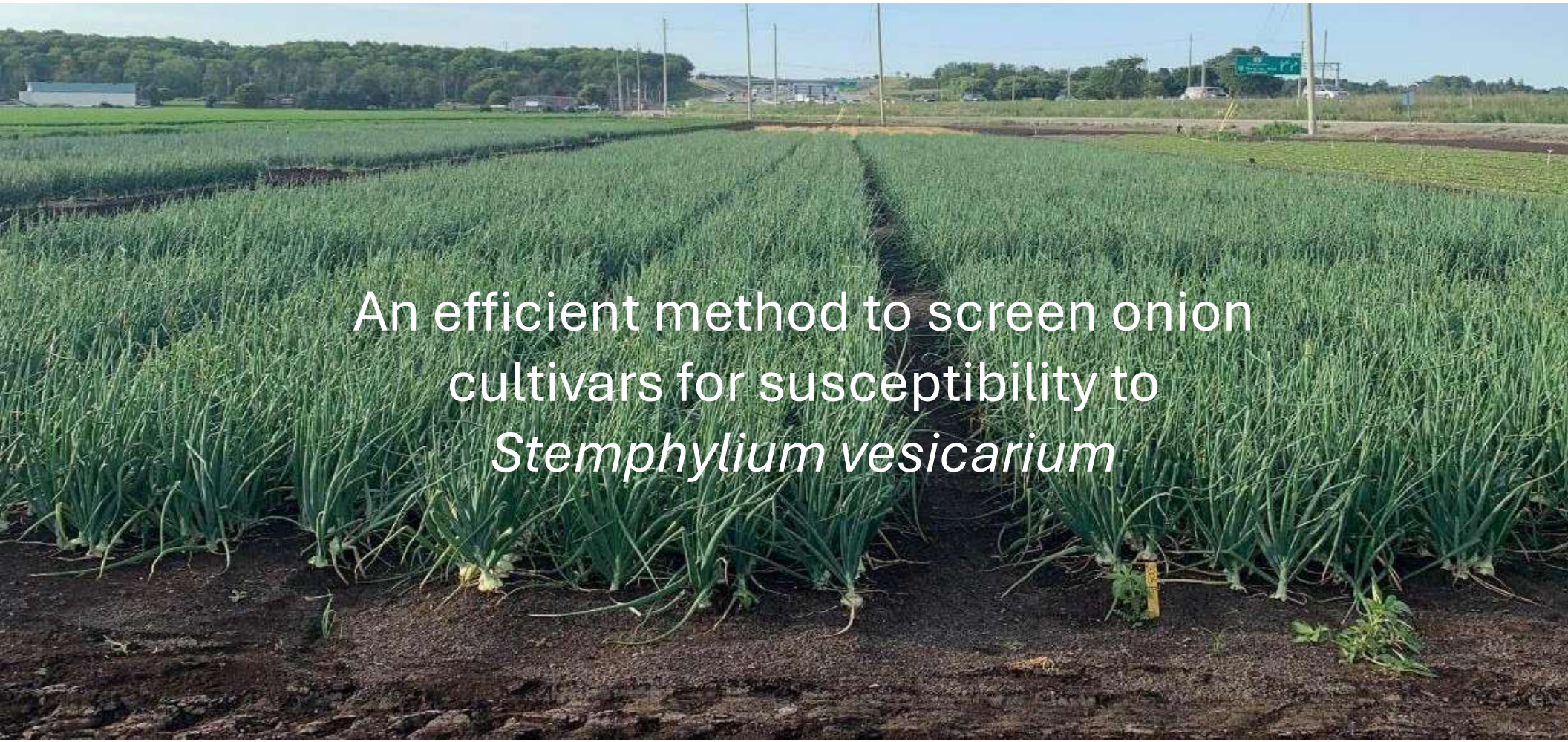
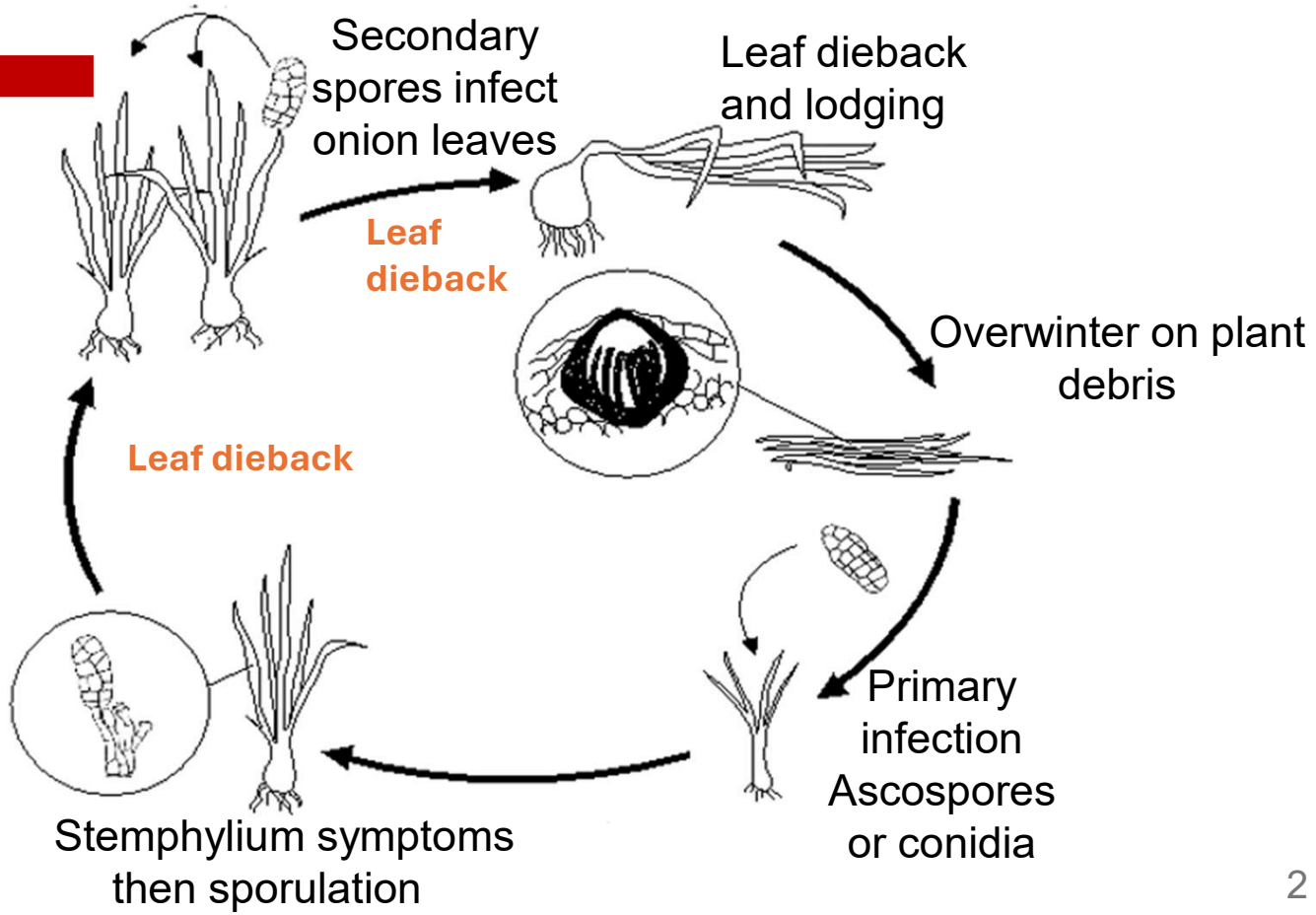


An efficient method to screen onion cultivars for susceptibility to *Stemphylium vesicarium*

Julia Scicluna, Emily McFaul, Bruce. D. Gossen and Mary Ruth McDonald

S. vesicarium lifecycle

Long leaf wetness at warm temperatures contribute to infection

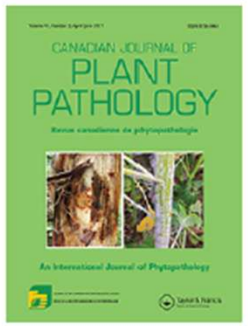


Hay et al. 2021

S. vesicarium toxins

- Leaf dieback is the most common symptom in Ontario
  - Likely caused by host specific toxins (9 identified)
    - SV toxin I and II
    - Phytotoxin metabolites
      - Stemphylin
      - Stemphyperlenol
      - Stemphyloxin (I and IV)
      - Stemphol
- Most of the research conducted on pear

Differences in susceptibility to SLB were observed in cultivar trials and in growers' fields. Results of cultivar trials published.



