Herbicide Tolerance Testing of Canola and Alfalfa

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Herbicide Bioassay

- Exposing seeds or seedlings to a diluted solution of herbicide to assess the level of tolerance.
- Glufosinate (Liberty), Glyphosate (RR or GT), Imazethapyr (IMI or Clearfield), STS (Chlorsulfuron)
- Typical methods:
  - spray
  - seed soak
  - substrate imbibition
  - combination of methods

Herbicide Bioassay

- Desirable to have check seeds with and without traits.
- Check seeds indicate whether concentration is correct and serve as comparison for classifying seedlings.
- It is common to color code equipment as to herbicide.
- Seedling Evaluation (Volume 4 of AOSA Rules) is typically the basis for classifying seedlings as normal or abnormal.
- Categories:
  - Normal Tolerant Seedling
  - Non-Tolerant (Susceptible) Seedling
  - Abnormal Seedling (Tolerant, but not counted)
  - Dead

Canola

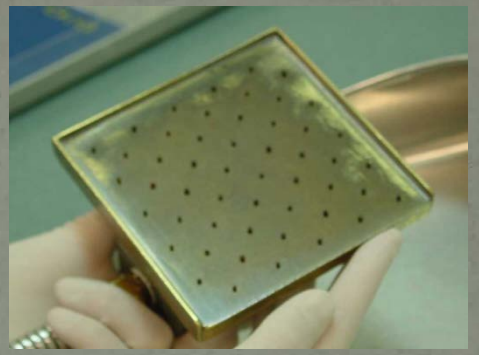
Brassica napus var. napus annual rape, winter rape

Options from Volume 1 of AOSA Rules

Substrates:	Between Blotters (B), Towels (T)
Temperature:	20-30°C; 15-25°C
First Count:	3 days
Final Count:	7 days

No specific requirements or Fresh & dormant seed notes

Herbicide bioassay - canola



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AOSA Rules Volume 4: Seedling Evaluation

BRASSICACEAE, MUSTARD FAMILY

Barbarea verna, upland cress
Brassica spp., mustards etc.
Crambe abyssinica, crambe
Eruca sativa, roquette
Lepidium sativum, garden cress
Nasturtium officinale, watercress
Raphanus sativus, radish
Sinapis alba, white mustard

GENERAL DESCRIPTION

Seedling type: Epigeal dicot.

Food reserves: Cotyledons that expand and become thin, leaf-like and photosynthetic. In *Brassica*, *Sinapis* and *Raphanus*, the cotyledons are bi-lobed and folded, with the outer cotyledon being larger than the inner.

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.

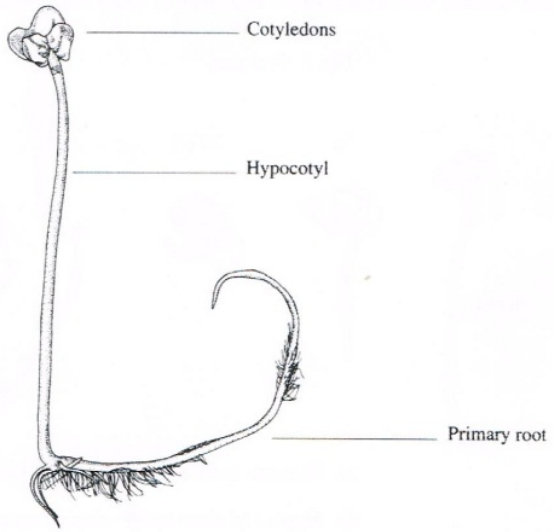


Fig. 1 ***Brassica.***

Canola from AOSA Rules Volume 4: Seedling Evaluation

ABNORMAL SEEDLING DESCRIPTION

Cotyledons

- decayed at point of attachment.
- less than half of the original cotyledon tissue remaining attached.
- less than half of the original cotyledon tissue free of necrosis or decay.

