Seed Drying Protocols

HSBP Information Resource Committee

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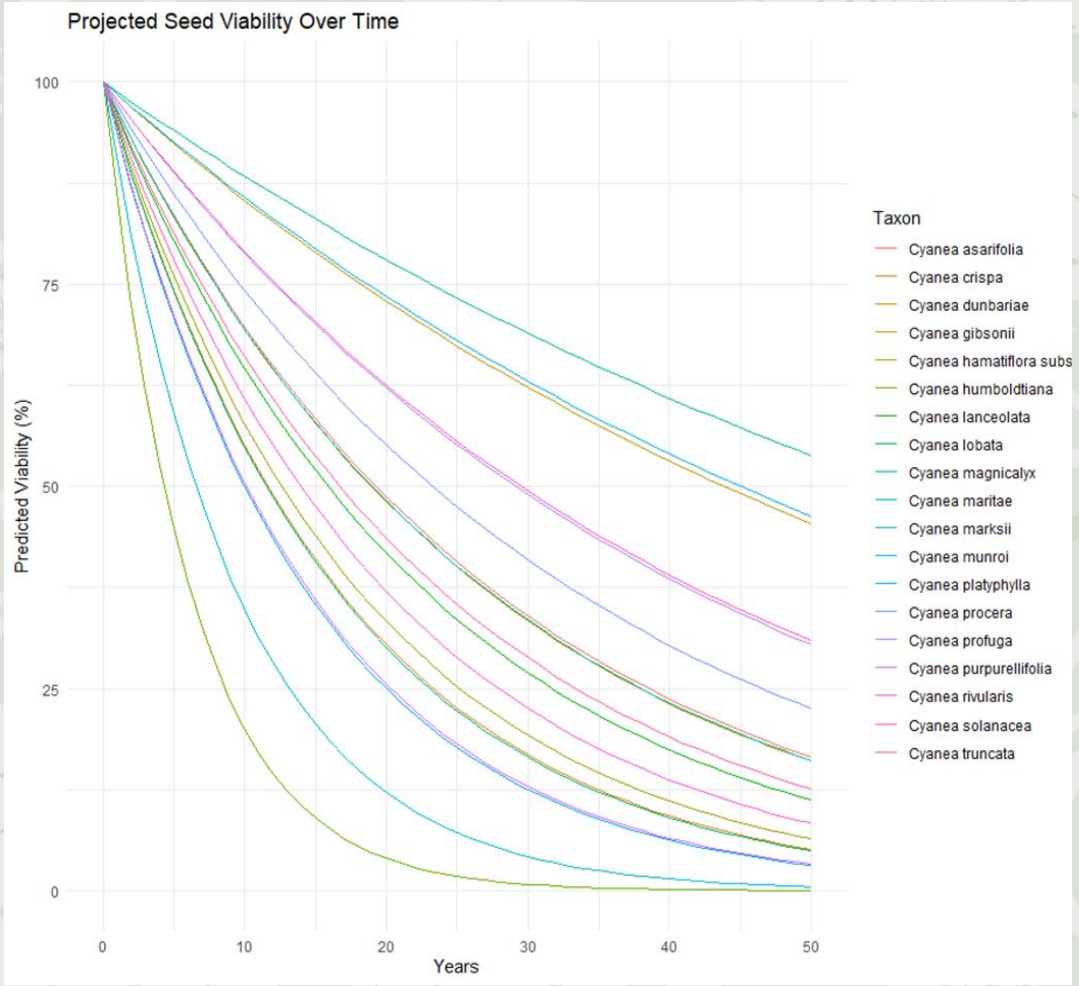
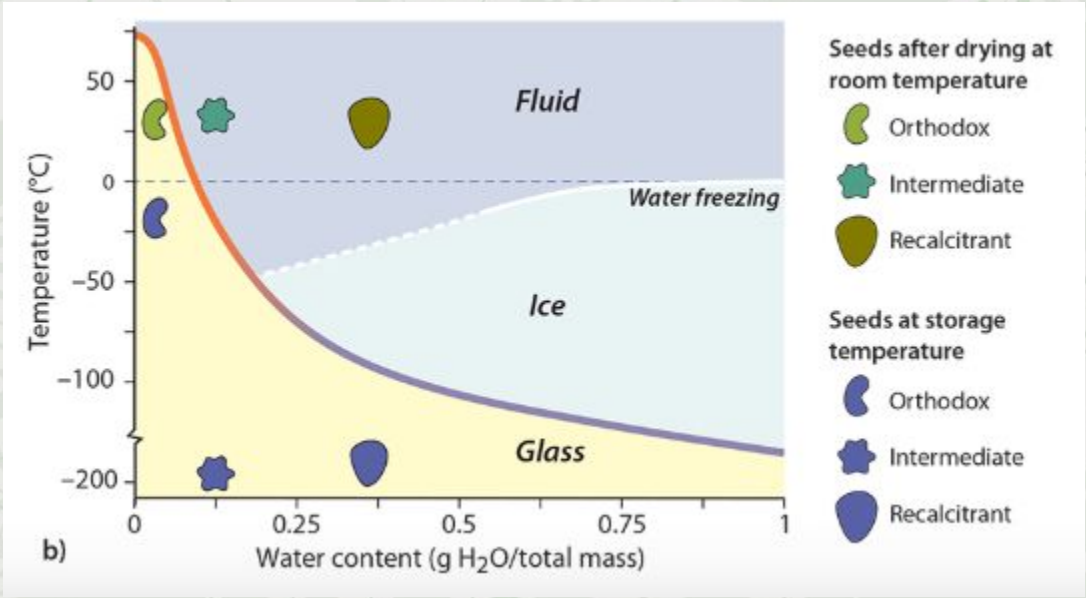
Seed Drying

