4th Mediterranean Plant Conservation Week

VALÈNCIA | 23-27 OCTOBER | 2023

Organized by:

VNIVERSITAT DE VALÈNCIA Jardí Botànic

GENERALITAT VALENCIANA Conselleria de Medi Ambient, Aigua, Infraestructures i Territori

CIEF CENTRE PER A LA INVESTIGACIÓ I L'EXPERIMENTACIÓ FORESTAL

Ex situ conservation of pteridophytes and bryophytes through spores

Daniel Ballesteros

University of Valencia, Spain

Royal Botanic Gardens, Kew, UK

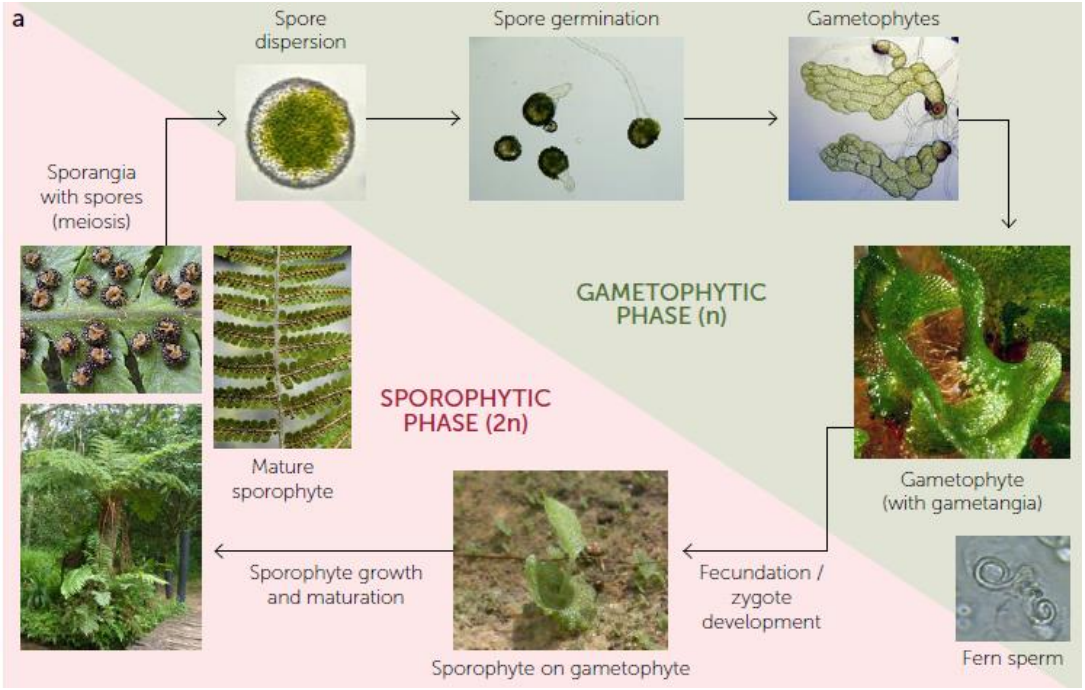
VNIVERSITAT DE VALÈNCIA

Royal Botanic Gardens Kew

Pteridophytes

Pteridophytes is a paraphyletic group of vascular plants that includes the **Lycophytes** (*club mosses, spike mosses, quillworts*) and the **Monilophytes** (*true ferns, whisk and fork ferns, grapeferns, horsetails,…*).

Pteridophytes **reproduce by means of spores** and lack flowers and seeds.

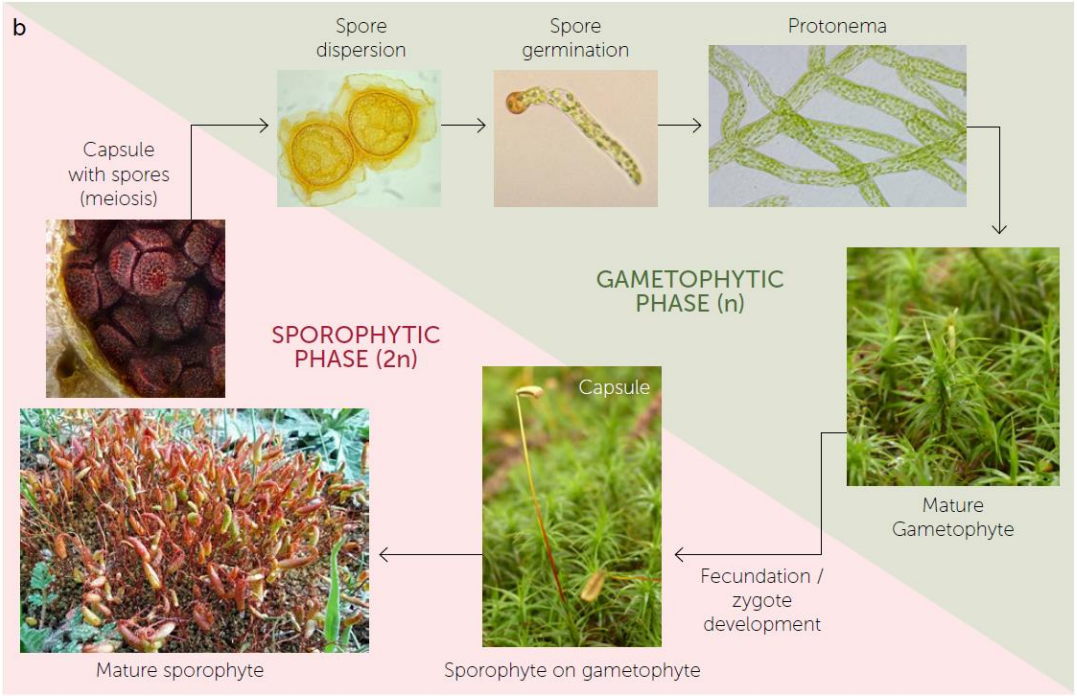


Plant Germplasm Conservation in Australia (2021)

Bryophytes

Bryophytes is considered a monophyletic group of non-vascular plants that includes *liverworts* (**Marchantiophyta**), *mosses* (**Bryophyta**) and *hornworts* (**Anthocerotophyta**).

Bryophytes also **reproduce by means of spores**.



