The generation of oxygen radicals during host plant responses to infection

MARK W. SUTHERLAND

School of Applied Science, University of Southern Queensland, Toowoomba, Queensland 4350, Australia

Recent evidence points to significant oxygen radical production by some plant tissues in response to pathogenic challenge. These findings have proved quite controversial, in part because of an inadequate appreciation of the behaviour of oxygen radicals in biological systems. This review critically discusses the evidence to date and outlines several potential roles for oxygen species in host-pathogen interactions. The production of oxygen radicals during plant defence responses is compared to the respiratory burst of mammalian phagocytic cells.

INTRODUCTION

The discovery that aerobic cells have evolved a group of enymes (superoxide dismutases) which catalyse the removal of the superoxide radical (0_2^-) from respiring tissue *[88],* has been a landmark in our developing understanding of the complex role of oxygen in cell metabolism. During the 1970s research following this discovery concentrated both on the nature, occurrence and role of superoxide dismutases in aerobic cells *[51-54]* as well as on the biochemistry of oxygen radical production and reactivity *[15, 20, 22, 84].* Undoubtedly the most exciting advance of this period was the discovery that mammalian phagocytic cells can be stimulated to produce a flux of oxygen radicals external to the cell membrane which is lethal towards pathogenic micro-organisms *[10,80].* Subsequently, the 1980s have witnessed a rapid expansion in medical research into the involvement of oxy-radicals and oxidation processes in an array of pathological conditions including arthritis, pulmonary oedema, reperfusion injury and atherosclerosis *[34,93].* A role for active oxygen species in ageing processes *[65]* also has become clearly established *[32, 66, 85].*

It was quickly realized that oxygen radical production and metabolism were significant issues in plant biochemistry *[40],* not just in mitochondrial electron transport processes *[16, 48, 86, 98, 103],* but more particularly in photosynthesis *[3, 42, 50, 91, 119].* However, it was not until after the implications of the work with mammalian phagocytes became known that plant pathologists began to speculate on connections between the rapid necrotic reactions observed in plants expressing resistance to pathogenic invaders (the hypersensitive response) and the possibility that cell wall- or membrane-located enzyme systems might produce a flux of oxygen radicals at the surface of the host cell, capable of initiating this necrosis. Subsequently a number of studies attempting to define the role of active oxygen species (and in particular the superoxide radical) in plant pathogenesis have been undertaken, investigating a wide range of host-pathogen interactions.

Before turning to a detailed review of this work, it would be appropriate to summarize some of the major features of oxygen radical production and chemistry relevant to plant tissues. Despite the availability of excellent and detailed reviews of the chemistry of these radicals *[21, 33, 57, 84, 112],* much of the discussion of oxygen radicals in the plant pathology literature displays an insufficient appreciation of the chemical behaviour and reactivity of these species.

This is perhaps understandable considering that much of this information is to be found in the literature of free-radical chemistry and radiation biology, unfamiliar territory for most plant biologists.

THE CHEMISTRY OF OXYGEN RADICALS IN CELLS

Superoxide / perhydroxyl radical

The superoxide anion (0_2~) is a free radical species (i.e. it bears an unpaired electron) which arises from the one electron reduction of molecular oxygen. In aerobic cells 0_2^- is routinely generated, in low concentrations, by electron transport systems, in particular those of respiring mitochondria *[86, 103]*. As much as 4% of oxygen taken up by mitochondria under normal physiological conditions is monovalently reduced to 0_2^- *[30]*. *Of* is also generated by the other major electron transport processes of eukaryotes, photosynthesis *[3, 42, 50, 91, 119]* and microsomal activity *[104]*. Aero-bically respiring prokaryotes are thought to generate 0_2~ at sites similar to those of mitochondria *[HI]*. 0_2~ is also produced by a number of enzymes which participate in oxidation-reduction processes *[63]*. The most studied of these is the enzyme xanthine oxidase, which is frequently used in *in vitro* experiments as a source of 0_2~ *[58]*.

In aqueous solution the superoxide radical exists in an acid-base equilibrium with its protonated form, the perhydroxyl radical ($H0_2$) (Equation 1).

