1. The purpose of this document is to present a proposal for a partial revision of the Test Guidelines for Lettuce (document TG/13/11 Rev. 2).

2. The Technical Working Party for Vegetables (TWV), at its fifty-seventh session, considered a proposal for a partial revision of the Test Guidelines for Lettuce (Lactuca sativa L.) on the basis of documents TG/13/11 Rev. 2 and TWV/57/12 “Partial revision of the Test Guidelines for Lettuce” and proposed the following changes (see document TWV/57/26 “Report”, paragraph 68):
   
   (a) Addition of “Resistance to Bremia lactucae (Bl) Isolate Bl: 36EU”, including example varieties;
   
   (b) Revision of explanation Ad. 38 to 52: Resistance to Bremia lactucae (Bl).

   (c) Inclusion of characteristics from the Table of Characteristics in the Technical Questionnaire

3. The proposed changes are presented below in highlight and underline (insertion) and strikethrough (deletion).

Proposed addition of “Resistance to Bremia lactucae (Bl) Isolate Bl: 36EU”

Proposed addition

<table>
<thead>
<tr>
<th></th>
<th>English</th>
<th>français</th>
<th>deutsch</th>
<th>español</th>
<th>Example Varieties Exemples Beispielssorten Variedades ejemplo</th>
<th>Note/ Nota</th>
</tr>
</thead>
<tbody>
<tr>
<td>53</td>
<td>QL</td>
<td>VG</td>
<td>(+)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>resistance to Bremia lactucae (Bl) Isolate Bl: 36EU</th>
<th>Résistance à Bremia lactucae (Bl) Isolat Bl: 36EU</th>
<th>Resistenz gegen Bremia lactucae (Bl) Isolat Bl: 36EU</th>
<th>Resistencia a Bremia lactucae (Bl) Aislado Bl: 36EU</th>
<th>Bartoli, RYZ2164</th>
<th>Design, Kibrille</th>
</tr>
</thead>
<tbody>
<tr>
<td>absent</td>
<td>absente</td>
<td>fehlend</td>
<td>ausente</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>present</td>
<td>présente</td>
<td>vorhanden</td>
<td>presente</td>
<td></td>
<td>Bartoli, RYZ2164</td>
<td>Design, Kibrille</td>
</tr>
</tbody>
</table>

4. It is proposed to include the new characteristic 53 in Chapter 10 “Technical questionnaire”, Section 5 “Characteristics of the variety to be indicated”.

1 held in Antalya, Türkiye, from May 1 to 5, 2023
**Proposed revision of explanation Ad. 38 to 52 "Resistance to Bremia lactucae (Bl)"

Ad. 38 to 52-53: Resistance to Bremia lactucae (Bl), several isolates

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pathogen</td>
<td><em>Bremia lactucae</em></td>
</tr>
<tr>
<td>2.</td>
<td>Quarantine status</td>
<td>no</td>
</tr>
<tr>
<td>3.</td>
<td>Host species</td>
<td>lettuce - <em>Lactuca sativa</em> L.</td>
</tr>
<tr>
<td>4.</td>
<td>Source of inoculum</td>
<td>GEVES(^2) (FR) or Naktuinbouw(^3) (NL)</td>
</tr>
<tr>
<td>5.</td>
<td>Isolate</td>
<td>Bl: 16EU, 17EU, 20-27EU, 29-31EU, 33EU, 35EU, 36EU</td>
</tr>
<tr>
<td>6.</td>
<td>Establishment isolate identity</td>
<td>test on differentials (see table below)</td>
</tr>
<tr>
<td>7.</td>
<td>Establishment pathogenicity</td>
<td>test on susceptible varieties</td>
</tr>
<tr>
<td>8.</td>
<td>Multiplication inoculum</td>
<td>susceptible variety, for example Green Towers. For isolates with a higher number than Bl: 16EU, a variety with defeated resistance may be preferable to keep the isolate fit.</td>
</tr>
</tbody>
</table>

### 8.2 Multiplication variety

2 hours at room temperature; 2 days in fridge |

### 9. Format of the test

#### 9.1 Number of plants per genotype

at least 20 |

#### 9.2 Number of replicates

- |

#### 9.3 Control varieties

(informative) differentials (see table below) |

#### 9.4 Test design

- |

#### 9.5 Test facility

climate room |

#### 9.6 Temperature

15°C-18°C |

#### 9.7 Light

adequate for good plant growth; seedlings should not etiolate. option: reduced light 24 hours after inoculation |