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Susceptibility to *Stemphylium vesicarium* of asparagus, onion, pear, and rye in Canada

Jennifer. M. Foster, C. Selasi Tayviah, Sara M. Stricker, Bruce D. Gossen & Mary Ruth McDonald

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To link to this article: https://doi.org/10.1080/07060661.2019.1574901

Part of Stemphylium cultivar trial - 2015

Screening onion cultivars for susceptibility to *S. vesicarium*

Field trials:

Wildtype pathogen- relevant to growers in that area

Field environment

Maturity of onion can affect the results- may be difficult to separate disease symptoms from natural senescence

There are years when little disease develops

Controlled environment trials

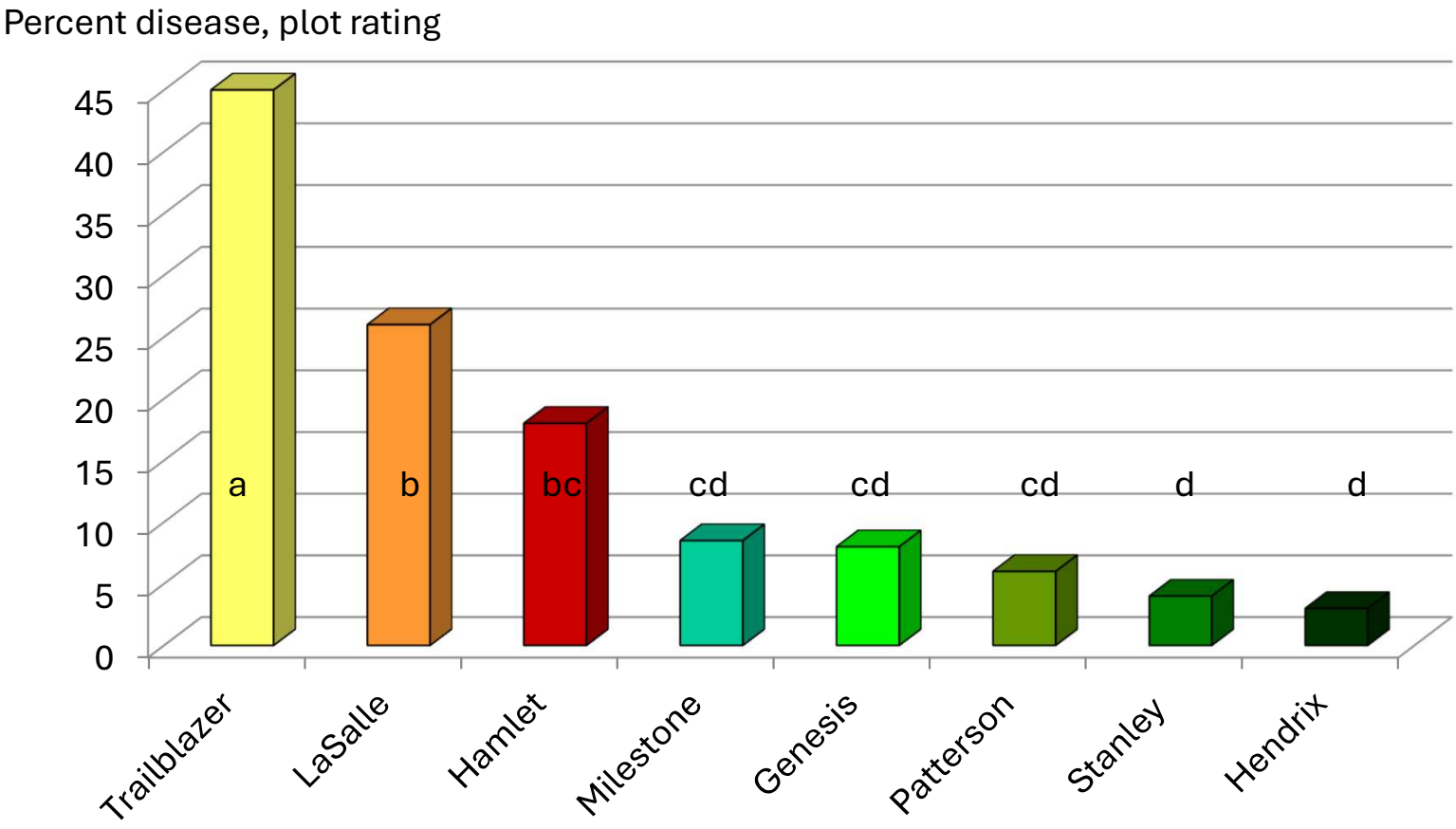
Can improve consistency of inoculum source and concentration

Control for senescence- noninoculated checks

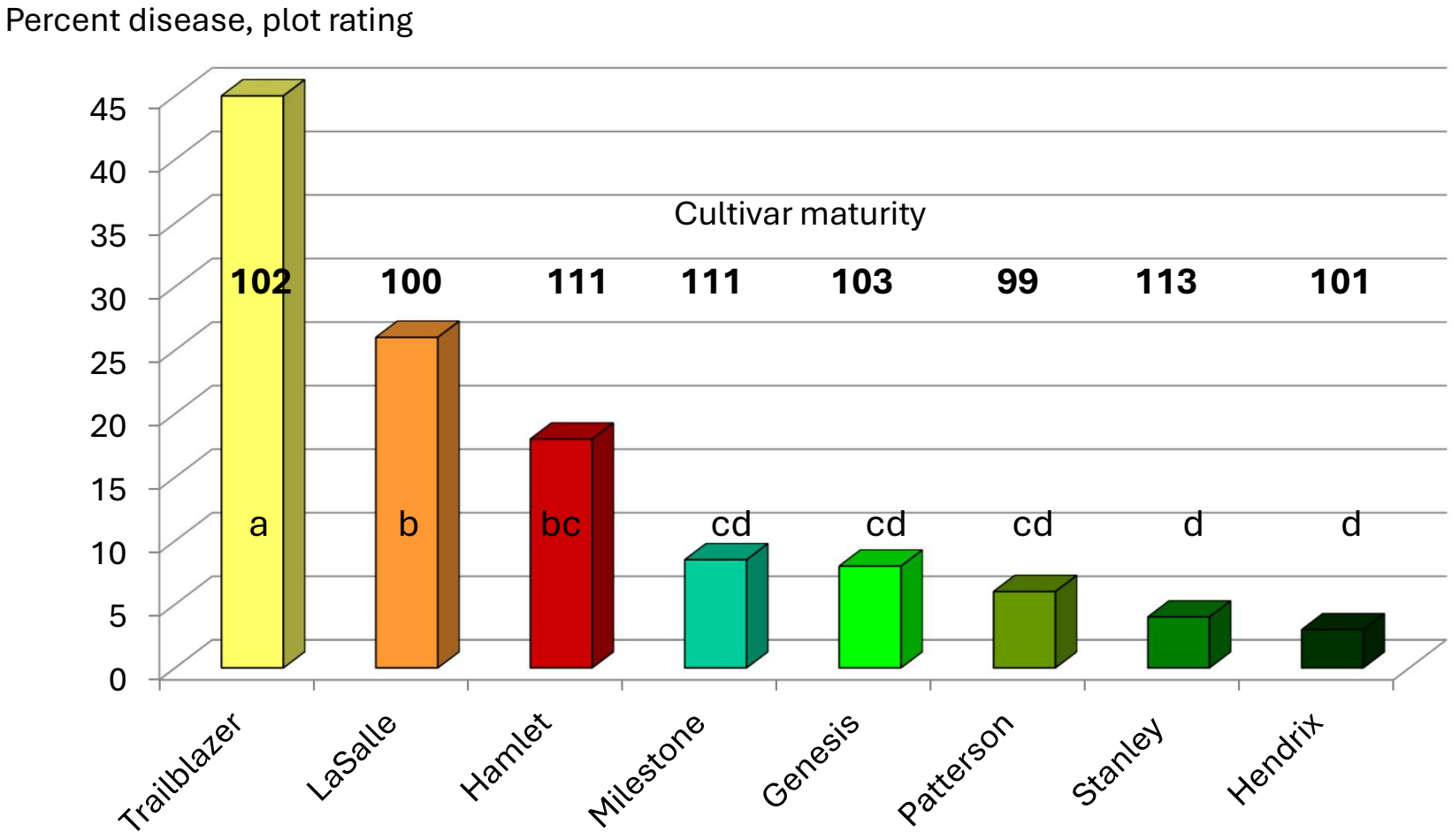
Repeat the trials several times in one year

