Interactions between seed moisture content and solvent damage in seed treatment of soybeans

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Abstract

Acetone has often been recommended as a suitable solvent for non-aqueous seed treatment when it is desirable to introduce chemicals such as pesticides, plant growth regulators or even antioxidants into dry seed. We have, however, detected toxicity problems with this solvent in a range of species. Seeds of a high vigour lot of soybean, cv. Amsoy, showed a small decrease in germinability when soaked in acetone for between 2 and 16 hours, but the resistance of acetone treated seeds to 3 days controlled deterioration (storage at 20% seed moisture, 40°C) was markedly impaired and became more severe with increasing duration of exposure to the solvent. Susceptibility to acetone damage varied considerably between seed lots; seeds showing visible signs of mechanical damage being particularly susceptible.

Further studies with cvs Amsoy and Davis showed that there is a considerable interaction between seed moisture content (SMC) and acetone toxicity, damage being considerably reduced as SMC is lowered. Six percent is recommended as being a safer SMC for acetone treatment. Seeds soaked in acetone at higher SMC's show significant levels of phospholipid deletion from the embryo axis during artificial ageing.

Seeds at higher moisture contents show much less sensitivity to solvent damage when soaked in hexane, a solvent with a lower dielectric constant than acetone.

Additional key words: *acetone, hexane, controlled deterioration, artificial ageing, phospholipid, seed germinability*

Introduction

Recently there has been renewed interest in the use of organic solvents - particularly acetone - for the introduction of chemicals into dry seeds. This approach would seem to have considerable potential as a seed treatment technique where the compounds to be applied are of low water solubility or the seeds are liable to suffer from soaking injury. Successes have been reported with a variety of species using pesticides (Ekenrode *et al.*, 1974), fungicides (Shortt and Sinclair, 1980), plant growth regulators (Petruzzelli and Taranto, 1985; Persson, 1988) and (our own special interest) anti-ageing compounds (e.g., Parrish and Bahler, 1983; Gorecki and Harman, 1987; Dey and Mukherjee, 1988).

Acetone is generally the favoured choice of solvent because of its properties as a good carrier (Ekenrode *et al.*, 1974; Persson, 1988) and its low toxicity to seeds (Milborrow, 1963; Dadlani and Agrawal, 1985; Persson, 1988). However, there is some uncertainty about the possibility of acetone causing damage to seeds and the nature of other factors which might interact with the solvent. Thus, while Milborrow (1963) reported no deleterious effects after soaking peas and several other species in acetone for three months or longer and Lewis *et al.* (1979) found no problems with 24h soaks of peas and soybeans, these latter workers did find injurious effects of acetone on snapbean, an observation confirmed by Muchovej and Dhingra (1980).

In some cases toxic effects of acetone have been reported to be associated with the presence of major impurities in the solvent (such as 10-20% water, Lewis *et al.*, 1979). In other work, solvent damage has only manifested itself in aged seeds (e.g., Khan *et al.*, 1973 and Gorecki and Harman, 1987, working with lettuce and peas, respectively).

Our aim in the study reported here was to characterise the toxic effects of acetone on soybean seeds

and undertake a preliminary investigation into the nature of the mechanisms involved. Attention was focused on interactions of toxicity with soaking duration, seed lot, ageing and initial seed moisture content. Recently published work on the storage of pollen grains (Jain and Shivanna, 1988) suggests that the toxicity problems with acetone on pollen arise from its relatively high dielectric constant. Accordingly, a comparison of acetone treatment with the much less polar solvent, hexane, was also undertaken.

Materials and Methods

Seed material

Five seed lots of cv. Amsoy and one lot of cv. Davis were used in this study. All lots were harvested in 1988, with the exception of Amsoy, lot A (1987 harvest). Amsoy lots A, B and C were all of high initial germinability and were, respectively, purchased from Wrightson's Ltd., (now Challenge Seeds Ltd.) Palmerston North, N.Z.; Corson Grain Ltd., Gisborne, N.Z. or grown in the Seed Technology Centre's own experimental plots. A low germination sample of cv. Amsoy (lot D) was also gifted to us by Corson Grain Ltd. Lot E was also obtained from Corson Grain Ltd., and was visually sorted into mechanically damaged or undamaged sub-lots. The seedlot of cv.Davis was imported from Wright Stephenson & Co. (Australia) Pty. Ltd.