Epicotyl

- missing (may be assumed to be present if the cotyledons are intact).

Hypocotyl

- deep open cracks extending into the conducting tissue.
- malformed, such as markedly shortened, curled or thickened.
- watery.

Root

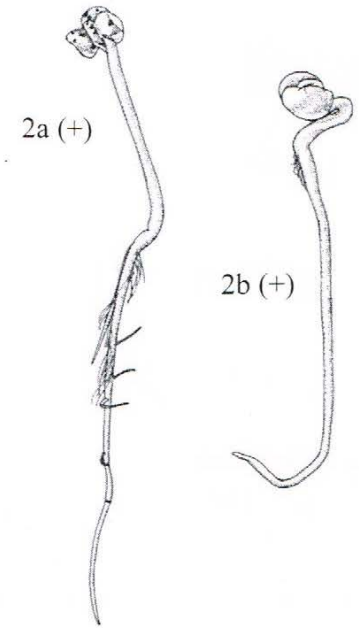
- weak, stubby or missing primary root (secondary roots will not compensate for a defective primary root).

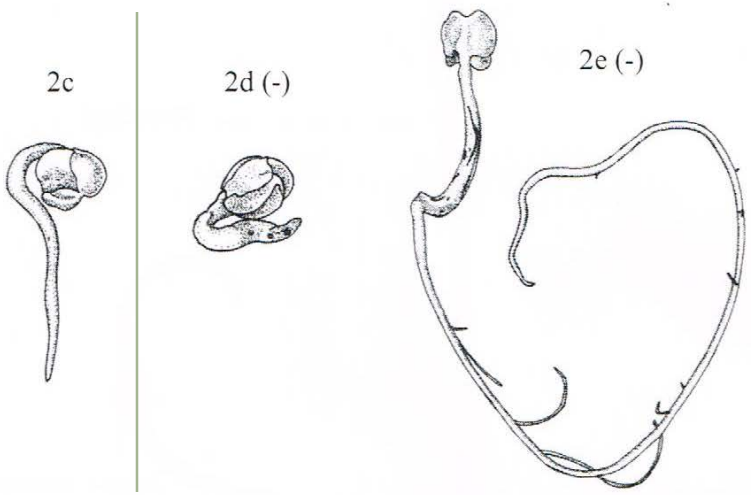
Seedling

- one or more essential structures impaired as a result of decay from primary infection
- albino

Canola AOSA Rules Volume 4: Seedling Evaluation

Fig. 2 ***Brassica.***





2a. Necrotic spots covering less than 50% of cotyledon area.

2b. Hypocotyl short but developing normally.

2c. Seedling too small to evaluate; extend test (see sec 3.5.1.b).

2d. Stubby primary root, poor hypocotyl development.

2e. Hypocotyl lesions.

MUSTARDS, etc. 34 - 2011

Non-Trait Characteristics - Dicots

- Roundup®: 1° root short, thickened and off-color. 2° roots very stubby (if present).
- STS®: Unifoliate leaves crossed.
- Liberty®: Reduced root development.

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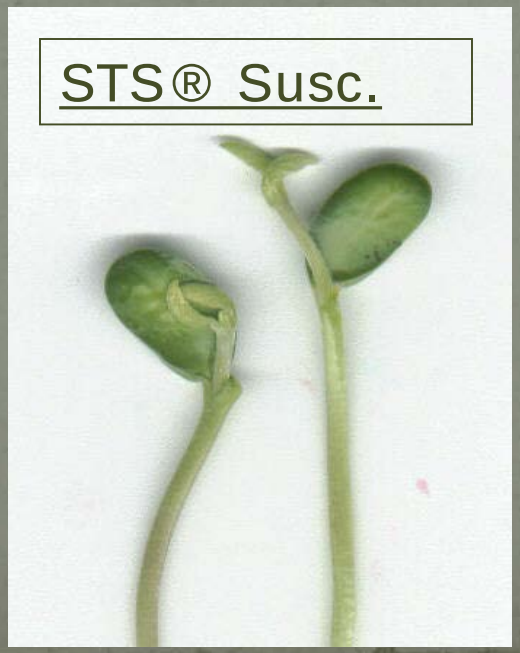