Cultivar rating for SLB disease severity

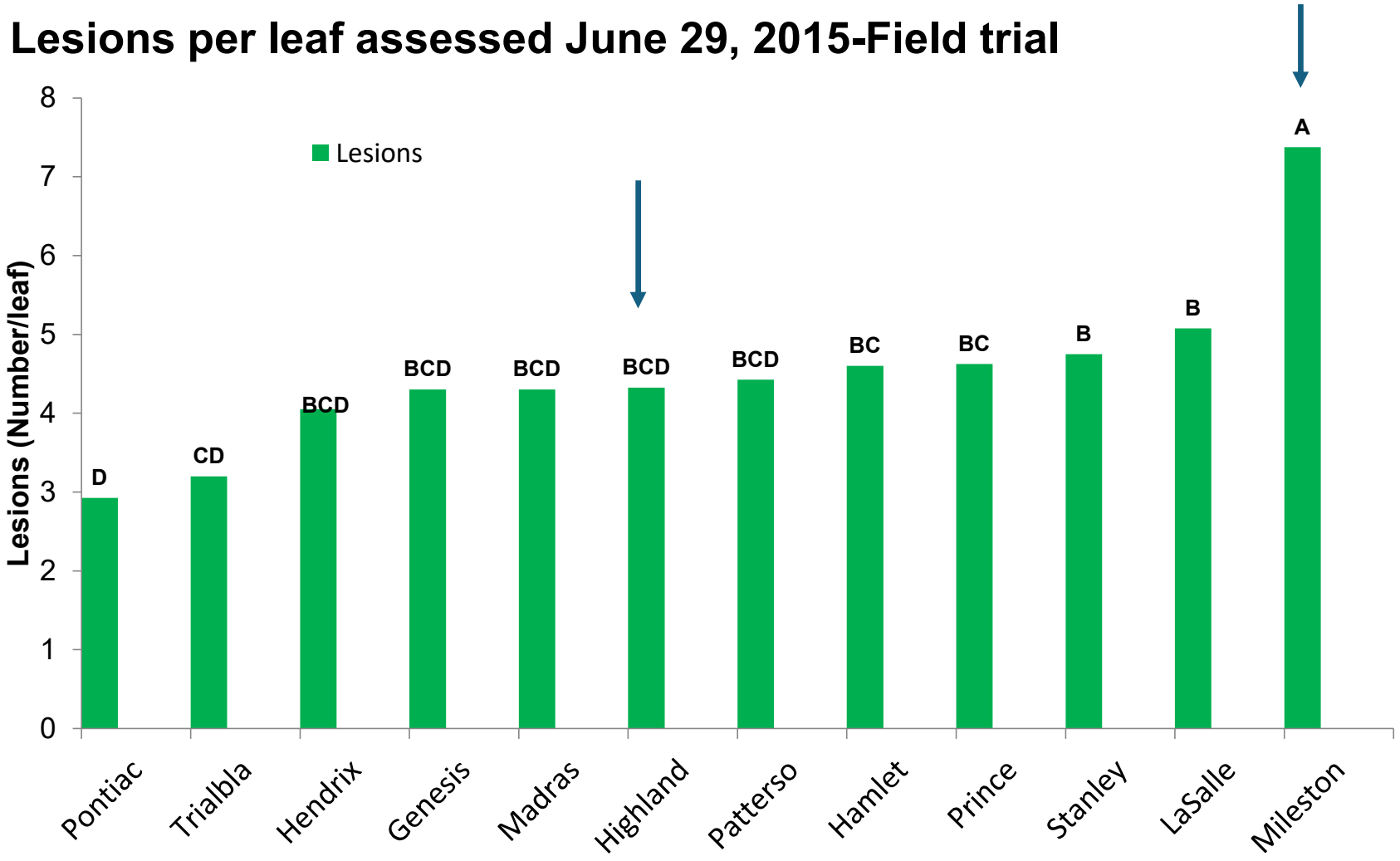
Aug.19, 2014

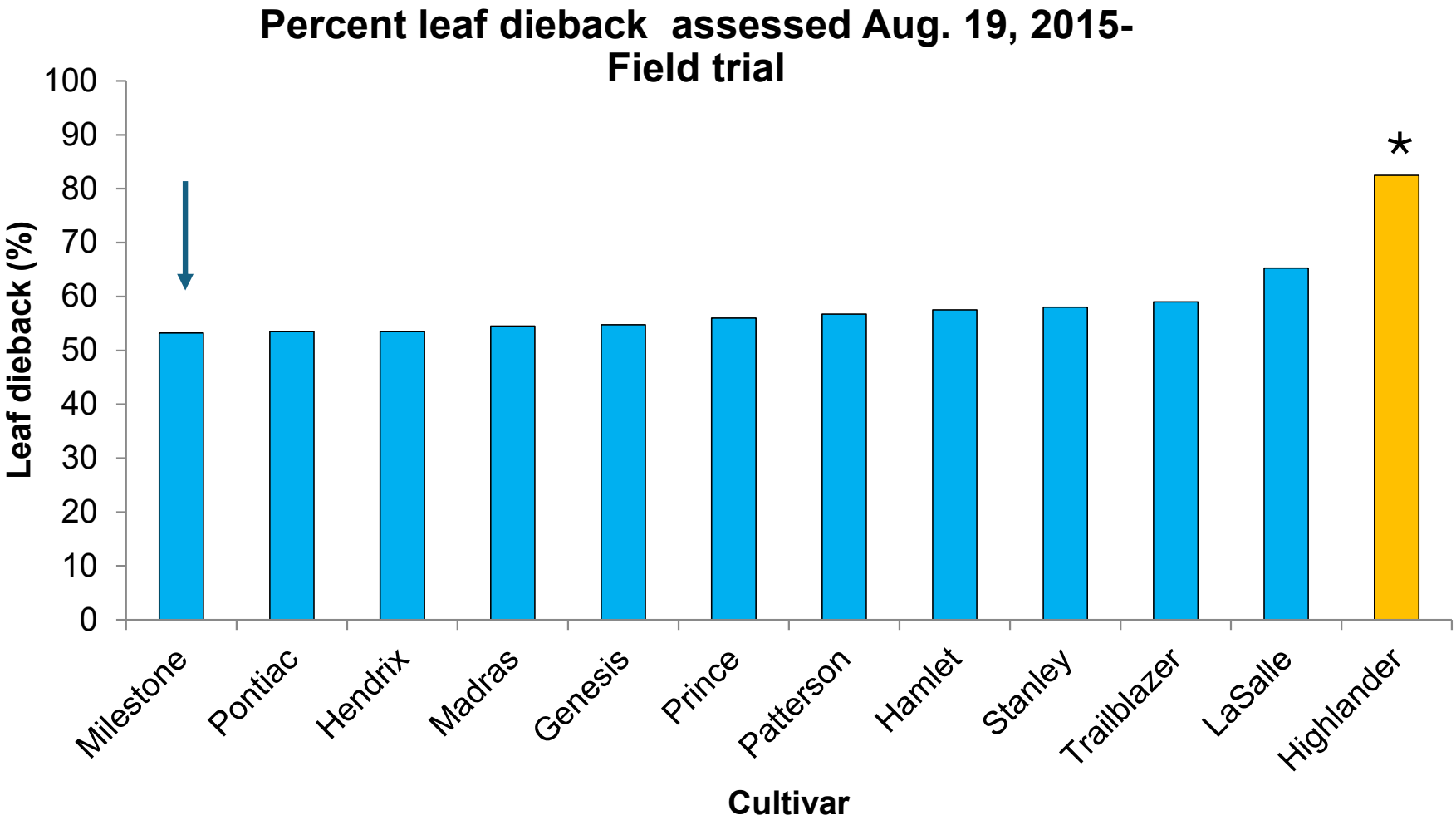


Disease severity and cultivar maturity Aug.19, 2014



No correlation between leaf dieback and days to maturity for any trial


Lesions per leaf assessed June 29, 2015-Field trial
Lesions
Lesions (Number/leaf)
0
1
2
3
4
5
6
7
8
D
CD
BCD
BCD
BCD
BCD
BCD
BC
BC
B
B
A
Pontiac
Trialbla
Hendrix
Genesis
Madras
Highland
Patterso
Hamlet
Prince
Stanley
LaSalle
Mileston