Solvent treatments

Analar grade acetone and Hipersolv hexane (BDH Chemicals, N.Z.Ltd.,) were used as supplied. A trial with peas had previously shown that redistilling did not ameliorate any toxicity problems found using the normal stock solvent (data not shown). Seeds were soaked by immersing each replicate in pure solvent for up to 16h at a constant 20°C. Following soaking, samples were spread out on absorbent paper and left to dry at 20°C for 24h, except in one experiment where seeds were dried at either 20°C or 35°C for up to 48h.

Ageing treatments and seed moisture adjustments

Accelerated ageing (AA) treatment was carried out using the technique described by Baskin (1987), holding seed at 100% RH, 40°C for up to 6 days. Controlled deterioration (CD) was carried out by a modification of the method of Matthews and Powell (1987). Seeds were brought up to a moisture content of 20% by adding the calculated amount of distilled water to a weighed sample of seeds of known initial moisture content and then equilibrating overnight at 10°C in heat-sealed, moisture proof, polyester-aluminium foil - polyethylene laminated packages. Following equilibration, seeds were held at 40°C for up to three days.

Where seed moisture content (SMC) required adjustment prior to acetone treatment, a similar method to the above was employed to raise the water content. Alternatively, to reduce the initial SMC, seeds were held over silica gel in desiccators at room temperature until the calculated water loss had been achieved. In all cases actual moisture contents were determined for each replicate sample in these experiments. SMC's were determined by the oven method according to Internatioal Seed Testing Association Rules (1985).

Seed germination testing

Germination trials were normally conducted on 50 seeds per replicate kept at a constant 25°C in the dark. The between paper method (ISTA, 1985) was used, recording percentage normal seedlings 8 days after sowing. Seeds were dusted with thiram prior to setting up the germination trials. Percentage seed viability (normal and abnormal germinants plus fresh non-germinated seeds) was also recorded, as were the fresh and dry weights of embryonic axes of normal seedlings 8 days from sowing.

Phospholipid analysis

The lipid fraction was extracted as described by Francis and Coolbear (1984). Twenty axes or an equivalent weight of cotyledons were boiled for 2 minutes in 1 ml water saturated butanol (WSB) containing 100 µg butylated hydroxytoluene. The tissue was then hand ground to a slurry and washed into a centrifuge tube with a further 1 ml WSB. After centrifuging for 10 min. at 1300 G, the supernatant was decanted and the precipitate re-extracted with a further 2 ml WSB and recentrifuged. The two supernatants were then combined and the volume of the fraction measured before taking a 200 µl aliquot for determining the phospholipid content according to the method of Bartlett (1959). Three replicate extractions were carried out for each treatment.

Data analysis

Four replications were used in all experiments with seed lots of cv. Amsoy, but, because of limited supplies of cv. Davis, only three replications were used. A split plot design was used to analyse the interactions between duration of acetone soaking and controlled deterioration, while randomised complete

block analyses were used for the phospholipid data. Otherwise individual standard errors of means were calculated. Where combined estimates of standard error were obtained an arcsine √% transform was employed.

Results

Effects of soaking seeds in acetone

Figure 1 shows the effects of duration of soaking on seeds of cv. Amsoy, Lot A. There was a small, but significant ($p<0.05$) toxic effect of acetone on unaged seeds after just 2h soaking, when the percentage of normal germinants fell from 95 to below 90%. Apart from that, there were no other clear toxic effects of acetone in unaged or 1d aged seeds. However, toxic effects were clearly evident on these seeds when they were subjected to 3d CD after acetone treatment. Damage increased dramatically with the duration of acetone treatment up to 16h.

In order to confirm that all the acetone in the seed was completely removed after soaking for 16h, a comparison was made between the germination behaviour of seeds dried at 20°C and 35°C for 24h or 48h after acetone treatment. Data are presented in Table 1 for unaged seeds and those subjected to 3d CD. Longer drying times or higher drying temperatures did not significantly reduce the level of acetone damage.

