

**AOSA/SCST**  
**Asteraceae, Sunflower family I**  
**Lettuce**  
**Seedling Evaluation Handbook Committee**

# **Lettuce Seedling Evaluation Webinar**

Presented by Elizabeth Bada, November 14, 2013

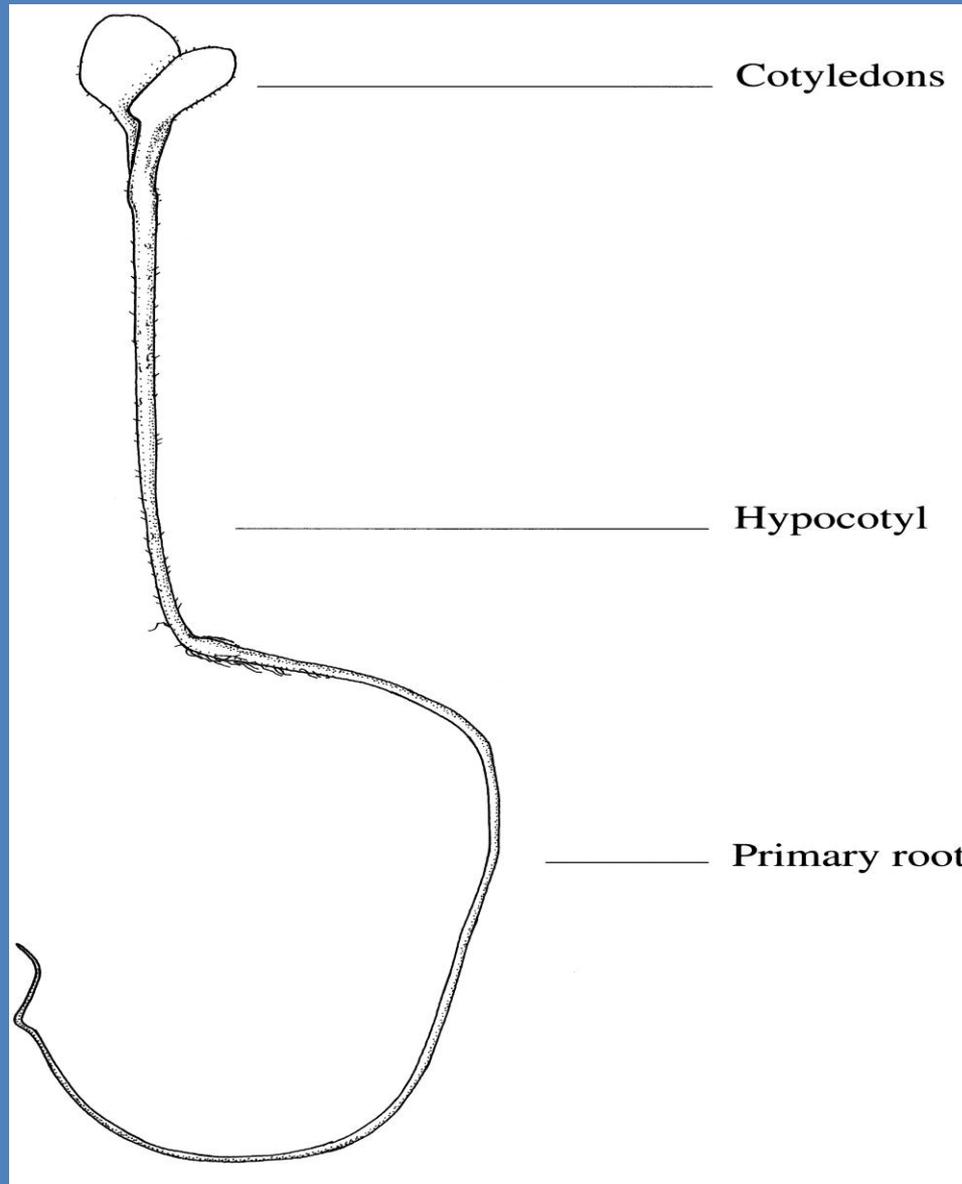
# AOSA RULES

*Lactuca sativa*, lettuce (The committee's proposed changes are in black font.)

## GENERAL DESCRIPTION

- Seedling type: Epigeal dicot.
- Food reserves: Cotyledons that expand and become thin, leaf-like and photosynthetic. Some varieties develop elongated petioles at the base of the cotyledons.
- 
- Shoot system: The hypocotyl elongates and carries the cotyledons above the soil surface. The epicotyl usually does not show any development within the test period.
- Root system: A long primary root.

# Essential seedling structures



# Abnormal Seedling Description

## Cotyledons :

- less than half of the original cotyledon tissue remaining attached.
- less than half of the original cotyledon tissue free of necrosis or decay (see notes 5, 6 and 8).
- **cotyledons that are swollen, curled, or deformed. (Added to harmonize with ISTA.)**

## Epicotyl:

- missing (may be assumed to be present if cotyledons are intact).
- any degree of necrosis, decay or damage at the point of cotyledon attachment.

## Hypocotyl :

- deep open cracks extending into the conducting tissue.
- severely twisted or grainy.
- watery.
- **malformed, such as markedly shortened or thickened. (Added to harmonize with ISTA.)**

# Abnormal Seedling Description

## Root:

- none.
- primary root tip blunt, swollen and discolored.
- primary root with splits or lesions.
- weak, stubby or missing primary root (secondary roots will not compensate for a defective primary root).

## Seedling:

- swollen cotyledons associated with extremely short hypocotyl and root.
- one or more essential structures impaired as a result of decay from primary infection.
- albino
- yellow (applies to individual discolored seedlings in the replicate.)  
(Added to harmonize with ISTA)

# Notes

- **1. Toxic materials in the substrate will cause short, blunt roots. The roots lift away from the substrate. Check media for toxicity and conduct retest if necessary.**
- **2. Seedlings grown on top of white filter paper will be shorter than those grown on dyed blotters. Retest if necessary.**
- **3. Remove attached seed coats for seedling evaluation.**
- **4. Seedlings with slight dormancy or light sensitivity may be slow to germinate. Extend test according to the rules.**
- **5. One type of necrosis on lettuce cotyledons is a physiological breakdown of the plant tissues, the cause of which has not been determined. It is manifested by discolored areas on the cotyledons, first appearing on or adjacent to the midrib and lateral veins, and should not be confused with the natural pigmentation of the different lettuce cultivars.**

# Notes

6. Seedlings with extensive physiological necrosis on the cotyledons may be slower in growth than those without such affected areas. Hypocotyl and root length may be affected by other factors such as proximity to light, delayed germination or dormancy.
7. Seedlings with three cotyledons should be considered as “normal”.
8. The 50% Rule must be followed to classify seedlings with mechanical damage (dark areas of discoloration or decay) as “abnormal” seedlings.