$H0_2 \wedge H^+ + CV, \quad p/T_a = 4\text{-}8$ (1)

This equilibrium is significant for two reasons. Firstly, these two radical species disappear by spontaneous dismutation (Equations 2, 3).

$H0_2 + H0_2 \text{---} > H_20, + 0_2$ (2)

$H0_2 + 0_2\text{-} + H_20 \text{---} * H_20_2 + 0_2 + OH\text{-}$ (3)

The rate constants for these two reactions are $8'3xl0^5$ and $9\text{-}7 \times 10^7$ 1 mol^{-1} s''^1, respectively *[19]*. While Equation 2 can be ignored in all but the more acidic cellular pH domains, Equation 3 will be the major (second order) route of decay under most physiological conditions. Hence as pH increases, the rate of radical dismutation will decrease due to the lower relative concentrations of $H0_2$.

Equation 4 describes the half life *(k)* of 0_2 at an initial concentration of *a* under second order decay *(k* = the observed pH-dependent rate constant, taken from figure 2 in ref. *[19]).* Thus the half-life of a 1-0 x $10\sim^6$ M solution of 0_2'' at pH 6-5 is 0-5 s, while the half-life at pH 8^-5 is 50 s. It should be noted that the rate of spontaneous dismutation of 0_2~ with itself (Equation 5) is negligible (rate constant < 0'3 1 mol^{-1}

s"[1]) *[17]*.

Of + *Of* + $2H_20$ - - -> H_20_2 + 0_2 + 20H" (5)

It is unfortunate that Equation 5, as written, is the form most frequently used in the biological literature to describe the spontaneous decay of $H0_2/0_2$~. This encourages a disregard for the dependence of the rate of radical decay upon local pH conditions surrounding cellular sites of generation.

The second reason for the importance of the equilibrium in Equation 1 is that the protonated perhydroxyl radical is generally much more reactive toward organic molecules than the anionic superoxide form *[19]*. This is particularly relevant with regard to the direct oxidation of polyunsaturated fatty acids [18], considered to be an important site of damage to cells by oxygen radicals *[41, 118]*.

The pH of the various environments in *in vivo* and *in vitro* experimental systems is thus of great significance in determining the lifetime and diffusion path in solution of generated radicals, and their potential for direct reaction with cellular constituents. The reactivity of $H0_2/0_2$~ in biological tissues has been extensively reviewed *[25, 34, 54, 55, 64, 93]* and will not be dealt with here.

Aerobic cells constitutively express metal-containing superoxide dismutases (SOD) which rapidly catalyse the dismutation of $H0_2/0_2$" *[53, 54, 88]*. Rate constants for this catalysed dismutation are typically of the order of 10^9 M^{-1} S^{-1} *[54]*. Much significance has been placed on the fact that although $H0_2/0_2$~ is only generated in low concentrations and disappears quite rapidly by reaction with its protonated form, almost all aerobic tissues contain an enzyme system which specifically hastens its dismutation. Those few aerobic micro-organisms which lack SOD appear to rely on less efficient non-enzymic mechanisms such as high internal Mn^{2+} levels to perform the same task *[54]*. Fridovich has argued that under typical physiological conditions, $H0_2/0_2$~ will be removed 10^{10} times more rapidly in the presence of the enzyme than in its absence *[54]*. One of the most important tests for $H0_2/0_2$~ involvement in any cellular phenomenon is the demonstration that either the addition of active SOD, or the inhibition of endogenous enzyme, alters the progress of events. Inactivated enzyme should be used as a control wherever possible *[58]*.

Hydrogen peroxide

Hydrogen peroxide is a stable, partially-reduced form of oxygen produced in cells by both the dismutation of $H0_2/0_2$" (Equations 2, 3) and by several enzymic routes *[63]*. While not a radical, H_20_2 has been shown to exhibit cytotoxicity *[63]*. H_20_2 is removed from the cell by

the action of catalase and various peroxidases (e.g. glutathione peroxidase) *[63]*. The collective term active oxygen species is frequently used in the literature in preference to oxygen radicals in order to include non-radical forms such as H_20_2.

