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Physiological Causes of Seed Deterioration

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Seed Deterioration

Definition: "Deteriorative changes occurring with time that increase the seed's vulnerability to external challenges and decrease the ability of the seed to survive."

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Seed Deterioration

- General observations
  1) Undesirable attribute of agriculture; provides a template for increasing agricultural profits
  2) Physiology is a separate event from development and/or germination
  3) Deterioration is cumulative; as seed aging increases, seed performance is compromised

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Seed Deterioration

"Seed deterioration is inexorable and the best that can be done is to control its rate."

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Predisposition for Seed Deterioration

- Factors that cause deterioration
  - Genetics

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Relative Storage Life of Flower Seeds

Short	Medium	Long
Anemone	Ageratum	Centaurea
Aster	Alyssum	Chrysanthemum
Begonia	Cycalmen	Shasta Daisy
Coneflower	Dusty miller	Morningglory
Coreopsis	Gaillardia	Sweet pea
Impatiens	Lisanthus	Zinnia
Pansy	Marigold	
Phlox	Nicotiana	
Salvia	Petunia	
Vinca	Snapdragon	
Viola	Verbena	

From McDonald and Kwong. 2006. Flower Seeds: Biology and Technology.

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Predisposition for Seed Deterioration

- Genetics
- Seed Structure
  - Size/surface area
  - Seed coat permeability

Zinnia, left; Verbena, middle; Petunia, right

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Predisposition for Seed Deterioration

- Genetics
- Seed Structure
- Seed Chemistry
  - Lipid vs. protein vs starch

TABLE III
APPROXIMATE MOISTURE CONTENT OF SEEDS IN EQUILIBRIUM WITH AIR AT VARIOUS RELATIVE HUMIDITIES[a]

Seeds	Relative humidity (%) 15	30	45	60	75
Cereals (starchy)					
Rye	7.0	8.5	10.5	12.0	15.0
Rice (milled)	6.5	9.0	10.5	12.5	14.5
Sorghum	6.5	8.5	10.5	12.0	15.0
Corn (maize)	6.5	8.5	10.5	12.5	14.5
Wheat	6.5	8.5	10.0	11.5	14.5
Barley	6.0	8.5	10.0	12.0	14.5
Oats	5.5	8.0	9.5	12.0	14.0
Vegetables (starchy)					
Spinach	7.0	8.0	9.5	11.0	13.0
Pea	5.0	7.0	8.5	11.0	14.0
Bean, snap	5.0	6.5	8.5	11.0	14.0
Oil seeds					
Soybean	–	6.5	7.5	9.5	13.0
Flaxseed	4.5	5.5	6.5	8.0	10.0
Vegetables (oily)					
Tomato	6.0	7.0	8.0	9.0	11.0
Carrot	5.0	6.0	7.0	9.0	11.5
Cucumber	6.0	7.0	7.5	8.0	9.5
Lettuce	4.0	5.0	6.0	7.0	9.0
Cabbage	3.5	4.5	6.0	7.0	9.0

[a] 25°C, moisture content wet basis, in percent.

From Harrington, J. 1973.

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Predisposition for Seed Deterioration

- Genetics
- Seed Structure
- Seed Chemistry
  - Lipid vs. protein vs. starch
  - mucilage

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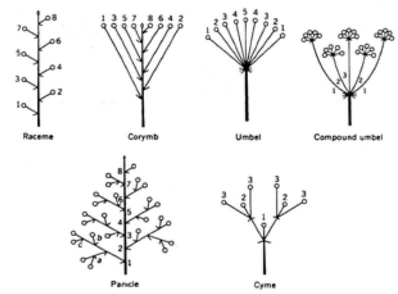
Predisposition for Seed Deterioration

- Genetics
- Seed Structure
- Seed Chemistry
- Physical/physio-logical seed quality
  - Maturity



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Predisposition for Seed Deterioration

- Genetics
- Seed Structure
- Seed Chemistry
- Physical/physio-logical seed quality
  - Maturity
  - Physical damage

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Predisposition for Seed Deterioration

- Genetics
- Seed Structure
- Seed Chemistry
- Physical/physio-logical seed quality
  - Maturity
  - Physical damage
  - Dormancy

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Predisposition for Seed Deterioration

- Genetics
- Seed Structure
- Seed Chemistry
- Physical/physiological seed quality
- Relative humidity and temperature of the storage environment
- External environmental factors

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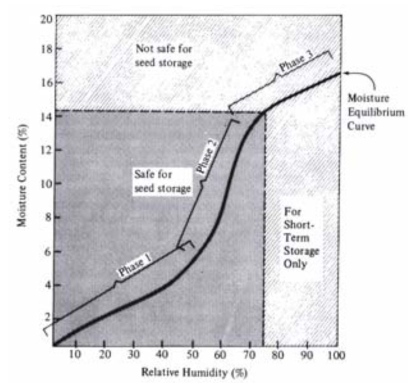
Causes of Seed Deterioration