Plant Germplasm Conservation in Australia (2021)

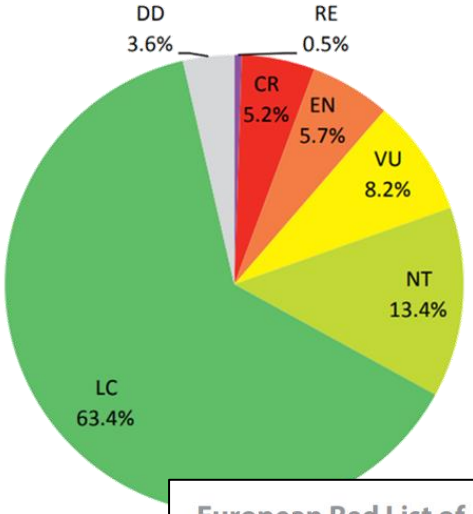
Fern conservation

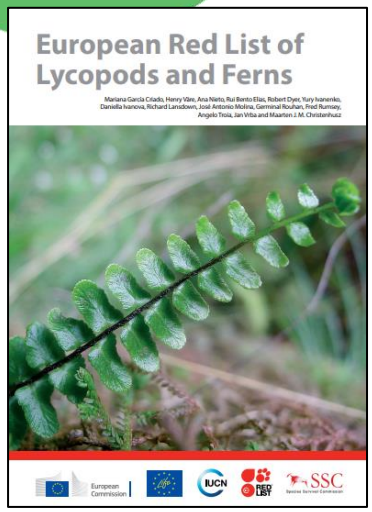
155 species of ferns (16%) are threatened with extinction globally (CR, EN, VU).

Priority conservation areas: tropical and subtropical montane forests.

37 species in Europe (20%) are considered CR, EN or VU in the IUCN Red List.

Brummitt et al (2016) J. Syst. Evol. 54

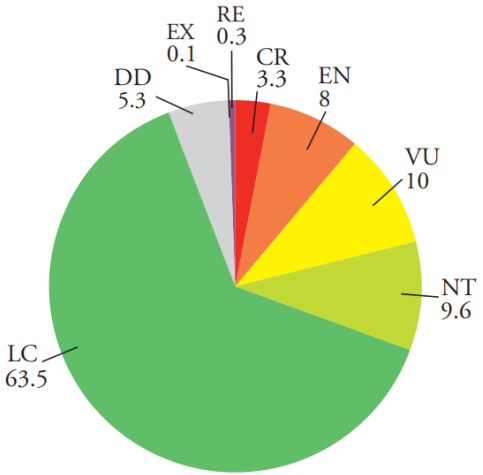


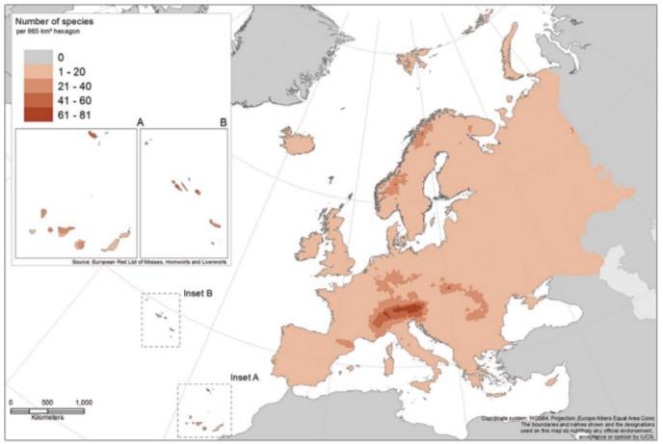


Garcia Criado et al (2017)

Bryophyte conservation

382 of bryophyte species (22,5%) are threatened with extinction in Europe (CR, EN, VU), with two species classified as Extinct and six assessed as Regionally Extinct (RE).

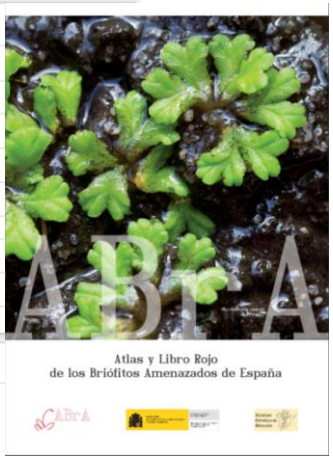




Hodgetts et al. (2019)

272 species in Spain (21%) are considered threatened (EX, RE,CR, EN or VU) in the Spanish Red List.

MUSGOS	ESPAÑA	Criterio	España Peninsular e Islas Baleares	Criterio	Islas Canarias	Criterio
Syntrichia abranchesii (Luisier) Ochyra	EX		EX			
Bryum arcticum (R. Br.) Bruch & Schimp.	RE		RE			
Campyliadelphus elodes (Lindb.) Kande	RE		RE			
Campylopus setifolius Wilson	RE		RE			
Discelium nudum (Dicks.) Brid.	RE		RE			
Hennediella heimii (Hedw.) Mitt.	RE		RE			
Hygrohypnum cochlearifolium (Venturi) Broth.	RE		RE			
Hypnum hamulosum Schimp.	RE		RE			
Isopterygium bottinii (Breidl.) Kindb.	RE		RE			
Ptychomitrium incurvum (Schwägr.) Spruce	RE		RE			
Ulota phyllantha Brid.	RE		RE			
Arctoa fulvella (Dicks.) Bruch & Schimp.	CR	B2a(ii, iv)	CR	B2a(ii, iv)		
Brachythecium cirrosum (Schwägr.) Schimp.	CR	B1ab(iii, iv)+2ab(iii, iv)	CR	B1ab(iii, iv)+2ab(iii, iv)		



Garilleti & Albertos (2012)

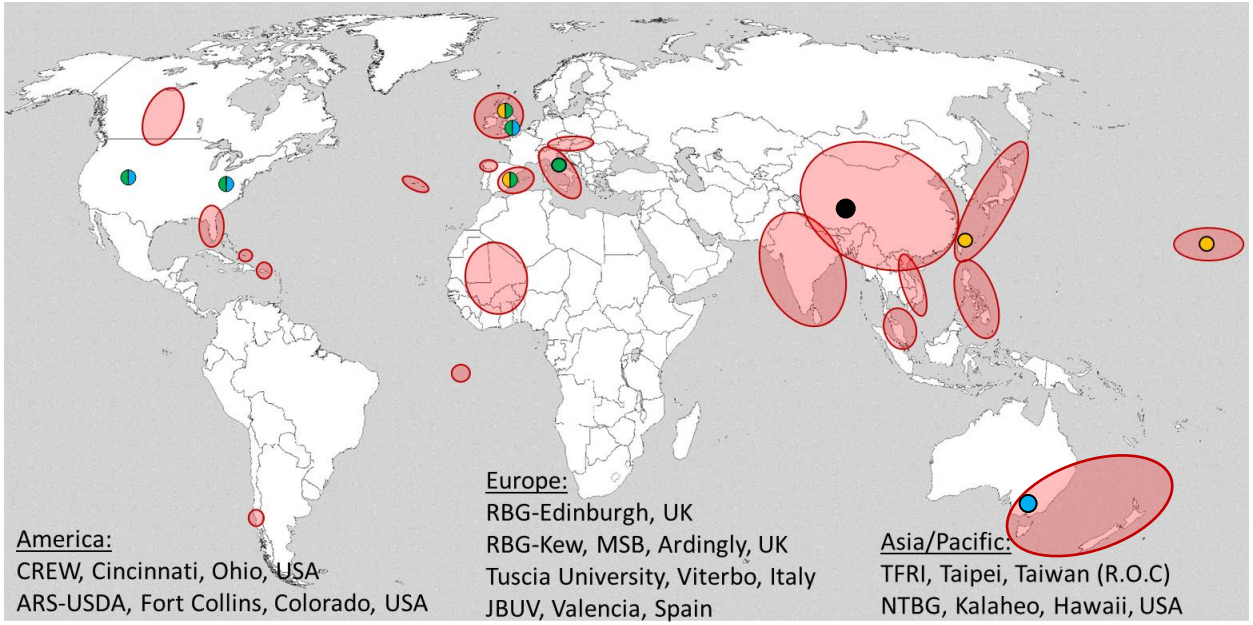
Fern spore banks

Are the preferred ex situ conservation tool

High genetic diversity can be preserved in a small space with minimal technical requirements.