Round up® Susc. - spray

Herbicide bioassay - canola

T nT T

Glufosinate ammonium (Liberty) bioassay

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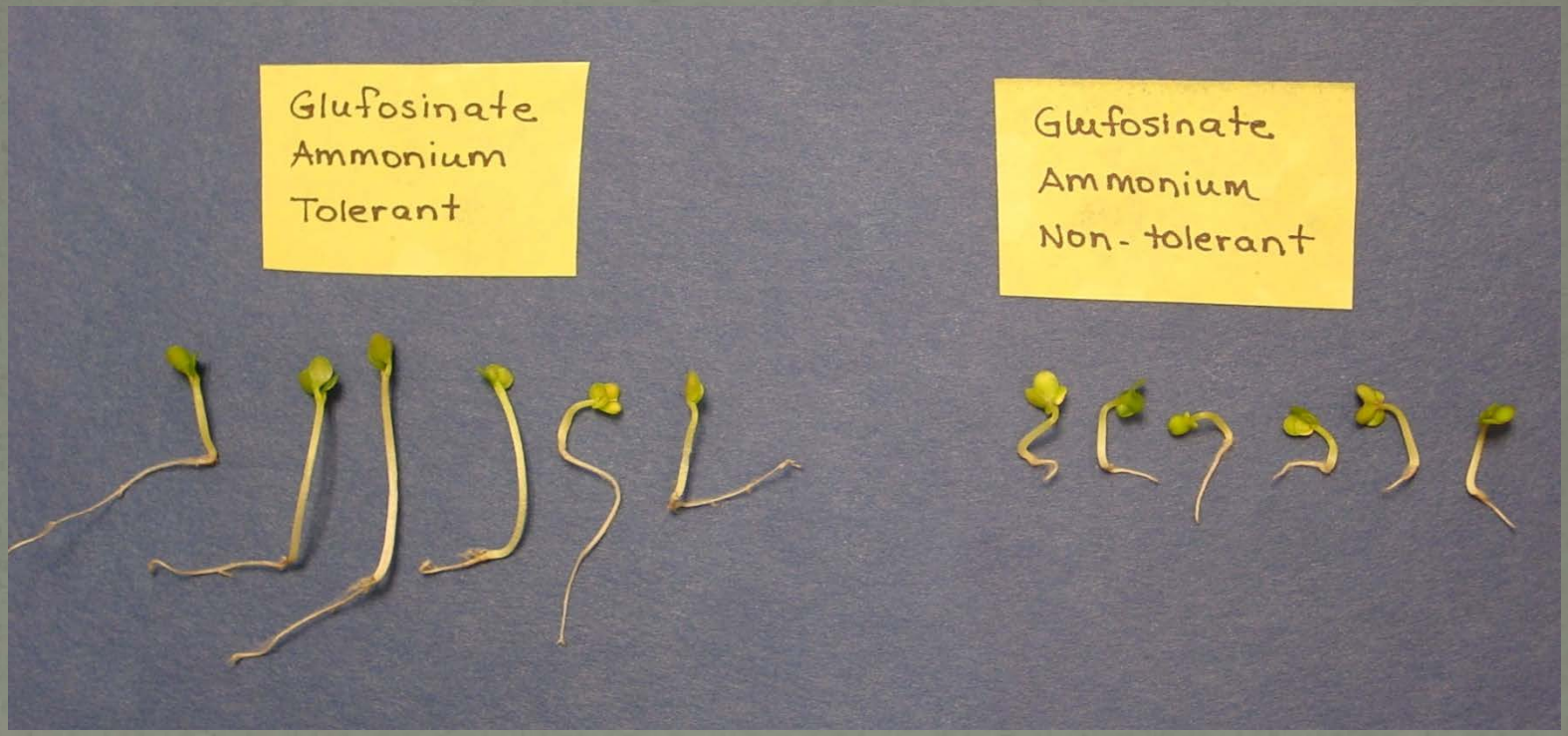
Canola seedlings
Glufosinate ammonium bioassay



