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.”

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
Seed Deterioration

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

Predisposition for Seed Deterioration

- Factors that cause deterioration
  - Genetics
Relative Storage Life of Flower Seeds

<table>
<thead>
<tr>
<th>Short</th>
<th>Medium</th>
<th>Long</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemone</td>
<td>Ageratum</td>
<td>Centaurea</td>
</tr>
<tr>
<td>Aster</td>
<td>Alyssum</td>
<td>Chrysanthemum</td>
</tr>
<tr>
<td>Begonia</td>
<td>Cycalmen</td>
<td>Shasta Daisy</td>
</tr>
<tr>
<td>Coneflower</td>
<td>Dusty miller</td>
<td>Morningglory</td>
</tr>
<tr>
<td>Coreopsis</td>
<td>Gaillardia</td>
<td>Sweet pea</td>
</tr>
<tr>
<td>Impatiens</td>
<td>Lisanthus</td>
<td>Zinnia</td>
</tr>
<tr>
<td>Pansy</td>
<td>Marigold</td>
<td></td>
</tr>
<tr>
<td>Phlox</td>
<td>Nicotiana</td>
<td></td>
</tr>
<tr>
<td>Salvia</td>
<td>Petunia</td>
<td></td>
</tr>
<tr>
<td>Vinca</td>
<td>Snapdragon</td>
<td></td>
</tr>
<tr>
<td>Viola</td>
<td>Verbena</td>
<td></td>
</tr>
</tbody>
</table>


Predisposition for Seed Deterioration

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

Zinnia, left; Verbena, middle; Petunia, right
Predisposition for Seed Deterioration

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

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TABLE III
APPROXIMATE MOISTURE CONTENT OF SEEDS UNDER EQUILIBRIUM WITH AIR OF VARIOUS RELATIVE HUMIDITIES

<table>
<thead>
<tr>
<th>Seeds</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canola (unshelled)</td>
<td>8.0</td>
<td>7.5</td>
<td>7.1</td>
<td>6.7</td>
<td>6.3</td>
</tr>
<tr>
<td>Rape (cotton)</td>
<td>7.5</td>
<td>7.0</td>
<td>6.5</td>
<td>6.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Sesame</td>
<td>6.0</td>
<td>5.5</td>
<td>5.0</td>
<td>4.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Oilseeds (unshelled)</td>
<td>8.0</td>
<td>7.5</td>
<td>7.1</td>
<td>6.7</td>
<td>6.3</td>
</tr>
<tr>
<td>Vegetables (unshelled)</td>
<td>8.0</td>
<td>7.5</td>
<td>7.1</td>
<td>6.7</td>
<td>6.3</td>
</tr>
<tr>
<td>Wheat</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Maize</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Soybeans</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Carrots</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Lettuce</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Cabbage</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

*At 25°C, moisture content wet basis, in percent.

Predisposition for Seed Deterioration

- Genetics
- Seed Structure
- Seed Chemistry
- Physical/physiological seed quality
  - Maturity

Predisposition for Seed Deterioration

- Genetics
- Seed Structure
- Seed Chemistry
- Physical/physiological seed quality
  - Maturity
  - Physical damage
Predisposition for Seed Deterioration

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

Predisposition for Seed Deterioration

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

- **Relative Humidity**
  - Equilibrium moisture content

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

<table>
<thead>
<tr>
<th>°C</th>
<th>g H₂O/kg air</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.9</td>
</tr>
<tr>
<td>10</td>
<td>7.6</td>
</tr>
<tr>
<td>20</td>
<td>14.8</td>
</tr>
<tr>
<td>30</td>
<td>26.4</td>
</tr>
<tr>
<td>40</td>
<td>41.4</td>
</tr>
</tbody>
</table>
“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:
  \[ \% \text{ RH} + ^\circ\text{C} \leq 45.5 \]

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

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 1: Effects of ageing and hydration on malondialdehyde (MDA), total peroxidase, and soluble protein content in seed and cotyledons of two cotton cultivars. Ageing and planting densities were calculated as described in the legend to Table 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age</th>
<th>MDA (g/g DM)</th>
<th>Total peroxidase (g/g DM)</th>
<th>Soluble protein (g/g DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lycopersicon</td>
<td>young</td>
<td>31</td>
<td>55</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>aged</td>
<td>21</td>
<td>42</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>young</td>
<td>38</td>
<td>59</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>aged</td>
<td>28</td>
<td>60</td>
<td>88</td>
</tr>
</tbody>
</table>


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
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?

- 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
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

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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."

