and programmed cell death (den Boer & Murray, 2000; Potters *et al.*, 2002; Pavet *et al.*, 2005).

The chloroplast's complement of photoprotective processes can be grouped into (1) pre-emptive processes preventing ROS formation and (2) detoxification processes that de-excite ROS once formed. (1) Pre-emptive prevention of ROS formation is achieved (Fig. 2) by harmless removal of excess amounts of ^{1}Chl*, via de-excitation, involving the PsbS protein (Li *et al.*, 2000) and xanthophyll pigments such as zeaxanthin, in the process of photoprotective thermal dissipation (estimated from nonphotochemical chlorophyll fluorescence quenching (NPQ); Demmig *et al.*, 1987; Niyogi *et al.*, 1997, 1998; Holt *et al.*, 2005; for reviews, see Demmig-Adams *et al.*, 1996; Niyogi, 2000; Adams *et al.*, 2004; Niyogi *et al.*, 2005; Demmig-Adams & Adams, 2006) and/or by harmless removal of excess amounts of ^{3}Chl* via de-excitation by various carotenoid pigments (see e.g. Telfer, 2005; Mozzo *et al.*, 2008), all before reactive oxygen can be formed. (2) Detoxification of already formed reactive oxygen species, such as $^1O_2^*$ or $O_2^{\cdot-}$, and other reactive species, such as peroxy radicals and lipid peroxides, occurs via de-excitation or re-reduction, respectively, to their respective nonreactive states by tocopherols (vitamin E; Munné-Bosch & Alegre, 2002; Havaux *et al.*, 2005; Munné-Bosch, 2007; Munné-Bosch *et al.*, 2007; Traber & Stevens, 2011) and/or zeaxanthin and possibly other carotenoids (Krinsky & Deneke, 1982; Conn *et al.*, 1991; Lim *et al.*, 1992; Packer, 1993; Jorgensen & Skibsted, 1993; Tinkler *et al.*, 1994; Hill *et al.*, 1995; for a review, see Beatty *et al.*, 2000). The elegant work of Havaux & Niyogi (1999) demonstrated an inhibitory effect of zeaxanthin on lipid peroxidation (see also Baroli & Niyogi, 2000; Havaux *et al.*, 2000, 2004, 2005, 2007; Baroli *et al.*, 2004) in addition to zeaxanthin's role in thermal energy dissipation.

In vitro, zeaxanthin protects lipids against photosensitized singlet oxygen-catalyzed peroxidation and this effect is enhanced in the presence of vitamin E (Wrona *et al.*, 2003, 2004). Vitamin E also scavenges lipid peroxy radicals and thereby terminates lipid peroxidation chain reactions (Schneider, 2005). In addition, vitamin E can inhibit LOX (the enzyme that catalyzes ALA peroxidation; several LOX isoforms are present in the chloroplast; Bachmann *et al.*, 2002) via reduction of the catalytic iron center from the active LOX-Fe^{3+} to the inactive LOX-Fe^{2+} (Maccarrone *et al.*, 1999). Furthermore, ROS are not only able to directly oxidize fatty acids, but are also needed to activate LOX (via oxidation of inactive LOX-Fe^{2+} to active LOX-Fe^{3+}; Maccarrone *et al.*, 1996). Zeaxanthin is located in the thylakoids, with some also present in the chloroplast envelope (Markwell *et al.*, 1992; see also Costes *et al.*, 1979). The potential of zeaxanthin to affect plant oxylipin production thus includes ROS suppression, ROS scavenging, and suppression of various aspects of fatty acid peroxidation – alone or by interaction with vitamin E (involving re-reduction of oxidized vitamin E by zeaxanthin) (for a review, see Baroli & Niyogi, 2000). The interaction of (1) pre-emptive prevention of reactive oxygen formation and (2) detoxification of any reactive species still formed

apparently counteracts formation of reactive species and their derivatives rather effectively.