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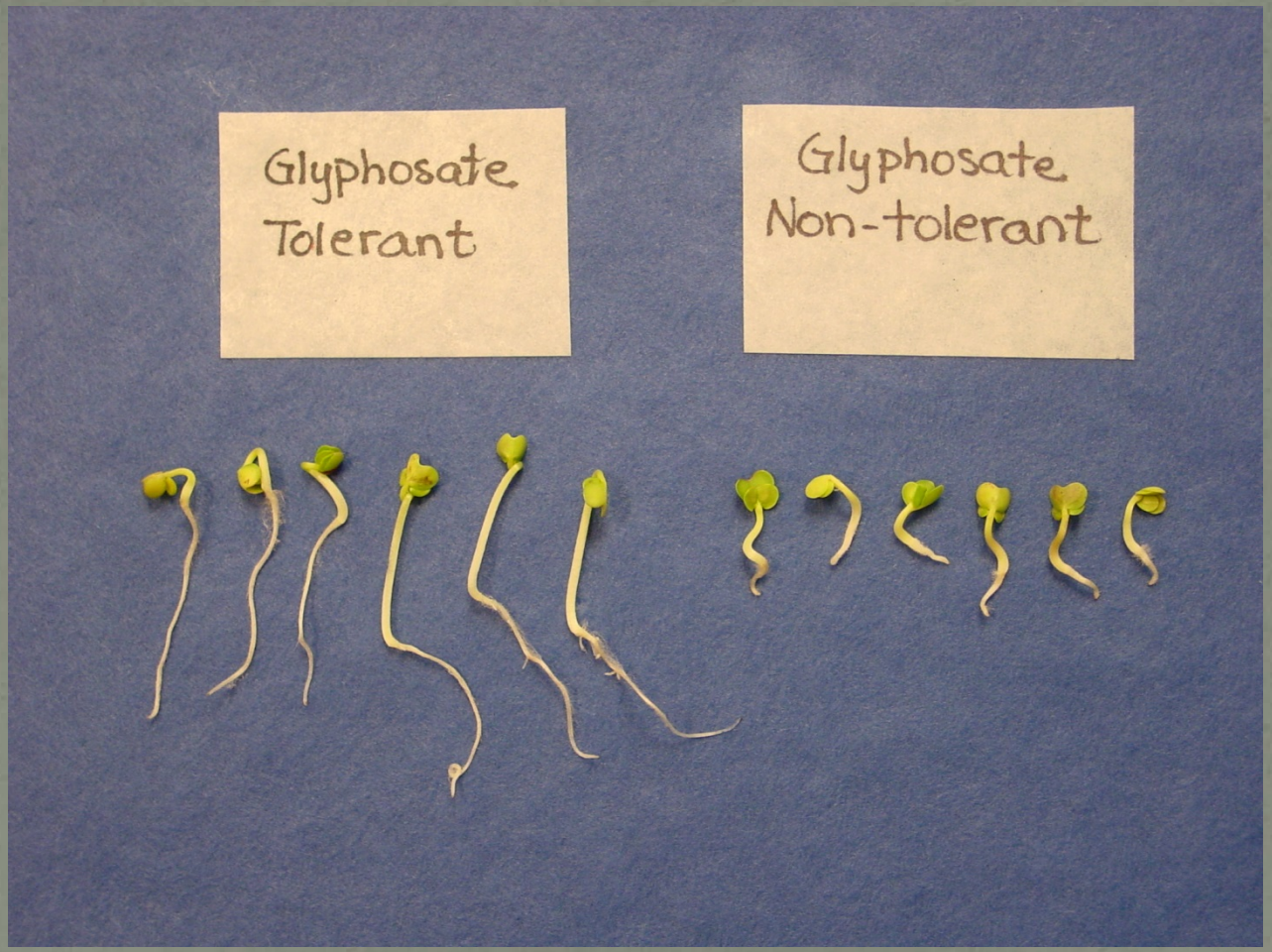
Canola Non-Trait Seedlings

