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Review

Climate Change, CO_2, and Defense: The Metabolic, Redox, and Signaling Perspectives

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Ongoing human-induced changes in the composition of the atmosphere continue to stimulate interest in the effects of high CO_2 on plants, but its potential impact on inducible plant defense pathways remains poorly defined. Recently, several studies have reported that growth at elevated CO_2 is sufficient to induce defenses such as the salicylic acid pathway, thereby increasing plant resistance to pathogens. These reports contrast with evidence that defense pathways can be promoted by photorespiration, which is inhibited at high CO_2. Here, we review signaling, metabolic, and redox processes modulated by CO_2 levels and discuss issues to be resolved in elucidating the relationships between primary metabolism, inducible defense, and biotic stress resistance.

Biotic Challenges in the Context of the Living, Breathing Cell

Plants growing in the field are surrounded by microorganisms keen to access compounds produced through photosynthesis and related processes. Entry and propagation of pathogenic invaders poses a serious threat to plant health, vigor, and productivity. Thus, plants have evolved barriers to restrict pathogen entry, as well as chemical compounds that can be deployed as needed to counter growth of the invader. The battery of defense compounds includes phytohormones, such as **salicylic acid** (SA; see Glossary), jasmonic acid (JA), and ethylene, as well as antimicrobial **phytoalexins** and **pathogenesis-related (PR) proteins**. Tremendous progress has been made in defining the genetic factors that determine whether a challenged plant successfully deploys this arsenal to resist disease. The qualitative outcome of an interaction (disease or resistance) depends on the interplay between pathogen-derived molecular patterns, plant surface receptors, pathogen effectors, and plant intracellular receptors that recognize effectors to allow **effector-triggered immunity (ETI)** [1].

Despite this body of knowledge, its application to improving crop resistance has so far met with limited success in the field [1]. Alternative strategies include stimulation of defense response time or amplitude without prior exposure to pathogens. This phenomenon is known as 'priming' and can be induced by treatment with certain chemicals [2]. There is also interest in genes and processes underlying quantitative resistance, which decreases disease symptoms without preventing them entirely [3]. It has become clear that defense signaling pathways are intricately woven into the metabolic fabric of the cell, with extensive communication between developmental programs, stress conditions, and nutritional status [4–9]. Fundamental factors, such as irradiance and photoperiod, as well as the basic oxidoreductive processes by which plants generate cellular matter and energy (i.e., photosynthesis and respiration), can influence the outcome of defense signaling [10–12]. Recently, it has been reported that growth at elevated

Trends

Pathogenesis-related (PR) responses are closely integrated with primary metabolism and influenced by simple compounds involved in plant nutrition, such as sugars, organic acids, and amino acids.

Photorespiratory metabolism linked to enhanced production of reactive oxygen species (ROS) in peroxisomes can induce typical PR responses.

Growing plants at increased CO_2 levels, which slows photorespiratory rates, can also induce defense metabolism and PR genes, effects that are accompanied by upregulation of ROS signaling.

Genes involved in CO_2 sensing and signaling in stomatal regulation are beginning to be elucidated but components that link CO_2 availability to biotic stress responses remain to be identified.

Emerging evidence suggests that metabolite and ROS signaling are likely to be important in linking CO_2 availability to the activation of defense metabolism.

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CO_2 is sufficient to induce plant defenses such as the SA and JA pathways, thereby increasing resistance to bacteria and fungi [13–15]. These reports appear paradoxical to observations that **photorespiration**, which is decreased when plants are grown at **high CO_2**, can also activate the same biotic stress pathways [12,16,17]. Beyond their academic appeal, these issues are relevant to efforts to prime plants for enhanced resistance to pathogens and to predict crop performance in the atmospheric conditions of the coming century [18]. Here, we review the latest findings in these areas and provide a critical discussion of current concepts as well as directions for future inquiry.

Photorespiratory H_2O_2 Production and Biotic Stress Responses

Photorespiration is an integral part of photosynthesis in C_3 plants, but is largely suppressed in C_4 plants, which maintain high CO_2 concentrations in certain cells. Although apparently dispensable for growth and a drag on agricultural yields, the photorespiratory carbon and nitrogen recycling pathway is tightly embedded in primary metabolism [19,20] and involves intricate metabolic communication between several subcellular compartments (Figure 1). The clearest potential impact of this pathway on biotic stress responses is through the production of **reactive oxygen species** (ROS), compounds that have key roles in plant perception of pathogens and subsequent events. While considerable attention has focused on plasmalemma-associated enzymes, notably NADPH oxidases and peroxidases [21,22], ROS can also be produced in abundant amounts in chloroplasts, mitochondria, and peroxisomes (summarized in Figure 1).

Peroxisomes house several types of protein that are important in pathogen defense signaling [23,24]. A major peroxisomal function is to convert chloroplast-derived glycolate to glycine and mitochondrion-derived serine to glycerate in the photorespiratory pathway (Figure 1), reactions that can involve rapid production of the key ROS, H_2O_2, through peroxisomal glycolate oxidase (GOX) activity [25,26]. Available knowledge suggests that GOX activity, and the overall flux through the photorespiratory pathway, is largely determined by the supply of glycolate from RuBP oxygenation in the chloroplast. Oxygenation rates will in turn be determined by irradiance and the relative stromal concentrations of oxygen and CO_2 [19,20]. Therefore, GOX activity should be favored by high light and conditions that limit CO_2 diffusion from the leaf exterior to photosynthetic cells by promoting stomatal closure. As well as decreased water potential, such conditions include challenge by certain pathogens.

How influential is photorespiratory metabolism in biotic stress responses? Both GOX and glyoxylate aminotransferases, which process the product of the GOX reaction, have been implicated in PR responses in studies conducted in arabidopsis (*Arabidopsis thaliana*) and melon (*Cucumis melo*) [17,27]. In arabidopsis, two genes encode GOX involved in photorespiration [28], although GOX1 may be the major player in H_2O_2 production [29]. It is not yet clear whether these photorespiratory GOX isoforms have significant roles during plant responses to biotic stress. A key issue is the role of a third peroxisomal enzyme, catalase, which removes the H_2O_2 produced by GOX and other peroxisomal enzymes [30]. In the presence of typical wild-type levels of catalase, peroxisomal H_2O_2 is kept relatively low (estimated at 10 μM [26]). However, in mutants with decreased catalase, enhanced H_2O_2 availability triggers a range of canonical PR responses in conditions that favor photorespiration and, therefore, GOX activity [12,16,31–33].

Activation of SA synthesis and signaling in catalase-deficient plants demonstrates the competence of photorespiratory H_2O_2 to drive biotic stress responses, but does not establish whether this source of ROS has any role in wild-type plants [30]. It is interesting that several mechanisms have been identified that could post-transcriptionally regulate catalase activity and, thereby,

affect H_2O_2 concentrations during pathogen challenge. These include uncharacterized metabolite inhibitors and well-studied compounds, such as SA and nitric oxide (NO) [34,35].

From an evolutionary perspective, high rates of photorespiration in plants that have not evolved C_4 photosynthesis are probably a recent phenomenon driven by the relatively low atmospheric CO_2 concentrations of the past 10–20 million years. Before this, for most of the timeline of plant evolution, atmospheric CO_2 was probably substantially higher, making photorespiration less important. This perhaps suggests that photorespiration is not likely to be essential to the core generic features of plant immune responses, which are considered to be evolutionarily ancient. However, factors such as pathogen effectors, which determine disease resistance in specific interactions, can evolve rapidly [1]. Therefore, it is interesting that bacterial and fungal effectors that may interact with catalase in the peroxisomes, cytosol, or nucleus have recently been described [36,37]. A bacterial effector delivered through the type III secretion system was targeted to peroxisomes when expressed in protoplasts and reported to interact with catalase, producing a modest decrease in enzyme activity [37]. Several endogenous plant proteins involved in the regulation of cell fate have also been reported to interact with catalase. An analysis of mutants for LESION SIMULATING DISEASE1 (LSD1), a plant protein that antagonizes cell death, pointed to a functional interaction with photorespiration [10]. More recently, this protein has been reported to interact directly with catalase and to maintain its activity [38]. Finally, the NO CATALASE ACTIVITY1 (NCA1) protein is involved in the regulation of autophagy, possibly linked to its chaperoning of the major leaf catalase [39,40].

High Growth CO_2 Modulates Defense Responses

While the impact of increased CO_2 on plant–pathogen interactions has been examined [41–47], there have been few studies on the influence of elevated CO_2 on PR metabolism. Within the past 5 years, several papers have described an upregulation of basal defense-related metabolism in plants grown at high CO_2. Since one of the best-documented effects of high CO_2 is to inhibit photorespiration, these observations stand in apparent contrast to those discussed in the previous section. They raise questions about the factors that link plant nutritional status to defense responses within the context of a changing environment.

