SANBI
Biodiversity for Life
South African National Biodiversity Institute

Research Findings in Advancing Cycad Propagation

"Is pollen germination the cause of low seed germination in a threatened African cycad genus, *Encephalartos*?"

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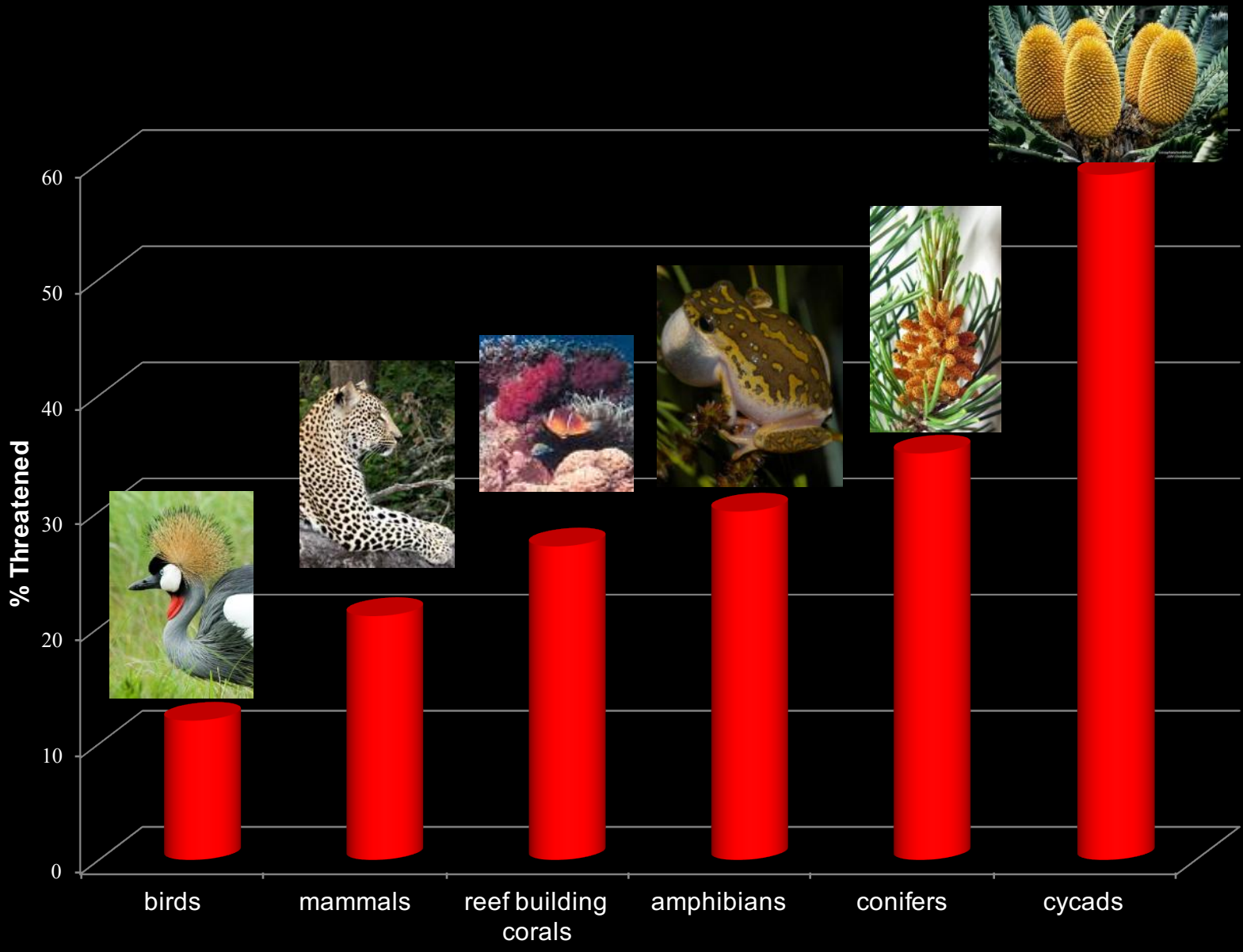
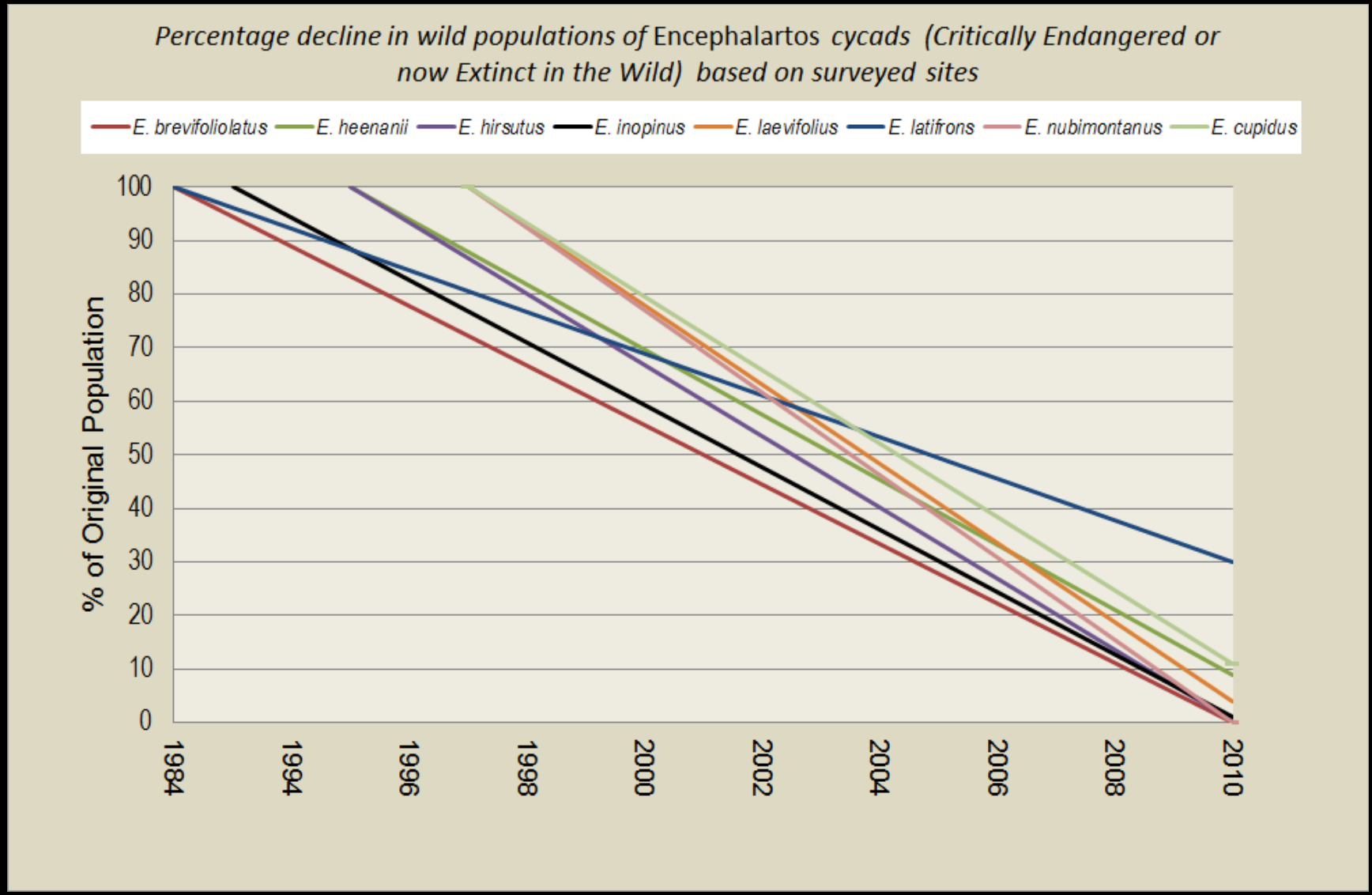
What are cycads?

