Plant Stress ©2007 Global Science Books

Hydrogen Peroxide and Plant Stress: A Challenging Relationship

John M. Cheeseman

Department of Plant Biology, University of Illinois, Urbana IL 61801, USA

Correspondence: **j-cheese@life.uiuc.edu**

ABSTRACT

The relationship between plants and hydrogen peroxide is a challenging one: H_2O_2 has many essential roles in plant metabolism but at the same time, accumulation related to virtually any environmental stress is potentially damaging. In this review, I consider H_2O_2 physiology broadly, both as a stress and as a developmentally and physiologically important metabolite, including its sources and mobility, and the vexing question of tissue level concentrations. I then consider problems associated with H_2O_2 as a signaling molecule, including mechanisms of H_2O_2 sensing, signaling, and response networks. Finally, I discuss recent advances in transcript network modeling, and complex systems approaches to understanding the interactions between the transcriptome, proteome and metabolome in responses to H_2O_2.

Keywords: amine oxidase, apoplast, complex systems modeling, NAD(P)H oxidase, peroxidase, transcriptome, photosynthesis, proteome, mitochondria, signaling cascade

Abbreviations: APX, ascorbate peroxidase; **CAT**, catalase; **DAO**, diamine oxidase; **GLP**, germin-like protein; **HR**, hypersensitive response; **MAO**, monoamine oxidase; **MAPK**, mitogen-activated protein kinase; **PAO**, polyamine oxidase; **POX**, peroxidase; **ROS**, radical oxygen species; **SOD**, superoxide dismutase

CONTENTS

INTRODUCTION

It is now well established that virtually all biotic and abiotic stresses induce or involve oxidative stress to some degree, and the ability of plants to control oxidant levels is highly correlated with stress tolerance. Whether the antioxidant approach to explaining tolerance will, in the long run, be any better as a single factor explanation than, say, the ability to exclude Na from shoots and maintenance of K/Na discrimination ratios has been for salt tolerance, or the ability to accumulate compatible osmotica has been for drought tolerance, remains to be seen. It is now clear, however, that any stress condition or significant change in environment is associated with up- or down-regulation of hundreds of genes, that some proteins important to oxidative metabolism may have high stabilities and low turnover rates, and that even the cell wall, once considered of little biological importance, contains hundreds of proteins and metabolites, many of which may be involved in oxidative metabolism.

At the same time, it is also well established that oxidative metabolism, and particularly H_2O_2, is involved in a wide variety of reactions and signaling cascades necessary for all aspects of plant growth and the integration of activity, ranging from the develop of individual root hairs, to xylem differentiation and lignification, to wall loosening and wall cross-linking, to root/shoot coordination and stomatal control. Thus, while the involvement of H_2O_2 in stress responses is of particular interest, it really must be considered in the context of, and even as a special case of, H_2O_2 involvement in "normal" growth and metabolism.

Overall, the current "fashion" in plant stress studies is to grow plants in controlled conditions, apply a stress rather suddenly after a period of unstressed growth, and then compare some aspect or aspects of response – ranging from activity of a single enzyme to whole genome transcript networks – at a fixed time thereafter. Unfortunately for plant biologists, but fortunately for plants, such environmental

Received: 6 March, 2007. Accepted: 30 April, 2007.

Special Feature

perturbations are not typical of the real world, and models built on them may be, as Manfred Eigen put it, "right, but irrelevant" (Eigen 1973). Much more difficult is understanding the small, minute-to-minute or day-to-day variations which preclude the necessity to respond dramatically, and developing relevant models that include them.

My objective for this review is to consider the physiology of H_2O_2 as it relates to plant stress responses, but to do so in a way that recognizes "normal" activities. To do this, I will acknowledge generality more than details of specific responses, and as many of the H_2O_2-related "tools" that plants have as possible, not just those for which the most detailed genetic models have been derived. This also means that important aspects of stress response that are only indirectly or distantly down-stream related to H_2O_2 will be emphasized less. This effort will, of necessity, leave out references to many reports: there have been more than 2100 journal articles on the topic of peroxide in plants since 2000 alone, and the number is increasing rapidly.

What is H_2O_2?

Hydrogen peroxide is the two electron reduction product of O_2. It is potentially reactive oxygen, but not a free radical (Halliwell *et al*. 2000). By comparison with superoxide, $O_2^{\bullet}$, and certainly by comparison with the hydroxyl radical, $^{\bullet}OH$, H_2O_2 is relatively "safe": in the absence of transition metals, it is stable and unreactive, even at concentrations much higher than a biological system would ever generate. Functionally, this imparts on it greater mobility within tissues, and potential utility not only as a substrate in a variety of reactions, but as a molecule for ROS-related signaling.

However, H_2O_2 is potentially quite reactive with molecules containing Fe^{2+} or other transition metals, through the Fenton reaction (Becana *et al*. 1998). The "evil" result of this reaction is the homolysis of H_2O_2 to 2 $^{\bullet}OH$, and H_2O_2 toxicity is most commonly associated with that action. For example, inhibition of Rubisco by exogenous H_2O_2 results from the fragmentation of the LSU at a glycine in the catalytic site (Ishida *et al*. 1999), dependent on the activation state of the enzyme. An identical fragmentation pattern occurred in intact chloroplasts when oxidant scavenging systems were inhibited (Ishida *et al*. 1999), or when cold-sensitive maize leaves were exposed to low temperatures (Kingston-Smith *et al*. 1999), as a consequence of the fact that chloroplasts contain as much as 80% of the Fe in a plant, and are a good source of radical oxygen species (ROS) generally. Similar Fenton reaction mechanisms have been associated with H_2O_2 (or actually, $^{\bullet}OH$) sensitivity of FeSOD (Bhattacharya *et al*. 2004), and glutamine synthase (Farber and Levine 1986), among other enzymes. By contrast, direct reaction of H_2O_2 with the –SH groups has been suggested as the mechanism by which H_2O_2 inactivates fructose bisphosphatase (Charles and Halliwell 1980, 1981) and sedohepulose bisphosphatase (Wise 1995; Tamoi *et al*. 2006) in chloroplasts, and cytosolic glyceraldehyde 3-phosphate dehydrogenase (Brodie and Reed 1987; Hancock *et al*. 2005). H_2O_2 toxicity is reduced by removing it enzymatically (i.e. by catalase or ascorbate peroxidase), or by complexing Fe(III) and Fe(II) with compounds such as tannic acid and proanthocyanidins, thus preventing $^{\bullet}OH$ generation (Toda 2005; Andrade *et al*. 2006).

WHAT ARE THE RELEVANT TISSUE LEVELS OF H_2O_2?

The basic question here is: how much H_2O_2 is there in plant tissue, and against what background might changes be useful signals? Given that there are so many good methods for assaying H_2O_2 in solutions, some of which are quite specific, it is surprising that there is such a wide range of estimates in plant tissues, spanning nearly seven orders of magnitude, and no apparent consensus concerning how large a stress or treatment related change is physiologically significant. At the low end, Hernández *et al*. (2001) reported tissue levels ranging from 10 to 150 pmol/gFW in the pea leaf apoplast with the difference (salt induced) being sufficient to cause oxidative lesions. At the other extreme, He *et al*. (2005) reported concentrations in *Poa pratensis* leaves as high as 1.3% of the dry weight, which, based on data in their report, was ca. 60 μmol/gFW or 100 mM on a leaf water basis. In maize, Tewari *et al*. (2004) reported concentrations of 20 μmol/gFW, rising to 75 μmol/gFW with N deficiency. Ben Amor *et al*. (2006), in an interesting study of the coastal halophyte, *Cakile maritima* also reported tissue H_2O_2 contents on the high end, as much as 45 μmol/gFW. Veljovik-Jovanovic *et al*. (2002) were the first to recognize possible interferences by plant constituents with H_2O_2 assay protocols, and suggested that leaf levels should generally be less than 0.1 μmol/gFW. On the other hand, an analysis of field grown plants, with care to account for potential interferences as well as continued metabolism of H_2O_2 after harvesting, suggested that values in the 1-5 μmol/gFW range might be normal (Cheeseman 2006).

Similarly confusing is what it means – in terms of tissue level H_2O_2 concentrations – to have an "oxidative burst". This issue is undoubtedly complicated by the rapidity of H_2O_2 turnover both *in planta* and after tissue harvesting (Cheeseman 2006). In response to an acute ozone exposure, (200 ppb/ 2 hr), Chen and Gallie (2005) reported tobacco (cv. 'Xanthi') leaf H_2O_2 levels had increased ca. 4x (in the 100 nmol/gFW range, using plants grown with as little prior ozone exposure and potential irradiance stress as possible), but increased another four to five-fold after 24 hr recovery. Karpinski *et al*. (1997) on the other hand, reported an oxidative burst in *Arabidopsis* with exposure to excess irradiance - in this case, ten-fold higher than their growth irradiance of 200 μmol $m^{-2}s^{-1}$ - that increased the leaf content from about 5 μmol/gFW to less than 7 μmol/gFW. The issue here is only partly the order of magnitude and the percent change. The more critical question – which I can not answer – is, what background levels and what sort of changes are needed to support the role of H_2O_2 in signaling, especially if measurements are limited, in practice, to bulk tissue levels?

WHAT PRODUCES H_2O_2?

H_2O_2, and ROS generally, are a fundamental fact of life in an aerobic environment (Moller 2001). Understanding the role of H_2O_2 in plant growth or stress responses requires models that accommodate the large number of ways in which it can be formed and degraded at any given time, and that ROS produced by one source may be the drivers or substrates for a second (Allan and Fluhr 1997). Major sources include misfires in the electron transport chains of chloroplasts and mitochondria, the Mehler reaction, a wide variety of limited substrate oxidases, type III peroxidases, and NAD(P)H oxidases (Halliwell and Gutteridge 1999). Some of these produce H_2O_2 directly, and others only via more reactive intermediates (e.g. $^{\Gamma}O$ or $O_2^{\bullet}$). Broadly, these events are enhanced by stresses (Alscher *et al*. 1997; Bolwell 1999), although they occur as an integral part of many facets of plant development.

Excess light and other energy imbalances

It is impossible to envision any environmental effect, whether or not we or plants recognize it as a stress, that does not reflect the energy available to respond or imbalances in energy availability, energy transduction and energy metabolism. This necessarily links ROS metabolism with all aspects of plant life. Oxidative stress associated with photosynthesis is a potential problem any time, but especially when the capacity for electron transport exceeds the capacity for recycling the NADPH and ATP which result. This is likely at irradiances above light saturation, but also at lower irradiance when stomates (for example) limit CO_2 supplies. This problem is reduced by activity of the xantho-

phyll cycle, (e.g. Demmig-Adams *et al*. 1999), but the effectiveness is not complete. In that case, the Mehler-Ascorbate Peroxidase, or water-water cycle also contributes to damage prevention (see Allen 1995; Asada 1999; Heber 2002). This has been modeled as operating through the sequential actions of superoxide dismutase (SOD – generating H_2O_2), ascorbate peroxidase (APX – reducing H_2O_2 at the expense of ascorbate) and glutathione reductase (GR – regenerating ascorbate at the expense of reduced glutathione). Oxidized glutathione, and the monodehydroascorbate radical can both be re-reduced by NADPH, both allowing the cycle to continue, and reducing the electron pressure for $O_2^{\bullet}$ generation.

That both the xanthophyll cycle and the water-water cycle are important under field conditions has been clearly demonstrated using cultivated (e.g. Logan *et al*. 1998a, 1998b) or wild-grown plants (Streb *et al.* 1997; Logan *et al*. 1998c; Streb *et al*. 1998). Moreover, the interplay between nutrient and CO_2 availability has been demonstrated using FACE (free-air CO_2 enrichment) studies (Polle *et al*. 1997). In the latter case (using three year old *Fagus sylvatica*), "intrinsic oxidative stress" was reduced when photosynthesis was favored over photorespiration at elevated CO_2, and modulated by relative nutrient resource availability and assimilation.

