

Bacterial Stem Blight (BSB) of Alfalfa: An under-recognized disease affecting forage yield and quality

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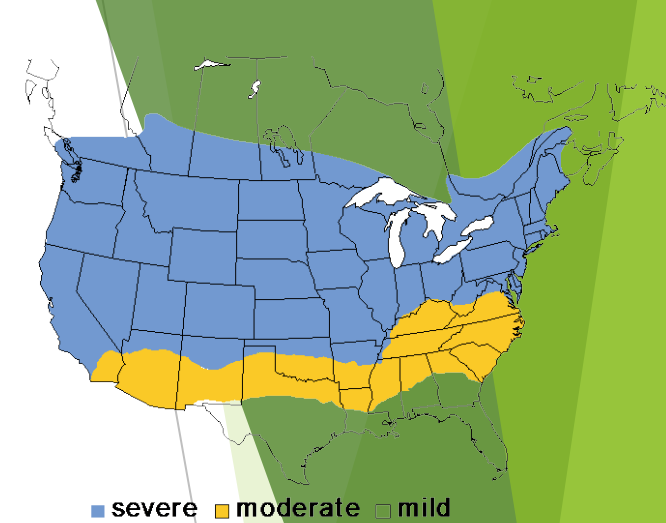
Outline

- ▶ Major bacterial diseases of alfalfa: how to identify BSB
- ▶ History of BSB in the US
- ▶ Pathogen characteristics and disease cycle
- ▶ Identifying resistance

Bacterial diseases of alfalfa

Bacterial wilt: *Clavibacter insidiosus*

Previous names: *Clavibacter michiganensis* subsp. *insidiosum*
Corynebacterium insidiosum



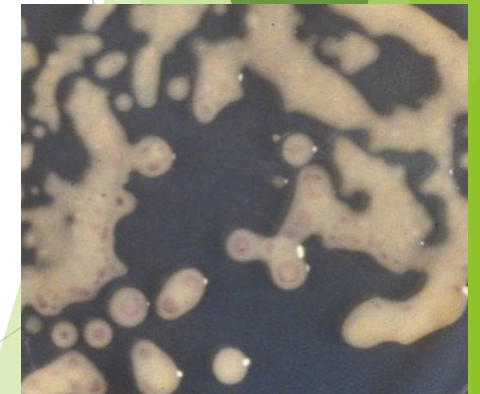
Stunting, yellowing, witch's broom
Seen in mid-late summer, older stands



Leaf cupping



Root vascular
discoloration



Bacterial colonies

Bacterial diseases of alfalfa

Bacterial leaf spot: *Xanthomonas euvesicatoria* pv. *alfalfae*

Previous names: *Xanthomonas alfalfa* subsp. *alfalfa*
Xanthomonas alfalfa
Xanthomonas campestris pv. *alfalfae*



Watersoaked, tan lesions, dark border, chlorosis.



Long stem lesions with bacterial exudate
Seen in hot rainy weather after windstorms.



Yellow rapidly growing colonies

Bacterial diseases of alfalfa

Bacterial stem blight: *Pseudomonas syringae* pv. *syringae* AND *Pseudomonas viridiflava*



Interveinal chlorosis



V-shaped necrosis



Stem lesions at nodes



Leaf loss

Symptoms in spring (before 1st harvest), after cold temperatures (near 32° F).

Bacterial stem blight identified in 1904



Bulletin 158

April, 1910

The Agricultural Experiment Station

OF THE

Colorado Agricultural College

UNIVERSITY OF ILLINOIS
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A BACTERIAL DISEASE OF ALFALFA

BY

WALTER G. SACKETT

The next year, however, 1900, the conditions were worse than ever before and the universal complaint among the farmers was that there was "something wrong with the alfalfa." Professor Paddock again visited Gypsum, and at this time the blackened stems were very abundant and much more conspicuous than when he was there before. He brought back specimens of this material to the

thousand acres, more than one half of which is in alfalfa.

The next year, however, 1906, the conditions were worse than ever before and the universal complaint among the farmers was

but they were unable to give any decisive answer as to the exact cause. Occasionally, worms were found in the crowns and in the roots, and by splitting the latter lengthwise, numerous dark streaks could be traced through the tissue. A few crowns were blackened, as well as some of the stems, but this discoloration was looked upon as due, possibly, to insect work, although no specific insects could

A BACTERIAL DISEASE OF ALFALFA.