Hydroxy radical

Of the biologically occurring oxygen radicals, HO • is by far the most reactive and is one of the strongest oxidizing agents known. HO • has a very brief half-life in the presence of most organic molecules due to its ability to participate in addition, **6-2**
hydrogen abstraction, and electron transfer reactions *[2J]*. This results in a rather non-selective attack on biological molecules by HO- *in vivo,* along a very short diffusion path from the site of production *[33, 115, 118]*.

The relatively poor reactivity of 0_2~ towards biological macromolecules has led to suggestions that 0_2~ is able to react with other intermediates to produce HO • which then initiates macromolecular damage. Early proposals *[44, 71]* centred on the possibility of a direct reaction of 0_2~ with H_20_2 (Equation 6).

0_2" $+H_20_2 + H^+ \text{ — } *0_2 + H_20 + HO-$ (6)

Careful kinetic studies *[89, 121]* have since indicated that the rate of this reaction is negligible. It has become apparent [67], however, that transition metals (e.g. iron) are able to catalyse HO' formation in the following manner:

$0_2- + Fe^{3+}\text{---}^0_2 + Fe^{2+}$ (7)

$Fe^{2+} + H_20_2\text{---}*Fe^{3+} + 0H- + H0-$ (8)

Chelation of the iron has a profound effect on its availability to participate in these reactions and the nature of the chelating agent is critical *[5, 13, 114]*. There is much debate concerning the nature of the damaging species produced by these metal-catalysed processes. It is not clear whether free HO ■ or a reactive iron-oxygen complex is formed in Equation 8 *[106, 117]*.

Transition metal contamination

Trace contamination of experimental systems with transition metals may significantly increase the rate of disappearance of $H0_2/0_2$~, rendering detection difficult. Contamination may also significantly increase the apparent rate of reaction (oxidation/reduction) of detector molecules or biological components with oxygen radicals, particularly in *in vitro* systems.

Two approaches have been used to overcome these problems. The first approach is to use only the best of analytical grade reagents in

aqueous solutions made with high purity water (typically triply distilled, or passed through a succession of reverse osmosis, activated carbon and mixed-bed ion-exchange treatments). This approach has limitations when dealing with crude biological extracts or tissue slices, due to the many trace "contaminants" they contain. The second approach is effectively to remove trace metals by adding organic metal-chelating compounds in order to complex metal ions in solution in inactive forms. However, care must be exercised, since some commonly used chelators such as EDTA may serve to increase the participation of metal ions in redox reactions, firstly by effectively maintaining higher solution concentrations of the ions, and secondly by altering their reactivity *[56, 117]*.

Singlet oxygen
Ground state oxygen bears two electrons in separate orbitals having parallel spins, in what is known as a triplet conformation. This configuration prevents triplet oxygen from being highly reactive biologically because of the spin restrictions imposed by the ground state singlet configuration of most organic molecules in which the outermost two electrons have opposed spins *[52,82]*. When sufficient energy is available, the triplet state ^{3}£g0_2 can be excited to one of two singlet configurations. However only one of these, $^1AgO_a$, appears to be formed in biological systems. While this excited singlet state is able to react rapidly with a broad spectrum of organic molecules, it is rapidly de-excited in aqueous solutions to the ground triplet state, with a half-life of approx. 2 us *[90]*.

The occurrence of singlet oxygen in biological systems has received wide attention *[46, 47, 78, 83]*. During the 1970s there were a number of claims that singlet oxygen was a product of the spontaneous dismutation of $H0_2/0_2$~ (Equations 2, 3) *[45, 77, 87, 102]*. Subsequent work, however, has demonstrated conclusively that the dismutation reactions yield only ground state oxygen *[47,97]*. It was also proposed that singlet oxygen arose from the reaction of $H0_2/0_2$" with H_20_2 (Equation 6), *[44, 70, 71]*, but this possibility was discounted when the rate of this reaction was shown to be negligible *[89, 121]*. It is now considered most unlikely that singlet oxygen plays a role in cellular damage arising from oxygen radical generation.