Intracellular ROS scavenging is both highly efficient and adaptable, and H_2O_2 related stress, or its prevention, resulting from activities in the chloroplasts, mitochondria or other organelles, reflects the integration of all cellular activities. For example, following application of the catalase (CAT) inhibitor, 3-aminotriazole in *Arabidopsis*, oxidant damage was limited by increases in the activities of the pre-existing APX1 and GR1 isoforms (Kang *et al*. 1999) or increased transcription of cytosolic APX (Morita *et al*. 1999). Pea also showed adjustments in the light-independent photosynthetic pathways – net photosynthesis, the RuBP regeneration rate and carboxylation efficiency all declined (Amory *et al*. 1992). Photorespiratory carbon flow was reduced (as indicated by an increase in the formate pool), preventing its return to the Calvin cycle. However, only when the capacity for H_2O_2 reduction was additionally challenged by enhanced photorespiratory conditions, did H_2O_2 concentrations increase. Similar manipulations have also been accomplished using antisense techniques. For example, tobacco was engineered to reduce APX and CAT expression, individually and together (Rizhsky *et al*. 2002). Double antisense plants compensated, preventing oxidative stress, by suppressing photosynthetic activity, up-regulating the pentose phosphate pathway, increasing monodehydroascorbate reductase activity, and inducing a chloroplast homologue of the mitochondrial alternative oxidase. Interestingly, the response network was less complete in plants that lacked only APX or CAT, rendering them more sensitive to oxidative stress.

The emphasis on scavenging may no longer be sufficient, however (Foyer and Noctor 2003). Rather, the involvement of H_2O_2 in signaling demands closer attention: redox cascades in both chloroplast and mitochondrial electron transport chains, and the redox states of compounds including thioredoxins, ascorbate and glutathione, carry information in addition to electrons. Indeed, the cellular redox state may have precedence over ATP production: a *Nicotiana sylvestris* mutant defective in mitochondrial complex I, for example, compensated through antioxidant crosstalk, a whole network response involving mitochondria and other organelles, maintaining whole cell redox balance (Dutilleul *et al*. 2003). This included markedly increased alternative oxidase (AOX) activity, and enhanced oxidative stress tolerance. Cytosolic APX and glutathione reductase, mitochondrial MnSOD, and two isoforms of CAT also showed substantial increases in transcript levels. Similarly, when low-light-acclimated (200 µmol $m^{-2}s^{-1}$) *Arabidopsis* plants were exposed to excess light (2000 µmol $m^{-2}s^{-1}$) for 1 hr, inducing reversible photoinhibition, signal transduction reflecting the redox status of the plastoquinone (PQ) pool, led to elevated expression of two cytosolic ascorbate peroxidases. Preventing the change in the PQ redox poise by supplying reduced glutathione enhanced photoinhibition and prevented the APX transcriptional changes (Karpinski *et al*. 1997).

In mitochondria, the alternative oxidase (AOX) provides an additional way of reducing ROS production which has too often been overlooked in stress response studies (Wagner 1995; Popov *et al*. 1997). Mitochondrial ROS production is particularly associated with electron transfer between the multiple Fe-S centers and cytochromes in Complexes I and III. Under conditions of surplus electron supply or limitations in ATP consumption, AOX and the non-proton-pumping NADH dehydrogenases on the matrix side of the inner membrane function to limit mitochondrial ROS production by keeping the electron transport chain relatively oxidized and minimizing the number of individual electron transfers (e.g. Baxter *et al*. 2007). Antioxidant enzymes in the matrix, together with small antioxidants such as glutathione, help remove ROS that are formed. The antioxidants are kept in a reduced state by matrix NADPH produced by NADP-isocitrate dehydrogenase and non-proton-pumping transhydrogenase activities (e.g. Purvis and Shewfelt 1993; Popov *et al*. 1997; Braidot *et al*. 1999; Maxwell *et al*. 1999; Casolo *et al*. 2000). AOX is induced by a number of stresses (e.g. Farrar and Rayns 1987; Parsons *et al*. 1999; Xie and Chen 1999). If these defenses are overwhelmed, as can occur during both biotic and abiotic stress, the mitochondria may be damaged. This can be induced, for example, by inhibition of AOX with salicyl hydroxamic acid (SHAM) or propyl gallate, stimulating H_2O_2 production with the same substrate dependence as inhibition of CN-insensitive respiration (Popov *et al*. 1997). Antisense suppression of AOX also leads to significantly higher ROS production, while AOX over-expression has the opposite effect (Maxwell *et al*. 1999; Parsons *et al*. 1999).

As important as chloroplast and mitochondrial electron transfer are in generation of ROS, they are not the only sources. Indeed, multiple sources may be involved in many, if not all, stress reactions, and different sources may be important in different species (e.g. Allan and Fluhr 1997; Bolwell *et al*. 1998).

Limited substrate oxidases

Limited-substrate oxidases such as glycolate oxidase in peroxisomes, and xanthine oxidase and urate oxidase in glyoxisomes, are flavin-containing enzymes which directly produce H_2O_2 (as opposed to indirect production via $O_2^{\bullet}$) (Delrio *et al*. 1992). Recently, a H_2O_2-producing sulfite oxidase has also been identified, localized to peroxisomes (Hansch *et al*. 2006). H_2O_2 produced in these organelles is usually quickly consumed by catalase, although isoforms of ascorbate peroxidase (APX3) localized to the organelles may also contribute to its control (Wang *et al*. 1999). Other flavin-containing oxidases important in specific compartments or tissues include a variety of monoamine (MAO) and polyamine (PAO) oxidases, and germin-like proteins. For example, in maize, flavin-containing polyamine oxidases have been identified especially in cell types destined for lignification (Cona *et al*. 2005; Paschalidis and Roubelakis-Angelakis 2005; Cona *et al*. 2006a, 2006b). Production of polyamines under water or low-temperature stress has also been correlated with protection against oxidative stress (e.g. in chickpea – Nayyar and Chander 2004). The maize PAO is, like many flavin-containing enzymes, DPI sensitive, but its activity can be differentiated from others sensitive to the inhibitor (such as NAD(P)H oxidases) by the fact that H_2O_2 is released on supply of spermidine or other polyamines, and that it is sensitive to phosphatase inhibitors. Diamine (copper-containing) oxidases, (DAO) using putrescine and cadaverine (diamines) or spermidine (triamine) in the apoplast as their substrates, are also important in H_2O_2 production for lignification, as well as being induced in response to fungal elicitors and wounding and in

cells destined for programmed cell death (Angelini *et al*. 1996; Moller and McPherson 1998; Laurenzi *et al*. 2001; Langebartels *et al*. 2002).

In tobacco (*Nicotiana tabacum* cv. 'Xanthi'), ornithine decarboxylase, genes involved in polyamine biosynthesis, and polyamine oxidase activities were up-regulated in response to tobacco mosaic virus infection. These were quantitatively related to the magnitude and size of the hypersensitive response (HR) and HR-like cell death (Yoda *et al*. 2003). Inhibiting polyamine biosynthesis with α-difluoromethyl-ornithine, or apoplast-localized PAO synthesis by RNAi, suppressed H_2O_2 production and prevented cell death (Yoda *et al*. 2006). Note, however, that amine oxidases are also constitutively present in the apoplast (Liu *et al*. 1995), and different enzymes (e.g. diamine vs. polyamine oxidases) show different patterns of constitutive and pathogen-induced expression (Asthir *et al*. 2004). In *Mesembryanthemum crystallinum*, NaCl shock activated both diamine oxidase and guaiacol peroxidase, as did exogenous cadaverine (Shevyakova *et al*. 2006). Thus, at least in some cases, it appears that synthesis of the amines themselves is the controlling factor in responses, rather than synthesis of the enzymes (Rea *et al*. 2004).

An alternative to polyamines as a substrate for extracellular H_2O_2 production is the organic acid, oxalate. Oxalate oxidase (germin or germin-like protein, GLP) functions in this role, generating H_2O_2 for the purpose of peroxidase-mediated wall cross linking (Caliskan *et al*. 2004), in association with wall formation by protoplasts, and in response to wounding (Bernier and Berna 2001; Le Deunff *et al*. 2004). It also increases resistance to certain pathogens, e.g. the oxalate producing *Sclerotinia sclerotiorum* (Cober *et al*. 2003; Hu *et al*. 2003), and in plants lacking the enzyme, oxalate has been shown to inhibit H_2O_2 production (Cessna *et al*. 2000). In transgenic sunflowers, however, in addition to increased resistance to the fungus, low constitutive expression of oxalate oxidase activated a suite of defense genes and higher expression led to HR-like lesions (Hu *et al*. 2003). Treatment of tobacco (cv. 'Petit Havana SR1') with 100 mM NaCl also led to increased apoplastic accumulation of the protein (Dani *et al*. 2005).

As the name suggests, GLP also plays a role in seed germination. Combining *in vitro* germination experiments with data on emergence potential of sugar beet (*Beta vulgaris*) in the field, de los Reyes and McGrath (2003) screened for germination-enhancing and stress-induced genes. In accessions with superior germination potential, GLP gene expression, oxalate oxidase activity, and H_2O_2 content (but not catalase activity), were induced under flooding, salt, osmotic, or oxalate treatment. In this case, H_2O_2 production promoted germination, and partially compensated for salt or osmotically-related inhibitions. Accessions with poorer rates of germination had correspondingly lower activity of oxalate oxidase.

Type III peroxidases

Unlike APX which is largely intracellular and involved in the control of cellular H_2O_2 levels (Veitch 2004), type III peroxidases (POX) are more frequently secreted into the apoplast and involved in phenolic metabolism using H_2O_2 as a substrate. Despite their classification in one group, they perform a wide diversity of functions, inspiring their comparison to a Swiss army knife (Passardi *et al*. 2005). In part, this is possible because of their large number. In *Arabidopsis*, for example, there are 73 POX genes and their products are found in the cytosol and vacuole as well as in the apoplast (Mittler *et al*. 2004). Peroxidases show tissue and developmental specificity (Kay and Basile 1987; Perez and Burgos 2004) and vary with respect to substrate specificity and pH optima (Bestwick *et al*. 1998). The presence of multiple peroxidase isoforms with different substrate specificities can affect cell wall composition, cell wall rigidity, the wall redox environment, signaling and defense. In addition, different oxidized substrates differ in their potential to be re-reduced by ascorbate, and presumably, other reducing agents (Pearse *et al*. 2005). However, other than, perhaps, in its role in lignification, the actual, *in vivo* substrates of peroxidase are unclear (Halliwell and Gutteridge 1999).

In addition to their role in oxidation of phenolics, some forms of POX, especially basic forms, can generate H_2O_2 coupled to oxidation of NADH (Ros Barceló 2000; Koutaniemi *et al*. 2005; Sukalovic *et al*. 2005). In such reactions, peroxidase acts as an oxidase, creating a substrate free radical ($XH^{\bullet}$) which reduces O_2 (Halliwell and Gutteridge 1999). This activity was first demonstrated with NADH using horse radish peroxidase (HRP) by Akazawa and Conn (1958), and 25 years ago, Mäder and Amberg-Fisher, showed that two cell wall peroxidases from tobacco differing in pI and in their ability to polymerize cinnamyl alcohols, could act similarly (Mäder and Amberg-Fisher 1982). It has received considerable attention since then. Details of the reaction and reaction mechanisms have been most intensively studied with respect to NADH, although an analogous mechanism has been postulated for stimulation of H_2O_2 production by salicylic acid (SA) (Kawano and Muto 2000). Whether or not NADH is a potential substrate *in vivo* clearly depends on whether or not it is present in the same compartment as the peroxidase. In the apoplast, this is doubtful (Otter and Polle 1997; Karkonen *et al*. 2002).

Type III peroxidases are also important in the responses of plants to pathogens, with distinct differences between isoenzyme effects: changes in the activity and distribution of the enzyme were examined during the development of a nonhost hypersensitive reaction (HR) to *Pseudomonas syringae* pv. phaseolicola and an hrp mutant of the bacterium in lettuce (Bestwick *et al*. 1998). Inoculation with water or with wild-type or hrp mutant strains of the bacteria caused an initial decline in total POX activity, followed by recovery dependent on the phenolic substrate. In tissues experiencing the HR, guaiacol peroxidase (pH_{opt} 6.0) recovered more rapidly, while recovery of tetramethylbenzidine peroxidase (pH_{opt} 4.5) was independent of the type of interaction, and chlorogenic acid peroxidase activity (pH_{opt} 6.0) was significantly higher in response to the hrp mutant. Direct involvement of wall peroxidases in H_2O_2 production in response to fungal elicitors has been demonstrated in French bean cell cultures (Bolwell *et al*. 1998), *Arabidopsis* (Bolwell *et al*. 2002; Bindschedler *et al*. 2006), cotton (Martinez *et al*. 1998), and other species, but there clearly appear to be species specific differences in this activity (Bolwell *et al*. 1998). The complexity of plant responses and H_2O_2 metabolism is especially clear with respect to pathogens: in the hypersensitive response of *Arabidopsis* responding to *Fusarium*, for example, H_2O_2 production via POX in the apoplast stimulated NAD(P)H oxidase activation, and apoplastic Ca, K, Cl and wall alkalinization were intimately associated with this in a signaling cascade (Davies *et al*. 2006).

