

Buffalo treehopper (*Stictocephala bisonia*) as a vector of Grapevine Red Blotch Virus (GRBV) in Ontario

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Brock University

Agriculture and Agri-Food Canada

GRBV is a major threat to the Canadian wine industry

Ontario grapes valued at $113M[1]

Grapevine red blotch virus (GRBV) causal agent of grapevine red blotch disease (GRBD)[2]

Circular, single strand DNA

Affects grape yield, quality, and vigor[3]

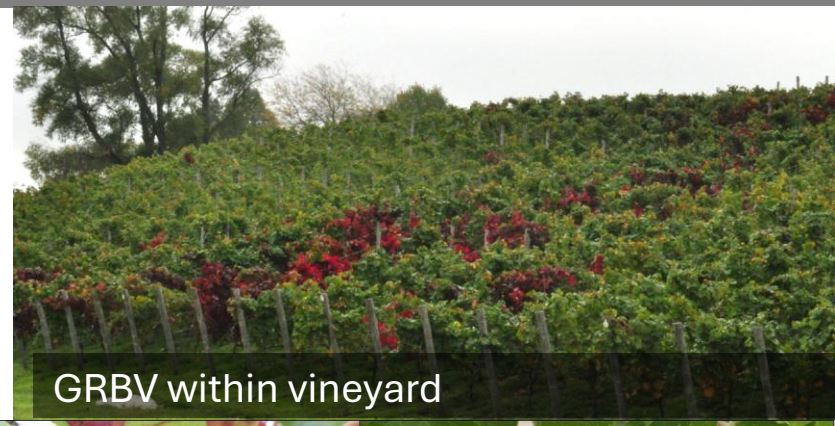
Reduces stomatal conductance and photosynthesis, delays fruit ripening[4]

Transmissible via insect vectors or grafting[5]





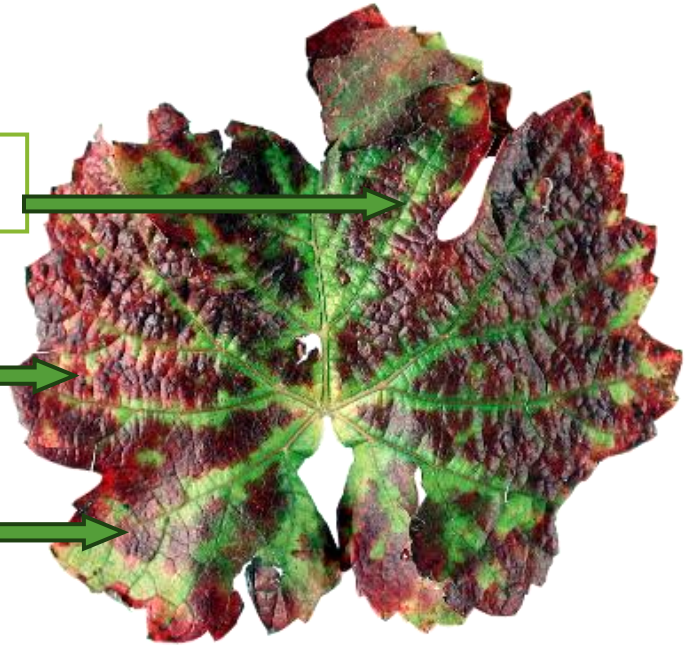
Advanced symptoms on Pinot noir





GRBV Symptoms

Red berried cultivar

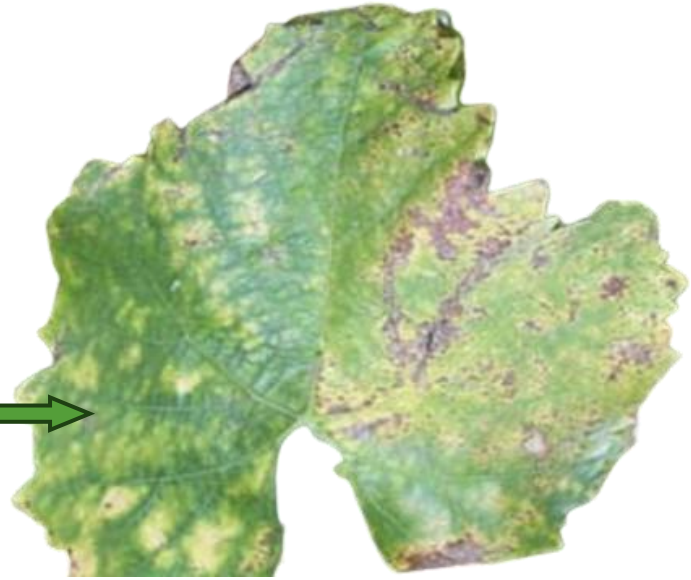


Red blotches

Marginal reddening

Red leaf veins

White berried cultivar



Chlorotic blotches

Red+white berried cultivars



Repressed ripening

Reduced sugar content (-1 to -4 brix)[4]