- Relative Humidity
  - Equilibrium moisture content



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Causes of Seed Deterioration

- **Temperature**
  - Increases air’s ability to suspend water
  - Enhances physiological speed of deterioration reactions

^oC	g H_2O/kg air
0	3.9
10	7.6
20	14.8
30	26.4
40	41.4

"Rules of Thumb"

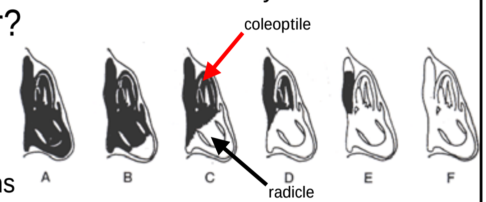
- Every 1% decrease in seed moisture content doubles seed storage life.
- Every 5°C decrease in storage temperature doubles seed storage life.
- Practical seed storage equation:

$$\% \text{ RH} + {}^{o}\text{C} \leq 45.5$$

The Physiology of Seed Deterioration

- Where does seed deterioration occur?
  - Does not occur uniformly in seed
    - Monocots: Deterioration begins in root tip
      - Causes radicle extension to be reduced more than coleoptile extension

Wheat embryos



from Das and Sen-Mandi. 1988. Plant Physiol. 88:983-986.

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The Physiology of Seed Deterioration

- Where does seed deterioration occur?
  - Does not occur uniformly in seed
    - Dicots: Deterioration begins in growing points (shoot and root) of embryonic axis

Table 2. Effects of ageing and hydration on malondialdehyde (MDA), total peroxides, and soluble protein contents in axes and cotyledons of two peanut cultivars. Ageing and priming treatments were conducted as described in the legend to Table 1.

		Hydration	MDA		Total peroxides		Soluble protein	
			Axis	Cotyle-don	Axis	Cotyle-don	Axis	Cotyle-don
			µmol/g DW				mg/g DW	
Li-chih-Tzae	unaged	−	44	21	264	42	103	34
		+	33	23	217	44	98	32
	aged	−	51	25	287	52	89	28
		+	40	27	254	53	84	26
Li-chih-Tzae	unaged	−	39	34	114	40	94	40
		+	34	36	88	43	89	38
	aged	−	43	39	238	57	83	33
		+	34	41	212	58	83	33
$LSD_{0.05}$			3	4	19	5	7	6

from Jeng and Sung. 1994. Seed Sci. & Technol. 22:531-539.

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The Physiology of Seed Deterioration

- Understanding seed deterioration
  - Process of seed deterioration is varied
    - Short-term deterioration in field different than long-term deterioration in storage
    - Mechanical damage different than uniform deterioration

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The Physiology of Seed Deterioration

- Understanding seed deterioration
  - Different methods used to study seed deterioration
    - Are accelerated aging conditions the same as long-term natural storage conditions?

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The Physiology of Seed Deterioration

- Understanding seed deterioration
  - Rate of seed deterioration influenced by confounding environmental and biological factors
    - High temperature, high moisture enhance seed deterioration
    - Interaction greater than the sum of the two
    - Storage fungi increase as seeds deteriorate and have separate effect on deterioration

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The Physiology of Seed Deterioration

- Understanding seed deterioration
  - Addition of seed treatments influences deterioration
    - Fungicides control storage fungi leading to increased germination under AA conditions

Table 1. The effect of seed infestation and fungicide seed treatment on the germination of soybean seed on rolled paper towels after accelerated aging.

Fungi	Spore rate[a]	Seed treatment: Vitavax 200	Captan 40	Thiram 30	Benlate 50 W	Control (No fungicide)	Mean[b]
		% normal seedlings					
A. niger	Low	38.2	74.2	75.0	83.0	9.0	64.32 B
A. niger	High	44.2	54.2	50.6	59.2	5.6	42.86 D
A. glaucus	Low	76.6	77.6	38.2	83.2	59.0	75.00 A
A. glaucus	High	50.6	53.0	49.2	58.0	30.0	48.20 C
Control (Non infested)		69.6	76.6	79.66	86.0	69.2	75.00 A
Mean fungi		63.928	66.88	69.28	74.2 A	36.6 C	

LSD (p = 0.05) for fungi = 3.2
for seed treatment = 3.2
for interaction = 7.2

[a] Low rate 8-10 spores/seed. High rate 50-60 spores/seed
[b] Means followed by same letters do not differ at p = 0.05% level of significance.