Apart from the enzyme-substrate relationship between POX and phenolics, phenolic metabolism is part of normal plant growth and responses to environment, and there are very large differences in the extent to which plants accumulate phenolics, including tannins, constitutively or following induction. It is important that phenolic accumulation not be considered indicative of pathology alone; phenolic acids are critical to normal leaf development and senescence (Tamagnone *et al*. 1998), and more broadly, are often critical to successfully integrating overall resource acquisition and allocation. This is reflected in "tissue quality", e.g. toughness and phenolic content, both of which are associated with H_2O_2 metabolism. As noted by Haslam (1985, 1986), when C and energy utilization are limited by lack of other resources, the resulting metabolic imbalance requires diversion of carbon from energy production to energy consuming pathways. This occurs within chloroplasts, in the glycolytic pathway, and within the mitochondria. For example, pyruvate, PEP, acetyl-CoA and 3-phosphogly-

cerate may be shunted into end products which are metabolically harmless, but ecologically useful in defensive roles. While this may reduce the potential for oxidative damage to mitochondria, it will not necessarily eliminate it. The response of oxidative (and anti-oxidative) metabolism to nutrient limitations is, thus, also closely tied to mitochondrial protection (see above). Recently, Baxter *et al*. (2007) demonstrated the extent of both transcriptome and metabolome changes associated with oxidative stress in heterotrophic *Arabidopsis* cells, confirming the extent to which rapid metabolic adjustments can occur.

Both abiotic and biotic stresses can cause shifts in phenolic metabolism. In bean (*Phaseolus vulgaris*) for example, Malusa *et al*. (2002) reported that induction of mild oxidative stress, lipid peroxidation, and an increase in phenolic production reflecting a redirection of carbon metabolism, all occurred under conditions of P-limitation, while Cakmak (1994) reported strong induction with either K or Mg deficiency. Similar induction has also been associated with other nutrient deficiencies, e.g. K (Shin and Schachtman 2004), Mg (Tewari *et al*. 2006), and Fe (Ranieri *et al*. 2001). In response to herbivory, POX responses are critical to reducing tissue palatability by wall cross linking (Brisson *et al*. 1994). It is important to note, however, that shifting phenolic metabolism does not necessarily mean induction of POX genes, at least in the short term. POX enzymes are frequently stable and long-lived, enabling rapid and flexible responses (Pearse *et al*. 2005).

NAD(P)H oxidases

These membrane proteins oxidize NADPH at the cytosolic surface of the plasmamembrane, and reduce O_2 to $O_2^{\bullet}$ at the outer surface (Sagi and Fluhr 2006). H_2O_2 is produced indirectly by spontaneous or SOD-mediated dismutation. Plant NAD(P)H oxidases were first identified in 1987 in purified plasmamembrane fractions from cauliflower (Askerlund *et al*. 1987) and the activity was attributed to a membrane bound peroxidase. *Trans*-plasmamembrane electron transport and NAD(P)H dehydrogenase activity were subsequently identified, not associated with peroxidases (Misra 1991; Serrano *et al*. 1994), and insensitive to POX inhibitors but sensitive to DPI, also an inhibitor of neutrophil NADPH oxidase (Murphy and Auh 1996). However, at least some enzymes with these characteristics were found to lack flavin cofactors, suggesting that they were mechanistically different from the mammalian enzyme (Murphy *et al*. 2000). In some cases, e.g. cultured soybean cells, NADH can be oxidized on either side of the plasmamembrane (de Hahn *et al*. 1997). Given that NADH has not been found in the apoplast, an alternative function has been suggested for this enzyme in protein disulfide-thiol interchange (Chueh *et al*. 1997; de Hahn *et al*. 1997). Note, too, that DPI inhibition is far from diagnostic of NAD(P)H oxidases: it also inhibits mitochondrial NADH-ubiquinone reductase, NO synthase, xanthine oxidase, and cytochrome P-450 reductase due to phenylation of a flavin co-factor or a haem (in the case of cytochrome P-450), during enzyme turnover (O'Donnell *et al*. 1993).

Plant homologs to neutrophyll NADPH oxidase make up a gene family identified as respiratory burst oxidase homologs (rboh) of which there are 10 members in *Arabidopsis*. All have significant similarity to one subunit [*gp91(phox)*] of the neutrophil enzyme (Keller *et al*. 1998), but plant transcripts are larger and have a hydrophilic N-terminal domain with binding sites suggesting Ca, and G protein stimulation of $O_2^{\bullet}$ production. Also unlike the mammalian enzymes, plant forms are not glycosylated. Different rboh family members are constitutively expressed or inducible, and expressed throughout the plant or limited to specific tissues. *AtrbohA*, for example, is constitutively expressed and largely restricted to roots, *atrbohD* is involved in ROS production during the hypersensitive response to pathogens, and *atrbohF* is associated with control of programmed cell death (Torres *et al*. 2002). In tobacco (*N. benthamiana*), *nbrbohA*, is expressed constitutively at a low level in leaves, but mere infiltration with buffer increases its expression. *NbrbohB*, on the other hand, was specifically induced by a protein elicitor from *Phytophthora infestans*. Based on virus-induced gene silencing, both are involved in programmed cell death responses (Yoshioka *et al*. 2003). A similar pattern was demonstrated in potato tubers (*strobhA* and *strobhB*) where the proteins were localized to the plasmamembrane by immunolocalization and the $O_2^{\bullet}$-generating capacity (sensitivity to DPI, but not azide) was shown (Kobayashi *et al*. 2006). Specificity of function of the enzymes in the absence of pathogen attack is suggested by expression of different homologues in the mesophyll, epidermis and guard cells in leaves and their association with darkness- and ABA-induced stomatal closure (Desikan *et al*. 2004).

The activity of rboh proteins as integrating agents between ROS production and plant responses to stress is suggested by evidence linking them to Ca-dependent signaling associated with such diverse activities as root hair growth (Preuss *et al*. 2004; Shin *et al*. 2005; Carol and Dolan 2006), abscisic acid (ABA) induced Ca-channel activation in guard cells (Kwak *et al*. 2003; Desikan *et al*. 2004; Bright *et al*. 2006; Desikan *et al*. 2006), and activation of a mitogen-activated protein kinase (MAPK) cascade (Desikan *et al*. 1999; Hancock *et al*. 2001; Mittler *et al*. 2004; Zhang *et al*. 2006). Recently, extracellular ATP, which unlike NAD(P)H has been demonstrated to occur, has been added to the list of agents interacting with the proteins as well as stimulating their expression (Song *et al*. 2006). The involvement of NAD(P)H oxidases in H_2O_2 production associated with lignification and the HR was concluded for cells in the xylem of *Zinnia elegans* based on sensitivity to a variety of NADPH oxidase inhibitors (Ros Barceló 1999). Interestingly, ROS, particularly $O_2^{\bullet}$, are also required for wall loosening and leaf extension (Rodriguez *et al*. 2002; Liszkay *et al*. 2004), and root elongation (Renew *et al*. 2005).

In some, but not all species, NAD(P)H oxidase has been implicated in responses to drought and other abiotic stresses. For example, in maize leaves subjected to a sudden stress by floating them on PEG, H_2O_2 production increased transiently by about 50% over a period of 2 hr (Jiang and Zhang 2002), preceded by an increase in ABA concentration and followed by increased activities of antioxidant enzymes. Pretreatment with non-enzymatic ROS scavengers or DPI prevented the increase, as did suppression of ABA accumulation with tungstate.

Others and unknowns

In addition to the major enzymatic sources of H_2O_2 discussed above, there are many physiologically interesting, interspecific differences, even differences between accessions of a single species, which are poorly characterized and which might shed light on the ecological breadth of H_2O_2 involvement in stress responses if they were pursued. A few examples will illustrate this: when the response to 100 mM NaCl stress was compared under controlled conditions in *Lycopersicon esculentum* and its wild relative, *L. pennellii*, the changes were substantially opposite (Mittova *et al*. 2003). *L. esculentum* showed oxidative stress in the form of increased lipid peroxidation and H_2O_2 levels while *L. pennellii* did not. The levels of antioxidant enzymes remained the same or decreased in the domesticated species, but increased in the wild relative. Or, consider the coastal halophyte, *Cakile maritime*, which is also adapted to oligotrophic conditions. Ben Amor *et al*. (2006) studied two accessions which, although their growth was different under non-saline conditions, showed no differences at salinities ranging from 100 to 400 mM NaCl. On the other hand, all measures of oxidant/antioxidant activity, from accumulation of ascorbate and H_2O_2, to lipid peroxidation and electrolyte leakage, to activities of antioxidant enzymes, differed significantly and substantially with increasing sal-

inity: in one accession, the damage related measures were unaffected by salinity while levels of antioxidants and enzyme activities increased. In the other, enzyme activities changed little while H_2O_2, electrolyte leakage and lipid peroxidation increased.

Drought tolerance is another area where more information is needed to understand the enzymatic basis for ROS generation and oxidative stress. Drought tolerance has been reported to be directly correlated with increases in antioxidant enzymes and inversely correlated with levels of lipid peroxidation and H_2O_2 accumulation (Zlatev *et al*. 2006). The potential complexity of the oxidant sources was also indicated in tomato and *Arabidopsis* under water stress (Yesbergenova *et al*. 2005), involving xanthine dehydrogenase ($O_2^{\bullet}$) and ascorbate oxidase (H_2O_2), neither of which was associated with the pathogen-induced HR. H_2O_2 production was insensitive to DPI and both ROS production and transcript levels for the two enzymes were upregulated by ABA and water stress. Interactions of water stress and factors such as mycorrhizal infection and the enhancement of tolerance associated with it (Gafur *et al*. 2004; Fester and Hause 2005; Wu *et al*. 2006) also deserve further consideration, if possible under field conditions.

HOW MOBILE IS H_2O_2? WHERE IS IT?

Although it was previously hypothesized that H_2O_2 produced intracellularly diffuses to other cells for use by POX and other defensive enzymes (Takahama and Oniki 1997; Yamasaki *et al*. 1997), it now appears more probable that intracellularly produced H_2O_2 is consumed quickly and locally, and that extracellular metabolism uses H_2O_2 produced extracellularly (Bestwick *et al*. 1998). Transmembrane movements of H_2O_2 (e.g. from the apoplast to the cytosol) probably involve controlled passage through aquaporins (Bienert *et al*. 2007). Expressed in yeast, for example, two *Arabidopsis* aquaporins decreased growth and survival when cells were challenged with H_2O_2, and blocking the channels reversed the effect. The effect also interacts with other stresses: low temperature exposure of cucumber roots led to extracellular accumulation of H_2O_2 in the millimolar range and reduced hydraulic conductivity (Lee *et al*. 2004), consistent with subsequent reports on applied H_2O_2 effects (Ye and Steudle 2006). On the other hand, at lower levels of H_2O_2, transport through aquaporins may be important in eliciting responses intracellularly, such as those reported by Allan and Fluhr (1997). This may be essential to H_2O_2-dependent signaling and in toxicity of extracellular oxidants (de Marco and Roubelakis-Angelakis 1996; Bestwick *et al*. 1997; Pellinen *et al*. 1999).