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

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The Physiology of Seed Deterioration

- Proposed mechanisms of seed deterioration
  - Enzyme activities
    - Free radical scavenging enzymes: SOD, catalase, etc.
  - Protein (decline) or amino acid (increase) content

*Table 1: Effect of aging and hydration on superoxide dismutase (SOD), catalase, peroxidase (POD) and anionic content of Vigna unguiculata L. by accelerated aging in a thermostatted cabinet at 45°C. values are mean ± SEM from triplicate samples of 3 replications.*

<table>
<thead>
<tr>
<th>Duration (Days)</th>
<th>SOD (U/mg)</th>
<th>Catalase (U/mg)</th>
<th>POD (U/mg)</th>
<th>Anion (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.5 ± 0.1</td>
<td>3.8 ± 0.2</td>
<td>2.3 ± 0.1</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>1</td>
<td>2.2 ± 0.1</td>
<td>3.5 ± 0.2</td>
<td>2.1 ± 0.1</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>7</td>
<td>2.0 ± 0.1</td>
<td>3.3 ± 0.2</td>
<td>1.9 ± 0.1</td>
<td>4.3 ± 0.2</td>
</tr>
</tbody>
</table>


*Table 2: Effect of aging and hydration on superoxide dismutase (SOD), catalase, peroxidase (POD) and anionic content of Vigna unguiculata L. by accelerated aging in a thermostatted cabinet at 45°C. values are mean ± SEM from triplicate samples of 3 replications.*

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</table>

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

- Increased membrane permeability

The Physiology of Seed Deterioration

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

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
The Physiology of Seed Deterioration

*Old Consensus “Models”*

DNA ↔ mRNA → Enzymes → No germination

stored

DNA → mRNA ↔ Enzymes → No germination

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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

Oxygen is essential for generation of three ATPs.

Free radical production, primarily initiated by oxygen, has been related to the peroxidation of lipids and other essential compounds found in cells.
Free radicals cause a host of undesired events
- Decreased lipid content
- Reduced respiratory competence
- Increased evolution of volatiles ranging from hexanal to aldehydes
**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.

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**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.

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[Image of free radical structure]
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

- 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
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

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

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

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
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

\[
\begin{align*}
\text{CH}_2(\text{CH}_2)_n\text{CH}=\text{CH} & \xrightarrow{\text{H}^+}\text{CH} \cdot (\text{CH}_2)_n\text{CH}=\text{CH}-\text{COOH} \\
\text{CH}_2(\text{CH}_2)_n\text{CH}=\text{CH} & \xrightarrow{\text{O}}\text{CH} \cdot (\text{CH}_2)_n\text{CH}=\text{CH} + \text{OH} \\
\text{Hydroperoxide} & \\
\text{Alkoxyl radical} & \\
\text{Hydroxyl radical} & 
\end{align*}
\]

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

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
The Physiology of Seed Deterioration

- Free radical assault on mitochondria
  - Prime “sink” for oxygen - leaks from membranes during respiration

\[
\text{O}_2 + 4\text{H}^+ + 4e^- \rightarrow 2\text{H}_2\text{O (about 95% of the time)}
\]

\[
2\text{O}_2^- \quad \text{(about 5% of the time)}
\]

Superoxide free radical


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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

![Graph showing decreased seed germination over time](image)

*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 germination trial of Ljekin (1927).*
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

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
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 peroxidase
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)

Figure 3. The oxidation-reduction pathways that involve glutathione. From Maloney et al.
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• + TOH → RH + TO•

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
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

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

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

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**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

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**Table 4**: Measurements, dehydrogenase activity and lipid peroxidation of eggplant (S) and radish (R) seeds subjected to simulated ageing. Table 4: Solution infiltration treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conductance (mS/cm)</th>
<th>Leakage of sugars (mg/g fresh weight)</th>
<th>Tocopherol (mg/g sample)</th>
<th>TBA index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current</td>
<td>100</td>
<td>0.100</td>
<td>0.175</td>
<td>0.230</td>
</tr>
<tr>
<td>10%</td>
<td>90</td>
<td>0.100</td>
<td>0.200</td>
<td>0.250</td>
</tr>
<tr>
<td>20%</td>
<td>80</td>
<td>0.150</td>
<td>0.250</td>
<td>0.300</td>
</tr>
<tr>
<td>30%</td>
<td>70</td>
<td>0.200</td>
<td>0.300</td>
<td>0.350</td>
</tr>
<tr>
<td>40%</td>
<td>60</td>
<td>0.300</td>
<td>0.400</td>
<td>0.450</td>
</tr>
<tr>
<td>50%</td>
<td>50</td>
<td>0.500</td>
<td>0.600</td>
<td>0.600</td>
</tr>
</tbody>
</table>

*Accelerated ageing was done at 50%, 60% and 70% for 32 days at room temperature 20°C. Values in parenthesis are 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
  - Free radical damage can be ameliorated by
    - Presence of antioxidants, particularly during storage
    - Hydration either during imbibition or priming
      - Permits repair

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

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
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
- 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:
