A Philosophy of Dormancy Testing in Natives

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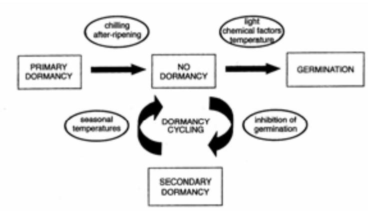
Why Break Seed Dormancy In Native Species?

- Natives are undomesticated and express high levels of seed dormancy
- Must determine the potential planting value of a seed lot
- Differences in germination and viability have significant economic value
- Breaking seed dormancy provides a more accurate appraisal of the viability (value) of the seed lot

Objectives

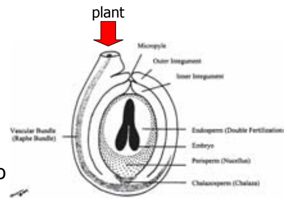
- To emphasize that dormancy breaking and germination are discrete physiological processes
- To demonstrate that traditional dormancy breaking treatments create stress and harm during seed germination
- To question the need for dormancy breaking treatments as useful measures of seed lot stand establishment in native species

Dormancy and Germination: Discrete Physiological Processes



Causes of Dormancy

- Physiological dormancy is determined by maternal parent
  - Stess induces ABA production
  - ABA translocated to seed through seed coat and to embryo



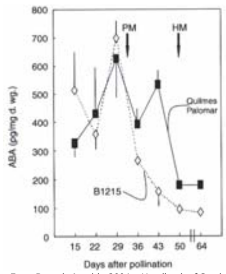
Drawing from Meyer, D. 2001. Seed Technologists Training Manual, SCST, Ithaca, NY.

Causes of Dormancy

- Dormancy expression is population based

Causes of Dormancy

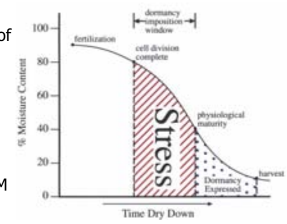
- Imposed between precocious germination and physiological maturity (??) – Dormancy Induction Window



From Benech-Arnold. 2004. *Handbook of Seed Physiology: Applications to Agriculture.* Pp.174.

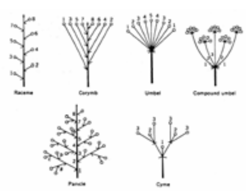
Causes of Dormancy

- Dormancy Imposition Window
  - Begins at completion of cell division
  - Encountering parent plant stress induces dormancy
  - Completed at physiological maturity
  - Expressed between PM and harvest



Causes of Dormancy

- Not uniform due to
  - Nature of inflorescence and time of anthesis
  - Variability in environment during seed development



Causes of Dormancy

- Stress conditions (usually drought and/or high temperatures) induce dormancy
- Often, more than one type of dormancy is present
- One dormancy breaking technique seldom results in 100% germination
- Seed analysts must recognize diversity in dormancy expressed by individuals in a seed lot

Definitions

- Dormant seeds: Viable seeds, other than hard seeds, which fail to germinate when provided the specified germination conditions for the kind of seed in question.
- Viable seeds: Seeds that germinate under favorable conditions in the absence of dormancy

Dormancy Breaking Techniques

- Prechill
- After-ripening (Predry)
- Scarification (piercing, seed coat removal)
- Leaching
- Light, KNO_3, GA_3

Prechill

- Seeds imbibed for long periods in cold, then optimum temperatures
  - Activates hydrolytic enzymes in cold
  - Cold temperatures prevent germination (create stress)
  - Warm temperatures allow germination

Prechill

- Physiological basis
  - Hydrolytic enzymes encourage synthesis and release of GAs and degradation of inhibitors under cold conditions over time
  - Changes in balance of promoters (GAs) and inhibitors is expressed physiologically during germination under warm temperatures

Prechill

ISSUES

- Not all seeds express the same intensity of dormancy
- Requires long periods (over a month) to break dormancy of most seeds
- Process equivalent to a cold test – seeds are stressed and low quality seeds die
- Storage fungi invade seeds and cause unwanted death