By WALTER G. SACKETT.

Bacterial stem blight in Intermountain West

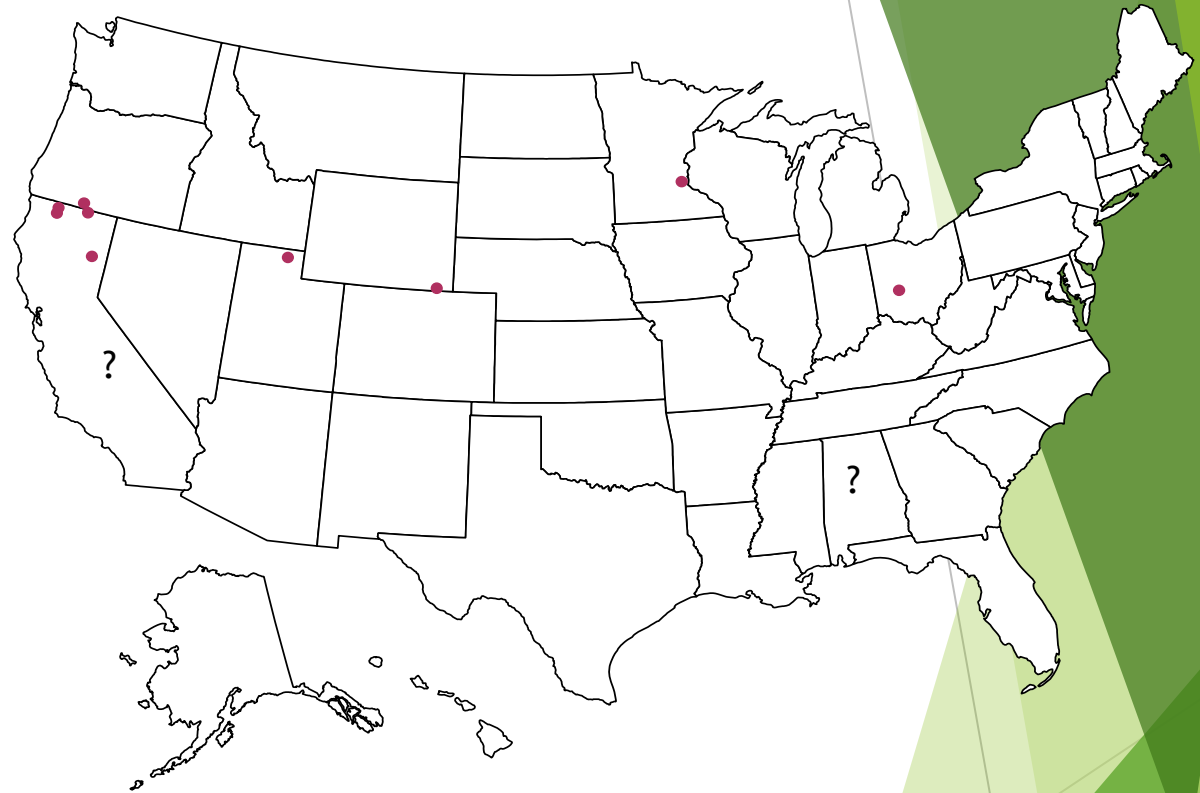
- ▶ 2014: Steve Orloff documented glyphosate injury on Roundup Ready alfalfa
 - ▶ Early spring glyphosate application followed by cold temperatures
 - ▶ Stunting and chlorosis
 - ▶ Possible increased frost damage
- ▶ 2016: *P. syringae* identified in symptomatic plants



