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Ex situ pollination and multiplication of *Encephalartos laurentianus* De Wild. (Zamiaceae, Cycadales)

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This paper reports the first case of *ex situ* pollination and multiplication of the Central African giant cycad *Encephalartos laurentianus* De Wild. (Zamiaceae, Cycadales), a rare and endangered species cultivated at the National Botanical Gardens of Meise, Belgium. A brief description of the discovery, distribution and morphology of the species is given; artificial pollination and seed germination techniques are described. About 160 seeds were produced by each of the two pollinated female cones. While most seeds were stored in order to use them for exchanges, ten seeds were sown; germinability was 60%.

Keywords: *Encephalartos*; cycads; conservation; pollination; Zaire

Introduction

The Cycadales are a very primitive and isolated group of seed plants consisting of three families (Cycadaceae, Strangeriaceae and Zamiaceae), 11 genera and about 190 species (Stevenson, 1990, 1992; Stevenson *et al.*, 1990; Stevenson and Osborne, 1993).

The fossil record of the Cycadales dates back to the Permian period (Zhifeng and Thomas, 1989); during the Mesozoic era they were widespread, playing a major role in the composition of the forests (Delevoryas, 1982). At the end of the Secondary era their range underwent a sudden contraction, and today they have a discontinuous, scanty distribution along the intertropical belt and subtropical areas in Africa, America, Asia and Oceania (Balduzzi *et al.*, 1982).

A large number of species belonging to this order are in danger of extinction. Reasons for their disappearance are climatic changes and competition with the angiosperms, as well as anthropogenic effects such as the environmental alterations (mainly in Mexico and Central Africa), bulk collection for commercial purposes, and use of pesticides (Vovides and Peters, 1987; Osborne, 1989). These chemicals cause the extinction of the snout weevils (Coleoptera, Curculionidae) which pollinate cycads.

The existence of widespread insect pollination in cycads is a subject of great interest: it is the only report of entomophily in living non-angiospermous plants, and shows that mutualistic relationships between plants and insects are more primitive than hypothesized before (Norstog *et al.*, 1986; Tang, 1987b; Norstog and Fawcett, 1989; Chadwick, 1993;

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Connell and Ladd, 1993; Fawcett and Norstog, 1993; Jones, 1993; Vovides *et al.*, 1993). However, entomophily in *Encephalartos* is still only suspected, not enough studies being available in this regard (Rattray, 1913; Goode, 1989).

Two cycad genera are endemic to Africa: the monospecific *Stangeria* T. Moore (Stangeriaceae), with *S. eriopus* (Kunze) Baillon, and *Encephalartos* Lehm. (Zamiaceae), with about 55 species (Dyer and Verdoorn, 1969; Giddy, 1984; Goode, 1989; Stevenson and Osborne, 1993). Most of the species of *Encephalartos* (about 35) are distributed in Southern Africa; but only about 20 can be found in Central Africa (Melville, 1957; Heenan, 1977; Goode, 1989). Knowledge of Central African *Encephalartos* species is poor compared with that on Southern African species. The reasons for the paucity of data on Central African species are numerous: isolation of ranges, difficulty in reaching them, deficiency of local botanical institutions supporting field reasearch, and difficulties encountered in botanical exploration of Central African countries, as a result of logistical and political problems.

Studies are made even more difficult by the increasing rarity of these species, which have never grown abundantly in their ranges, and now are even less widely dispersed. In this last respect, it can be noted that all *Encephalartos* species are classified as endangered, vulnerable, or rare by the Threatened Plant Unit (TPU) of the International Union for Conservation of Nature and Natural Resources (IUCN) (Gilbert, 1984). All *Encephalartos* species are also listed in Appendix I of the Washington Convention on International Trade in Endangered Species of Wild Fauna and Flora.

