Preliminary results from an evaluation of the use of an oxygen absorber for prolonging the storage life of packaged soybean seed

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Abstract

A preliminary study has been conducted to evaluate the potential of using 'Ageless' (an oxygen absorber developed for the food industry) to increase the preservation of germinability and vigour of packaged seeds during storage. Seeds of soybean (*Glycine max* (L.) Merrill) cv. Davis were stored for up to 28 days in a simulated ageing system (35°C and seed moisture contents (SMC's) of 9.5, 13 or 18%). During this time all seeds were stored in water and oxygen impermeable plastic-aluminium foil laminate packages.

The inclusion of 'Ageless' in these packages gave a small protective effect in seed germinability at 13% and 18% SMC after 7 days storage, but little or no advantage thereafter. At 9.5% there was little loss of germinability after 28 days in either treatment, but losses in seed vigour were apparent. The presence of 'Ageless' significantly protected the mean seedling dry weight of normal germinants at this SMC.

Effects of the oxygen absorber on conductivity of seed leachate and fat acidity both showed interactions with seed moisture and storage time. At 18% SMC, seeds stored for 7 days with 'Ageless' showed significantly reduced conductivity and free fatty acid levels compared to controls. However, over longer storage periods at this moisture content, anaerobic conditions in the package significantly increased the levels of both these parameters.

This preliminary study suggests that there may be scope for using oxygen absorbers in packaging systems for seeds for sowing, but interactions with seed moisture and the possible proliferation of anaerobic micro-organisms need careful characterisation.

Additional key words: *conductivity, fat acidity, germination, viability, vigour*

Introduction

Seed packaging is an appropriate option for small to medium scale seed storage, especially when the maintenance of suitable storage conditions is expensive or impossible. The efficiency of packaging may be increased by modifying the atmosphere inside the package. Such modifications may be used to slow down tissue metabolism and thus delay deterioration (Zagory and Kader, 1988). Furthermore, many deteriorative reactions in seeds during storage are known to be oxidative ones (e.g., Harman and Mattick, 1976; Priestley, 1986).

Accordingly, it is not surprising that ambient oxygen levels have been reported as deleterious to seed survival for a range of species (e.g., Roberts and Abdalla, 1968; Justice and Bass, 1979; Pixton, 1980; Ohlrogge and Kernan, 1982). There is, however, some question as to whether complete removal of oxygen may itself be detrimental to seed survival in store (e.g., Justice and Bass, 1979).

Nevertheless, there have been reports (e.g., vacuum storage of soybean, New, 1988; or storage of grains under nitrogen, Di Maggio, 1980; Bason *et al.*, 1985) showing successful enhancement of seed storage when ambient oxygen levels are low or zero. One key factor to emerge from this type of study has been the importance of interacting variables such as moisture content and microfloral contamination of the stored seed.

This paper reports our preliminary evaluation of the novel approach of using a chemical oxygen absorber for the preservation of the germinability and vigour of stored seeds using packaged soybean (*Glycine max* (L.) Merrill) as a model system. Seeds were stored in simulated ageing environments at various moisture contents in the presence of a ferrous oxide based oxygen absorber, 'Ageless' (© Mitsubishi Gas Chemical Inc.). This oxygen absorber was developed for the food industry and has the capacity to reduce the oxygen content inside an oxygen proof package to less than 100 ppm.

Materials and Methods

Seed material

Seeds of soybean, cultivar Davis were purchased from Wright-Stephenson and Co. (Australia) Pty.Ltd., Sydney. Initial germination was 86%. The same seed lot was used throughout.

Packaging and oxygen absorbers

The packages used in this study were 12/20/50 µm polyester/aluminium foil/polythene laminates (Printpak-UEB, N.Z.) which are expected to be oxygen proof when properly sealed (Mitsubishi Gas, 1989).

'Ageless' S-50 was selected as the most appropriate version of the product for the SMC of the seeds and the amount of airspace within the package. This can be expected to remove the oxygen from the sealed package within 12-48 hours. Its selection was based on the estimated water activity of the seeds and the calculated airspace inside the package (Mitsubishi Gas, 1989). As a check, 'Ageless-Eye', an oxygen indicator was also added to each package containing 'Ageless'. These tablets remain pink at oxygen concentrations less than ≤0.1% and change to blue when levels rise to ≥0.5%. The condition of the tablets on opening all but one of the packages indicated that the presence of 'Ageless' maintained the ambient oxygen level at ≤0.1%. Where the tablet indiciated that oxygen levels were above 0.1% in one of the packages stored for three weeks, it is likely that the problem was caused by an incomplete heat seal or pin-hole damage to the packet.

