The longevity of cycad pollen in storage

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An investigation was made into the effects of temperature, headspace atmosphere and humidity on the longevity of stored pollen from several *Encephalartos* species and from *Cycas thouarsii*. Samples were assayed by *in vitro* germination tests and by *in vivo* pollination trials. *Encephalartos* pollen rapidly lost germinability at ambient temperatures but retained 50% germinability for approximately two years at 0°C and three years at –15°C. Storage in ambient atmosphere resulted in better germinability than storage in a nitrogen atmosphere. Pre-drying for 24 h was beneficial but storage over a desiccant was disadvantageous. Pollen stored for 2 years at 0 – 4°C gave rise to a 58% seed germination when used to pollinate a female *Encephalartos transvenosus* cone. Pollen of *Cycas thouarsii* stored at reduced temperatures exhibited a pronounced seasonal variation in germinability with reduction in maxima over a 3-year period, implying the operation of a hitherto undocumented biological clock.

Die invloed van temperatuur, bodamp-atmosfeer en humiditeit op die langlewendheid van gebergde stuifmeel van verskillende *Encephalartos*-spesies en *Cycas thouarsii* is ondersoek. Stuifmeelkiemkragtigheid is bepaal deur middel van *in vitro*-ontkiemingstoetse en *in vivo*-bestuiwingsproewe. *Encephalartos*-stuifmeel het vinnig hul kiemkragtigheid by heersende omgewingstemperature verloor, maar het ongeveer 50% kiemkragtigheid behou na opberging vir twee jaar by 0°C en drie jaar by –15°C. Die stuifmeel het beter ontkiem na berging by heersende atmosferiese toestande in vergelyking met berging onder stikstofatmosfeer. Voorafdroging vir 24 h was voordelig, terwyl berging van stuifmeel oor 'n droogmiddel nadelig was. Stuifmeel wat vir twee jaar by 0 – 4°C geberg is, het 58% kiemkragtige sade tot gevolg gehad nadat dit gebruik is om 'n vroulike *Encephalartos transvenosus*-keël te bestuif. Stuifmeel van *Cycas thouarsii* wat by verlaagde temperature geberg is, het 'n duidelike seisoenale variasie in kiemkragtigheid getoon met 'n uiteindelike verlaging in maksimum kiemkragtigheid oor 'n periode van drie jaar, wat die werking van 'n tot nog toe ongedokumenteerde biologiese klok impliseer.

Keywords: Cycadales, cycads, pollen longevity, pollen storage.

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Introduction

Of the world's approximately 180 cycad taxa (Stevenson *et al.* 1990), about one half are considered endangered, vulnerable or rare (Gilbert 1984). Since *in situ* conservation measures are not always possible or practical, the maintenance of gene pools in botanic gardens and in private collections, and propagation from these resources, are important (Osborne 1990). The most effective method of cycad propagation is from seed, but for several genera, seed collected from both habitat and garden sources is often infertile and hence artificial pollination is usually necessary to ensure a fertile seed crop (Kiem 1972; Giddy 1984; Tang 1986a). Since fresh pollen of a corresponding male species is often not readily available when female cones are receptive, it is desirable to maintain private or collective pollen banks.

Evidence from storage trials on non-cycad material indicates that low temperature and/or humidity commonly extends pollen longevity (Stanley & Linskens 1974; Shivanna & Johri 1985) but, although there have been significant recent advances in our understanding of cycad biology (Norstog 1987), there has been very little work published on the longevity of cycad pollen. Charles Joseph Chamberlain, pioneer in the field of cycad biology, regarded a month as the limit of life of *Ceratozamia* pollen under ambient conditions (Chamberlain 1926). Preliminary work by Tang (1986b) indicated that cycad pollen stored over silica gel in a domestic refrigerator maintained at least some viability for up to one year; different genera showing somewhat different quantitative responses when assessed by a nitroblue tetrazolium staining technique.

In order to more fully investigate factors affecting pollen longevity, we carried out two sets of experiments and report our findings below.

Materials and methods

Experiment 1

Pollen of *Encephalartos ferox* Bertolini f., *E. natalensis* R.A. Dyer and Verdoorn, *E. princeps* R.A. Dyer, *E. woodii* Sander and *Cycas thouarsii* Gaudichaud was harvested between February and July in 1988. Small amounts of the pollen were stored in the dark under different conditions of temperature (ambient, 0°C and –15°C), atmosphere (normal and under nitrogen) and humidity (pre-dried, stored over a desiccant or stored under ambient humidity). Sub-samples were removed every 3 months and incubated in darkness for 72 h at 20°C on a medium comprising 2% sucrose, 1% agar and 0.01% boric acid, a medium used for assessment of *Pinus* pollen viability (Stanley & Linskens 1974). Sub-samples from these cultures were examined using light

microscopy. Triplicate counts of the numbers of germinating and non-germinating pollen grains were made and the percentage germinability was calculated.