- Dioecious gymnosperm “naked seed plants”
- Ancient seed plants 300 MYA linage
- 300 species world wide (Tropical & Sub-Tropical regions)
- Africa Genus *Encephalartos* with > 60 species, Stangeria and Cycas (one species each)
- 38 occur in South Africa

Cycads are globally the most threatened group of organisms





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Background

Kirstenbosch Cycad Living Plants Collection was Established in 1913.

The collection consists mainly of *Encephalartos* species (41) from South Africa and neighbouring African countries.

It's objectives are:

RESEARCH

CONSERVATION

EDUCATION

HORTICULTURAL DISPLAY

Securing a future for *Encephalartos latifrons*

1913 → 1980's

Harold Pearson

" This species seem to be on the verge of extinction. It is only known to occur in two localities, in which the plants are now very hard to find" (1916, Pearson). Thus collected 16 plants.

John Winter

Reported low seed viability in *E. latifrons.*

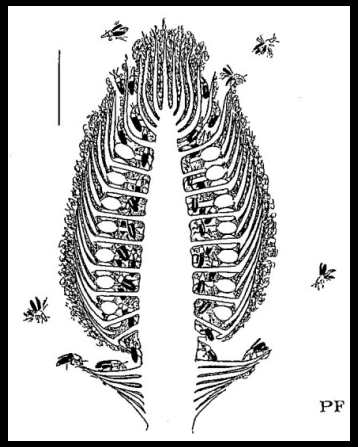
Collection Flagship Species

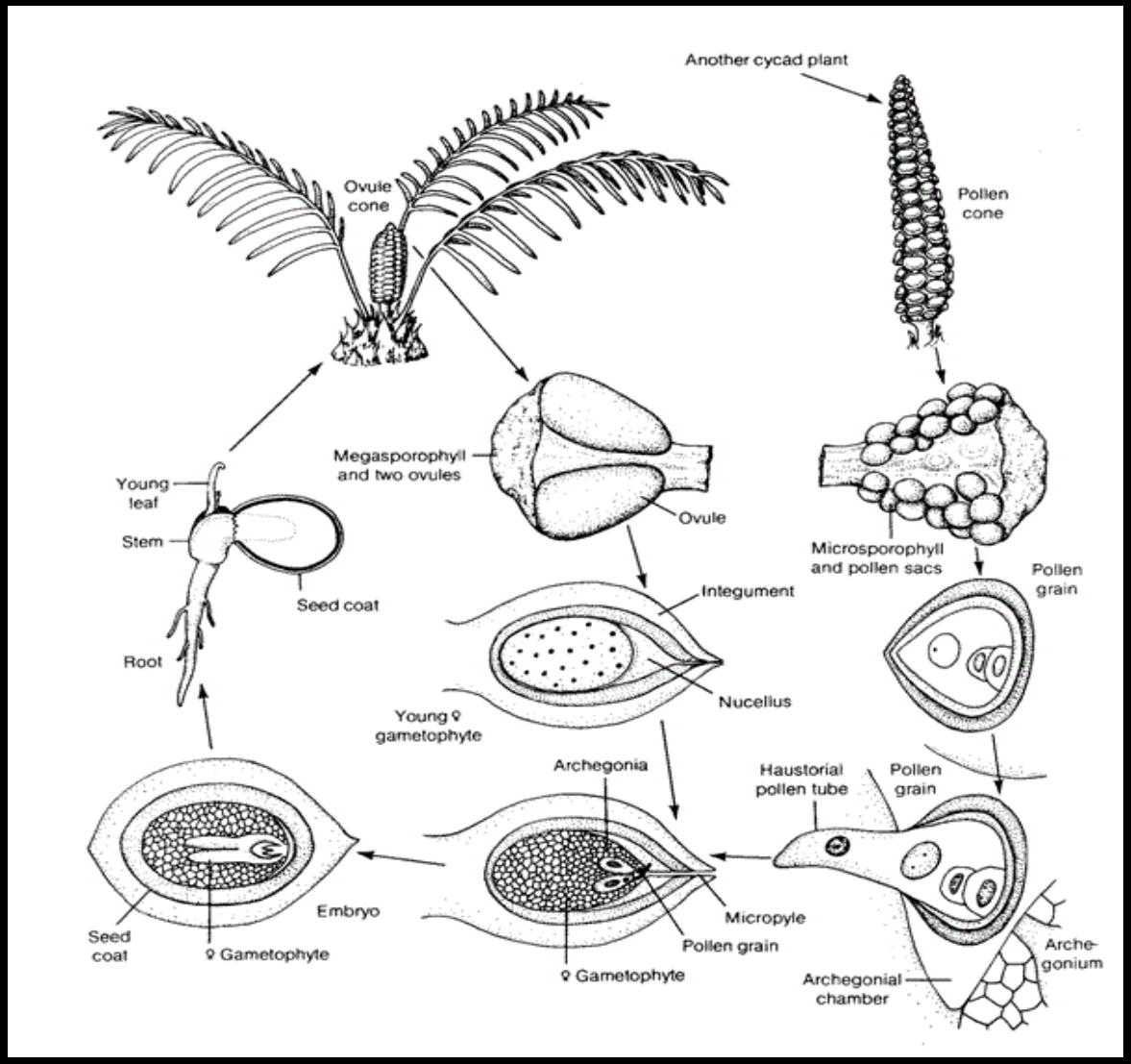
Critically Endangered *Encephalartos latifrons* **Lehm 1838,** with < 60 plants remaining in the wild.

