EPR Studies of Milk Products Tested for Grapevine Powdery Mildew

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The fungal disease powdery mildew can be controlled by introducing free radicals to its environment. Some years ago, the Adelaide group[b] proposed milk products could do this, and asked the Monash group[a] to verify the presence of free radicals in these products This was done using EPR in the *solid* state, to save time and money, and gave positive results. These results, with more recent ones, are presented here.

1. Introduction

The solid, powdered milk products originally presented, and acquired recently to verify the original results were: full cream milk, no fat milk, whey powder, whey proteins, lactoferrin and lactose. The extra compounds were holo- (full) and apo- (empty) lactoferrin. This glycoprotein, molecular weight 80 kiloDaltons, with some 400 proteins in it, is a well known antioxidant, antifungal and antibacterial agent. It contains two inequivalent Fe^{3+} sites. EPR, and Mossbauer experiments [1] were carried out on it in 1970 – 80, for the iron sites. The lactoferrin is probably the origin of the idea that all the milk products (except lactose!) might contain or generate free radicals. After the verification that this was so, successful field trials and greenhouse trials against powdery mildew on wine grapevines were carried out.

These trials and their results have been described elsewhere [2]. This paper describes the EPR work and results which helped justify the trials, together with some further work.

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2 Materials and Methods

The materials have been well described in the introduction. Each powder sample was placed in its own special quartz EPR tube (Wilmad), to a depth which ensured that the active space in the microwave cavity was filled.The apparatus used in the initial investigations was a Varian E-12 EPR X-Band (~9.1 GHz) spectrometer. The results were verified, and new measurements made, with a Bruker EPR spectrometer (~9.4 GHz). All initial tests were carried out at room temperature. Verification tests were carried out at room temperature, or at ~ 120 K, with the Bruker,

The initial choice of solid state sample testing at room temperature was a gamble, to save time, &c, instead of looking at solutions. So we had some luck.

3 Results

All examined samples, including to our surprise lactose, gave free radical radical signals, at the initial and subsequent tests. Lactoferrins (LF) gave the expected Fe^{3+} signal, except for the apo- molecules. To save space, only the room temperature Fe^{3+} and free radical spectra of apo-LF, and 'average' LF are shown, together with the free radical signal of 'average' LF alone (Figs 2, 1, and 3). Note the asymmetry of the free radical signal. At room temperature, all the lines are broadened by spin-lattice relaxation effects. A saturation study of the free radical line showed that it did not consist of a single radical species, but of a number of different species.

4. Discussion

The free radicals in LF may not be entirely due to the Fe^{3+} actions, because the line is present in apo-LF. There is no detectable line at g = 6, showing that there is negligible contribution of Fe^{3+} to the g = 2 line, which is therefore due maily to free radicals. Since there are some 400 amino acids in LF, molecular weight 80 kiloDaltons, it would be surprising if some of the radicals were not due to Sulphur proteins. Back to square one, in a way.

It was not the object of this article to discuss how the introduction of free radicals into the powdery mildew environment destroys the fungus. A brief account of this, and of the earlier EPR work, can be found in [1].

Acknowledgements.

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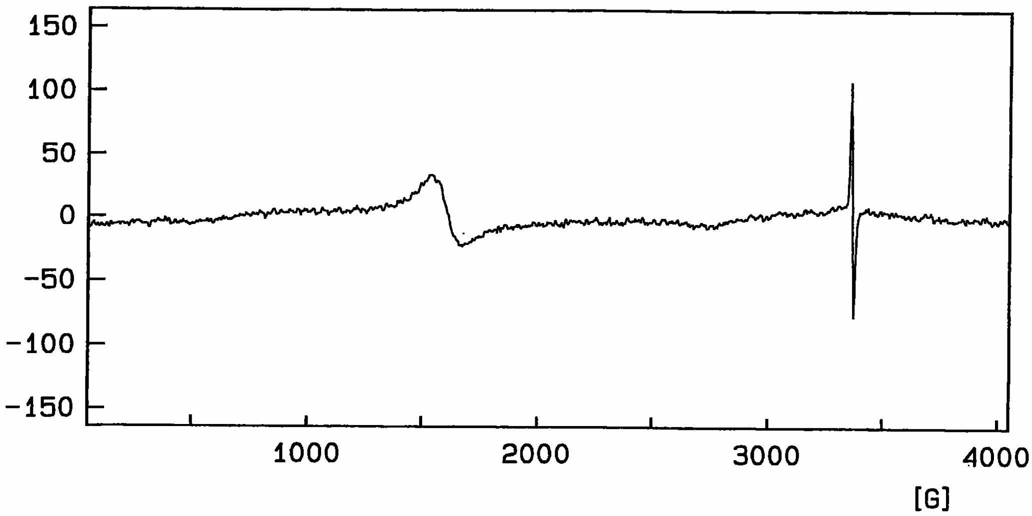
References