Box 1 summarizes studies in which SA and related factors were analyzed in model species and crop plants grown either in air or at a higher CO_2 concentration. There is variation between the findings, probably related to taxonomic specificity or differences in conditions such as growth photoperiod, irradiance, or nutrient supply. Nevertheless, an overarching picture emerges. Elevated growth CO_2 significantly increases basal levels of SA or related defense compounds in many species (Box 1). This effect is accompanied by upregulation of SA-associated gene expression, and enhanced resistance to viruses, bacteria, or fungi. These broad outcomes were particularly evident for tomato (*Solanum lycopersicum*), arabidopsis, and common bean (*Phaseolus vulgaris*) [14,15,48–50]. A study of six cultivars of soybean (*Glycine max*) grown in field conditions at modestly increased CO_2 (550 $\mu l.l^{-1}$) also reported that SA was increased (up to twofold) in most of them [51]. The picture for other biotic defense pathways is less clear. While some authors have reported decreased JA in plants at high CO_2, effects were less evident in other studies [14,48–51], and JA-associated gene expression was upregulated along with the SA pathway in arabidopsis grown at high CO_2 [15].

While further work is required, the available data suggest that SA accumulation in response to high CO_2 may be less evident in monocotyledonous plants. Although the study focused on stems rather than leaf tissue, no effect of high CO_2 on SA contents was observed in the C_4 species, maize (*Zea mays*) [52]. When grown at high CO_2, only modest increases in leaf SA were observed in the C_3 monocots, wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*),

Glossary

Abscisic acid (ABA): an important phytohormone in plant development and environmental responses, notably with a role in stomatal closure in response to water deficit.
Effector-triggered immunity (ETI): a type of immunity that depends on recognition by intracellular immune receptors of pathogen effectors that otherwise suppress defense responses.
Group VII ethylene response factors (ERFVII): a subset of transcription factors that are important in stress responses.
High CO_2: concentrations of CO_2 above air levels (380-400 $\mu L.L^{-1}$). In many studies 'high CO_2' is defined as a concentration between 550 and 1000 $\mu L.L^{-1}$, corresponding to predicted atmospheric conditions in the year 2100.
Isopentenyl pyrophosphate (IPP): the building-block for terpenoids.
Jasmonic acid (JA): a defense phytohormone derived from fatty acids that is involved in responses to tissue-damaging stresses such as wounding by herbivores and necrosis-inducing microorganisms.
Methylerythritol phosphate (MEP) pathway: plastidial route of IPP synthesis.
NONEXPRESSOR OF PATHOGENESIS-RELATED GENE 1 (NPR1): a redox-regulated protein that activates PR gene expression downstream of SA.
Pathogenesis-related (PR) responses: induced in plants challenged by pathogens. Canonical markers include SA- and JA-dependent PR genes.
Phosphoenolpyruvate carboxylase (PEPc): an enzyme that is an essential for CO_2 concentration mechanisms in C_4 plants. In C_3 plants, PEPc is implicated in the production of carbon skeletons for amino acid synthesis and pH regulation.
Photorespiration: light-dependent O_2 uptake and CO_2 evolution initiated by oxygenation of RuBP. Closely linked to photosynthesis, photorespiration is more rapid in C_3 plants than in C_4 plants, and is inhibited by high CO_2.
Phytoalexins: antimicrobial compounds that show a significant degree of taxonomic specificity (e.g., indoles in Brassicaceae, terpenoids

Box 1. Summary of Effects Observed in Five Studies of Growth at High CO_2 on Pathogenesis-Related Metabolism in Leaves of C_3 Plants

All five studies reported data on defense metabolites, notably SA and related compounds, in the absence of pathogen challenge at two CO_2 levels, which were 350–400 $\mu l.l^{-1}$ (air) and 750–1000 $\mu l.l^{-1}$ (high CO_2). Where more than one variety or ecotype were analyzed, the names are given in parentheses. If tested, the effect of high CO_2 on leaf resistance to pathogens is also indicated.

- Tobacco: increased phenylpropanoids, PAL activity, chlorogenic acid (up to twofold), scopol(et)in (three–sixfold); no increase in SA; increased viral resistance [50].
- Tomato (YF 8): twofold increase in SA; twofold decrease in JA; some decrease in abscisic acid (ABA); decreased incidence of viral symptoms [48].
- Tomato (Zheza 205): fourfold increase in SA; no effect on JA; increased PR gene expression; increased resistance to bacteria and virus; enhanced susceptibility to fungus [14].
- Tomato (Moneymaker): fourfold increase in SA; twofold decrease in JA; increased resistance to virus [49].
- Arabidopsis (Col-0 and Ws); SA increased approximately 14-fold in both ecotypes; *PR* gene expression increased markedly; increased resistance to bacteria and fungi; SA produced through the isochorismate pathway [15].
- Common bean (BAT93 and JaloEEP558): four–fivefold increase in SA in both cultivars [15].
- Wheat: slight increase in SA [15].
- Barley: no increase in SA [15].

contrasting with the more marked effects observed in common bean and arabidopsis in the same conditions [15].

CO_2 Signaling: Insights from Studies of Stomatal Regulation

Can plants sense CO_2 levels? Progress towards answering this question has been favored by genetic screens for arabidopsis mutants that show altered stomatal regulation in response to a variety of factors [53]. Key findings are summarized in Box 2. The OST1-SLAC1 pathway regulates stomatal closure in response to a range of external triggers, including water availability, darkness, and CO_2 [54–56]. OST1 is a protein kinase that phosphorylates and activates both SLAC1, a slow anion channel, and QUAC1, a rapid anion channel [57]. Another protein kinase, HT1, antagonizes stomatal closure by phosphorylating and inactivating OST1 [58,59]. When activated, SLAC1 and QUAC1 transfer anions out of the cell [57,60]. Mutants for either transporter show decreased stomatal closure in response to high CO_2 [56,61]. Another transporter, ABCB14, may act to import malate from the apoplast: loss of its function promotes CO_2-induced stomatal closure [62].

All of the above proteins function in the stomatal response to various factors, not only CO_2. Hence, additional factors are likely to be needed for the primary sensing steps that allow specific information on CO_2 concentrations to be transmitted. A MATE-type transporter, RESISTANT TO HIGH CO2 1 (RHC1) was recently reported to operate upstream of HT1, OST1, and SLAC1 in CO_2-dependent stomatal closure [59]. This protein, which interacts with HT1 at the plasmalemma, has been proposed to act as a bicarbonate sensor [53,59]. Consistent with this idea, the enzymatic activity of at least one of two carbonic anhydrases (CA1 in the chloroplast, CA4 at the plasmalemma) is required for high CO_2-induced stomatal closure [63,64]. These enzymes are also involved in CO_2 regulation of stomatal development [65]. A physical interaction at the plasma membrane between RHC1 and the two carbonic anhydrases was found in mesophyll protoplasts [59]. Hence, these components may cooperate in CO_2 sensing to feed information into the OST1-

in Solanaceae, and isoflavonoids in Fabaceae).

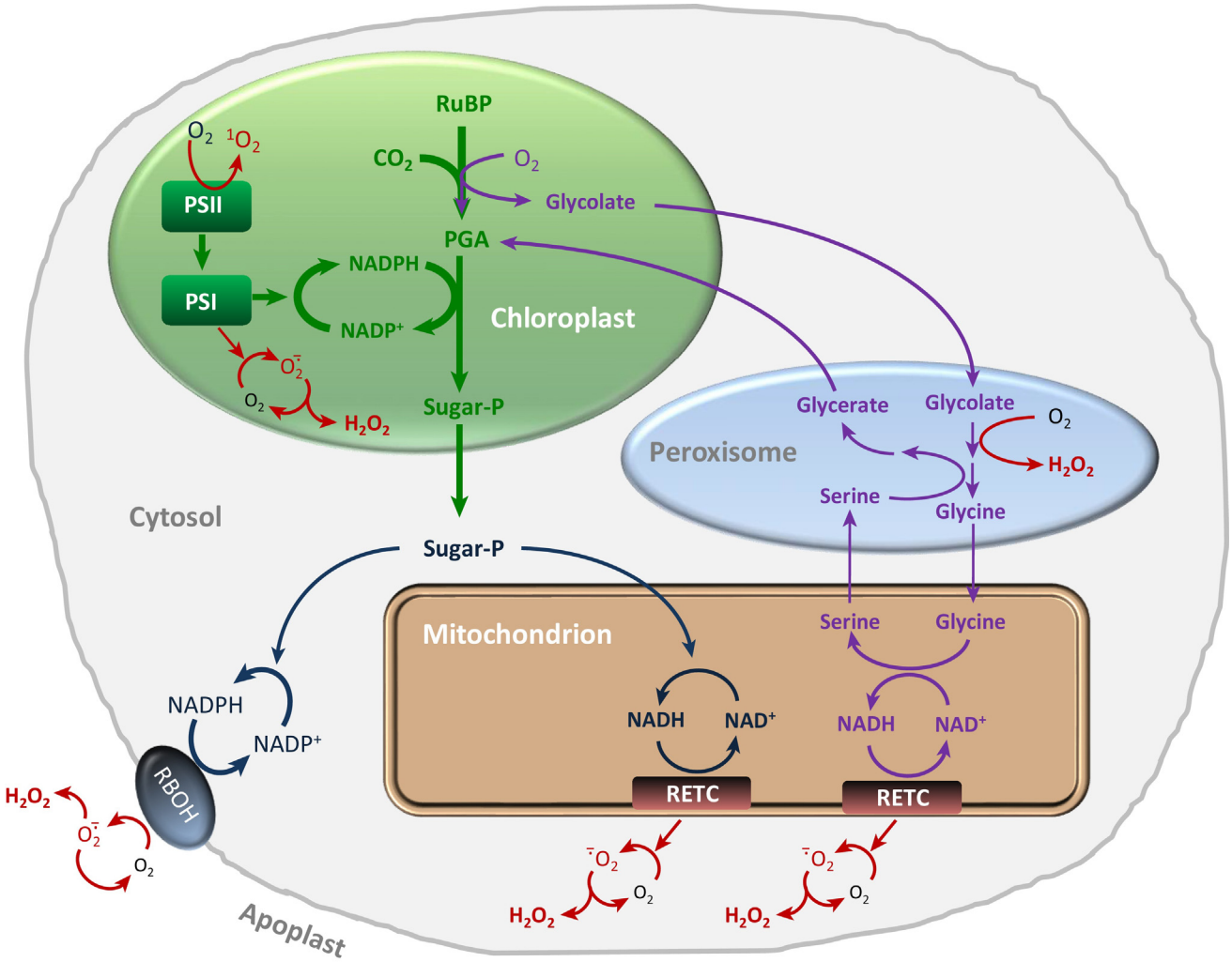
Reactive oxygen species (ROS): derivatives of oxygen such as singlet oxygen, superoxide, and hydrogen peroxide (H_2O_2); important signaling and regulatory molecules in plants challenged by pathogens and other stress factors.