# Virtual Lettuce Referee Review

- ❖ The AOSA/SCST Seedling Evaluation Handbook Committee conducted a lettuce referee in 2012. The referee results were presented in Boise in May 2013. The presentation is available on the SCST website.
- ❖ The referee photos have been classified by the Seedling Evaluation Handbook committee and will be reviewed along with some additional photos and evaluation criteria, in this webinar.
- ❖ The Lettuce webinar will soon be available on the SCST website to view or download.
- ❖ The different types of abnormal seedlings as described in the rules are represented in the photos.

# Lettuce germination test



# Assessment of Seedlings

## Laboratory definition of germination;

- ❖ In seed laboratory practice, germination is the emergence and development of the essential structures of the seed embryo that, for the kind of seed in question, are indicative of the ability to produce a normal plant under favorable conditions. The objective of the germination test is to determine the percentage of normal seedlings in the test. (See section 6.2 a. of the AOSA Rules for Testing Seeds Vol. 1.)

# Assessment of Seedlings

## Normal seedlings;

- ❖ In general, seedlings are classified as normal if they have no defects or only slight defects that will not impair the continued development of the seedling or plant when grown in soil under favorable conditions.  
(See section 6.2 b. of the AOSA Rules Vol. 1.)

# Image 2

Distribution of referee responses:

- Normal: 100%
- Abnormal: 0%

All essential structures are healthy and normal.



# Image 22

Distribution of  
referee responses:

- Normal: 97.2%
- Abnormal: 2.8%

All essential structures are  
healthy and normal



# Image 11

Distribution of referee responses:

- Normal: 90.1%
- Abnormal: 9.9%

All essential structures are healthy and normal.

❖The hypocotyl is out of proportion to the root possibly due to its proximity to the light source. The replicates of a single test should be placed at an equal distance from the light source to avoid causing any discrepancy between replicates. Tests placed too far from the light will have seedlings that lean and reach toward the light causing an un-uniform stand with leggy hypocotyls and out of proportion seedlings.

❖This is a test condition and is to be considered normal.



# Image 26

Distribution of  
referee responses:

- Normal: 56.3%
- Abnormal: 43.7%

Normal with 50% functional  
cotyledon tissue, free of  
necrosis or decay, remaining  
attached.

❖ The growing point is not  
visible and would need to be  
free of necrosis or decay to be  
normal. This classification is  
based on the cotyledons in this  
case.



# Assessment of seedlings

## Additional photo

Abnormal seedling with a decayed root.

- ❖ This may be an effect of toxicity, possibly from the pelleting material. If the presence of these is 5% or more, consider a retest in a different media.



# Assessment of Seedlings

## Abnormal seedlings;

- ❖ **Seedlings are classified as abnormal if they have defects that will prevent them from developing into mature plants when grown in soil under favorable conditions. These defects are not to be considered abnormalities if they are caused by test conditions. To classify a seedling as abnormal an analyst must judge the defect to be so severe that further development of the seedling would be unlikely. Specific abnormalities are listed in seedling descriptions of Part II. (See section 6.2c of the AOSA Rules for Testing Seeds Vol.1.)**

# Image 21

Distribution of referee responses:

- Normal: 2.8%
- Abnormal: 97.2%

Abnormal due to decay at the point of attachment and a short, grainy or lesioned hypocotyl.

- ❖ The hypocotyl surface appears grainy due to the abnormal cell division, creating a lesion during the development of the hypocotyl tissues.



# Image 25

Distribution of referee responses:

- Normal: 42.3%
- Abnormal: 57.7%

Abnormal due to a short, weak and watery hypocotyl.

- ❖ This seedling also has less than 50% necrosis.



# Image 13

Distribution of referee responses:

- Normal: 2.8%
- Abnormal: 97.2%

Abnormal seedling with a swollen hypocotyl and a short, blunt root with a decayed tip.



# Image 8

Distribution of referee responses:

- Normal: 1.4%
- Abnormal: 98.6%

Albino is considered abnormal.

❖ All tissues are white due to the absence of pigments. Cotyledons without chlorophyll cannot perform photosynthesis.



# Image 19

Distribution of referee responses:

- Normal: 21.1%
- Abnormal: 78.9%

Abnormal seedling due to the split root.

- ❖ The hypocotyl is swollen and lesioned at the transition point to the split root.