<10 spore banks are active globally.

Ballesteros and Pence (2018)



Royal Botanic Gardens Victoria:
https://www.youtube.com/watch?v=ERUrtcJQNTg

Ohlsen & Miller (2023)

Fern spore conservation guidelines

(2018)

Chapter 11
Fern Conservation: Spore, Gametophyte, and Sporophyte Ex Situ Storage, In Vitro Culture, and Cryopreservation

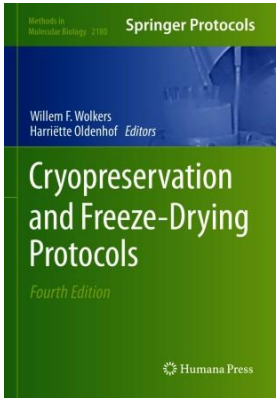
Daniel Ballesteros and Valerie C. Pence

(2020)

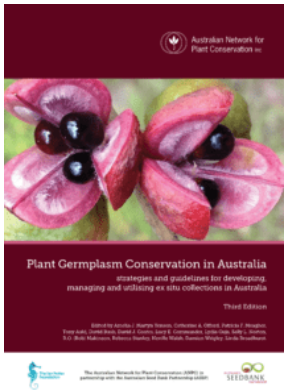
Chapter 33

Cryopreservation of Fern Spores and Pollen

Anna Nebot, Victoria J. Philpott, Anna Pajdo, and Daniel Ballesteros



(2021)



Chapter 13
Special collections and under-represented taxa in Australasian ex situ conservation programs

Tom North, Caroline Chong, Adam Cross, Karin van der Walt, Daniel Ballesteros

(2023)

CHAPTER 4
Culture Preservation and Storage Methods

Carolina Corrales, Marco Thines, Laura Forrest, Filip Vandelook, Jackie Mackenzie-Dodds, Elspeth Haston, María Paz Martín, Manuela Nagel, Daniel Ballesteros, and Jonas J. Astrin

CHAPTER 5
Cryopreservation

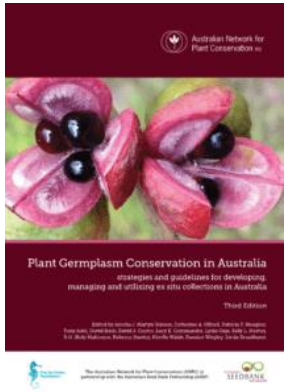
Carolina Corrales, Frederik Leliaert, Laura Forrest, María Paz Martín, Filip Vandelook, Marco Thines, Péter Poczai, Gila Kahila, Daniel Mulcahy, Elisabeth Haring, Luise Krukenhauser, Jackie Mackenzie-Dodds, Manuela Nagel, Daniel Ballesteros, and Jonas J. Astrin

Bryophte spore banks

Tiloca et al (2022)

Bryophyte spore conservation guidelines

(2021)



Chapter 13

Special collections and under-represented taxa in Australasian ex situ conservation programs

Tom North, Caroline Chong, Adam Cross, Karin van der Walt, Daniel Ballesteros

(2022)

plants

MDPI

Article

Bryophyte Spores Tolerate High Desiccation Levels and Exposure to Cryogenic Temperatures but Contain Storage Lipids and Chlorophyll: Understanding the Essential Traits Needed for the Creation of Bryophyte Spore Banks

Giuseppe Tiloca [1,2], Giuseppe Brundu [2] and Daniel Ballesteros [1,3,*]

[1] Seed and Stress Biology, Royal Botanic Gardens Kew, Wakehurst Place, Ardingly RH17 6TN, West Sussex, UK; tilocagiuseppe93@gmail.com
[2] Dipartimento di Agraria, Università degli Studi di Sassari, 07100 Sassari, Sardinia, Italy; gbrundu@uniss.it
[3] Departamento de Botánica y geología, Universitat de València, 46100 Burjassot, Valencia, Spain
* Correspondence: daniel.ballesteros@uv.es

(2023)

CHAPTER 4
Culture Preservation and Storage Methods

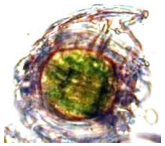
Carolina Corrales, Marco Thines, Laura Forrest, Filip Vandelook, Jackie Mackenzie-Dodds, Elspeth Haston, María Paz Martín, Manuela Nagel, Daniel Ballesteros, and Jonas J. Astrin

CHAPTER 5
Cryopreservation

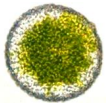
Carolina Corrales, Frederik Leliaert, Laura Forrest, María Paz Martín, Filip Vandelook, Marco Thines, Péter Poczai, Gila Kahila, Daniel Mulcahy, Elisabeth Haring, Luise Krukenhauser, Jackie Mackenzie-Dodds, Manuela Nagel, Daniel Ballesteros, and Jonas J. Astrin

Longevity of fern spores

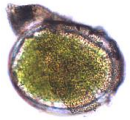
Green spores



Equisetum



Osmunda



Matteuccia

Onoclea

Green spores age fast and should be processed **within a week**

1 week | 1 month | 1 year | 10 years | 50 years

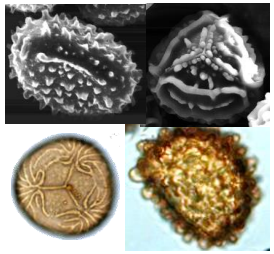
Non-Green spores

Culcita macrocarpa

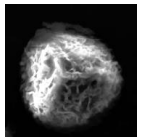
Thyrsopteris elegans

Gleichenaceae

Most fern species



Cheilanthes mysurensis



Pellaea

Marsilea

Both spore types tolerate desiccation

Longevity of Bryophyte spores

Green spores age fast?