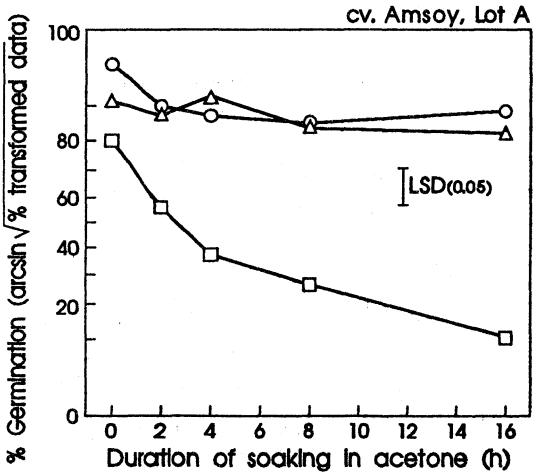

Figure 1: **The effects of duration of soaking in acetone on normal germination of soybean seeds, cv. Amsoy, Lot A. Data are presented as arcsin √% transformed means of germination after 0 (O), 1 (Δ) and 3 days (□) CD following acetone treatment. The initial SMC of the seeds before treatment was 10.9%.**

Seed lot variation

Figure 2 shows that, although all three high germination lots show susceptibility to acetone damage, there was considerable variation in responses between seed lots. Unaged seeds of the high vigour lots A and B do not show significant loss of germination after 16h acetone treatment. Seed lot C was of medium vigour (as assessed by preliminary germination counts and seedling growth evaluation, data not shown) and unaged seeds of this lot were severely damaged by acetone. In all three lots there were highly significant toxic effects of the solvent on soaked seeds which were then subjected to 3 days CD. Interestingly, no effects of acetone were detected on the small numbers of unaged germinable seed remaining in the poor quality seed lot D.

In another experiment (Fig. 3) evidence was obtained showing that one factor affecting the toxicity of acetone was the extent of mechanical damage in the seeds. Cracked seeds were much more sensitive to acetone damage. The addition of methyl red to the solvent showed that in intact seed there was little or no movement of dye beyond the seed coat, compared to extensive penetration in mechanically damaged material.

Table 1: **The effects of extended and higher temperature drying on normal germination of seeds of cv. Amsoy, lot A after 16h soaking in acetone. Data are means of four replications and are presented as backtransformed values with arcsine √% transformed data (radians) in brackets.**

Acetone treatment	Drying conditions	% normal germination unaged	After 3d CD
Control	none	98 (1.43)	86 (1.18)
Acetone	20°C, 24h	84 (1.16)	0
	20°C, 48h	85 (1.17)	0
	35°C, 24h	80 (1.11)	0
	35°C, 48h	91 (1.26)	2 (0.04)
$LSD_{0.05}$		(0.17)	(0.10)

Effect of changing seed moisture content

While it can be concluded from Figure 2 that variation between seed lots in initial seed moisture contents had little impact in determining a seed lot's behaviour, variation of SMC within a seed lot was of crucial importance in determining the resistance to acetone damage of that lot, as was the level of mechanical damage (Fig. 3). As can be seen from the figure the extent of damage caused by solvent treatment was much reduced if initial seed moisture content was lowered to around 6% before soaking in acetone. This effect has now been shown in a total of four seed lots (3 cv. Amsoy and 1 cv. Davis). Of course, the extent of damage varied between lots and

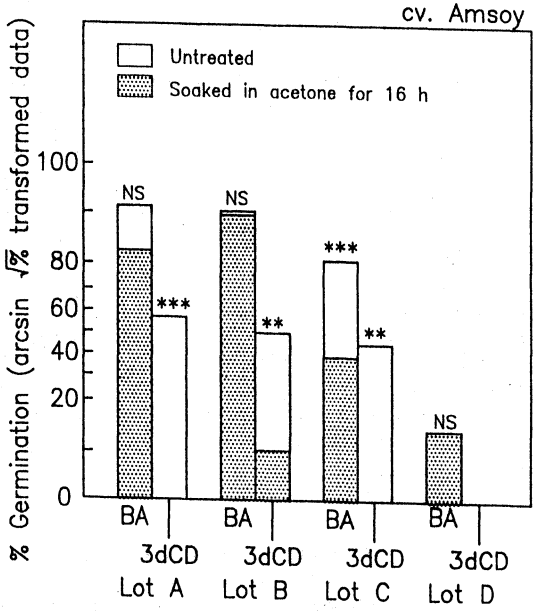

Figure 2: The responses of four different seed lots to 16h soaking in acetone either before ageing (BA) or with a subsequent controlled deterioration treatment (CD) for 3 days at 40°C, 20% SMC. Data presented are the arcsin √% transforms of normal germination. NS: not significant; ** and * significant differences between untreated and acetone treated seeds (p<0.01 and p<0.001, respectively). Initial SMC's of the four lots, A-D, before soaking were 15.1, 11.1, 10.1 and 11.1%, respectively.**