Ageing technique

Artificial ageing was carried out using a modification of the controlled deterioration technique proposed by Matthews and Powell (1987b). The moisture contents of seeds were raised by adding the calculated amounts of water required to bring approximately 60g of seeds up to 9.5, 13 or 18% SMC in each package. The laminate pouches were then heat-sealed and the seeds equilibrated at 10°C for 48h to ensure an even distribution of moisture before storage for up to 21 (for 18% SMC) or 28 days at 35°C. One sachet of 'Ageless' S-50 and one tablet of 'Ageless-Eye' were included in half the packages before sealing. Each treatment was replicated four times. At the end of each storage period SMC's were checked for each seed sample. Moisture contents were determined by oven drying at 103°C (International Seed Testing Association, 1985).

Seed germination after storage

Standard germination testing was carried out according to ISTA Rules (1985) using the between paper method at 30°C. Seeds were dusted with thiram before sowing. Seed germinability (% normal germinants), seed viability and dry weights of normal seedlings (by oven drying 65°C for 4 days) were determined after 8 days.

Indirect measurements of seed vigour

Conductivity testing of seed leachate was carried out according to Matthews and Powell (1987a), having allowed weighed samples of seed to moisture equilibrate for 24h at 20°C before weighing and then soaking in deionised water for 24h.

Fat acidity was determined by a modification of the methods of Baker *et al.*, (1957) and Association of Official Analytical Chemists (1984). Whole seeds were pre-dried for short periods at 103°C to remove excess moisture so a fine flour could be prepared by grinding. Twenty grams of sample were then blended with 50 ml benzene for 5 minutes and 20ml of the resulting filtrate mixed with an equal volume of 95% (v/v) ethanol containing 0.1% phenolpthalein. The mixture was then titrated with KOH. Percentage fat acidity was calculated as mg KOH required to neutralise the free fatty acids from 100g dry weight of seeds.

Results

Data in Table 1 show the results for initial SMC's (after equilibration at 10°C) and SMC's after the longest period of storage used for each treatment. Deviations from the nominal SMC at the start of storage may indicate pre-equilibration during the 10°C incubation period, but are not believed to affect the interpretation of the results.

TABLE 1: Moisture contents of soybean seeds after storage at 35°C.

Nominal SMC	Initial SMC[1]		Final SMC[2]	
	-'Ageless'	+'Ageless'	-'Ageless'	+'Ageless'
9.5%	9.5 (±0.04)[3]	9.4 (±0.10)	9.5 (±0.05)	9.5 (±0.06)
13%	12.5 (±0.25)	11.9 (±0.12)	13.0 (±0.03)	12.9 (±0.09)
18%	17.3 (±0.60)	17.2 (±0.25)	17.0 (±0.17)	17.8 (±0.12)

[1] After 48h equilibration at 10°C
[2] After 28 days at 35°C for 9.5% and 13% SMC, but only 21 days at 35°C for 18% SMC
[3] Values are means of four replications (±SEM).

Seed germinability and seedling growth

The effects of controlled deterioration on the germination performance of seeds after storage at 35°C, 13% or 18% SMC are shown in Figure 1. Seeds lost germinability rapidly at 18% SMC, there being less than 10% normal germinants after 14 days and none after 21 days. 'Ageless' seemed to have a small protective effect up to 7 days ($p<0.10$), but none thereafter. At 13% SMC, where deterioration was slower, seeds in packages containing 'Ageless' consistently performed marginally better than controls, but a significant advantage ($p<0.05$) was noted only after 7 days storage. These increases in germinability are a function of increased proportions of normal seedlings. There was no clear evidence of a protective effect of 'Ageless' on overall seed viability, although, after 28 days storage at 13% SMC, seeds stored with 'Ageless' retained 85% viability compared to 75% for controls ($p<0.05$). In contrast, the presence of 'Ageless' was detrimental to seeds held for 14 days at 18% SMC where mean viability was 69% for controls and only 50% for seeds stored in the presence of the oxygen absorber ($p<0.01$).