Locations with Bacterial Stem Blight

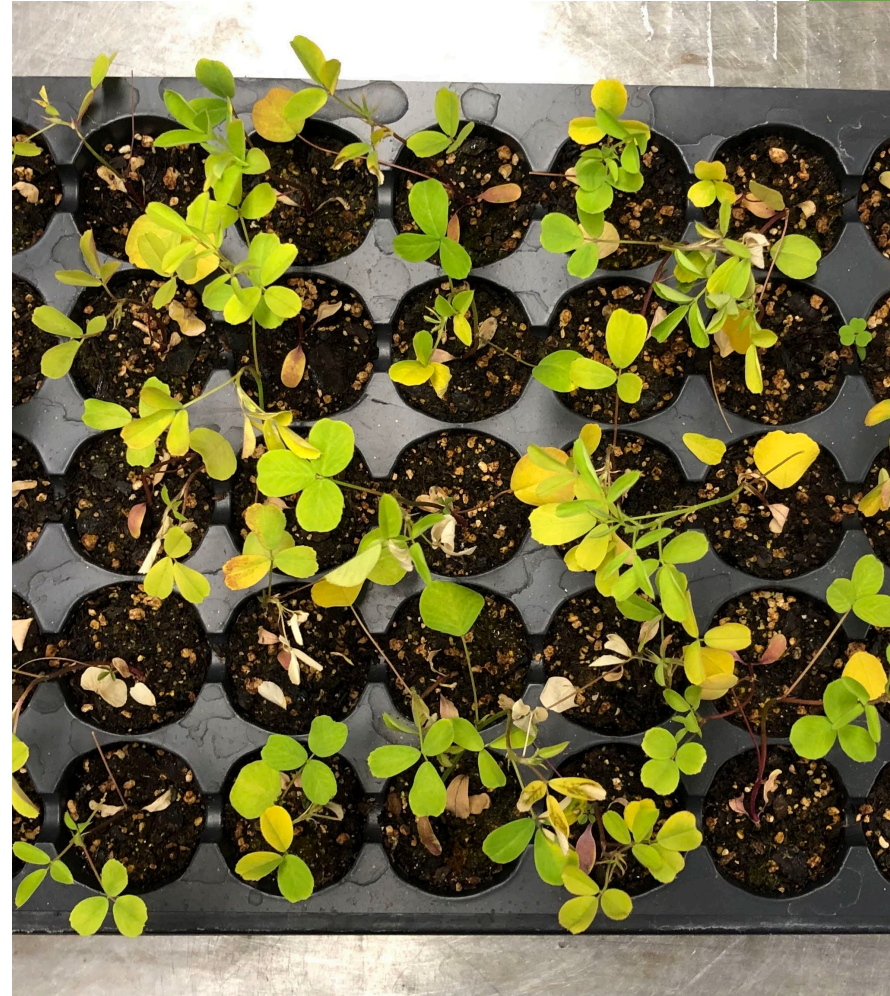
- ▶ Southern Wyoming: 2004
- ▶ Northern California: 2016
- ▶ Northern Utah: 2016
- ▶ Southern Oregon: 2019
- ▶ St. Paul, MN: 2019
- ▶ Western OH: 2019

- ▶ Five Points, CA: 2019; Possible (*P. viridflava*)
- ▶ Auburn, AL: 2021; Possible (symptoms)



Two bacteria cause bacterial stem blight

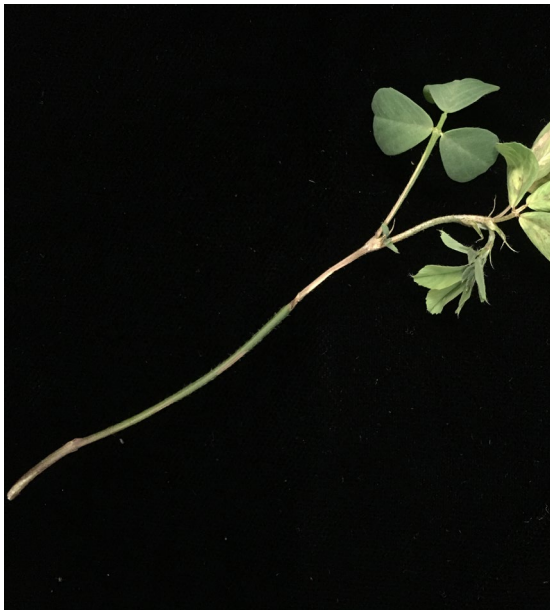
- ▶ *Pseudomonas syringae* pv. *syringae* (phylogroup 2b)
 - ▶ Wide host range
 - ▶ Ubiquitous in nature and agriculture
 - ▶ Plant toxins
 - ▶ Ice nucleation active



