Accelerated Aging Test

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Global Vegetable Seed Production Projects
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Agenda

1. Background and Principles
2. Equipment and Supplies
3. Procedures
4. Considerations related to Precision
5. Interpretation of Results
Background and Principles

- **Background**
  - Utilizes same environmental factors impacting seed deterioration
    - Temperature
    - Relative Humidity
    - Duration
  - Initially developed to estimate seed longevity to predict storage of soybeans
  - Test has been expanded to several crops
  - Approved by ISTA for soybeans
Background and Principles

• Principles
  – Seed is exposed to high temperature and relative humidity for a short period of time.
  – The seed aging process becomes “accelerated” causing deterioration.
  – Seeds are subjected to the standard germination test and evaluated for normal seedlings.
  – High vigour seed lots demonstrate higher germination following aging.
  – Lower vigour seed lots have lower germination following aging.
Materials and Supplies

- Analytical balance – weigh to 0.001 g
- Plastic AA boxes
- Seed Moisture Containers – as described by ISTA
- Distilled or deionized water source.
- Dispenser with range from 0 – 100 ml for water.
- AA Chamber
- NIST Thermometer
- Germination testing facilities
  - With equipment, supplies and capability for standard germination (ISTA, 1999).
Materials and Supplies

• Plastic AA Boxes
  – With lids
  – 11.0 x 11.0 x 3.5 cm
  – Wire trays, 10.0 x 10.0 x 3.0 cm
  – Available commercially
    • Hoffman Mfg.
Materials and Supplies

- **AA Chamber**
  - Water jacketed
  - Must maintain temperature +/- 0.3°C
  - Desired range of 41 – 45°C, depending on species.
  - Plastic or stainless steel tray to be placed at bottom of chamber with distilled water.
Materials and Supplies

- Multiple chambers
  - May be required for higher capacity
  - Can designate different temperatures.
Procedures – Preparation

1. Set temperature and preheat chamber to precise temperature.
   - Add distilled or deionized water in bottom tray (4 cm depth)
   - Use NIST thermometer to confirm temperature
   - Confirm temperature is maintained for 24-48 hours

2. Thoroughly wash AA boxes, lids and screens
   - Use a 15% hypochlorite solution
   - Wash and dry before each use
   - Can use a commercial dishwasher

3. Determine initial seed moisture content of seed lots.
   - Moisture range should be 10-14%.
   - Refer to ISTA Handbook to adjust as needed
Procedures – Filling Boxes

4. Dispense 40 ml of distilled or deionized water in each plastic box
   • Insert screens in each box
   • Avoid splashing any water on surface of the screens

5. Weigh seed and place on screen for each AA box.
   • For soybeans, this 42 g (~ 200 seeds)
   • Use more than one AA box for large seeded seed lots
   • Level seeds to a one layer depth
   • Include one “control sample” to check for seed moisture after the aging period

6. Place lids on each AA box
   • Lids should fit firmly
   • Replace any warped lids
Procedures – Placement in Chamber

7. Arrange boxes on tray and transfer to the AA Chamber
   • Allow at least 2.5 cm between each box for air movement
   • Avoid splashing any water on the screen surface
   • Place only one or two screens containing the AA boxes in the middle or upper portion of the chamber
   • Record the exact time when AA boxes are placed in the chamber
   • Close the door and do not open it until the end of the aging period

8. Monitor the temperature during the aging period
   • Maintain temperature at +/- 0.3C
   • Confirm temperature has been maintained during the aging period
8. After the aging period, remove AA boxes from the chamber
   • Remove the trays (i.e. 72 hours, +/- 15 min.)
   • Leave lids on each box until the aged seed is planted
   • Remove the screens with the seed from the AA box
   • Planting should be completed within 1-2 hours after removal from the chamber
   • Weigh the control sample of the imbibed seed and compare to target range for species

9. Proceed to plant the aged seed following ISTA Rules.
   • Refer to ISTA Handbook and adjust as needed

10. For many seed lots, stagger the placement of samples into the AA Chamber in 1-2 hour intervals
    • Use separate aging chambers
## Control Sample – After Aging Seed Weights

<table>
<thead>
<tr>
<th>Species</th>
<th>Seed Wt</th>
<th>Aging Temp</th>
<th>Duration</th>
<th>M% after Aging</th>
<th>Seed Weight Range After Aging (10% M)</th>
<th>Seed Weight After Aging (14% M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean</td>
<td>42 g</td>
<td>41C</td>
<td>72 h</td>
<td>27-30%</td>
<td>51.8 – 54.0 g</td>
<td>49.5 – 51.6 g</td>
</tr>
<tr>
<td>Corn, Field</td>
<td>40 g</td>
<td>43C</td>
<td>72 h</td>
<td>26-29%</td>
<td>49.3 – 51.5 g</td>
<td>47.1 – 49.1 g</td>
</tr>
</tbody>
</table>