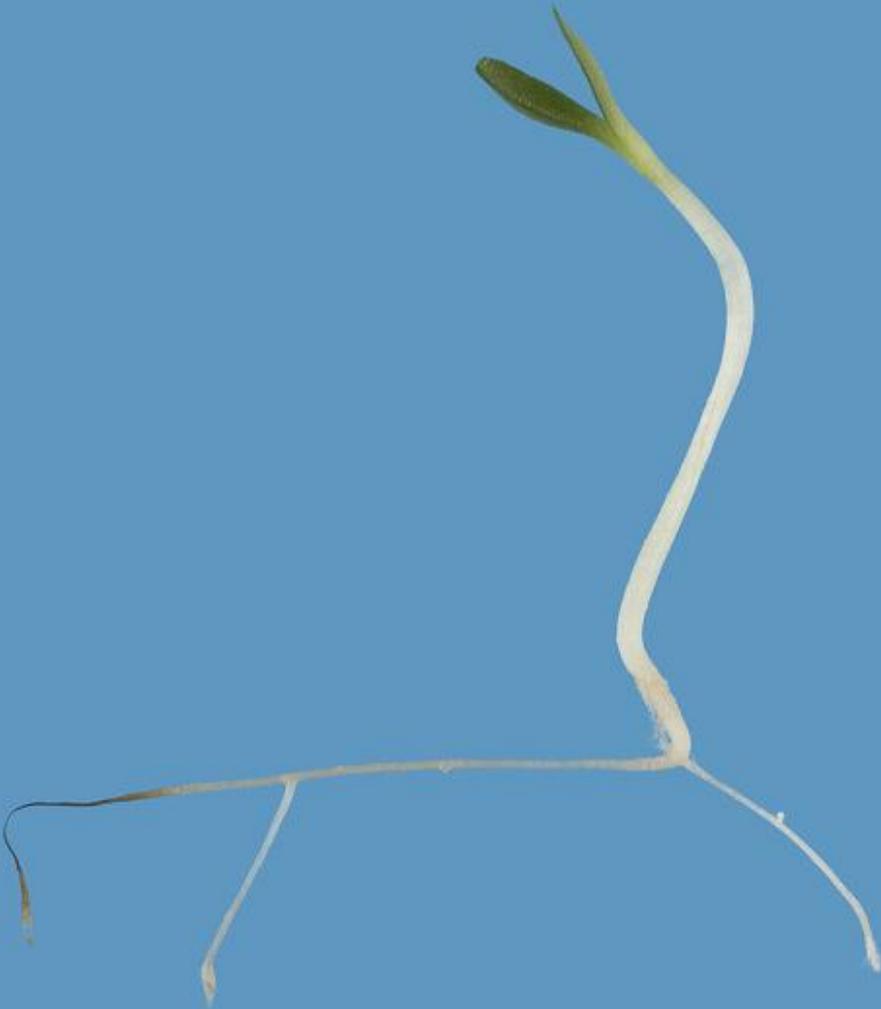
# Image 7

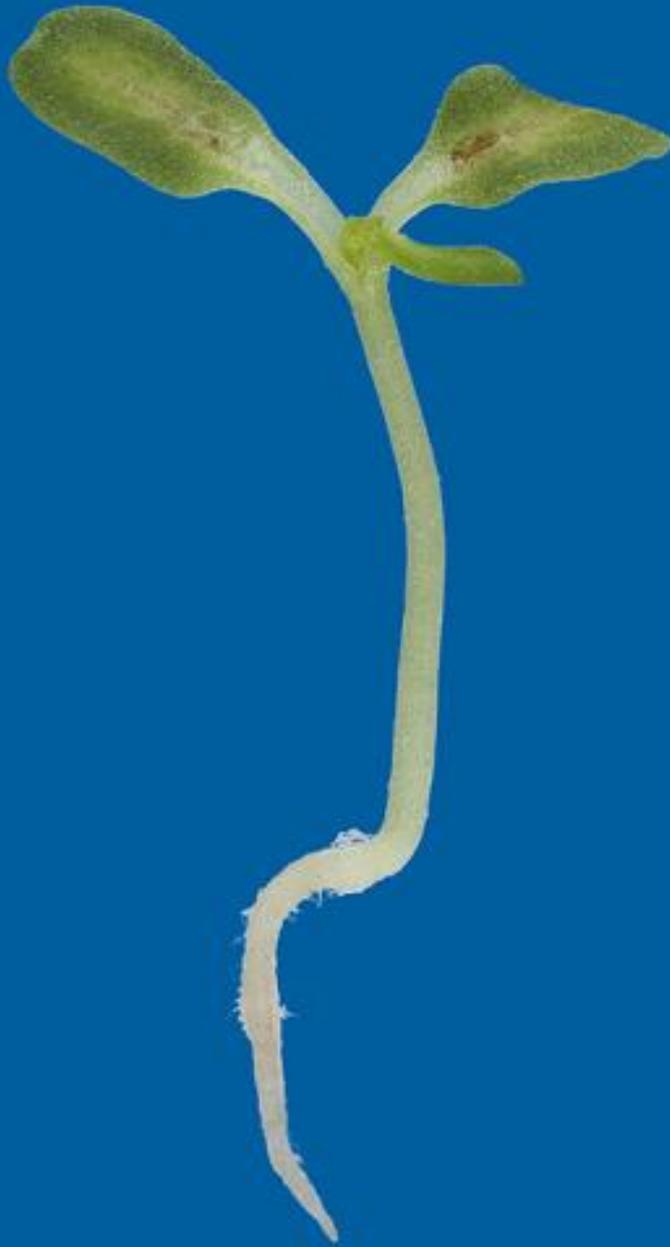
Distribution of referee responses:

- Normal: 44.4%
- Abnormal: 55.6%

Abnormal with a decayed root. This may be an effect of toxicity.

❖ Secondary roots will not compensate for a defective primary root.





## Image 16

Distribution of referee responses:

- Normal: 20%
- Abnormal: 80%

Abnormal seedling with a short, blunt root and a watery hypocotyl.

❖ Less than 50% necrosis.

# Image 27

Distribution of referee responses:

- Normal: 0%
- Abnormal: 100%

A late germinating , undeveloped seedling with decay at the point of attachment and a blunt watery root is abnormal.



# Assessment of seedlings

**Diseased and decayed seedlings;**

- ❖ **Secondary infection is infection that does not originate with the seed or seedling itself, but rather from other diseased seeds or seedlings. Seedlings with any degree of secondary infection are to be classified as normal provided that all essential structures are present and otherwise normal (See section 3.5.5, part 1. of the AOSA Rules for Testing Seeds, Vol. 4.)**

# Assessment of seedlings

**Diseased and decayed seedlings;**

- ❖ **Primary infection originates from the seed or seedling itself. Seedlings with slight primary infection are considered normal provided development of none of the essential structures has been impaired. Seedlings with primary infection sufficient to impede development of one or more essential structures are to be classified as abnormal. (See section 3.5.5, part 1. of the AOSA Rules for Testing Seeds, Vol. 4.)**