H_2O_2 diffusion not involving membrane transit is also restricted to short distances, although much longer than movements of other ROS which are even more restricted by their greater reactivity. H_2O_2 localization within tissues, sometimes to portions of cell walls in root hairs (Carol and Dolan 2006), or in epidermal cells in association with wounding or stomatal movements (Allan and Fluhr 1997), indicates the extent to which plants control their internal environments, as does compartmentation at the tissue level within leaves (Doulis *et al*. 1997; Pastori *et al*. 2000), in vascular tissues (Ogawa *et al*. 1997; Moller and McPherson 1998), and in areas of regeneration (rhizogenesis) (Neves *et al*. 1998). As exemplified by comparative ozone studies, resistance may be determined by the extent to which H_2O_2 can be kept, first, out of cells, and then, out of chloroplasts (Pellinen *et al*. 1999; Oksanen *et al*. 2004). Localization with respect to lignification and xylem differentiation in "normal" growth and development has been particularly well established (Olson and Varner 1993; Richardson *et al*. 1997; Ros Barceló 1998; Repka 1999; Ros Barceló 1999; Paschalidis and Roubelakis-Angelakis 2005; Ros Barceló 2005), although the actual mechanism of H_2O_2 production at the cell surface remains unclear; this reflects, in part, differences between species or even within single plants responding to different environmental stimuli (Ros Barceló and Ferrer 1999).

Using tissue printing techniques in a variety of plants, Schopfer (1994) and Olson and Varner (1993) demonstrated longitudinal and radial gradients during hypocotyl growth, response to ethylene, association with lignification, with light mediated inhibition of elongation and with wounding, while Neves (1998) documented a progression of tissue level changes in H_2O_2 localization during auxin-induced rooting of grapevine cuttings. K-deprivation led to ROS accumulation in regions of *Arabidopsis* roots which were active in K uptake and transport, and this accumulation was suppressed, independent of the induction of high affinity transporters, by mutation of the *atrbohC* NADPH oxidase gene (Shin and Schachtman 2004). Interestingly, the application of H_2O_2 induced those transporters even under K-sufficient conditions. The same authors examined the role of ROS in *Arabidopsis* root hair mutants in response to N and P deprivation (Shin *et al*. 2005). The patterns of increased ROS production indicated that root hairs were important in N and K responses, but that P responses were localized in cortical layers. Even with respect to bacterial attack, H_2O_2 production can be highly localized to bacterial attachment sites in cell walls (Bestwick *et al*. 1998).

SIGNALING AND RESPONSE NETWORKS

The production of H_2O_2 is seldom if ever the end of the story; frequently, if not always, it is associated with additional responses and plays a major role in signaling. There is much better understanding of the fact that H_2O_2 is involved in signaling and for some of the intricacies of downstream processing than there is for what the meaning of the signal is, how a plant decides what the threat is, or how a decoded signal is interpreted for a particular stress or other metabolic need. In part this is due to uncertainty about what actual concentrations of H_2O_2 are in tissues; as noted above, a reported range of seven orders of magnitude is really too great to fit into any model. In part it is also due to the cellular and tissue-level, spatial restrictions of some responses. And in many ways, considering the concentration of H_2O_2 in tissue and its relationship to stress or defense is analogous to the situation with calcium. Bulk calcium levels in leaf tissues are much higher than could be tolerated intracellularly and indicate nothing about its activity in signaling and metabolic control. Some of the most exciting advances in integrative plant biology in recent years have been directed at understanding sensing, signaling and response networks, and it is appropriate to conclude this review with a brief consideration of those results. In the end, however, there are many similarities in the response networks to different environmental stimuli and developmental states, crosstalk between them seems certain, and understanding how plants avert problems inherent with that complexity remains a daunting but exciting challenge.

Sensing H_2O_2

If, for now, we accept the "normal" tissue level concentrations of H_2O_2 in, for example, leaves, to be between about 0.1 and 5 μmol/gFW (Veljovic-Jovanovic *et al*. 2002; Cheeseman 2006), that H_2O_2 turnover is rapid, and that it and other ROS have a number of important roles in development and physiology in the absence of stress, then sensing is clearly a complex problem. Instead of simple presence or absence, cells would need to be able to sense change, perhaps even qualitative change (e.g. Spiro *et al*. 1998). Chandra and Low (1995) presented one model for this, involving protein phosphorylation. They reported that kinase inhibitors blocked the oxidative burst in cultured soybean cells, and if added once the burst were underway, terminated it. Phosphatase inhibitors, on the other hand, stimulated it in the absence of other stimuli. They concluded that the kinases involved may be constitutively active and that the burst was signaled when their phosphorylated

forms were stabilized. On the other hand, Hancock *et al*. (2006) recently noted that the small size of the H_2O_2 molecule made it unlikely that there would be specific receptor proteins involved in its sensing. They presented an alternative suggestion that ROS perception in general was moderated by proteins with other roles, but sharing the characteristic of having active thiol groups as redox targets. That is, sensing was a result of oxidation of the thiol groups by H_2O_2 and other ROS, including NO, perhaps even involving competition of the two types of oxidants for the same modification sites (see also Foyer *et al*. 1997; Neill *et al*. 2002). One example of this would be the histidine kinase receptor, ETR1, essential for sensing H_2O_2 leading to stomatal closure (Desikan *et al*. 2005). Interestingly, the kinase domain itself was not required for this, but a single cysteine (Cys65) was. Kolbe *et al*. (2006) have presented results using both genomic and proteomic analysis to support this model.

Yet another integrating hypothesis has come from analysis of heat shock responses, involving interactions between H_2O_2 and heat shock promoter elements as sensors (Volkov *et al*. 2006). Miller and Mittler (2006) have argued that heat shock transcription factors are the molecular sensors of ROS, and that their complexity, flexibility and specialization allow them to control the expression of a wide range of stress response genes, not only those involving heat shock.

Signaling

The connection between H_2O_2 and signaling networks has been extensively documented for a number of stress responses, including to pathogen elicitors, insect feeding, wounding, high temperature and ABA associated stomatal closure (Larkindale and Knight 2002; Apel and Hirt 2004; Peng *et al*. 2004; Mateo *et al*. 2006). These share many common features, including the relationship between H_2O_2 and Ca, and rather than attempt to review each of them here, my approach will be to focus first on that link, then on initial aspects of the response cascade, and finally on some aspects of the problem which pose the greatest challenges and opportunities.

The link between H_2O_2, Ca, and stomatal closure was clearly established by Pei *et al*. (2000) using patch clamp techniques, showing that the well-established signaling cascade connecting ABA to stomatal closure runs through H_2O_2 and is mediated by calcium channels. Using fluorescent Ca-sensitive dye, the Ca current was shown to lead to increased Ca_{cyt}. The sensitivity of ABA-induced stomatal closure to DPI suggested that the increased Ca_{cyt} stimulated NAD(P)H oxidase activity, leading to extracellular release of $O_2^{\bullet}$, followed by dismutation to H_2O_2. Subsequently, Chen and Gallie (2004) showed that stomatal responsiveness reflects the internal redox state of the guard cells and diurnal variations in ascorbate levels and H_2O_2 production.

Response networks

In the last five years, this response network has been repeatedly extended and summarized, not only for stomatal responses but for responses to other biotic and abiotic stresses, and the signaling cascades have been shown to have many similarities (e.g. Desikan *et al*. 1999; Taylor *et al*. 2001; Mittler *et al*. 2004; Baier *et al*. 2005; Kalbina and Strid 2006; Kotchoni and Gachomo 2006; Mishra *et al*. 2006; Suzuki and Mittler 2006; Zhang *et al*. 2006). Commonalities include both early steps involving Ca or phosphatidic acid activated serine/threonine protein kinase (OXI1), a mitogen activated protein kinase (MAPK3/6) cascade, and downstream transcription factors which influence both transcription of scavenging enzymes and NAD(P)H oxidase. As a result, the initial response to H_2O_2 can both reduce and amplify the oxidative signal, allowing graded or controlled response to particular elicitation events (Suzuki and Mittler 2006).

The availability of much-of-the-genome microarrays, especially for *Arabidopsis*, has led to even greater extension of this response network. In 2004, Mittler *et al*. (2004) annotated 152 genes involved in ROS control in *Arabidopsis*, while Hancock *et al*. (2006) expanded this to at least 400. The network is both redundant and dynamic, as should be expected because of the involvement of ROS in development (which is cell and tissue specific), metabolism and defense, and the need to maintain a steady-state on which critical signals can be registered. Even more complex and complete models, including analysis of gene response clusters, has been possible with shared *Arabidopsis* microarray result databases (e.g. http://www.arabidopsis.org/info/expression/ATGenExpress.jsp) (Ma *et al*. 2006; Schreiber and Baumann 2007).

Although relevant studies have included numerous experimental conditions, ozone responses provide a convenient illustration both of the extent of the networks and their dependence on the conditions of the experiments (e.g. Olbrich *et al*. 2005; D'Haese *et al*. 2006; Lee and Yun 2006; Li *et al*. 2006a; Tosti *et al*. 2006). Ozone toxicity has been recognized for more than 50 years, but neither the mechanism of action nor the response of organisms at the molecular level is well understood (Rao *et al*. 2000a). An early role was recognized for ascorbic acid in ozone detoxification (Tanaka *et al*. 1985), and mathematical modeling (Chameldes 1989) as well as studies using ascorbate deficient or enhanced mutants suggested that it could account for a substantial portion of ozone defense (Conklin *et al*. 1996; Chen and Gallie 2005). This model has not, however, gone unchallenged (D'Haese *et al*. 2005).

At the molecular level, initial studies of the response network showed that it had extensive similarities to the networks for other oxidative stresses (Baier *et al*. 2005). A recent study by Li *et al*. (2006a), however, illustrates the extent of differences possible even within one species and with close relatives of differing overall stress tolerance. These authors used microarrays to study the response of *Arabidopsis* and its stress-tolerant relative, *Thellungiella halophila* to ozone. Uniquely, they performed the study in the field. Ozone was manipulated using free air concentration enrichment (FACE) conditions, comparing plants at ambient levels and at a mere 1.2-times ambient (dynamically adjusted). Three *Arabidopsis* ecotypes were included: Columbia-0 (Col-0), Cape Verde Islands (Cvi-0), and Wassilewskija (WS). Even within this small range of genotypes, the responses at both the physiological and transcription levels were significantly different. The number of genes responding (up- or down-regulation) to elevated ozone ranged from 320 in *Thellungiella* to more than 2900 in WS. Among *Arabidopsis* ecotypes, WS was also the most affected, i.e. showed the greatest actual damage to leaves, and transcriptome responses included photosynthetic light reaction genes, genes in the phenylpropanoid pathway, ROS scavenging, photorespiration and the reductive pentose phosphate pathway, and hormone biosynthesis and response (ethylene, jasmonic acid and salicylic acid). Interestingly, but underscoring the importance of conducting field studies rather than relying on highly contrived controlled conditions, the relative ozone resistance of the *Arabidopsis* genotypes was reversed under FACE conditions to what it had been reported with acute exposure (see Rao *et al*. 2000b). The authors emphasized, in addition, the dependence of the results on local weather conditions, time of growth and harvesting, and potential biotic stresses during the experiment. The "take home lesson" from this is that any "definitive" model of a stress response should be accepted very cautiously.

Complex systems modeling and proteomics

Returning to stomatal responses – this phenomenon provides the basis for models extending in a different direction. Stomatal closure can mediated by external H_2O_2 directly, without prior activation of the NAD(P)H oxidase or ABA;

it happens without preceding changes in gene expression. In this case, the H_2O_2 can be produced by apoplastic POX or amine oxidases (Allan and Fluhr 1997; Kawano *et al*. 2000), the former being stimulated by SA, and involving $SA^{\bullet}$ as an intermediate (Kawano *et al*. 1998; Kawano and Muto 2000; Mori *et al*. 2001). Alternately, the Ca-dependent changes can be mediated by other extracellular elicitors such as oligogalactouronic acid (Hu *et al*. 2004). As a generalization, whether H_2O_2 production leads or follows the increase in Ca_{cyt} depends on the signal. In the case of ABA, for example, H_2O_2 follows (Pei *et al*. 2000). In the case of extracellular elicitation, the bulk of the H_2O_2 production leads (Hu *et al*. 2004) followed by other responses dependent on the type of insult (Orozco-Cárdenas *et al*. 2001; Miles *et al*. 2002; Mur *et al*. 2005). In sum, it includes activities and responses, simultaneously, of the proteome, the metabolome and the transcriptome.