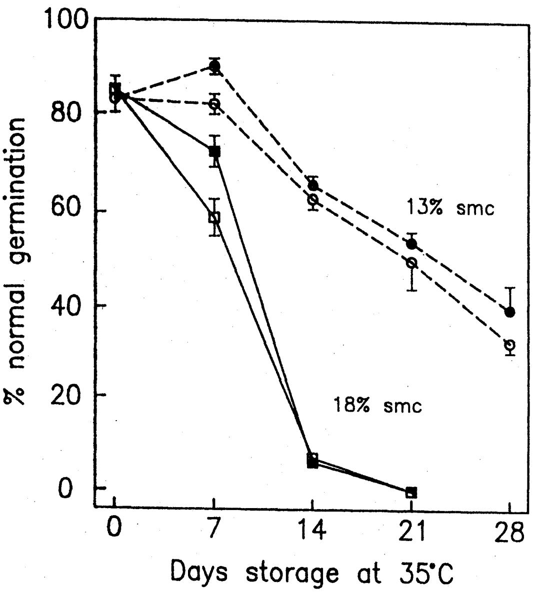

Figure 1: Changes in % normal germination (germinability) of seeds stored at 13% or 18% SMC at 35°C with (closed symbols) or without (open symbols) the oxygen absorber 'Ageless'. Data are means of four replications. Individual SEM's are shown where larger than the symbols used.

Seeds stored at 9.5% SMC for 28 days retained high germinability and viability irrespective of whether 'Ageless' was present or not (data not shown). However, there was an indication that 'Ageless' helped preserve some seedling growth capacity as the seeds deteriorated. Mean normal seedling dry weights were 46 (±0.4) and 43 (±0.5)mg, stored with and without 'Ageless' respectively ($p<0.01$). At 13% or 18% 'Ageless' had no significant effect on seedling dry weight after storage (data not shown).

Indirect measurements of seed vigour

'Ageless' had little effect on the changing conductivity of leachate from seeds stored at 13% SMC, but at 18% there was a significantly ($p<0.01$) smaller increase in conductivity as a result of the inclusion of 'Ageless' in the seed package for 7 days storage (Fig. 2). However, after 14 or 21 days at this SMC, the presence of the oxygen absorber resulted in a significant increase ($p<0.01$) in the leachate conductivity. For all four treatments (storage at 13% or 18% SMC, ± 'Ageless') there were highly significant negative correlations between conductivity of seed leachate and seed germinability (minimum R^2 = 82.8%).

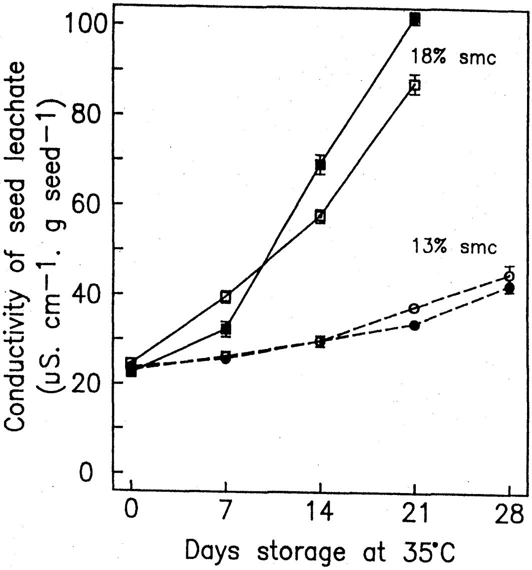

Figure 2: Changes in conductivity of seed leachate from seeds after storage at 13% or 18% SMC at 35°C with (closed symbols) or without (open symbols) the oxygen absorber, 'Ageless'. Data are means of four replications. Individual SEM's are shown where larger than the symbols used.

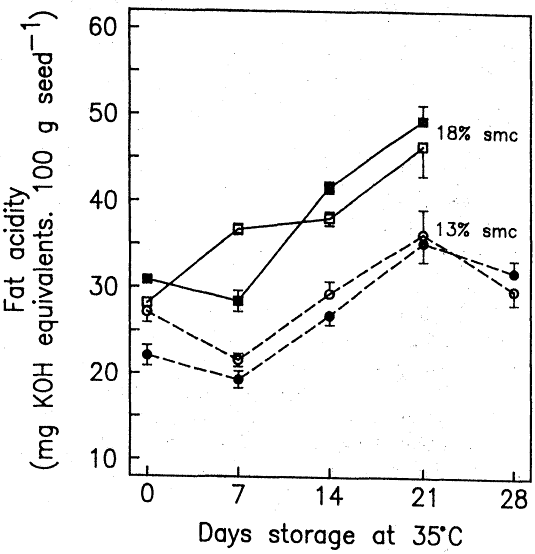

Figure 3: Changes in fat acidity of seeds after storage at 13% or 18% SMC at 35°C with (closed symbols) or without (open symbols) the oxygen absorber, 'Ageless'. Individual SEM's are shown where larger than the symbols used.