Spissitilus festinus: confirmed GRBV vector

- Three-cornered alfalfa treehopper
- Feeds on grapevine petioles creating ‘girdle’ scars
- Transmitted GRBV within caged greenhouse assays after a 2-week controlled exposure to GRBV[6,7]
- Vector of epidemiological importance in California and Oregon vineyards
  - No established populations in Canada

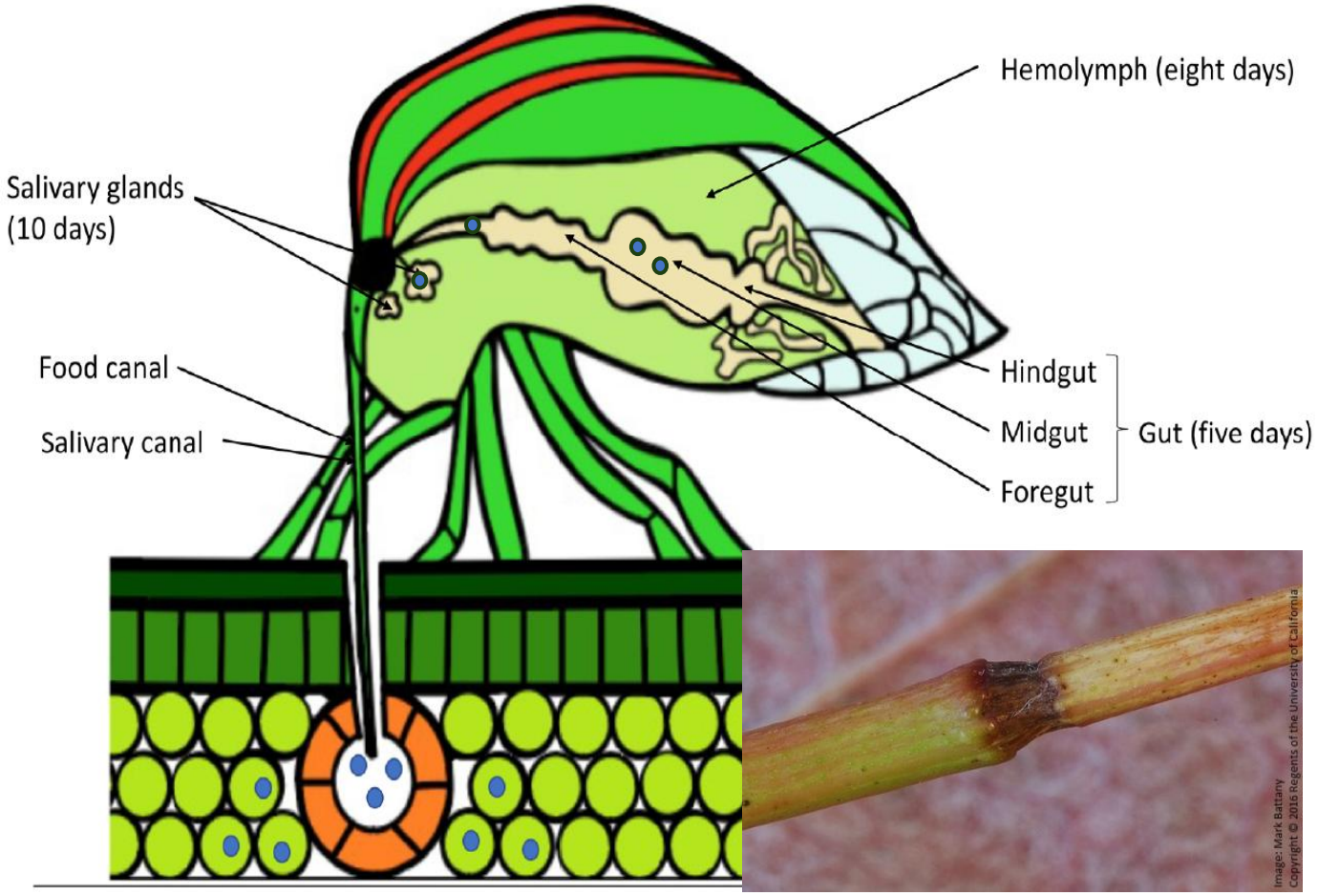