THE RESPIRATORY BURST OF MAMMALIAN PHAGOCYTES

It was recognized as early as 1933 that neutrophils significantly increase their consumption of oxygen during phagocytosis *[14]*. Only

many years later did it become clear that this burst of oxygen consumption is not due to an increased mitochondrial electron transport, but rather to the production of H_20_2, linked to a surge in hexose monophosphate shunt activity *[68, 108]*.
It has now been established *[9, 11]* that this activity results from the activation of a cell membrane-located enzyme system which catalyses the single electron reduction of oxygen to form $H0_2/0_2$~, using NADPH as the reductant.

$$20_2 + NADPH \text{---}> 20_2\text{-} + NADP^+ + H^+ \quad (9)$$

Spontaneous dismutation of $H0_2/0_2$~ then yields H_2O_a (Equation 3). Consumption of glucose via the hexose monophosphate shunt regenerates the NADPH consumed by the activated membrane-bound NADPH oxidase system *[12]*. This pathway is present in a number of phagocytic cell types, both polynuclear (e.g., neutrophils and eosinophils) and mononuclear (e.g., macrophages). While many stimuli which activate the respiratory burst are known, the mechanism of activation is the subject of continuing debate *[12, 123]*. A detailed discussion of the activation of the respiratory burst is not possible here, but can be found in many of the published reviews of phagocyte action *[12, 31, 49, 105, 116, 123]*.

The activated oxygen species are released externally to the cell membrane, usually into a phagocytic vacuole formed around the invading foreign body, where they contribute to microbial killing. In neutrophils, but not in all phagocytes, the enzyme myeloperoxidase is also released and catalyses a reaction between H_20_2 and chloride ions to form hypochlorite (Equation 10) *[80]*.

$$C1\text{-} + H_20_2 \text{---}> 0C1\text{-} + H_20 \quad (10)$$

Hypochlorite, strongly bacteriocidal in its own right, can also react with amines to form chloramines, a much more stable group of antimicrobials. A detailed examination of the production and reactions of neutrophil oxidants has recently been published *[123]*.

Fungal pathogens
In 1983, Doke published the first of a series of papers which invoked the generation of $H0_2/0_2$~ by tissues responding hypersensitively to pathogenic infection. This initial work *[36, 37]* concentrated on the infection of potato tuber tissue with races of the potato blight fungus *Phytophthora infestans*. Doke observed that aged discs of tuber tissue inoculated with an incompatible fungal race were stimulated to reduce both cytochrome *c* and the dye nitroblue tetrazolium (NBT) when they were present in the bathing solution *[36]*. Elicitors of hypersensitivity, such as hyphal wall components, also had this effect. The addition of SOD to the assay solution inhibited these reductions, while the

vacuum infiltration of the enzyme into tuber discs appeared to delay both hypersensitive cell death and phytoalexin production. SOD-inhibitable reduction of cytochrome *c* or NBT are commonly used tests for the presence of $H0_2/0_2$~ *[58]*. Similar findings were obtained with protoplasts isolated from tuber tissue *[37]*. Protoplasts were rapidly stimulated by a non-specific elicitor from hyphal cell walls of the fungus to reduce cytochrome *c* or NBT in the external medium. Reducing equivalents in the form of exogenous NADPH were required for significant stimulation to occur. Again SOD prevented the reduction of cytochrome *c* and NBT. On the basis of this body of evidence, Doke concluded that 0_2~-generation may be directly involved in host cell hypersensitivity.

Subsequent studies have suggested the existence of a membrane-located superoxide-generating NADPH oxidase sensitive to digitonin stimulation *[38, 39]*. In leaf discs exposed to compatible and incompatible infections *[29]*, 0_2~ generation was observed as a two-stage process consisting firstly of a Step I response which occurred non-selectively in host tissue prior to fungal penetration of the leaf epidermis. The post-penetration Step II response was seen only in incompatible interactions, in association with hypersensitive cell death. Exogenous calcium appeared to enhance the Step II response *[28]*.

Recently there has been criticism of these studies by Moreau & Osman [96] who have correctly drawn attention to errors made by Doke's group in calculating the yields of $H0_2/0_2$~ produced by host tissues reacting to either infection or to elicitors present in germination fluids. Furthermore Moreau & Osman *[96]* did not observe any significant $H0_2/0_2$~ generation by either potato tuber or leaf discs challenged with germination fluids from *P. infestans.* Unfortunately, these authors did not clearly demonstrate that the radical detection system they employed was sensitive enough to scavenge oxygen radicals produced by host tissues at the lower concentrations suggested by their revised calculations. Further work to clarify this situation is required.

In an investigation of the disruption of cell membranes during the hypersensitive response of potato leaf tissue to *P. infestans,* Jordan & DeVay *[69]* concluded that in addition to $H0_2/0_2$" generation, there was a significant hydroxyl radical flux leaching from the tissue into the assay solution in both infected and healthy tissues. However, it seems certain that the observed fluorescence due to the oxidation of benzoate in the external solution was caused by reaction with factors

other than OH •. The reactivity of this species (see above) is such that it will react indiscriminately with cellular components long before it is able to diffuse away from any tissue slice to react with an external scavenger. The claim that the benzoate-reactive component released from tissue is stable for an hour ([69], figure 17) confirms that it is not the hydroxyl radical.

Oxygen radical involvement has also been proposed in resistance expression by rice cultivars towards rice blast disease. Sekizawa *et al. [109, 110]* have presented evidence for the generation of $H0_2/0_2$" in rice leaves infected by rice blast fungus *(Pyricularia oryzae).* While the time-course for this process appears curiously complex, 0_2~-generation occurred earlier and was larger in magnitude in response to incompatible infections compared to compatible infections.
Aver'yanov and co-workers [7, *8]* have also observed a SOD-inhibitable adrenalin reduction by rice leaves which is coincident with disease resistance expression towards the blast fungus. However, the observation that this activity is still present in leaf diffusates some time after removal from inoculated resistant leaves *[8],* suggests that the active species in this study is not a short-lived oxygen radical.
Buonaurio *et al. [24]* have taken a different approach and have explored variations in SOD and peroxidase levels during fungal infection. They observed significant increases in cyanide-sensitive Cu-ZnSOD during the hypersensitive response of bean *[Phaseolus vulgaris)* to bean rust *(Uromyces phaseoli)* which were not observed in compatible infections. In tobacco leaves infected with tobacco mosaic virus, hypersensitively-reacting tissues showed higher levels of both the Cu-Zn and Mn forms of SOD than susceptible leaves *[95].* However, in both investigations, the relative enhancement of SOD activity in hypersensitive tissue was greatly exceeded by an increase in peroxidase activity, suggesting that despite the rise in SOD levels, a net increase in the level of cellular oxidants, due to the increased peroxidase activity, was likely. Increased MnSOD expression has also been reported for cultured tobacco cells during conditions of stress caused by both pathogenic and non-pathogenic factors *[23].* This induction of MnSOD activity by stress factors accompanied an increase in mitochondrial cytochrome oxidase activity.
In contrast, Zacheo & Bleve Zacheo *[124-126],* investigating the resistance of tomato varieties towards infection by the nematode *Meloidogyne incognita,* have reported a fall in SOD activity in hypersensitively responding tomato roots. This fall is accompanied by an apparent increase in oxygen radical generation, as detected by both NBT and cytochrome *c* reduction. Resistance expression is not the only instance in which oxygen radical generation by root tissues has

been invoked. Aver'yanov *[6]* has observed a superoxide-generating system in intact pea roots which is enhanced following wounding. $H0_2/0_2$~ may also be involved in the reduction of extracellular transition metal ions by some dicotyledon roots under conditions of nutrient deficiency *[26, 27]*.