After 28 days at 9.5% SMC, the conductivity of leachate from seeds stored in the presence of 'Ageless' was similar to that of unaged seeds, being 24.720 (±0.28) µs $cm^{-1}.g\ seed^{-1}$. That of the aged controls was significantly higher: 28.120 (±0.96) µs $cm^{-1}.g\ seed^{-1}$ ($p<0.05$).

Changes in fat acidity of seeds stored at 18% SMC followed a broadly similar pattern to conductivity changes (Fig. 3). Levels of free fatty acids in the seeds rose with time, but the presence of 'Ageless' inhibited their formation in seeds stored for 7 days ($p<0.01$). However, after 14 days seeds stored with 'Ageless' had significantly higher levels of fat acidity than the controls ($p<0.05$). At 9.5 (data not shown) or 13% SMC the presence of 'Ageless' had no significant effects on free fatty acid levels of seeds stored at 35°C.

At 18% SMC there were close and highly significant correlations between fat acidity and conductivity in aged seeds ($R^2 = 90.8$ and 82.3% for plus or minus 'Ageless' respectively). At 13% SMC correlations were lower, that for seeds stored without 'Ageless' having a coefficient of determination of less than 20% (n.s.).

Discussion

The results of this preliminary study show only marginal protective effects of 'Ageless' on the germination performance of soybeans, with evidence that the presence of the oxygen absorber increases the level of seed deterioration during prolonged storage at high moisture contents. Nevertheless, we feel that this project merits further development and continued evaluation of the system. This discussion centres on issues which might form the focus of future research.

One problem with artificial ageing systems such as those used here is that the deterioration process is very rapid and when sampling is on a weekly basis it is often easy to miss short periods during storage when significant benefits become evident. There has also been considerable discussion about whether the physiological mechanisms of deterioration in this kind of system parallel events occurring during seed storage in commercial practice (e.g., Priestley, 1986), despite the fact that such systems have proved very useful in the construction of predictive models of seed storage life (e.g., Ellis and Roberts, 1981). It should be noted that 8% is the recommended safe sealed moisture content for soybean (Justice and Bass, 1979), although the seed moisture and temperature conditions used here are similar to those routinely encountered in the tropics (e.g., Nour/Jantan *et al.*, 1988).

Both the conductivity of seed leachate and fat acidity measurements are indirect assessments of seed quality, but both are at least partly related to membrane damage (e.g., Powell and Matthews, 1981 and Earnshaw *et al.*, 1970). We take this as preliminary evidence that, at least for a short time, storage of seeds with 'Ageless' has the capacity to protect seed viability by inhibiting the oxidation of lipids. Nevertheless, it is clear that there are complex interactions with other deteriorative events in the seed and that interactions with seed moisture content are crucially important. For example, one possibility might be at 18% SMC, 'Ageless' may be effective on atmospheric oxygen but cannot control dissolved oxygen already present in significant quantities in the seed at high moisture levels. In any case, further work will require more careful monitoring of oxygen levels in the system.

One key area for exploration is the observation that after storage for 14 or 21 days at 18% SMC, inclusion of 'Ageless' is deleterious to the seed. In his studies on storage of grains under nitrogen, Di Maggio (1980) reported that advantages at high seed moisture were only short term because seeds were prone to mould attack. It is likely that a similar situation is occurring here in that these conditions are favourable for the proliferation of anaerobic micro-organisms. Microbial lipase action may be responsible for both the increased membrane leakage and free fatty acid production observed under these conditions. One circumstantial piece of evidence for this view is the apparent increase in seed moisture content incurred by seeds stored at 18% SMC in the presence of ageless (Table 1). Clearly a major thrust for future work must be attempts to evaluate and control the effects of any anaerobes.

One final area of research that could be usefully developed, which is worth mentioning here, centres on the package itself. By using a high value laminate we have eliminated any problems of oxygen leakage into the stored seeds. However, the costs of such laminates may be prohibitive for many agricultural systems. The feasibility of using 'Ageless' in conjunction with cheaper oxygen-permeable materials also warrants further investigation.

Acknowledgements

Mrs Wanna Tungjaroenchai was funded by an Official Development Assistance postgraduate study award granted by the New Zealand Ministry of External Relations and Trade as part of the country's bilateral aid programme.

We are indebted to Mitsubishi New Zealand Ltd., for their interest in this project and their generous gifts of 'Ageless' and 'Ageless-Eye'.

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