No correlation between lesions and leaf dieback

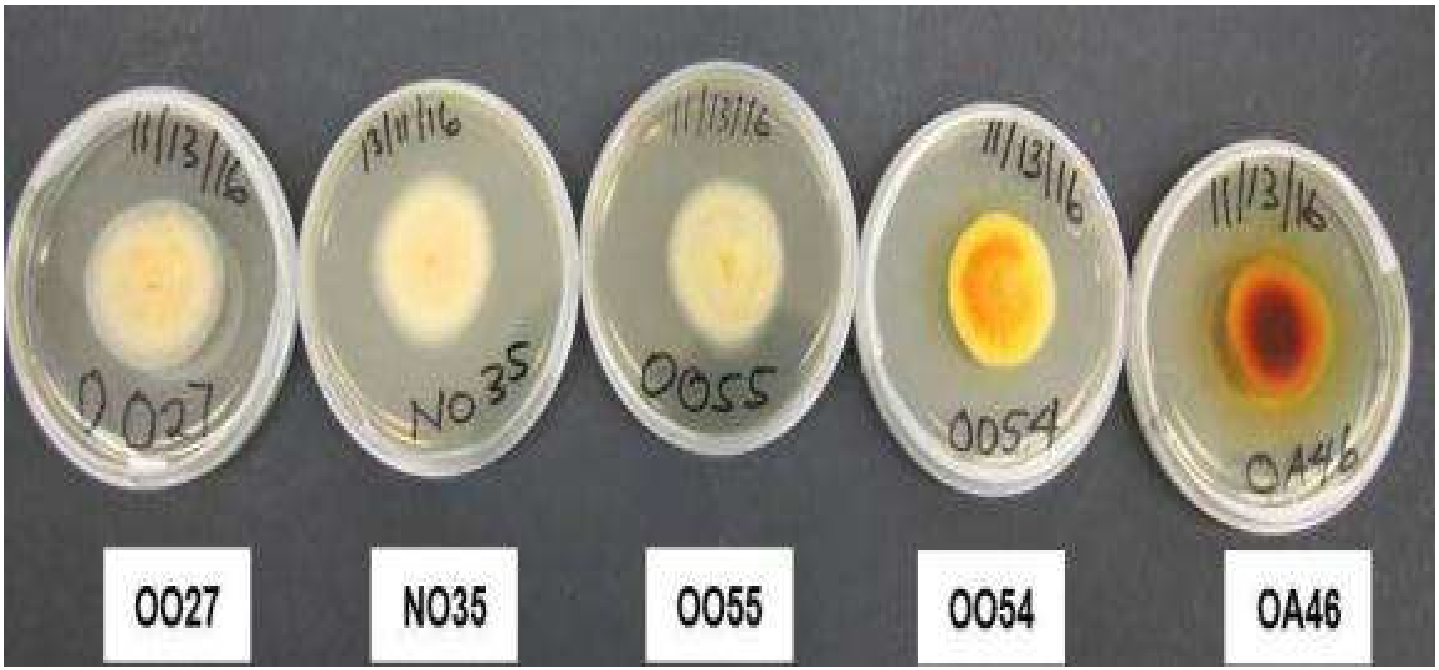
Controlled environment trials

Inoculate 2 x 10^6 conidia/ml, keep leaves wet for ~ 48 hours

- Disease assessment:
  - Lesions per leaf
  - Leaf dieback (%)
- Experiment analysed as a factorial.
- Repeated twice

Inoculated at 3-4 leaf stage, 5 isolates including a mock inoculated control.

First incidence of SLB was seen 10-12 days after inoculation

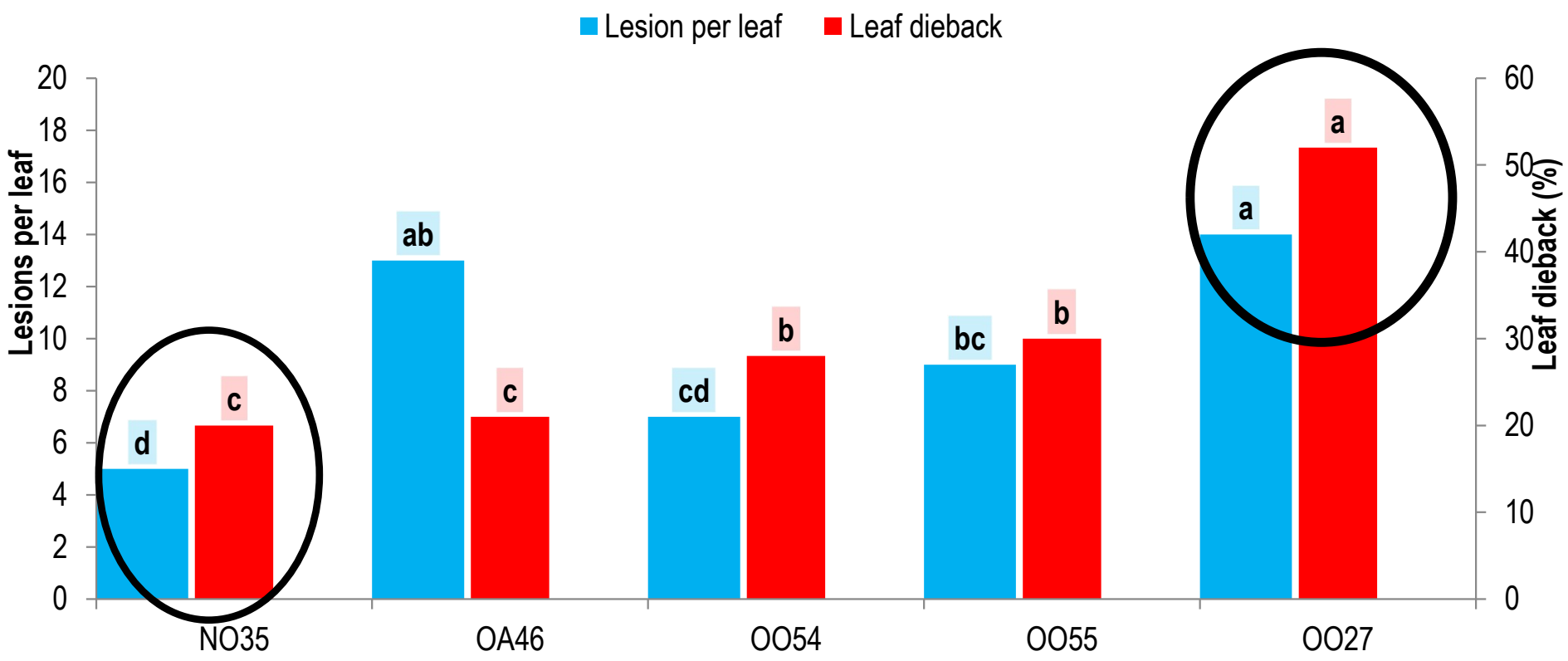


Different isolates of *S. vesicarium*

- Grown on PDA
- Confirmed with PCR and sequencing
- Sporulation on V8 agar
- Conidia 2 x 10^6 for inoculation