The effect of seed infestation and fungicide seed treatment on the germination of soybean seed on rolled paper towels after accelerated aging (Gupta, et al. 1993)

Fungi	Spore Rate	Seed Treatment		
		Captan	Benlate	Non-treated
		% normal seedlings		
A. niger	8/seed	74.2	83.0	9.0
A. niger	50/seed	54.2	59.2	5.6
A. glaucus	8/seed	77.6	83.2	59.0
A. glaucus	50/seed	53.0	58.0	30.0

After-ripening (Predry)

- Non-imbibed seeds subjected to
  - Long periods (often years) of dry storage
  - High temperatures (35, 40^{o}C) for 5 to 7 days
  - Changes in seed quality occur during these conditions

After-ripening

- Physiological basis
  - Over time, enzymes encourage synthesis and release of GAs and degradation of inhibitors
  - Under high temperatures, these changes occur more rapidly
  - Changes in balance of promoter (GAs) and inhibitors expressed during germination

Constant temperature of accelerated after-ripening treatments of cuphea seeds over time (Widrlechner and Kovach, 2000)

	Duration of Treatment			
Temperature	1 weeks	2 weeks	3 weeks	4 weeks
	% Germination			
30C	40	19	2	6
25C	4	13	0	3
20C	4	17	15	22
15C	11	30	34	52
10C	8	29	45	55
5C	29	32	42	48

After-ripening and Seed Age

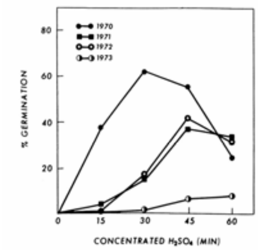


Fig. 2. Influence of concentrated H_2SO_4 on the germination of 1, 2, 3, and 4-year-old Indian ricegrass seeds.

From McDonald and Khan, 1977

Influence of exogenous hormonal treatments on the germination of 1- and 2-year-old intact and scarified Indian Ricegrass seeds after 72 hours soaking (McDonald and Khan, 1977).

	1-Year		2-Year	
Treatment	Intact	Scarified	Intact	Scarified
H_2O	0	31	5	67
GA_3	0	65	5	76
Kinetin	0	54	6	72
ABA	0	1	2	2

After-ripening

ISSUES

- Long periods of dry storage cause loss in seed quality and vigor
- High temperatures stress low quality seeds making them non-germinable over time

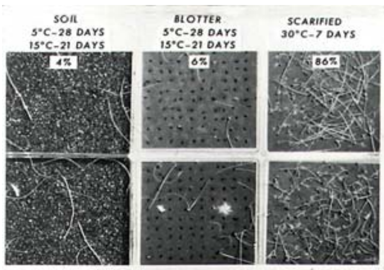
Scarification

- Seeds subjected to
  - Mechanical abrasion (sandpaper)
  - Chemical digestion (H_2SO_4)
  - Piercing
  - Seed coat removal
  - Cutting

Scarification

- Physiological basis
  - Hard seeds must imbibe water
  - Inhibitors localized in
    - seed coat can be abraded away
    - seed can be leached away during imbibition
  - Gas exchange is enhanced
  - Mechanical restriction is minimized

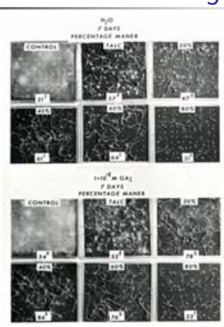
Scarified vs. Intact Seeds



From McDonald, 1976

Storage Fungi on Scarified Seeds

Scarification + GA_3



Protein Repair During Germination

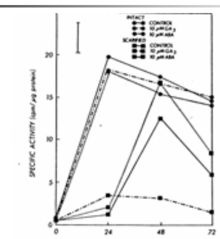


Fig. 1. Incorporation of ^{14}C-leucine into soluble protein of 1-year-old intact and scarified Indian ricegrass seeds as influenced by 10 µM GA_3 and 10 µM ABA during soaking. Vertical bar denotes LSD (0.05) to compare any two treatment means.