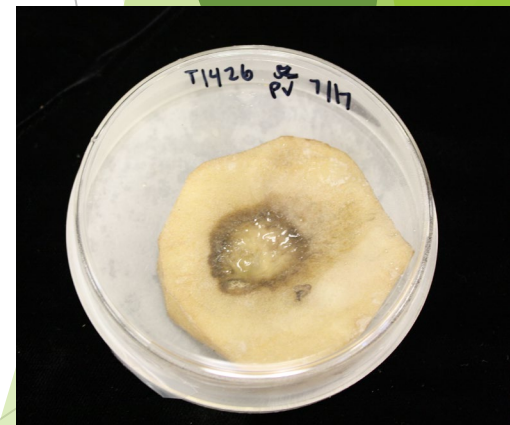
Two bacteria cause bacterial stem blight

Pseudomonas viridiflava (phylogroup 7a)

Lipps, S.M., Lenz, P., and Samac, D.A. (2019) First report of bacterial stem blight of alfalfa caused by *Pseudomonas viridiflava* in California and Utah. *Plant Disease*, 103, 3274.

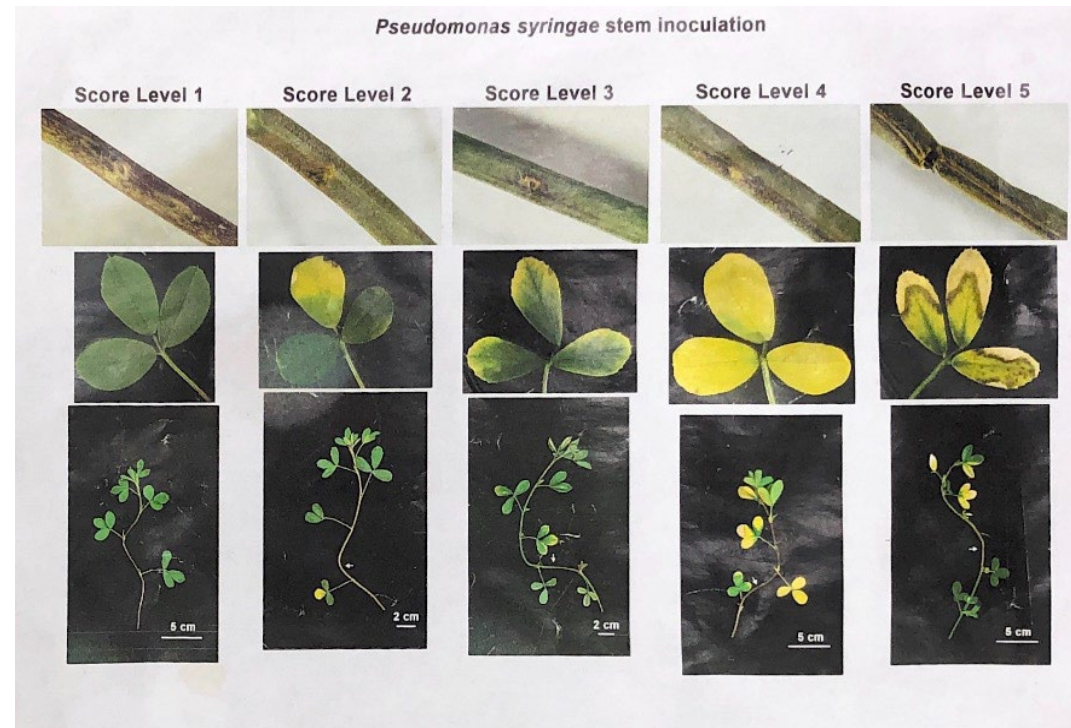


- Reported as crown rot pathogen of alfalfa.
- Little INA
- Strong cell wall degrading activity