Ribulose-1,5-*bis*phosphate carboxylase/oxygenase (Rubisco): the carboxylase of the Calvin-Benson cycle that also catalyzes the initiating reaction of photorespiration.

RuBP oxygenation: the initiating reaction in the photorespiratory pathway, catalyzed by Rubisco and involving incorporation of O_2 into ribulose-1,5-*bis*phosphate (RuBP) to form 3-phosphoglycerate and 2-phosphoglycolate. Its rate is generally favored by increases in irradiance and the chloroplast O_2:CO_2 ratio.

Salicylic acid (SA): a defense phytohormone that is particularly important in responses to (hemi) biotrophic organisms.

***S*-nitrosoglutathione (GSNO) reductase:** a key player in regulating *S*-nitrosylation of protein Cys residues by GSNO.



Trends in Plant Science

Figure 1. Major Sources of Reactive Oxygen Species (ROS) in Photosynthesizing Cells. In photosynthesizing cells, ROS are produced in the light by the chloroplast electron transport chain, which generates singlet O_2 at PSII and superoxide at several sites (top left). In C_3 species, the photorespiratory pathway generates H_2O_2 at high rates through the glycolate oxidase reaction (right). Other sources of superoxide and H_2O_2 that can operate in both the light and the dark are the mitochondrial electron transport chain (bottom) and several enzyme systems located in the apoplast, such as NADPH oxidases involved in pathogenesis-related, hormonal, and systemic signaling (bottom left). Photosynthesis is shown by green arrows, respiration by blue arrows, and the photorespiratory pathway by purple arrows. ROS-producing reactions are indicated in red. Arrows may indicate reactions involving more than one step. Abbreviations: PGA, 3-phosphoglycerate; PSI, photosystem I. PSII, photosystem II. RBOH, respiratory burst oxidase homolog (NADPH oxidase); RETC, respiratory electron transport chain; RuBP, ribulose-1,5-*bis*phosphate; sugar-P, sugar-phosphate.

SLAC1 signaling module. However, mutants for PYR/RCAR proteins that act as **abscisic acid** (ABA) receptors upstream of OST1 also show decreased stomatal closure in response to high CO_2 [66]. Thus, CO_2 responses may also partly be mediated by ABA [53]. Interestingly, microarray analysis of arabidopsis whole-leaf tissue revealed that ABA-related transcripts were upregulated in air compared with high CO_2 [67].

Although many stomatal signaling components are strongly expressed in guard cells, some are also found in other cells, raising the possibility that they may participate more generally in CO_2 signaling. Are these (or related) components involved in linking high CO_2 to PR responses, such as activation of the SA pathway? As yet, information is limited, but certain signaling

Box 2. Examples of Components Involved in CO_2 Signaling in Arabidopsis

Most of the information available has been obtained by analysis of mutants with altered stomatal responses to CO_2, although some were originally identified in screens for other responses (e.g., ozone or drought). The function in stomatal closure in response to high CO_2 is described for the wild-type protein. Components that may be specifically involved in CO_2 sensing are in bold type.

- PYR/RCAR proteins: ABA receptors acting upstream of OST1 [66]; promotes stomatal closure.
- OST1: protein kinase that phosphorylates SLAC1 and QUAC1 [54,57]; promotes stomatal closure.
- SLAC1: slow anion channel that ensures efflux from the cell [55,56,60]; promotes stomatal closure.
- QUAC1: rapid anion channel that probably ensures efflux from the cell [61]; promotes stomatal closure.
- HT1: protein kinase that phosphorylates and inhibits the OST1-SLAC1 pathway [58,59,70]; antagonizes stomatal closure.
- ABCB14: ABC transporter that imports anions into the cell [62]; antagonizes stomatal closure.
- RHC1: MATE transporter protein that interacts with HT1, possible bicarbonate sensor [59]; promotes stomatal closure.
- MPK4/MPK12: Mitogen-activated protein kinases that phosphorylate and inhibit HT1 activity [70,71]; promote stomatal closure.
- CA1, CA4: Carbonic anhydrases that produce bicarbonate, possibly the form in which CO_2 is perceived [63,64]; promote stomatal closure.

components are known to have dual roles in stomatal regulation and biotic stress responses. As well as specific NADPH oxidases (discussed further below), MPK4, a mitogen-activated protein kinase known to be involved in PR responses in arabidopsis [68,69], has been shown to act in guard cell signaling. Both MPK4 and MPK12 can phosphorylate HT1, leading to its inactivation [70]. Loss-of-function *mpk12* mutants show both slower stomatal closure in response to high CO_2 and slower stomatal opening in low CO_2 [71]. Expression data suggest that carbonic anhydrases are involved in biotic stress responses [72,73]. Furthermore, the chloroplastic CA1 was identified in a screen for SA-interacting proteins in arabidopsis, and named SABP3, for SA-Binding Protein 3 [74]. Stomatal closure through an SA-dependent mechanism is one way in which plants protect themselves against pathogens [75]. However, loss-of-function *ost1* and *slac1* mutants showed wild-type accumulation of SA when grown at elevated CO_2 [15]. This observation suggests, first, that neither OST1 nor SLAC1 is essential for high CO_2-induced PR responses and, second, that upregulation of defense metabolism in high CO_2 is not necessarily coupled to stomatal closure [15].

Carbonic anhydrases have roles in the regulation of pH, which is decreased by carbonic acid formation as CO_2 increases. The impact of CO_2 could be particularly significant in the weakly buffered apoplastic compartment, where pH changes are implicated in regulation of physiological processes, including growth and pathogenesis responses. The most common pH response to pathogen challenge is alkalinization. For example, the apoplastic pH increased from 4.8 to 5.3 within 12 h following inoculation of common bean with an ETI-eliciting strain of *Pseudomonas* [76]. However, transcriptomics analysis of hydroponically grown arabidopsis shifted from pH 6.0 to pH 4.5 revealed the induction of several SA-associated genes, including *ICS1* [77]. Acidification to such an extent would probably require higher external CO_2 concentrations than those that are used in most studies. In young barley plants exposed to 800 $\mu l.l^{-1}$ CO_2 for 2 weeks, apoplast pH was maintained at just above 6.0 and was only marginally

lower than in controls kept in air [78]. Therefore, induction of PR pathways by moderately increased CO_2 is unlikely to be caused solely by pH changes.

Metabolic Links between High CO_2 and Defense Metabolism

A large array of plant secondary compounds has antimicrobial activity. Commonly known as phytoalexins, their chemical nature often shows some taxonomic specificity. In the Brassicaceae, the best-studied phytoalexin is the indole compound, camalexin [79], which is upregulated alongside SA in arabidopsis [15]. Observations in tobacco (*Nicotiana tabacum*), poplar (*Populus* × *euramericana*), and arabidopsis suggest that the phenylpropanoid pathway is activated in plants grown at high CO_2 [15,50,80]. Abrogation of SA and camalexin accumulation in the arabidopsis *sid2* mutant abolishes high CO_2-induced resistance to bacteria and fungi [15]. Thus, upregulation of defense metabolism is required for increased basal resistance. How does high CO_2 induce secondary metabolism? The most obvious link is that more substrate becomes available for biosynthesis when plants fix more CO_2 in photosynthesis (Figure 2). Although many secondary pathways are regulated at the transcriptional level to allow appropriate activation as needed, close interactions between primary and secondary metabolism have been documented in plants with decreases in the Calvin-Benson cycle enzyme, transketolase [81]. The major direct effect of increased CO_2 concentrations on metabolism is mediated by **Ribulose-1,5-*bis*phosphate carboxylase/oxygenase** (Rubisco), but fixation of CO_2 can also be catalyzed by **cytosolic phosphoenolpyruvate carboxylase** (PEPc), which initiates the synthesis of C_4 acids. Since PEP is at a metabolic crossroads (Figure 2), PEPc activity might compete to some degree with the biosynthesis of defense-related compounds.

