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Recalcitrant Seed Storage Physiology in Three Aquatic Grasses (*Zizania palustris, Spartina anglica* and *Porteresia coarctata*)

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ABSTRACT

The moisture content/probit viability relationship for stored seeds of *Zizania palustris* L. and *Spartina anglica* C. E. Hubbard was linear and independent of the rate of embryo drying. These results provide firm evidence of recalcitrant storage physiology in these taxa. Preliminary tests strongly suggest that fresh seeds of *Porteresia coarctata* (Roxb.) Tateoka are also intolerant of desiccation.

In *Z. palustris* apparent differences in desiccation tolerance between individuals can be partly explained by wide variation in individual embryo moisture contents during desiccation. Long-term storage experiments in solutions of polyethylene glycol 6000 (PEG) suggest that the actual variation in desiccation tolerance is confined to a narrow range of embryo water potentials in the range −2 to −3 MPa.

Despite the presence of prolonged dormancy in seeds of *Z. palustris* and *S. anglica* there is no evidence of a significant effect of dormancy or storage period (up to the point of visible germination) on the limits of desiccation tolerance.

Key words: Aquatic grasses, seeds, storage, desiccation intolerance.

INTRODUCTION

In a survey of the seed storage behaviour of 43 aquatic plant species representing 30 genera and 20 families Muenscher (1936) reported that desiccation actually killed the embryos in only four of the genera studied. Changes in germination behaviour during storage in the majority of species included in the survey were apparently associated with changes in dormancy. Of the two grasses included in Muenscher's paper results obtained for *Zizania aquatica* L. supported earlier observations (Brown and Scofield, 1903; Duvel, 1906) that seeds of this species are intolerant of desiccation. Results obtained for the second species (*Glyceria striata* Hitchc.), however, showed that some seeds remained viable even after 7 months of dry storage in the laboratory. These results strongly suggest that seeds of *Glyceria* are in fact orthodox and the inclusion of this species in a list of putative recalcitrant seeds (King and Roberts, 1980*a*) is therefore probably erroneous.

There have been several well reported cases where orthodox seeds have been misclassified as recalcitrant (for a review see Roberts, King and Ellis, 1984). Whilst there are at least nine possible causes which underlie the misinterpretation of results; loss of viability during the desiccation phase due to slow drying or the development of a germination constraint (in some cases dormancy) are amongst the most important. Kotobuki (1978) reported that the viability of Japanese persimmon seeds was dependent on moisture content irrespective of the rate of desiccation. King and Roberts (1980*b*) suggested that the use of different rates of desiccation would be a useful approach in the study of recalcitrant seeds. Use of such methodology enables a precise characterization of the relationship between moisture content and viability. For recalcitrant seeds this relationship should be independent of drying rate and loss of viability can be expected at a fairly high critical moisture content. Orthodox seeds – by definition – characteristically display an inverse relationship between moisture content and longevity.

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As a result the use of an increased rate of desiccation would be expected to cause a downward shift in the moisture content/viability relationship for an orthodox seed.

Using the above criteria detailed studies using different rates of desiccation have been used to quantify the storage physiology of a wide range of tropical timber species in the *Dipterocarpaceae* (Tompsett, 1985,1987) and *Araucariaceae* (Tompsett, 1982, 1984) families. For recalcitrant species in these taxonomic groups analysis has revealed a direct linear relationship between the probit of germination and moisture content and a wide variation in desiccation tolerance between individual seeds within populations has been implicated.

In the grasses, germination studies on seeds of *Z. aquatica* and the closely related species *Z. palustris* have tended to concentrate on dormancy problems (Simpson, 1966; Cardwell, Oelke and Elliot, 1978; Atkins, Thomas and Stewart. 1987) and although it is widely accepted that seeds of these species are intolerant of desiccation. we are not aware of any detailed storage studies providing unequivocal evidence that they are truly recalcitrant.

There is some evidence in the literature that other aquatic grass species may produce recalcitrant seeds. In a report on the germination of *Spartina* × *townsendii* H. and J. Groves (*S. anglica* C. E. Hubbard, *sensu stricto*), Nelson and Munro (1934) clearly demonstrated that storage treatments which resulted in a significant decline in caryopsis moisture content also resulted in greatly reduced viability. Similar effects of desiccation were also reported for seeds of the parental species *S. alterniflora* Loisel. (Mooring, Cooper and Seneca, 1971). It is worthy of note that whilst the *Zizania* and *Spartina* species described here are temperate in origin and occupy broadly similar ecological habitats the two genera have been ascribed to different subfamilies of the *Gramineae* and are therefore considered to be phylogenetically unrelated (Clayton and Renvoize, 1986).