Girdles left from *S. festinus* feeding



Spissistilus festinus

How *Spissistilus festinus* transmits GRBV

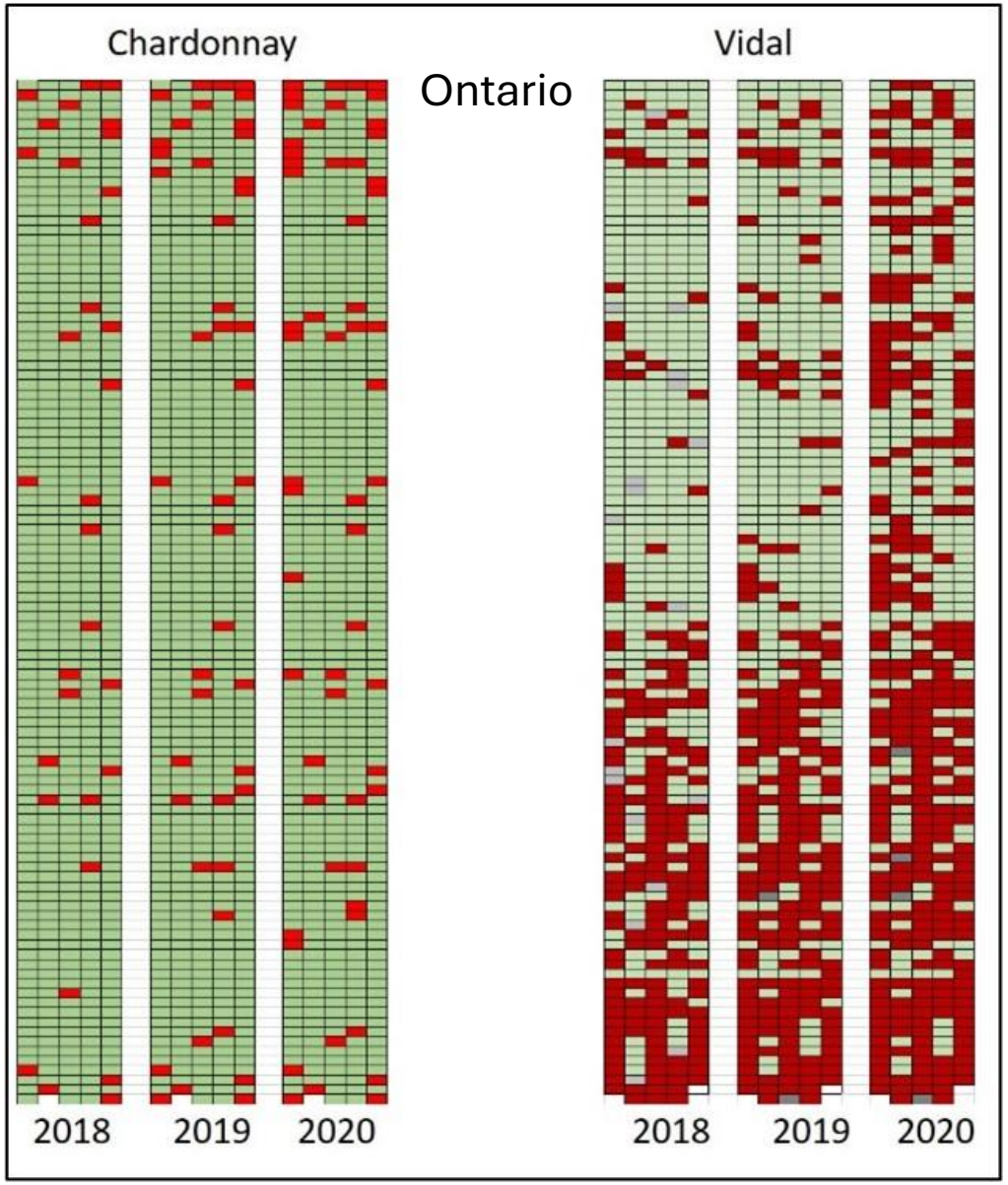


1. Feeds on phloem sap via food canal
2. GRBV moves into gut epithelium (5 days)
3. GRBV circulates in hemolymph (8 days)
4. GRBV moves into the salivary glands (10 days) where it gains transmission potential