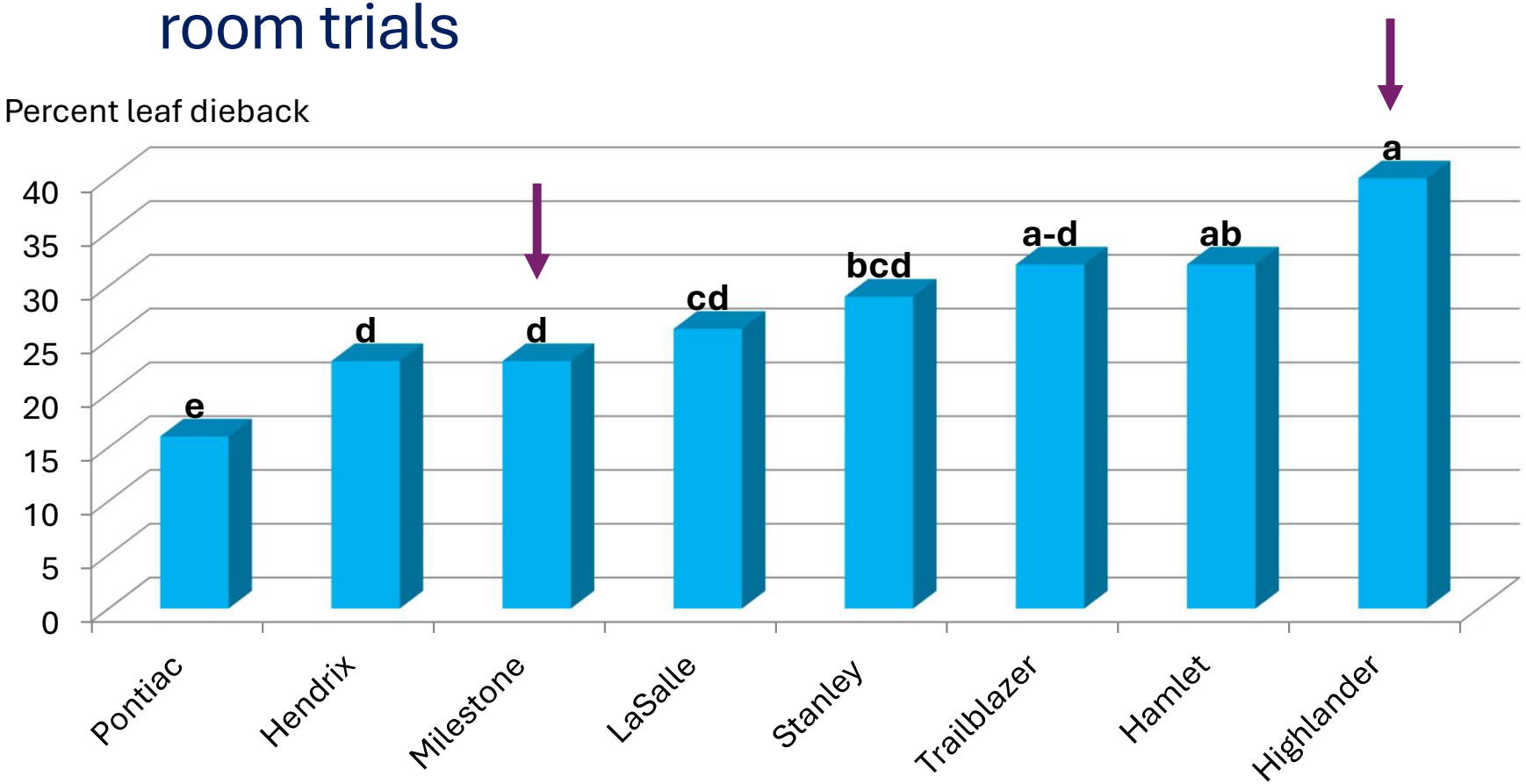
Tested for virulence and aggressiveness on onion
S.vesicarium loses virulence with repeated subculturing

Differences among isolates of S. vesicarium



N or O: Nova Scotia or Ontario; O or A: onion or asparagus

Leaf dieback in inoculated onions in growth room trials



Susceptibility to Stemphylium leaf blight

- All cultivars were susceptible to Stemphylium and developed symptoms
- Susceptibility to *S. vesicarium* in the field may be partly related to maturity of the cultivars:
  - Some (but not all) early maturing cultivars have higher susceptibility.

- Differences in susceptibility also identified in controlled environment trials
- Not consistent with the field trials in all cases (r = 0.68)
- It could be even more efficient (and reproduceable?) to apply a known amount of Stemphylium toxin= culture filtrate

Production of toxins in culture filtrate:

Select virulent isolates (3)
Culture in potato dextrose broth on orbital shaker for 21 days under diffuse light

Filter with filter paper to remove any mycelium or conidia
- Test to be sure it is ‘clean’

Some mycelium was still getting through the filter

Concentrated by solvent extraction with methanol or ethyl acetate and evaporation and resuspension

Another treatment: the toxin is not heat sensitive, so the culture filtrate was autoclaved to kill any mycelium

Methods:

Toxin treatments

1.a ethyl acetate water layer,

1.b solvent layer

2. autoclaved

Controls: sterile broth, water

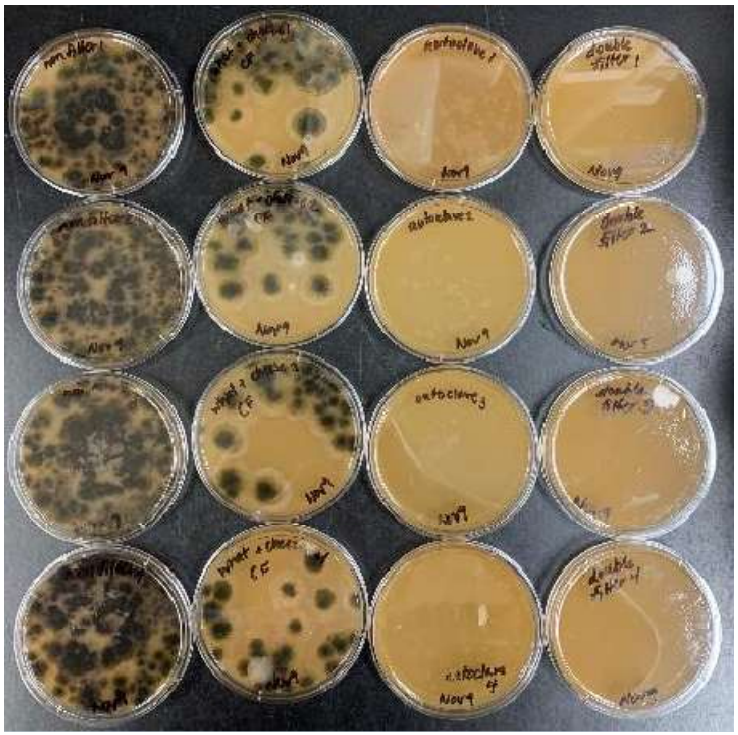
Plants at the 5–6 leaf stage

Injected with 1 mL of filtrate 2nd and 3rd oldest leaves using a 3 mL syringe. Injection site marked

Assessed 7, 14 and 21 days after injection

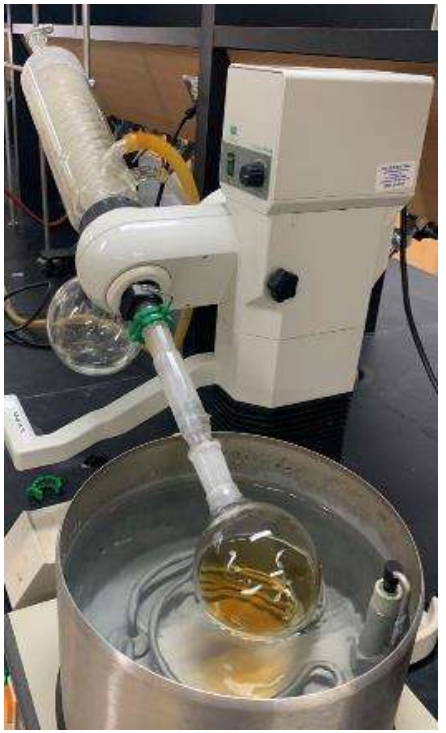
Leaf length and length of dieback was measured, percent leaf dieback calculated. Subtract any dieback that occurred on controls