Experiment 2

Pollen from *Encephalartos transvenosus* Stapf and Burtt Davy was collected from male cones in 1987 and 1988 and stored in sealed containers at 0 – 4°C. Fresh pollen from *E. woodii* Sander cones was harvested in 1989. Sub-samples of the pollen were suspended in hanging drops of solution containing 5, 10 or 15 % sucrose and 0.005% boric acid and the viability was assessed by counting the germinating pollen grains after 48 h at 28°C. Each result represents the mean of six counts.

In addition to the germination counts described above, an aqueous suspension of fresh *E. woodii* pollen was used to pollinate a female cone of *E. natalensis* by four successive pollen applications in June 1989. Stored *E. transvenosus* pollen was similarly used to pollinate female cones of that species in August 1989. The seeds were allowed to develop naturally and were harvested at the time of cone fragmentation. A preliminary assessment of the seed viability was carried out by determining the percentage of seeds which sank when immersed in water; thereafter the seeds were planted according to normal horticultural practice (Giddy 1984) and the ultimate percentage germination recorded.

Results

Experiment 1

The effect of storage temperature

Figure 1 shows the effect of storage at three different temperature conditions on the germinability of *Encephalartos* pollen. The samples stored at ambient temperatures rapidly lost apparent viability although a small germination percentage was recorded for several months. The samples stored at 0°C or –15°C showed a much slower decrease in germinability, with half-lives of approximately 2 and 3 years, respectively.

An anomalous result was obtained with *Cycas thouarsii* pollen, as shown in Figure 2. All samples appeared to loose viability rapidly within the first 6 months of storage but those stored at reduced temperatures subsequently showed an unanticipated 'recovery' in germinability. This reached a maximum approximately 1 year after initial pollen harvesting. The cycle was then repeated: a sharp decline in germinability followed by a second apparent recovery 2 years after the initial harvesting. An indication of a third such cycle, peaking at 3 years, is also present.

The effect of storage atmosphere

Figure 3 shows the results of a comparison of *Encephalartos* pollen samples stored under a normal atmospheric headspace against those stored in an inert atmosphere of nitrogen. There appears to be no benefit to storing pollen in a nitrogen atmosphere; on the contrary, the inert atmosphere appears to be generally disadvantageous.

There is no clear-cut advantage or disadvantage to the inert atmosphere for storage of *Cycas* pollen (Figure 4). However, the seasonal dormancy effect, described previously, is again apparent.

The effect of storage humidity

Figure 5 summarizes the results obtained when pollen is either pre-dried (by drying the sample in a desiccator with

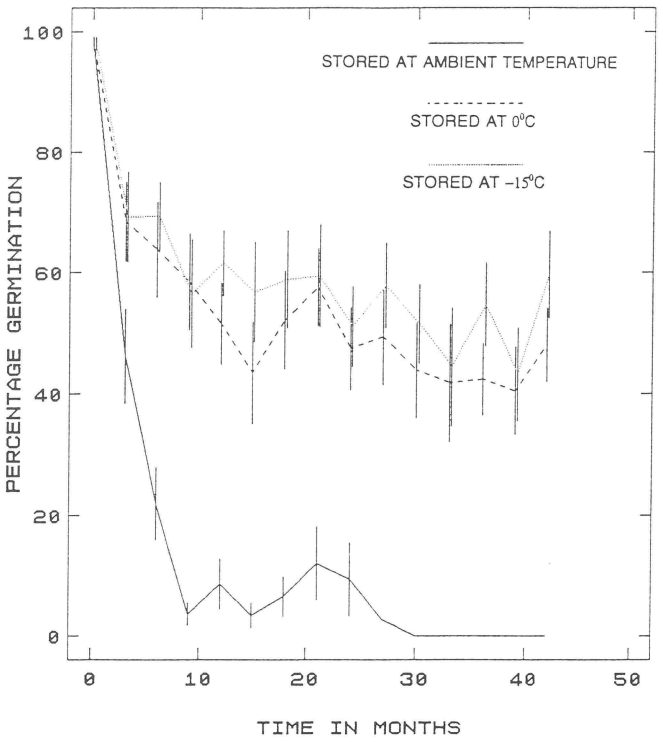

Figure 1 The effect of storage temperature on the germinability of *Encephalartos* pollen. The results from four species have been averaged. Vertical bars represent the standard error values at each observation.