Li *et al*. (2006b) combined more than 40 pathway and physiological components known to be involved in stomatal responses into a single, dynamic model using a complex systems approach. It allowed simulation of stimulus and response, as well as inhibitor effects and gene disruptions. In addition, individual components of physiological pathways could be manipulated – ion fluxes, electrophysiological parameters, signaling cascades or cellular characteristics – and the results were very largely in keeping with experimental observations. This type of modeling essentially says, "if we know what we think we know, then we ought to be able to predict responses to manipulations, or even design novel, new experiments." Perhaps most significant is the fact that this type of complex systems modeling allows quantitative simulation based on limited quantitative background information. It allows, for example, "manipulation" of the apoplastic proteome and metabolome, otherwise poorly understood, to generate testable predictions.

Direct proteome analysis is much more difficult than transcriptome analysis because of the greater difficulty of protein isolation and sequencing, and the difficulty of extracting the apoplast without contamination by other compartments (Watson *et al*. 2004; Zhu *et al*. 2006), although the tools are developing rapidly. The promise of wall proteome studies was shown by analysis of tobacco leaves and their response to salt stress (Dani *et al*. 2005). Using two-dimensional electrophoresis of apoplastic fluids, they identified 150 polypeptide spots, 20 of which changed in abundance with salt stress, but other than identification of one germin-like protein, the results were still somewhat disappointing. That the activity of apoplastic enzymes can be influenced by biotically and abiotically-induced oxidative stress (Diaz-Vivancos *et al*. 2006), and be modified by stress-related hormones, e.g. methyl jasmonate (Maksymiec and Krupa 2002), is clear. As importantly, some apoplastic enzymes are well known for their stability (e.g. peroxidases), which means a comparative proteomic analysis would not identify them as responding. Moreover, the correlation between enzyme activity and expression of the associated mRNAs is, in other cases, demonstrably poor (e.g. DAO - Angelini *et al*. 1996).

While these problems can be addressed at one level using the systems modeling approach, experimentally, as was the case for many of the other responses discussed in this review, cell cultures have the advantage of simplicity that can provide initial models if not appropriately represent the responses of intact organisms. Chivasa *et al*. (2005), for example, used maize cell cultures to elucidate responses of the wall proteome to pathogen elicitors. The responses included changes in phosphorylation status (extracellular peroxidases), disappearance of some proteins (e.g. a putative extracellular β-N-acetylglucosamonidase), and accumulation of others (a secreted putative xylanase inhibitor), and appearance of some classically cytosolic proteins (e.g. glyceraldehyde-3-phosphate dehydrogenase).

CONCLUSION

Understanding the integrated responses of plants to their environment throughout their life cycles which enable them to acquire and allocate resources, grow, and reproduce in the face of serious and dynamic environmental constraints is as challenging now as it has been throughout the history of plant biology. In this review, I have focused on one metabolite which is both a constraint and an essential element of physiology, and the tools plants have at their disposal to deal with it. While it would be naïve to think that recent advances in analytical techniques, standardized experimental systems and modeling put us on the threshold of fully understanding the role of H_2O_2 in plant metabolism, it is certain that they will open new levels of exciting uncertainty.

REFERENCES

Akazawa T, Conn EE (1958) Oxidation of reduced pyridine nucleotides by peroxidase. *The Journal of Biological Chemistry* **232**, 403-415

Allan AC, Fluhr R (1997) Two distinct sources of elicited reactive oxygen species in tobacco epidermal cells. *Plant Cell* **9**, 1559-1572

Allen RD (1995) Dissection of oxidative stress tolerance using transgenic plants. *Plant Physiology* **107**, 1049-1054

Alscher R, Donahue J, Cramer CL (1997) Reactive oxygen species and antioxidants: Relationships in green cells. *Physiologia Plantarum* **100**, 224-233

Amory AM, Ford L, Pammenter NW, Cresswell CF (1992) The use of 3-amino-1,2,4-triazole to investigate the short-term effects of oxygen toxicity on carbon assimilation by *Pisum sativum* seedlings. *Plant, Cell and Environment* **15**, 655-663

Andrade RG, Ginani JS, Lopes GKB, Dutra F, Alonso A, Hermes-Lima M (2006) Tannic acid inhibits *in vitro* iron-dependent free radical formation. *Biochimie* **88**, 1287-1296

Angelini R, Rea G, Federico R, Dovidio R (1996) Spatial distribution and temporal accumulation of mRNA encoding diamine oxidase during lentil (*Lens culinaris* Medicus) seedling development. *Plant Science* **119**, 103-113

Apel K, Hirt H (2004) Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology* **55**, 373-399

Asada K (1999) The water-water cycle in chloroplasts: Scavenging of active oxygen and dissipation of excess photons. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 601-639

Askerlund P, Larsson C, Widell S, Moller IM (1987) NAD(P)H oxidase and peroxidase activities in purified plasma membranes from cauliflower inflorescences. *Physiologia Plantarum* **71**, 9-19

Asthir B, Spoor W, Duffus CM (2004) Involvement of polyamines, diamine oxidase and polyamine oxidase in resistance of barley to *Blumeria graminis* f. sp *hordei*. *Euphytica* **136**, 307-312

Baier M, Kandlbinder A, Golldack D, Dietz KJ (2005) Oxidative stress and ozone: perception, signalling and response. *Plant Cell and Environment* **28**, 1012-1020

Baxter CJ, Redestig H, Schauer N, Repsilber D, Patil KR, Nielsen J, Selbig J, Liu JL, Fernie AR, Sweetlove LJ (2007) The metabolic response of heterotrophic *Arabidopsis* cells to oxidative stress. *Plant Physiology* **143**, 312-325

Becana M, Moran JF, Iturbe-Ormaetxe I (1998) Iron-dependent oxygen free radical generation in plants subjected to environmental stress: toxicity and antioxidant protection. *Plant and Soil* **201**, 137-147

Ben Amor N, Jimenez A, Megdiche W, Lundqvist M, Sevilla F, Abdelly C (2006) Response of antioxidant systems to NaCl stress in the halophyte *Cakile maritima*. *Physiologia Plantarum* **126**, 446-457

Bernier F, Berna A (2001) Germins and germin-like proteins: Plant do-all proteins. But what do they do exactly? *Plant Physiology and Biochemistry* **39**, 545-554

Bestwick C, Brown I, Bennett M, Mansfield J (1997) Localization of hydrogen peroxide accumulation during the hypersensitive reaction of lettuce cells to *Pseudomonas syringae* pv *phaseolicola*. *Plant Cell* **9**, 209-221

Bestwick CS, Brown IR, Mansfield JW (1998) Localized changes in peroxidase activity accompany hydrogen peroxide generation during the development of a nonhost hypersensitive reaction in lettuce. *Plant Physiology* **118**, 1067-1078

Bhattacharya J, Ghosh Dastidar K, Chatterjee A, Majee M, Majumder AL (2004) *Synechocystis* Fe superoxide dismutase gene confers oxidative stress tolerance to *Escherichia coli*. *Biochemical and Biophysical Research Communications* **316**, 540-544

Bienert GP, Moller ALB, Kristiansen KA, Schulz A, Moller IM, Schjoerring JK, Jahn TP (2007) Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. *The Journal of Biological Chemistry* **282**, 1183-1192

Bindschedler LV, Dewdney J, Blee KA, Stone JM, Asai T, Plotnikov J, Denoux C, Hayes T, Gerrish C, Davies DR, Ausubel FM, Bolwell GP (2006) Peroxidase-dependent apoplastic oxidative burst in *Arabidopsis* required for

pathogen resistance. *Plant Journal* **47**, 851-863

Bolwell GP (1999) Role of active oxygen species and NO in plant defence responses. *Current Opinion in Plant Biology* **2**, 287-294

Bolwell GP, Bindschedler LV, Blee KA, Butt VS, Davies DR, Gardner SL, Gerrish C, Minibayeva F (2002) The apoplastic oxidative burst in response to biotic stress in plants: a three-component system. *Journal of Experimental Botany* **53**, 1367-1376

Bolwell GP, Davies DR, Gerrish C, Auh CK, Murphy TM (1998) Comparative biochemistry of the oxidative burst produced by rose and French bean cells reveals two distinct mechanisms. *Plant Physiology* **116**, 1379-1385

Braidot E, Petrussa E, Vianello A, Macri F (1999) Hydrogen peroxide generation by higher plant mitochondria oxidizing complex I or complex II substrates. *FEBS Letters* **451**, 347-350

Bright J, Desikan R, Hancock JT, Weir IS, Neill SJ (2006) ABA-induced NO generation and stomatal closure in *Arabidopsis* are dependent on H_2O_2 synthesis. *The Plant Journal* **45**, 113-122

Brisson L, Tenhaken R, Lamb C (1994) Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. *Plant Cell* **6**, 1703-1712

Brodie AE, Reed DJ (1987) Reversible oxidation of glyceraldehyde-3-phosphate dehydrogenase thiols in human-lung carcinoma-cells by hydrogen-peroxide. *Biochemical and Biophysical Research Communications* **148**, 120-125

Cakmak I (1994) Activity of ascorbate-dependent H_2O_2-scavenging enzymes and leaf chlorosis are enhanced in magnesium- and potassium-deficient leaves, but not in phosphorus-deficient leaves. *Journal of Experimental Botany* **45**, 1259-1266

Caliskan M, Turet M, Cuming AC (2004) Formation of wheat (*Triticum aestivum* L.) embryogenic callus involves peroxide-generating germin-like oxalate oxidase. *Planta* **219**, 132-140

Carol R, Dolan L (2006) The role of reactive oxygen species in cell growth: lessons from root hairs. *Journal of Experimental Botany* **57**, 1829-1834

Casolo V, Braidot E, Chiandussi E, Macri F, Vianello A (2000) The role of mild uncoupling and non-coupled respiration in the regulation of hydrogen peroxide generation by plant mitochondria. *FEBS Letters* **474**, 53-57

Cessna SG, Sears VE, Dickman MB, Low PS (2000) Oxalic acid, a pathogenicity factor for *Sclerotinia sclerotiorum*, suppresses the oxidative burst of the host plant. *Plant Cell* **12**, 2191-2199

Chameldes W (1989) The chemistry of ozone deposition in plant leaves: role of ascorbic acid. *Environmental Science and Technology* **23**, 595-600

Chandra S, Low PS (1995) Role of phosphorylation in elicitation of the oxidative burst in cultured soybean cells. *Proceedings of the National Academy of Sciences USA* **92**, 4120-4123

Charles SA, Halliwell B (1980) Effect of hydrogen peroxide on spinach (*Spinacia oleracea*) chloroplast fructose bisphosphatase. *Biochemical Journal* **189**, 373-376

Charles SA, Halliwell B (1981) Light activation of fructose bisphosphatase in isolated spinach chloroplasts and deactivation by hydrogen peroxide - a physiological role for the thioredoxin system. *Planta* **151**, 242-246

Cheeseman JM (2006) Hydrogen peroxide concentrations in leaves under natural conditions. *Journal of Experiment Botany* **57**, 2435-2444

Chen Z, Gallie D (2004) The ascorbic acid redox state controls guard cell signaling and stomatal movement. *Plant Cell* **16**, 1143-1162

Chen Z, Gallie D (2005) Increasing tolerance to ozone by elevating foliar ascorbic acid confers greater protection against ozone than increasing avoidance. *Plant Physiology* **138**, 1673-1689

Chivasa S, Simon WJ, Yu XL, Yalpani N, Slabas AR (2005) Pathogen elicitor-induced changes in the maize extracellular matrix proteome. *Proteomics* **5**, 4894-4904

Chueh PJ, Morre DM, Penel C, de Hahn T, Morré DJ (1997) The hormone-responsive NADH oxidase of the plant plasma membrane has properties of a NADH:protein disulfide reductase. *The Journal of Biological Chemistry* **272**, 11221-11227

Cober ER, Rioux S, Rajcan I, Donaldson PA, Simmonds DH (2003) Partial resistance to white mold in a transgenic soybean line. *Crop Science* **43**, 92-95

Cona A, Moreno S, Cenci F, Federico R, Angelini R (2005) Cellular re-distribution of flavin-containing polyamine oxidase in differentiating root and mesocotyl of *Zea mays* L. seedlings. *Planta* **221**, 265-276

Cona A, Rea G, Angelini R, Federico R, Tavladoraki P (2006a) Functions of amine oxidases in plant development and defence. *Trends in Plant Science* **11**, 80-88