One would thus predict that higher concentrations of the above thermal dissipation catalysts and/or antioxidants in the chloroplast should lower oxylipin production, while low concentrations or the absence in knock-out lines of these same photoprotective compounds should increase oxylipin production (cf. Fig. 2). Genetically altered *Arabidopsis* lines are available that are deficient in the PsbS protein (the nonphotochemical-quenching-impaired *npq4* line; Li *et al.*, 2002; Fig. 2); zeaxanthin (*npq1*, deficient in the enzyme violaxanthin de-epoxidase that forms zeaxanthin under excess light; Niyogi *et al.*, 1998; Fig. 2); or vitamin E (*vte1*, deficient in tocopherol cyclase, an enzyme catalyzing a key step of vitamin E biosynthesis; Porfirova *et al.*, 2002; Fig. 2). The present review summarizes existing literature on oxylipin concentrations in *npq4* and *vte1* and presents new data on oxylipin concentrations in *npq1*, all compared with oxylipin production in wild-type (WT) *Arabidopsis*. In addition, the actual or apparent biotic defense potential in these lines of *Arabidopsis* is addressed.

Oxylipins and biotic defense in PsbS-deficient plants

Studies from the group of Stefan Jansson have focused on the performance of the PsbS-deficient *Arabidopsis npq4* line under outdoor/field conditions (Külheim *et al.*, 2002). A follow-up study from the Jansson group (Frenkel *et al.*, 2009) assessed the concentrations of the oxylipin stress hormone JA, and reported augmented concentrations of JA in *npq4* vs WT under field conditions where plants experienced herbivory, but not under control field conditions where herbivory was not allowed to occur (Fig. 3a). This result suggests that the absence of PsbS, and PsbS-dependent photoprotective thermal dissipation of ^{1}Chl*, leads to greater reactive oxygen formation and greater levels of JA formation, but only under the conditions produced by herbivore attack.

The finding that the PsbS-deficient mutant produces more JA than WT plants supports the view that PsbS-dependent photoprotective thermal dissipation lowers the level of production of oxylipins such as JA. Herbivore attack may synergistically further augment the levels of reactive oxygen formed in PsbS-deficient *npq4* leaves. Moreover, growth of plants under field conditions (including natural exposure to herbivores) resulted in a greater fraction of WT plants than *npq4* plants that were attacked by herbivores (Fig. 3b). This latter finding suggests that the greater concentration of the plant defense hormone JA in PsbS-deficient (photoprotective-energy-dissipation-deficient) plants was involved in deterring herbivore attack. This latter observation furthermore suggests that the greater levels of photoprotective thermal energy dissipation in WT vs *npq4* made WT plants more susceptible to herbivore damage. The next section will further explore such a connection for another photoprotective process, detoxification via vitamin E.

Oxylipins in vitamin E-deficient plants

The group of Munné-Bosch had previously suggested that the antioxidant vitamin E (tocopherol) not only has direct redox-modulating effects in photosynthesis, but may also 'indirectly affect jasmonic acid accumulation by controlling the extent of lipid peroxidation in chloroplasts' (Munné-Bosch & Falk, 2004). Munné-Bosch *et al.* (2007) explored the effect of the vitamin E-deficient *Arabidopsis* mutant *vte1* on JA formation under high light and low temperature. Figure 4 shows that *vte1* plants produced greater concentrations of JA than WT plants. Greater JA concentrations in the *vte1* mutant vs WT were also documented in a subsequent study by the Munné-Bosch group (Cela *et al.*, 2011). These observations indicate that detoxification of ROS and/or other oxidized species by vitamin E lowers oxylipin production, while vitamin E deficiency increases oxylipin production – just as was observed by Jansson's group for plants deficient in PsbS-dependent photoprotective thermal dissipation (cf. Fig. 3a). Both photoprotective processes – thermal dissipation and detoxification – thus apparently suppress oxylipin production and have the potential to increase plant sensitivity to biotic stress. The following section 'Oxylipins and structural biotic defense in zeaxanthin-deficient plants' will visit the effect of yet another component involved in photoprotection, the carotenoid zeaxanthin.