In this report we present unequivocal evidence that stored seeds of *Z. palustris* and *S. anglica* are truly recalcitrant. Using different rates of desiccation the relationship between embryo moisture content and viability is accurately defined for both species. Variation in the moisture content of individual embryos is identified as a key source of apparent variability in desiccation tolerance between seeds, and the influence of dormancy and storage period on the limits of desiccation tolerance are considered.

Preliminary results which confirm recent observations (T. J. Flowers pers. comm., Ray, 1973) that freshly harvested seeds of the tropical grass *Porteresia coarctata* are intolerant of desiccation are also presented.

MATERIALS AND METHODS

Seed sources and routine handling

Seed of *Z. palustris* var. Netum was obtained from the University of Minnesota, USA, and regenerated at Wakehurst Place out of doors in irrigated concrete tanks. Plants were grown to maturity in a loam substrate which was flooded to a depth of approximately 10 cm. Following harvest seeds were routinely stored at 2 °C fully immersed in distilled water held in sealed plastic buckets. Every 2–3 weeks the water was drained off, seeds were rinsed and fresh water added. Under such conditions seeds remained viable for several months during which time dormancy steadily declined with germination occurring at 2 °C in the least dormant individuals after 6–7 months storage.

Seeds of *S. anglica* originally harvested from a wild population at Pagham Harbour Nature Reserve, West Sussex, were also regenerated at Wakehurst Place. Plants were raised in polythene pots filled with standard potting compost (John Innes), and supported on capillary matting which provided continuous irrigation. After harvest, seeds were surface sterilized for 5 mins in a 25% (V/V) solution of commercial bleach ('Domestos', Lever Bros.. UK) followed by rinsing with at least five changes of sterilized water and then allowed to surface dry for about 0·5 h in a flow of sterile air in a laminar flow cabinet. Seeds were then lightly dusted with Thiram wettable powder (Fargro Ltd) and then stored at 2 °C in plastic sandwich boxes (Stewart Plastics), which were floated on water in sealed polythene containers. Under these conditions ± 100% relative humidity (RH) was maintained and seeds remained viable for the duration of the present study during which time a steady decline in dormancy was recorded.

A small sample of seeds of *Porteresia coarctata* were obtained from T. J. Flowers, Sussex University, and regenerated in a warm glasshouse (night minimum 21 °C). Plants were raised in polythene pots filled with potting compost which were stood in trays of water up to a depth of approx. 5 cm. Following harvest seeds were transferred directly to experimental conditions.

Moisture content determination

Moisture content determined gravimetrically involved oven drying for 1 h at 130 °C according to ISTA recommendations for non-oily seeds

TABLE 1. *Summary of storage regimes*

Description of storage conditions	Temperature (°C)	Effective relative humidity (% RH)
Seeds air dried: Open storage, dry room	15	15
In sealed polythene box, silica gel	6	—
Over saturated salt solutions:		
$CaBr_2 . 2H_2O$	6	22
NaI	6	42
NaBr	6	62·5
NH_4Cl	6	82
$ZnSO_4 . 7H_2O$	6	94·8
KNO_3	6	96·5
Seeds dried fully immersed in sterile polyethylene glycol (PEG) 6000		
−10 MPa	1	92·4
− 8 MPa	1	93·9
− 6 MPa	1	95·4
− 4 MPa	1	96·9
− 3 MPa	1	97·7
− 2 MPa	1	98·4
− 1 MPa	1	99·2

(International Rules for Seed Testing, 1985) and is expressed throughout on a f. wt basis.

Germination

For *Z. palustris* and *S. anglica* drying experiments were carried out on seeds previously stored at 2 °C and as a result levels of embryo dormancy were low. Despite this some residual coat imposed dormancy was encountered in *Z. palustris* and it was necessary to remove the paleas and lemmas and to slit the pericarp above the embryo with a scalpel to ensure maximum possible germination. Following this treatment caryopses were immersed in approx. 40 cm^3 of distilled water held in 9 cm diameter Sterilin Petri dishes. Treatments were incubated at 16 °C.

S. anglica seeds were sown intact (paleas, lemmas and glumes attached) onto a plain agar substrate (approx. 30 cm^3) held in 9 cm Petri dishes. In this case treatments received daily alternating temperature cycles of 21/11 °C (12 h/12 h).

P. coarctata seeds were also sown intact with paleas and lemmas attached and held on a plain agar substrate. As *P. coarctata* is a tropical species a high temperature requirement for germination was anticipated and treatments received daily alternations of 33/19 °C (12 h/12 h).