Green spores

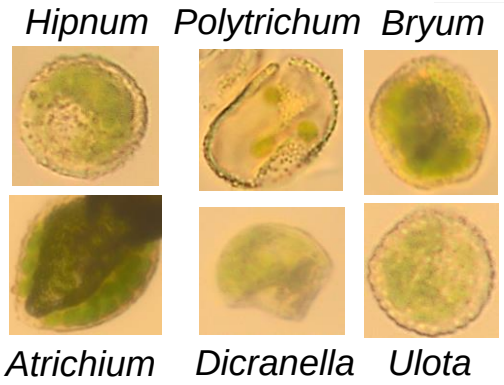
1 week 1 month 1 year 10 years 50 years

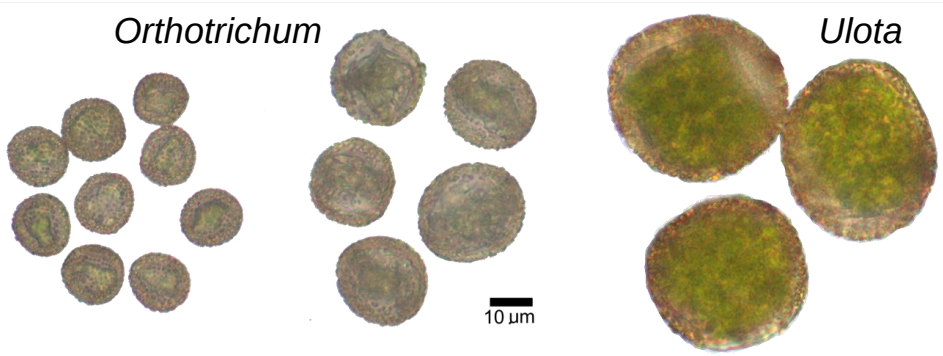
Lewinskya tasmanica
Andrewsiamthus
Gimnocoleopsis
Anastrophyllum

Frullaria
Jungermannia
Funaria hygrometrica

Physcomitrium turbinatum

Funaria hygrometrica
Ceratodon purpureus





Spores are desiccation tolerant

Expanding longevity of spores

Spores' longevity increases as temperature decreases

It was predicted that green fern spores of *Osmunda regalis* could maintain germination capacity >75% for about 55 years (-80 °C) and 1666 years (in LN).

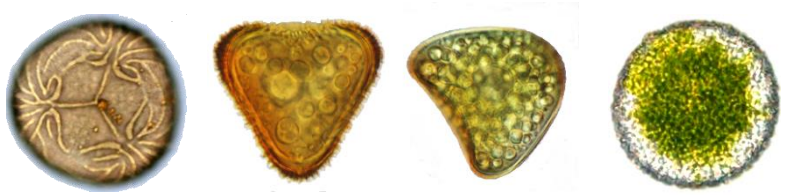
Ballesteros et al (2011)

But complete viability loss was measured in some green spores (*Equisetum hyemale*) within 12 years of LN storage.

Ballesteros et al (2019)

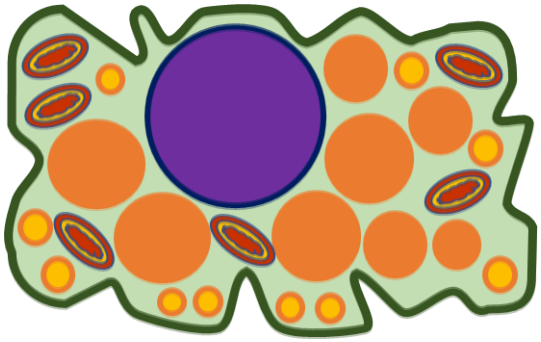
It has been predicted that non-green fern spores can maintain germination capacity for centuries or millennia when stored at -80 °C and in LN.

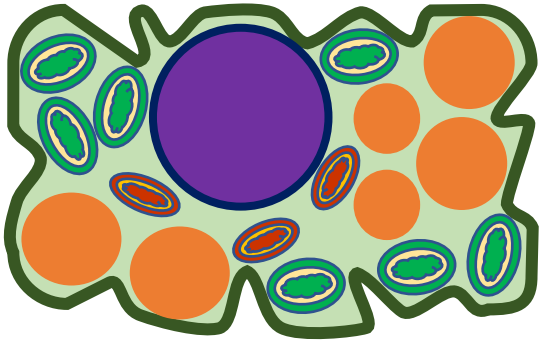
Ballesteros et al (2019)



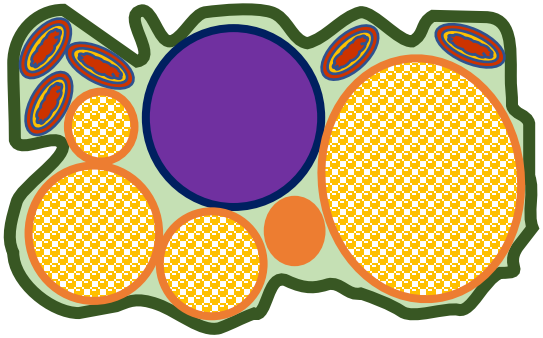
Unicellular model for seed research

Ageing mechanisms of diverse dry architectures





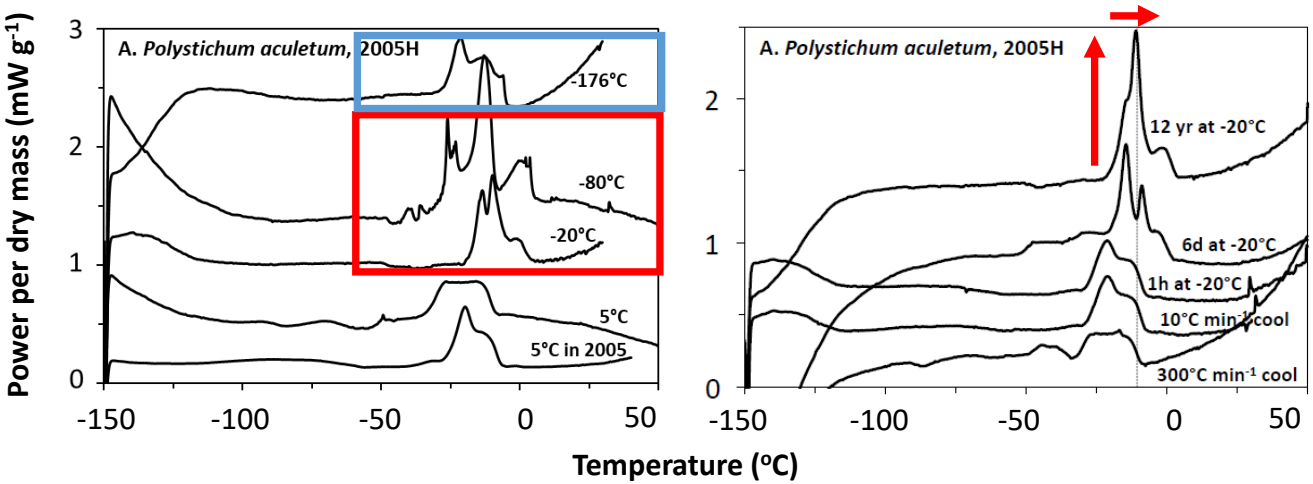
Fast ageing at all temperatures



Fast ageing at -20°C

Ballesteros, Pritchard, Walters (2020)

Molecular mobility in glassy matrices



Dry *P. aculeatum* spores aged faster at -18 than at 5°C (12 years storage experiment).