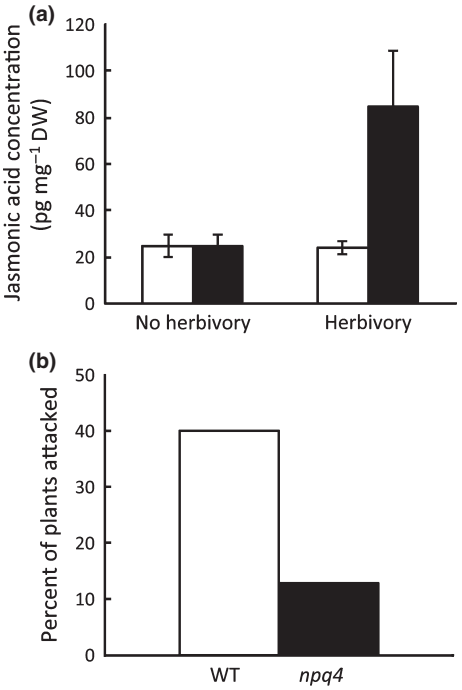

Fig. 3 Differences in (a) the foliar concentration of jasmonic acid in the absence or presence of herbivory (mean ± SE) and (b) the percentage of plants attacked by herbivores between wild-type *Arabidopsis* (WT) and mutants deficient in PsbS (an intrinsic chlorophyll-binding protein of photosystem II) (*npq4*) under outdoor/field conditions. Data are from Frenkel *et al.* (2009).

Oxylipins and structural biotic defense in zeaxanthin-deficient plants

Zeaxanthin is involved in photoprotection via a role in thermal energy dissipation and/or antioxidation. Low-light-grown leaves of the zeaxanthin-deficient *Arabidopsis* mutant *npq1-1* exhibited higher concentrations of the oxylipin, and JA precursor, OPDA (cf.

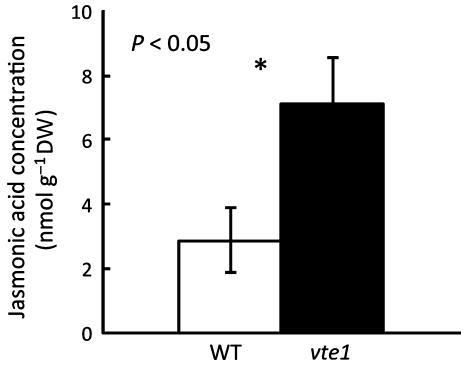

Fig. 4 Difference (mean ± SE) in foliar concentrations of jasmonic acid between wild-type *Arabidopsis* (WT) and mutants deficient in vitamin E (*vte1*) under outdoor/field conditions. Data are from Munné-Bosch *et al.* (2007). The asterisk indicates significance (at the $P < 0.05$).