- Prevents Ice Crystal Damage: Seeds with high moisture content risk ice formation when temperatures drop, which can damage cells and reduce germination.
- Slow Metabolic Activity: Seeds with a lower moisture content retain vigor longer, and cool, dry conditions are crucial in maximizing shelf life.
- Extend Longevity: Preserve by removing moisture

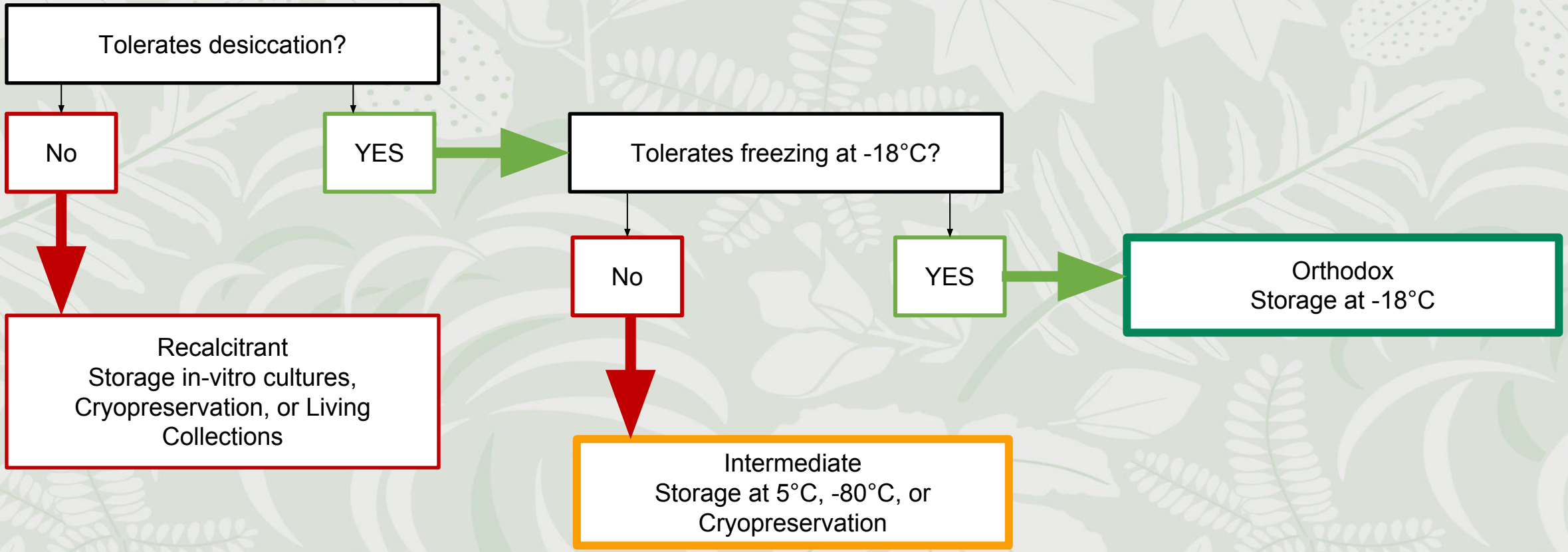


Seed Storage Behavior

- **Orthodox**
  - Desiccation Tolerant
  - Freeze Tolerant at -18°C
  - Conventional Seed Storage
- **Intermediate**
  - Desiccation Tolerant or Sensitive
  - Freeze Sensitive at -18°C
  - Alternative Seed Storage (5°C, -80°C, or Cryopreservation)
- **Recalcitrant**
  - Desiccation Sensitive
  - Water loss is lethal
  - Alternative Ex-situ Storage (Living Collections, Cryopreservation, or Micropropagation)