The terpenoid biosynthesis pathway is important for the production of phytoalexins in groups such as the Solanaceae. Furthermore, metabolites involved in the initial steps of the chloroplastic **methylerythritol (MEP) pathway** of **isopentenyl pyrophosphate** (IPP) synthesis can influence retrograde signaling to the nucleus [82]. Accumulation of 2-methylerythritol

2,4-cyclopyrophosphate (MEcPP) in arabidopsis mutants was associated with the accumulation of SA and enhanced resistance to biotrophic pathogens [82]. Isoprene emission can constitute a significant part of the carbon budget of some plants and may be important in resistance to heat or oxidative stress [83]. High CO_2 tends to inhibit isoprene production, possibly by stimulating PEPc activity [83]. Links between high CO_2, the MEP pathway, and PR responses remain to be elucidated.

While primary metabolites might influence PR responses as substrates for secondary biosynthesis, they could also be important as signals. Sugars, organic acids, and amino acids are among compounds elaborated by plants that are keenly sought by invading microorganisms. Metabolites within all these classes have been implicated in plant defense. Evidence that sugars are influential comes from the analysis of plants with altered invertase activities [84]. Several studies, notably of arabidopsis mutants, have drawn attention to the potential importance of free amino acids [4,6]. Pretreating rice (*Oryza sativa*) leaves with a subset of the amino acids found in proteins can confer resistance to challenge with *Magnaporthe oryzae* [9]. These include key players in nitrogen assimilation (Glu, Gln, Asp, and Asn) as well as amino acids involved in photorespiration (Gly and Ser [9]). How will sugar and amino acid contents be affected by photorespiration or by high CO_2? In the case of sugars, increased CO_2 generally favors accumulation. For amino acids, the situation is more complicated. Metabolites that were increased with the SA pathway at high CO_2 include Glu, which along with other amino acids may signal through Glu receptors, and proline, which has been implicated in PR responses [85,86]. Only Gly was clearly decreased in arabidopsis grown in these conditions [15], probably due to the inhibition of photorespiration [87].

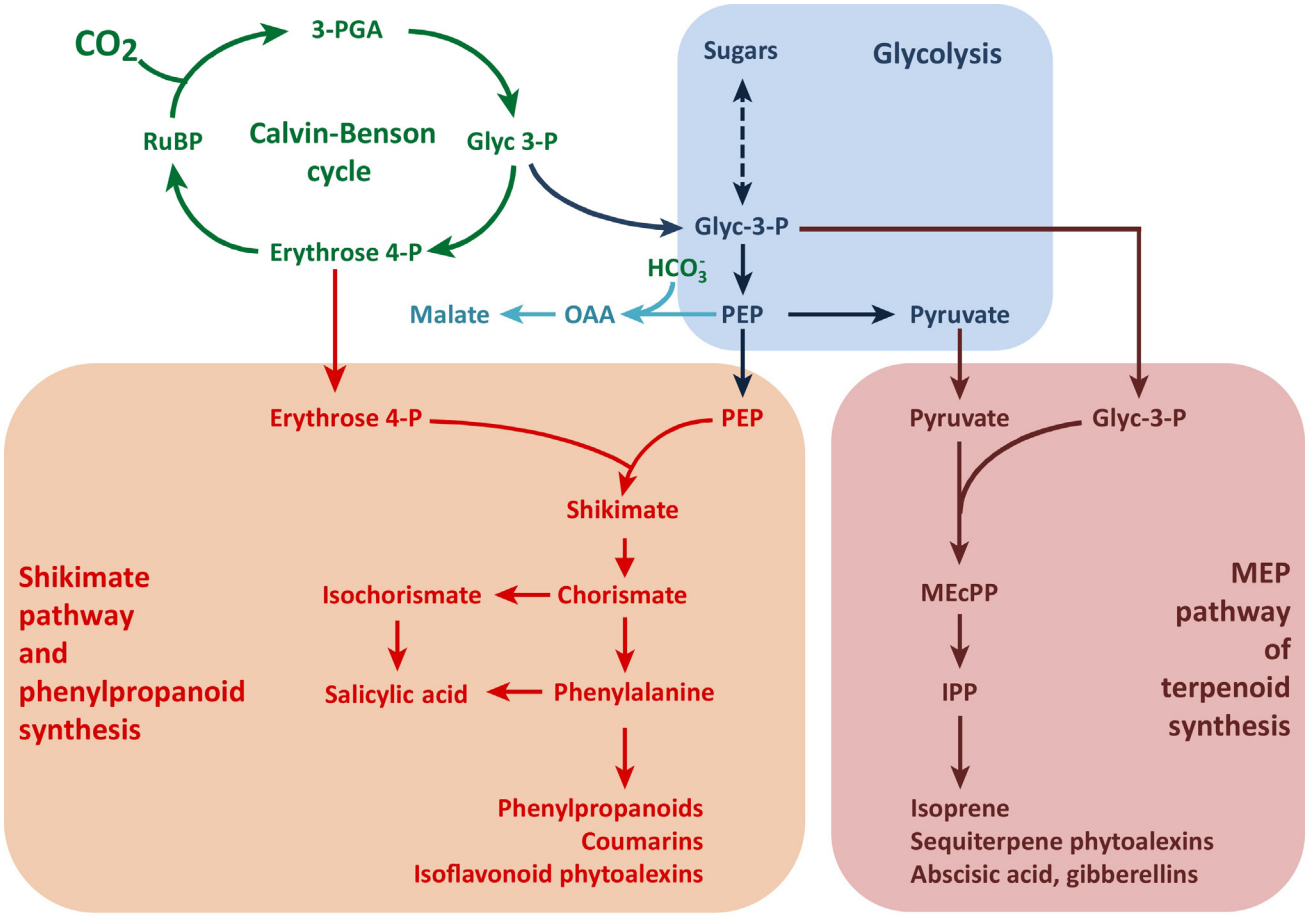


Figure 2. Photosynthetic and Respiratory Origin of Secondary Defense Compounds. Phenylpropanoids and terpenoids are two of the most important classes of secondary compounds in plants, and include several vital defense compounds. Both types of metabolite are synthesized from simpler building-block compounds produced through primary metabolic pathways, such as photosynthetic CO_2 fixation in the Calvin-Benson cycle (top left, green) and glycolysis (top right, blue). From these pathways, the shikimate pathway produces aromatic amino acids, notably phenylalanine, from which phenylpropanoids are generated (bottom left), while terpenoid compounds are generated from IPP, which is synthesized via either the mevalonate pathway or the chloroplastic MEP pathway (bottom right). Hence, through its impact on basic primary metabolic processes, the atmospheric CO_2 concentration may influence the synthesis of secondary metabolites involved in defense against biotic threats. Abbreviations: Glyc 3-P, glyceraldehyde 3-phosphate; IPP, isopentenyl pyrophosphate; MecDP, 2-methylerythritol 2,4-cyclodiphosphate; MEP, methylerythritol phosphate; OAA, oxaloacetate; PEP, phospho*enol*pyruvate; 3-PGA, 3-phosphoglycerate; RuBP, ribulose 1,5-*bis*phosphate.

Several simple organic acids that are central to plant primary metabolism have been implicated in defense against biotic stress. Citrate accumulated strongly alongside SA in arabidopsis grown at high CO_2 [15] and this organic acid was increased in the apoplastic space of barley following bacterial challenge [76]. Both citrate and enzymes involved in its metabolism have been linked to biotic stress signaling in arabidopsis [5,8,88]. Malate concentration is another candidate that could link carbon status to PR responses. While stomatal regulation by CO_2 and other factors involves signaling and malate transport within the guard cells, it may also be influenced by signals originating in the mesophyll cells [89]. Malate has been proposed to act as one such signal [90]. Whether analogous intercellular signaling might be involved in activating PR responses in plants grown at high CO_2 is a question that merits further study. Mutants for respiratory enzymes that are both upstream and downstream of PEPc and malate attenuated the activation of the SA pathway and associated pathogen resistance by high CO_2 [15].

Redox Signaling and ROS at High CO_2

Redox signaling involves post-translational oxidation or reduction of proteins triggered by components such as oxygen, H_2O_2, NO, glutathione, ***S*-nitrosoglutathione** (GSNO), or thioredoxins (TRX). Several important redox signaling mechanisms in plants involve the modulation of protein Cys residues. Notable examples include light-dependent regulation of chloroplast metabolism by TRX systems [91], suppression of the hypoxia response by oxygen- and enzyme-dependent degradation of **Group VII ethylene response factors** (ERFVII) transcriptional factors [92,93], and regulation of **nonexpressor of pathogenesis-related gene 1** (NPR1) redox status by cytosolic TRX [94]. NPR1 is instrumental in SA signaling, and is entrained following ROS produced in pathogen-induced oxidative bursts.