In all cases seeds were incubated in temperature controlled incubators accurate to ±1·5 °C. illumination for 12 h each day (coincident with the warm phase in alternating temperature cycles) was provided in each incubator by a single 13 W Philips warm white fluorescent tube.

In the case of *S. anglica* and *P. coarctata* germination was recorded when the primary root and coleoptile were clearly visible. For *Z. palustris*, seedlings were allowed to develop further in germination tests and only those with normal primary and secondary root development and healthy shoots with well-formed first leaves were recorded.

Sample sizes for moisture content determination, and replicates per treatment varied according to the requirements of individual experiments and are detailed below.

Tetrazolium test

In a preliminary drying experiment on freshly harvested seeds of *P. coarctata*, viability was monitored using a buffered solution of 2,3,5-triphenyltetrazolium chloride (Ellis, Hong and Roberts, 1985). Caryopses were imbibed on wetted filter paper for 24 h at 26 °C following removal of the palea and lemma and slitting of the pericarp above the embryo. Caryopses were then transferred to tetrazolium solution and incubated in darkness for a further 24 h at 26 °C before evaluation of the distribution of staining by longitudinal disection of the caryopses through the embryo, and examination under a microscope.

In a drying experiment on dormant seeds of *Z. palustris* a similar procedure was followed except that the pericarp was scraped to expose the

TABLE 2. *The moisture content of component structures in seeds of* Zizania palustris *(fresh) and* Spartina anglica *(stored at 100% RH)*

	Moisture content (% of f. wt.)	
	Zizania ($n = 10$)	*Spartina* ($n = 5$)
Covering structures	31·7	22·8
Endosperm	26·8	27·2
Embryo	52·5	62·1
Whole seed	28·3	41·2

TABLE 3. *The contribution of component structures to total f. wt in hydrated seeds of* Zizania palustris *and* Spartina anglica

	Proportion of whole seed f. wt (%)*	
	Zizania	*Spartina*
Embryo (axis + scutellum)	8	35
Endosperm	72	20
Covering structures	20	45

Estimates based on a single 10-seed sample of each species.

embryo prior to imbibition and caryopses were imbibed for 48 h at 21 °C.

Experimental storage conditions

In order to study the effect of embryo moisture content on viability, using different rates of desiccation and to evaluate the relationship between equilibrium moisture content and relative humidity, a wide range of different storage regimes were used (Table 1). Seeds were either stored as intact seeds or as caryopses following the removal of the outer covering structures.

Concentrations of PEG required to produce the water potential (ψ) values indicated in Table 1 were calculated according to the equations of Michel and Kaufman (1973). Water potential was converted to percentage relative humidity using the equation:

$$\mathrm{RH} = \exp(\psi \bar{V}_w / RT) \times 100,$$

where ψ is the water potential expressed in megapascals (MPa), $\bar{V}_w$ is the partial molar volume of water (= 18 $m^3\ mol^{-1}$), R is the gas constant (8·31 $JK^{-1}\ mol^{-1}$) and T is the absolute temperature.

RESULTS AND DISCUSSION

The possibility of considerable differences in moisture content between seed parts has been reported previously (Grout, Shelton and Pritchard, 1983; Berjak, Dini and Pammenter, 1984). Since we were interested in embryo viability an assessment was made of the variation in moisture content between component tissues using freshly harvested seeds of *Z. palustris* and seeds of *S. anglica* previously stored at 100% RH.

In both species considerable variation was recorded (Table 2) and it was clear that measurements of whole seed moisture content would greatly underestimate the moisture content of the embryo. Moreover, large differences between the two species in the relative contribution of component tissues to the total f. wt (Table 3) precluded meaningful comparisons if whole seed moisture content determinations were used. In view of these findings embryo moisture contents were measured routinely. Quoted moisture content values for storage experiments refer to the mean of samples of individual embryos which were dissected from caryopses or intact seeds.

Storage experiments

The effect of drying on the viability of *Z. palustris* seeds was investigated in three separate experiments. Contrasting rates of desiccation were achieved by the use of different effective relative humidities (Fig. 1). A direct linear relationship between probit viability and moisture content was recorded which was independent of the rate of embryo desiccation (Fig. 2). The mean embryo moisture content below which a decline in viability was recorded was approx. 45%. These results provide unequivocal evidence that stored seeds of *Z. palustris* are truly recalcitrant. The wide variation in desiccation tolerance between individual seeds suggested by the data presented in Fig. 2 broadly agree with trends reported for other recalcitrant seeds (Tompsett, 1986).