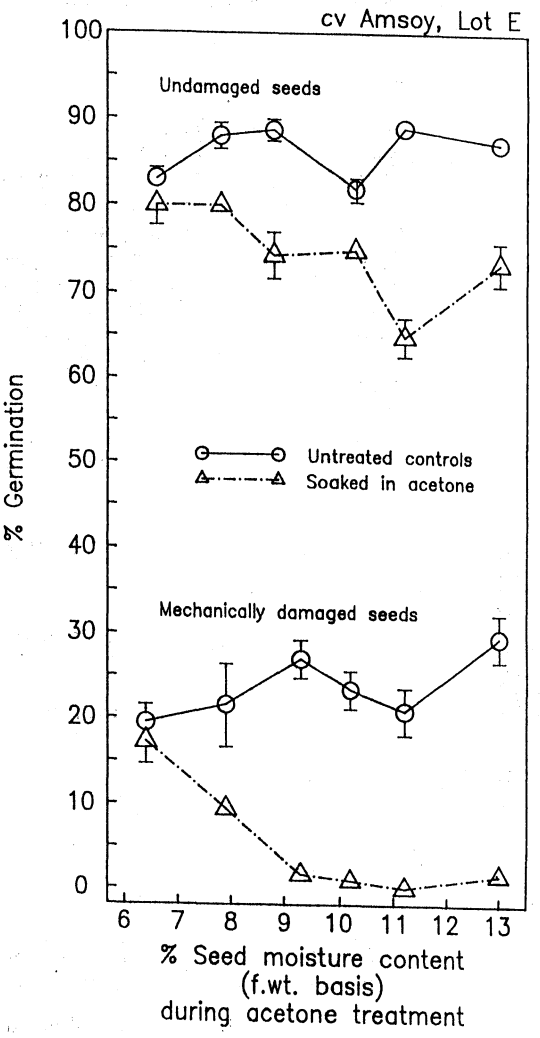

Figure 3. The effects of initial seed moisture content and visible mechanical damage on the germinability of unaged seeds after a 16h soak in acetone. Data are the means of four replications. Individual SEM's are shown where larger than the symbols used.

level of cracking within a seed lot, but in each case the pattern of response was similar. Careful seed drying before treatment also offered some protection to soaked seeds of cv. Davis on subsequent accelerated ageing (data not shown).

The mechanisms of acetone toxicity

Analysis of the phospholipid (PL) content of untreated or acetone soaked seeds showed that acetone treatment greatly increased the rate of PL deletion from both axes and cotyledons during accelerated ageing (AA), (Fig. 4). Untreated seeds showed no significant losses of phospholipid from axes and only a small significant ($p<0.05$) decrease (~20%) in cotyledonary PL after 6 days AA. Acetone soaked seed showed significant losses ($p<0.01$) in this tissue after 4 days AA and more than 35% deletion after 6 days. A similar rate of loss was also detected in axis tissue. Under these storage conditions, untreated seeds lost nearly 40% of their germinability after 4 days AA, whereas acetone treated material lost nearly 75% germinability (Fig. 5a). After 6 days AA untreated seeds had only 12% normal germinants, acetone treated seeds none.

Figure 5 also presents a comparison of the effects of hexane and acetone treatment on seeds of cv. Davis. Hexane is less deleterious to the seeds and there is no interaction with ageing.

Discussion

Factors affecting acetone toxicity

It is clear from these results that, despite reports to the contrary (e.g., Lewis *et al.*, 1979), there is a toxicity problem when acetone is used as a solvent to deliver chemicals to soybean seeds. This work shows that the level of damage depends on variations between seedlots, level of mechanical damage, initial seed moisture content prior to acetone treatment, duration of acetone treatment and also on subsequent storage after treatment.