### 9.8 Season

- |

#### 9.9 Special measures

plants may grow on wet blotting paper with or without a nutrient solution, on sand or on potting soil (see point 13). high humidity (>90%) is essential for infection and sporulation. |

### 10. Inoculation

#### 10.1 Preparation inoculum

washing off from leaves by vigorous shaking in a closed container |

#### 10.2 Quantification inoculum

counting spores; spore density should be 3 x 10\(^4\) - 1 x 10\(^5\) |

#### 10.3 Plant stage at inoculation

cotyledon stage |

#### 10.4 Inoculation method

spraying till run-off. option: reduced light 24 hours after inoculation |

#### 10.5 First observation

beginning of sporulation on susceptible varieties (around 7 days after inoculation) |

#### 10.6 Second observation

3-4 days after first observation (around 10 days after inoculation) |

#### 10.7 Final observations

14 days after inoculation two of these three observations may be sufficient, the third notation is optional for observation of evolution of symptoms in case of doubt. the day of maximum sporulation should occur in this period.

---

\(^2\) matref@geves.fr  
\(^3\) resistentie@naktuinbouw.nl
### 11. Observations

#### 11.1 Method

Visual observation of sporulation and necrotic reaction to infection.

#### 11.2 Observation scale

<table>
<thead>
<tr>
<th>Resistant (no sporulation, no necrosis)</th>
<th>Susceptible (reduced sporulation, no necrosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 0: no sporulation, no necrosis</td>
<td>Class 5: reduced sporulation (compared to susceptible control) without necrosis</td>
</tr>
<tr>
<td>Class 1: no sporulation, necrosis present</td>
<td>Class 6: normal sporulation without necrosis</td>
</tr>
<tr>
<td>Class 2: weak sporulation (much less than susceptible control) with necrosis</td>
<td></td>
</tr>
<tr>
<td>Class 3: weak sporulation (less than susceptible control and not evolving between second and third observation) with necrosis</td>
<td></td>
</tr>
<tr>
<td>Class 4: very sparse sporulation (not evolving between second and third observation) without necrosis</td>
<td></td>
</tr>
</tbody>
</table>

#### 11.3 Validation of test on standards

In case of normal sporulation (same level as susceptible control) with necrosis, another test on bigger plants or other substrate must be undertaken.

### 12. Interpretation of data in terms of UPOV characteristic states

Class 0, 1, 2, 3 and 4: resistant  
Class 5 and 6: susceptible

### 13. Critical control points

Reaction of standards (the infection pressure may vary between experiments, leading to slight differences in sporulation intensity); when the reactions are not clear, the experiment should be repeated.

The sowing on soil can be used to see necrosis, but weak sporulation (much less than susceptible control) can appear; when testing on sand, spores can be confused with grains of sand.

In case of use of nutritive solution on blotting paper, a fungicide can be added to avoid contamination by saprophytes.
For reference: The International Bremia Evaluation Board (IBEB) produces regular updates of the host differential reaction table. The most recent table is available through ISF at http://www.worldseed.org/our-work/plant-health/other-initiatives/ibeb/. Pictures for the observation scale are also provided.

Resistance is indicated with – or (-), susceptibility is indicated with + or (+). The brackets indicate a lower and sometimes variable level of expression of the phenotype.

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<thead>
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<tbody>
<tr>
<td>D. bracteata</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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</tr>
</tbody>
</table>

Resistance is indicated with – or (-), susceptibility is indicated with + or (+). The brackets indicate a lower and sometimes variable level of expression of the phenotype.
Proposed inclusion of characteristics from the Table of Characteristics in the Technical Questionnaire

5. The TWV is invited to consider the inclusion of the following characteristics in the TQ (characteristics for inclusion indicated in highlight and underline):