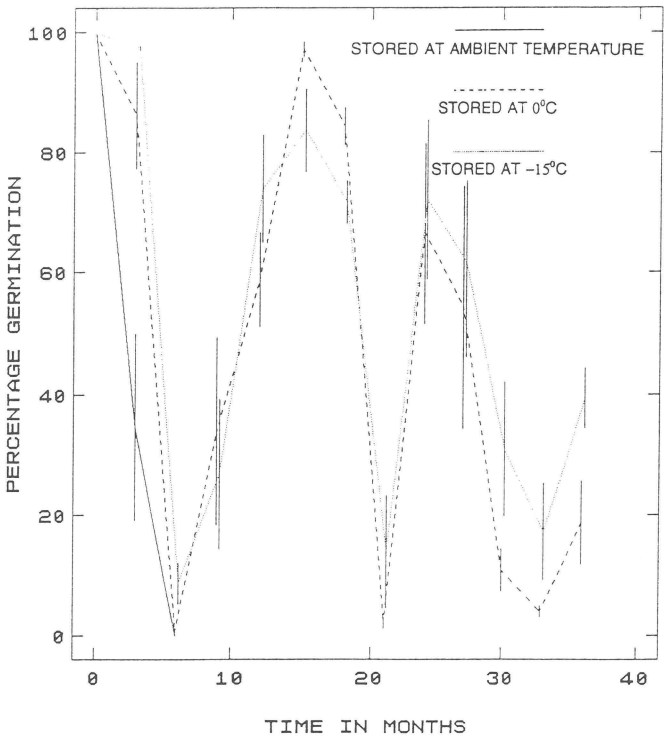

Figure 2 The effect of storage temperature on the germinability of *Cycas thouarsii* pollen. Vertical bars represent the standard error values at each observation. Note also that the 'ambient temperature' treatment was discontinued after six months.

silica gel for 24 h immediately after harvesting) or storing the material continuously over silica gel (*i.e.* in near zero humidity conditions). There is significant benefit to the pre-drying process but continuous storage over the desiccant appears to be marginally disadvantageous.

In Figure 6 the cyclical dormancy pattern for *Cycas thouarsii* pollen is illustrated again. Pre-treatment drying, or storage over silica gel, both appear to be somewhat disadvantageous.

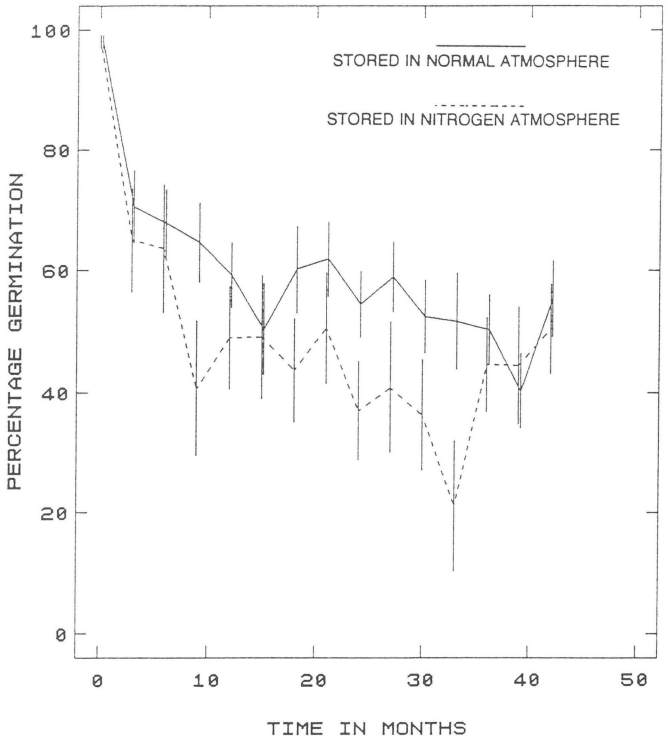

Figure 3 The effect of storage atmosphere on the germinability of *Encephalartos* pollen. The results from three species have been averaged. Vertical bars represent the standard error values at each observation.

