**Rule Proposal #16**

**1. PURPOSE OF PROPOSAL:**

Improve uniformity of evaluating cotyledons of lettuce seedlings with various degrees and patterns of necrotic tissue damage. Color pictorial examples, with a range of symptoms, are proposed as supplementary material to the ‘ASTERACEAE, SUNFLOWER FAMILY I – Lettuce’ section of Vol. 4 of the AOSA Rules. Black and white seedling drawings depicting necrosis are inadequate due to their lack of coloration and detail. No changes in evaluation rules are proposed.

**2. PRESENT RULE:**

None. Currently there are no photos in AOSA Rules Vol. 4

**3. PROPOSED RULE:**

Proposed supplementary material for addition to the ‘ASTERACEAE, SUNFLOWER FAMILY I – Lettuce’ section

**Supplemental Evaluation Guide**

|  |  |
| --- | --- |
|  | **Figure 1**  **Normal**  **Healthy cotyledons. Lighter coloration is normal for lettuce at point of attachment to hypocotyl. Intact epicotyl.**  **Figure 2** |
| A green cucumber on a white background  Description automatically generated with medium confidence | **Normal**  **Figure 2**  **Lighter areas (sometimes tissue appears transparent) at the point of attachment to hypocotyl and cotyledon tips are normal for lettuce.** |
| A green leaf with a white background  Description automatically generated with low confidence | **Normal**  **Figure 3**  **Minor necrosis on one cotyledon. Apparent blotchiness on surface is outer epidermal cells of cotyledons.**  **Figure 4** |
|  | **Normal**  **Necrosis or decay affects less than 50% of cotyledon tissue. No necrosis at point of attachment to hypocotyl. Healthy epicotyl.** |
|  | **Figure 5**  **Normal**  **Physical necrosis affecting less than 50% of cotyledon tissue; no necrosis at point of attachment to hypocotyl.** |
| A picture containing insect  Description automatically generated | **Figure 6**  **Abnormal**  **Necrosis at the point of cotyledon attachment to the hypocotyl.** |
|  | **Figure 7**  **Abnormal**  **Severe necrosis at the point of cotyledon attachment to the hypocotyl as well as other pre-necrotic (light green or brown) cotyledonary tissue.** |
|  | **Abnormal**  **Figure 8**  **Necrosis or decay at point of attachment, extending into the hypocotyl and the epicotyl.** |
| A picture containing blue, cloth, pool, swimming  Description automatically generated | **Abnormal**  **Figure 9**  **Necrosis at the point of cotyledon attachment to the hypocotyl. Same seedling is depicted from two angles.** |
| A picture containing ocean floor  Description automatically generated | **Figure 10**  **Abnormal**  **Necrosis at the point of cotyledon attachment to the hypocotyl and extending to the rest of the cotyledons.** |
| A picture containing blue, mollusk, ocean floor  Description automatically generated | **Figure 11**  **Abnormal**  **Necrosis affecting more than 50% of cotyledonary tissue. Light-green tissue surrounding dark necrotic area is an example of early stages of necrosis.** |
| A close up of a cucumber  Description automatically generated with low confidence | **Figure 12**  **Abnormal**  **Necrosis and pe-necrosis affecting most cotyledonary tissue. ‘Transparent’ tissue of the cotyledon-hypocotyl juncture is normal for lettuce.** |
| A green leaf on a blue surface  Description automatically generated with medium confidence | **Figure 13**  **Normal**  **Physical necrosis, usually due to insect or mechanical damage. Epicotyl is healthy. ‘Transparent’ tissue at the hypocotyl juncture is normal.** |
| A close up of a cucumber  Description automatically generated with medium confidence | **Figure 14**  **Normal**  **Example of physical rather than physiological necrosis due to insect or mechanical damage, affecting less than 50% of cotyledon tissue.** |
|  | **Figure 15**  **Normal**  **Another example of physical necrosis due to insect or mechanical damage, affecting less than 50% of cotyledon tissue.** |
| A close-up of a leaf  Description automatically generated with medium confidence | **Figure 16**  **Abnormal**  **Decayed epicotyl. Without the aid of magnification, epicotyl deterioration might not be apparent. Healthy cotyledons with normal semi-transparent tissue at the hypocotyl juncture-normal for lettuce.** |

**4. HARMONIZATION AND IMPACT STATEMENT:**

No changes in evaluation rules or criteria are proposed, so this proposal has no impact on harmonization. However, the pictorial illustrations enhance uniformity among AOSA, ISTA, and Canadian M&P by demonstrating similarities in evaluating necrosis at the cotyledon-hypocotyl juncture.

**5. SUPPORTING EVIDENCE:**

Authors reviewed three lettuce referee results which, to our knowledge, were the only lettuce referees in the past 10 years (attached). Review results indicated that necrosis evaluation remains a major area of concern and lack of uniformity. Some referee responses specific to necrosis sometimes diverged by more than 40% among respondents. The reviewed referees were:

* 2013 Virtual Lettuce Referee; Seedling Evaluation Handbook Committee (henceforth referred to as VLR)
* 2016 Southwest Region Referee (henceforth referred to as SWRR)
* 2022 CFIA Referee Study on Lettuce Seedling Evaluation (henceforth referred to as CFIA).

Our review identified four common themes:

1. General agreement when evaluating obvious cases of either intact or severely defective seedlings. Examples include VLR images 2, 4 and 5; and CFIA images 2, 6, 7 and 8.
2. A moderate to high degree on variation among analysts when evaluating necrosis at the point of attachment of cotyledons to the hypocotyl. Examples include VLR images 1 and 25; SWRR images 2, 5 and 9; and CFIA images 9, 12 and 13. In the case of CFIA images 9 and 12, it was apparent that many AOSA analysts do not consider necrosis at point of attachment as an abnormality if less than 50% of the cotyledonary tissue is defective,
3. Difficulty in sometimes distinguishing pre-necrotic tissue, usually apparent around darker necrotic areas, from light (low chlorophyl) coloration of normal tissue, which in the case of lettuce is highly dependent on test conditions. Examples include VLR images 18 and 28; SWRR images 2, 4, 7 and 10; and CFIA images 1, 5 11 and 14.
4. It was not possible to draw any reliable conclusions regarding pigmentation evaluations, but it seems likely that pigmentation differences influenced cotyledon evaluations. In most cases, this tissue appears lighter in color or transparent (lacks pigmentation), which is a normal condition for lettuce and is light- and variety-dependent. Based on some referee results, this lack or reduced pigmentation could have been regarded as a defect. Examples include VLR images 3, 15, 18 and 26.

The proposed supplementary material provides examples and descriptions of cotyledon evaluations to address and clarify the above issues. No modifications to evaluation criteria are proposed.

**NOTE: THIS PROPOSAL IS CONTINGENT ON PROPOSAL #15 PASSING**

**6. SUBMITTED BY:**

Riad Baalbaki, CSA Germination - Dormancy and Germination Subcommittee Co-Chair

Senior Seed Botanist

California Department of Food & Agriculture

Plant Pest Diagnostics Branch

Email: [riad.baalbaki@cdfa.ca.gov](mailto:riad.baalbaki@cdfa.ca.gov)

David M. Johnston, CSA/RST - Dormancy and Germination Subcommittee Co-Chair

Program Coordinator Seed Programs

Louisiana Dept. of Agriculture and Forestry

Email: [djohnston@ldaf.state.la.us](mailto:djohnston@ldaf.state.la.us)

**7. DATE SUBMITTED:** October 14, 2022