From McDonald and Khan, 1978

Conductivity Following Scarification

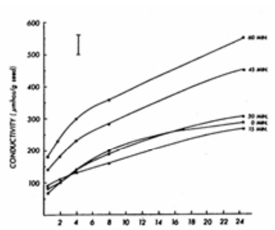


Fig. 4. Seed leachate levels of 1-year-old Indian ricegrass seeds scarified for various times as expressed by conductivity (µmhos/g seed). Vertical bar denotes LSD (0.05) to compare any two treatment means.

From McDonald and Khan, 1983

Scarification

ISSUES

- Not all seeds express the same intensity of dormancy
- Small seeds damaged, large seeds not sufficiently abraded
- Damaged seeds slow to germinate and must effect repair
- Storage fungi invade seeds and cause death
- After-ripening requirement often present for maximum results

Leaching

- Seeds exposed to flowing water
- Physiological basis
  - Water-soluble inhibitors removed from seed or seed coat
  - Germination occurs when seed returned to favorable conditions

Leaching

ISSUES

- Treatment similar to a conductivity vigor test
- Low quality seeds poor in membrane structure leach more elements, some essential for germination
- Low quality seeds less able to germinate when returned to favorable conditions

Light, KNO_3, GA_3

- Light enhances the germination of some seeds due to the photoreversible pigment phytochrome
- KNO_3
  - Active component is nitrate
  - Naturally occurring in soils
  - Most nitrate-dependent seeds are light sensitive suggesting a physiological interaction

Kinds responding to light and KNO_3 to break seed dormancy as listed in the AOSA Rules for Seed Testing

Treatment	Number of Kinds
Light only	148
KNO_3 only	25
Light and KNO_3	98

Light, KNO_3, GA_3

- GA_3 seldom used in seed testing
- Naturally occurring plant hormone
- Breaks dormancy of light, prechill and after-ripening requiring seeds
- Sometimes it does not get into seed, large molecule (If seed must be cut or pierced for TZ test, then it is likely GA_3 is excluded)
- Should be used in seed testing more often for optimum response

Seed Viability

- Important in native species because of high incidence of dormancy
- Determined by a TZ test
- TZ conducted before germination test to determine % viability
- Germination test (including dormancy breaking approaches) conducted followed by a TZ test of non-germinating seeds

Example

- TZ before germination test = 90% viable
- Germination test = 40%
- TZ test of non-germinating seeds = 30%
- Practical consequence: 20% loss of viable seeds
- Is this an accurate appraisal of seed quality? Does it have an economic consequence?

Possibilities

- Is it possible that the 20% loss is due to stress encountered during dormancy breaking treatments?
- If so, what is the best way to report seed viability results for native grasses?
- TZ first, germinate under optimum conditions (do not break dormancy), obtain % germination?

Conclusions

- Can we successfully break dormancy of all seeds in a seed lot that may possess multiple forms of dormancy and consists of a heterogeneous population of individuals?
- Do dormancy breaking tests provide a more accurate appraisal of the viability of a seed lot?

Conclusions

- Is it sufficient to report only the TZ results obtained before the germination test?
- What is the most accurate reflection of potential seed performance in native species from the growers perspective?

TZ Concerns for Natives

But, "Not so fast my friends…."

TZ Concerns for Natives

- How do you interpret staining patterns?
- Development of a common and accepted resource is important

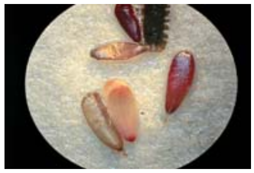
Coreopsis TZ

Photograph courtesy of J. Norcini, U. Florida.

TZ Concerns for Natives

- How do you evaluate seeds with dormancy?
- Seed coat in Coreopsis prevents TZ from entering embryo
- Methods need to be developed to fully understand TZ results

Coreopsis TZ



Photograph courtesy of J. Norcini, U. Florida.

TZ Concerns for Natives

- Until staining patterns and dormancy issues are resolved, TZ interpretations in natives becomes problematic
- Techniques and illustration/photographs must be developed
- These must be accepted by experts before TZ replaces germination in native species