- Round up®: 1° root short, thickened and off-color (brown root tip). 2° roots very stubby (if present).
- Liberty®: Reduced root development, shortened hypocotyl

Images: Midwest Seeds Inc. (SGS)

Canola seedlings
Glyphosate bioassay



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Alfalfa

Medicago sativa alfalfa

Options from Volume 1 of AOSA Rules

Substrates:	Between Blotters (B), Towels (T), Sand (S)
Temperature:	20°C
First Count:	4 days
Final Count:	7 days

Specific requirements : See sec. 6.9c.

Hard seeds: see sec. 6.2d and 6.9m(6)

Alfalfa Herbicide Bioassay for RR

Two methods:

1) Greenhouse Spray
2) Substrate Imbibition (towels, blotters)

Potential Problems

Hard seed:

- May germinate late or not at all.
- Tolerance of hard seed in proportion to rest of seedlings?
- Scarify?

Separating Normal from Susceptible Seedlings

- Root development; Where does hypocotyl end?

Getting Seed for Controls

Greenhouse Spray Method

pre-2008:

- Greenhouse seedling grow-out approved by Executive Committee of the North American Alfalfa Improvement Conference.

- Tolerant check seed may only be used in the NAAIC Standard Test.

Greenhouse/Growth Chamber Method

- Seed germination flats with drain holes
- Peat-based, potting soil mix, etc.
- 20-24°C; >16 hour day length
- 200 seedlings per entry per rep, min.
- Four reps, minimum.
- Duration: 4 to 6 weeks from planting.
- Apply 2X field label rate. Use only Monsanto Roundup WeatherMAX® at 2nd trifoliate and then 7 to 10 days after first spraying.

From handout by Fitzpatrick, Reisen, and Teuber

Greenhouse/Growth Chamber

Scoring:

- Evaluate when > or =99% non-tolerant check plants have symptoms.
- Tolerant: Plants have normal vigor & growth. May have temporary chlorosis, … .
- Non-Tolerant: Plants initially exhibit stem tip wilt and systemic chlorosis, followed by total collapse, necrosis, and death.
- Killed seedlings rapidly decompose amd may become very difficult to see within a few days.

Possible Substrate Imbibiton Method

- May use blue blotters or rolled towels. Towels would be preferred.
- Four reps of 100 seed.
- Moisten media with WeatherMAX®.

  400 PPM = 1.64 mL glyphosate/2 L water

  600 PPM = 2.46 mL glyphosate/2 L water
- Plant and place in 20°C for seven days with light.
- Susceptible seedlings will have reduced root development. See AOSA Rules Volume 4: Seedling Evaluation for normal alfalfa seedling growth.

Alfalfa

FABACEAE, LEGUME FAMILY V - Small-seeded

Alysicarpus vaginalis, alyceclover
Astragalus cicer, cicer milkvetch
Crotalaria spp., crotalaria
Cyamopsis tetragonoloba, guar
Desmodium tortuosum, beggarweed
Hedysarum boreale, northern sweetvetch
Indigofera hirsuta, hairy indigo
Kummerowia spp., lespedeza
Lespedeza spp., lespedeza
Lotus spp., trefoil
Medicago arabica, spotted burclover
Medicago lupulina, black medic
Medicago orbicularis, button clover
Medicago polymorpha, California bur-clover
Medicago sativa, alfalfa
Melilotus indicus, sourclover
Melilotus spp., sweetclover
Onobrychis viciifolia, sainfoin
Pueraria montana var. *lobata*, kudzu
Securigera varia, crownvetch
Sesbania exaltata, sesbania
Trifolium spp., clover

GENERAL DESCRIPTION

Seedling type: Epigeal dicot.