Seed Storage Behavior



Types of Desiccants

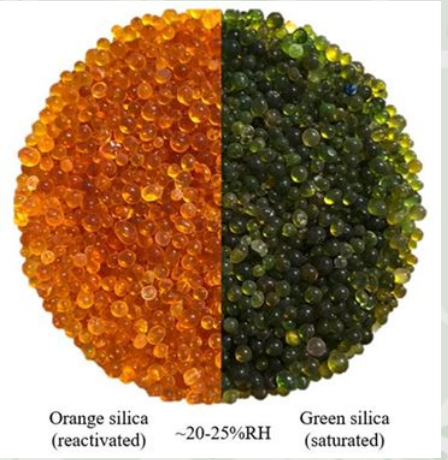
Comparisons of RH Values of Select **Saturated Salt** Solutions

Salt	Humidity at 25 °C (%)	Salt (g)	Water (mL)
Lithium Chloride	11.3	150	85
Potassium Acetate	22.5	200	65
Magnesium Chloride	32.8	200	25
Potassium Carbonate	43.2	200	90
Magnesium Nitrate	52.9	200	30
Sodium Bromide	57.6	200	80
Strontium Chloride	70.85	200	60
Sodium Chloride	75.3	200	60
Potassium Chloride	84.3	200	80

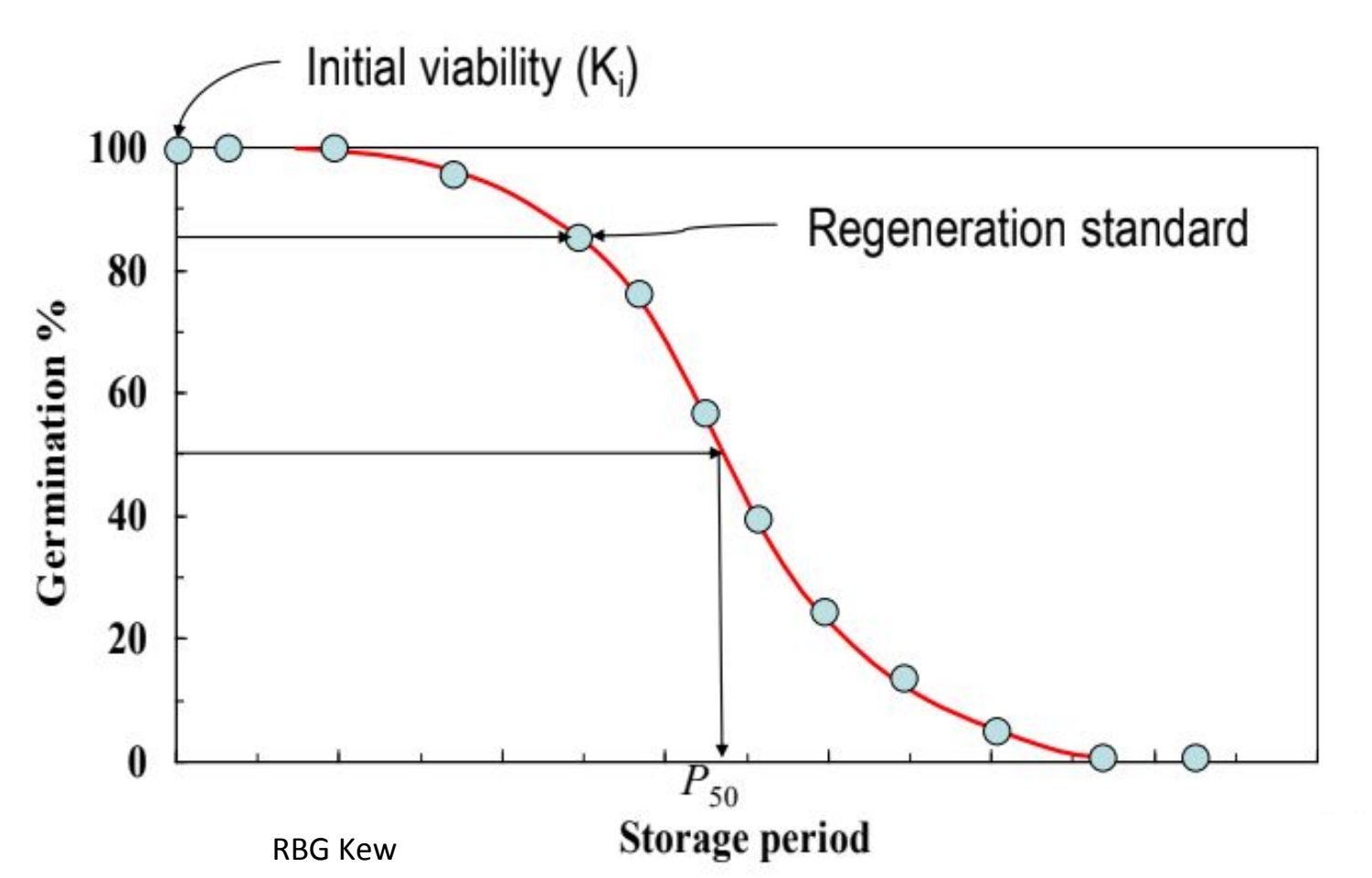
R. Jowitt and P.J. Wagstaffe, "The Certification of the Water Content of Microcrystalline Cellulose at 10 Water Activities", Commission of the European Communities, EUR 12429, Luxembourg, 1989

Silica Gel

- High adsorption capacity <15% RH
- Color-indicating: change according to moisture status
- Can be dried and re-used as a desiccant until it no longer absorbs moisture.