Lipids crystals conformation changed as function of storage temperature.

Higher preservation in cryopreserved spores, yet lipids still mobile at LN.

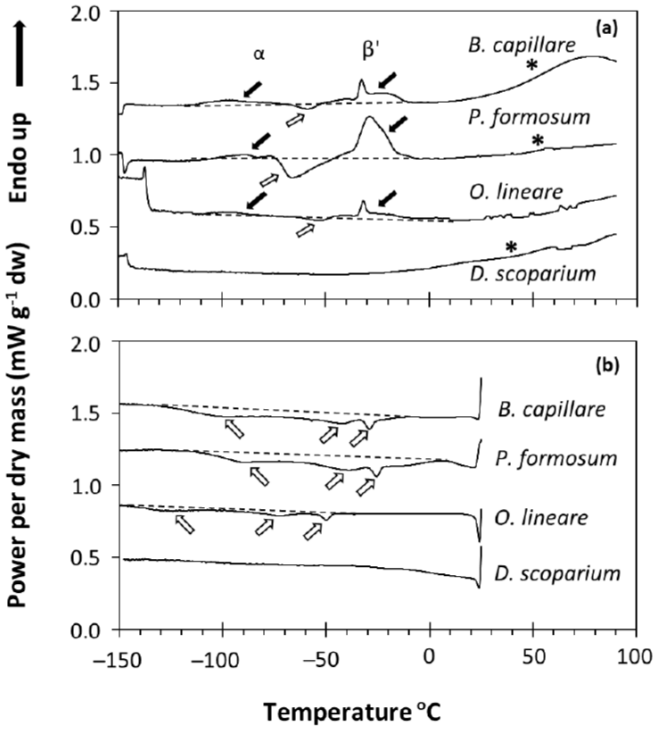
Lipids crystals conformation changed over storage time, particularly at -20 °C.

Ballesteros et al. 2019. PC&P

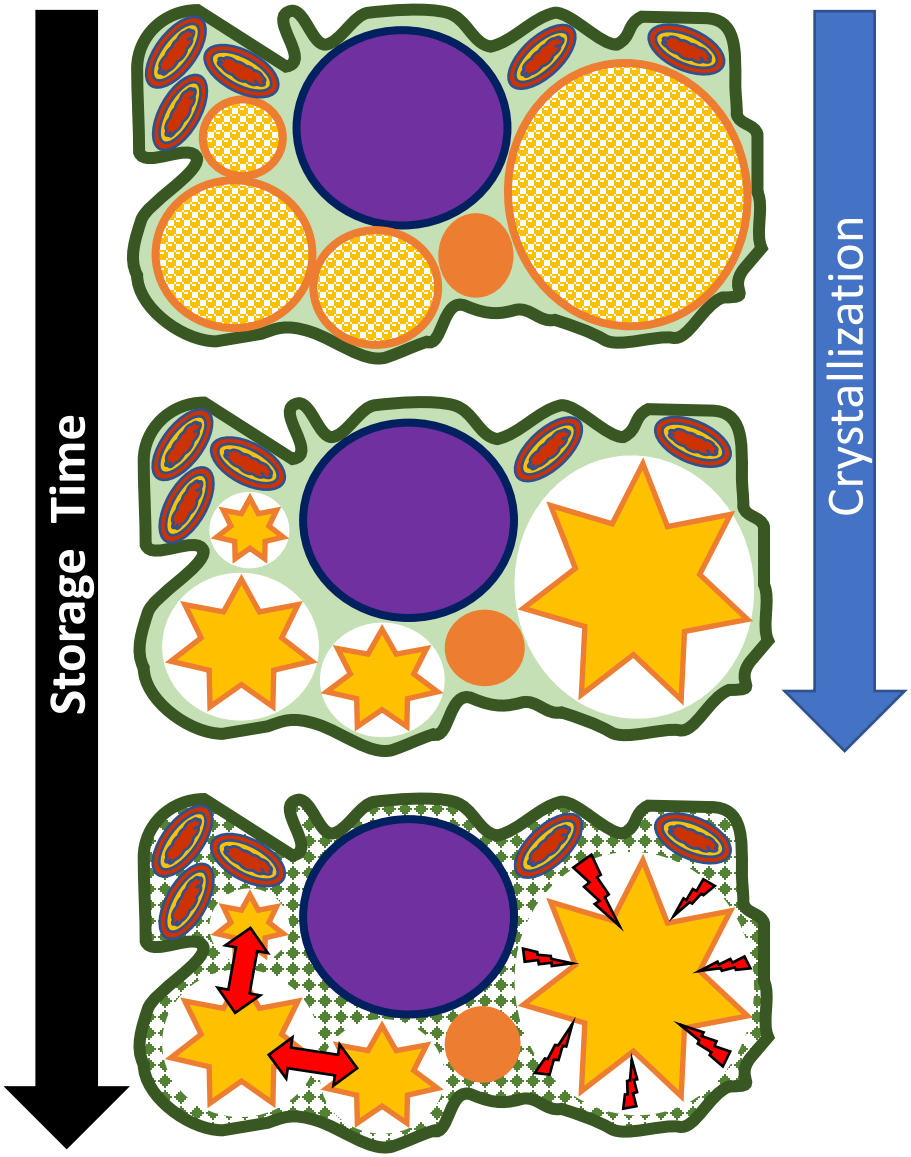
Ageing mechanisms

Proposed ageing mechanism of some oilseeds and fern spores stored at -20°C.