Food reserves: Cotyledons that are small and fleshy; they expand, and become photosynthetic.

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 tapering primary root, usually with roots hairs. Most of the included species do not normally develop secondary roots within the test period.

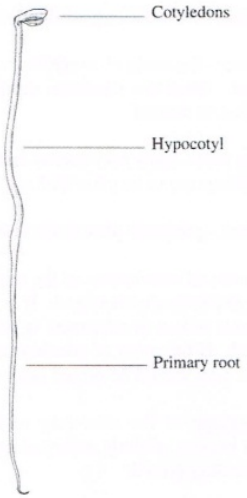


Fig. 1 Alfalfa.

Alfalfa

ABNORMAL SEEDLING DESCRIPTION

Cotyledons:

- less than half of the original cotyledon tissue remaining attached (see note 2).
- less than half of the original cotyledon tissue free of necrosis or decay.

Epicotyl:

- missing (may be assumed to be present if cotyledons are intact).

Hypocotyl:

- deep open cracks extending into the conducting tissue.
- malformed, such as markedly shortened, curled or thickened.
- watery.

Root:

- none.
- primary root stubby (for sweetclover and crownvetch, or for roots bound by the seed coat see note 1).
- split extending into the hypocotyl.

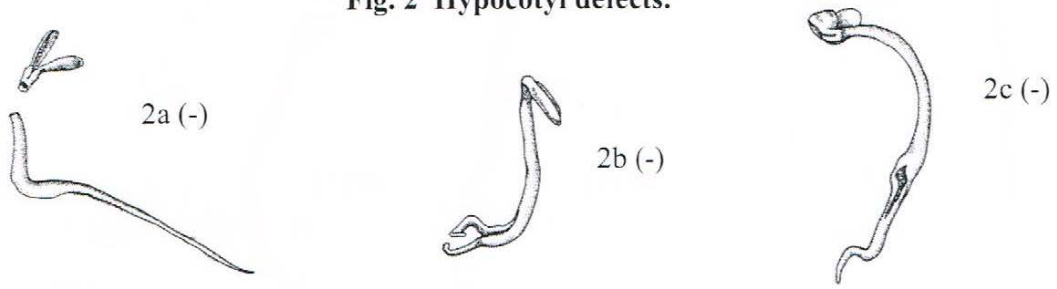
Seedling:

- one or more essential structures impaired as a result of decay from primary infection.
- albino.

Alfalfa

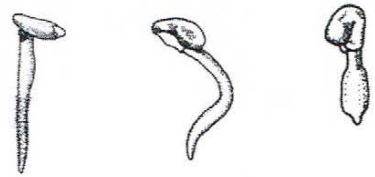
5. The percentage of hard seeds must be determined at the end of the test period for all genera in this group. Swollen seeds that fail to germinate by the end of the test should be allowed an additional five days as provided in the AOSA Rules Vol. 1 (Sec. 6.9 d (6)). For swollen seeds of alyceclover, see AOSA Rules Vol. 1 Sec. 6.8 a and for crownvetch, see Sec. 6.8 o. Swollen seeds are an indication of dormancy and can be induced by incorrect temperatures.

Fig. 2 Hypocotyl defects.



2a. Broken hypocotyl.
2b. Split root and hypocotyl.
2c. Deep lesion of hypocotyl.