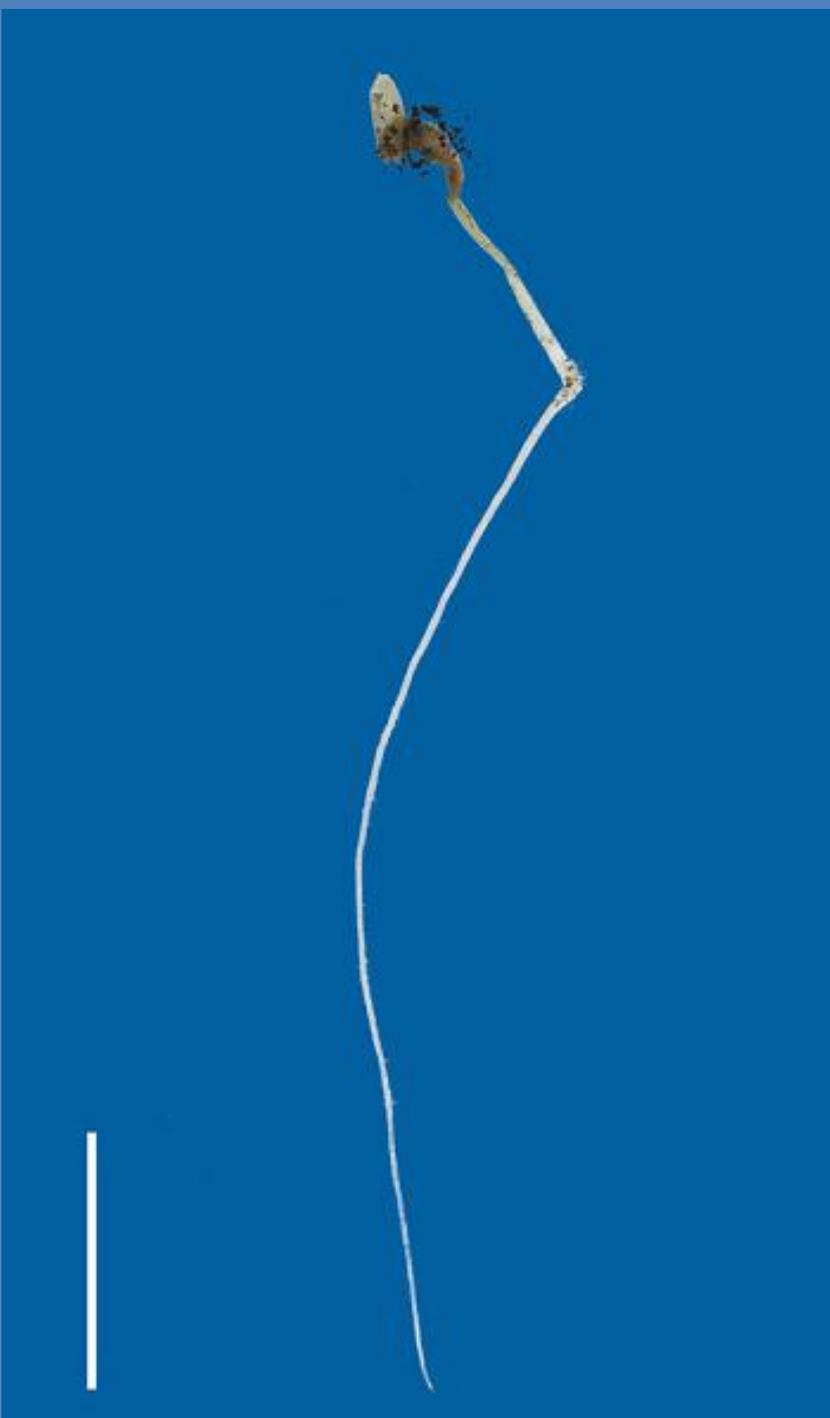
# Image 4

Distribution of referee responses:

- Normal: 0%
- Abnormal: 100%

Abnormal with the essential structures decayed from primary infection.

❖The structures of the seedlings effected by the fungi turn mushy and decayed.



# Image 5

Distribution of referee responses:

- Normal: 0%
- Abnormal: 100%

Abnormal with decay at the point of attachment.

❖ When the point of attachment is decayed the seedling is abnormal and no further assessment is needed.



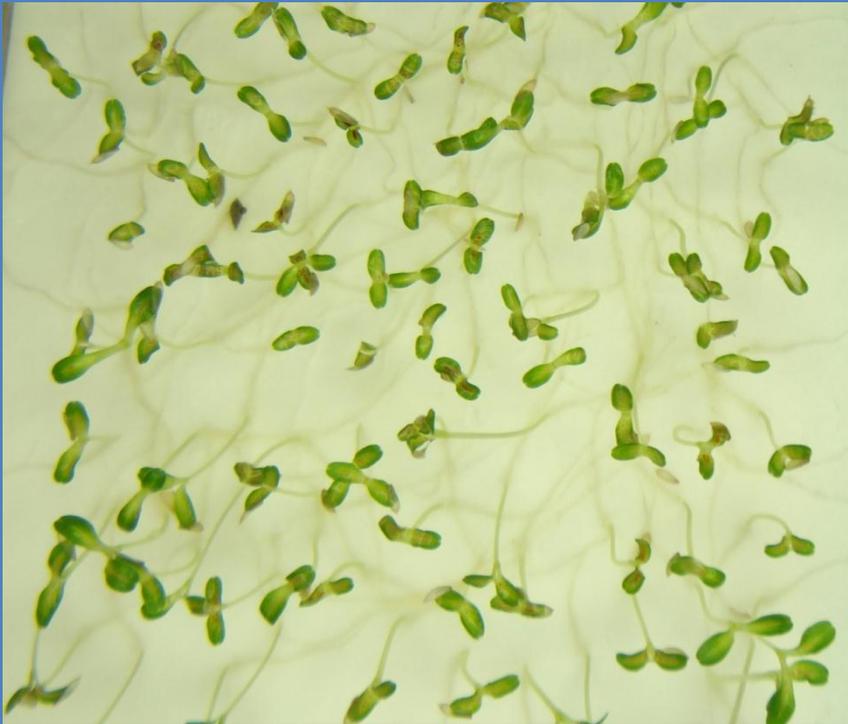
# Assessment of seedlings

## Physiological necrosis;

- ❖ **Physiological necrosis is an effect of ageing in lettuce. The exact cause is unknown. A seed lot with necrosis will continue to decline in vitality and shelf life. Poor storage conditions or stressing the seed will contribute to this decline. The presence of necrosis in the seed lot will increase over time.**
- ❖ **Due to a physiological breakdown of the plant tissues, the cells die off and turn dark. Necrosis usually starts along the midrib and progresses up the lateral veins of the cotyledons from the base to the tip of the leaf.**
- ❖ **The dark areas caused by physiological necrosis can appear as bubbly and bumpy or sunken leaf tissues.**

# Assessment of seedlings

## Physiological necrosis



- ❖ **Physiological necrosis can affect the entire seed lot whereas mechanical damage or decay affects the individual seeds or seedlings.**

## Necrosis

- ❖ **The word necrosis means dead cells. It is important to determine if the dead or dying spot is physiological necrosis, mechanical, in other words physical damage, or decay.**

# Image 28

Distribution of referee responses:

- Normal: 35.7%
- Abnormal: 64.3%

Abnormal due to less than 50% functional cotyledon tissue, free of necrosis or decay, remaining attached.