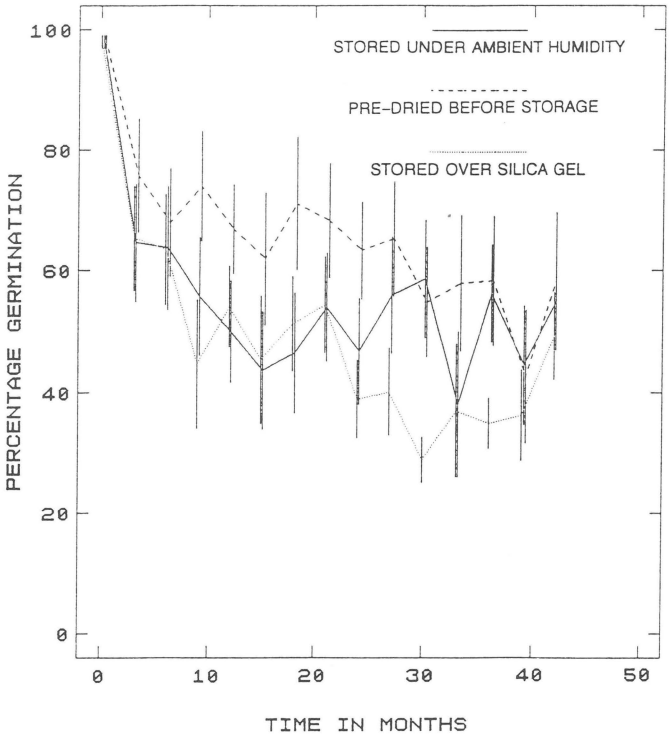

Figure 5 The effect of storage humidity on the germinability of *Encephalartos* pollen. The results from four species have been averaged. Vertical bars represent the standard error values at each observation.

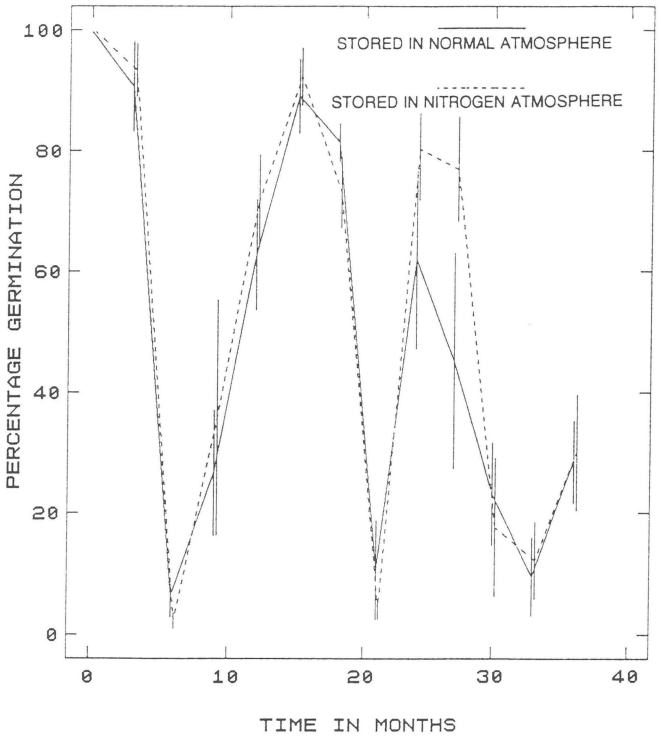

Figure 4 The effect of storage atmosphere on the germinability of *Cycas thouarsii* pollen. Vertical bars represent the standard error values at each observation.