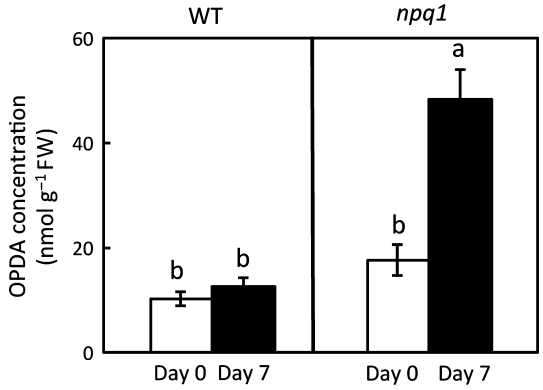

Fig. 5 Differences in foliar concentrations of the oxylipin, and jasmonic acid precursor, 12-oxo-phytodienoic acid (OPDA) between wild-type (WT) *Arabidopsis* (Columbia) and mutants deficient in zeaxanthin (*npq1*), all grown at low light intensities (day 0; 100 μmol photons m^{-2} s^{-1}) and transferred to high light (1000 μmol photons m^{-2} s^{-1}) for 7 d (day 7). Where indicated by different lowercase letters above the bars, means (± SD) were significantly different at $P < 0.05$, using a Student's *t*-test. For lipid extraction, samples were freeze-dried and homogenized in 10 mM phosphate-buffered saline, followed by addition of cool methanol-chloroform (v/v, 2 : 1). After shaking and centrifugation, the organic phase was dried and the remaining residue dissolved in 300 μl of methanol and diluted with 800 μl of water and applied to a solid-phase extraction column (OASIS HLB; 30 mg; Waters, Etten-Leur, the Netherlands). The column was washed with 1 ml of water and fatty acid metabolites were eluted with 1 ml of methanol. To the sample 4.0 nmol 13-hydroxyoctadecanoic acid, as internal standard, was added and the sample was dried under a gentle stream of nitrogen gas and re-dissolved in 100 μl of methanol. An aliquot was analyzed by RP-HPLC (Hewlett-Packard 1090 LC equipped with a Hewlett-Packard 1040A diode array detector; Amstelveen, the Netherlands) on a Cosmosil 5C18 ARII column (5 μm; 250 × 4.6 mm; Nacalai Tesque, Kyoto, Japan) at a flow rate of 1 ml min^{-1} with a 10-min linear gradient from 75 : 25 : 0.1 (v/v/v) to 95 : 5 : 0.1 (v/v/v) methanol:water:acetic acid and held at these conditions for 5 min before returning to the initial conditions. 12-oxo-phytodienoic acid was identified by GC/MS (van Zadelhoff *et al.*, 1998) and quantified on the basis of its absorption at 206 nm.

Fig. 1) compared with WT *after* 7 d of exposure to high light, but not before high light exposure (Fig. 5). This latter observation suggests that zeaxanthin-dependent photoprotection prevented elevated oxylipin production under high light. This finding is quite similar to what was observed for PsbS-dependent photoprotection (see section 'Oxylipins and biotic defense in PsbS-deficient plants' above) and vitamin E-dependent photoprotection (see section 'Oxylipins in vitamin E-deficient plants' above). The effects of zeaxanthin, PsbS, and vitamin E, respectively, in suppressing reactive oxygen formation (and/or resulting oxidation events) all apparently acted to suppress oxylipin formation.

For the case of *npq1* mutants, just as was the case for *npq4* mutants examined in the field, evidence is furthermore available that plant defense potential is indeed affected. Plant pathogens, such as viruses and fungi, frequently employ a path of attack, and spread, throughout the plant through the long-distance sugar-transporting phloem (sieve element) tubes (see e.g. Vuorinen *et al.*, 2011). As a counterbalance, plant defense processes are expected to, and can apparently, counter this pathogen attack strategy by reinforcing barriers to pathogen invasion of the phloem's long-distance-transport sieve tubes (Amiard *et al.*, 2007, and reference therein).

Fig. 6 shows images of phloem ultrastructure in *Arabidopsis* with cell wall-reinforcing ingrowths between sieve elements (SEs) and their surrounding phloem parenchyma cells (PCs), with these cell wall thickenings being minimal in low-light-grown plants and significantly enhanced in high-light-grown plants – and also in continuously low-light-grown plants experimentally treated with methyl jasmonate (LL+MeJA). These presumably protective cell wall thickenings are thus apparently triggered by oxylipin, and high light probably acts to generate reactive oxygen to promote endogenous oxylipin formation which stimulates cell wall thickening.