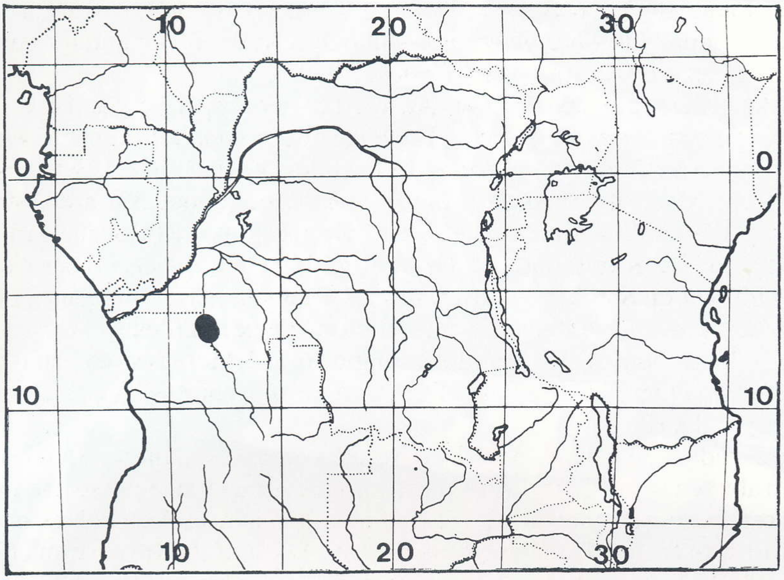
Since 1967 we have been carrying out research on Central African species both to improve information on their biology and to contribute to their conservation (Malaisse, 1969; Lisowski and Malaisse, 1971; Malaisse *et al.*, 1990; 1992; 1993). As a part of this work, we present here results obtained in the study of the *ex situ* pollination and multiplication of *E. laurentianus*, a species particularly rare in nature at the present time.

Encephalartos laurentianus: Discovery, distribution and morphology

In August 1902, L. Gentil discovered a population formed of several hundred plants of this species downstream from the old hamlet of Kasongo-Lunda, along the sandstone cliffs of the Kwango river in Zaire (Map 1). He collected two hundred young plants and sent them to the Colonial Garden of the Congo State at Laeken near Brussels (Belgium). This new species was described in 1903 by De Wildeman. In January 1930, J. Lebrun rediscovered the locality and determined the restricted distribution of the species, by reporting occurrence of plants in the Kwango valley, in an area delimited northwards by the confluence of the Kwango river with the Fufu and southwards by the confluence with the Kikasu. The plants occurred along river banks in gallery forest at the borders of the Kwango and of most of the tributaries of the two above-mentioned rivers. In October 1948, W. Robyns visited this locality and took a photograph of a female plant; this picture was used as a model for the colour painting present in the 'Flore du Congo Belge et du Ruanda-Urundi' (Robyns, 1948).

The specific epithet *laurentianus* was chosen in honour of Professor Emile Laurent (Agronomic Institute of Gembloux, Belgium), who introduced the first *Encephalartos* plants (*E. poggei* Ascherson) from the Belgian Congo (now Zaire) to Belgium.

Encephalartos laurentianus is a giant species with stems up to 15 m long and 1–1.25 m in diameter. Leaves are very long, usually 4–6 m but occasionally up to 7 m. Median leaflets



Map 1. Distribution of *Encephalartos laurentianus*.

are 35–50 cm long and 4–7 cm wide and are heavily toothed. Male plants produce 2–6 cylindrical to ellipsoid cones, 20–35 cm long and 6–10 cm wide. Female plants produce 2–4 oblong to ovoid cones, 35–45 cm long and about 21 cm in diameter. Seeds are bright red.

Ex situ pollination in *E. laurentianus*

At the beginning of September 1991, in the greenhouses at the National Botanical Gardens of Meise, Belgium, we noted the presence of a male and a female plant of *E. laurentianus*. The female plant had two ovoid cones and the male plant three cylindrical cones. The simultaneous occurrence of the two kinds of cones in *Encephalartos*, and in other cycads as well, is a very rare event both in the field and in cultivation (Giddy, 1984; Goode, 1989). This unusual finding stimulated us to carry out the artificial pollination of these plants.