In terms of embryo water relations the data presented for *Z. palustris* suggest that some embryos die when the water potential (ψ)* drops below about −3 MPa[1] whereas other individuals at the opposite end of the tolerance distribution can withstand a ψ 1–2 order of magnitude greater than this (Fig. 3). Such wide variation in desiccation tolerance within a single seed population

* ψ Values were calculated from relative humidity values at equilibrium moisture content (Fig. 3).

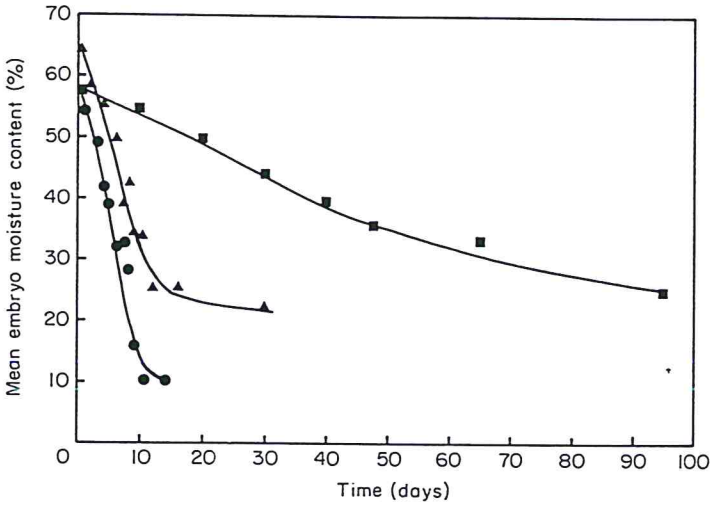

FIG. 1. Embryo drying curves for seeds of *Zizania palustris*. Caryopses (n = 10) immersed in polyethylene glycol, −10 MPa (92·4% RH) at 1 °C (■); caryopses (n = 20) air-dried over saturated NH_4Cl (82% RH) at 16 °C (▲); intact seeds (n = 5) air-dried in a dry room (15% RH) at 15 °C (●).

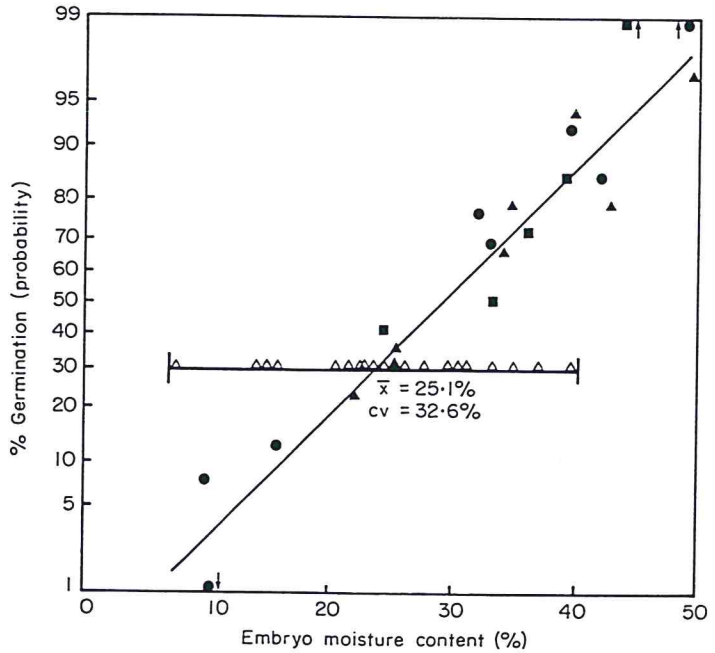

FIG. 2. Probit viability/embryo moisture content relationship for seeds of *Zizania palustris*. Symbols correspond to the three different embryo desiccation rates indicated in Fig. 1. Sample sizes in germination tests were: two replicates of 20 seeds (●), one replicate of 20 seeds (■) and one replicate of 35 seeds (▲). Open triangles refer to individual embryo moisture content values (n = 20).