Treatment with MeJA induces the formation of extensive cell wall ingrowths in specific phloem cells (Fig. 6; see Amiard *et al.*, 2007). Jasmonates such as JA and MeJA have been shown to stimulate expression of nuclear genes related to synthesis of wall components and to modulate several aspects of cell wall structure and signaling (Ellis *et al.*, 2002; Uppalapati *et al.*, 2005). Offler *et al.* (2003) had already hypothesized that JA might be responsible for inducing phloem transfer cell formation and cell wall invagination. We (Amiard *et al.*, 2007) subsequently used *Arabidopsis* – which shows increased wall invaginations exclusively in phloem PCs and not in companion cells (CCs) – as well as pea (*Pisum sativum*) (with cell wall invaginations in CCs only) and yet another species (*Senecio vulgaris*) that exhibits cell wall ingrowths in both PCs and CCs. In high light relative to low light, wall invagination was greater in all three plant species in CCs and/or PCs (Amiard *et al.*, 2007; cf. Fig. 6). We furthermore demonstrated that MeJA treatment of plants growing in low light induced cell wall ingrowths in the phloem PCs of *Arabidopsis* and *S. vulgaris* but not in phloem CCs (Amiard *et al.*, 2007; see Fig. 6 for *Arabidopsis*). These latter results are consistent with a role of PC wall ingrowths in defense, with PCs having been shown to be the primary target of insect and pathogen attack on the phloem (Ding *et al.*, 1995; Heller & Gierth, 2001; Zhou *et al.*, 2002).

The conclusion that oxylipins are involved in triggering putatively protective phloem PC wall reinforcement is further corroborated by the suppression of these phloem PC wall