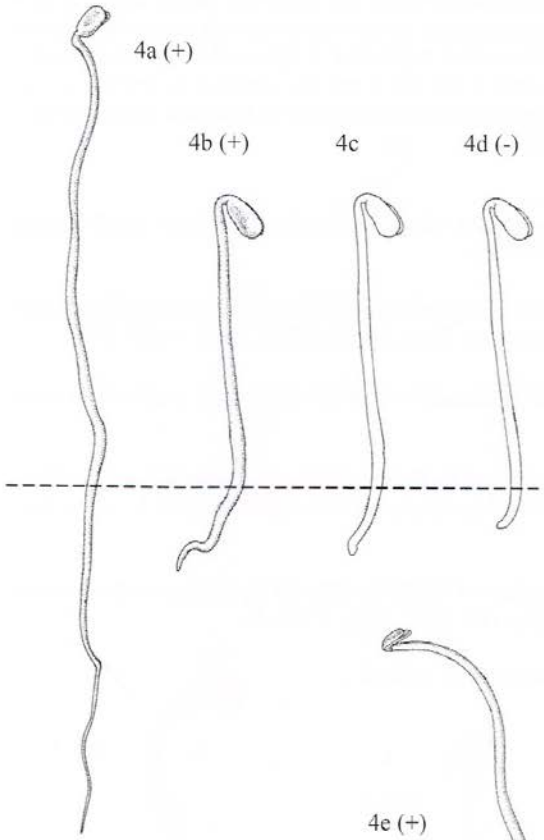
Fig. 3 Small seedlings.



3a. Late-germinating seedling (at final count; see 3.5.1.b).
3b. Seedling too small to evaluate; extend test (see 3.5.1.b).
3c. Swollen hypocotyl, stubby root.

Alfalfa

Fig. 4 Root defects.

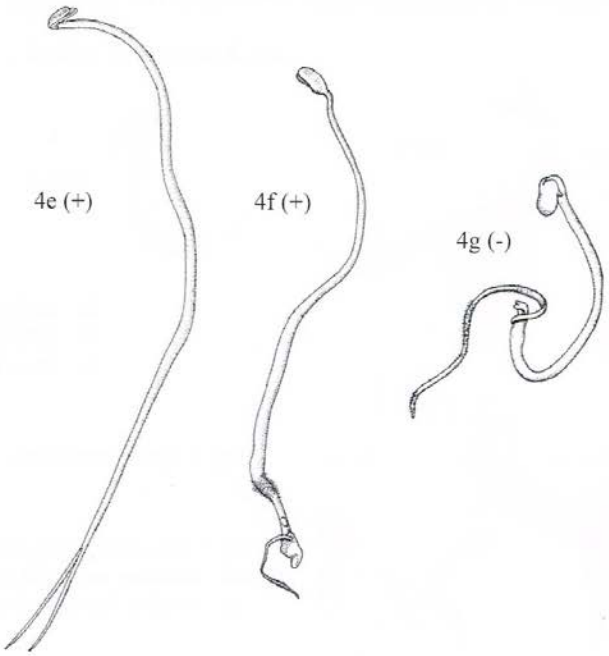


4a. Normal seedling.

4b. Short root.

4c. Stubby root, normal for sweetclover and crownvetch, abnormal for others.

4d. Stubby root.



4e. Split root, not extending into hypocotyl.

4f. Root trapped in seed coat.

4g. Primary root missing, adventitious root developing.