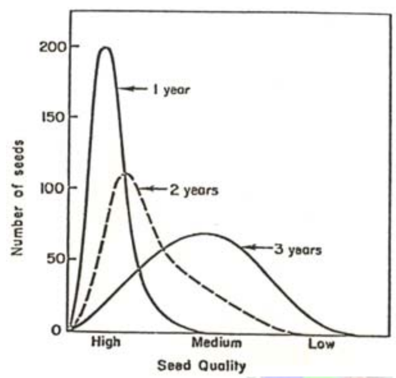
from Gupta, et al. (1993). Seed Sci. & Technol. 21:581-591.

The Physiology of Seed Deterioration

- Understanding seed deterioration
  - Most studies examine whole seeds
    - Specific portions of the seed may deteriorate more rapidly than others
    - 80% of corn seed is non-respiring endosperm

The Physiology of Seed Deterioration

- Understanding seed deterioration
  - Seed deterioration is an individual event
    - Most studies bulk seed samples (both viable and nonviable)
    - Report single result suggesting it is representative of the population of seeds



"A seed lot is a population of individuals – each with its own ability to withstand deterioration." – McDonald and Wilson. 1980. J. Seed Technol. 5:56-66.

The Physiology of Seed Deterioration

- Proposed mechanisms of seed deterioration
  - Enzyme activities
    - Markers of germination: α-amylase

Wheat seeds

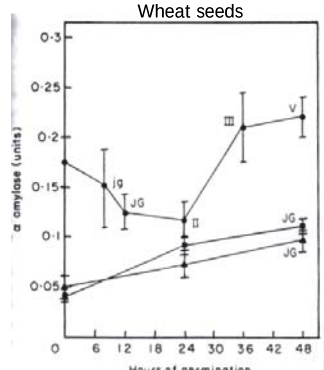


FIG. 1. Scutellar α amylase in dry and imbibed seeds of unaged and aged wheat stocks up to 48 h of imbibition. (●——●) 99% viability (unaged) stock; (■——■) 29% viability stock (subjected to accelerated ageing); (▲——▲) 27% viability (naturally aged) stock. Growth stages, jg, JG, II, III, V, are as cited in Table 2. The values are obtained from data of ten extractions. Vertical bars represent ±s.e. of the mean.

from Das and Sen-Mandi. 1992. Anal. Bot. 69:497-450.

The Physiology of Seed Deterioration

- Proposed mechanisms of seed deterioration
  - Enzyme activities
    - Free radical scavenging enzymes: SOD, catalase, etc.

Peanut

Table 3. Effects of ageing and hydration on superoxide dismutase (SOD), catalase, peroxidase (POD) and ascorbate POD in axes and cotyledons in two peanut cultivars. Ageing and priming treatments were conducted as described in the legend to Table 1.

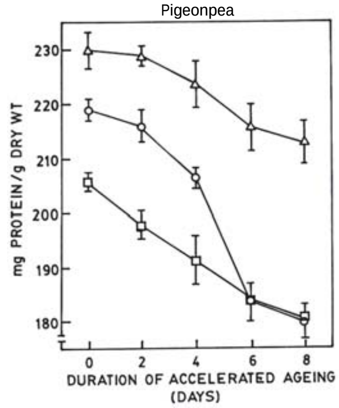
		Hydration	SOD		Catalase		Ascorbate POD		POD
			Axis	Cotyledon	Axis	Cotyledon	Axis	Cotyledon	Axis
			— unit/mg DW —		— µmol/g DW/min —				
TN.11	unaged	−	2.68	0.83	1.10	0.49	21.63	2.35	7.19
		+	3.55	0.74	2.01	0.55	31.61	3.91	20.09
	aged	−	2.49	0.67	1.05	0.50	16.85	1.83	4.51
		+	3.10	0.70	1.54	0.56	23.12	2.36	5.84
Li-Chih-Tzae	unaged	−	1.88	1.08	0.74	0.49	16.50	3.42	5.71
		+	2.81	0.99	1.26	0.56	22.84	4.35	10.78
	aged	−	1.68	0.86	0.73	0.51	14.30	2.45	4.04
		+	2.12	0.79	1.27	0.53	22.51	2.96	6.34
$LSD_{0.05}$			0.25	0.11	0.13	0.03	3.79	0.39	0.67

from Jeng and Sung. 1994. Seed Sci. & Technol. 22:531-539.

