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LETTER TO THE EDITOR

DO EPR SPECTRA SHOW THE PRESENCE OF A UNIQUE AND UBIQUITOUS QUINONE-DERIVED FREE RADICAL THAT IS ASSOCIATED WITH SENESCENCE IN PLANTS?

As part of an extensive programme of research aimed at establishing links between senescence processes in plants and the formation of a stable free radical that can be detected routinely by electron paramagnetic resonance (EPR) spectroscopy, Atherton *et al.*[1] report to have 'established beyond doubt that there is a unique and probably ubiquitous free radical species which reflects damage in senescent plant material and ... postulate that this radical derives from a quinone'. This is in contrast to previous work[2] where it was shown that two components could be observed in the spectra from embryonic axes of *Quercus robur* L. seeds that had been subjected to desiccation stress.

The more precise interpretations proposed in reference 1 contain two fundamental assumptions, the validity of which we question. These assumptions are (i) that the EPR spectra of desiccated plant tissues correspond to a single free radical species, and (ii) that this free radical is characteristic of senescence processes. Here, we critically examine these assumptions and review the evidence for a quinone-derived radical as characteristic of senescence processes in desiccated plant tissue.

(i) Do the EPR spectra of desiccated plant tissues correspond to a single free radical?

Because of the high degree of overlap in the ranges of their g-values,[3] it is often difficult to distinguish between oxygen- and carbon-centred radicals in the absence of hyperfine structure. For radicals that give EPR spectra with low levels of anisotropy, the range of peak positions for the centres of their spectra at the commonly-used X-band frequencies is comparable to the linewidths observed in the solid state. Thus, the observation of a single peak in an EPR spectrum does not, on its own, demonstrate that there is a single radical species or even a single major component present in the sample. As a consequence, the initial assumption that the X-band spectra of dried beech and tea leaves[1] and desiccated moss[4] originated from a single free radical is questionable.

In a study of the effects of desiccation on the EPR spectra of embryonic axes of *Q. robur* seeds, Hendry *et al.*[2] have reported the presence of two peaks whose relative intensities varied with sample history. This strongly suggests that more than one free radical species is involved in the desiccation process. Although Hendry *et al.*[2] did not

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report g-values for their spectra, we have made measurements with similar material and found g-values of 2.0051 ± .0005 and 2.0026 ± .0010. These values are comparable to those reported by Atherton *et al.*[1] for desiccated moss, which suggests that that sample might contain more than one component.

(ii) Are the EPR spectra of desiccated plants characteristic of senescence processes?

In any population of naturally senescing cells, there will be examples of different stages of deterioration, including death, but senescing material is on the whole viable. The dried leaves studied by Atherton *et al.*[1] were not viable tissue and should not, therefore, be considered senescent. Nevertheless, the possibility cannot be excluded that radicals formed in this material as a result of senescence processes before drying and death.

It is also difficult to distinguish between senescence-induced free radicals and those produced during normal and/or stress metabolism. Indeed it has been reported that these latter processes yield EPR spectra[5] with g-values similar to those reported by Atherton *et al.*[1] at X-band frequencies. For example, unstressed developing wheat roots produced a single peak X-band EPR spectrum with g = 2.0045, whose intensity was dependent on the oxygen concentration around the roots. Moreover, stress-induced senescence can result in a decrease in EPR spectral intensity as seen in the response of wheat roots to heavy metal toxicity induced by exposure to high levels of Cu(II) ions.[5]

A respiratory origin has also been reported by Leprince *et al.*[6] for the EPR signal in radicles of *Zea mays* L., where a direct correlation was observed between O_2 consumption and EPR signal amplitude in subsequently desiccated tissue. The free radical was reported to be located primarily in the defatted cellular debris and mitochondrial fractions, leading to the conclusion that it was derived from ubiquinone that had been damaged in the desiccation process.

Stable free radicals with single peak EPR spectra can also be formed after tissue death, an extreme example of which is the dramatic increase in spectral intensity that is seen in ground spices after gamma-irradiation.[7] There is also the possibility of changes in free radical contents of such specimens during subsequent storage, as has been observed with roasted coffee.[8]

In any interpretation of EPR signals in plant tissue it is important to recognise that free radicals can be formed either as a result of normal metabolic processes and after death. On the evidence available at the present time, the free radicals produced during both of these events appear to have EPR spectra that are similar, at least at X-band frequencies, to those assigned by Atherton *et al.*[1] specifically to senescence processes.

(iii) Does the W-band EPR spectrum of moss correspond to a quinone-derived radical?

Identification of the quinone-derived radical by Atherton *et al.*[1] is based almost exclusively on similarities in the g-values of a desiccated moss (*Dicranella palustris* Dicks.) and those of model semiquinone radicals. The moss spectrum contained two peaks with g-values of 2.0054 and 2.0023 flanking the third peak of a sextet from Mn^{2+}, which is commonly found in biological specimens. The positions of these peaks are similar to those of the outermost peaks in the spectra of two simple semiquinone radical anions (derived from 1,4-benzoquinone and 7,8-dimethyl-1,4-naphthoquinone) and on the basis of this observation it was concluded that they represented a semiquinone

radical whose central peak was obscured by the Mn^{2+} peak. No consideration was given to any alternative assignment of the spectrum, especially the possibilities that it might correspond either to the $g_{\perp}$ and $g_{//}$ features of a radical with axial symmetry or to two distinct radicals. In view of the contrasting results of Hendry *et al.*[2] on desiccated seeds, the latter would appear to be at least possible and maybe even the most probable assignment of the desiccated moss spectrum. The possibility of a semiquinone radical making a *contribution* to the EPR spectrum is not, however, completely excluded, because of the possibility of anisotropy in a minor component. Indeed, because of the dramatic decrease in spectral height with increasing anisotropy, it is *possible* that such a radical could account for a considerable proportion of the total organic free radical content, but there is as yet no *spectral* evidence to support this contention.

There is clearly a need for more extensive investigations if the roles of free radicals in plant senescence processes are to be fully understood. Although there is overwhelming chemical evidence to support the involvement of free radicals in senescence processes in plants,[9] we do not believe that the current EPR spectral evidence is sufficient to demonstrate the production of a stable, unique and ubiquitous free radical that reflects damage in senescent tissue, nor to identify this radical as a semiquinone. Indeed, current evidence suggests that a number of different types of free radical with similar EPR parameters can be formed in plant tissue.

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