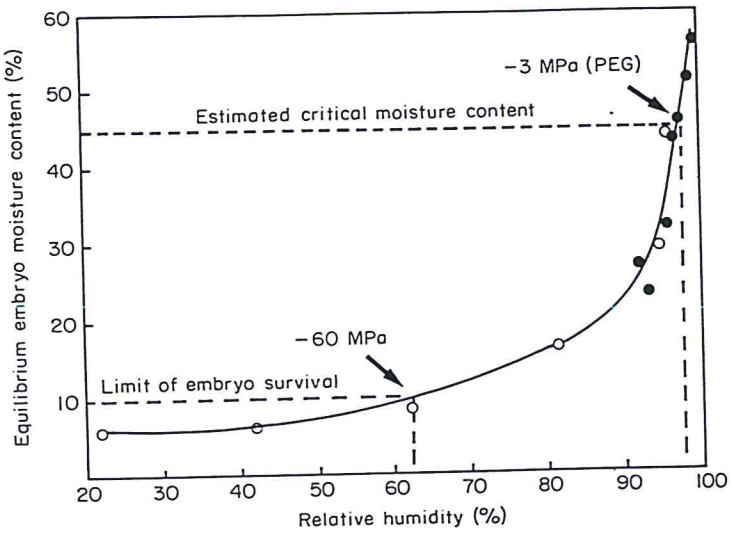

FIG. 3. The equilibrium embryo moisture content/relative humidity relationship for seeds of *Zizania palustris* stored in solutions of polyethylene glycol at 1 °C (●), or in air over saturated salt solutions at 6 °C (○) (detailed in Table 1).

seems implausible. The large coefficient of variation (32·6 %) on the moisture content estimate of a sample of 20 individual embryos (Fig. 2) strongly suggests that a large proportion of the apparent variation in desiccation tolerance could be accounted for by differences in embryo moisture content between individual seeds during desiccation.

During long-term storage in polyethylene glycol the duration of a lag phase before viability declined was directly related to water potential (Fig. 4). Evidence suggests that this lag phase was related to the rate of embryo desiccation. Since there was no measurable change in the viability of caryopses stored for 365 d at −2 MPa it may be concluded that the critical embryo ψ below which viability declines is between −2 and −3 MPa. The corresponding ψ value from the estimated critical mean embryo moisture content in short-term storage experiments (Fig. 2) closely agrees with these findings (Fig. 4). Mexal *et al.* (1975) pointed out that oxygen availability may be severely limiting in solutions of PEG 6000 even at a ψ of −0·7 MPa. Over the range of ψ used in the present study oxygen availability would have been extremely low and differences between solutions small. Therefore, differences in the storage behaviour of seeds of *Z. palustris* stored in PEG solutions cannot be explained by differences in oxygen availability.

On the one hand the results presented here strongly suggest that the embryos of *Z. palustris* die when the ψ drops significantly below −2 to −3 MPa which corresponds to an embryo moisture content in the range 55–45 %. On the other hand the moisture content values of a sample of 20 individual embryos after 12 d storage at 80 % RH (Fig. 2) indicate that a significant proportion of embryos remained viable even when their moisture content had dropped well below 40 %. The likely explanation for these findings is that tissue survival below a critical ψ is related to time of exposure and that irreversible damage will occur only when some critical point in time has been reached which will be dependent on the combined effects of desiccation rate, temperature, and genotype.

Two separate experiments investigated the effects of drying on the viability of *S. anglica* seeds. Differences in the rate of embryo drying were relatively small (Fig. 5). Despite this the moisture content/probit viability relationship was similar to that of *Z. palustris* (Fig. 6) and there can be little doubt that stored *S. anglica* seeds are likewise truly recalcitrant. The data presented indicate that the critical embryo moisture content for survival in *S. anglica* (50–55 %) was slightly higher than that recorded for *Z. palustris* (45–50 %). Future studies of the equilibrium moisture content/relative humidity relationship including direct measurement of tissue ψ using thermocouple psychrometry will reveal whether these differences reflect actual differences in desiccation tolerance between the two species.

Preliminary tests on small samples of freshly

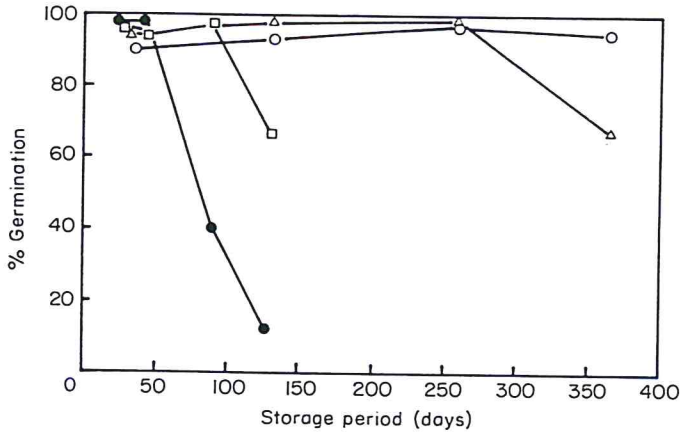

FIG. 4. Viability decline curves for caryopses of *Zizania palustris* stored in solution of polyethylene glycol at 1 °C. −10 MPa (92·4% RH) (●); −4 MPa (96·9% RH) (□); −3 MPa (97·7% RH) (△); −2 MPa (98·4% RH) (○). Symbols represent the mean of either of single sample or in most cases two replicate samples of 25 seeds.