❖ This seedling has more than 50% necrosis and chlorosis.



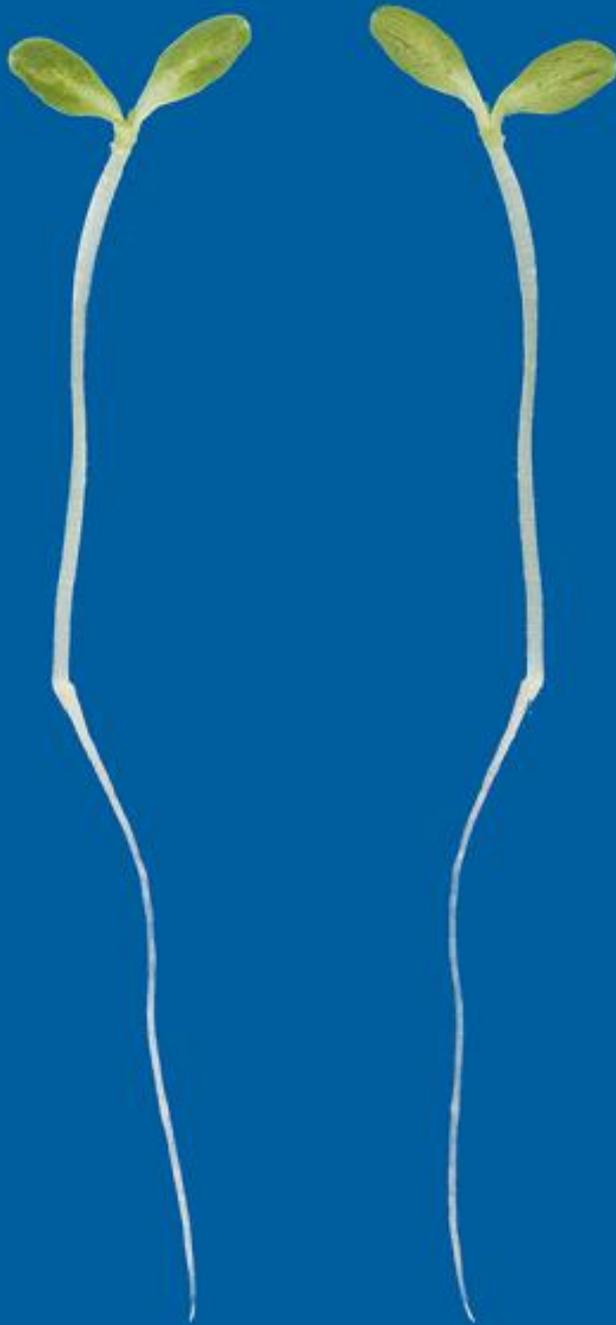
## Image 23

Distribution of referee responses:

- Normal: 85.9%
- Abnormal: 14.1%

Normal seedling with more than 50% functional cotyledon tissue, free from necrosis or decay, remaining attached.

❖ Acceptable defect in a slightly twisted hypocotyl.



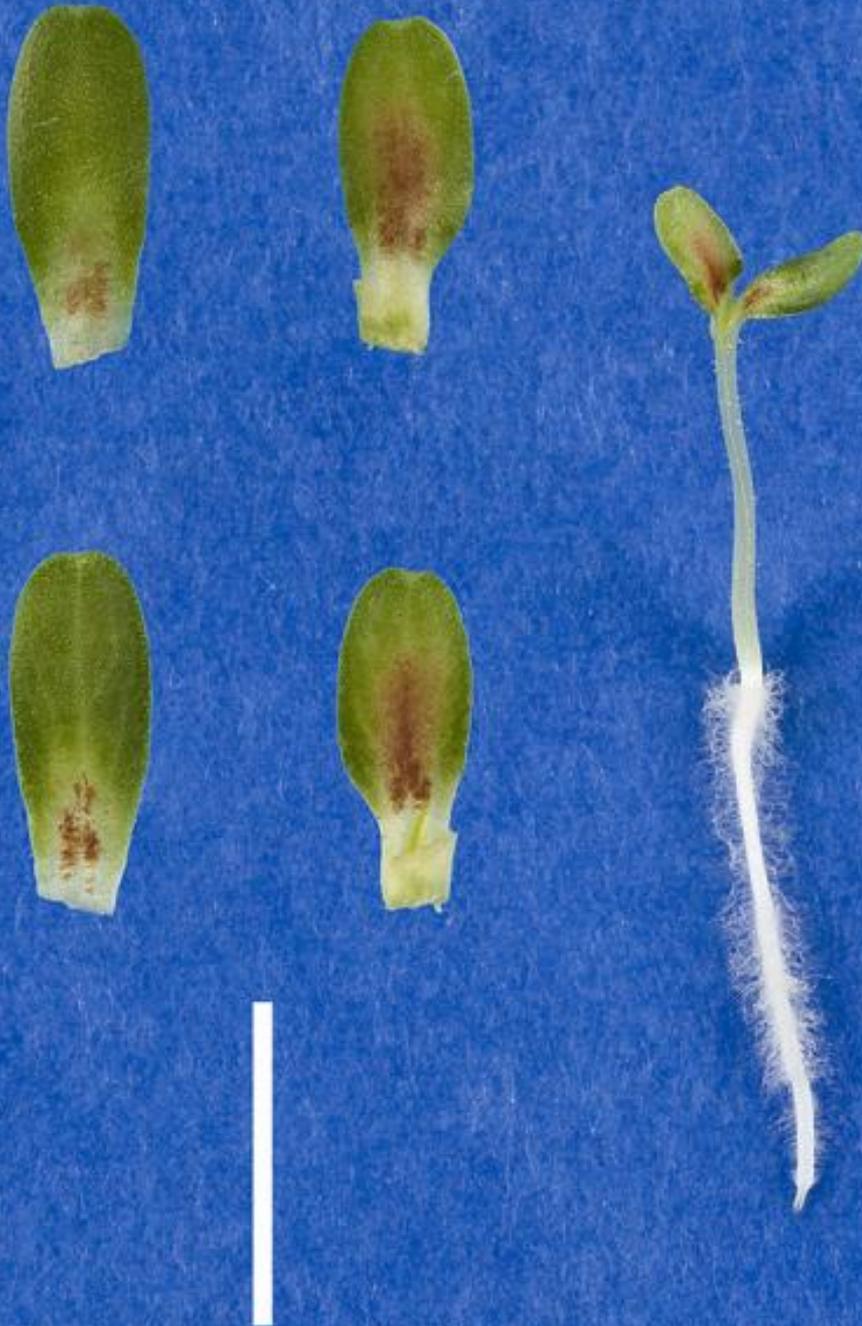
# Image 18

Distribution of referee responses:

- Normal: 32.4%
- Abnormal: 67.6%

Abnormal due to the watery hypocotyl and the deformed root tip.