We periodically checked cone maturation stages, waiting for the best period to attempt pollination. In the middle of October, male cones were 29 cm long and 7 cm wide; female cones were 36 cm long and 18 cm wide (sizes represent the average of measurements of all cones). Male cone scales were slightly separated showing a coloured internal part, and initial shedding of mature pollen was apparent (Fig. 1). In the meantime, clear slits among scales appeared on female cones, a condition indicating possible receptivity.

On 16 October, we carried out a first pollination experiment, following the procedure suggested by Giddy (1990). We gently shook male cones and collected pollen on smooth paper (Fig. 1); by using a knife, we removed a scale from the top of each female cone, so that the central axis was visible, and then introduced some pollen inside the cones by blowing it into the opening created by removing scales (Fig. 2). We also introduced pollen in the slits among scales by using a sable brush (Fig. 3).

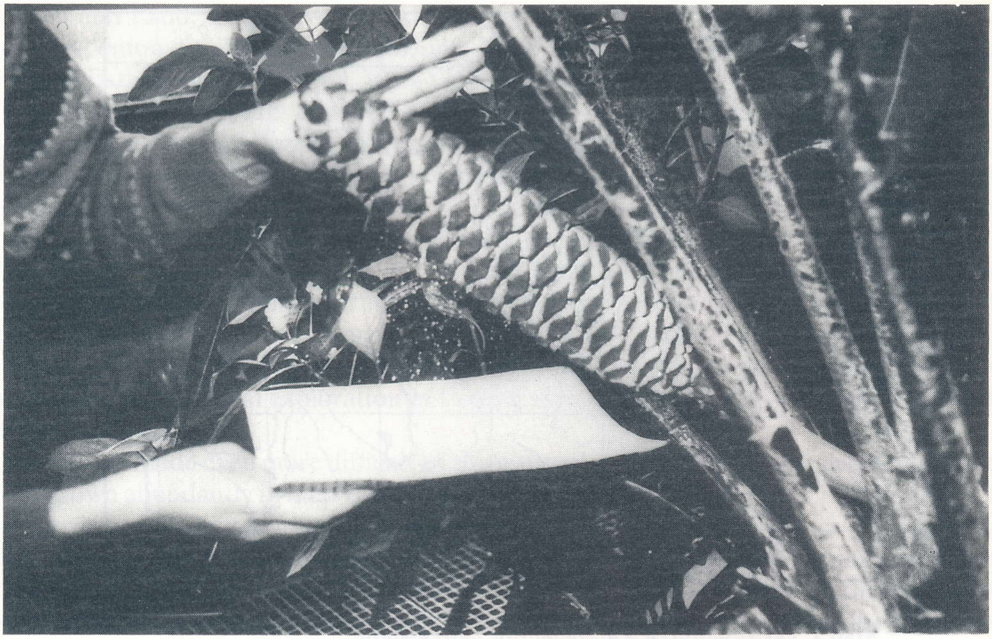


Figure 1. Collecting pollen by shaking a male cone of *Encephalartos laurentianus*.

As pollen receptivity by *Encephalartos* female cones lasts 6–8 days (Giddy, 1990), we repeated pollination twice during the week. Due to short viability of cycad pollen (3–4 days, at room temperature) (Giddy, 1984), we collected a large amount of pollen before the first pollination and stored it at 4°C, in order to use it for two additional pollinations. In this way, pollen viability can be prolonged up to 8–10 days (Giddy, 1984), a time long enough to complete our manipulations.

Female cones began to release seeds only in August 1992, ten months after pollination. A comparable time was reported by Tang (1987a); this author artificially pollinated 18 cultivated cycad species and showed that the time elapsing between pollination and seed ripening ranged from four (in *Encephalartos hildebrandtii*) to 13 months (in *Dioon edule*).