Culture in broth



Unfiltered filter paper autoclaved

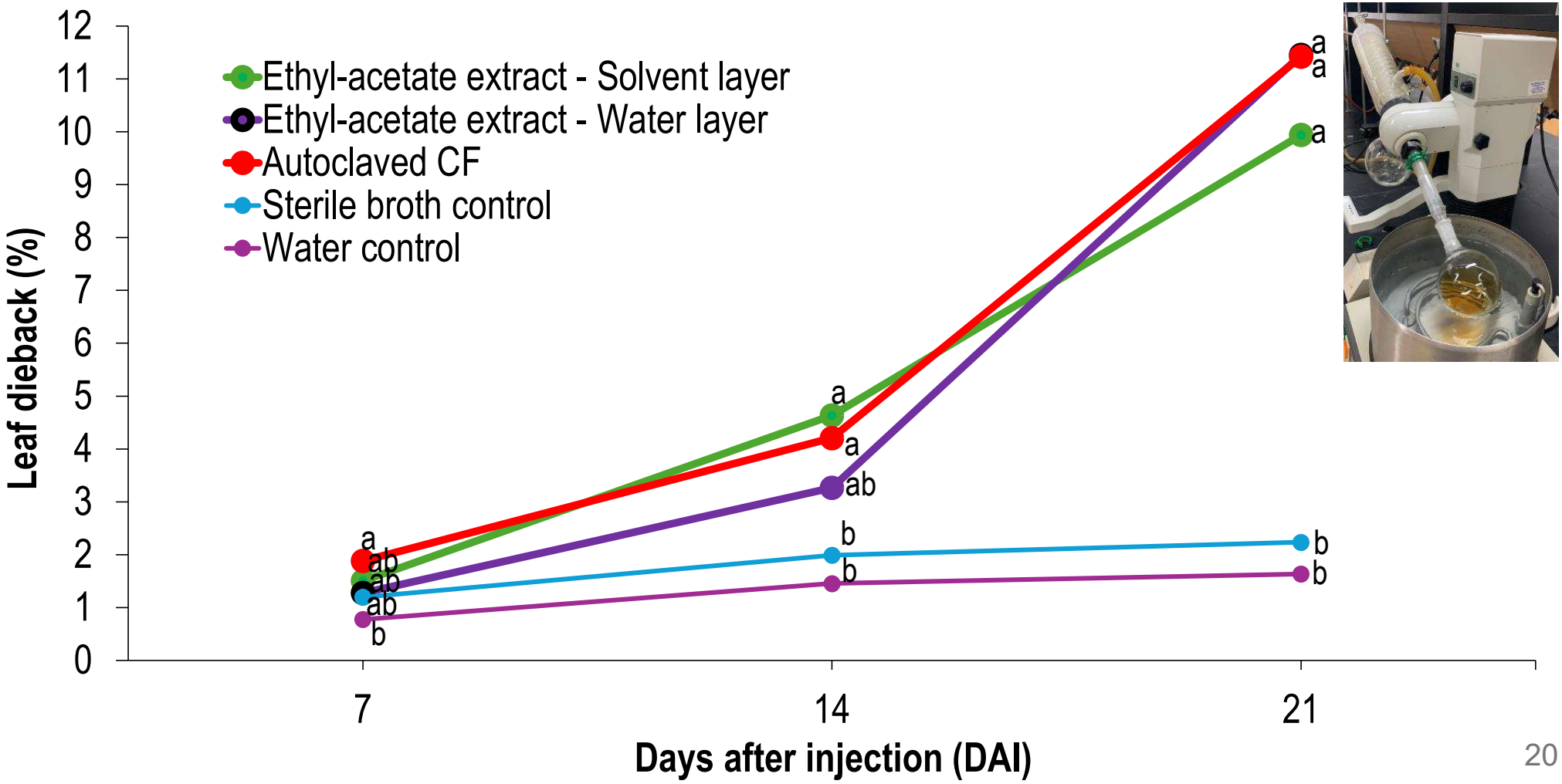
double filter



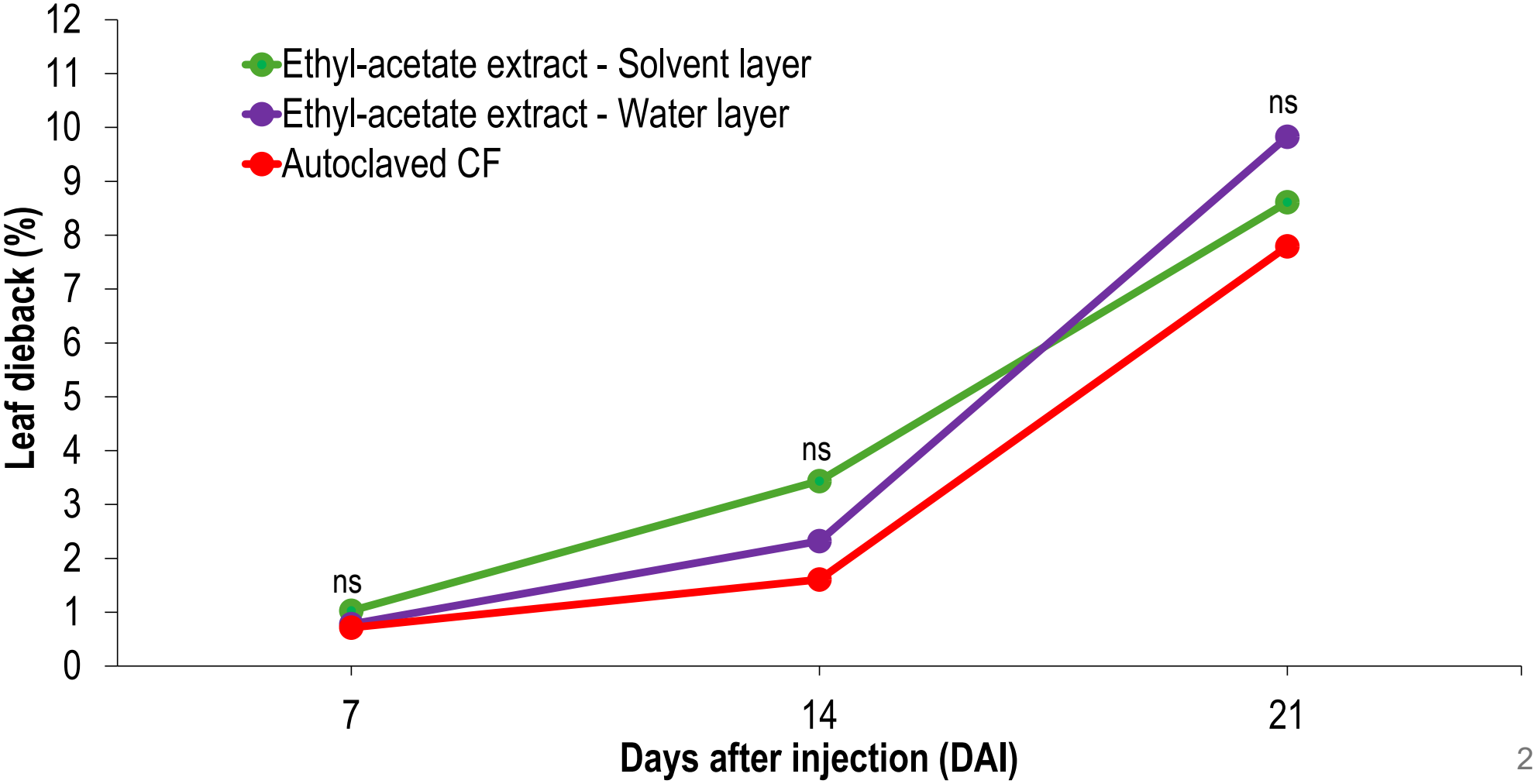
Producing toxins in culture filtrate

Toxin injection

Toxin filtrate



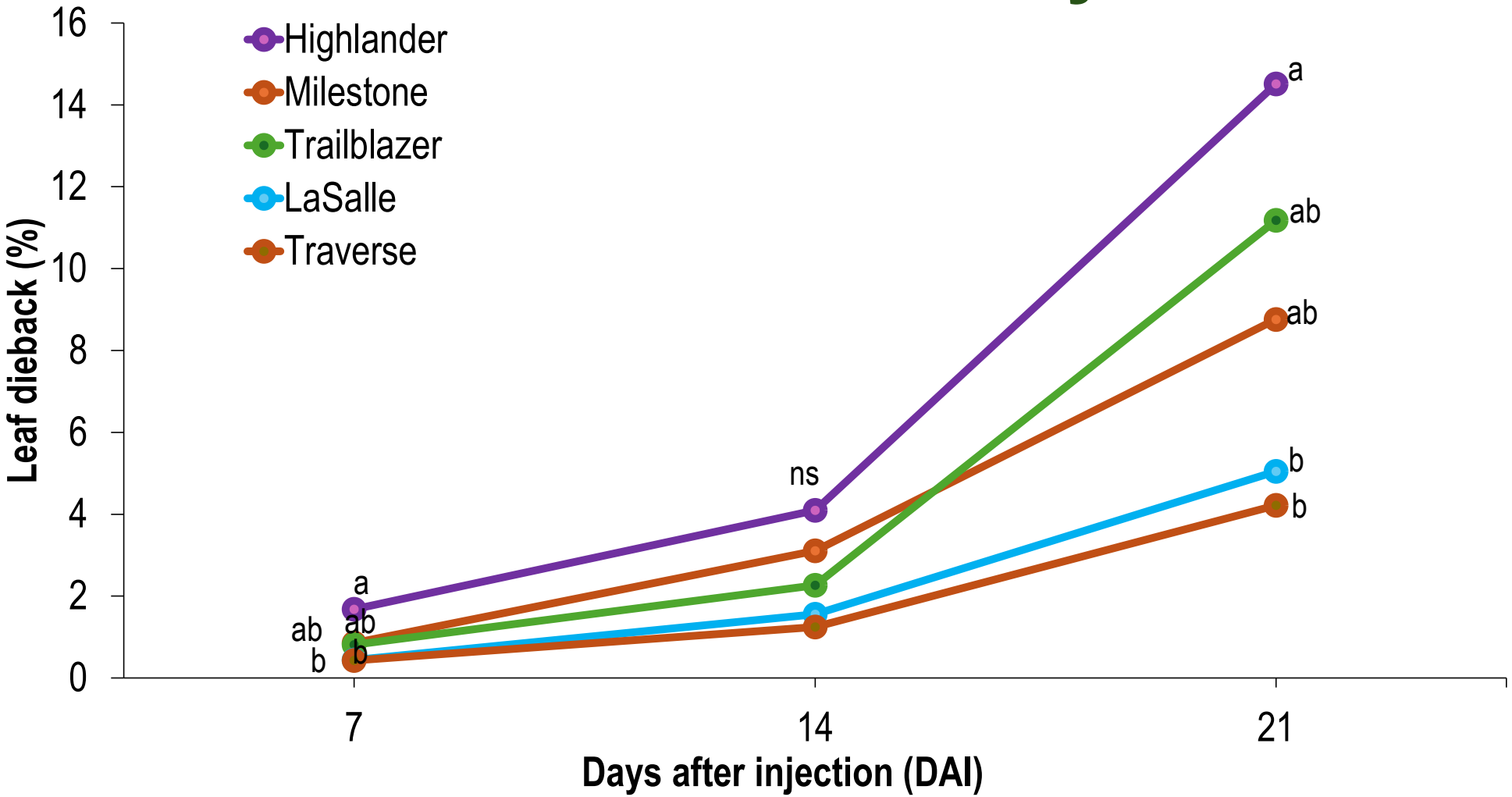
Toxin filtrate



Cultivar	Source	Days to maturity[1]
Highlander	American Takii	92
Traverse	Stokes Seeds	105
Trailblazer	American Takii	106
La Salle	Seminis Vegetable Seeds	106
Milestone	American Takii	110

In previous trials, Highlander had more leaf dieback, Milestone more lesions and low leaf dieback

Cultivar sensitivity



Autoclaving the culture filtrate was as effective as extracting and resusupending - much faster if you have access to an autoclave