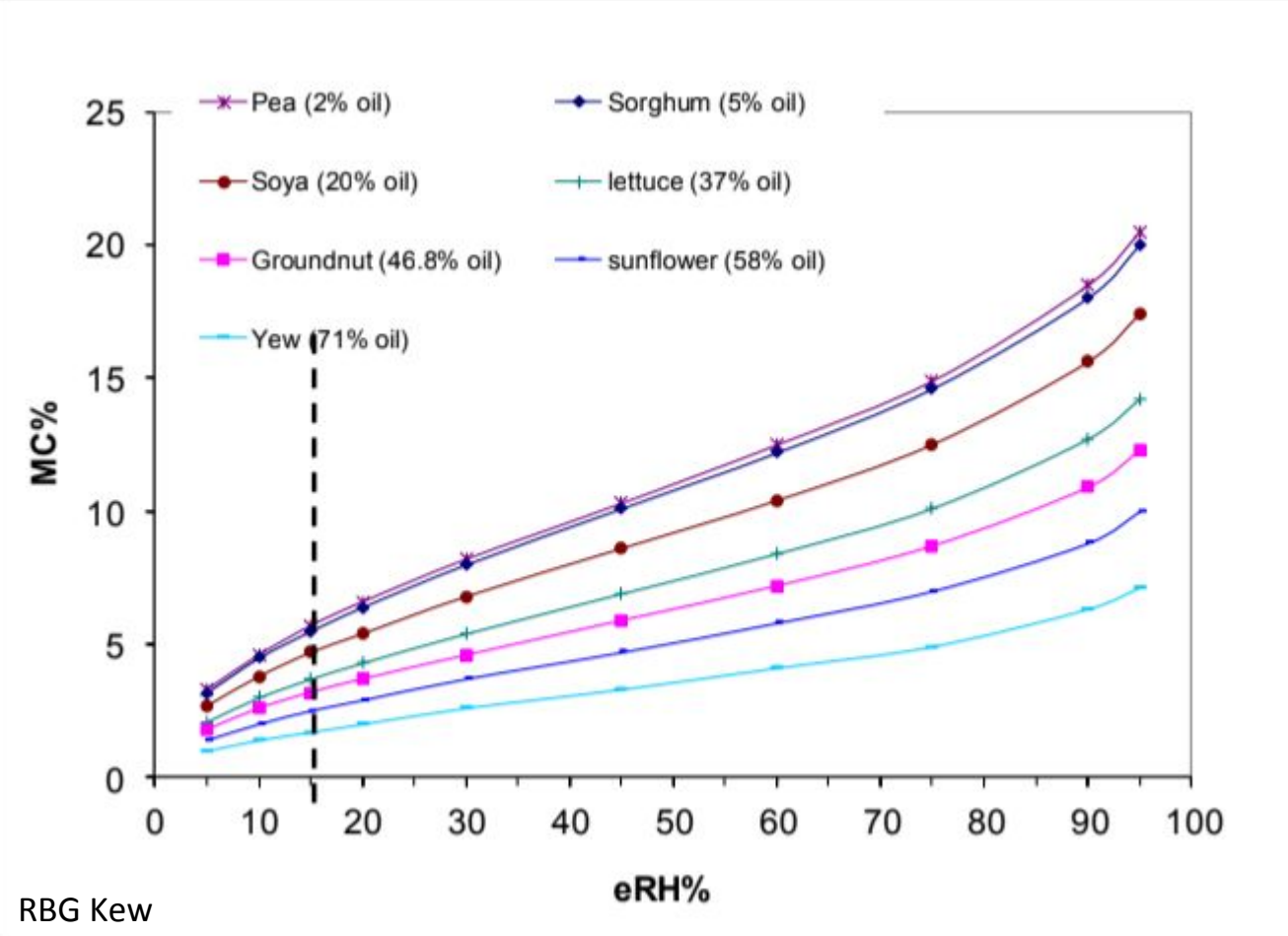


Seed Survival Curve



- For every 1% reduction in moisture content, or 10% reduction in RH, seed life span doubles
- For every 5°C drop in temperature seed life span doubles
- Initial quality has a significant impact on seed longevity

Seed Moisture Content



RBG Kew

Equilibrium Relative Humidity (eRH) refers to the relative humidity of air in equilibrium with a material's moisture content.