Cona A, Rea G, Botta M, Corelli F, Federico R, Angelini R (2006b) Flavin-containing polyamine oxidase is a hydrogen peroxide source in the oxidative response to the protein phosphatase inhibitor cantharidin in *Zea mays* L. *Journal of Experimental Botany* **57**, 2277-2289

Conklin PL, Williams EH, Last RL (1996) Environmental stress sensitivity of an ascorbic acid-deficient *Arabidopsis* mutant. *Proceedings of the National Academy of Sciences USA* **93**, 9970-9974

D'Haese D, Horemans N, De Coen W, Guisez Y (2006) Identification of late O_3-responsive genes in *Arabidopsis thaliana* by cDNA microarray analysis. *Physiologia Plantarum* **128**, 70-79

D'Haese D, Vandermeiren K, Asard H, Horemans N (2005) Other factors than apoplastic ascorbate contribute to the differential ozone tolerance of two clones of *Trifolium repens* L. *Plant Cell and Environment* **28**, 623-632

Dani V, Simon WJ, Duranti M, Croy RRD (2005) Changes in the tobacco leaf apoplast proteome in response to salt stress. *Proteomics* **5**, 737-745

Davies DR, Bindschedler LV, Strickland TS, Bolwell GP (2006) Production of reactive oxygen species in *Arabidopsis thaliana* cell suspension cultures in response to an elicitor from *Fusarium oxysporum*: implications for basal resistance. *Journal of Experimental Botany* **57**, 1817-1827

de los Reyes BG, McGrath JM (2003) Cultivar-specific seedling vigor and expression of a putative oxalate oxidase germin-like protein in sugar beet (*Beta vulgaris* L.). *Theoretical and Applied Genetics* **107**, 54-61

de Hahn T, Barr R, Morré DJ (1997) NADH oxidase activity present on both the external and internal surfaces of soybean plasma membranes. *Biochimica et Biophysica Acta* **1328**, 99-108

Delrio LA, Sandalio LM, Palma JM, Bueno P, Corpas FJ (1992) Metabolism of oxygen radicals in peroxisomes and cellular implications. *Free Radical Biology and Medicine* **13**, 557-580

de Marco A, Roubelakis-Angelakis KA (1996) The complexity of enzymic control of hydrogen peroxide concentration may affect the regeneration potential of plant protoplasts. *Plant Physiology* **110**, 137-145

Demmig-Adams B, Adams WWI, Ebbert V, Logan BA (1999) Ecophysiology of the xanthophyll cycle. In: Frank H, Young A, Cogdell R (Eds) *The Photochemistry of Carotenoids*, Kluwer Academic, Dordrecht, pp 245-269

Desikan R, Cheung MK, Clarke A, Golding S, Sagi M, Fluhr R, Rock C, Hancock J, Neill S (2004) Hydrogen peroxide is a common signal for darkness- and ABA-induced stomatal closure in *Pisum sativum*. *Functional Plant Biology* **31**, 913-920

Desikan R, Clarke A, Hancock JT, Neill SJ (1999) H_2O_2 activates a MAP kinase-like enzyme in *Arabidopsis thaliana* suspension cultures. *Journal of Experimental Botany* **50**, 1863-1866

Desikan R, Hancock JT, Bright J, Harrison J, Weir L, Hooley R, Neill SJ (2005) A role for ETR1 in hydrogen peroxide signaling in stomatal guard cells. *Plant Physiology* **137**, 831-834

Desikan R, Last K, Harrett-Williams R, Tagliavia C, Harter K, Hooley R, Hancock JT, Neill SJ (2006) Ethylene-induced stomatal closure in *Arabidopsis* occurs via AtrbohF-mediated hydrogen peroxide synthesis. *Plant Journal* **47**, 907-916

Diaz-Vivancos P, Rubio M, Mesonero V, Periago PM, Ros Barceló A, Martínez-Gómez P, Hernández JA (2006) The apoplastic antioxidant system in *Prunus*: response to long-term plum pox virus infection. *Journal of Experimental Botany* **57**, 3813-3824

Doulis AG, Debian N, Kingston Smith AH, Foyer CH (1997) Differential localization of antioxidants in maize leaves. *Plant Physiology* **114**, 1031-1037

Dutilleul C, Garmier M, Noctor G, Mathieu C, Chetrit P, Foyer CH, de Paepe R (2003) Leaf mitochondria modulate whole cell redox homeostasis, set antioxidant capacity, and determine stress resistance through altered signaling and diurnal regulation. *Plant Cell* **15**, 1212-1226

Eigen M (1973) The origin of biological information. In: Mehra J (Ed) *The Physicist's Conception of Nature*, D. Reidel Publishing Co., Dordrecht, pp 594-632

Farber JM, Levine RL (1986) Sequence of a peptide susceptible to mixed-function oxidation. Probable cation binding site in glutamine synthetase. *The Journal of Biological Chemistry* **261**, 4574-4578

Farrar JF, Rayns FW (1987) Respiration of leaves of barley infected with powdery mildew: Increased engagement of the alternative oxidase. *New Phytologist* **107**, 119-126

Fester T, Hause G (2005) Accumulation of reactive oxygen species in arbuscular mycorrhizal roots. *Mycorrhiza* **15**, 373-379

Foyer CH, Lopez Delgado H, Dat JF, Scott IM (1997) Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. *Physiologia Plantarum* **100**, 241-254

Foyer CH, Noctor G (2003) Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiologia Plantarum* **119**, 355-364

Gafur A, Schutzendubel A, Langenfeld-Heyser R, Fritz E, Polle A (2004) Compatible and incompetent *Paxillus involutus* isolates for ectomycorrhiza formation *in vitro* with poplar (*Populus* × *canescens*) differ in H_2O_2 production. *Plant Biology* **6**, 91-99

Halliwell B, Clement M, Long L (2000) Hydrogen peroxide in the human body. *FEBS Letters* **486**, 10-13

Halliwell B, Gutteridge J (1999) *Free Radicals in Biology and Medicine* (3rd Edn), Oxford University Press, Oxford, 936 pp

Hancock J, Desikan R, Harrison J, Bright J, Hooley R, Neill S (2006) Doing the unexpected: proteins involved in hydrogen peroxide perception. *Journal of Experimental Botany* **57**, 1711-1718

Hancock JT, Desikan R, Neill SJ (2001) Role of reactive oxygen species in cell signalling pathways. *Biochemical Society Transactions* **29**, 345-350

Hancock JT, Henson D, Nyirenda M, Desikan R, Harrison J, Lewis M, Hughes J, Neill SJ (2005) Proteomic identification of glyceraldehyde 3-phosphate dehydrogenase as an inhibitory target of hydrogen peroxide in *Arabidopsis*. *Plant Physiology and Biochemistry* **43**, 828-835

Hansch R, Lang C, Riebeseel E, Lindigkeit R, Gessler A, Rennenberg H, Mendel RR (2006) Plant sulfite oxidase as novel producer of H_2O_2 - Combination of enzyme catalysis with a subsequent non-enzymatic reaction step. *The Journal of Biological Chemistry* **281**, 6884-6888