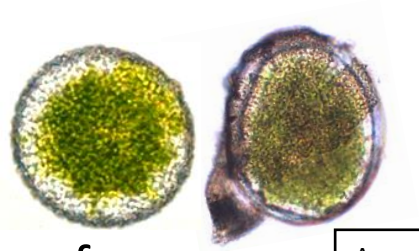
Ballesteros, Pritchard, Walters (2020)



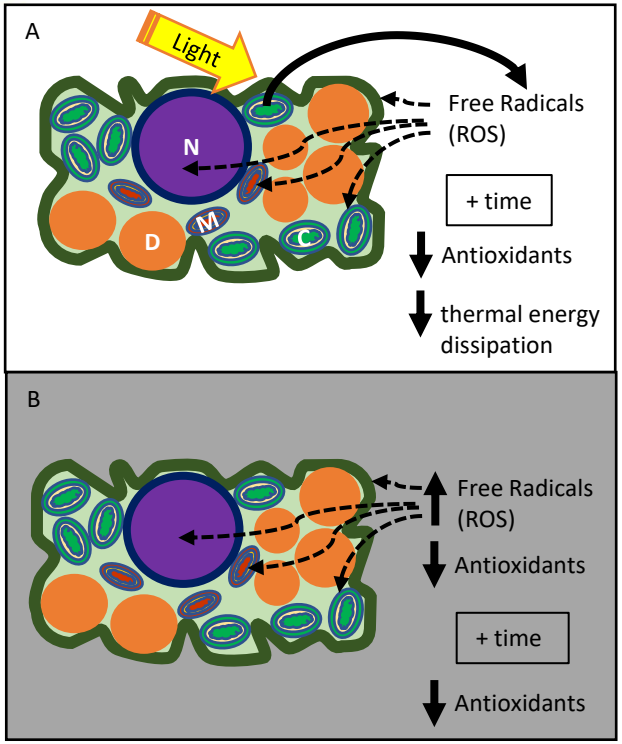
Tiloca et al. (2022)



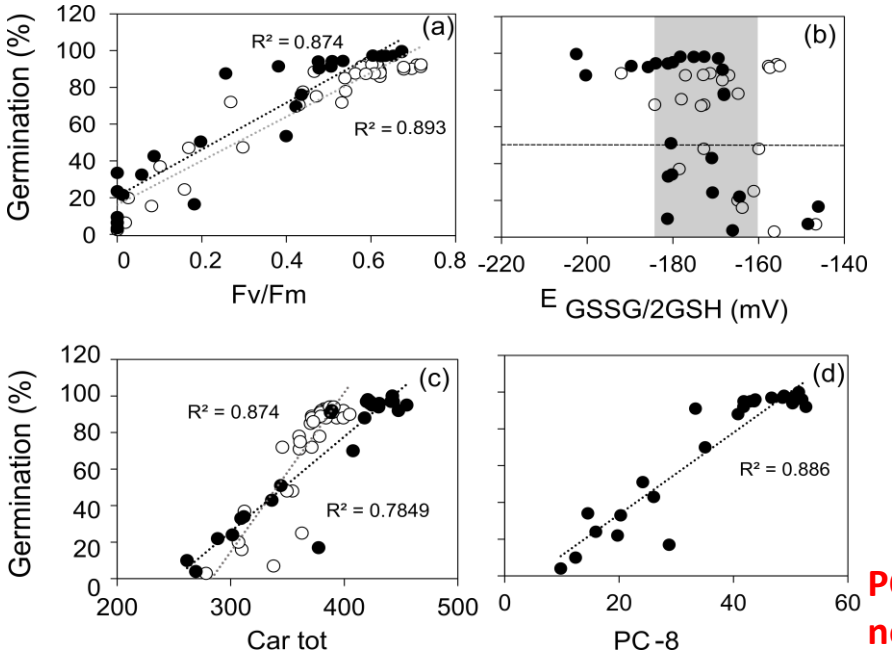
Ageing mechanisms



Antioxidants differ among green fern spores and regulate their lifespan in the dry state by preserving the photosynthetic activity in both light and dark conditions.



Ballesteros, Pritchard, Walters (2020)

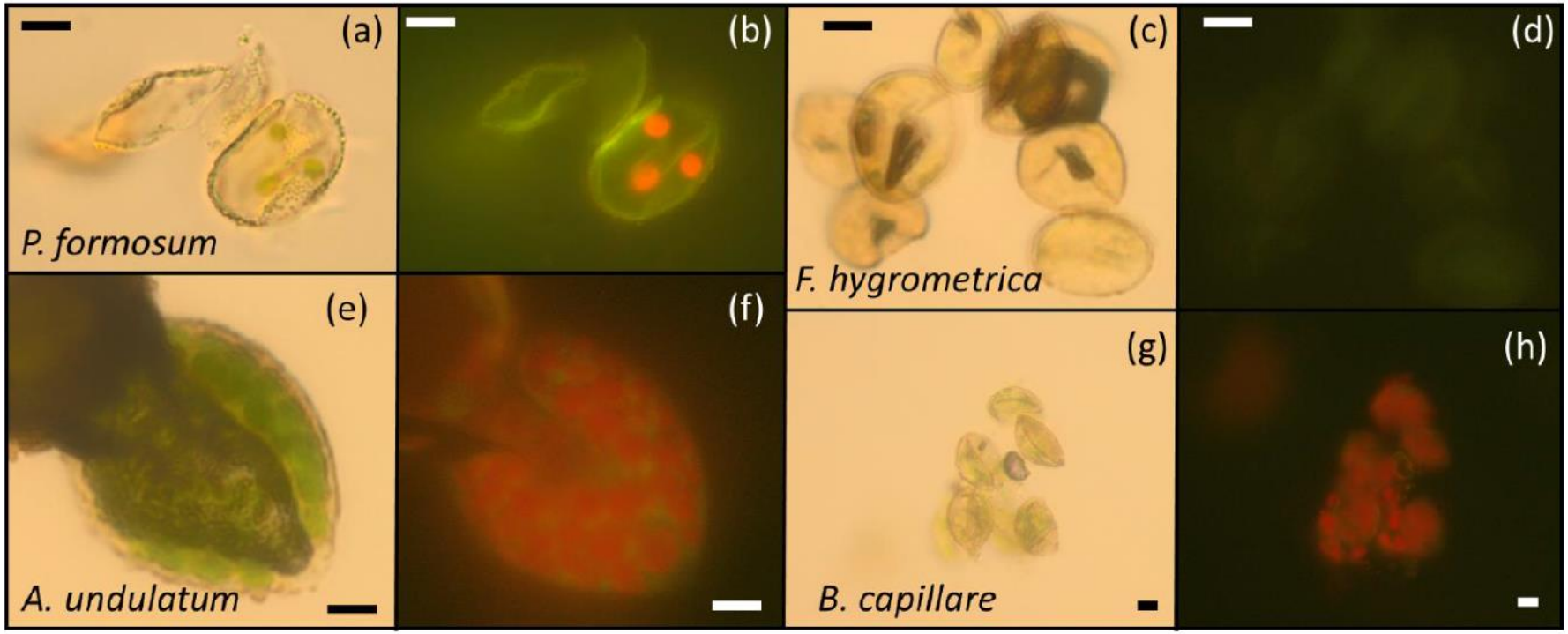


López-Pozo et al (2019)

López-Pozo et al. (2023)

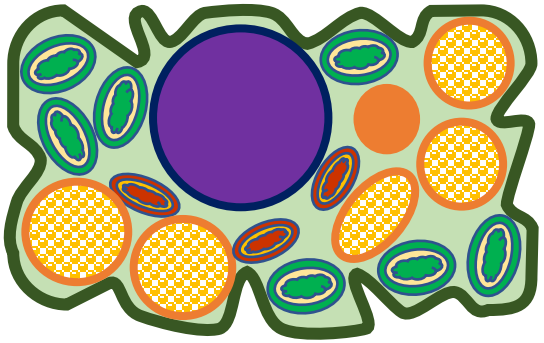
PC-8 is found in *Matteuccia* (long-lived) but not in *Osmunda* (short-lived).