The Physiology of Seed Deterioration

- Proposed mechanisms of seed deterioration
  - Protein (decline) or amino acid (increase) content

Pigeonpea



from Kalpana and Madhava Rao. 1997. Seed Sci. & Technol. 25:271-279

The Physiology of Seed Deterioration

- Proposed mechanisms of seed deterioration
  - Nucleic acids: Decreased DNA synthesis and increased degradation leads to faulty translation and transcription of enzymes essential for germination

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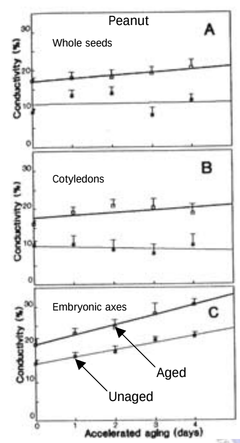
The Physiology of Seed Deterioration

- Proposed mechanisms of seed deterioration
  - Increased membrane permeability



from Perez and Arguello. 1995. Seed Sci. & Technol. 23:439-445.

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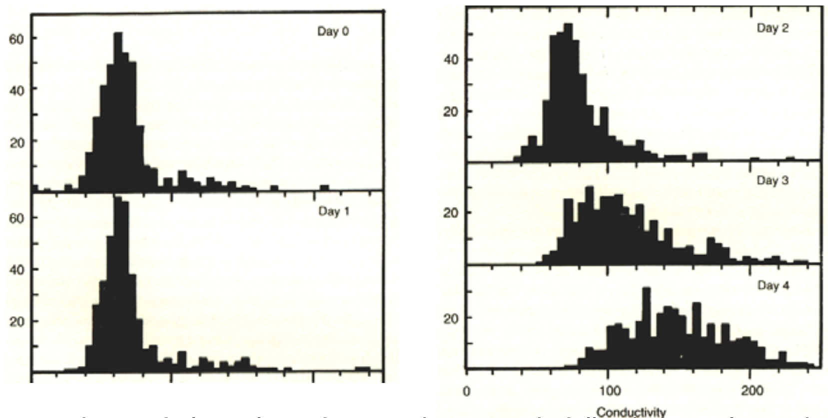
The Physiology of Seed Deterioration



Leakage of electrolytes from soybean seeds following accelerated aging (McDonald and Wilson. 1980. J. Seed Technol. 5:56-66).

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The Physiology of Seed Deterioration

- Old consensus
  - DNA degraded leading to impaired transcription causing faulty translation of enzymes
  - Possible degradation of “long-lived” mRNA programmed for enzymes responsible for first stages of germination

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The Physiology of Seed Deterioration

Old Consensus "Models"

DNA —✕→ mRNA - - → Enzymes - - → No germination

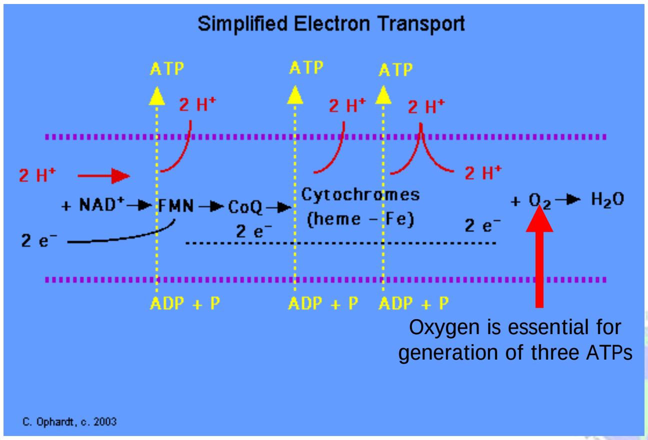
stored

DNA ——→ mRNA ✕→ Enzymes - - → No germination

The Physiology of Seed Deterioration

- New consensus
  - Free radicals cause profound cellular damage
  - Greatest free radical "sink" is the mitochondrion
  - Most mitochondria found in meristematic cells
  - mtDNA replication is hindered
    - Fewer mitochondria
    - Less ATP
    - Slower seedling growth

The Physiology of Seed Deterioration



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The Physiology of Seed Deterioration

"Free radical production, primarily initiated by oxygen, has been related to the peroxidation of lipids and other essential compounds found in cells."

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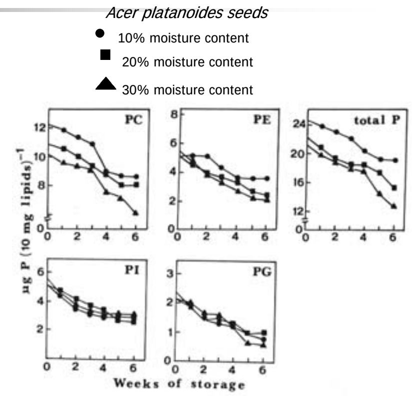
The Physiology of Seed Deterioration

- Free radicals cause a host of undesired events
  - Decreased lipid content



from Pukacka and Kuiper. 1988. Physiol. Plant. 72:89-93.