Haslam E (1985) *Metabolites and Metabolism: A Commentary on Secondary Metabolism*, Clarendon Press, Oxford, 161 pp
Haslam E (1986) Secondary metabolism - fact and fiction. *Natural Product Reports* **4**, 217-249
He YL, Liu YL, Cao WX, Huai MF, Xu BG, Huang BG (2005) Effects of salicylic acid on heat tolerance associated with antioxidant metabolism in Kentucky bluegrass. *Crop Science* **45**, 988-995
Heber U (2002) Irrungen,Wirrungen? The Mehler reaction in relation to cyclic electron transport in C_3 plants. *Photosynthesis Research* **73**, 223-231
Hernández J, Ferrer M, Jiménez A, Ros Barceló A, Sevilla F (2001) Antioxidant systems and $O_2^{\cdot}/H_2O_2$ production in the apoplast of pea leaves. Its relation with salt-induced necrotic lesions in minor veins. *Plant Physiology* **127**, 817-831
Hu X, Bidney DL, Yalpani N, Duvick JP, Crasta O, Folkerts O, Lu GH (2003) Overexpression of a gene encoding hydrogen peroxide-generating oxalate oxidase evokes defense responses in sunflower. *Plant Physiology* **133**, 170-181
Hu X-Y, Neill S-J, Cai W-M, Tang Z-C (2004) Induction of defence gene expression by oligogalacturonic acid requires increases in both cytosolic calcium and hydrogen peroxide in *Arabidopsis thaliana*. *Cell Research* **14**, 234-240
Ishida H, Makino A, Mae T (1999) Fragmentation of the large subunit of ribulose-1,5-bisphosphate carboxylase by reactive oxygen species occurs near Gly-329. *The Journal of Biological Chemistry* **274**, 5222-5226
Jiang M, Zhang J (2002) Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. *Journal of Experimental Botany* **53**, 2401-2410
Kalbina I, Strid A (2006) The role of NADPH oxidase and MAP kinase phosphatase in UV-B-dependent gene expression in *Arabidopsis*. *Plant Cell and Environment* **29**, 1783-1793
Kang KS, Lim CJ, Han TJ, Kim JC, Jin CD (1999) Changes in the isozyme composition of antioxidant enzymes in response to aminotriazole in leaves of *Arabidopsis thaliana*. *Journal of Plant Biology* **42**, 187-193
Karkonen A, Koutaniemi S, Mustonen M, Syrjanen K, Brunow G, Kilpelainen I, Teeri TH, Simola LK (2002) Lignification related enzymes in *Picea abies* suspension cultures. *Physiologia Plantarum* **114**, 343-353
Karpinski S, Escobar C, Karpinska B, Creissen G, Mullineaux PM (1997) Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in *Arabidopsis* during excess light stress. *Plant Cell* **9**, 627-640
Kawano T, Muto S (2000) Mechanism of peroxidase actions for salicylic acid-induced generation of active oxygen species and an increase in cytosolic calcium in tobacco suspension culture. *Journal of Experimental Botany* **51**, 685-693
Kawano T, Pinontoan R, Uozumi N, Morimitsu Y, Miyake C, Asada K, Muto S (2000) Phenylethlamine-induced generation of reactive oxygen species and ascorbate free radicals in tobacco suspension culture: mechanism for oxidative burst mediating Ca^{2+} influx. *Plant and Cell Physiology* **41**, 1259-1266
Kawano T, Sahashi N, Takahashi K, Uozumi N, Muto S (1998) Salicylic acid induces extracellular superoxide generation followed by an increase in cytosolic calcium ion in tobacco suspension culture: The earliest events in salicylic acid signal transduction. *Plant and Cell Physiology* **39**, 721-730
Kay L, Basile D (1987) Specific peroxidase isoenzymes are correlated with organogenesis. *Plant Physiology* **84**, 99-105
Keller T, Damude HG, Werner D, Doerner P, Dixon RA, Lamb C (1998) A plant homolog of the neutrophil NADPH oxidase gp91(phox) subunit gene encodes a plasma membrane protein with Ca^{2+} binding motifs. *Plant Cell* **10**, 255-266
Kingston-Smith AH, Harbinson J, Foyer CH (1999) Acclimation of photosynthesis, H_2O_2 content and antioxidants in maize (*Zea mays*) grown at sub-optimal temperatures. *Plant Cell and Environment* **22**, 1071-1083
Kobayashi M, Kawakita K, Maeshima M, Doke N, Yoshioka H (2006) Subcellular localization of Strboh proteins and NADPH-dependent O_2^--generating activity in potato tuber tissues. *Journal of Experimental Botany* **57**, 1373-1379
Kolbe A, Oliver SN, Fernie AR, Stitt M, van Dongen JT, Geigenberger P (2006) Combined transcript and metabolite profiling of *Arabidopsis* leaves reveals fundamental effects of the thiol-disulfide status on plant metabolism. *Plant Physiology* **141**, 412-422
Kotchoni SO, Gachomo EW (2006) The reactive oxygen species network pathways: an essential prerequisite for perception of pathogen attack and the acquired disease resistance in plants. *Journal of Biosciences* **31**, 389-404
Koutaniemi S, Toikka MM, Karkonen A, Mustonen M, Lundell T, Simola LK, Kilpelainen IA, Teeri TH (2005) Characterization of basic p-coumaryl and coniferyl alcohol oxidizing peroxidases from a lignin-forming *Picea abies* suspension culture. *Plant Molecular Biology* **58**, 141-157
Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JDG, Schroeder JI (2003) NADPH oxidase AtrbohD and AtrbohF genes function in ROS-dependent ABA signaling in Arabidopsis. *The EMBO Journal* **22**, 2623-2633
Langebartels C, Wohlgemuth H, Kschieschan S, Grun S, Sandermann H (2002) Oxidative burst and cell death in ozone-exposed plants. *Plant Physiology and Biochemistry* **40**, 567-575
Larkindale J, Knight M (2002) Protection against heat stress-induced oxidative damage in *Arabidopsis* involves calcium, abscisic acid, ethylene, and salicylic acid. *Plant Physiology* **128**, 682-695
Laurenzi M, Tipping AJ, Marcus SE, Knox JP, Federico R, Angelini R, McPherson MJ (2001) Analysis of the distribution of copper amine oxidase in cell walls of legume seedlings. *Planta* **214**, 37-45
le Deunff E, Davoine C, Le Dantec C, Billard JP, Huault C (2004) Oxidative burst and expression of germin/oxo genes during wounding of ryegrass leaf blades: comparison with senescence of leaf sheaths. *The Plant Journal* **38**, 421-431
Lee S, Yun SC (2006) The ozone stress transcriptome of pepper (*Capsicum annuum* L.). *Molecules and Cells* **21**, 197-205
Lee SH, Singh AP, Chung GC (2004) Rapid accumulation of hydrogen peroxide in cucumber roots due to exposure to low temperature appears to mediate decreases in water transport. *Journal of Experimental Botany* **55**, 1733-1741
Li PH, Sioson A, Mane SP, Ulanov A, Grothaus G, Heath LS, Murali TM, Bohnert HJ, Grene R (2006a) Response diversity of *Arabidopsis thaliana* ecotypes in elevated $[CO_2]$ in the field. *Plant Molecular Biology* **62**, 593-609
Li S, Assmann SM, Albert R (2006b) Predicting essential components of signal transduction networks: A dynamic model of guard cell abscisic acid signaling. *Plos Biology* **4**, 1732-1748
Liszkay A, van der Zalm E, Schopfer P (2004) Production of reactive oxygen intermediates ($O_2^{\cdot}$, H_2O_2, and (OH)-O$^{\cdot}$) by maize roots and their role in wall loosening and elongation growth. *Plant Physiology* **136**, 3114-3123
Liu L, Eriksson KEL, Dean JFD (1995) Localization of hydrogen-peroxide production in *Pisum sativum* L. using epi-polarization microscopy to follow cerium perhydroxide deposition *Plant Physiology* **107**, 501-506
Logan BA, Demmig Adams B, Adams WW, III (1998a) Antioxidants and xanthophyll cycle-dependent energy dissipation in *Cucurbita pepo* L. and *Vinca major* L. upon a sudden increase in growth PPFD in the field. *Journal of Experimental Botany* **49**, 1881-1888
Logan BA, Demmig Adams B, Adams WW III, Grace SC (1998b) Antioxidants and xanthophyll cycle-dependent energy dissipation in *Cucurbita pepo* L. and *Vinca major* L. acclimated to four growth PPFDs in the field. *Journal of Experimental Botany* **49**, 1869-1879
Logan BA, Grace SC, Adams WW III, Demmig Adams B (1998c) Seasonal differences in xanthophyll cycle characteristics and antioxidants in *Mahonia repens* growing in different light environments. *Oecologia* **116**, 9-17
Ma SS, Gong QQ, Bohnert HJ (2006) Dissecting salt stress pathways. *Journal of Experimental Botany* **57**, 1097-1107
Mäder M, Amberg-Fisher V (1982) Role of peroxidase in lignification of tobacco cells. *Plant Physiology* **70**, 1128-1131
Maksymiec W, Krupa Z (2002) The *in vivo* and *in vitro* influence of methyl jasmonate on oxidative processes in *Arabidopsis thaliana* leaves. *Acta Physiologiae Plantarum* **24**, 351-357
Malusa E, Laurenti E, Juszczuk I, Ferrari RP, Rychter AM (2002) Free radical production in roots of *Phaseolus vulgaris* subjected to phosphate deficiency stress. *Plant Physiology and Biochemistry* **40**, 963-967
Martinez C, Montillet JL, Bresson E, Agnel JP, Dai GH, Daniel JF, Geiger JP, Nicole M (1998) Apoplastic peroxidase generates superoxide anions in cells of cotton cotyledons undergoing the hypersensitive reaction to *Xanthomonas campestris* pv. *malvacearum* race 18. *Molecular Plant-Microbe Interactions* **11**, 1038-1047
Mateo A, Funck D, Muhlenbock P, Kular B, Mullineaux PM, Karpinski S (2006) Controlled levels of salicylic acid are required for optimal photosynthesis and redox homeostasis. *Journal of Experimental Botany* **57**, 1795-1807
Maxwell DP, Wang Y, McIntosh L (1999) The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. *Proceedings of the National Academy of Sciences USA* **96**, 8271-8276
Miles GP, Samuel MA, Ellis BE (2002) Suramin inhibits oxidant signalling in tobacco suspension-cultured cells. *Plant Cell and Environment* **25**, 521-527
Miller G, Mittler R (2006) Could heat shock transcription factors function as hydrogen peroxide sensors in plants? *Annals of Botany* **98**, 279-288
Mishra NS, Tuteja R, Tuteja N (2006) Signaling through MAP kinase networks in plants. *Archives of Biochemistry and Biophysics* **452**, 55-68
Misra PC (1991) Transplasma membrane electron transport in plants. *Journal of Bioenergetics and Biomembranes* **23**, 425-441
Mittler R, Vanderauwera S, Gollery M, van Breusegem F (2004) Reactive oxygen gene network of plants. *Trends in Plant Science* **9**, 490-498
Mittova V, Tal M, Volokita M, Guy M (2003) Up-regulation of the leaf mitochondrial and peroxisomal antioxidative systems in response to salt-induced oxidative stress in the wild salt-tolerant tomato species *Lycopersicon pennellii*. *Plant Cell and Environment* **26**, 845-856
Moller IM (2001) Plant mitochondria and oxidative stress: Electron transport, NADPH turnover, and metabolism of reactive oxygen species. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**, 561-591
Moller SG, McPherson MJ (1998) Developmental expression and biochemical analysis of the *Arabidopsis atao1* gene encoding an H_2O_2-generating diamine oxidase. *The Plant Journal* **13**, 781-791
Mori I, Pinontoan R, Kawano T, Muto S (2001) Involvement of superoxide

generation in salicylic acid-induced stomatal closure in *Vicia faba*. *Plant and Cell Physiology* **42**, 1383-1388

Morita S, Kaminaka H, Masumura T, Tanaka K (1999) Induction of rice cytosolic ascorbate peroxidase mRNA by oxidative stress; the involvement of hydrogen peroxide in oxidative stress signalling. *Plant and Cell Physiology* **40**, 417-422

Mur LAJ, Kenton P, Draper J (2005) *In planta* measurements of oxidative bursts elicited by avirulent and virulent bacterial pathogens suggests that H_2O_2 is insufficient to elicit cell death in tobacco. *Plant Cell and Environment* **28**, 548-561

Murphy TM, Auh CK (1996) The superoxide synthases of plasma membrane preparations from cultured rose cells. *Plant Physiology* **110**, 621-629

Murphy TM, Vu H, Nguyen T, Woo CH (2000) Diphenylene iodonium sensitivity of a solubilized membrane enzyme from rose cells. *Protoplasma* **213**, 228-234

Nayyar H, Chander S (2004) Protective effects of polyamines against oxidative stress induced by water and cold stress in chickpea. *Journal of Agronomy and Crop Science* **190**, 355-365

Neill S, Desikan R, Hancock J (2002) Hydrogen peroxide signalling. *Current Opinion in Plant Biology* **5**, 388-395

Neves C, Sa MC, Amancio S (1998) Histochemical detection of H_2O_2 by tissue printing as a precocious marker of rhizogenesis in grapevine. *Plant Physiology and Biochemistry* **36**, 817-824

O'Donnell BV, Tew DG, Jones OT, England PJ (1993) Studies on the inhibitory mechanism of iodonium compounds with special reference to neutrophil NADPH oxidase. *Biochemical Journal* **290 (Pt 1)**, 41-9

Ogawa K, Kanematsu S, Asada K (1997) Generation of superoxide anion and localization of Cu/Zn-superoxide dismutase in the vascular tissue of spinach hypotcotyls: their association with lignification. *Plant and Cell Physiology* **38**, 1118-1126

Oksanen E, Haikio E, Sober J, Karnosky DF (2004) Ozone-induced H_2O_2 accumulation in field-grown aspen and birch is linked to foliar ultrastructure and peroxisomal activity. *New Phytologist* **161**, 791-799

Olbrich M, Betz G, Gerstner E, Langebartels C, Sandermann H, Ernst D (2005) Transcriptome analysis of ozone-responsive genes in leaves of European beech (*Fagus sylvatica* L.). *Plant Biology* **7**, 670-676

Olson PD, Varner JE (1993) Hydrogen peroxide and lignification. *Plant Journal* **4**, 887-892

Orozco-Cárdenas M, Narváez-Vásquez J, Ryan C (2001) Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. *Plant Cell* **13**, 179-191

Otter T, Polle A (1997) Characterisation of acidic and basic apoplastic peroxidases from needles of Norway spruce (*Picea abies* L. Karsten) with respect to lignifying substrates. *Plant and Cell Physiology* **38**, 595-602

Parsons HL, Yip JYH, Vanlerberge GC (1999) Increased respiratory restriction during phosphate-limited growth in transgenic tobacco cells lacking alternative oxidase. *Plant Physiology* **121**, 1309-1320

Paschalidis KA, Roubelakis-Angelakis KA (2005) Sites and regulation of polyamine catabolism in the tobacco plant. Correlations with cell division/ expansion, cell cycle progression, and vascular development. *Plant Physiology* **138**, 2174-2184

Passardi F, Cosio C, Penel C, Dunand C (2005) Peroxidases have more functions than a Swiss army knife. *Plant Cell Reports* **24**, 255-265

Pastori G, Foyer CH, Mullineaux P (2000) Low temperature-induced changes in the distribution of H_2O_2 and antioxidants between the bundle sheath and mesophyll cells of maize leaves. *Journal of Experimental Botany* **51**, 107-113

Pearse I, Heath KD, Cheeseman JM (2005) A partial characterization of peroxidase in *Rhizophora mangle*. *Plant, Cell and Environment* **28**, 612-622

Pei Z, Murata Y, Benning G, Thomine S, Kluesener B, Allen GJ, Grill E, Schroeder JI (2000) Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. *Nature* **406**, 731-734

Pellinen R, Palva T, Kangasjarvi J (1999) Subcellular localization of ozone-induced hydrogen peroxide production in birch (*Betula pendula*) leaf cells. *Plant Journal* **20**, 349-356

Peng JY, Deng XJ, Huang JH, Jia SH, Miao XX, Huang YP (2004) Role of salicylic acid in tomato defense against cotton bollworm, *Helicoverpa armigera* Hubner. *Zeitschrift Fur Naturforschung C-A Journal of Biosciences* **59**, 856-862

Perez FJ, Burgos B (2004) Alterations in the pattern of peroxidase isoenzymes and transient increases in its activity and in H_2O_2 levels take place during the dormancy cycle of grapevine buds: the effect of hydrogen cyanamide. *Plant Growth Regulation* **43**, 213-220

Polle A, Eiblmeier M, Sheppard L, Murray M (1997) Responses of antioxidative enzymes to elevated CO_2 in leaves of beech (*Fagus sylvatica* L.) seedlings grown under a range of nutrient regimes. *Plant Cell and Environment* **20**, 1317-1321

Popov VN, Simonian RA, Skulachev VP, Starkov AA (1997) Inhibition of the alternative oxidase stimulates H_2O_2 production in plant mitochondria. *FEBS Letters* **415**, 87-90

Preuss ML, Serna J, Falbel TG, Bednarek SY, Nielsen E (2004) The *Arabidopsis* Rab GTPase RabA4b localizes to the tips of growing root hair cells. *Plant Cell* **16**, 1589-1603

Purvis AC, Shewfelt RL (1993) Does the alternatie pathway ameliorate chilling injury in sensitive platn tissues? *Physiologia Plantarum* **88**, 712-718