Most bryophyte spores are chlorophyllous…



Tiloca et al. (2022)

…and oily…



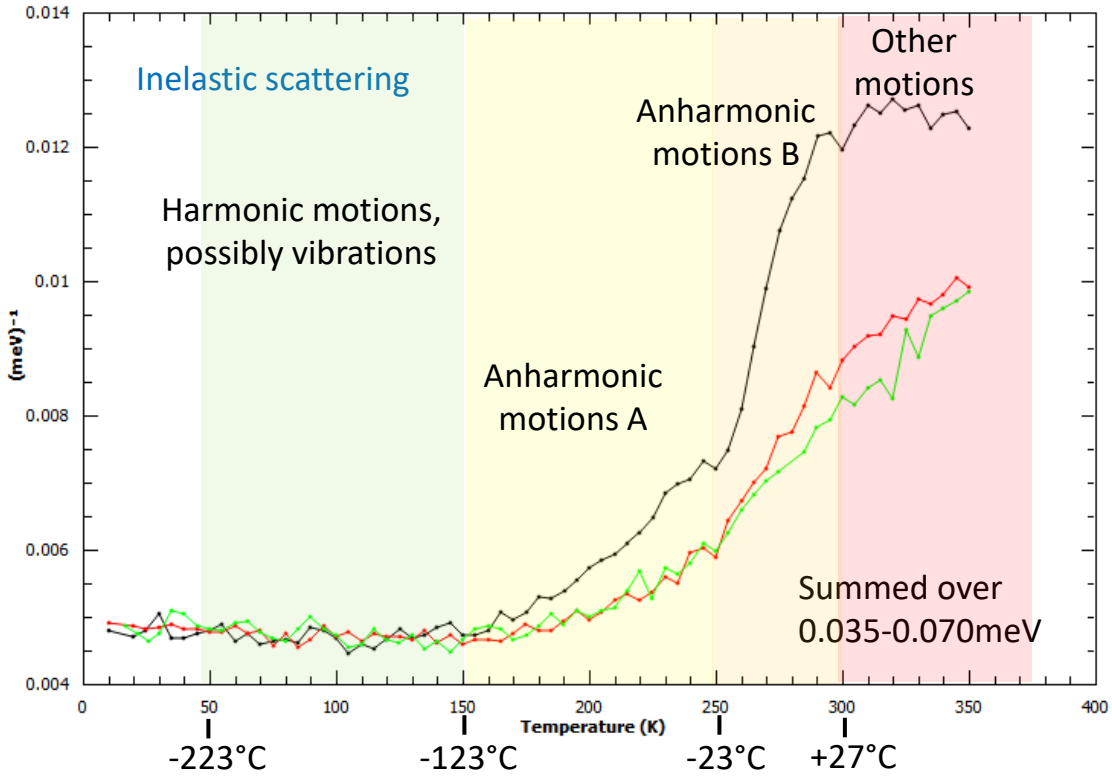
Additional dry architecture

Molecular mobility in dry fern spores by Neutron Scattering

Fern spores showed four distinct dynamic regimes that were related to different molecular mobility within the glassy state.

Results indicated that molecular motion is still possible at LN temperatures and below.

Science & Technology Facilities Council
ISIS Neutron and Muon Source



Ballesteros, Garcia-Sakai, Pritchard (2020)

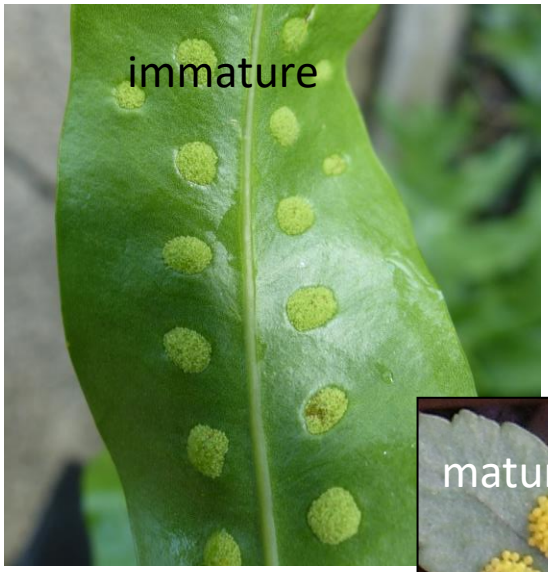
Maturity of fern spores

Always collect **mature spores**, as immature or old spores won't tolerate drying or will show short lifespan.

Dryopteris sp.

Mature spores at different stages

Maturity of fern spores



POLYPODIACEAE

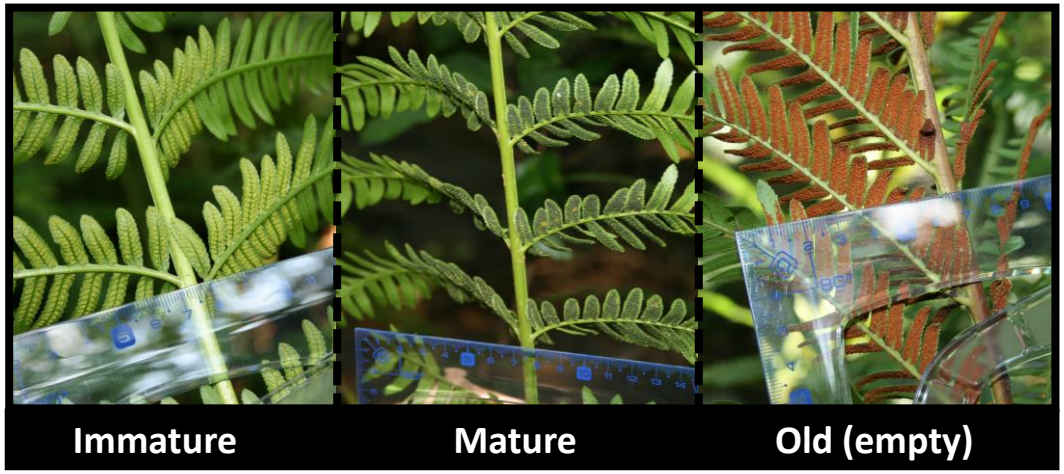
The color and aspect of the sorus and the sporangia is a good guide but can vary among some taxa.