Despite abundant evidence that ROS act as signaling molecules, particularly during biotic stress responses, a mechanistic description of all steps from perception to response is yet to be developed. It is generally assumed that ROS action must be related to changes in concentrations or production rates at specific locations [26]. Increases in ROS are sufficient to trigger SA accumulation in the absence of pathogens, as shown for catalase-deficient mutants and plants exposed to ozone [16,95].

How will high CO_2 affect different cellular sources of ROS production, such as those shown in Figure 1? Although it is currently impossible to monitor rates of ROS production through these specific reactions, the effects of high CO_2 can be estimated from what is known about the metabolic physiology of photosynthetic cells (Box 3). Key factors determining ROS production are substrate availability, enzyme activation state, and, in the case of electron transport chains, redox status. The supply of glycolate is probably the major factor determining peroxisomal GOX activity and so photorespiratory H_2O_2 will be produced more slowly at high CO_2. In the case of the chloroplast electron transport chain, one view is that high CO_2 should ensure the optimal availability of major electron acceptors in the stroma. The availability of oxidants such as $NADP^+$ should minimize ROS formation by competing with oxygen for electrons from ferredoxin and

Box 3. Gray Areas: How the Metabolic Effects of High CO_2 Might Impact ROS Sources

Chloroplast Electron Transport Chain

- Lower ROS production because of: (i) increased electron acceptor availability when CO_2 is less limiting; and (ii) greater metabolic demand for electrons relative to ATP (lower ATP:NADPH requirement when photorespiration is decreased).

Mitochondrial Electron Transport Chain

- Lower ROS production linked to: (i) decreased glycine oxidation when photorespiration is decreased; and (ii) decreased respiratory control linked to higher cytosolic ATP sinks at high CO_2.
- Higher ROS production linked to possible increase in TCA cycle activity.

Peroxisomal Photorespiratory Metabolism

- Lower ROS production because of decreased glycolate oxidase activity.

NADPH Oxidases

- Possible higher ROS production linked to higher NADPH availability when carbon is plentiful.

Peroxidase/Oxidase Functions

- Higher ROS production linked to increased substrate?

upstream components (Figure 1). This effect might be reinforced by the lower ATP:NADPH requirement of photosynthetic metabolism when photorespiration is decreased, contributing to a decreased stromal reduction state at high CO_2 [96–98]. Analogous considerations could apply to mitochondrial ROS production, which will be related to not only the rate of respiration but also the strength of respiratory control. In conditions where ATP sinks decrease, the proton motive force will increase, restricting ubisemiquinone oxidation and favoring superoxide production at complex III [99]. Sucrose synthesis, which should be promoted at high CO_2, is considered to be one of the major sinks for mitochondrial ATP in the light [100]. Therefore, enhanced sucrose synthesis in the cytosol might lower the probability of mitochondrial ROS production, an effect that could be reinforced by decreased oxidation of photorespiratory glycine [101].

Although the above discussion is speculative, it suggests that increases in CO_2 should attenuate ROS production by most of the major light-dependent metabolic sources in leaves. Thus, it is surprising that several reports point to activation of ROS signaling at high CO_2. When soybean was grown at modestly increased CO_2, leaf H_2O_2 contents were increased by approximately 40% [102]. Protein carbonylation, a marker of oxidative stress, was more intense in leaves of soybean and arabidopsis grown in high CO_2 conditions [103] and ROS signals were increased in both root and mesophyll cells of tomato grown at higher (twofold) air levels of CO_2

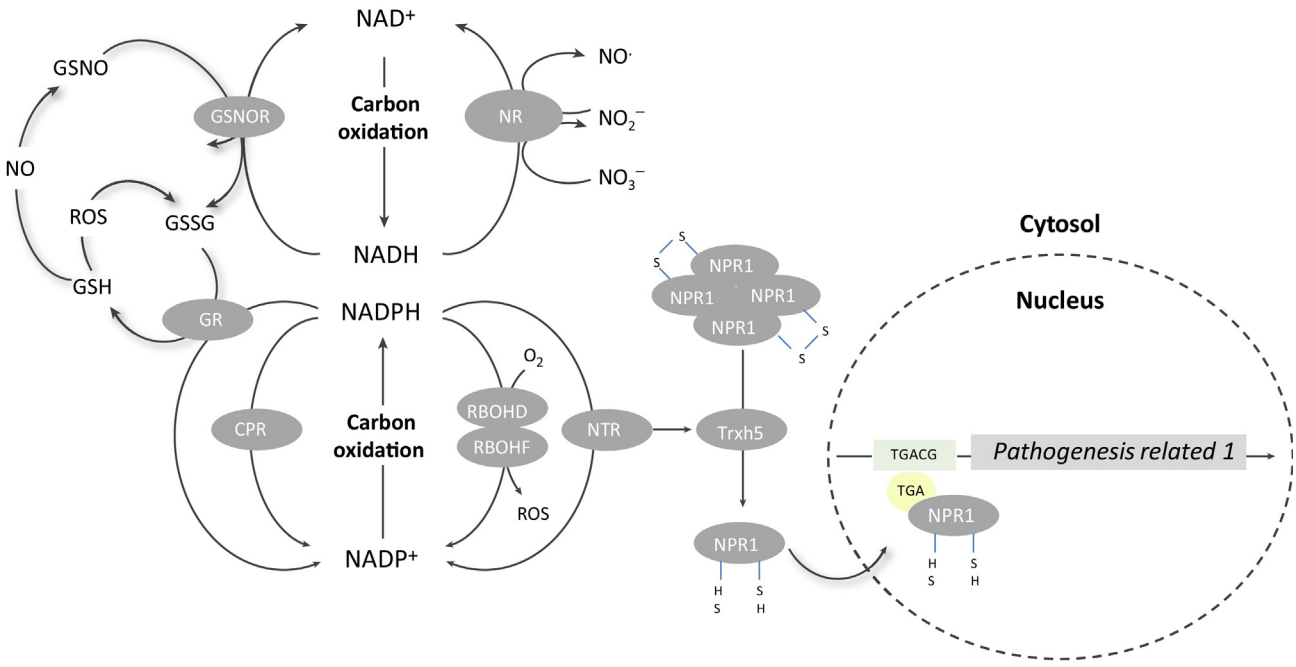


Figure 3. Cytosolic NAD(P)H-Dependent Systems Link Carbon Status to Pathogenesis-Related (PR) Signaling. Redox changes are a key part of cellular signaling in response to biotic stress, and ultimately depend on NADPH and NADH generated by oxidation of carbon substrates, such as sugars, sugar-phosphates, and organic acids. NADPH and NADH underpin various signaling processes such as ROS production by RBOH-type enzymes, NO generation by NR or other enzymes, and GSNO metabolism by GSNOR. As a cofactor for GR and NTR, NADPH is also critical in regulating protein thiol status through its effect on glutathione and thioredoxin redox states. One well-known redox-regulated cytosolic protein is NPR1, a co-activator of *PR* gene expression. NPR1 thiol-disulfide status, which is influenced by Trxh5 (as shown) as well as by related factors, such as GSH:GSSG and GSNO, determines the subcellular localization of NPR1 through masking/unmasking of a nuclear localization sequence. Alterations in NADPH status mediated by CO_2-dependent changes in primary metabolism could also affect defense through other mechanisms, such as altered CPR activity. Abbreviations: CPR, cytochrome P450 reductase; GR, glutathione reductase; GSH, reduced glutathione; GSNO, *S*-nitrosoglutathione; GSSG, glutathione disulfide;. GSNOR, GSNO reductase; NO, nitric oxide; NPR1, NONEXPRESSOR OF PATHOGENESIS-RELATED GENE 1; NR, nitrate reductase; NTR, NADPH-thioredoxin reductase; RBOHD/F, Respiratory burst oxidase homolog D/F; ROS, reactive oxygen species; Trx, thioredoxin.

[104]. In arabidopsis grown at 1000 $\mu l.l^{-1}$ CO_2, several marker transcripts for increased ROS were more abundant than in plants grown in air [15].

These observations raise questions about the source of increased ROS at high CO_2. Further work will be required to elucidate this question, but there are indications that RBOH-type NADPH oxidases are stimulated when CO_2 is increased. This could reflect enhanced expression, as observed for *AtRBHOD* and *AtRBOHF*, the major isoforms in arabidopsis leaves [15]. NADPH oxidases encoded by these genes function in stomatal closure as well as immune responses [105]. Thus, the cell specificity of their induction by high CO_2 is worth investigating. In tomato, transcripts for *RBOH1* accumulated more strongly during salt stress when the stress was imposed at high CO_2 [104]. Silencing *RBOH1* decreased ROS signals and prevented the improved salt tolerance relative to plants stressed in air, possibly by compromising ROS-dependent stomatal closure [104]. As well as effects on expression, or altered post-translational modification, the activities of such enzymes could be stimulated by enhanced availability of cytosolic NADPH at high CO_2. Cytosolic concentrations of NAD(H) and NADP(H), key players that link metabolism to redox signaling, underpin the activity of several enzymes or other proteins that are important in PR responses (Figure 3).