The percent leaf dieback was relatively low compared to the field

- Only injected once, in the field the pathogen would keep producing toxin as it grows

Highlander had higher leaf dieback, consistent with field assessments.
Trailblazer also had high disease in early plot assessments
Milestone not different from other cultivars

Repeated with a wider range of onion species and cultivars

Second toxin trials: methods

- *S. vesicarium* isolates grown in incubated in culture broth to produce toxins
- 5 recent isolates from onion
- Filtered (0.2um) to remove live pathogen
- Onions 5- 6 leaf stage
- Filtrate injected in 4 leaves of each onion
- 3 points per leaf
- 11 cultivars assessed:

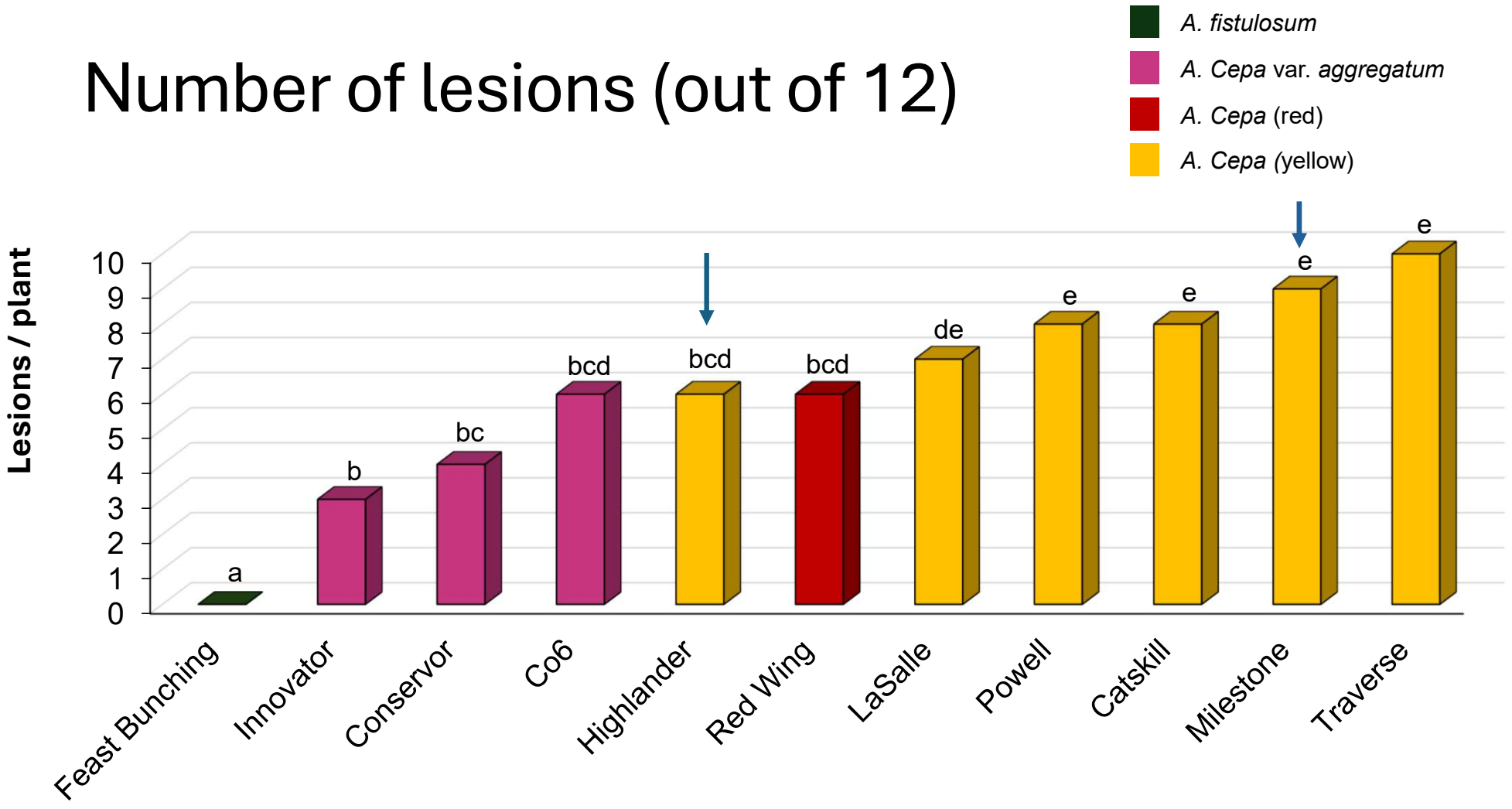
- bunching onion, shallot, red and yellow bulb onions