The observation that mechanically damaged seeds were more prone to acetone damage is in agreement with Shortt and Sinclair (1980) who found that acetone killed cotyledon tissue in soybean with cracked seedcoats. Halloin (1977) similarly reported that while damaged cottonseed or excised embryos were injured by acetone, the germinability of intact seeds was unimpaired. Nevertheless, it is clear that this is not the only factor involved. In the studies detailed here lots A-C and cv. Davis had low levels of visible mechanical damage. Similarly, Tao and Khan (1974) reported that although acetone reached the embryos of cucumber or squash seed, it did not cause any apparent damaging effects on seeds germinated immediately after treatment.

Our data on the interaction between acetone and initial seed moisture contents complement similar results found for peas (Coolbear, McGill and Sakunnarak, unpublished data). These findings appear to be novel in that no other workers have identified this interaction. Only two relevant reports have been found in the literature, both mentioning increased toxicity of solvents when they have 10% or more water added to them (Lewis *et al.*, 1979; Persson, 1988).

Possible mechanism of action

The results in Figure 4 show that the interaction between acetone toxicity and ageing is related to losses of phospholipid from axis and cotyledon tissue, a mechanism originally suggested by Halloin (1977), although he presented no substantiating evidence apart from increased conductivity of leachate from acetone soaked embryos. It should be noted that our data present no evidence of PL losses in unaged acetone treated seed. It is worth speculating that penetration of acetone into cell membranes results in a disruption of the crystalline lipid bilayer which exists in dry tissue (Crowe *et al.*, 1989). This disruption could be exacerbated in higher SMC seeds if the solvent can bring water molecules into the apolar region of the membrane. High vigour seed may have some capacity for repair of this kind of damage, but this may become increasingly difficult for low vigour or ageing seed.

This hypothesis explains many of the observations reported here and merits further investigation. Nevertheless, the evidence obtained on PL losses to date is purely correlative, although Swanson *et al.*, (1973) reported from their electron microscope work that acetone disrupted membranes in the cells of tobacco leaves. However, it could be that acetone causes damage via other effects. For example, Meyer and Mayer (1971) found that acetone depressed oxygen uptake of lettuce seeds while Eldan and Meyer (1974) reported a decrease in invertase activity. In neither case, however, was germinability affected.

The use of a solvent with a lower dielectric constant such as hexane (Fig. 5b) could be safer than acetone because of its ability to penetrate dry membranes without disrupting the amphoteric structure of the bilayer. We are unaware of any other reports of its use as a solvent for seed treatment, but its cost and toxicity to humans may prohibit its routine use.