Ranieri A, Castagna A, Baldan B, Soldatini GF (2001) Iron deficiency differently affects peroxidase isoforms in sunflower. *Journal of Experimental Botany* **52**, 25-35

Rao M, Koch J, Davis K (2000a) Ozone: a tool for probing programmed cell death in plants. *Plant Molecular Biology* **44**, 345-358

Rao MV, Lee H, Creelman RA, Mullet JE, Davis KR (2000b) Jasmonic acid signaling modulates ozone-induced hypersensitive cell death. *Plant Cell* **12**, 1633-1646

Rea G, de Pinto MC, Tavazza R, Biondi S, Gobbi V, Ferrante P, De Gara L, Federico R, Angelini R, Tavladoraki P (2004) Ectopic expression of maize polyamine oxidase and pea copper amine oxidase in the cell wall of tobacco plants. *Plant Physiology* **134**, 1414-1426

Renew S, Heyno E, Schopfer P, Liszkay A (2005) Sensitive detection and localization of hydroxyl radical production in cucumber roots and *Arabidopsis* seedlings by spin trapping electron paramagnetic resonance spectroscopy. *The Plant Journal* **44**, 342-347

Repka V (1999) Improved histochemical test for in situ detection of hydrogen peroxide in cells undergoing oxidative burst or lignification. *Biologia Plantarum* **42**, 599-607

Richardson A, Stewart D, McDougall GJ (1997) Identification and partial characterization of a coniferyl alcohol oxidase from lignifying xylem of Sitka spruce (*Picea sitchensis*). *Planta* **203**, 35-43

Rizhsky L, Hallak-Herr E, Van Breusegem F, Rachmilevitch S, Barr JE, Rodermel S, Inzé D, Mittler R (2002) Double antisense plants lacking ascorbate peroxidase and catalase are less sensitive to oxidative stress than single antisense plants lacking ascorbate peroxidase or catalase. *The Plant Journal* **32**, 329-342

Rodriguez AA, Grunberg KA, Taleisnik EL (2002) Reactive oxygen species in the elongation zone of maize leaves are necessary for leaf extension. *Plant Physiology* **129**, 1627-1632

Ros Barceló A (1998) Hydrogen peroxide production is a general property of the lignifying xylem from vascular plants. *Annals of Botany* **82**, 97-103

Ros Barceló A (1999) *In situ* inactivation of the oxidase activity of xylem peroxidases by H_2O_2 in the H_2O_2-producing xylem of *Zinnia elegans*. *Journal of Plant Research* **112**, 383-390

Ros Barceló A (2000) Copper-containing xylem oxidases: a phantom vision beyond the lignification front? *Plant Peroxidase Newsletter* (http://www.unige.ch/LABPV/newsletters/newsl15/n15p3.html), **No. 15**, 3-11

Ros Barceló A (2005) Xylem parenchyma cells deliver the H_2O_2 necessary for lignification in differentiating xylem vessels. *Planta* **220**, 747-756

Ros Barceló A (1999) Some properties of the H_2O_2/O_2^- generating system from the lignifying xylem of *Zinnia elegans*. *Free Radical Research* **31**, S147-+ Suppl S

Ros Barceló A, Ferrer MA (1999) Does diphenylene iodonium chloride have any effect on the O_2^--generating step of plant peroxidases? *FEBS Letters* **462**, 254-256

Sagi M, Fluhr R (2006) Production of reactive oxygen species by plant NADPH oxidases. *Plant Physiology* **141**, 336-340

Schopfer P (1994) Histochemical demonstration and localization of H_2O_2 in organs of higher plants by tissue printing on nitrocellulose paper. *Plant Physiology* **104**, 1269-1275

Schreiber AW, Baumann U (2007) A framework for gene expression analysis. *Bioinformatics* **23**, 191-197

Serrano A, Cordoba F, Gonzalezreyes JA, Navas P, Villalba JM (1994) Purification and characterization of two distinct NAD(P)H dehydrogenases from onion (*Allium cepa* L.) root plasma membrane. *Plant Physiology* **106**, 87-96

Shevyakova NI, Rakitin VY, Stetsenko LA, Aronova EE, Kuznetsov VV (2006) Oxidative stress and fluctuations of free and conjugated polyamines in the halophyte *Mesembryanthemum crystallinum* L. under NaCl salinity. *Plant Growth Regulation* **50**, 69-78

Shin R, Berg RH, Schachtman DP (2005) Reactive oxygen species and root hairs in *Arabidopsis* root response to nitrogen, phosphorus and potassium deficiency. *Plant and Cell Physiology* **46**, 1350-1357

Shin R, Schachtman DP (2004) Hydrogen peroxide mediates plant root cell response to nutrient deprivation. *Proceedings of the National Academy of Sciences USA* **101**, 8827-8832

Song CJ, Steinebrunner I, Wang XZ, Stout SC, Roux SJ (2006) Extracellular ATP induces the accumulation of superoxide via NADPH oxidases in *Arabidopsis*. *Plant Physiology* **140**, 1222-1232

Spiro M, Ridley B, Eberhard S, Kates K, Mathieu Y, O'Neill M, Mohnen D, Guern J, Darvill A, Albersheim P (1998) Biological activity of reducing-end-derivatized oligogalacturonides in tobacco tissue culture. *Plant Physiology* **116**, 1289-1298

Streb P, Feierabend J, Bligny R (1997) Resistance to photoinhibition of photosystem II and catalase and antioxidative protection in high mountain plants. *Plant Cell and Environment* **20**, 1030-1040

Streb P, Shang W, Feierabend J, Bligny R (1998) Divergent strategies of photoprotection in high-mountain plants. *Planta* **207**, 313-324

Sukalovic VHT, Vuletic M, Vucinic Z (2005) The role of *p*-coumaric acid in oxidative and peroxidative cycle of the ionically bound peroxidase of the

maize root cell wall. *Plant Science* **168**, 931-938

Suzuki N, Mittler R (2006) Reactive oxygen species and temperature stresses: A delicate balance between signaling and destruction. *Physiologia Plantarum* **126**, 45-51

Takahama U, Oniki T (1997) A peroxidase/phenolics/ascorbate system can scavenge hydrogen peroxide in plant cells. *Physiologia Plantarum* **101**, 845-852

Tamagnone L, Merida A, Stacey N, Plaskitt K, Parr A, Chang C, Lynn D, Dow J, Roberts K, Martin C (1998) Inhibition of phenolic acid metabolism results in precocious cell death and altered cell morphology in leaves of transgenic tobacco plants. *Plant Cell* **10**, 1801-1816

Tamoi M, Nagaoka M, Miyagawa Y, Shigeoka S (2006) Contribution of fructose-1,6-bisphosphatase and sedoheptulose-1,7-bisphosphatase to the photosynthetic rate and carbon flow in the Calvin cycle in transgenic plants. *Plant and Cell Physiology* **47**, 380-390

Tanaka K, Suda Y, Kondo N, Sugahara K (1985) O_3 tolerance and the ascorbate-dependent H_2O_2 decomposing system in chloroplasts. *Plant and Cell Physiology* **26**, 1425-1431

Taylor ATS, Kim J, Low PS (2001) Involvement of mitogen-activated protein kinase activation in the signal-transduction pathways of the soya bean oxidative burst. *Biochemical Journal* **355**, 795-803

Tewari R, Kumar P, Tewari N, Srivastava S, Sharma P (2004) Macronutrient deficiences and differential antioxidant responses - influence on the activity and expression of superoxide dismutase in maize. *Plant Science* **166**, 687-694

Tewari RK, Kumar P, Sharma PN (2006) Magnesium deficiency induced oxidative stress and antioxidant responses in mulberry plants. *Scientia Horticulturae* **108**, 7-14

Toda S (2005) Antioxidative effects of polyphenols in leaves of *Houttuynia cordata* on protein fragmentation by copper-hydrogen peroxide *in vitro*. *Journal of Medicinal Food* **8**, 266-268

Torres MA, Dangl JL, Jones JDG (2002) *Arabidopsis* gp91(phox) homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. *Proceedings of the National Academy of Sciences USA* **99**, 517-522

Tosti N, Pasqualini S, Borgogni A, Ederli L, Falistocco E, Crispi S, Paolocci F (2006) Gene expression profiles of O_3-treated *Arabidopsis* plants. *Plant Cell and Environment* **29**, 1686-1702

Veitch N (2004) Horseradish peroxidase: a modern view of a classical enzyme. *Phytochemistry* **65**, 249-259

Veljovic-Jovanovic S, Noctor G, Foyer CH (2002) Are leaf hydrogen peroxide concentrations commonly overestimated? The potential influence of artefactual interference by tissue phenols and ascorbate. *Plant Physiology and Biochemistry* **40**, 501-507

Volkov RA, Panchuk, II, Mullineaux PM, Schoffl F (2006) Heat stress-induced H_2O_2 is required for effective expression of heat shock genes in *Arabidopsis*. *Plant Molecular Biology* **61**, 733-746

Wagner AM (1995) A role for active oxygen species as second messengers in the induction of alternative oxidase gene expression in *Petunia hybrida* cells. *FEBS Letters* **368**, 339-342

Wang J, Zhang H, Allen RD (1999) Overexpression of an *Arabidopsis* peroxisomal ascorbate peroxidase gene in tobacco increases protection against oxidative stress. *Plant and Cell Physiology* **40**, 725-732

Watson BS, Lei ZT, Dixon RA, Sumner LW (2004) Proteomics of *Medicago sativa* cell walls. *Phytochemistry* **65**, 1709-1720

Wise RR (1995) Chilling-enhanced photooxidation: the production, action and study of reactive oxygen species produced during chilling in the light. *Photosynthesis Research* **45**, 79-97

Wu QS, Zou YN, Xia RX (2006) Effects of water stress and arbuscular mycorrhizal fungi on reactive oxygen metabolism and antioxidant production by citrus (*Citrus tangerine*) roots. *European Journal of Soil Biology* **42**, 166-172

Xie ZX, Chen ZX (1999) Salicylic acid induces rapid inhibition of mitochondrial electron transport and oxidative phosphorylation in tobacco cells. *Plant Physiology* **120**, 217-225

Yamasaki H, Sakihama Y, Ikehara N (1997) Flavonoid-peroxidase reaction as a detoxification mechanism of plant cells against H_2O_2. *Plant Physiology* **115**, 1405-1412

Ye Q, Steudle E (2006) Oxidative gating of water channels (aquaporins) in corn roots. *Plant, Cell and Environment* **29**, 459-470

Yesbergenova Z, Yang GH, Oron E, Soffer D, Fluhr R, Sagi M (2005) The plant Mo-hydroxylases aldehyde oxidase and xanthine dehydrogenase have distinct reactive oxygen species signatures and are induced by drought and abscisic acid. *The Plant Journal* **42**, 862-876

Yoda H, Hiroi Y, Sano H (2006) Polyamine oxidase is one of the key elements for oxidative burst to induce programmed cell death in tobacco cultured cells. *Plant Physiology* **142**, 193-206

Yoda H, Yamaguchi Y, Sano H (2003) Induction of hypersensitive cell death by hydrogen peroxide produced through polyamine degradation in tobacco plants. *Plant Physiology* **132**, 1973-1981

Yoshioka H, Numata N, Nakajima K, Katou S, Kawakita K, Rowland O, Jones JDG, Doke N (2003) *Nicotiana benthamiana* gp91(phox) homologs NbrbohA and NbrbohB participate in H_2O_2 accumulation and resistance to *Phytophthora infestans*. *Plant Cell* **15**, 706-718

Zhang AY, Jiang MY, Zhang JH, Tan MP, Hu XL (2006) Mitogen-activated protein kinase is involved in abscisic acid-induced antioxidant defense and acts downstream of reactive oxygen species production in leaves of maize plants. *Plant Physiology* **141**, 475-487

Zhu JM, Chen SX, Alvarez S, Asirvatham VS, Schachtman DP, Wu YJ, Sharp RE (2006) Cell wall proteome in the maize primary root elongation zone. I. Extraction and identification of water-soluble and lightly ionically bound proteins. *Plant Physiology* **140**, 311-325

Zlatev ZS, Lidon FC, Ramalho JC, Yordanov IT (2006) Comparison of resistance to drought of three bean cultivars. *Biologia Plantarum* **50**, 389-394