Kirstenbosch has the largest *ex situ* collection of 32 adults.

Encephalartos latifrons at KNBG experiences an unknown cause of low seed germination (<10). This is negatively impacting on the conservation programme of this species.

Pollination Biology of Cycads





The hypothesis is that low seed germination is due to problems with pollen viability or germination. The sub hypotheses are that:

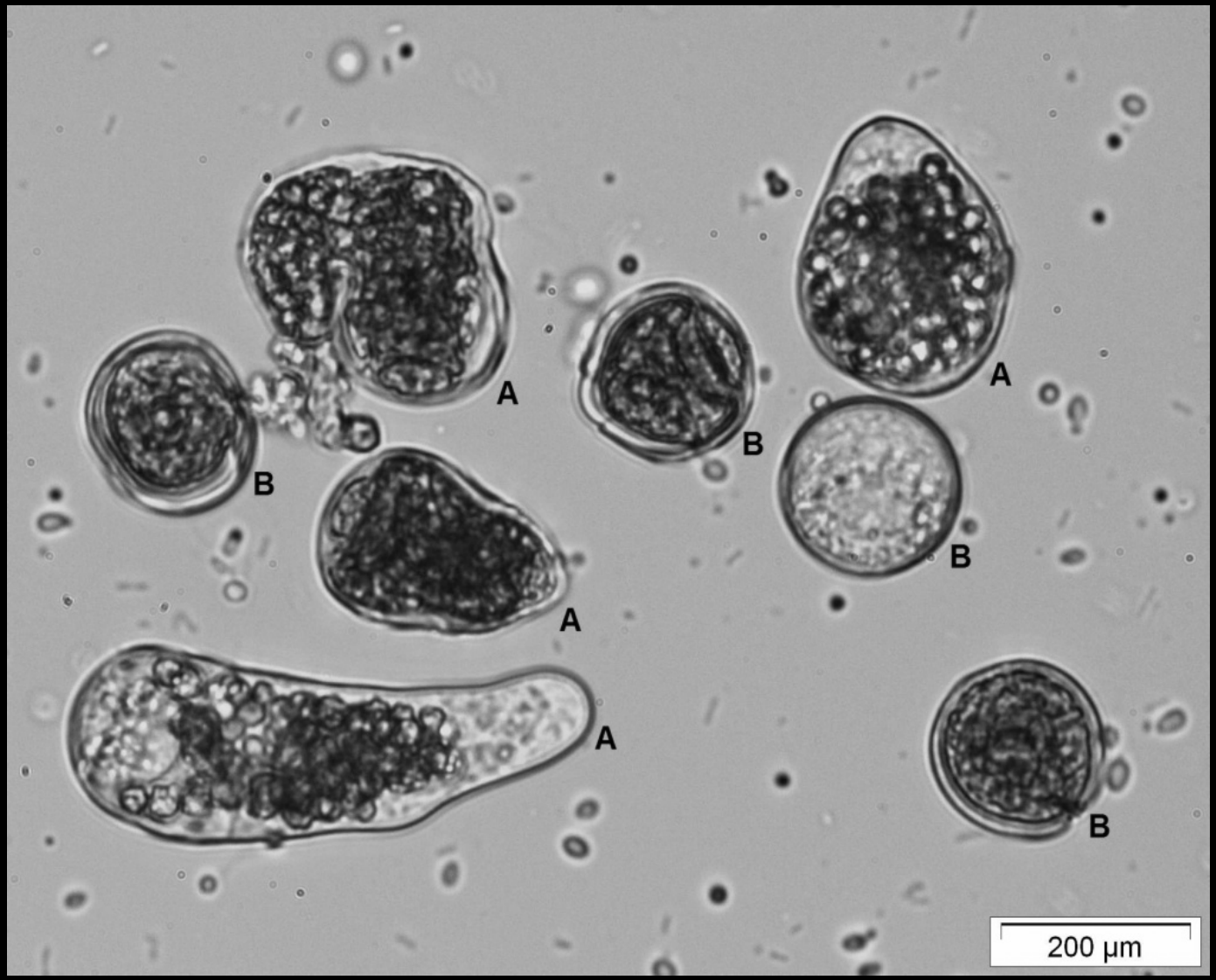
- ***Pollen viability in E. latifrons is lower than in other species with higher levels of seed germination and therefore accounts for low seed viability.***

- ***Viable pollen does not germinate due to a lack of favourable environmental conditions for germination, thus resulting in low seed viability.***

This study compared E. latifrons to a more common cycad E. altensteinii with high seed viability (> 60%) at Kirstenbosch.