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The Physiology of Seed Deterioration

- Free radicals cause a host of undesired events
  - Decreased lipid content
  - Reduced respiratory competence
  - Increased evolution of volatiles ranging from hexanal to aldehydes

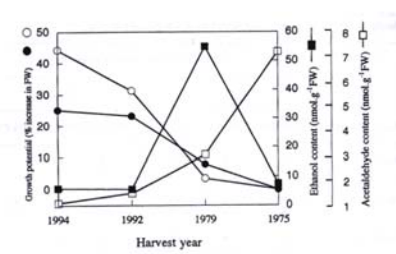

Figure 3. Growth potentials of axial (○) and cotyledonary (●) tissues of cocklebur seeds harvested in different years and the contents of ethanol (■) and Ald (□)

from Esashi, et al. 1997. *Basic and Applied Mechanisms of Seed Biology*, pp. 489-498.

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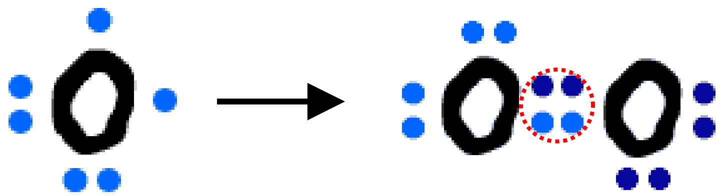
The Physiology of Seed Deterioration

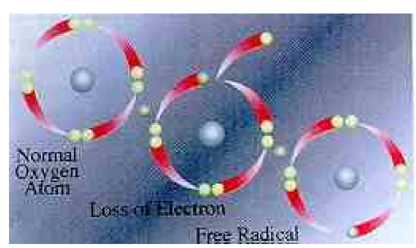


Oxygen has 6 outer electrons--it forms 2 pair on its own and seeks two other partners

The gas **Oxygen** (O_2) shares TWO pairs (a double bond) of electrons with another oxygen

The Physiology of Seed Deterioration

- What are free radicals?
  - All atoms contain orbitals that occupy zero, one or two electrons
  - An atom or molecule that possesses an unpaired electron is a free radical
  - An unpaired electron in an atom or molecule carries more energy than a paired electron



The Physiology of Seed Deterioration

- Why are free radicals important?
  - The energetic "lonely electron" can
    - Detach from its host atom or molecule and move to another atom or molecule
    - Pull another electron (which may not have been lonely) from another atom or molecule

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The Physiology of Seed Deterioration

- Why are free radicals important?
  - Most common reaction is when one free radical and one non-free radical transfer one electron between them leaving the free radical as a non-free radical, but now the non-free radical is a free radical

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The Physiology of Seed Deterioration

- Why are free radicals important?
  - This initiates a chain of similar reactions causing substantial rearrangement of molecules that alters their structure and function

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The Physiology of Seed Deterioration

- Why are free radicals important?
  - If these are proteins (enzymes), lipids (membranes), or nucleic acids (DNA), normal biological function is compromised and deterioration increased

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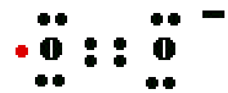
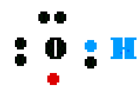
The Physiology of Seed Deterioration

- Most important free radicals in seeds
  - Superoxide anion ($O_2^{\bullet -}$): Generated by oxygen and autoxidation of hydroquinones, etc.
  - Hydroxyl radicals ($\bullet OH$): Most damaging. Generated from $O_2^{\bullet -}$ and H_2O_2



Superoxide anion



Hydroxyl radical

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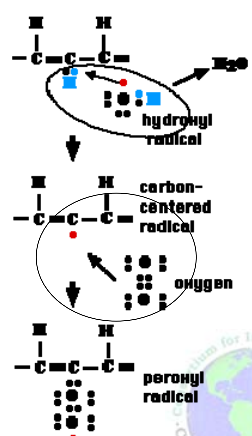
The Physiology of Seed Deterioration

- How do free radicals cause lipid peroxidation?
  - Initiated by $\bullet OH$ around oleic and linoleic acids that have a double bond
  - Release a free carbon-centered radical that combines with oxygen leaving a peroxy-free radical ($ROO\bullet$)
  - ...and so on



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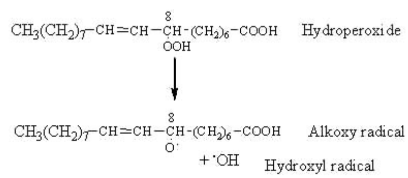
The Physiology of Seed Deterioration

- How do free radicals cause lipid peroxidation?
  - Long chain fatty acids broken into smaller and smaller compounds, ultimately releasing volatiles
  - Final consequence: loss of membrane structure and increase in leakiness