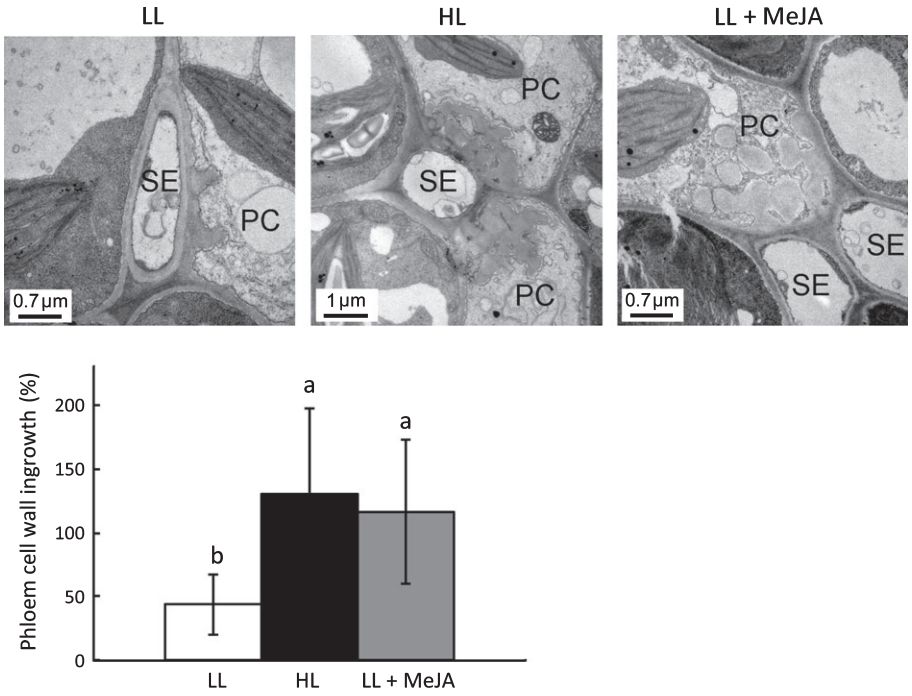

Fig. 6 Increases in cell wall length (due to wall ingrowths) in phloem parenchyma cell (PC) of minor loading veins of *Arabidopsis* before and after transfer from low light (LL) to high light (HL; 100–1000 µmol photons m^{-2} s^{-1}) for 1 wk. Percentages are relative to hypothetical (without any ingrowths) cell wall lengths as assessed from electron microscopic images. Where indicated by different lowercase letters above the bars, means (± SE) were significantly different at $P < 0.05$, based on an ANOVA followed by a Tukey–Kramer comparison for honestly significant differences. Data are from Amiard *et al.* (2007). Plants treated with methyl jasmonate (LL+MeJA) were grown under low light and sprayed daily with a solution of 10 µM MeJA in water and 0.05% Tween 20 for 1 wk. Control plants (LL) were sprayed daily with water and 0.05% Tween 20 for 1 wk. Cells labeled as SE are the sugar-transporting sieve elements.

thickenings under high light conditions in an *Arabidopsis* mutant deficient in fatty acid desaturation (Fig. 7). We quantified the level of phloem PC wall ingrowths in the *Arabidopsis fad7-1 fad8-1* double mutant lacking two fatty acid desaturases that generate the polyunsaturated chloroplast fatty acid ALA serving as an oxylipin precursor (Fig. 1; Falcone *et al.*, 2004). There was significantly less wall ingrowth in the *fad7-1 fad8-1* mutant compared with WT (Fig. 7), which further supports a role of oxylipins as signals in generating phloem PC wall ingrowths.

The connection between oxylipins and phloem parenchyma cell wall thickening under high light was exploited to further address the role of chloroplast photoprotection in modulating oxylipin-dependent plant responses. We hypothesized that zeaxanthin, which is able to suppress lipid peroxidation (via multiple mechanisms), should also suppress oxylipin formation and thereby counteract PC wall ingrowth formation. If this were indeed the case then, compared with WT plants, zeaxanthin-deficient mutants should produce a greater level of phloem PC wall ingrowths upon transfer to high light. A small data set in which *npq1* was compared with WT yielded means consistent with the hypothesis, but not significantly different: the mean per cent increase in PC wall ingrowth following transfer from 100 to 1000 µmol photons m^{-2} s^{-1} was 64 ± 21% for WT, 103 ± 22% for *npq1-1*, and 78 ± 24% for *npq4-1* (all data given as mean per cent increase ± SE). We therefore conducted a low light to high light transfer with an *Arabidopsis* double mutant deficient not only in zeaxanthin, but also in the zeaxanthin isomer lutein (*npq1-2, lut2-1*; Niyogi *et al.*, 2001); the double mutant indeed exhibited significantly greater phloem PC wall thickening than WT in response to a transfer to elevated light intensities (Fig. 8). The xanthophyll lutein has been shown to further augment zeaxanthin-dependent photoprotective thermal energy dissipation and the double mutant deficient in both zeaxanthin and lutein shows an even more complete suppression of thermal dissipation than the zeaxanthin-deficient single mutant *npq1* (Niyogi *et al.*, 2001).

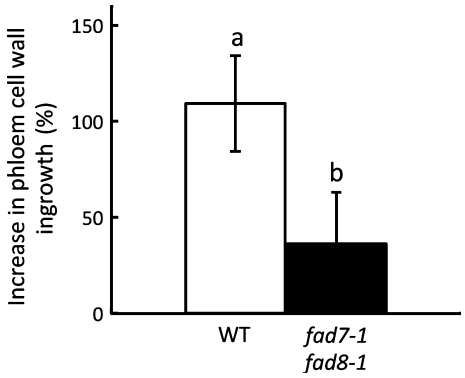

Fig. 7 Increases in cell wall ingrowths in minor vein phloem parenchyma cells of wild-type (WT) *Arabidopsis* and the *fad7-1 fad8-1* double mutant of *Arabidopsis*. Plants were grown in low light (100 µmol photons m^{-2} s^{-1}) then transferred to high light (1000 µmol photons m^{-2} s^{-1}) for 1 wk. Values represent the percentage of actual measured wall length relative to hypothetical wall length of cells without ingrowths, as assessed from electron micrographic images. Where indicated by different lowercase letters above the bars, means (± SE) were significantly different at $P < 0.05$, using a Student's *t*-test. Data are from Amiard *et al.* (2007).