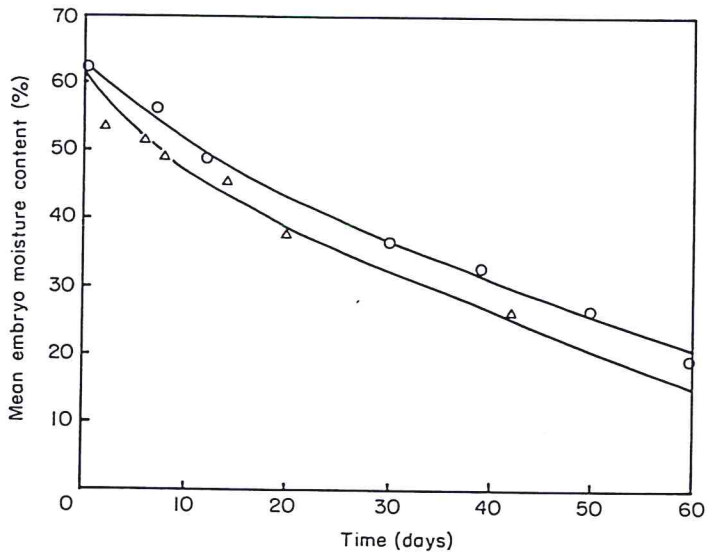

FIG. 5. Embryo drying curves for intact seeds ($n = 5$) of *Spartina anglica* dried in air at 6 °C, over saturated NaI (42% RH) (○); and over silica gel (relative humidity not recorded) (△).

harvested seed of *P coarctata* using different rates of desiccation strongly suggest the existence of recalcitrant storage physiology (Fig. 7). The slope of the line describing the relationship between embryo moisture content and probit viability was similar to that recorded for *Z. palustris* (Fig. 2) and *S. anglica* (Fig. 6). This similarity between the three species used in the present study could be explained by similar distributions of variation in embryo desiccation rates. Future studies will seek to confirm this.

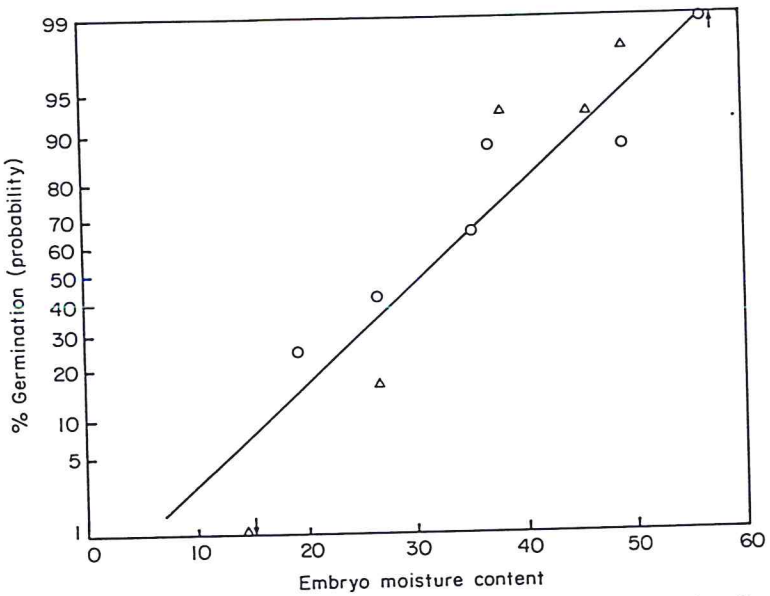

FIG. 6. Probit viability/embryo moisture content relationship for seeds of *Spartina anglica*. Symbols correspond to the two embryo desiccation rates indicated in Fig. 5. Two replicate samples of 25 seeds were used in germination tests.