[1] P.Crisp, E.S.Scott, T.Wicks and G.Troup, Proc.4th Workshop on Grapevine Powdery and Downy Mildew (Eds. D.M.Gadoury et al.), University of California Davis, California USA (2002) p.19.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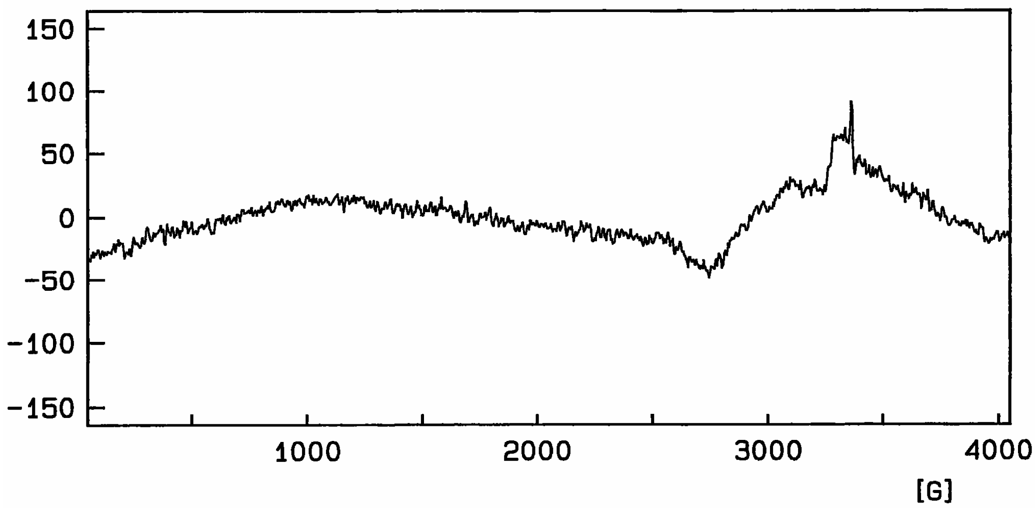
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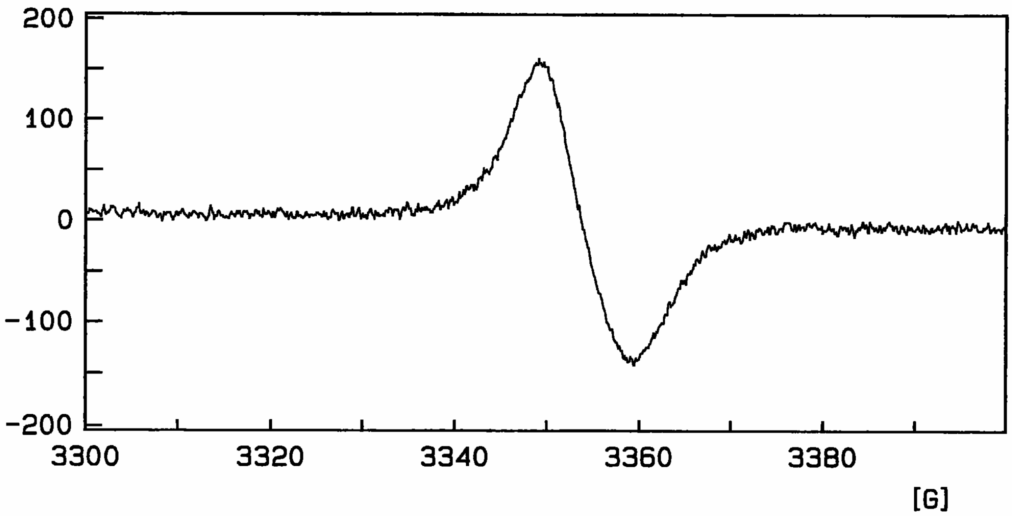
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