- Moisture content is expressed as the weight of water removed, divided by either the fresh or dry weight of seeds x100. The disadvantage of this method is its destructive nature.
- Moisture holding capacity of air increases as temperature increases, so relative humidity is reduced
- At a given RH, oily seeds will have a lower moisture content than non-oily seeds
- At a given RH, seed moisture content will be lower at higher temperatures

https://ser-sid.org/viability/moisture-equilibrium

Ultra-Dry

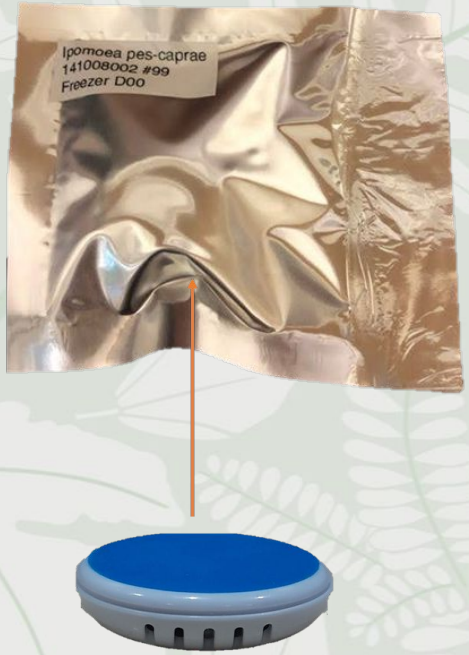
Low moisture content storage is a technique for decreasing seed moisture content below 5-6% or <10%RH

- Alternative seed storage to reduce or avoid refrigeration in seed banks with resource limitations
- Critical Water Content
  - Below which seed longevity is not improved
  - Below which seed longevity is reduced