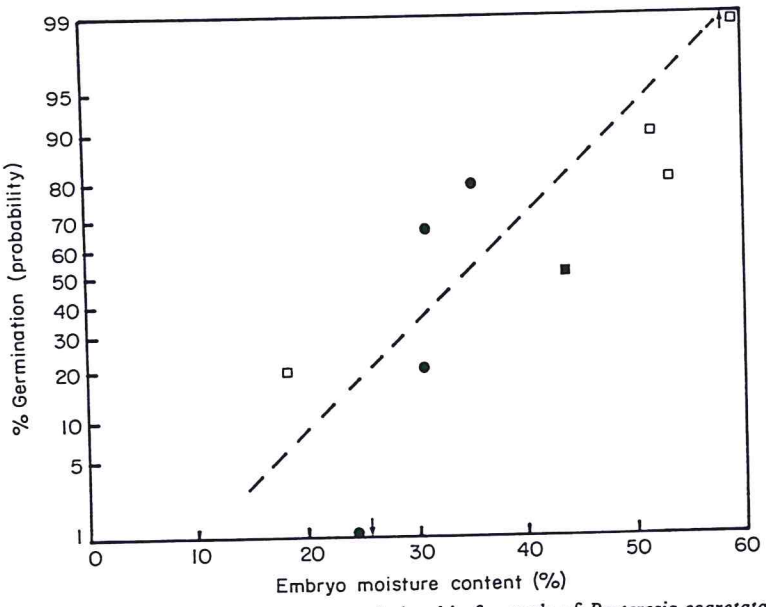

FIG. 7. Probit viability/embryo moisture content relationship for seeds of *Porteresia coarctata*. Seeds were dried in air at 80% RH (circles) or at 15% RH (squares). Viability was monitored using the tetrazolium test (open symbols) where $n = 10$, or using a germination test (closed symbols) where $n = 20$.

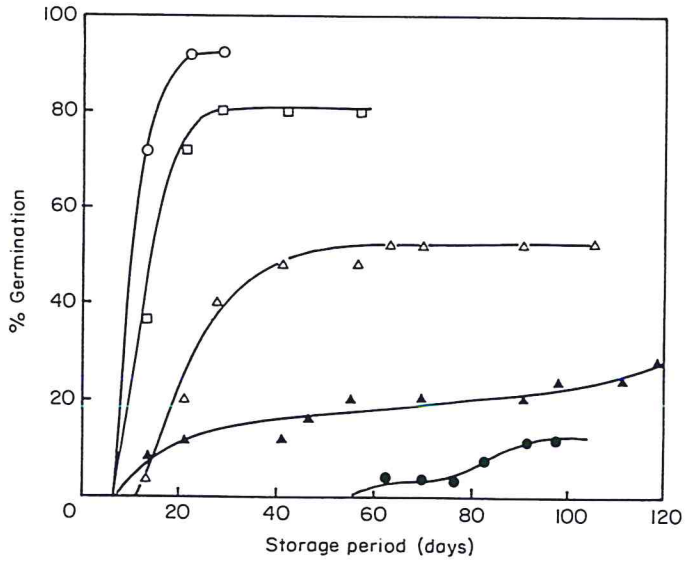

FIG. 8. Loss of dormancy in seeds of *Zizania palustris* during storage in distilled water at 2 °C. Germination tests using single samples of 20 or 25 seeds were set up immediately after harvest (●) and repeated after 9 weeks (▲), 13 weeks (△), 20 weeks (□), and 26 weeks (○).

Dormancy

Despite removal of a portion of the lemma and scraping of the pericarp directly above the embryo to eliminate the effects of coat-imposed dormancy freshly harvested seeds of *Z. palustris* did not exceed 8% germination even after 98 d incubation at 16 °C (Fig. 8). During storage in water at 2 °C dormancy progressively declined. After 26 weeks a high proportion of individuals (> 90%) were capable of rapid uniform germination. Despite quantitative differences, these results are in broad agreement with those reported previously for this species (Simpson, 1966; Cardwell *et al.*, 1978).

Although fewer samples were taken, changes in germination capacity of *S. anglica* seeds during storage at 2 °C were similar to that of *Z. palustris* (Fig. 9). Whereas it may be argued that loss of dormancy in *Z. palustris* could be attributable in part to the leaching of water soluble inhibitors from the covering structures, as suggested previously (Cardwell *et al.*, 1978) this argument cannot be applied to *S. anglica* because seeds were stored at 100% RH and were therefore not in contact with free water.

In a study of non-dormant propagules of the recalcitrant mangrove species *Avicennia marina* (Forssk.) Vierh., the initiation of germination processes during storage were associated with a concomitant upward shift in the critical moisture content for embryo survival (Farrant, Pammenter and Berjak, 1986).