Experiment 1: In vitro testing of pollen viability and longevity

- The handing drop technique was used for *in vitro* pollen testing.
- Pollen germination experiments were repeated on 17 *Encephalartos* species from KNBG zero and eight years stored.
- Wild specimens of *E. latifrons* stored for two to three years.
- In total there were 35 pollen samples.
- Analysed using a regression least square fit, since it was the best biological fit model and ANOVA (Statistica7®).



A = Viable Pollen B = Non Viable Pollen

In Vitro Pollen Germination of *Encephalartos latifrons*

Experiment 2:
Testing of pollen response to **desiccation**

- Pollen was mixed in distilled H_20, dried for 24h before testing, and also for 15 days then tested.

- Results were tested using factorial ANOVA (Statistica7®).

Experiment 3:
In vitro ***testing for temperature effects on pollen germination***

- Germination was tested at nine temperatures from 5°C - 45°C with a 5°C increment.

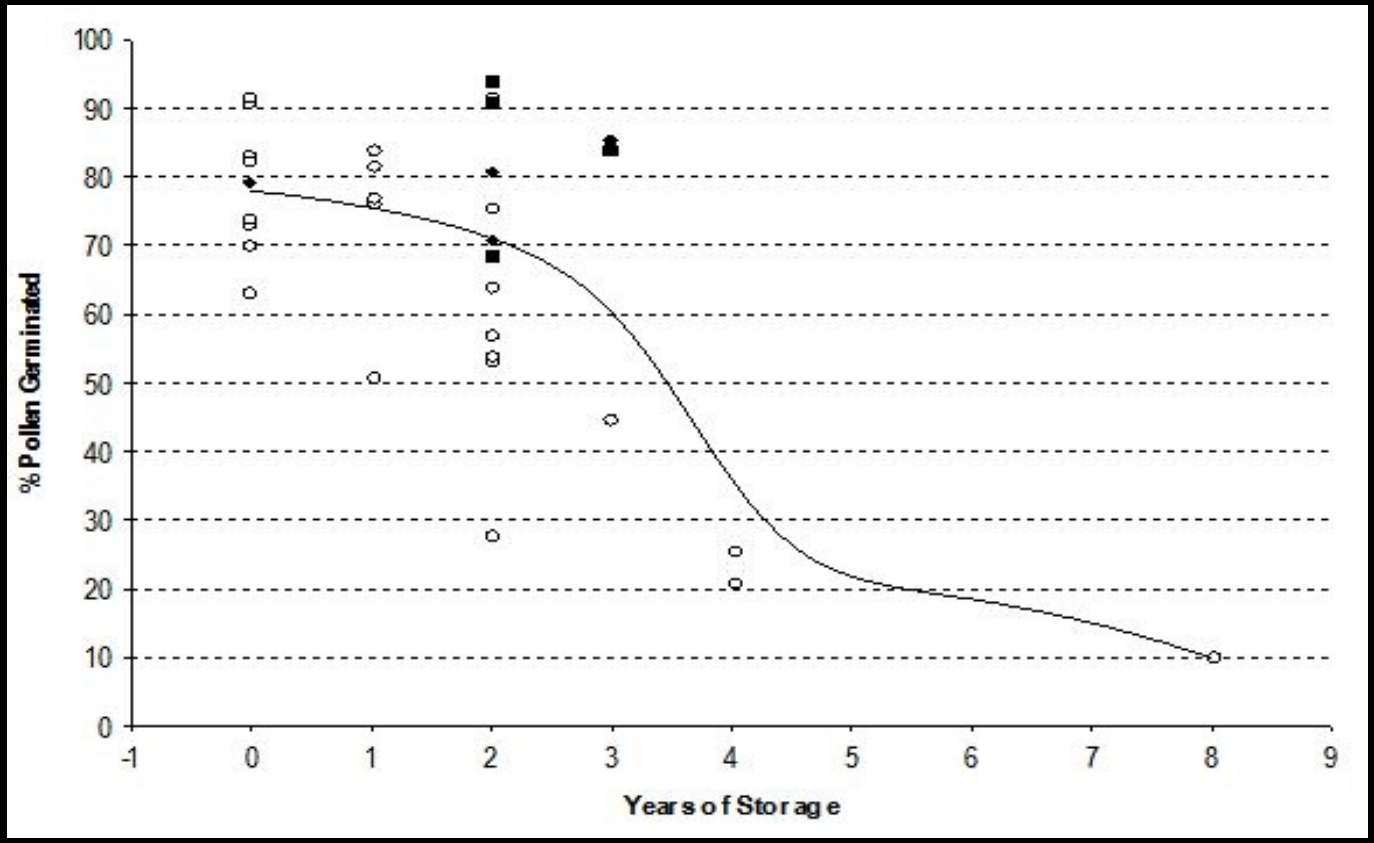
- Results were statistically analysed using factorial ANOVA (Statistica7®).

Experiment 4:
In vivo ***pollen germination in relation to ovulate cone temperature***

- Temperature of female cones was monitored on plants growing outdoors at KNBG using ibutton data loggers set to record temperature at 15 min intervals.