❖ This seedling also has less than 50% necrosis.



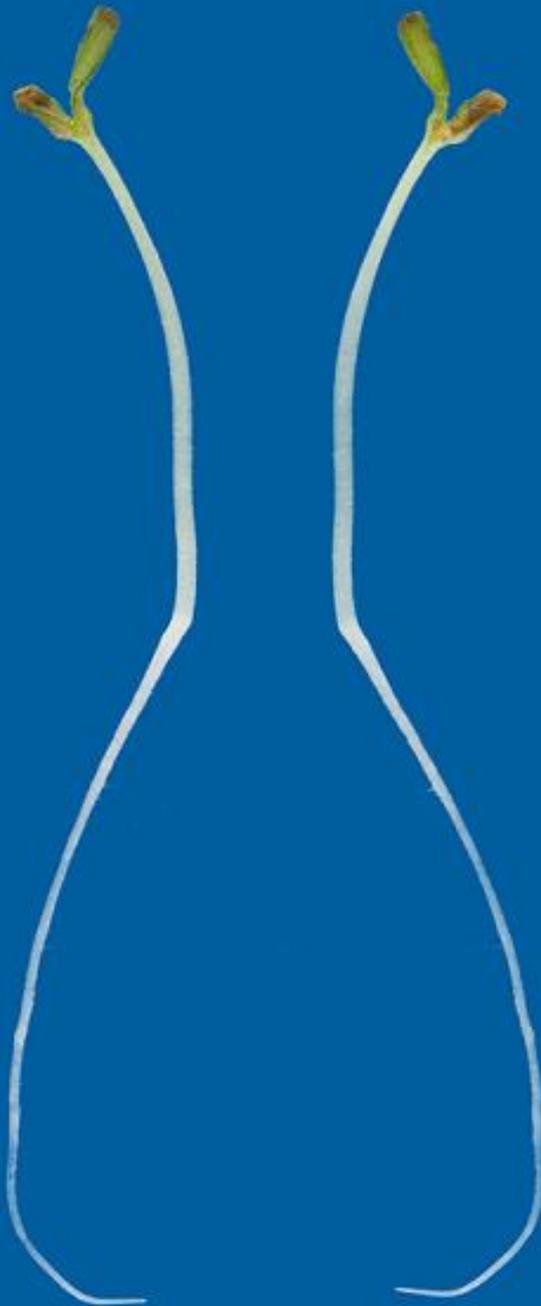
# Image 20

Distribution of referee responses:

- Normal: 4.3%
- Abnormal: 95.7%

Abnormal due to less than 50% functional cotyledon tissue, free of necrosis or decay, remaining attached.

- ❖ More than 50% physiological necrosis and mechanical damage.
- ❖ This abnormal seedling has physiological necrosis at the growing point .
- ❖ More than 50% mechanical damage on the cotyledons.



# Image 12

Distribution of referee responses:

- Normal: 78.9%
- Abnormal: 21.1%

Normal seedling with more than 50% functional cotyledon tissue, free of necrosis or decay, remaining attached.

- ❖ This seedling also has less than 50% necrosis.



# Assessment of seedlings

- **Mechanical damage;**
  - ❖ **Handling during harvesting, processing or by rough handling with forceps in the laboratory can cause damage to seeds and therefore the embryonic plant.**
  - ❖ **Mechanical damage causes injured and bruised spots, which result in decayed areas on the seedling.**
  - ❖ **A mechanical damaged area should not be confused with decay from an infection which can appear wet and rotting. By comparison mechanical damage looks more like a crimped or pinched dark area of dead cells similar to a dried up leaf. (See section 3.4.4, part 1. of the AOSA Rules for Testing Seeds, Vol. 4.)**

# Image 24

Distribution of referee responses:

- Normal: 12.7%
- Abnormal: 87.3%

This seedling is abnormal due to the watery hypocotyl. (The pinched spot on the hypocotyl is from handling the seedling.)

- ❖ The cotyledons have mechanical damage.



# Image 14

Distribution of referee responses:

- Normal: 98.6%
- Abnormal: 1.4%

Normal with more than 50% functional cotyledon tissue, free of necrosis or decay, remaining attached.

- ❖ One cotyledon is broken, the damaged edge is bruised from mechanical damage.



# Image 15

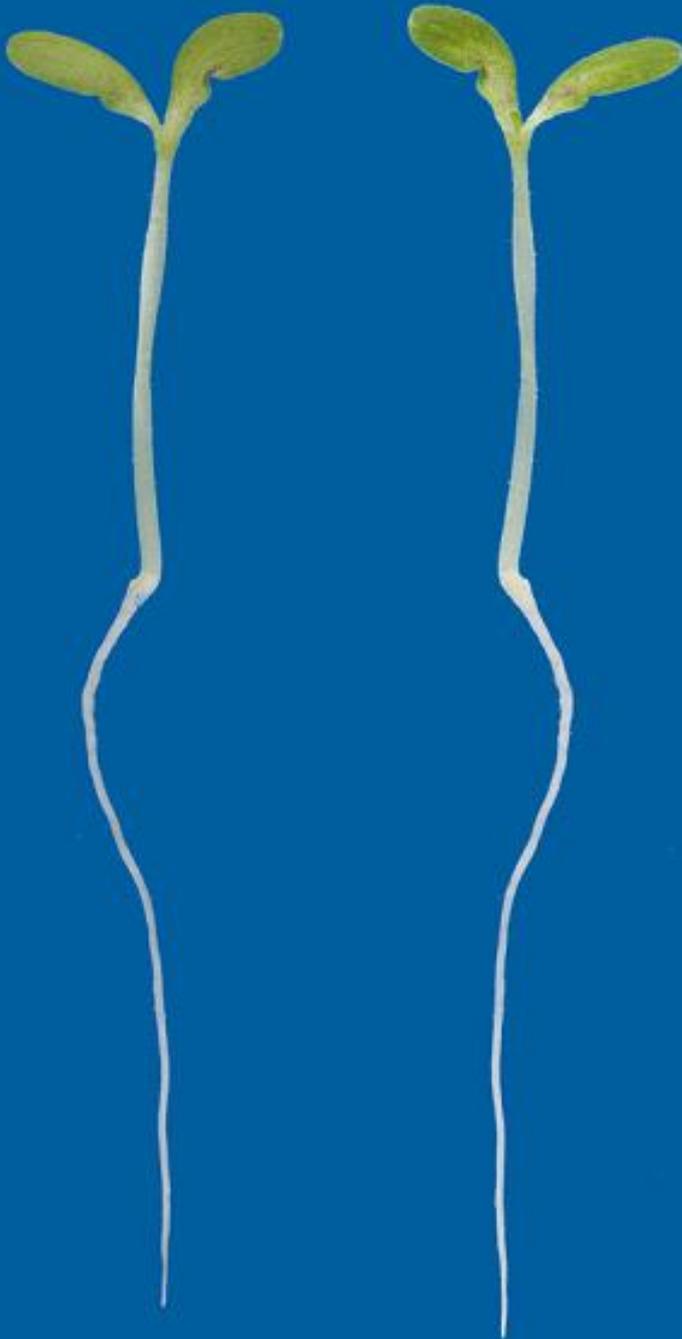
Distribution of referee responses:

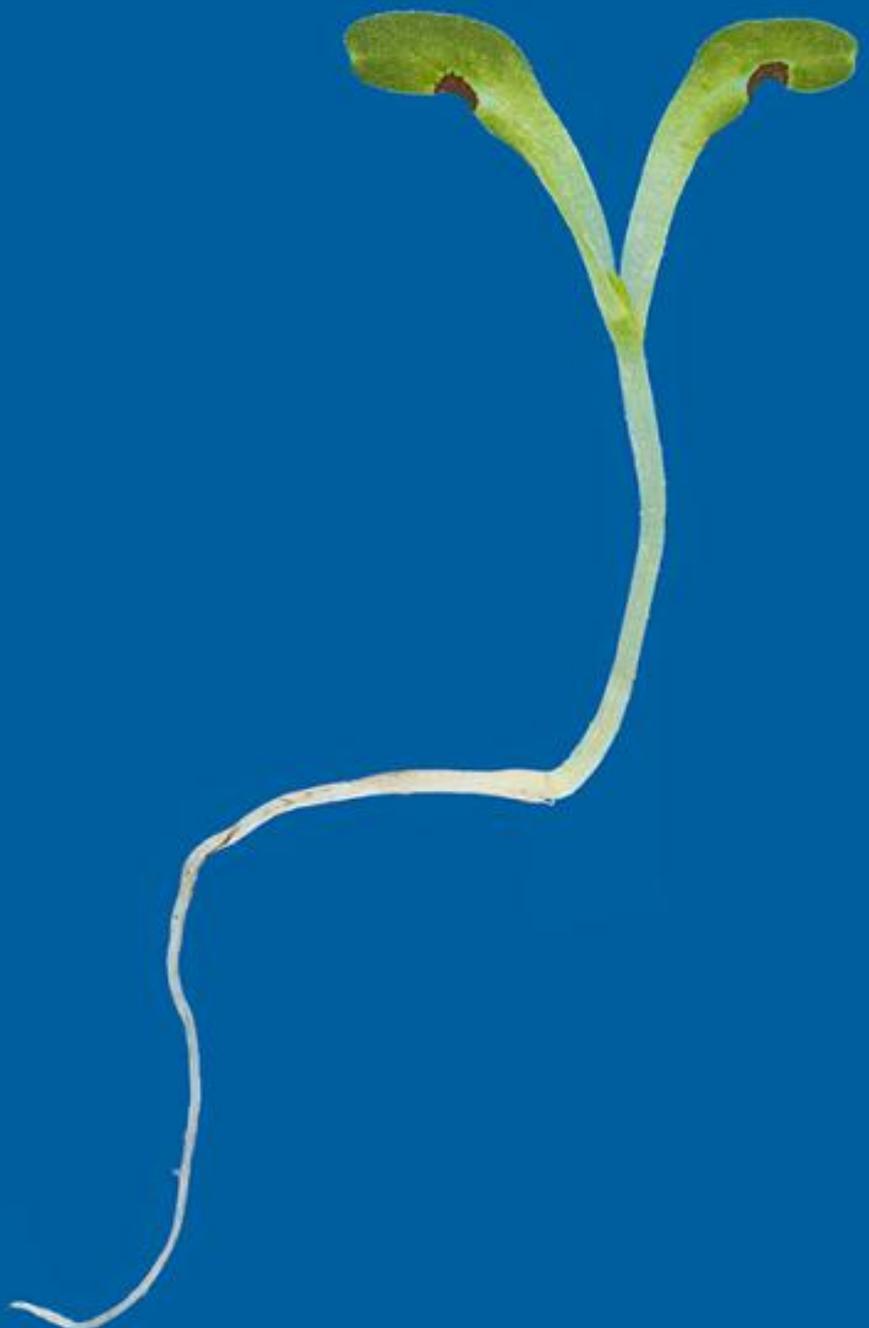
- Normal: 78.9%
- Abnormal: 21.1%

Normal seedling with more than 50% functional cotyledon tissue, free of necrosis or decay, remaining attached.

❖ The cotyledons have less than 50% mechanical damage.

❖ This seedling also has an acceptable defect in a slightly twisted hypocotyl.





# Image 17

Distribution of referee responses:

- Normal: 66.2%
- Abnormal: 33.8%

Normal due to more than 50% functional cotyledon tissue, free of necrosis and decay, remaining attached.

❖ Less than 50% mechanical damaged.

# Assessment of seedlings

## Chlorosis;

- ❖ A chlorophyll deficiency causing a lack of pigmentation which inhibits photosynthesis.
- ❖ Chlorotic leaves look pale and greenish-yellow in color.
- ❖ Can be in combination with necrosis.

# Image 9

Distribution of referee responses:

- Normal: 8.3%
- Abnormal: 91.7%

Abnormal with less than 50% functional cotyledon tissue, free of necrosis or decay, remaining attached.

❖ More than 50% chlorosis and necrosis.

❖ The hypocotyl is watery, swollen and damaged.