To enable the use of the germination test to monitor viability, drying experiments on *Z. palustris* and *S. anglica* used relatively non-dormant seed samples which had been previously stored for several months (Figs 2 and 6). Evidence of a shift in desiccation tolerance in *Avicennia* during storage raises the question as to whether desiccation tolerance in *Z. palustris* and *S. anglica* may be related to depth of dormancy and/or storage period. Detailed experiments which specifically address this question are planned for the future. In the meantime, three pieces of evidence suggest that any effect of storage period and dormancy (before the onset of visible germination) on the limits of desiccation tolerance must be small. Firstly, in a drying experiment on *Z. palustris* seeds at 80% RH, after 8 weeks storage, when approx. 80% of the seed population were completely dormant the relationship between decline in viability (measured by loss of embryo staining in tetrazolium tests) and moisture content, was consistent with that recorded in experiments on non-dormant seed in which the germination test was used. Using samples of 20 seeds tetrazolium staining of the complete embryo declined from 100 to 20% of individuals when the embryo moisture content was reduced from 36 to 15%. After 35 d storage although some seeds retained some capacity for staining in the shoot end of the embryo axis, all seeds failed to stain in the region of the primary root meristem. Secondly, in a number of

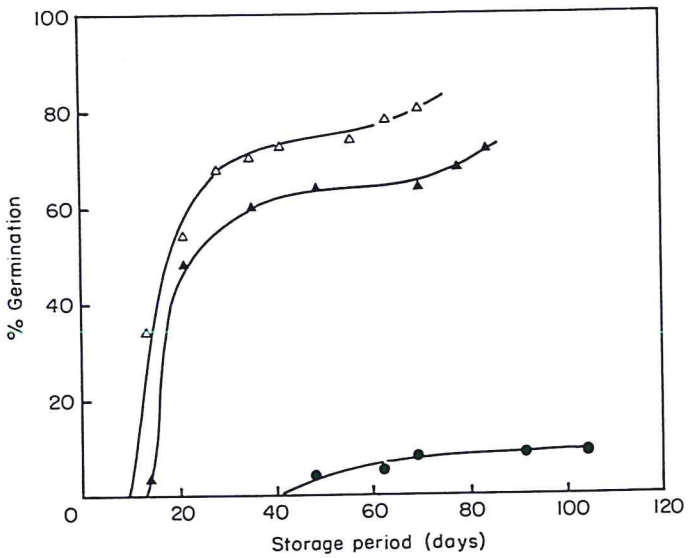

FIG. 9. Loss of dormancy in seeds of *Spartina anglica* during storage at 100% RH at 2 °C. Germination tests using single samples of 25 seeds were set up immediately after harvest (●) and repeated after 23 weeks (▲), and 37 weeks (△).

preliminary tests on unstored seed, set up shortly after harvest, using different rates of desiccation severe microbial contamination (indicative of death) always quickly developed in germination tests of dried seed. In contrast, undried seeds remained fresh even during incubation periods of 4–5 months. Finally, there is good evidence that desiccation tolerance in freshly harvested seeds of *P. coarctata* is similar to that of stored seed of *Z. palustris* and *S. anglica*. *P. coarctata* seeds, of course, do not exhibit dormancy. During storage under optimum conditions there is therefore no evidence of a major effect of storage period or dormancy (up to the point of visible germination) on desiccation tolerance in recalcitrant aquatic grasses.

CONCLUSIONS

It has been clearly shown that the relationship between mean embryo moisture content and viability in stored seeds of *Z. palustris* and *S. anglica* and freshly harvested seeds of *P. coarctata* is independent of the rate of desiccation. In the case of *Z. palustris* and *S. anglica* there is no evidence of a significant effect of dormancy or storage period on the limits of desiccation tolerance. These results, therefore, provide firm evidence that seeds of these species are truly recalcitrant. Earlier reports that drying resulted in loss of viability are therefore confirmed and the possible existence of orthodox storage physiology masked by slow desiccation or the development of dormancy is rejected.

Wide variation in desiccation tolerance between individual seeds suggested by the relationship between probit viability and mean moisture content is an artifact which can be accounted for at least in part by wide differences in embryo moisture content between individual seeds during the drying phase. This explanation may be applied to other recalcitrant species where similar relationships have been described (e.g. Tompsett, 1986).

Long-term storage experiments on *Z. palustris* using solutions of PEG suggest that the actual variation in desiccation tolerance between individuals is confined to a narrow range of ψ values around -2 to -3 MPa. Despite this there is good evidence that embryos may be capable of surviving a ψ significantly lower than this for limited periods. It is feasible that this time (and probably genotype) dependent survival could be exploited for long-term practical conservation purposes when combined with cryopreservation techniques. For medium-term storage, simple techniques using sterile solutions of PEG with ψ values above the critical level for embryo survival but sufficiently low to prevent germination, combined with near freezing temperatures can be used successfully.

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