The Physiology of Seed Deterioration

- Influence of seed moisture on free radical assault?
  - Below 6%, autoxidation is favored
  - Above 14%, oxidative enzymes such as lipoxygenase function that generate free radicals
  - Between 6 and 14%, lipid peroxidation is at a minimum

The Physiology of Seed Deterioration

- Do free radicals attack only lipids?
  - Changes in protein structure occur, modifying their function, e.g., enzymes
    - cleavage of protein to yield lower-molecular weight product
    - cross-linkage of protein to yield higher-molecular weight product
    - loss of catalytic and structural function by distorting its secondary and tertiary structure

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The Physiology of Seed Deterioration

- Do free radicals attack only lipids?
  - DNA assaulted leading to strand and deoxyribose sugar breakage
    - May explain increased propensity for genetic mutations as seeds age
    - May delay mitosis necessary for cell division and germination

Hydroxyl radical attacks sugar on backbone of DNA molecule

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The Physiology of Seed Deterioration

- Free radical assault on mitochondria
  - Prime "sink" for oxygen – leaks from membranes during respiration

$O_2 + 4H^+ + 4e^- \rightarrow 2\,H_2O$ (about 95% of the time)

$\rightarrow 2\,O_2^{\cdot -}$ (about 5% of the time)

Superoxide free radical

From Mignone, J. 2002. Webpage.

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The Physiology of Seed Deterioration

- Free radical assault on mitochondria
  - Prime "sink" for oxygen – leaks from membranes during respiration
  - Indispensable for normal cell function
  - Reduced seedling growth from poor quality seed may be a consequence of less efficient mitochondrial function

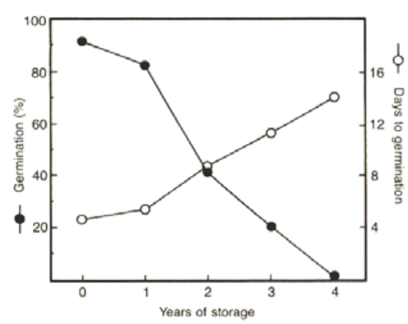

Figure 12. Decreased speed of germination as a function of aging. The average lag time to the start of germination for Norway spruce seeds (*Picea abies*) was determined in a laboratory germinator. Data of Lipkin (1927).

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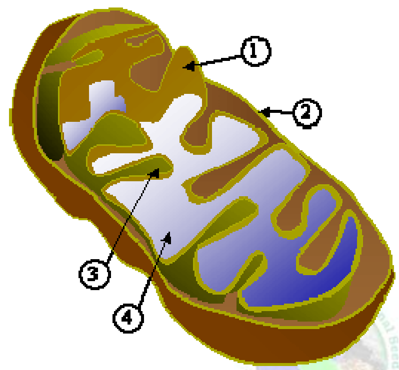
The Physiology of Seed Deterioration

- Effects of free radicals on mitochondria
  - Inner membrane (cristae) has great surface area and is the site of electron transfer
  - Lipid peroxidation of this structure would compromise energy (ATP) production
  - Mitochondria also contain their own DNA



Cross-section of a mitochondrion, showing:
(1) inner membrane, (2), outer membrane, (3) crista, (4) matrix

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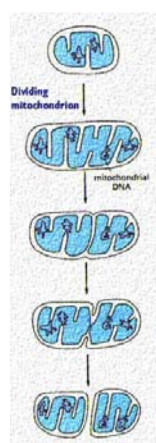
The Physiology of Seed Deterioration

- Effects of free radicals on mtDNA
  - Can divide in an active cell, important for the production of new mitochondria
  - Enzymes encoded on mtDNA are absolutely essential for oxidative phosphorylation; if compromised, energy production is reduced



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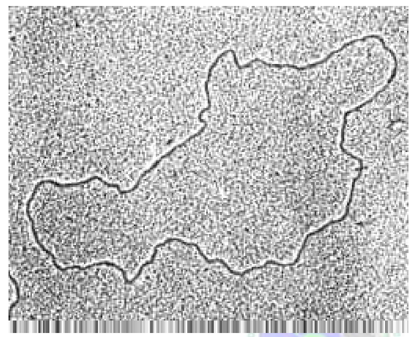
The Physiology of Seed Deterioration

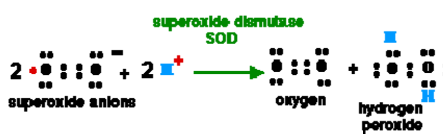
- mtDNA is more susceptible to free radical assault than nuclear DNA
  - Greater level of free radical production in mitochondria
  - mtDNA is naked, no protective histones
  - Repair of nuclear DNA is more successful than mtDNA – less repair enzymes in mitochondria