- **Seed Moisture Calculation (10-14%):**
  - \((100 – \text{initial seed MC} / 100 – \text{desired MC}) \times \text{weight of sample}\)
Procedures – Standard Germination

11. Complete test according to ISTA Rules
12. Evaluate test according to ISTA Rules
## Relationship with Field Emergence

Correlation Coefficients of SG and AA results and with 25 Field Emergence trials over 10 years

<table>
<thead>
<tr>
<th>Year</th>
<th>Seed Lots</th>
<th>1st Planting</th>
<th>2nd Planting</th>
<th>3rd Planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SG</td>
<td>AA</td>
<td>SG</td>
</tr>
<tr>
<td>1980</td>
<td>40</td>
<td>0.45</td>
<td>0.90</td>
<td>0.60</td>
</tr>
<tr>
<td>1981</td>
<td>52</td>
<td>0.28</td>
<td>0.66</td>
<td>0.27</td>
</tr>
<tr>
<td>1984</td>
<td>29</td>
<td>0.41</td>
<td>0.64</td>
<td>0.59</td>
</tr>
<tr>
<td>1985</td>
<td>12</td>
<td>0.85</td>
<td>0.40</td>
<td>0.82</td>
</tr>
<tr>
<td>1987</td>
<td>16</td>
<td>0.90</td>
<td>0.88</td>
<td>0.87</td>
</tr>
<tr>
<td>1988</td>
<td>33</td>
<td>0.84</td>
<td>0.80</td>
<td>0.85</td>
</tr>
<tr>
<td>1989</td>
<td>17</td>
<td>0.69</td>
<td>0.62</td>
<td>0.88</td>
</tr>
<tr>
<td>1991</td>
<td>15</td>
<td>0.09</td>
<td>0.74</td>
<td>0.48</td>
</tr>
<tr>
<td>1992</td>
<td>38</td>
<td>0.58</td>
<td>0.90</td>
<td>0.52</td>
</tr>
<tr>
<td>1993</td>
<td>20</td>
<td>0.89</td>
<td>0.91</td>
<td>-</td>
</tr>
</tbody>
</table>
Interpretation of Results

- Results are not “absolute”

- Rank seed lots to make decisions
  - Consider
    - Standard germination
    - Age of seed
    - Expected market conditions

- Relationship with field emergence.
  - Hampton and TeKrony, 1995
  - Egli and TeKrony, 1995, 1996
  - Wolz and TeKrony, 2001
  - Torres, Vieira and Panobianco, 2004

- Relationship with storage potential
  - Delouche and Baskin, 1973
  - Hampton and TeKrony, 1995

Correlation Coefficients ($r^2$) among Accelerated Aging test results and Field Emergence over Three Years*

<table>
<thead>
<tr>
<th>Planting</th>
<th>1993</th>
<th>1994</th>
<th>1997</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Planting</td>
<td>0.50**</td>
<td>0.50**</td>
<td>0.77**</td>
</tr>
<tr>
<td>2nd Planting</td>
<td>0.76**</td>
<td>0.74**</td>
<td>0.80**</td>
</tr>
<tr>
<td>3rd Planting</td>
<td>0.71**</td>
<td>0.72**</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Precision in the AA test is critical

- Why is “Precision” important?
  - Changes in seed vigour can occur rapidly
  - Small changes in aging conditions can have a major changes in results.
  - Improper equipment and supplies will influence results
Considerations related to Precision

- Water Jacketed Chamber – is it needed?

AA Results for three types of chambers

<table>
<thead>
<tr>
<th>Seed Lot</th>
<th>Water-Jacketed</th>
<th>Dry Incubator</th>
<th>Immersed Element</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>96</td>
<td>95</td>
<td>96</td>
</tr>
<tr>
<td>2</td>
<td>94</td>
<td>94</td>
<td>92</td>
</tr>
<tr>
<td>3</td>
<td>85</td>
<td>82</td>
<td>76</td>
</tr>
<tr>
<td>4</td>
<td>85</td>
<td>83</td>
<td>82</td>
</tr>
<tr>
<td>5</td>
<td>52</td>
<td>73</td>
<td>48</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>66</td>
<td>38</td>
</tr>
<tr>
<td>Mean</td>
<td><strong>72</strong></td>
<td><strong>82</strong></td>
<td><strong>72</strong></td>
</tr>
</tbody>
</table>

- Use a water-jacketed aging chamber to promote precise temperature control.
- Severe condensation occurs in the chamber with the immersed heating element resulting in higher seed moisture and lower results.
Considerations related to Precision

• How important is temperature control?