NO and glutathione interact closely with ROS in PR signaling. *S*-nitrosylation of SABP3 (CA1), which is implicated in CO_2 sensing [63,64] and which is an SA-binding protein [74], compromised both the enzyme activity and plant immune responses [106]. Interestingly, SA accumulation driven by photorespiratory H_2O_2 is partly dependent on glutathione [107] but SA that accumulates in response to high CO_2 is not [15]. Thus, the two effects appear to involve distinct paths of ROS signaling. NO availability could be affected by changes in NAD(H) redox state that may occur at high CO_2 [108]. An influential factor could be metabolic dialog through shuttles that link organellar redox states to the cytosol [109–111], where nitrate reductase and GSNO reductase both depend on NADH (Figure 3).

Concluding Remarks and Future Perspectives

Several recent findings point to a role for photorespiration in biotic stress responses. If photorespiration makes an indispensable contribution, PR responses should be compromised in plants growing at high CO_2. However, growing some plants at high CO_2 activates PR responses and enhances resistance to pathogens. These conflicting observations raise issues that are relevant to several areas of plant biology at both the fundamental and applied level (see Outstanding Questions).

Induction of defense responses by high CO_2 may involve mechanisms related to priming through compounds such as azelaic acid and pipecolic acid [112,113]. These metabolites accumulate in leaves when oxidative stress signaling is activated [12,114], but their response to high CO_2 is not yet known. The roles of ROS in conditions where photorespiration is more or less active is a key question. Ultimately, if oxidative processes are key players in the induction of defense pathways by high CO_2, defense induction by photorespiratory H_2O_2 may simply reflect the plasticity of ROS metabolism. It is possible that different ROS sources within the plant cell have specific roles in interactions with different microorganisms. Pathogen effectors have diverse mechanisms of action [115], and compartment-specific targeting of redox-homeostatic enzymes, such as catalase, may be among emerging effector mechanisms [36,37].

It is possible that any condition that perturbs redox homeostasis induces stress responses [26], and that exposing plants to increased CO_2 concentrations is one such condition. Gradual increases in CO_2, to which plants become adapted over many generations, might not produce the same effect. C_4 photosynthesis involves maintenance of CO_2 concentrations in the bundle sheath cells that are higher than those in mesophyll cells of either C_3 or C_4 species. Despite this,

Outstanding Questions

How important is photorespiration in biotic stress responses? Do mechanisms that downregulate catalase have roles in certain plant–pathogen interactions? If so, does this occur to allow the accumulation of H_2O_2 produced in photorespiration or from another source? Does this accumulation benefit the host or the pathogen?

Which sources are responsible for ROS production at high CO_2? What are the mechanisms by which they are influenced by high CO_2?

Is the activation of PR responses in plants grown at high CO_2 a 'shock to the system'? Are these responses activated because displaced metabolic homeostasis is somehow perceived as an attack? If so, perhaps the ongoing, gradual increases in atmospheric CO_2 (currently approximately 3 $\mu l.l^{-1}$ per year) will activate PR metabolism to a lesser extent. What are the potential consequences of engineering C_3 plants to express the C_4 pathway of photosynthesis, in which certain cells might have to adapt more quickly to operating at higher CO_2 concentrations?

Is there taxonomic specificity in PR responses triggered by high CO_2? How has the CO_2 response been conserved during recent angiosperm evolution, given that it is thought to be between 1 and 20 million years since atmospheric CO_2 was as high as current levels?

What are the triggers that upregulate PR responses at high CO_2? How are CO_2 levels transduced into PR responses? Are some of the sensing and signaling circuits shared with those involved in stomatal regulation? How important is metabolite signaling? What are the roles of sugars and organic acids, such as malate and citrate? Could protein remobilization at high CO_2, and the possible increase in free amino acids, be factors in eliciting similar responses to those observed during pathogen attack?

Does the activation of biotic stress responses by high CO_2 involve a yield penalty? Would plants in which key defense pathways are disabled better exploit the fertilization effect of increased CO_2?

reports of the cell specific transcriptomes of C_4 plants have not described an overexpression of PR-associated components in the bundle sheath [116,117]. One possibility is that defense pathways might be relatively insensitive to increased CO_2 in maize or other monocot plants on which most research attention in this area has focused. Alternatively, certain CO_2 signaling components could have been disabled during the evolution of C_4 photosynthesis. It will be interesting to see whether engineering the C_4 pathway into C_3 plants [118] has any impact on biotic stress responses.

Downregulation of photosynthetic capacity can limit the fertilizing effect of higher CO_2 on plant production [18]. Could the induction of defense metabolism also have an impact? Activated defenses can be advantageous if pathogens are present but, in the absence of biotic threats, they may entail a yield penalty. Elucidation of the mechanisms that link CO_2 levels to PR responses could provide information that can be exploited to optimize plant growth and resistance in a higher-CO_2 world.

References

1. Dangl, J.L. *et al.* (2013) Pivoting the plant immune system from dissection to deployment. *Science* 341, 746–751
2. Conrath, U. *et al.* (2015) Priming for enhanced defense. *Annu. Rev. Phytopathol.* 53, 97–119
3. Poland, J.A. *et al.* (2009) Shades of gray: the world of quantitative disease resistance. *Trends Plant Sci.* 14, 21–29
4. Liu, G. *et al.* (2010) Amino acid homeostasis modulates salicylic acid-associated redox status and defense responses in *Arabidopsis*. *Plant Cell* 22, 3845–3863
5. Mhamdi, A. *et al.* (2010) Cytosolic NADP-dependent isocitrate dehydrogenase contributes to redox homeostasis and the regulation of pathogen responses in *Arabidopsis* leaves. *Plant Cell Environ.* 33, 1112–1123
6. Stuttmann, J. *et al.* (2011) Perturbation of Arabidopsis amino acid metabolism causes incompatibility with the adapted biotrophic pathogen *Hyaloperonospora arabidopsidis*. *Plant Cell* 23, 2788–2803
7. Kangasjärvi, S. *et al.* (2012) Photosynthesis, photorespiration, and light signalling in defence responses. *J. Exp. Bot.* 63, 1619–1636
8. Finkemeier, I. *et al.* (2013) Transcriptomic analysis of the role of carboxylic acids in metabolite signaling in Arabidopsis leaves. *Plant Physiol.* 162, 239–253
9. Kadotani, N. *et al.* (2016) Exogenous proteinogenic amino acids induce systemic resistance in rice. *BMC Plant Biol.* 16, 60
10. Mateo, A. *et al.* (2004) LESION SIMULATING DISEASE1 is required for acclimation to conditions that promote excess excitation energy. *Plant Physiol.* 136, 2818–2830
11. Foyer, C.H. and Noctor, G. (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *Plant Cell* 17, 1866–1875
12. Chaouch, S. *et al.* (2010) Peroxisomal hydrogen peroxide is coupled to biotic defense responses by ISOCHORISMATE SYNTHASE1 in a daylength-related manner. *Plant Physiol.* 153, 1692–1705
13. Li, X. *et al.* (2015) Tomato-*Pseudomonas syringae* interactions under elevated CO_2 concentration: the role of stomata. *J. Exp. Bot.* 66, 307–316
14. Zhang, S. *et al.* (2015) Antagonism between phytohormone signaling underlies the variation in disease susceptibility of tomato plants under elevated CO_2. *J. Exp. Bot.* 66, 1951–1963
15. Mhamdi, A. and Noctor, G. (2016) High CO_2 primes plant biotic stress responses through redox-linked pathways. *Plant Physiol.* 172, 929–942
16. Chaouch, S. *et al.* (2012) AtRbohF is a crucial modulator of defence-associated metabolism and a key actor in the interplay between intracellular oxidative stress and pathogenesis responses in Arabidopsis. *Plant J.* 69, 613–627
17. Rojas, C.M. *et al.* (2012) Glycolate oxidase modulates reactive oxygen species-mediated signal transduction during nonhost resistance in *Nicotiana benthamiana* and Arabidopsis. *Plant Cell* 24, 336–352
18. Long, S.P. and Ort, D.R. (2010) More than taking the heat: crops and global change. *Curr. Opin. Plant Biol.* 13, 241–248
19. Foyer, C.H. *et al.* (2009) Photorespiratory metabolism: genes, mutants, energetics, and redox signaling. *Annu. Rev. Plant Biol.* 60, 455–484
20. Bauwe, H. *et al.* (2010) Photorespiration: players, partners and origin. *Trends Plant Sci.* 15, 330–336
21. Torres, M.A. *et al.* (2006) Reactive oxygen species signaling in response to pathogens. *Plant Physiol.* 141, 373–378
22. O'Brien, J.A. *et al.* (2012) A peroxidase-dependent apoplastic oxidative burst in cultured Arabidopsis cells functions in MAMP-elicited defense. *Plant Physiol.* 158, 2013–2027
23. Hu, J. *et al.* (2012) Plant peroxisomes: biogenesis and function. *Plant Cell* 24, 2279–2303
24. Sørhagen, K. *et al.* (2013) The emerging role of photorespiration and non-photorespiratory peroxisomal metabolism in pathogen defence. *Plant Biol.* 15, 723–736
25. Noctor, G. *et al.* (2002) Drought and oxidative load in the leaves of C_3 plants: a predominant role for photorespiration? *Ann. Bot.* 89, 841–850
26. Foyer, C.H. and Noctor, G. (2016) Stress-triggered redox signalling: what's in pROSpect? *Plant Cell Environ.* 39, 951–964
27. Taler, D. *et al.* (2004) Plant eR genes that encode photorespiratory enzymes confer resistance against disease. *Plant Cell* 16, 172–184
28. Dellero, Y. *et al.* (2016) Decreased glycolate oxidase activity leads to altered carbon allocation and leaf senescence after a transfer from high CO_2 to ambient air in *Arabidopsis thaliana*. *J. Exp. Bot.* 67, 3149–3163
29. Kerchev, P. *et al.* (2016) Lack of GLYCOLATE OXIDASE1, but not GLYCOLATE OXIDASE2, attenuates the photorespiratory phenotype of CATALASE2-deficient Arabidopsis. *Plant Physiol.* 171, 1704–1719
30. Mhamdi, A. *et al.* (2012) Plant catalases: peroxisomal redox guardians. *Arch. Biochem. Biophys.* 525, 181–194
31. Chamnongpol, S. *et al.* (1998) Defense activation and enhanced pathogen tolerance induced by H_2O_2 in transgenic plants. *Proc. Natl. Acad. Sci. U. S. A.* 95, 5818–5823
32. Li, S. *et al.* (2014) The protein phosphatase subunit PP2A-B'γ is required to suppress day length-dependent pathogenesis responses triggered by intracellular oxidative stress. *New Phytol.* 202, 145–160
33. Kaurilind, E. *et al.* (2015) A genetic framework for H_2O_2 induced cell death in *Arabidopsis thaliana*. *BMC Genom.* 16, 837
34. Vlot, A.C. *et al.* (2009) Salicylic acid, a multifaceted hormone to combat disease. *Annu. Rev. Phytopathol.* 47, 177–206