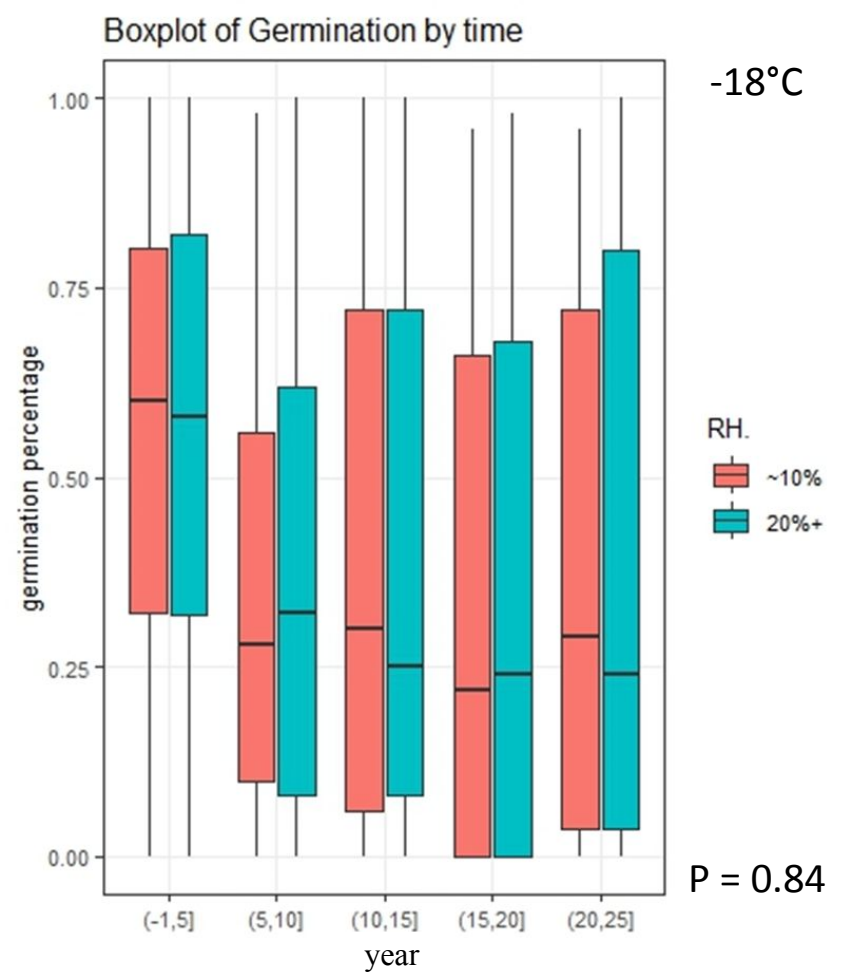
Lyon Drying Analysis

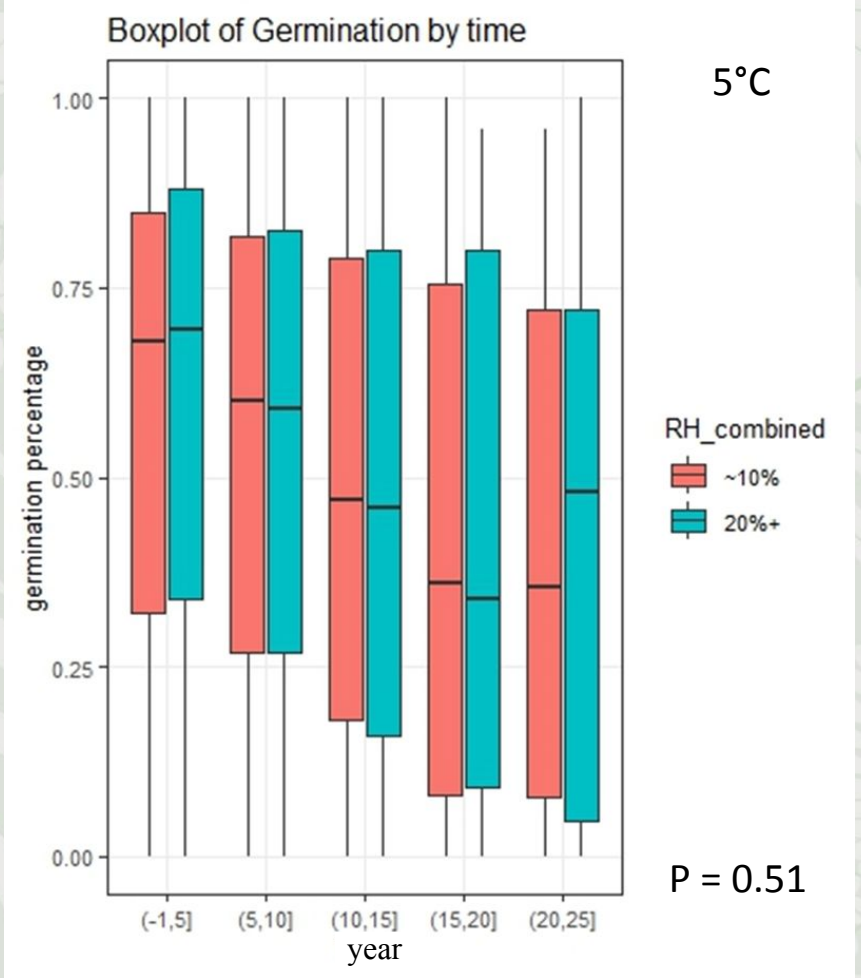
Storage code	Species	Accession number	RH (in %)	Temp (°C)
C20	Clermontia calophylla	240522004	40.00	5.5
C20	Cyanea marksii	240522003	39.10	5.5
C20	Cyanea stictophylla	240517002	38.10	5.5
C20	Joinvillea ascendens spp. ascendens	240516003	30.70	5.5
C20	Kadua fluviatis	240214002	35.80	5.2
C20	Lobelia oahuensis	221123003	46.90	5.8
C20	Myoporum stellatum	210723001	37.70	5.7
C20	Polyscias bisattenuata	161117011	27.80	5.8
C20	Santalum ellipticum	91005001	25.30	5.1
D20-A	Abutilon menziesii	130522007	38.70	-19.5
D20-A	Argyroxiphium sandwicense ssp. macrocephalum	140108001	28.50	-18
D20-A	Bidens torta	120301003	39.40	-18
D20-A	Bonamia menziesii	161117001	45.00	-18
D20-A	Colubrina oppositifolia	200804003	38.90	-20.1
D20-A	Cyperus trachysanthos	70601001	31.10	-18.7
D20-A	Erythrina sandwicensis	131112003	34.80	-18.7
D20-A	Hibiscus brackenridgei sp. brackenridgei	170613001	28.50	-18.1
D20-A	Ipomoea pes-caprae	141008002	34.00	-18.1
D20-A	Lysimachia filifolia	240214004	27.80	-18

BlueMaestro sensor data indicated that CPC drying protocols (~30-40% RH at ambient temperature) do not achieve the target ~20% eRH in storage and were extremely variable across taxa



Lyon Drying Analysis





As expected, germination decreased as storage time increased. However, there was no significant difference in germination response between the ultra-dry and traditional drying treatments.

HSBP Storage Recommendations

For long-term storage (10+ years), we recommend drying at ~20(±3)% RH at ~20°C to ensure optimal conditions for storage at both 5°C and -18°C (i.e., dry to the RH you want to achieve in storage).*

- Ultra-dry can maintain (not deleterious to) longevity
- Negative effects of ultra-drying may be masked by viability decline resulting from under-drying via CPC's methods.
- Germination rates highest when closer to target 20% eRH

Taxon	Year	Ultra-Dry Germ	CPC Stnd Germ	Ultra-Dry RH%	CPC Stnd RH%
L. mauritiania	22	30%	15%	13%	38%
M. polymorpha	24	40%	22%	9%	40%
P. tetraphylla	22	92%	96%	14%	27%
R. albescens	21	74%	50%	11%	35%
T. kaalae	23	78%	48%	12%	33%
V. reticulatum	24	20%	4%	7%	40%

Cyrtandra grandiflora (5°C)

Germination rate (%) conventional	Year	Germination rate (%) ultra-dry
73.00	0	88.00
82.00	1	76.00
92.50	2	82.00
83.00	5	89.00
75.00	10	82.00
11.00	12	75.00
1.00	15	42.00
0.00	17	44.00
0.00	19	3.00
0.00	21	0.00