Alfalfa Seedlings from Imbibition Test

Normal

Non-Tolerant

Abnormals

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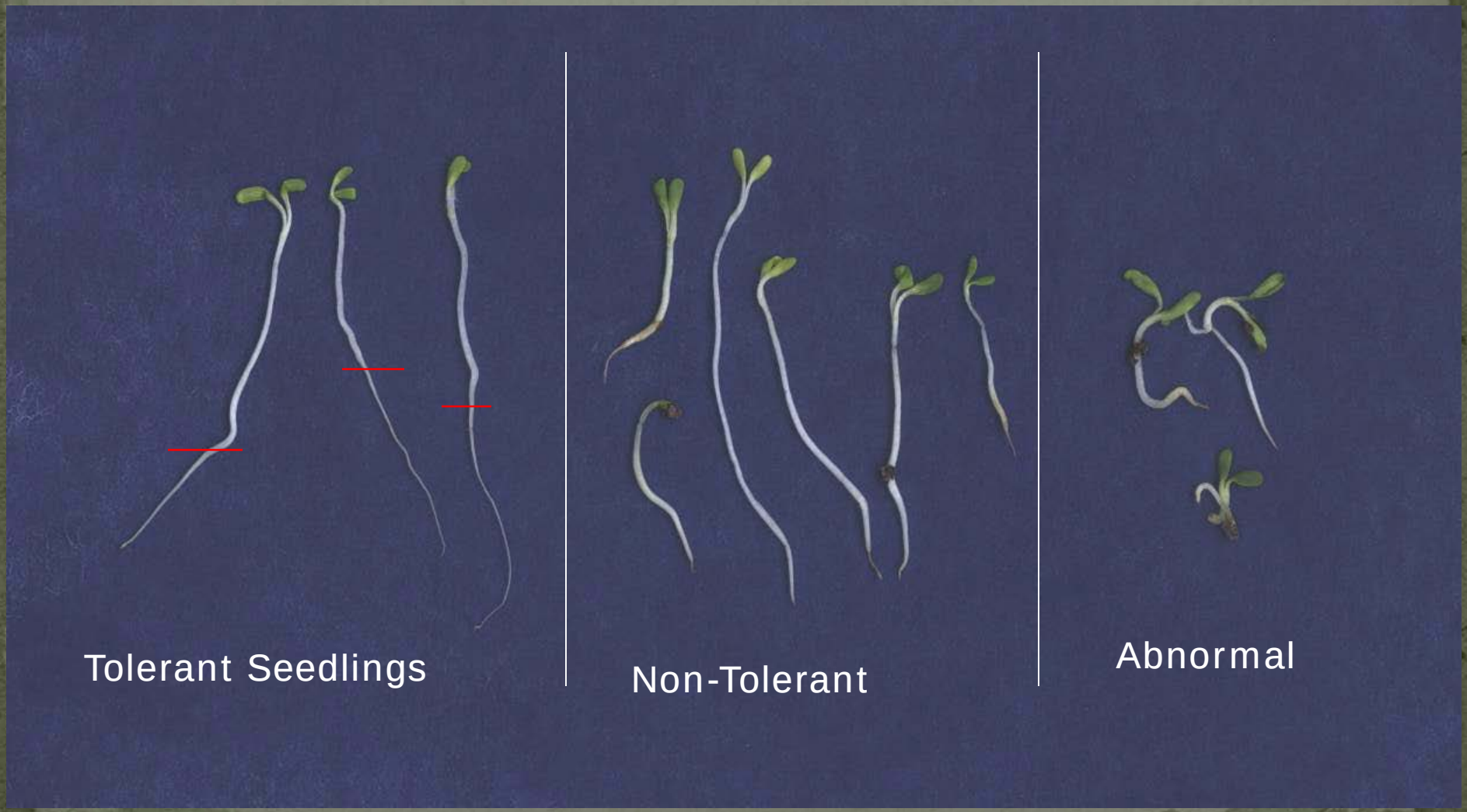
Testing Challenges – RR Alfalfa

Distinguishing tolerant seedlings from non-tolerant seedlings.



Herbicide Bioassay

Questionable seedlings of most species and for most herbicide traits can be checked with a lateral flow strip.

- Strips can be expensive if purchased in smaller quantities and in any case it isn't usually practical to check many seedlings.

Herbicide Tolerance Formula

- $$\frac{\text{\# Normal Tolerant}}{(\text{\# Normal Tolerant} + \text{\# Susceptible})} \times 100$$

Resources

- SCST Seed Technologist Training Manual
- SCST HB Working Group's "Non-Tolerant Seedling Web Page" (www.seedtechnology –Resouces)