This important finding, of zeaxanthin/lutein-dependent photoprotection suppressing a putative plant biotic defense response, is consistent with, and further corroborates, the results of Jansson's group on the suppression of plant herbivore defense via PsbS-dependent thermal dissipation (Frenkel *et al.*, 2009). All of these results provide evidence for a close link between plant abiotic defense, in the form of chloroplast photoprotection, and plant biotic defenses.

Similar to the role suggested here for zeaxanthin in a signaling pathway that targets the phloem, a link was recently established

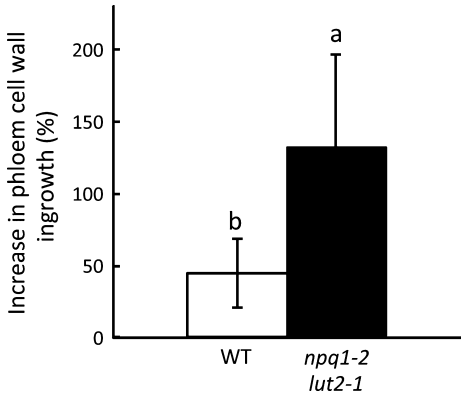

Fig. 8 Increase in phloem parenchyma cell wall ingrowth (quantified as in Fig. 6) in response to a transfer from 150 to 350 μmol photons m^{-2} s^{-1} for 1 wk in wild-type (WT) *Arabidopsis* and the double mutant *npq1-2 lut2-1* (background Col-0). This mutant was made available to us by Prof. Kris Niyogi. Where indicated by different lowercase letters above the bars, means (± SE) were significantly different at $P < 0.05$ using a Student's *t*-test. *npq1*, mutant deficient in violaxanthin de-epoxidase (and thus deficient in zeaxanthin formation); *lut2*, mutant deficient in lutein (structural isomer of zeaxanthin).

between tocopherol (vitamin E) synthesis in the chloroplast and inhibition of sucrose export into the phloem (Hofius & Sonnewald, 2003; Hofius *et al.*, 2004; see also Provencher *et al.*, 2001) involving callose deposition into plasmodesmatal cell wall openings (thereby 'plugging' plasmodesmata; Botha *et al.*, 2000), and into phloem PC walls adjacent to sieve tubes (Maeda *et al.*, 2006). Barriers at both of these sites can be expected to provide defense – albeit at the expense of efficient carbon distribution throughout the plant – against plant pathogens, whose movement employs both routes, that is, long-distance sieve tube transport (see earlier; Vuorinen *et al.*, 2011) and cell-to-cell movement through plasmodesmata (see e.g. Lee & Lu, 2011). Furthermore, a mutant deficient in vitamin C (ascorbate), *vtc1*, was shown to affect a host of plant developmental and defense responses, including the timing of senescence and the susceptibility to several pathogens (Barth *et al.*, 2004) as well as the susceptibility to ozone and other abiotic factors (Conklin *et al.*, 1996). Vitamin C synthesis can also be induced by MeJA treatment (Wolucka *et al.*, 2005).

Conclusions and future directions

A clear picture begins to emerge from all of the results reviewed and newly presented here. Chloroplast photoprotection, serving to prevent potentially damaging oxidative events, apparently simultaneously suppresses the formation of oxylipin hormones (as the oxidatively modified derivatives of polyunsaturated chloroplast fatty acids) that modulate key plant responses including development and defense. The present report provides evidence for such a role of chloroplast photoprotection for (1) pre-emptive prevention of reactive oxygen formation via thermal energy dissipation (involving PsbS and/or zeaxanthin and lutein) as well as (2) detoxification (involving vitamin E and, again, zeaxanthin) of ROS and other reactive species.