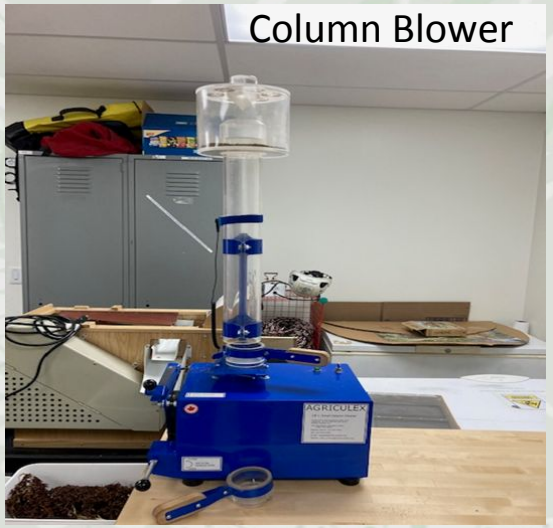
*These protocols assume the species is not desiccation-sensitive, or extremely short-lived.

Factors to Consider

- Are your seeds desiccation tolerant?
- Are your seeds mature?
- What are the ambient conditions?
- Volume of seeds
  - Spread seeds out in thin layer for even drying
- Time frame for storage
  - Long-term vs. short-term
- Materials
  - Facilities, drying cabinets, drying racks, desiccants, etc.

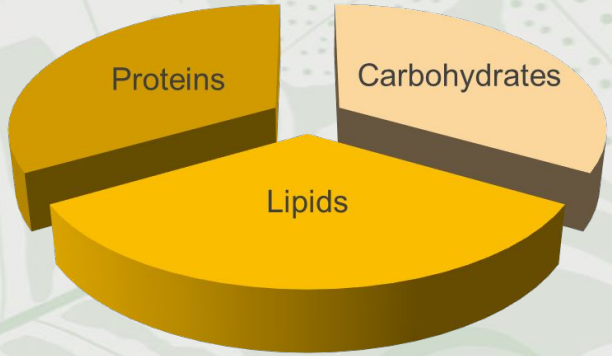
Seed Processing Prior to Drying

- Processing dependent on fruit type
  - Pods (e.g., Fabaceae) –Hand Process, rub over a framed wire, or cut with a clipper
  - Caryopsis (e.g., Poaceae) – Rub inflorescence across stair tread; peel or cut w/ clipper
  - Capsules (e.g, Myrtaceae, Papaveraceae) – Rub with stair tread, parse chaff w/ column blower
  - Berries, Drupes, & Pomes (e.g., Ericaceae, Rubiaceae, Rosaceae) – Macerate using a stair tread, dry seeds, and separated pulp, and parse seeds with a column blower
  - Achenes (e.g., Asteraceae) – Rub seed heads over stair tread, separate using a column blower or clipper
  - Utricles & Schizocarps (e.g., Amaranthaceae, Malvaceae) – Rub over stair tread and pass material through the clipper
- Remove pests or pathogens
  - Manually
  - Fumigation: CO_2, No-Pest strips
  - 10% Clorox
- Inventory
  - Weigh portion of seed lot (25-200) divided by entire lot

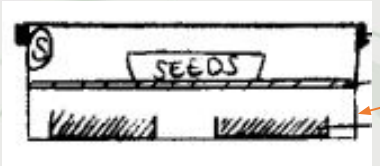


How to Achieve Target Relative Humidity

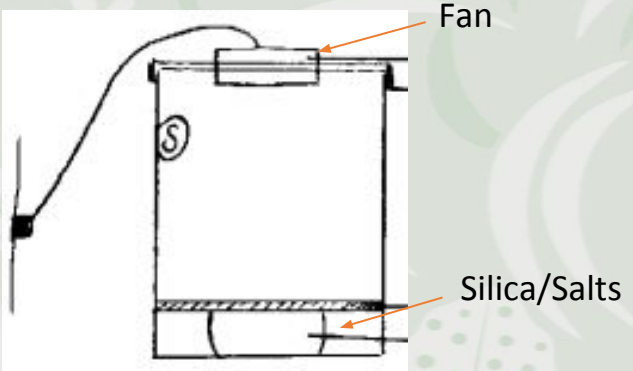
- Moisture content
  - 4-8% by seed weight
  - 15-20% equilibrium RH @ storage temp (-18°C & 5°C)
  - Relationship between MC & eRH varies by seed composition
- If drying @ ~20°C
  - ~17-23% RH for both storage temps
- Incubator-drying
  - Cooled incubators
- Drying Cabinets
  - MCS300, Cleatech, & X-treme Auto Dry