Bacterial pathogens

In addition to studies with fungal pathogens, investigations of host responses to pathovars of the bacterium *Pseudomonas syringae* have similarly implicated $H0_2/0_2$~ as a component of the hypersensitive response. Early in the attempted infection of cotyledons of an incompatible host (cotton) by *P. syringae* pv. *tabaci*, a depolarization of the host plasma membrane diffusion potential has been observed *[101]*. Furthermore, in cucumber cotyledons challenged with *P. syringae* pv. *pisi*, this depolarization was accompanied by membrane lipid peroxidation and preceded electrolyte leakage from the cell *[74]*. SOD infiltrated into cotyledon tissue before bacterial infection produced a significant reduction in both membrane lipid oxidation and subsequent electrolyte leakage, thus, implicating $H0_2/0_2$" production in these events *[75]*. SOD also lowered lipid peroxidation and electrolyte leakage in paraquat-treated tissues *[75]*. Paraquat is known to stimulate superoxide initiated lipid peroxidation in plant cells *[35]*. In both the hypersensitive response and paraquat treatment, a lowering in the level of membrane unsaturation was observed *[76]*, consistent with the oxidation and subsequent removal of unsaturated fatty acids from the bilayer. These findings suggest an oxygen radical mediated disruption of host cell membranes during the expression of hypersensitivity towards infection by incompatible bacterial pathovars. In addition, observations *[72]* of the hypersensitive response of tobacco suspension cultures towards *P. syringae* pv. *syringae*, imply that $H0_2/0_2$~ may also be involved in the direct killing of the pathogen.

These conclusions are in conflict with those of Minardi & Mazzucchi *[92]* who failed to observe exogenous cytochrome *c* reduction by discs cut from tobacco leaves reacting hypersensitively to a strain of *P. syringae* isolated from pear. However, this latter study first sampled leaf incubation medium and then added the cytochrome in a dilution step, by which time any $H0_2/0_2$~ produced by the hypersensitively reacting tissue is likely to have disappeared by spontaneous dismutation.

Some reports have invoked the production of oxygen species other than $H0_2/0_2$~ to explain aspects of plant response to pathogenic attack.

It is still being suggested *[73, 107]* that singlet oxygen, generated from $H0_2/0_2$~ via Equation (6) or by spontaneous dismutation, might play an important role in plant resistance. As discussed earlier in this review, singlet oxygen generation by these routes has been discounted *[47, 89, 97, 121]*. Not surprisingly, a recent study into the effects of singlet oxygen scavengers on the hypersensitive reaction of tobacco cells towards *P. syringae* pv. *pisi* found that singlet oxygen was not generated during the host response *[107]*.
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While the hydroxyl radical has been proposed as the radical species most likely to be responsible for initial lesions created in the presence of superoxide-generating systems *[55, 62]*, there have been very few studies directed specifically at investigating this possibility in the case of plant-pathogen interactions. One such study, based on the use of exogenous hydroxyl radical scavengers, has concluded that the hydroxyl radical is an important signal in triggering phytoalexin production in soybean cotyledons treated with abiotic elicitors *[43]*. Notably both SOD and catalase failed to inhibit this phytoalexin production, implying that $H0_2/0_2^-$ and H_20_2 may not be critical intermediates in the generation of the signal, and that HO • is generated by some other means.

The role of hydrogen peroxide

Relatively little is known concerning the mechanisms responsible for generating oxygen radicals during resistance responses. One possibility is that a specific membrane-located NAD(P)H oxidase catalyses the single electron reduction of molecular oxygen to 0_2^- *[38, 120]* in a manner roughly analogous to the oxidase found in neutrophils. Evidence for NAD(P)H-dependent generation of hydrogen peroxide by several routes has been put forward *[59, 60, 113]*. It is likely that one or more of these pathways to H_20_2 production proceed via 0_2^- generation *[1, 113]*. Peroxidases have also come under scrutiny as possible sources of oxygen radicals in plants *[60]*. This is a particularly attractive hypothesis in the case of host responses to pathogenic attack, since cell wall peroxidases are induced *[94]* under conditions where lignification of host cell walls is employed defensively. Thus, peroxidases might have a double role in both structural and physiological protection of the host.

H_20_2 is an important substrate for peroxidases in the oxidation of coniferyl alcohol to initiate the lignification chain reaction. In one pathway, NAD(P)H is oxidized in the presence of peroxidase and Mn^{2+} to reduce molecular oxygen to 0_2^- *[60]*, which undergoes dismutation either spontaneously or enzymically to form H_20_2. H_20_2 is

further reduced by peroxidase to water at the expense of electrons from coniferyl alcohol. The alcohol radical then initiates the lignification chain reaction *[59]*.