Monthly germination after following storage at 10C and aged at 41C for 72 hours

<table>
<thead>
<tr>
<th>Seed Lot</th>
<th>Feb</th>
<th>Mar</th>
<th>April</th>
<th>June</th>
<th>July</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>86</td>
<td>82</td>
<td>86</td>
<td>91</td>
<td>91</td>
<td>87</td>
</tr>
<tr>
<td>2</td>
<td>68</td>
<td>70</td>
<td>60</td>
<td>84</td>
<td>66</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>93</td>
<td>89</td>
<td>92</td>
<td>83</td>
<td>89</td>
</tr>
<tr>
<td>4</td>
<td>84</td>
<td>83</td>
<td>89</td>
<td>96</td>
<td>89</td>
<td>88</td>
</tr>
<tr>
<td>Mean</td>
<td>82</td>
<td>82</td>
<td>81</td>
<td>91</td>
<td>82</td>
<td></td>
</tr>
</tbody>
</table>

– Water-jacketed aging chamber provided repeatable results with exception of month of June.
– Chamber temperature declined to 40.5C in June which contributed to significantly higher results.
– Chamber temperature must be maintained +/- 0.3C
Considerations related to Precision

• Why use seed weight rather than seed number?

Effect of seed size on seed moisture content after aging

<table>
<thead>
<tr>
<th>Seed Size (mg/seed)</th>
<th>40 g</th>
<th>200 Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seed No.</td>
<td>SMC %</td>
</tr>
<tr>
<td>112</td>
<td>357</td>
<td>33.3</td>
</tr>
<tr>
<td>163</td>
<td>248</td>
<td>32.7</td>
</tr>
<tr>
<td>225</td>
<td>178</td>
<td>33.0</td>
</tr>
<tr>
<td>297</td>
<td>135</td>
<td>31.2</td>
</tr>
<tr>
<td>LSD (P=0.5)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

- Using 40 g provides consistent seed moistures after aging.
- Using seed consistent seed number (200) leads to lower seed moisture after aging as seed size increases, thus influencing results.
Considerations related to Precision

- What is impact of opening the door during the aging period?

<table>
<thead>
<tr>
<th>Door Open (min)</th>
<th># Trays</th>
<th>Boxes / Tray</th>
<th>Box (°C)</th>
<th>Chamber (°C)</th>
<th>Box (Min)</th>
<th>Chamber (Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>5</td>
<td>3</td>
<td>0.4</td>
<td>3.4</td>
<td>160</td>
<td>84</td>
</tr>
<tr>
<td>1.0</td>
<td>5</td>
<td>9</td>
<td>0.4</td>
<td>4.2</td>
<td>140</td>
<td>105</td>
</tr>
</tbody>
</table>

- Opening door reduces the time at the desired temperature for aging
Considerations related to Precision

- Effect of planting delays following aging on AA germination

<table>
<thead>
<tr>
<th>Seed Lot</th>
<th>Lids on 0 h</th>
<th>Lids on 2 h</th>
<th>Lids on 4 h</th>
<th>Lids Off 2 h</th>
<th>Lids Off 4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>74</td>
<td>68</td>
<td>71</td>
<td>70</td>
<td>59*</td>
</tr>
<tr>
<td>2</td>
<td>84</td>
<td>83</td>
<td>77</td>
<td>81</td>
<td>60*</td>
</tr>
<tr>
<td>3</td>
<td>93</td>
<td>90</td>
<td>89</td>
<td>88</td>
<td>86</td>
</tr>
</tbody>
</table>

- Keep lids on after the aging period until the seed is planted
- Plant within 2 hours after the aging period.
Considerations related to Precision

- Effect of seed treatment on AA germination

<table>
<thead>
<tr>
<th></th>
<th>Seed Lot 1</th>
<th></th>
<th>Seed Lot 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aging Temp</td>
<td>Untreated</td>
<td>Treated</td>
<td>Untreated</td>
<td>Treated</td>
</tr>
<tr>
<td>41 °C</td>
<td>84</td>
<td>86</td>
<td>81</td>
<td>82</td>
</tr>
</tbody>
</table>

- LSD @ 0.5 = 7.16
Considerations related to Precision

• Key points:
  – Maintain uniform temperature within the chamber
    • Temperature variation should be no more than +/- 0.3C
  – High humidity should be maintained within the chamber to minimize evaporation from the AA boxes.
  – Use a water-jacketed aging chamber to promote precise temperature control.
    • It is critical to avoid condensation within the aging boxes
    • Keep door closed during the aging period
Equipment Sources

- Hoffman Manufacturing
  - sales@hoffmanmfg.com
  - Basic Water-Jacketed CO2 Incubator SC06WE / SC06WE2
    - Single unit: $5200 - $5600 USD
  - CO2 Water Jacketed Incubator SCO5W
    - Single unit - $6,033 USD
    - Dual unit – $13,500 USD

- Sheldon Manufacturing, Inc.
  - sheldonmanufacturing.com
  - Same as above

- VWR International
  - vwr.com
  - VWR® Water Jacketed CO2 Incubators
    - Single unit: $8382 - $9203 USD
    - Dual units: $16,707 - $18,349 USD
Thank You for your Attention!