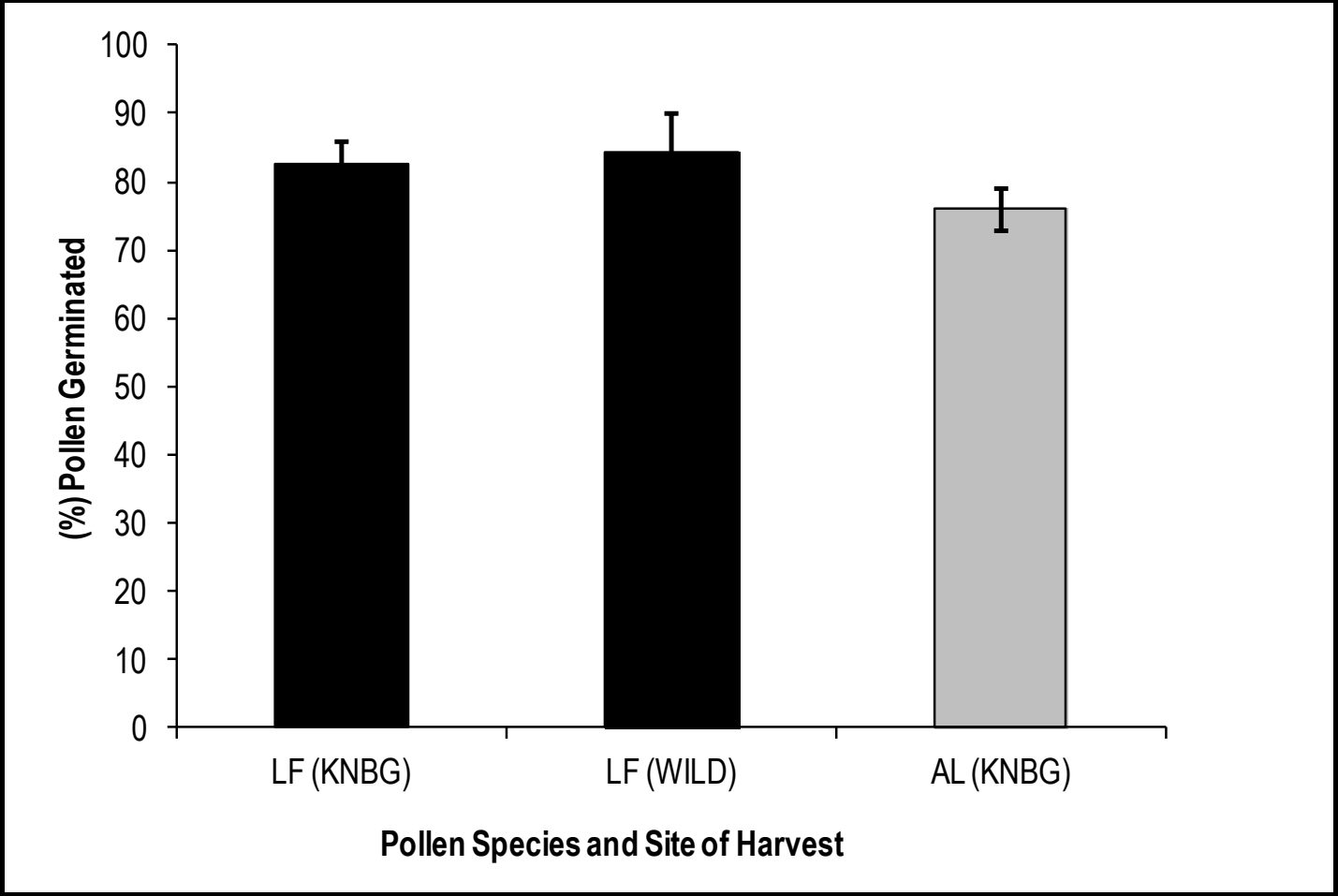
- One ibutton was inserted in the middle of the cone in between the sporophylls. Two ibuttons were hung outside the cone to compare cone and ambient temperature .

Experiment 1: In vitro testing of pollen viability and longevity



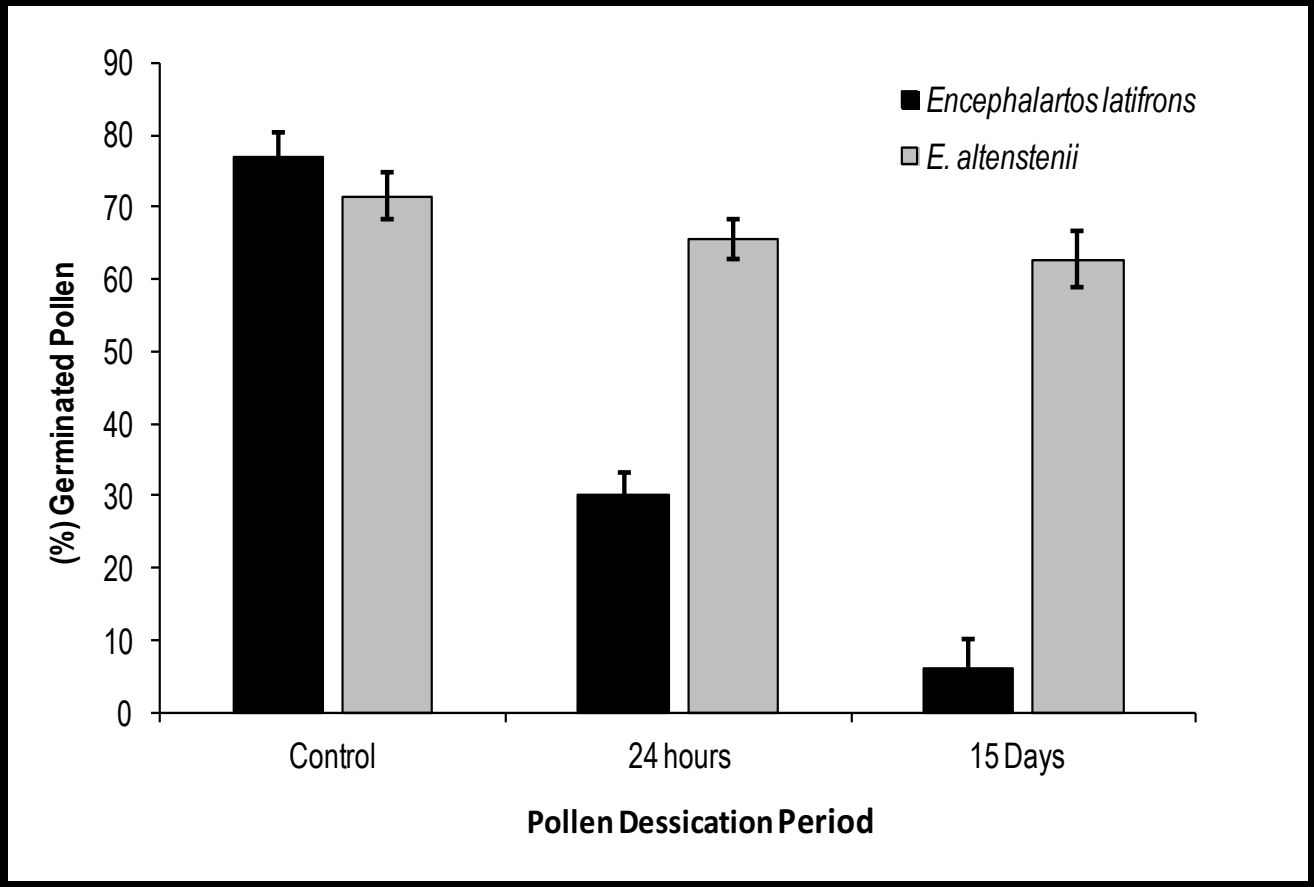
Viability of pollen of *Encephalartos* that was tested when fresh (0 years) or stored for up to eight years at subzero temperature (-15°C). The graph represents 17 *Encephalartos* species with (*n* = 32) samples. Squares represent *E. latifrons* from the wild, diamonds represent *E. latifrons* at KNBG and circles represent all other *Encephalartos* species from KNBG.