Cultivar	Reasoning for inclusion
Feast Bunching	*Allium fistulosum*
Innovator	Shallot (less susceptible) resistant to DM in 2021
Conservor	Standard shallot susceptible to DM in 2021 trial
Indian C06	In previous field trials it appeared
Powell	Yellow onion resistant to DM in 2021 trial
Red Wing	Common red onion
Highlander	Yellow onion - earlier trial
Traverse	Yellow onion - earlier trial
La Salle	Yellow onion - earlier trial
Milestone	Yellow onion - earlier trial
Catskill	Common yellow onion

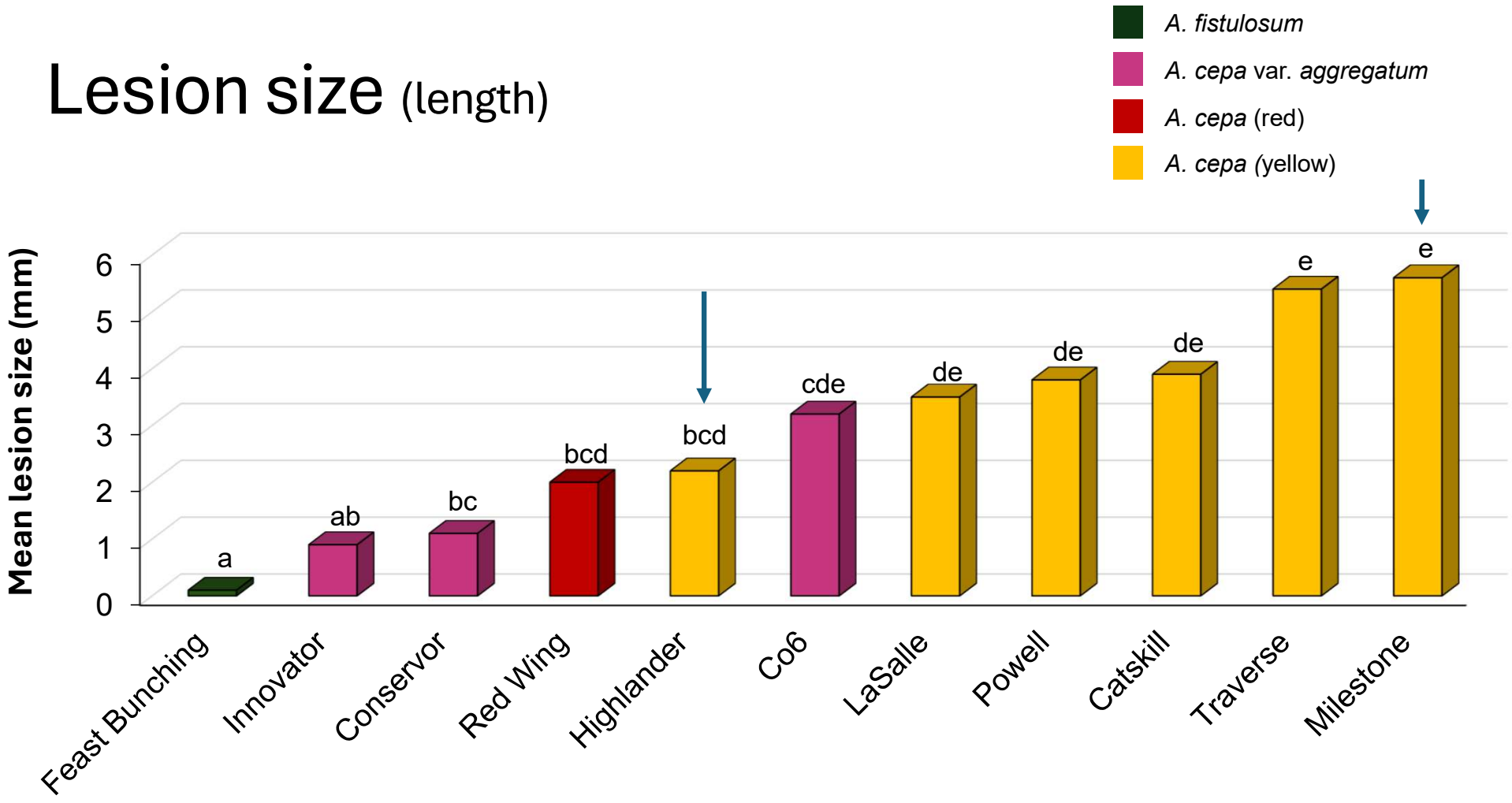
Lesions (chlorotic spots) developed within a few days- no leaf dieback

Counted enlarged chlorotic spots – max 12/plant and length of each spot

Number of lesions (out of 12)



Lesion size (length)



We are still uncertain why there was leaf dieback in the first set of experiments and chlorotic spots in the second set

Concentration of the toxins must be a factor

Next steps:
Compare autoclaved to 0.2 um filtered culture filtrate, amount of filtrate per injection site

Assess more yellow onion cultivars

Cultivar sensitivity summary

- All bulb onion cultivars were sensitive but some variability in sensitivity to toxins, the red onion was less sensitive than some
- *A. fistulosum* and shallots were least sensitive, as expected
- Toxins produced by *S. vesicarium* cause leaf dieback and chlorotic lesions (related to concentration?).
- Cultivar sensitivity to toxins was similar to the range of susceptibility to *S. vesicarium* in a previous study, with cv. Highlander having the more leaf dieback, but fewer lesions
- Milestone had more lesions and larger lesions than Highlander

Cultivar sensitivity summary

Resistance to symptoms appears to be closely related to sensitivity to the toxins

Is it better to have less leaf dieback or fewer lesions?

There are only subtle differences among several cultivars, probably not important for production

This approach could be used for evaluating new cultivars in breeding for resistance

Which is faster and easier? Inoculating or producing and injecting the culture filtrate?

Going forward…

- Develop a more repeatable method of producing the toxin solution
- Sort out why the differences between leaf dieback and chlorotic lesion symptoms
- Test more cultivars for sensitivity
- Conduct another field cultivar trial with more of the current cultivars

Acknowledgements

Funding was provided by:

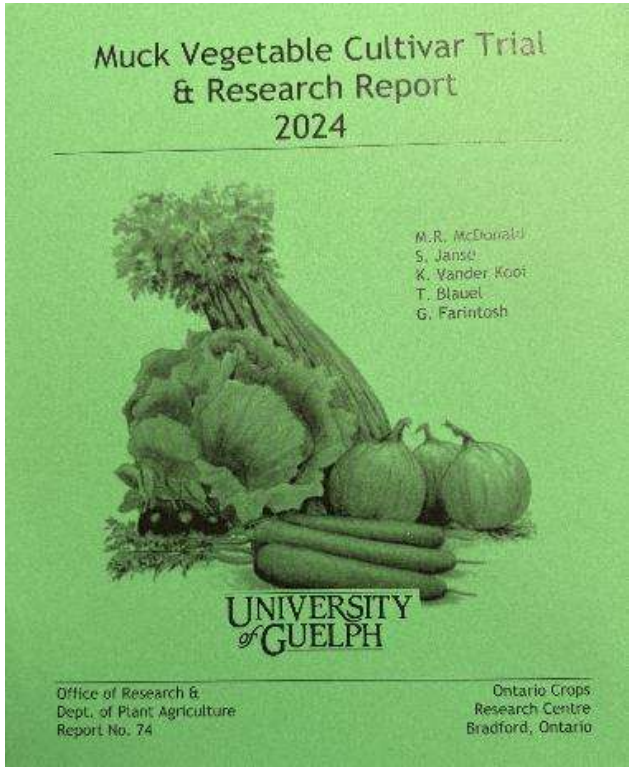
- The Fresh Vegetable Growers of Ontario
- Ontario Agri-Food Innovation Alliance

All research trials are summarized in the Greenbook – Annual Report

Note new name: Ontario Crops Research Center- Bradford

Download at the Research Station web site

https://bradford-crops.uoguelph.ca/




Questions?

Number	Cultivar Name	Days Maturity	Source
01	Stanley	105	Solar Seed
02	Prince	105	Bejo Seed
03	Highlander	85-90	American Takii
04	Hendrix	108	Bayer seed
05	Hamlet	108	Stoke
06	Trailblazer	95	America Takii
07	Madras	102	Bejo Seed
08	Patterson	104	Bejo Seed
09	Milestone	110	American Takii
10	Genesis	108	Crookham
11	La Salle	103	Stokes
12	Pontiac	115	Crookham