<table>
<thead>
<tr>
<th>Char. No.</th>
<th>(*) Characteristic Number</th>
<th>Characteristic Name</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(*)</td>
<td>Seed: color</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>(*)</td>
<td>Leaf: number of divisions</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>(*)</td>
<td>Leaf: anthocyanin coloration</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>(*)</td>
<td>Leaf: hue of anthocyanin coloration</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>(*)</td>
<td>Leaf: area covered by anthocyanin coloration</td>
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</tr>
<tr>
<td>14</td>
<td>(*)</td>
<td>Leaf: color</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>(*)</td>
<td>Leaf: intensity of green color</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td></td>
<td>Only varieties with Plant: degree of overlapping of upper part of leaves: medium or strong: Time of harvest maturity</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td></td>
<td>Time of beginning of bolting</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td></td>
<td>Resistance to <em>Bremia lactucae</em> (Bl) Isolate Bl: 16EU</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td></td>
<td>Resistance to <em>Bremia lactucae</em> (Bl) Isolate Bl: 17EU</td>
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<td>Resistance to <em>Bremia lactucae</em> (Bl) Isolate Bl: 20EU</td>
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<tr>
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<td>Resistance to <em>Bremia lactucae</em> (Bl) Isolate Bl: 21EU</td>
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<td>Resistance to <em>Bremia lactucae</em> (Bl) Isolate Bl: 22EU</td>
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<td>Resistance to <em>Bremia lactucae</em> (Bl) Isolate Bl: 23EU</td>
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<td>44</td>
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<td>Resistance to <em>Bremia lactucae</em> (Bl) Isolate Bl: 24EU</td>
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<td>Resistance to <em>Bremia lactucae</em> (Bl) Isolate Bl: 25EU</td>
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<td>Resistance to <em>Bremia lactucae</em> (Bl) Isolate Bl: 26EU</td>
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<td>Resistance to <em>Bremia lactucae</em> (Bl) Isolate Bl: 27EU</td>
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<td>Resistance to <em>Bremia lactucae</em> (Bl) Isolate Bl: 29EU</td>
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<td>Resistance to <em>Bremia lactucae</em> (Bl) Isolate Bl: 30EU</td>
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<tr>
<td>50</td>
<td></td>
<td>Resistance to <em>Bremia lactucae</em> (Bl) Isolate Bl: 31EU</td>
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<tr>
<td>51</td>
<td></td>
<td>Resistance to <em>Bremia lactucae</em> (Bl) Isolate Bl: 33EU</td>
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<tr>
<td>52</td>
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<td>Resistance to <em>Bremia lactucae</em> (Bl) Isolate Bl: 35EU</td>
<td></td>
</tr>
<tr>
<td>53</td>
<td></td>
<td>Resistance to <em>Bremia lactucae</em> (Bl) Isolate Bl: 36EU</td>
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</tr>
<tr>
<td>54</td>
<td></td>
<td>Resistance to Lettuce mosaic virus (LMV) Pathotype II</td>
<td></td>
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<tr>
<td>55</td>
<td></td>
<td>Resistance to <em>Nasonovia ribisnigri</em> (Nr) Biotype Nr: 0</td>
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<tr>
<td>56</td>
<td></td>
<td>Resistance to Fusarium oxysporum f. sp. lactucae (Fol) Race 1</td>
<td></td>
</tr>
</tbody>
</table>
5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the note which best corresponds).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Example Varieties</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5.1 Seed: color</strong></td>
<td></td>
<td></td>
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<tr>
<td></td>
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</tr>
<tr>
<td>white</td>
<td>Verpia</td>
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</tr>
<tr>
<td>yellow</td>
<td>Durango</td>
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<tr>
<td>brown</td>
<td>Oaklin</td>
<td>3 [ ]</td>
</tr>
<tr>
<td>black</td>
<td>Kograner Sommer 2</td>
<td>4 [ ]</td>
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<tr>
<td><strong>5.2 Leaf: number of divisions</strong></td>
<td></td>
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<td></td>
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<tr>
<td>absent or very few</td>
<td>Fiorella, Lollo rossa</td>
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</tr>
<tr>
<td>very few to few</td>
<td></td>
<td>2 [ ]</td>
</tr>
<tr>
<td>few</td>
<td>Curletta, Rodagio</td>
<td>3 [ ]</td>
</tr>
<tr>
<td>few to medium</td>
<td>Ezabel, Jadigon</td>
<td>4 [ ]</td>
</tr>
<tr>
<td>medium</td>
<td></td>
<td>5 [ ]</td>
</tr>
<tr>
<td>medium to many</td>
<td>Expedition, Multired 54</td>
<td>6 [ ]</td>
</tr>
<tr>
<td>many</td>
<td></td>
<td>7 [ ]</td>
</tr>
<tr>
<td>many to very many</td>
<td>Lollo rossa, Luana</td>
<td>8 [ ]</td>
</tr>
<tr>
<td>very many</td>
<td>Excite, Ezfrill, Telex</td>
<td>9 [ ]</td>
</tr>
<tr>
<td><strong>5.3 Leaf: anthocyanin coloration</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>absent or very weak</td>
<td>Clarion</td>
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<td>very weak to weak</td>
<td>Du bon jardinier</td>
<td>2 [ ]</td>
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<tr>
<td>weak</td>
<td></td>
<td>3 [ ]</td>
</tr>
<tr>
<td>weak to medium</td>
<td>Lollo rossa, Luana</td>
<td>4 [ ]</td>
</tr>
<tr>
<td>medium</td>
<td></td>
<td>5 [ ]</td>
</tr>
<tr>
<td>medium to strong</td>
<td>Merveille des quatre saisons</td>
<td>6 [ ]</td>
</tr>
<tr>
<td>strong</td>
<td>Iride</td>
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<td>8 [ ]</td>
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<tr>
<td>very strong</td>
<td>Iride</td>
<td>9 [ ]</td>
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<tr>
<td><strong>5.4 Leaf: hue of anthocyanin coloration</strong></td>
<td></td>
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<tr>
<td>reddish</td>
<td>Lollo rossa</td>
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<tr>
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<td>Iride</td>
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</tr>
<tr>
<td>brownish</td>
<td>Luana, Maravilla de Verano</td>
<td>3 [ ]</td>
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<tr>
<td>Characteristics</td>
<td>Example Varieties</td>
<td>Note</td>
</tr>
<tr>
<td>-----------------------------------------</td>
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</tr>
<tr>
<td><strong>5.5 Leaf: area covered by anthocyanin coloration</strong></td>
<td></td>
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</tr>
<tr>
<td>very small</td>
<td>Steirer Krauthauptel</td>
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<td>very small to small</td>
<td>Diablo</td>
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<td>3</td>
</tr>
<tr>
<td>small to medium</td>
<td>Luana</td>
<td>4</td>
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<tr>
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<td>5</td>
</tr>
<tr>
<td>medium to large</td>
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<td>6</td>
</tr>
<tr>
<td>large</td>
<td>Merveille des quatre saisons</td>
<td>7</td>
</tr>
<tr>
<td>large to very large</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>very large</td>
<td>Bijou, Revolution</td>
<td>9</td>
</tr>
<tr>
<td><strong>5.6 Leaf: color</strong></td>
<td></td>
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<td><strong>5.7 Leaf: intensity of green color</strong></td>
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<td>light to medium</td>
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<td>medium to dark</td>
<td>Expedition, Verpia</td>
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<td>dark</td>
<td>Clarke, Calmar</td>
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<td>dark to very dark</td>
<td>Expedition, Verpia</td>
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<td>Pascal, Verdetrix</td>
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**5.8 Only varieties with Plant: degree of overlapping of upper part of leaves: medium or strong: Time of harvest maturity**