Experiment 1: In vitro testing of pollen viability and longevity



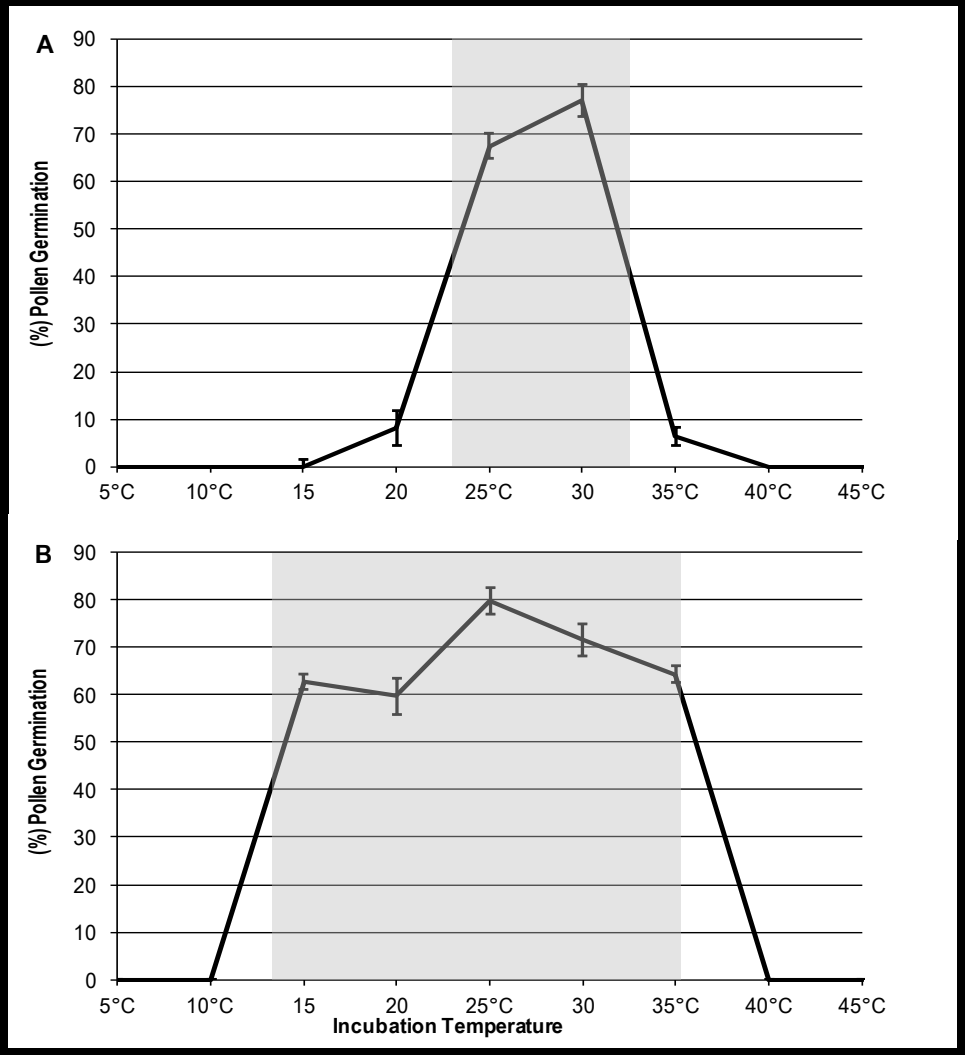
Viability of pollen sampled from the KNBG. Data represent the means (± SE) for *Encephalartos latifrons* (black) from KBNG (*n = 2*), wild (*n =* 4) and *E. altensteinii* (grey) (*n = 6*) from KNBG. (ANOVA, *p* > 0.05)