35. Beffagna, N. and Riva, M.A. (2011) Fusicoccin-induced catalase inhibitor is produced independently of H^+–ATPase activation and behaves as an organic acid. *Physiol. Plant.* 142, 144–156
36. Zhang, M. *et al.* (2015) Two cytoplasmic effectors of *Phytophthora sojae* regulate plant cell death via interactions with plant catalases. *Plant Physiol.* 167, 164–175
37. Sun, Y. *et al.* (2017) The *Ralstonia solanacearum* effector RipAK suppresses plant hypersensitive response by inhibiting the activity of host catalases. *Cell Microbiol.* 19, e12736
38. Li, Y. *et al.* (2013) LESION SIMULATING DISEASE1 interacts with catalases to regulate hypersensitive cell death in Arabidopsis. *Plant Physiol.* 163, 1059–1070
39. Hackenberg, T. *et al.* (2013) Catalase and its regulator NO CATALASE ACTIVITY 1 (NCA1) promote autophagy-dependent cell death in Arabidopsis. *Plant Cell* 25, 4616–4626
40. Li, J. *et al.* (2015) The chaperone function of NO CATALASE ACTIVITY 1 is required to maintain catalase activity and for multiple stress responses in Arabidopsis. *Plant Cell* 27, 908–925
41. Manning, W.J. and Tiedemann, A.V. (1995) Climate change: potential effects of increased atmospheric carbon dioxide (CO_2), ozone (O_3), and ultraviolet-B (UV-B) radiation on plant diseases. *Environ. Pollut.* 88, 219–245
42. Hibberd, J.M. *et al.* (1996) Effect of elevated concentrations of CO_2 in infection of barley by *Erysiphe graminis*. *Physiol. Mol. Plant Pathol.* 48, 37–53
43. Lake, J.A. and Wade, R.N. (2009) Plant–pathogen interactions and elevated CO_2: morphological changes in favour of pathogens. *J. Exp. Bot.* 60, 3123–3131
44. Eastburn, D.M. *et al.* (2011) Influence of atmospheric and climatic change on plant–pathogen interactions. *Plant Pathol.* 60, 54–69
45. Kane, K. *et al.* (2013) Long-term growth under elevated CO_2 suppresses biotic stress genes in non-acclimated, but not cold-acclimated winter wheat. *Plant Cell Physiol.* 54, 1751–1768
46. Melloy, P. *et al.* (2014) The influence of increasing temperature and CO_2 on *Fusarium* crown rot susceptibility of wheat genotypes at key growth stages. *Eur. J. Plant Pathol.* 140, 19–37
47. Pangga, I.B. *et al.* (2011) Pathogen dynamics in a crop canopy and their evolution under changing climate. *Plant Pathol.* 60, 70–81
48. Huang, L. *et al.* (2012) Lower incidence and severity of tomato virus in elevated CO_2 is accompanied by modulated plant induced defence in tomato. *Plant Biol.* 14, 905–913
49. Guo, H. *et al.* (2016) The contrasting effects of elevated CO_2 on TYLCV infection of tomato genotypes with and without the resistance gene, Mi-1.2. *Front. Plant Sci.* 7, 1680
50. Matros, A. *et al.* (2006) Growth at elevated CO_2 concentrations leads to modified profiles of secondary metabolites in tobacco cv. SamsunNN and to increased resistance against infection with potato virus Y. *Plant Cell Environ.* 29, 126–137
51. Casteel, C.L. *et al.* (2012) Elevated carbon dioxide increases salicylic acid in *Glycine max*. *Environ. Entomol.* 41, 1435–1442
52. Vaughan, M.M. *et al.* (2014) Effects of elevated [CO_2] on maize defence against mycotoxigenic *Fusarium verticillioides*. *Plant Cell Environ.* 37, 2691–2706
53. Engineer, C.B. *et al.* (2016) CO_2 sensing and CO_2 regulation of stomatal conductance: advances and open questions. *Trends Plant Sci.* 21, 16–30
54. Mustilli, A.C. *et al.* (2002) Arabidopsis OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. *Plant Cell* 14, 3089–3099
55. Negi, J. *et al.* (2008) CO_2 regulator SLAC1 and its homologues are essential for anion homeostasis in plant cells. *Nature* 452, 483–486
56. Vahisalu, T. *et al.* (2008) SLAC1 is required for plant guard cell S-type anion channel function in stomatal signaling. *Nature* 452, 487–491
57. Imes, D. *et al.* (2013) Open stomata 1 (OST1) kinase controls R-type anion channel QUAC1 in Arabidopsis guard cells. *Plant J.* 74, 372–382
58. Matrosova, A. *et al.* (2015) The HT1 protein kinase is essential for red light-induced stomatal opening and genetically interacts with OST1 in red light and CO_2-induced stomatal movement responses. *New Phytol.* 208, 1126–1137
59. Tian, W. *et al.* (2015) A molecular pathway for CO_2 response in Arabidopsis guard cells. *Nat. Commun.* 6, 6057
60. Geiger, D. *et al.* (2009) Activity of guard cell anion channel SLAC1 is controlled by drought-stress signaling kinase-phosphatase pair. *Proc. Natl. Acad. Sci. U. S. A.* 106, 1425–21430
61. Meyer, S. *et al.* (2010) AtALMT12 represents an R-type anion channel required for stomatal movement in Arabidopsis guard cells. *Plant J.* 10, 1217–1223
62. Lee, M. *et al.* (2008) The ABC transporter AtABCB14 is a malate importer and modulates stomatal response to CO_2. *Nat. Cell Biol.* 10, 1217–1223
63. Hu, H. *et al.* (2010) Carbonic anhydrases are upstream regulators of CO_2-controlled stomatal movements in guard cells. *Nat. Cell Biol.* 12, 87–93
64. Hu, H. (2015) Distinct cellular locations of carbonic anhydrases mediate carbon dioxide control of stomatal movements. *Plant Physiol.* 169, 1168–1178
65. Engineer, C.B. *et al.* (2014) Carbonic anhydrases, EPF2 and a novel protease mediate CO_2 control of stomatal development. *Nature* 513, 246–250
66. Merilo, E. *et al.* (2013) PYR/RCAR receptors contribute to ozone-, reduced air humidity-, darkness-, and CO_2-induced stomatal regulation. *Plant Physiol.* 162, 1652–1668
67. Queval, G. *et al.* (2012) Daylength is a key regulator of transcriptomic responses to both CO_2 and H_2O_2 in *Arabidopsis*. *Plant Cell Environ.* 35, 374–387
68. Peterson, M. *et al.* (2000) Arabidopsis map kinase 4 negatively regulates systemic acquired resistance. *Cell* 103, 1111–1120
69. Broderson, P. *et al.* (2006) Arabidopsis MAP kinase 4 regulates salicylic acid- and jasmonic acid/ethylene-dependent responses via EDS1 and PAD4. *Plant J.* 47, 532–546
70. Hõrak, H. *et al.* (2016) A dominant mutation in the HT1 kinase uncovers roles of MAP kinases and GHR1 in CO_2-induced stomatal closure. *Plant Cell* 28, 2493–2509
71. Jakobson, L. *et al.* (2016) Natural variation in *Arabidopsis* Cvi-0 accession reveals an important role of MPK12 in guard cell CO_2 signaling. *PLoS Biol.* 14, e2000322
72. Restrepo, S. *et al.* (2005) Gene profiling of a compatible interaction between *Phytophthora infestans* and *Solanum tuberosum* suggests a role for carbonic anhydrase. *Mol. Plant Microb. Int.* 18, 913–922
73. Sun, C. *et al.* (2014) Proteomic analysis of non-heading Chinese cabbage infected with *Hyaloperonospora parasitica*. *J. Proteomics* 98, 15–30
74. Slaymaker, D.H. *et al.* (2002) The tobacco salicylic acid-binding protein 3 (SABP3) is the chloroplast carbonic anhydrase, which exhibits antioxidant activity and plays a role in the hypersensitive defense response. *Proc. Natl. Acad. Sci. U. S. A.* 99, 11640–11645
75. Melotto, M. *et al.* (2008) Role of stomata in plant innate immunity and foliar bacterial diseases. *Annu. Rev. Phytopathol.* 46, 101–122
76. O'Leary, B.M. *et al.* (2016) Early changes in apoplast composition associated with defence and disease in interactions between *Phaseolus vulgaris* and the halo blight pathogen *Pseudomonas syringae* Pv. Phaseolicola. *Plant Cell Environ.* 39, 2172–2184
77. Lager, I. *et al.* (2010) Changes in external pH rapidly alter plant gene expression and modulate auxin and elicitor responses. *Plant Cell Environ.* 33, 1513–1528
78. Wang, L. *et al.* (2013) Elevated atmospheric CO_2 decreases the ammonia compensation point of barley plants. *J. Exp. Bot.* 64, 2713–2725
79. Glawischnig, E. (2007) Camalexin. *Phytochemistry* 68, 401–406
80. Tallis, M.J. *et al.* (2010) The transcriptome of *Populus* in elevated CO_2 reveals increased anthocyanin biosynthesis during delayed autumnal senescence. *New Phytol.* 186, 415–428