Since *Encephalartos* ovules increase their volume and reach their definitive size regardless of whether they are pollinated or not, any measurement of seed or cone size variation is not indicative of pollination effectiveness and seed fertility. The observation of the embryo located at the extremity of a suspensor is certain evidence of seed fertility. We observed this structure (Fig. 4).

About 160 seeds were produced by each female cone. The seeds were orange red in colour, 4.7 cm long and 2.4 cm wide. Fertile seeds, verified through the sinking/floating method (seeds which float in water are sterile, seeds which sink are fertile) (Giddy, 1984), were 40%.

Germination

Most seeds were stored in order to use them for exchanges with other scientific institutions involved in a cycad conservation programme. Ten seeds (presumed fertile) were placed in

Figure 2. Female cone of *Encephalartos laurentianus* showing the removed scale in the upper part.

pots in a greenhouse at the National Botanical Gardens of Meise, on 1 October 1992. Initial emergence of various plantlets was observed in a period ranging from 15 December 1992 to 5 January 1993. Seed germinability was 60%. Figure 5 shows one plantlet in April 1994, 18 months after the sowing; corraloid roots are already present.

Discussion

This paper reports the first known case of *ex situ* pollination in *E. laurentianus*. The specimens cultivated at the National Botanical Gardens of Meise since the beginning of this century, according to our knowledge, have never produced fertile seeds. Various hypotheses can be used to explain this: asynchronous coning in male and female

Figure 3. Introduction of pollen in the slits among scales of a female cone of *Encephalartos laurentianus*.

specimens, plants located too far apart to allow pollination, and absence of specific pollinators. The last factor would be of particular relevance, if the necessity of insects for *Encephalartos* pollination could be demonstrated.

This experiment assumes even more relevance because of the rarity of natural populations of *E. laurentianus*. During a recent exploration (1988) in Zaire, populations reported for areas where this species was originally described were not found (J.P. Sclavo and A. Moretti, personal communication).

Although protecting cycads means protecting their habitats, as well as preserving and reintroducing the plants *in situ* to ensure genetic variability, an easier and more practical interim solution is afforded by *ex situ* conservation. Collecting and transferring to botanical institutions plants of special rarity and interest, artificial pollination, and establishment of pollen and seed banks are valid alternatives when their habitats have been irrevocably altered. *Ex situ* conservation, in addition to ensuring plant survival, gives information on cultural requirements, reproductive biology and propagation techniques. It has potential also for plant reintroduction to the original habitats.

A good example of *ex situ* conservation is represented by *Encephalartos woodii* Sander, a species extinct in nature and only surviving in cultivation in a few botanical gardens and private collections. Similarly, *Microcycas calocoma* (Miq.) A. DC., whose pollinators as already indicated are extinct, has been frequently reproduced at the Fairchild Tropical Garden of Miami, Florida, USA, and seeds have been supplied to numerous international scientific institutions.

Cycads produce cones every year in botanical gardens, private collections and nurseries. We sincerely hope that our experience will stimulate other researchers to similar attempts, also considering the relative feasibility of artificial pollination, in order to avoid loss of

Figure 4. Fertile seed of *Encephalartos laurentianus* showing the presence of the embryo.

potentially fertile seeds (300–450 seeds can be produced by a megastrobilate cone each year).

Finally, development of pollen banks is highly desirable. In this respect, several scientific societies have already recognized this necessity and have organized cycad pollen banks

Figure 5. Eighteen months old plantlet of *Encephalartos laurentianus*.

(e.g. the Cycad Society of USA, the Cycad Society of South Africa and the Palm & Cycad Society of Australia and New Zealand). Unfortunately, due to short pollen viability (2–3 days), the usefulness of such enterprise is restricted only to users from nearby areas. Use of liquid nitrogen or similar preservation techniques should be investigated, in order to provide pollen storage methods of comparable efficiency to those already available for animal gametes.

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