In addition to its role in initiating lignification chemistry, hydrogen peroxide may act directly as an antifungal or bacteriocidal agent. This strategy has recently been observed in cultures of the potential biocontrol agent *Talaromyces flavus* which produces large quantities of glucose oxidase, an enzyme which oxidizes glucose to produce H_2O_a in the surrounding medium, inhibiting growth of competitors such as *Verticillium dahliae [79]*.

Most recently, a further role for hydrogen peroxide has been suggested. Apostol *et al.* [2], studying the responses of cell suspension cultures from soybean *{Glycine max)* to a preparation from *Verticillium dahliae* which elicited the phytoalexin glyceollin, noted the rapid destruction of fluorescent probes used to monitor the progress of elicitation. This destruction could be attributed to the action of cell wall peroxidase catalysing the oxidation of the probes. These oxidation reactions were dependent on a flux of exogenous H_20_2, produced by the plant cells within minutes of exposure to the elicitor. Surprisingly, added H_20_2, in the absence of the elicitor, was also effective at eliciting glyceollin production in the soybean cells. If catalase was added to the cultures prior to elicitor addition, glyceollin production was partially inhibited. The authors reasonably concluded that H_20_2, which is able to diffuse rapidly across the cell membrane to the interior, may act as a second messenger in eliciting the resistance response. At present the mechanism of H_20_2 production in the cell membrane in response to elicitation is unknown, nor is the possible involvement of $H0_2/0_2$~ as an intermediate in this production process clear.

The suggestion that H_20_2 is able to act as a second messenger in eliciting host plant resistance may represent an exciting development in our understanding of early events in this interaction. H_20_2 has been implicated in signal transduction during insulin action in animal systems *[67,81]*. Significantly, plasma membrane calcium influx, another recognized component of mammalian cell signal-transduction *[122]*, has been observed as an early event in the bacteria-induced hypersensitive reaction of tobacco suspension cells *[4]*.

CONCLUDING REMARKS

There are emerging at least three potential roles for active oxygen

species in plant defence responses: (i) the oxidation of host components, consisting of at least two distinct processes: the oxidation of membrane lipids during cell necrosis (the hypersensitive response), and the initiation of cell wall lignification reactions; (ii) direct injury of the pathogen; and (iii) signal transduction.

It may be that important parallels exist between the respiratory burst of mammalian phagocytes and oxygen metabolism during plant responses to infection. However, there are several important factors to be considered in drawing such comparisons. Structurally, the existence of cell walls and in some cases, intercellular air spaces in plant tissues has significant consequences for the spatial geometry of infection processes. Mammalian phagocytes are highly differentiated specialized cells which enclose their prey in a phagocytic vacuole where high concentrations of oxidants can be maintained. In contrast, the plant tissues which appear to produce oxygen radicals are poorly-differentiated tissues such as storage parenchyma, root cortex, root epidermis and leaf mesophyll. In plant tissues there is as yet no evidence for any compartmentalization of pathogens in an oxidizing environment, although for defence against biotrophic fungi, it may be that the host cell-haustorium interface presents such an opportunity.

Due to the conflicting nature of the available evidence it is not possible at this time to assess the broader significance of oxygen radical generation in plant cell defence responses. While there is a great need for continuing investigation into every aspect of this field, several avenues appear particularly worthwhile:

(i) Further studies, under well-defined conditions in suspension culture, of the significance of free radical generation by host cells of both resistant and susceptible genotype. The primary advantage of this approach is to remove some of the complexities associated with the diffusion path of radicals and soluble reactants in intact tissues or tissue slices.
(ii) Thoughtful application of the knowledge gained from extensive studies of oxygen radical generation during pathogenic infection of animal systems.
(iii) A clarification of the importance of H_20_2 generation, particularly in signal transduction during pathogen recognition and in subsequent resistance expression by host plant cells.
In pursuing these avenues, plant pathologists will need to draw on the expertise of free radical biochemists and the accumulated experience of cell physiologists studying mammalian phagocytic cells. Experimentally, investigations into free-radical generation in

biological systems are vulnerable to many pitfalls. In this regard several extensive reviews of methodology *[58, 93, 99, 100]* are available.

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