81. Henkes, S. *et al.* (2001) A small decrease of plastid transketolase activity in antisense tobacco transformants has dramatic effects on photosynthesis and phenylpropanoid metabolism. *Plant Cell* 13, 535–551
82. Xiao, Y. *et al.* (2012) Retrograde signaling by the plastidial metabolite MEcPP regulates expression of nuclear stress-response genes. *Cell* 149, 1525–1535
83. Sharkey, T.D. *et al.* (2008) Isoprene emission from plants: why and how. *Ann. Bot.* 101, 5–18
84. Siemens, J. *et al.* (2011) Extracellular invertase is involved in the regulation of clubroot disease in *Arabidopsis thaliana*. *Mol. Plant Pathol.* 12, 247–262
85. Cecchini, N.M. *et al.* (2011) Proline dehydrogenase contributes to pathogen defense in Arabidopsis. *Plant Physiol.* 155, 1947–1959
86. Manzoor, H. *et al.* (2013) Involvement of the glutamate receptor AtGLR33 in plant defense signaling and resistance to *Hyaloperonospora arabidopsidis*. *Plant J.* 76, 466–480
87. Novitskaya, L. *et al.* (2002) How does photorespiration modulate leaf amino acid contents? A dual approach through modelling and metabolite analysis. *Plant Cell Environ.* 25, 821–835
88. Anderson, J.C. *et al.* (2014) Decreased abundance of type III secretion system inducing signals in Arabidopsis *mkp1* enhances resistance against *Pseudomonas syringae*. *Proc. Natl. Acad. Sci. U. S. A.* 111, 6846–6851
89. Mott, K.A. (2009) Opinion: stomatal responses to light and CO_2 depend on the mesophyll. *Plant Cell Environ.* 32, 1479–1486
90. Araújo, W.L. *et al.* (2011) Control of stomatal aperture. A renaissance of the old guard. *Plant Signal. Behavior* 6, 1305–1311
91. Buchanan, B.B. and Balmer, Y. (2005) Redox regulation: a broadening horizon. *Annu. Rev. Plant Biol.* 56, 187–220
92. Weits, D.A. *et al.* (2014) Plant cysteine oxidases control the oxygen-dependent branch of the N-end rule pathway. *Nat. Commun.* 5, 3425
93. Gibbs, D.J. *et al.* (2015) Group VII ethylene response factors coordinate oxygen and nitric oxide signal transduction and stress responses in plants. *Plant Physiol.* 169, 23–31
94. Tada, Y. *et al.* (2008) Plant immunity requires conformational charges of NPR1 via S–nitrosylation and thioredoxins. *Science* 321, 952–956
95. Vainonen, J.P. and Kangasjärvi, J. (2015) Plant signalling in acute ozone exposure. *Plant Cell Environ.* 38, 240–252
96. Noctor, G. and Foyer, C.H. (1998) A re-evaluation of the ATP: NADPH budget during C_3 photosynthesis. A contribution from nitrate assimilation and its associated respiratory activity? *J. Exp. Bot.* 49, 1895–1908
97. Backhausen, J.E. and Scheibe, R. (1999) Adaptation of tobacco plants to elevated CO_2: influence of leaf age on changes in physiology, redox states and NADP-malate dehydrogenase activity. *J. Exp. Bot.* 50, 665–675
98. Foyer, C.H. *et al.* (2012) Photosynthetic control of electron transport and the regulation of gene expression. *J. Exp. Bot.* 63, 1637–1661
99. Huang, S. *et al.* (2016) The roles of mitochondrial reactive oxygen species in cellular signaling and stress response in plants. *Plant Physiol.* 171, 1551–1559
100. Krömer, S. (1995) Respiration during photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46, 45–70
101. Igamberdiev, A.U. *et al.* (1997) Involvement of cyanide-resistant and rotenone-insensitive pathways of mitochondrial electron transport during oxidation of glycine in higher plants. *FEBS Lett.* 412, 265–269
102. Cheeseman, J.M. (2006) Hydrogen peroxide concentrations in leaves under natural conditions. *J. Exp. Bot.* 57, 2435–2444
103. Qiu, Q.S. *et al.* (2008) Increased protein carbonylation in leaves of Arabidopsis and soybean in response to elevated $[CO_2]$. *Photosynth. Res.* 97, 155–166
104. Yi, C. *et al.* (2015) High atmospheric carbon dioxide-dependent alleviation of salt stress is linked to RESPIRATORY BURST OXIDASE 1 (RBOH1)-dependent H_2O_2 production in tomato (*Solanum lycopersicum*). *J. Exp. Bot.* 66, 7391–7404
105. Kwak, J.M. *et al.* (2003) NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in Arabidopsis. *EMBO J.* 22, 2623–2633
106. Wang, Y.Q. *et al.* (2009) *S*-Nitrosylation of AtSABP3 antagonizes the expression of plant immunity. *J. Biol. Chem.* 284, 2131–2137
107. Han, Y. *et al.* (2013) Functional analysis of Arabidopsis mutants points to novel roles for glutathione in coupling H_2O_2 to activation of salicylic acid accumulation and signaling. *Antioxid. Redox Signal.* 18, 2087–2090
108. Chamizo-Ampudia, A. *et al.* (2017) Nitrate reductase regulates plant nitric oxide homeostasis. *Trends Plant Sci.* 22, 163–173
109. Hebbelmann, I. *et al.* (2012) Multiple strategies to prevent oxidative stress in *Arabidopsis* plants lacking the malate valve enzyme NADP-malate dehydrogenase. *J. Exp. Bot.* 63, 1445–1469
110. Vogel, M.O. *et al.* (2014) Fast retrograde signaling in response to high light involves metabolite export, MITOGEN-ACTIVATED PROTEIN KINASE6, and AP2/ERF transcription factors in Arabidopsis. *Plant Cell* 26, 1151–1165
111. Noctor, G. and Foyer, C.H. (2016) Intracellular redox compartmentation and ROS-related communication in regulation and signaling. *Plant Physiol.* 171, 1581–1592
112. Jung, H.W. *et al.* (2009) Priming in systemic plant immunity. *Science* 324, 89–91
113. Návarová, H. *et al.* (2012) Pipecolic acid, an endogenous mediator of defense amplification and priming, is a critical regulator of inducible plant immunity. *Plant Cell* 24, 5123–5141
114. Noctor, G. *et al.* (2015) The metabolomics of oxidative stress. *Phytochemistry* 112, 33–53
115. Toruño, T.Y. *et al.* (2016) Plant–pathogen effectors: cellular probes interfering with plant defenses in spatial and temporal manners. *Annu. Rev. Phytopathol.* 54, 419–441
116. Chang, Y.M. *et al.* (2012) Characterizing regulatory and functional differentiation between maize mesophyll and bundle sheath cells by transcriptomic analysis. *Plant Physiol.* 160, 165–177
117. John, C.R. *et al.* (2014) Evolutionary convergence of cell-specific gene expression in independent lineages of C_4 grasses. *Plant Physiol.* 165, 62–75
118. Kajala, K. *et al.* (2011) Strategies for engineering a two-celled C_4 photosynthetic pathway into rice. *J. Exp. Bot.* 62, 3001–3010