The conclusions drawn here are based largely on the finding that oxylipin (OPDA and/or JA) concentrations are increased in photoprotection mutants (deficient in components involved in thermal dissipation and/or detoxification). For further evaluation, the photoprotection mutants should be crossed with oxylipin biosynthesis mutants and/or oxylipin perception mutants.

Much effort has gone into over-expression of various components of the chloroplast photoprotection system with the goal to engineer plant lines with superior abiotic stress tolerance. For the example of zeaxanthin-dependent photoprotection, mutant lines engineered to over-express xanthophyll cycle components did indeed exhibit increased abiotic stress tolerance, while zeaxanthin-depleted lines exhibited decreased abiotic stress tolerance (e.g. Davison *et al.*, 2002 Du *et al.*, 2010; Gao *et al.*, 2010; Wang *et al.*, 2010; Chen *et al.*, 2011).

However, the results reported here suggest that there may be an important trade-off between abiotic stress tolerance and biotic defense. Plant lines featuring superior abiotic stress tolerance may simultaneously suffer from suppression of oxylipin production and a potential increased susceptibility to herbivore and/or pathogen attack. At the same time, all plants appear to be constantly faced with a trade-off between efficient carbon translocation throughout the plant and barricading these same transport routes for the purpose of limiting pathogen movement through the plant, and thereby achieving superior pathogen defense.

Furthermore, the relationship between abiotic and biotic defenses appears to be complex. Initial exposure to abiotic stress intermittently increases oxylipin production, probably because existing antioxidant concentrations are insufficient to keep ROS in check. Oxylipin production itself subsequently triggers up-regulation of antioxidant production via a feedback loop. Lastly, augmentation of antioxidant concentrations presumably suppresses further oxylipin production. Jasmonate treatment has indeed been found to enhance overall antioxidant capacity (Wang & Zheng, 2005): accumulation of the antioxidant ascorbate (Sasaki-Sekimoto *et al.*, 2005; Wolucka *et al.*, 2005), a key defense component against ozone stress, provides defense in the cell wall against O_3 entry into the cell (Baier *et al.*, 2005); jasmonates provide protection against ozone injury (Overmyer *et al.*, 2000; Rao *et al.*, 2000; Tuominen *et al.*, 2004; Sasaki-Sekimoto *et al.*, 2005); and jasmonate treatment has been shown to increase the concentrations of the antioxidant vitamin E (Gala *et al.*, 2005; see also Munné-Bosch, 2005) and to stimulate carotenoid synthesis (Saniewski & Czapski, 1983).

Further studies are now urgently needed that comprehensively assess responses of both engineered and naturally varying plant lines (crop varieties, land races, and ecotypes; see e.g. Newton *et al.*, 2010) to the abiotic environment as well as to biotic attack. For plant lines with differing abiotic stress tolerance, the hypothesis should be tested that those varieties with superior abiotic stress tolerance will possess an inferior biotic stress tolerance and vice versa.

Studies assessing the role of photoprotection in plant productivity sometimes extrapolate potential gains in plant productivity for scenarios where losses from abiotic stresses such as drought and unfavorable temperatures were to be avoided. The present report

cautions that such extrapolations must take potentially enhanced losses in biomass to herbivores and pathogens into consideration.

Lastly, the connections and potential trade-offs highlighted here resonate well with a sweeping paradigm shift in the medical arena concerning the understanding of the roles of ROS (as involved in essential signaling events) and antioxidants (as potential suppressors of the latter signaling events).

Acknowledgements

We thank Krishna K. Niyogi for making *npq1-1* and the *npq1-2 lut2-1* double mutants available to us. This work was supported by the National Science Foundation (Award Numbers IBN-0235351, IOS-0841546, and DEB-1022236 to B.D-A. and W.A.) and the University of Colorado at Boulder, CO, USA.

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