Experiment 2: In vitro testing of pollen germination



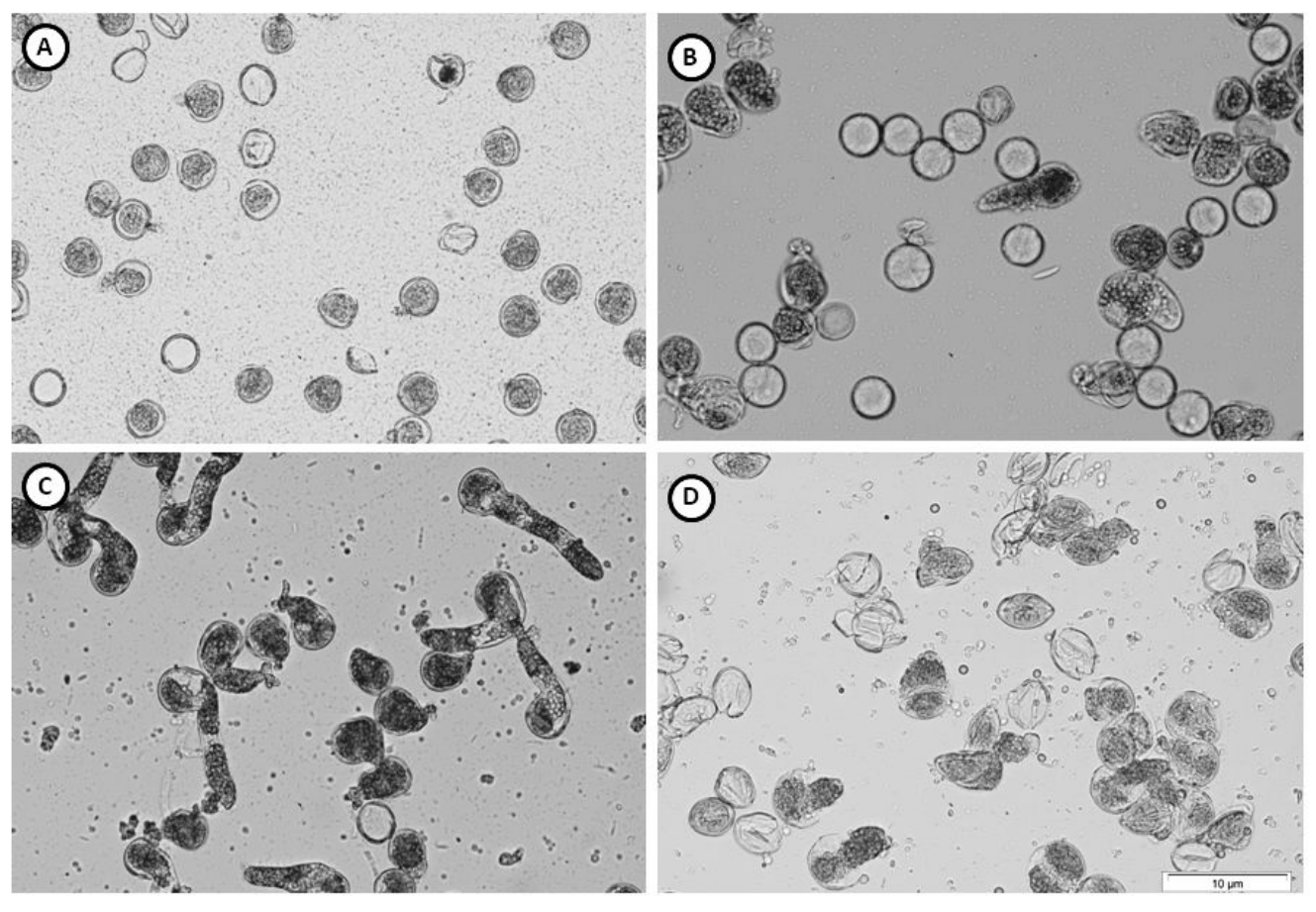
Germination response in fresh *Encephalartos* pollen after hydration for five minutes, then drying for 24h or 15 d before incubation. Bars represent the mean (± SE) for *E. latifrons* (n = 4) and *E. altensteinii* (n = 7). (ANOVA , $p < 0.05$).

Experiment 3: In vitro testing of temperature effects on pollen germination



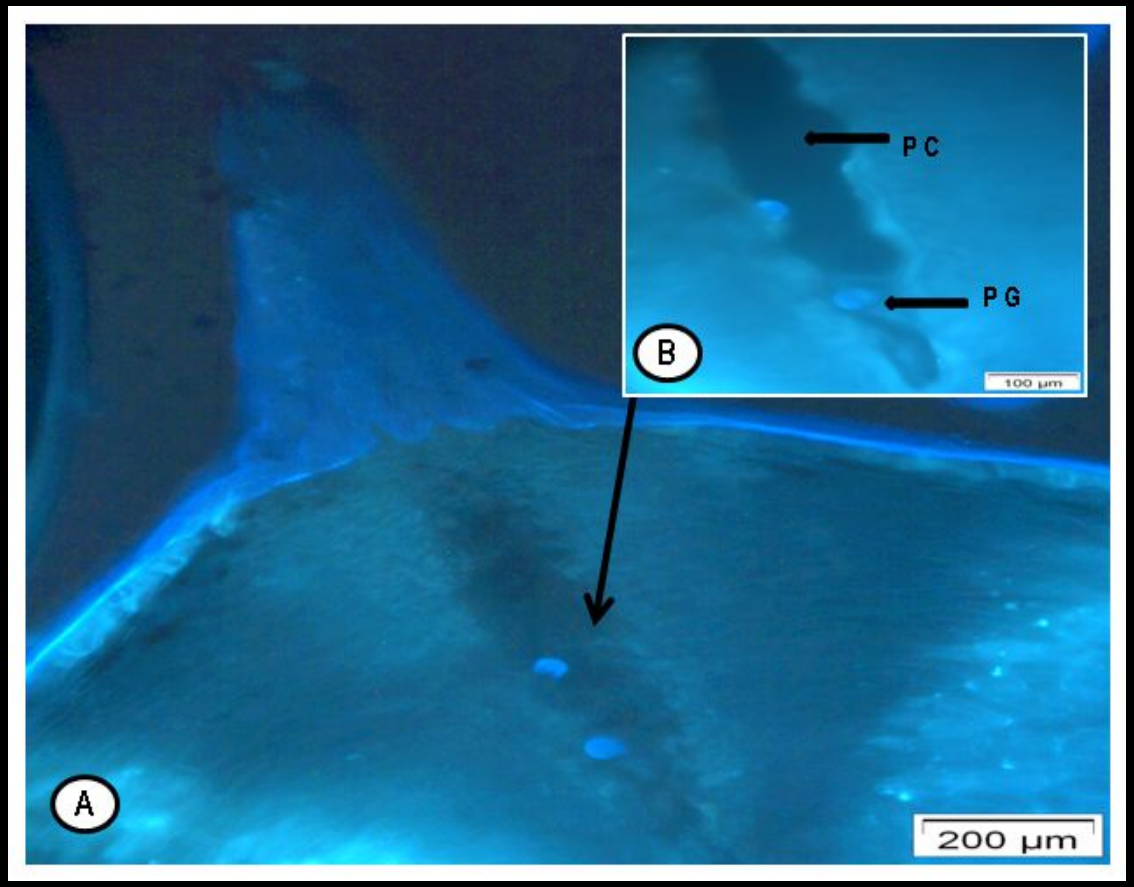
Pollen germination after incubation at different temperatures. *Encephalartos latifrons* (A) and *E. altensteinii* (B).The grey bar highlights the range in which pollen germination was > 40%. Lines represent the means (± SE) *(n =10).* (ANOVA, $p < 0.05$).