GRBV is circulative and non-propagative[7]

GRBV is spreading in Ontario vineyards

	2018	2019	2020
% GRBV+	5.8	8.3	10.8
% increase		2.5	2.5



	2018	2019	2020
% GRBV+	35.2	43.4	53.9
% increase		8.2	10.5

Data: Wendy McFadden-Smith



Stictocephala bisonia: potential GRBV vector

S. bisonia acquired GRBV from infected grapevines and transmitted GRBV to diet solution[7]

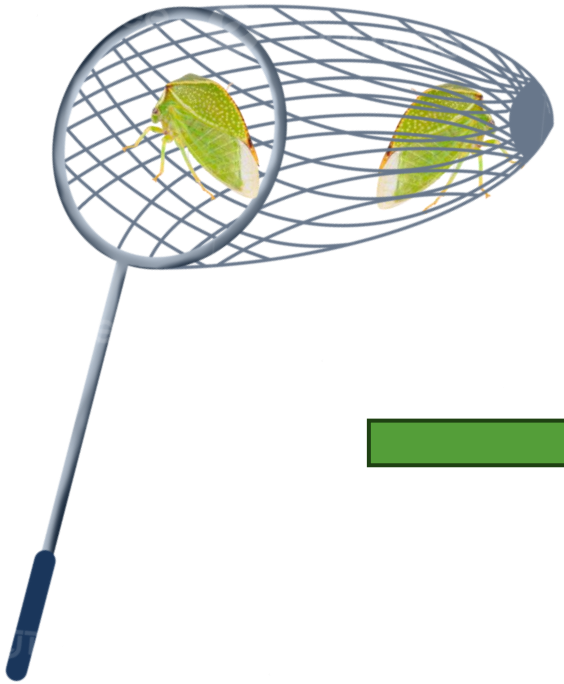
Common in edge habitats around vineyards in Ontario

Vine-to-vine GRBV transmission potential has not been tested

Objective: Determine if *Stictocephala bisonia* can transmit GRBV from an infected to a non-infected grapevine

GRBV Transmission Bioassay Methodology

1. Collection



Caught *S. bisonia* in field from July-August 2024/2025 with sweep net

2. Acquisition (14 days)



Separated *S. bisonia* into 6-12(2024)/6-8(2025) and placed within a sleeve of a GRBV+ grapevine

3. Gut Clear (2 days)



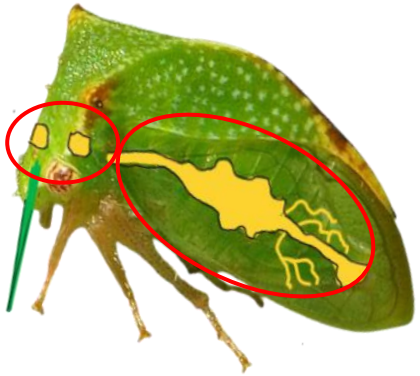
Placed *S. bisonia* 2-7(2024)/1-5(2025) inside tent with fava bean (2024)/ alfalfa(2025) to clear gut of GRBV+ material