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<td><strong>5.9</strong> Time of beginning of bolting</td>
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| 5.10 Resistance to *Bremia lactucae* (Bl) Isolate Bl: 16EU | | |
|----------------------------------------------------------|-------------------------------|
| absent | Green Towers | 1 | |
| present | Argelès | 9 | |
| not tested | | | |

| 5.11 Resistance to *Bremia lactucae* (Bl) Isolate Bl: 17EU | | |
|----------------------------------------------------------|-------------------------------|
| absent | Green Towers | 1 | |
| present | Argelès | 9 | |
| not tested | | | |

| 5.12 Resistance to *Bremia lactucae* (Bl) Isolate Bl: 20EU | | |
|----------------------------------------------------------|-------------------------------|
| absent | Green Towers | 1 | |
| present | FrRsal-1 | 9 | |
| not tested | | | |

| 5.13 Resistance to *Bremia lactucae* (Bl) Isolate Bl: 21EU | | |
|----------------------------------------------------------|-------------------------------|
| absent | Green Towers | 1 | |
| present | Argelès, Colorado | 9 | |
| not tested | | | |

| 5.14 Resistance to *Bremia lactucae* (Bl) Isolate Bl: 22EU | | |
|----------------------------------------------------------|-------------------------------|
| absent | Green Towers | 1 | |
| present | FrRsal-1 | 9 | |
| not tested | | | |

<p>| 5.15 Resistance to <em>Bremia lactucae</em> (Bl) Isolate Bl: 23EU | | |
|----------------------------------------------------------|-------------------------------|
| absent | Green Towers | 1 | |
| present | Colorado | 9 | |
| not tested | | | |</p>
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<th>Characteristics</th>
<th>Example Varieties</th>
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<td>5.26 Resistance to <em>Lettuce mosaic virus</em> (LMV) Pathotype II</td>
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<td>5.28 Resistance to <em>Fusarium oxysporum f.sp. lactucae</em> (Fol) Race 1</td>
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