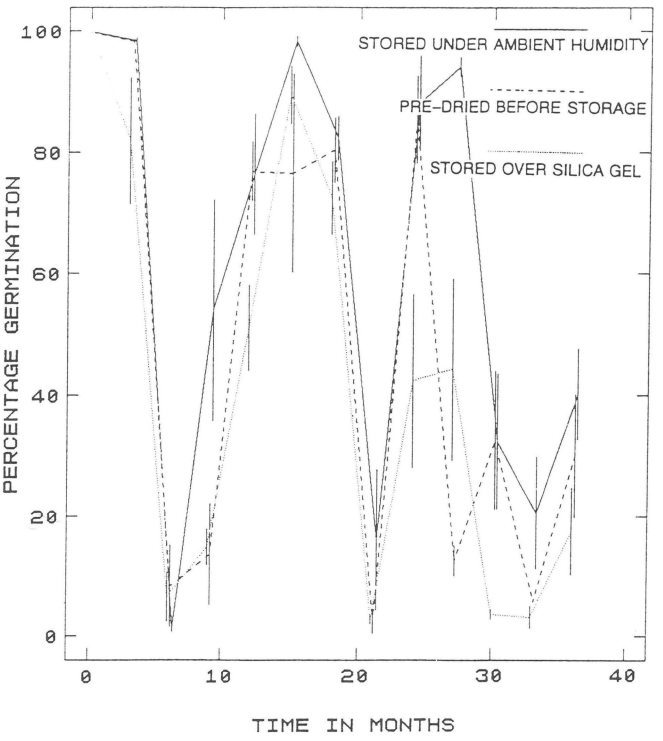

Figure 6 The effect of storage humidity on the germinability of *Cycas thouarsii* pollen. Vertical bars represent the standard error values at each observation.

Table 1 Pollen and seed viability tests for two species of *Encephalartos*

Species tested	Date pollen collected	Date pollen tested and cones pollinated	Percentage viability by the hanging-drop method using sucrose at			Percentage seeds sinking	Percentage seeds germinating
			5%	10%	15%		
E. transvenosus	1987-06-15	1989-08-16	19.0	65.6	66.2	97.2	58
E. transvenosus	1988-08-11	1989-08-16	6.4	71.8	44.9	96.5	42
*E. woodii**	1989-05-16	1989-06-07	29.7	60.8	17.8	56.3	21

*This pollen was used on a cone of *E. natalensis*.

Experiment 2

The results, summarized in Table 1, show that there is a significant difference in the germination when different concentrations of sucrose are used in the hanging drop suspension medium. The results obtained when 15% sucrose was used, appear best to approximate the percentage germination of the seeds in the *in vivo* test situation. The *E. transvenosus* pollen gave satisfactory results after both 1 and 2 years of storage, whereas the fresh pollen of *E. woodii*, used on the female cone of *E. natalensis*, gave somewhat poorer results.

Discussion

Pollen grains of the extant Cycadales are boat-shaped, monosulcate and bilaterally symmetrical and show intergeneric differences in their morphology (Dehgan & Dehgan 1988; Marshall *et al.* 1989). These morphological differences now appear to be paralleled by a variation in longevity responses, first observed by Tang (1986b) and now seen in our results as a striking difference in behaviour between *Encephalartos* and *Cycas* pollen.

In *Encephalartos*, the results demonstrate a general trend of decreasing germinability with time. Pollen can be stored successfully for periods of at least 3 and probably up to 5 years in cold conditions. The colder temperatures attained in a domestic deepfreeze (–15°C) are more advantageous than those of a refrigerator (0 – 4°C). Optimal results were obtained with *Encephalartos* pollen when the material was pre-dried for 24 h over silica gel before storage, but there is no advantage to storing pollen in an inert atmosphere. Both the reduced temperature and the pre-drying process may serve to prolong the integrity of the pollen cell membrane and also to inhibit the development of fungal infections in the stored material. From this information, a series of recommendations have been drawn up for the information of persons wishing to maintain cycad pollen banks (Osborne *et al.* 1991).

The results from Experiment 2 show that the percentage viability of *Encephalartos* pollen is best predicted by the 'hanging drop' method when 15% sucrose is used in the suspension medium. The fact that pollen of *E. transvenosus* stored at 0 – 4°C for 1 – 2 years produced an acceptable seed crop demonstrates that the results from the *in vitro* germinability tests can serve as a reliable index of viability. The somewhat poorer seed germination achieved with the *Encephalartos natalensis* × *E. woodii* hybrid may indicate that inter-specific seed crops in cycads generally have a lower viability than those of true species.

Our experimentation shows that pollen of *Cycas thouarsii* undergoes an unusual cyclic behaviour in its apparent viability. The results suggest that a biological clock mechanism operates in signalling alternating phases of dormancy and potential vigour, a phenomenon which has not previously been documented in pollen. If this behaviour proves to be consistent within the genus as a whole, it may confer a selectional advantage to *Cycas*, several species of which inhabit (and may have had their origin in) climates colder than those experienced by their more tropical *Encephalartos* counterparts (Pant 1973). With respect to the comparison between the different storage temperatures for *Cycas* pollen, –15°C is advantageous over 0°C, just as with *Encephalartos*. However, there is no major significant difference in the responses when *Cycas* pollen is stored in an ambient or a nitrogen atmosphere, nor when pre-dried or stored over a desiccant or under ambient humidity.

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