4. Inoculation (4 days)



S. bisonia moved to sleeves of GRBV- grapevines (1-5) 2024/(1-4) 2025

5. DNA extraction



DNA from salivary gland & head extracted separately from *S. bisonia*



DNA from GRBV- grapevines extracted before and after dormancy

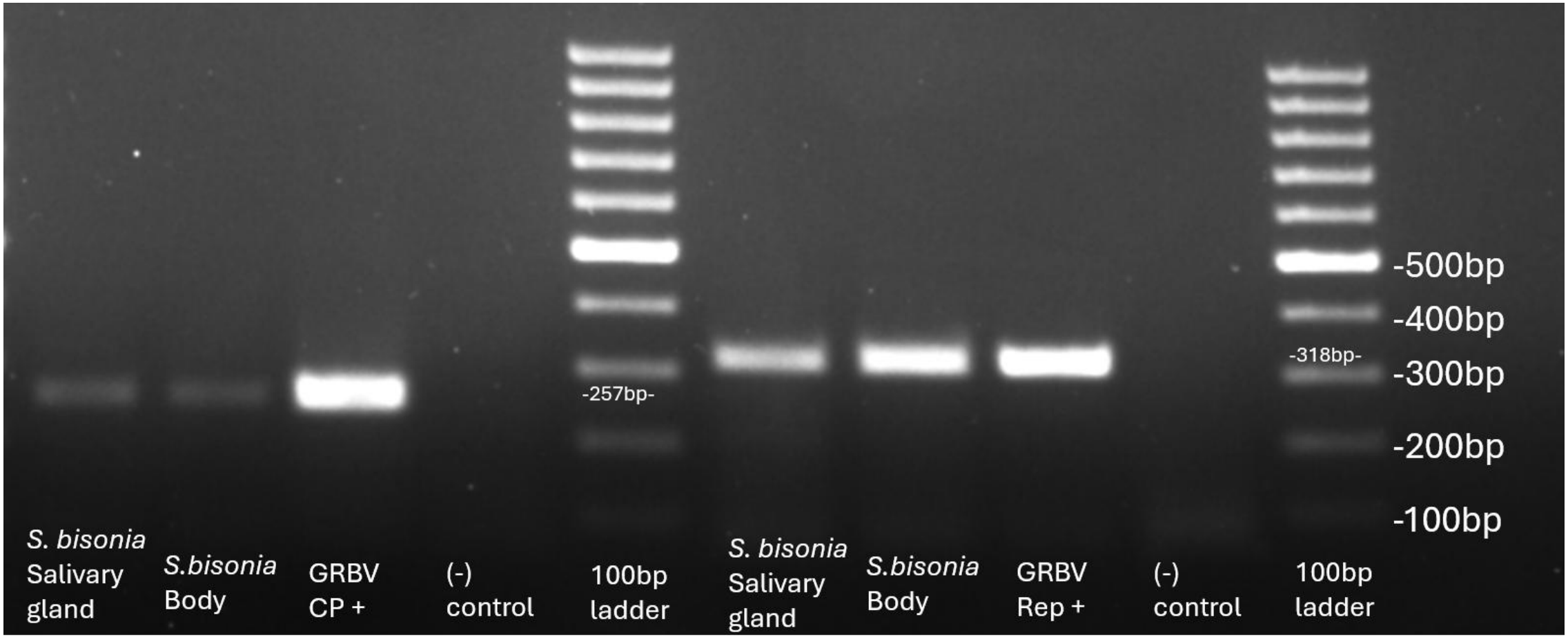
5. PCR Analysis & Gel Visualization

Two primers:
GRBV Replication
GRBV Coat Protein
If GRBV exists in the sample

PCR products visualized on an agar gel to determine presence/absence of GRBV

Reference genes were used for Grapevines and *S. bisonia* to verify DNA[6]

Gel results sample *S. bisonia*:



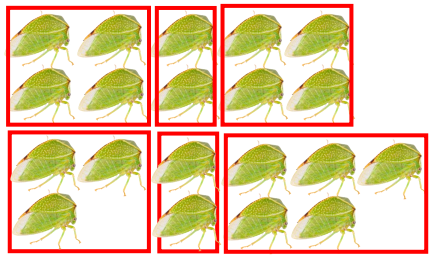
Results: *S. bisonia*

2024

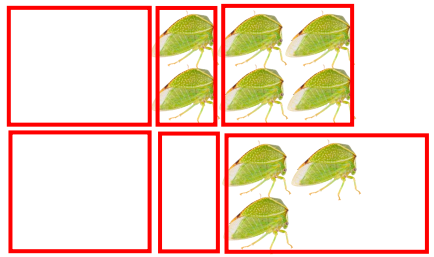


44 total, avg. 7.3/sleeve

45% survival



20 total, avg. 4/sleeve



9

0

 # S. bisonia start of acquisition → *# S. bisonia* end of inoculation → GRBV+ Bodies → GRBV+ Heads

red square = one sleeve = one replicate

Results: Grapevines

2024



0/6 GRBV+

1/3 months post-assay



0/6 GRBV+

8 months post-assay



0/6 GRBV+

1-year post-assay

red square = one sleeve = one replicate

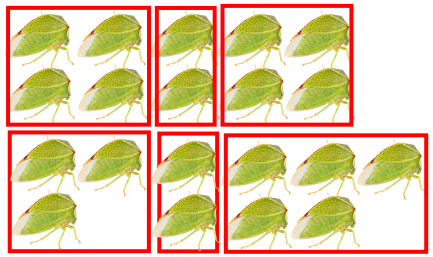
Results: *S. bisonia*

2024

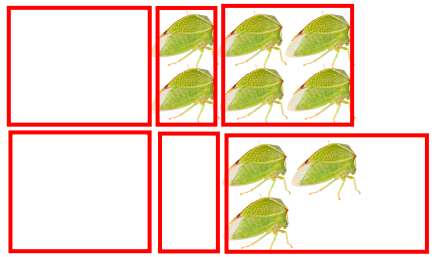


44 total, avg. 7.3/sleeve

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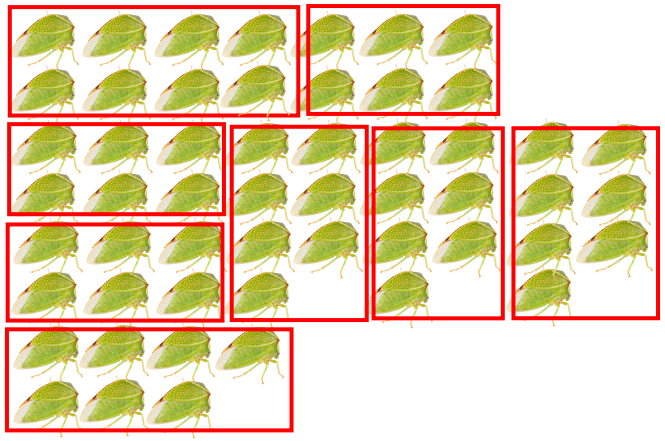


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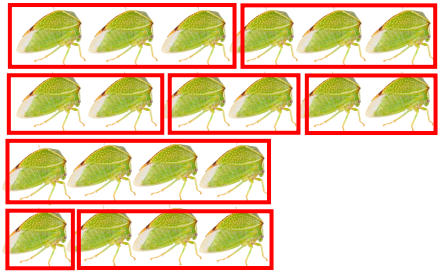
 # S. bisonia start of acquisition → *# S. bisonia* end of inoculation → GRBV+ Bodies → GRBV+ Heads

2025

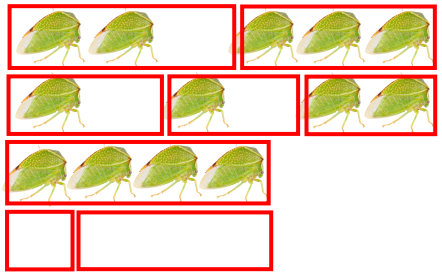


54 total, avg. 6.8/sleeve

37% survival



20 total, avg. 2.5/sleeve



13



4

Results: Grapevines

2024



0/6 GRBV+

1/3 months post-assay



0/6 GRBV+

8 months post-assay



0/6 GRBV+

1-year post-assay

2025



0/8 GRBV+



0/8 GRBV+



0/8 GRBV+

GRBV in *S. bisonia* salivary glands – but not in grapevines?

1. *S. bisonia* does not transmit GRBV
2. Not enough viral load
    - One salivary gland+ *S. bisonia* feeding on a grapevine petiole may have reduced chances of transmission
    - Virus titer too low to detect in standard PCR
        - If virus was present, should have replicated over time
3. Too many petioles for feeding choice
    - Should *S. bisonia* be tested with only one GRBV+/GRBV- leaf/ rep?



Future Directions

If grapes are not a preferred feeding host, is there a way to quantify feeding on grapes?

Can *S. bisonia* be reared?

Are other insects potential GRBV vectors?



Thank you!

AAFC Entomology Lab Technician:

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UNESCO Chair

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Text References

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7. Flasco MT, Hoyle V, Cieniewicz EJ, et al. The Three-Cornered Alfalfa Hopper, Spissistilus festinus , Is a Vector of Grapevine Red Blotch Virus in Vineyards. *Viruses*. 2023;15(4):927. doi:10.3390/v15040927
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Photo References

Slide 1-3: Marc Fuchs, Professor, Cornell AgriTech
Slide 4:
Girdles: Flasco MT, Hoyle V, Cieniewicz EJ, et al. The Three-Cornered Alfalfa Hopper, Spissistilus festinus , Is a Vector of Grapevine Red Blotch Virus in Vineyards. *Viruses*. 2023;15(4):927. doi:10.3390/v15040927
S. festinus: Johnny N. Dell, Iowa State University
Slide 5: Krenz B, Fuchs M, Thompson JR. Grapevine red blotch disease: A comprehensive Q&A guide. *PLOS pathogens*. 2023;19(10):e1011671-e1011671. doi:10.1371/journal.ppat.1011671
Slide 6: Mark Brown, Professor Emeritus, University of Georgia