Experiment 3: In vitro testing of temperature effects on pollen germination



***In vitro* germination of *Encephalartos latifrons* pollen incubated at different temperatures. A = 10°C no germination, B = 20°C some germination, C = 30°C optimum germination and D = 40°C pollen sporoderm ruptured.**

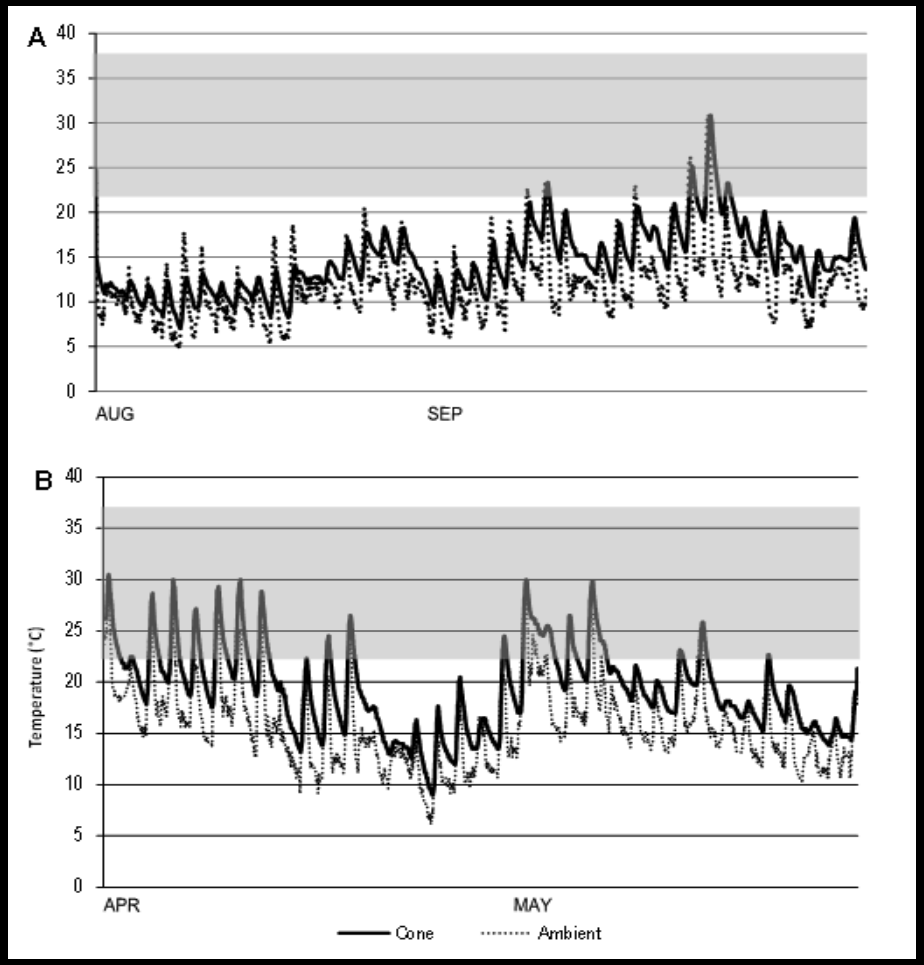
Experiment 3: In vivo pollen germination in relation to ovulate cone temperature



Pollen germination in *E. latifrons* ovules was significantly lower than in *E. altensteinii* (χ^2, $p < 0.001$).

Germinating *Encephalartos latifrons* pollen in the pollen chamber of a sectioned nucellus 20 days after pollination. A = Entire image of the sectioned nucellus and B = magnified view of (A) pollen chamber area. PC = Pollen chamber and PG = Pollen germination into the nucellus.

Experiment 4: In vivo pollen germination in relation to ovulate cone temperature



Grey horizontal bands show the temperature zone for >40% germination derived from *in vitro* pollen germination.

Ovulate cone temperature of two *Encephalartos* species during pollination receptivity. Solid lines represent mean cone temperatures for (A) *E. latifrons (n = 2)* and (B) *E. altensteinii (n = 3)* and dotted lines represent ambient temperatures for the corresponding period.

Discussion

- 17 *Encephalartos* species tested for pollen viability generally declined after three years.
- Fresh and stored *E. latifrons* pollen from KNBG has relatively high viability and is higher than the 40% threshold.
- Therefore, the hypothesis that low seed germination is a result of poor pollen viability in *ex situ* cycad collections at KNBG is therefore not supported by the results.

Discussion

- *Encephalartos latifrons* and *E. altensteinii* are similar in morphology, however differ in response to temperature and desiccation.

Discussion

Two variables at KNBG that could explain differences in pollen germination between species:

i) The time of year (Autumn and Winter) when reproduction takes place, and

ii) The method of pollination (wet and dry).

Conclusions

- The lower germination of pollen using the wet pollination method in *E. latifrons* suggests that wetting pollen may result in desiccation if not resorbed into the ovule rapidly.

- These results give some support to the idea that ambient conditions prevailing at the time of pollination may influence the effectiveness of pollination. Despite the apparent similarities in cycad pollen, observations of different pollen germination responses between *Encephalartos* species are not unique to this study, but were also noted in a previous study by Mostert (2000).

- Perhaps the seemingly minor morphological and sporoderm differences play a greater role than suspected in cycads, in particular for *Encephalartos.* These questions need to be further investigated.

- Nevertheless, the lower pollen germination using the wet artificial pollination method in *E. latifrons* suggests that the pollination method used at KNBG may be the cause of low seed germination. For this reason, the effects of wet and dry pollination are more intensively investigated.

Acknowledgements

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