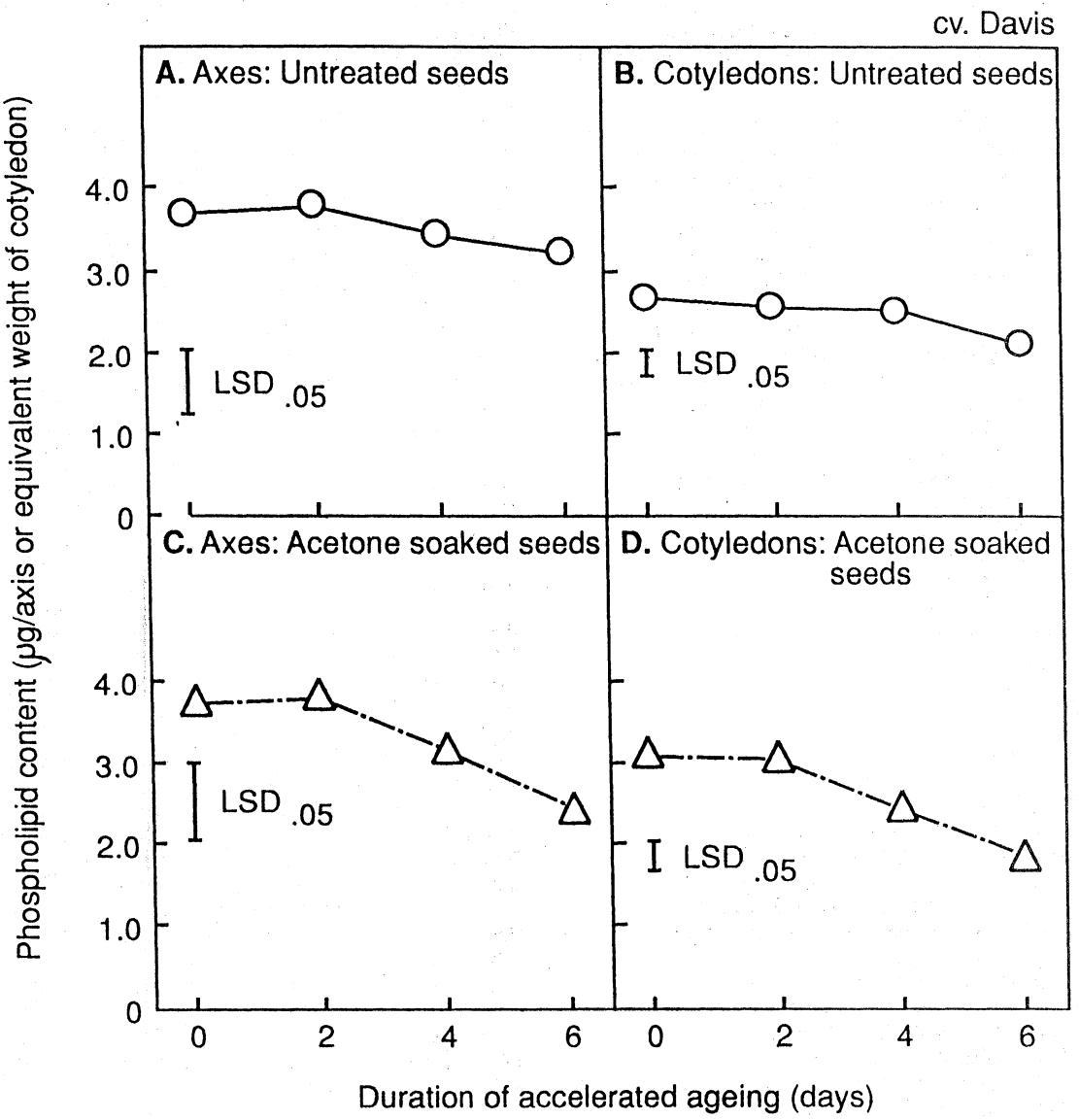

Figure 4: **The effects of acetone soaking on the phospholipid content of axis and cotyledonary tissue of soybean seeds cv. Davis subsequently subjected to accelerated ageing treatment. The initial SMC of seeds before acetone treatment was 9.3%.**

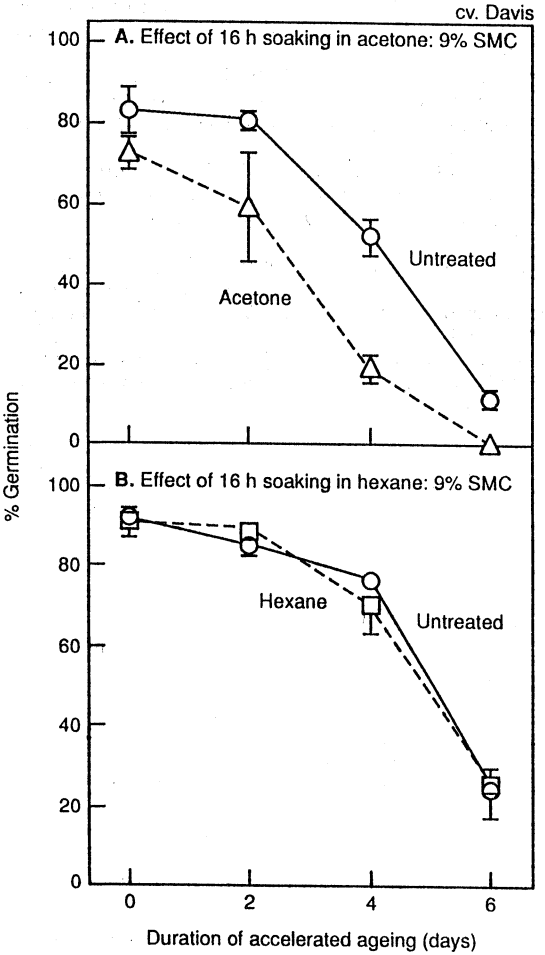

Figure 5. The effects on germination of soaking seeds of cv. Davis in either acetone (A) or hexane (B) for 16h followed by accelerated ageing treatment (100% R.H., 40°C) for up to 6 days. Individual SEM's are shown when larger than the symbols used.

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