Sealed Container



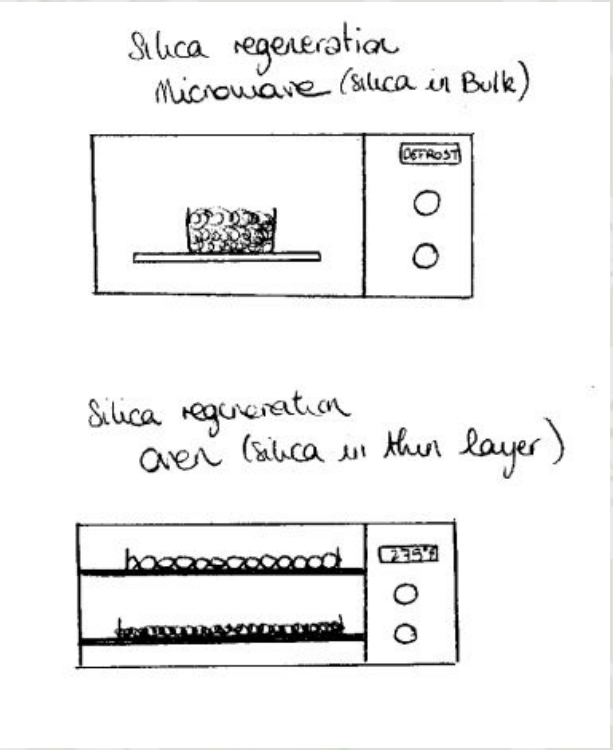
Drying Cabinet



*Never freeze collections until the seeds are fully dry

How to Achieve Target RH Cont.'

- Using Desiccants in an Air-tight Container or Cabinet
  - Saturated salts
    - Lithium Chloride – 130g to 200ml H2O
    - Potassium Acetate – 215g to 100ml H2O
  - Silica gel
    - Most resource and cost-effective
    - Color-indicating beads begin changing from wet to dry ~20% RH
    - Can be dried and re-used when RH rises above 15-20%
      - In Oven: no more than 100°C, for 1-2 hours (or until green beads turn orange).
      - In Microwave: defrost setting for 12 minutes
    - Maintain ~1:1 weight ratio of seeds to silica



Alternative Drying RH

The target drying RH outlined in the previous slides (~20(±3)% RH at ~20°C) provides a simple, unified approach to improving seed longevity.

However, for facilities with fewer resources or those focusing on short- to mid-term storage, the CPC recommended ~30-40% RH at room temperature for cold storage remains a viable option.

TABLE 1.1 Recommended drying conditions for seeds stored in moisture-proof containers at various temperatures. The given drying temperature and RH combinations give a storage RH of 20% at the indicated storage temperature.

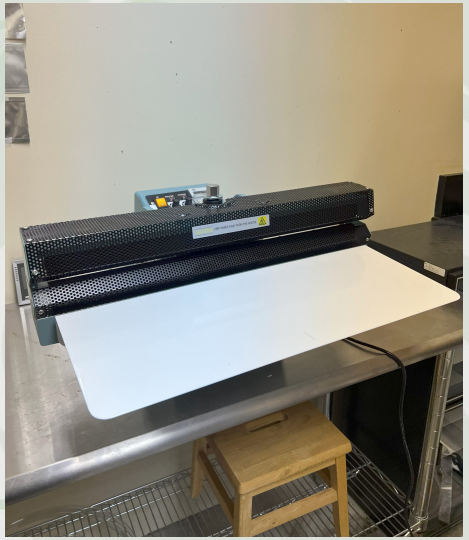
Drying Temperature (°C)	Drying Relative Humidity for Storage at 15°C	Drying Relative Humidity for Storage at 5°C	Drying Relative Humidity for Storage at -20°C
25	28%	33%	35%
15	20%	26%	35%
5	14%	20%	32%

Measuring Seed Moisture Status (eRH)

- Hygrometer – monitor temp and RH% of drying environment
  - BlueMaestro
  - Robotronic HC2-AW-USB
  - Tinytag Data Loggers
- Equilibration
  - Time to reach equilibrium dependent on:
    - Temp of sample and sensor
    - Initial H2O differential between seeds and air
    - Seed Composition
      - Permeability, size, oil content
- How long to dry seeds?
  - Depends on volume, seed coat, and drying conditions
  - Recommended minimum: ~4-6 weeks

Re-acclimating Seeds Post-Storage

- Let the storage container (e.g., laminate foil packets) come up to room temp before opening
- Re-humidify dry seeds before coming into contact with water to avoid imbibition damage
  - 24-48 hours in humid environment



Questions about Drying?

- Additional questions can be directed to the HSBP Information Resource Committee:
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  - Tim Chambers, Army Natural Resources Program Oʻahu – tchambers.oanrp@gmail.com
  - Dustin Wolkis, National Tropical Botanical Garden - dwolkis@ntbg.org
  - Alvin Yoshinaga, (Retired) Lyon Arboretum – alviny@hawaii.edu
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  - Lauren Weisenberger, U.S. Fish & Wildlife – lauren_weisenberger@fws.gov