## Image 3

Distribution of referee responses:

- Normal: 77.5%
- Abnormal: 22.5%

Normal with more than 50% functional cotyledon tissue, free of necrosis or decay, remaining attached.

❖The cotyledons have less than 50% necrosis

# Image 6

Distribution of referee responses:

- Normal: 33.3%
- Abnormal: 66.7%

Abnormal seedling with yellow cotyledons due to chlorosis.

❖ The chlorosis causes a chlorophyll deficiency.



# Image 10

Distribution of referee responses:

- Normal: 59.7%
- Abnormal: 40.3%

Abnormal with less than 50% functional cotyledon tissue, free of necrosis or decay, remaining attached.

❖ More than 50% chlorosis and mechanical damage on the deformed cotyledons.



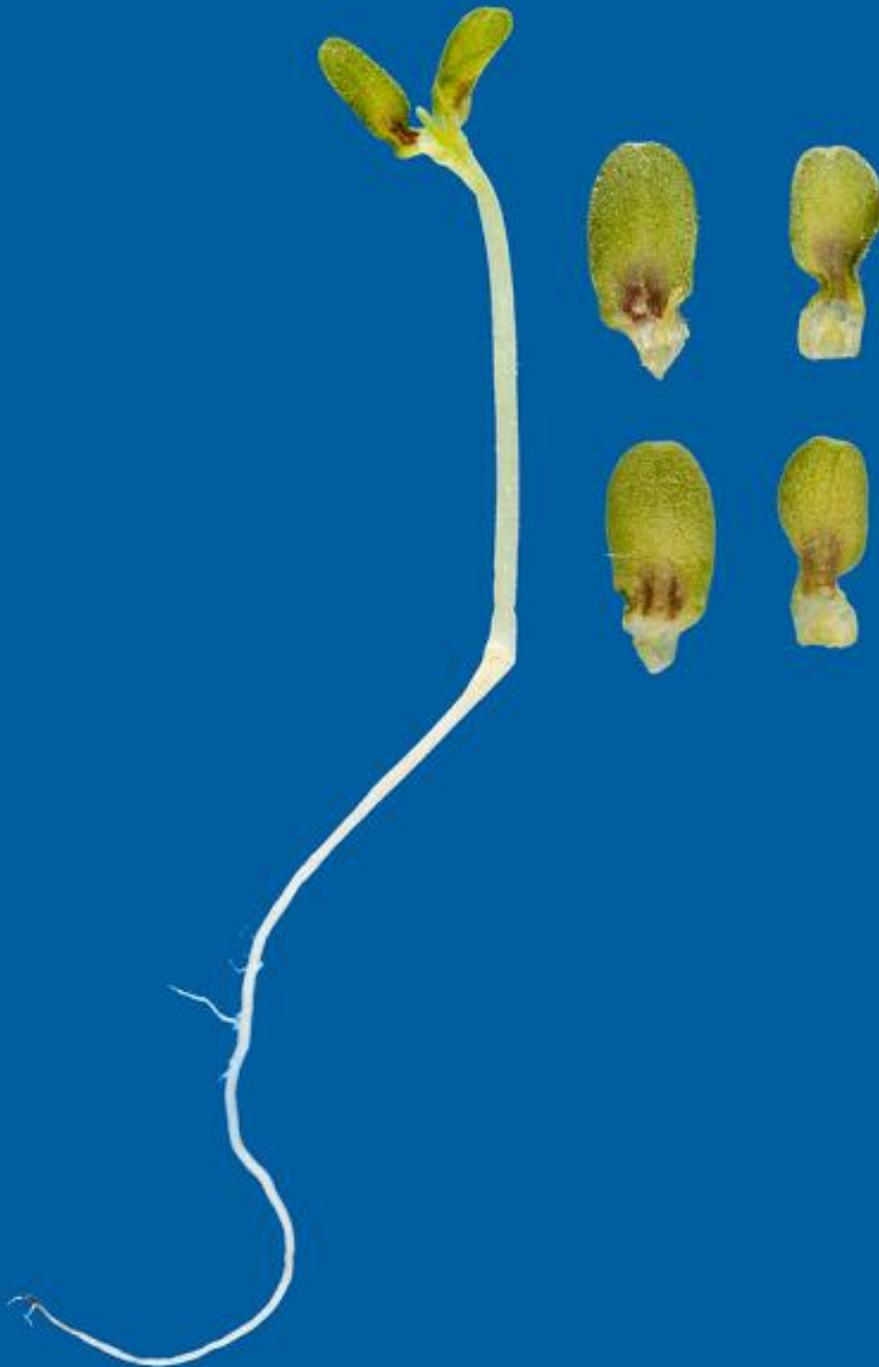
# Image 1

Distribution of referee responses:

- Normal: 54.9%
- Abnormal: 45.1%

Abnormal with less than 50% functional cotyledon tissue, free of necrosis and decay, remaining attached.

❖ Necrosis and watery at the point of attachment.



# Image 30

Distribution of referee responses:

- Normal: 53.7%
- Abnormal: 46.3%

Normal seedling with a natural varietal pigmentation on the cotyledons.

❖Varietal pigmentation can appear as a raised freckling of color, which can be in shades of red or brown. Not to be confused with necrosis.

❖Seedlings from a greenhouse test may display varietal pigmentation, as shown in this photo, more often than a standard germination test due to the increased level of light exposure.



# Conclusions

- ❖ The referee results showed that on those seedlings with borderline plus or minus 50% necrosis the classifications being made are not uniform.
- ❖ Once a seed lot has physiological necrosis the quality of the crop will be questionable only to decline further from there.

# Conclusions

- ❖ The referee results also showed that some analysts are classifying most of the dark areas on the lettuce cotyledons as necrosis and are not classifying the areas which have decay or mechanical damage as such. The decay or mechanical damage effects the individual seed or seedling whereas the physiological necrosis effects the entire seed lot.
- ❖ In the new Seedling Evaluation Handbook the digital photos will offer clarification of the differences between decay, mechanical damage and physiological necrosis.
- ❖ The new Seedling Evaluation Handbook will have adequate pictures with well defined parameters that will be easy for the analysts to correlate to their real life seedlings.

# **AOSA/SCST**

**Asteraceae, Sunflower family I, lettuce**

**Seedling Evaluation Handbook Committee**

**Members:**

**Elizabeth Bada**

**Jane Kohn**

**Ha Ung**

**David Johnston**

**Connie O'Brien**

**Riad Baalbaki**