Maturity of fern spores

Sporangia in ferns with chlorophyllous spores tend to dark green when spores are mature, and become brown after spore release

Nebot et al (2021)

Todea barbara



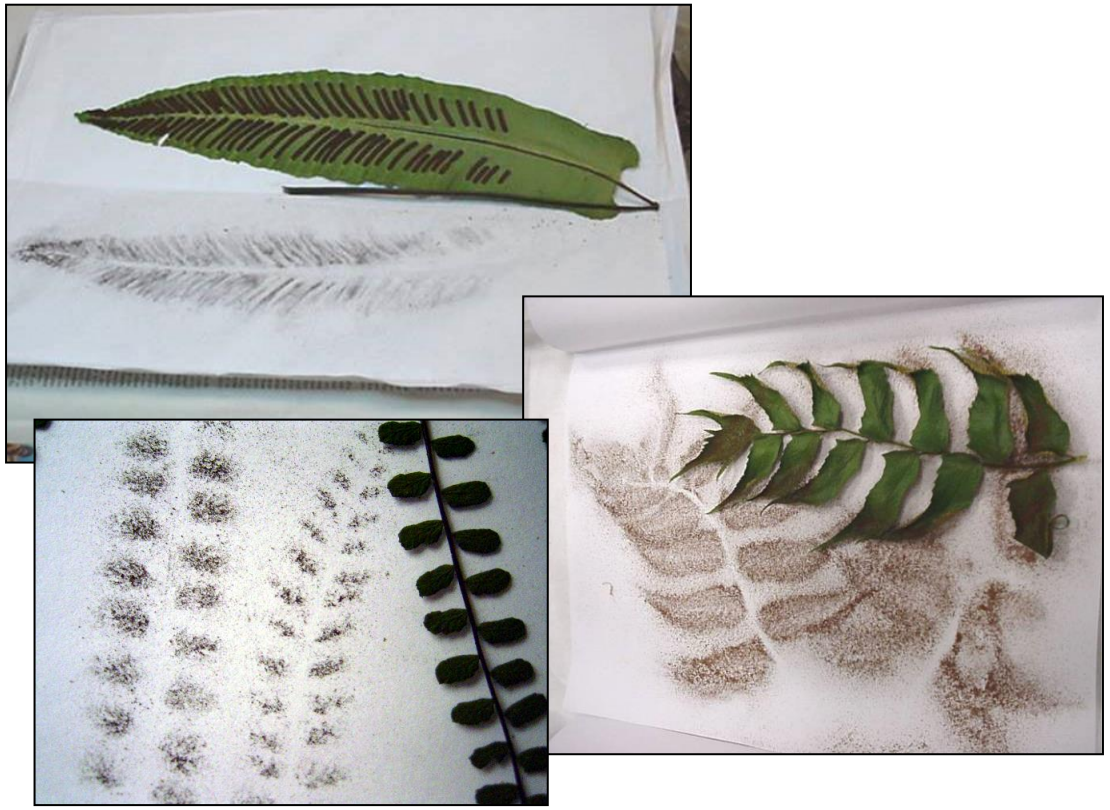
North et al (2021)

Spore collection

Fern fronds are put in glossy paper sheets.

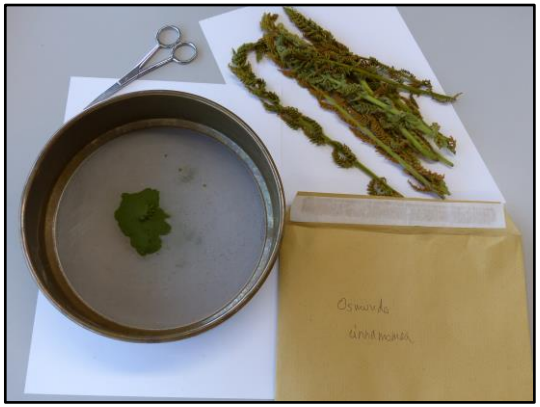
Dry at room conditions for 1-7 days if RH<75% (ideally 30-50%).

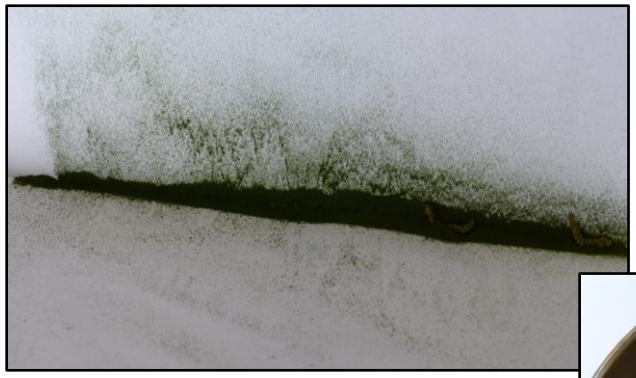
Sporangia will open, and spores will fall on the paper.



Spore collection

Collect spores from the paper sheet and sieve to eliminate frond and paleae rests.





Spore drying

Spores are desiccation tolerant, like orthodox seeds, but sensitive to RH<10%.

For storage at 5°C (fridge) or -20°C (freezer), drying at 20-35% RH is recommended.

For cryogenic storage at -80°C or in liquid nitrogen, drying between 30–50 % RH is suggested.

Dry green spores for 1-3 days and non-green spores for 5-7 days.

Spore storage

Store spores immediately after drying.

Long-term storage (>10 years) is recommended at cryogenic conditions (<-80°C).

Medium and short-term storage 1-10 years may be done in the fridge (5°C) or the freezer (-20°C) but avoiding freeze/thaw cycles.

Conclusions

- Fern and bryophyte **spore banks** can provide a high quality germplasm not only for fern **ex situ conservation** but also for fundamental seed science **research**.
- Fern and bryophyte spores tolerate similar drying to orthodox seeds and could be curated and stored at the **standard conditions of seed banks**.
- However, due to the **short life-span** of chlorophyllous spores at all storage temperatures and some non-chlorophyllous spores at -20°C, fast processing and **cryogenic storage** is recommended for their long-term conservation.

THANK YOU

VNIVERSITAT DE VALÈNCIA
Jardí Botànic

GENERALITAT VALENCIANA
Conselleria de Medi Ambient, Aigua, Infraestructures i Territori

CIEF
CENTRE PER A LA INVESTIGACIÓ I L'EXPERIMENTACIÓ FORESTAL

4th Mediterranean Plant Conservation Week
VALÈNCIA | 23-27 OCTOBER | 2023





Belen Albertos (UV)
Ricardo Garilleti (UV)
Carlos Eced (UV)
Aleksandra Ruzic (UV)
Giuseppe Tiloca (UV)
Marina López Pozo (UPV/EHU)
Victoria Garcia-Sakai (S&TRC)
Hugh Pritchard (Kew, CAS)
Christina Walters (USDA)
Elena Estrelles (JBUV)
And many other colleagues

VNIVERSITAT DE VALÈNCIA

BOKU
BPhys
Institute of Biophysics

Royal Botanic Gardens Kew

USDA ARS
Agricultural Research Service

UKRI
Science and Technology Facilities Council