Circular, naked mitochondrial DNA

The Physiology of Seed Deterioration

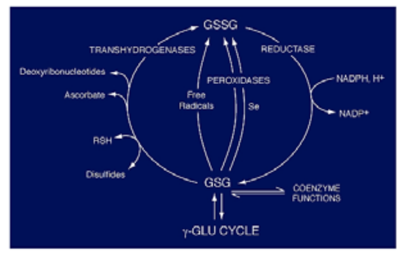
- How are seeds protected against free radicals?
  - Array of protective enzymes
    - Superoxide dismutase
    - Catalase
    - Glutathione peorxidase



The Physiology of Seed Deterioration

- How are seeds protected against free radicals?
  - Array of protective enzymes
  - Nonenzymatic compounds that react with free radicals
    - Glutathione

Figure 3. The oxidation-reduction pathways that involve glutathione. From Meister.[12]



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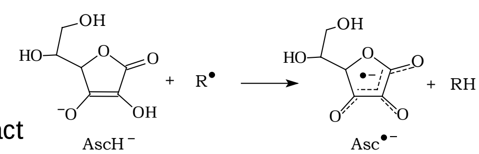
The Physiology of Seed Deterioration

- How are seeds protected against free radicals?
  - Array of protective enzymes
  - Nonenzymatic compounds that react with free radicals
    - Glutathione
    - Vitamin C (ascorbic acid)



Vitamin C (ascorbate) terminating a free radical

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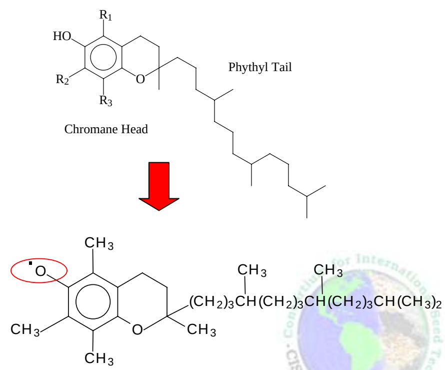
The Physiology of Seed Deterioration

- How are seeds protected against free radicals?
  - Array of protective enzymes
  - Nonenzymatic compounds that react with free radicals
    - Glutathione
    - Vitamin C (ascorbic acid)
    - Vitamin E (tocopherol)

$$R^{\bullet} + TOH \longrightarrow RH + TO^{\bullet}$$



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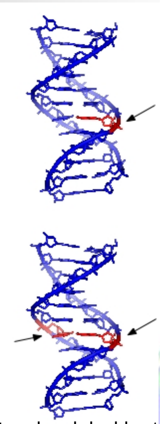
The Physiology of Seed Deterioration

- How are seeds protected against free radicals?
  - Array of protective enzymes
  - Nonenzymatic compounds that react with free radicals
  - Enzymes that repair damage
    - Base excision
    - Nucleotide excision
    - DNA mismatch



Single strand and double strand DNA damage

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The Physiology of Seed Deterioration

- Approaches to minimizing lipid peroxidation
  - Change proportion of fatty acids to favor saturated fatty acids and/or reduce lipoxygenase levels
  - Reduce oxygen around seeds
  - Increase level of antioxidants

The Physiology of Seed Deterioration

- Repair of seed damage

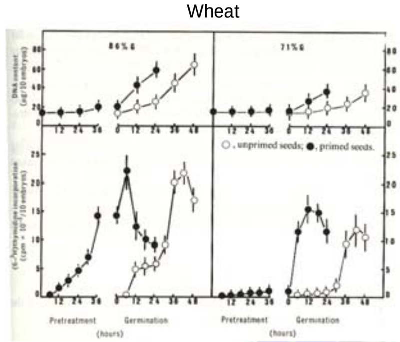
The Physiology of Seed Deterioration

- Repair of seed damage
  - When?
    - Hydration causes activation of repair enzymes
    - Drying phase permits repair and stabilize seed
    - May be the mechanisms responsible for improved performance of “primed” seeds

The Physiology of Seed Deterioration

- Repair of seed damage
  - Where?
    - Meristematic axes containing the most mitochondria
    - Priming results in a faster resumption of cell division and DNA synthesis on subsequent reimbibition

Wheat



from Dell’Aquila and Taranto. 1986. Seed Sci. & Technol. 14:333-342.

The Physiology of Seed Deterioration

- Repair of seed damage
  - How? Priming does the following:
    - Lower conductivity readings, perform better in diseased soils
    - Increases enzyme activity
    - Reduces lipid peroxidation

Table 4. Membrane functions, dehydrogenase activity and lipid peroxidation of eggplant (E) and radish (R) seeds subjected to accelerated ageing* after hydration-dehydration treatments.

Treatments	Conductance of leachate after one hour (μmho/g seed weight)		Leakage of sugar (μg glucose equiv./ml)		Tetrazolium index (OD 470)		TBA index (OD 520)	
	E	R	E	R	E	R	E	R
Control	110	63	41	45	0.100	0.175	0.230	0.239
ME-D(hours)								
24	90	54	22	36	0.141	0.246	0.210	0.216
48	84	49	13	29	0.168	0.255	0.198	0.210
72	88	50	15	32	0.155	0.250	0.208	0.212
D-D	60	36	7	9	0.165	0.252	0.202	0.211
S-D	36	23	5	11	0.163	0.249	0.206	0.216

* Accelerated ageing was done at 100% RH and 42 °C for 12 days (eggplant) and 93% RH and 42 °C for 10 days (for radish). Abbreviations as for table 1.

from Rudrapal and Nakamura. 1988. Seed Sci & Technol. 16:123-130

The Physiology of Seed Deterioration

- Model of seed deterioration
  - Lipid peroxidation is the central cause of seed deterioration.
  - Storage. Four types of damage
    - Mitochondrial dysfunction
    - Enzyme inactivation
    - Membrane perturbations
    - Genetic damage

The Physiology of Seed Deterioration

- Model of seed deterioration
  - Lipid peroxidation is the central cause of seed deterioration
  - Storage
  - Free radical damage can be ameliorated by
    - Presence of antioxidants, particularly during storage
    - Hydration either during imbibition or priming
      - Permits repair

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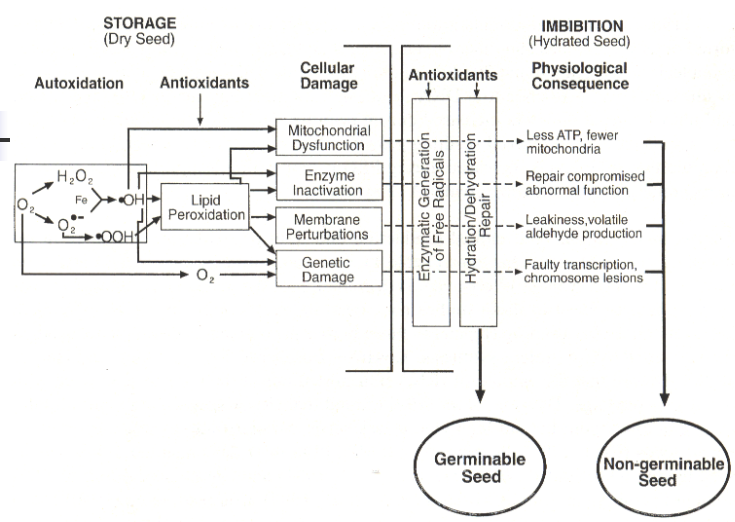

A model of seed germination and its physiological consequences during seed storage and imbibition

(from McDonald, M. B. 1999. Seed Sci. & Technol. 27:177-237)

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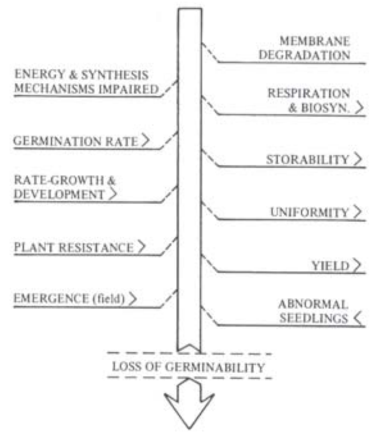
Consequences of Seed Deterioration

- Possible seed deterioration sequence
- Based on this model, membrane degradation first event of deterioration
- Final event is loss of germination



From Delouche and Baskin (1973)

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The Physiology of Seed Deterioration

- Conclusions
  - Seed moisture content and temperature have important roles influencing the biochemistry of deterioration
  - Seed deterioration is neither uniform among seeds nor among seed parts

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The Physiology of Seed Deterioration

- Conclusions
  - Mitochondria play a central role in deterioration
    - They are oxygen "sinks"
    - Crista prone to free radical assault and lipid peroxidation
    - Speed and uniformity of germination compromised

The Physiology of Seed Deterioration

- Conclusions
  - Mitochondria play a central role in deterioration
    - Repair can occur during imbibition
      - Antioxidants present in abundance in seeds
      - "repair" enzymes exist
      - May be related to success of priming as a seed enhancement

The Physiology of Seed Deterioration

- More information:

McDonald, M. B. 1999. Seed deterioration: Physiology, repair and assessment. Seed Sci. & Technol. 27:177-237

Bailly, C. 2004. Active oxygen species and antioxidants in seed biology. Seed Sci. Res. 14:93-109

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