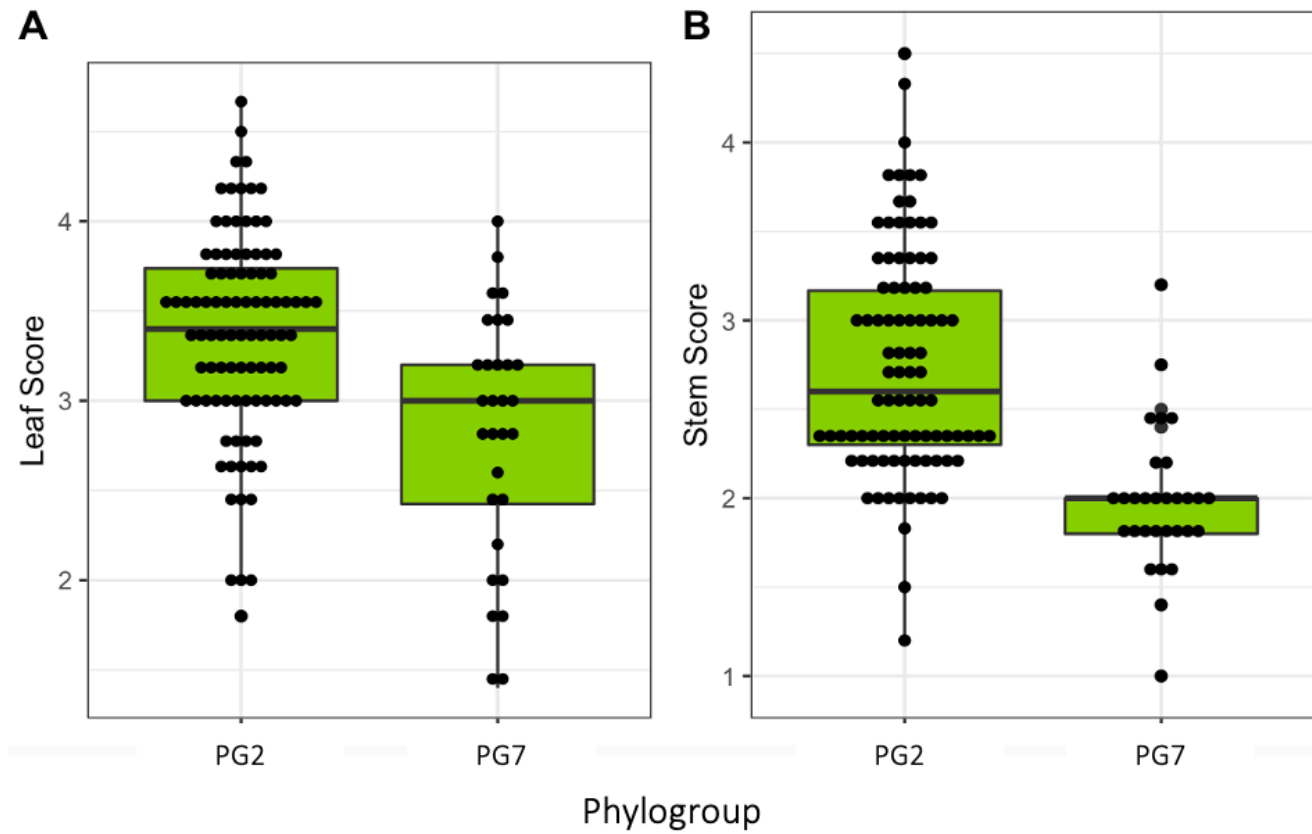


Pathogenicity Assay Methods

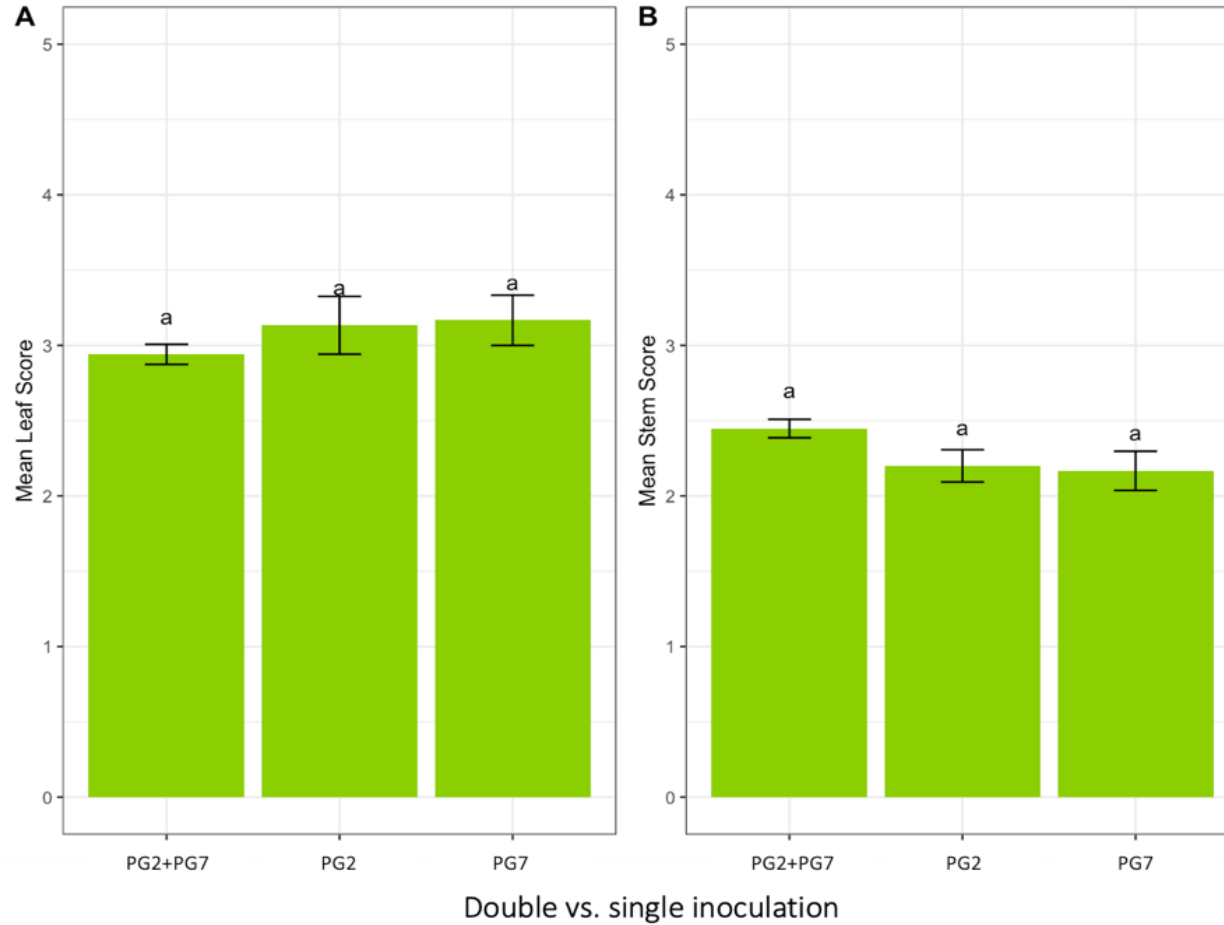
- Plants grown 3 weeks in 16-hour light cycle
- Stems wounded with a needle (to mimic frost damage wound) a bacterial suspension ($OD_{600}=0.1$ for single inoculations, $OD_{600}=0.05$ per strain for combined inoculum) was applied with a sponge
- Symptoms (leaf and stem) rated 7-10 days post-inoculation

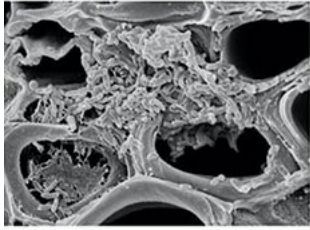


Results- single inoculations



Results- double inoculations





Bacteria in vasculature (endophytic stage)



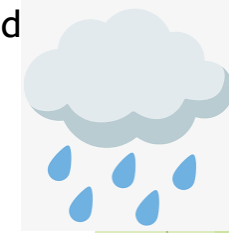
Systemic symptoms

P. syringae pv. *syringae*

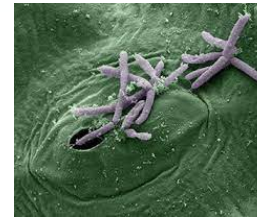


Infected plants or infected debris

Bacteria are rain splashed or wind borne



Bacteria multiply on plant foliage (epiphytic stage)



Frost damage creates entry wounds


Leaf blighting
Stem lesions



“My number one wish for alfalfa variety improvement is frost tolerance.”



Dennis Strom
Hay and Forage Grower
[https://hayandforage.com
/print-article-765-
permanent.html](https://hayandforage.com/print-article-765-permanent.html)



Can we increase frost tolerance in alfalfa by increasing resistance to bacterial stem blight?

Will resistance reduce bacterial populations so that frost damage is less likely to occur?

Identifying resistance to BSB

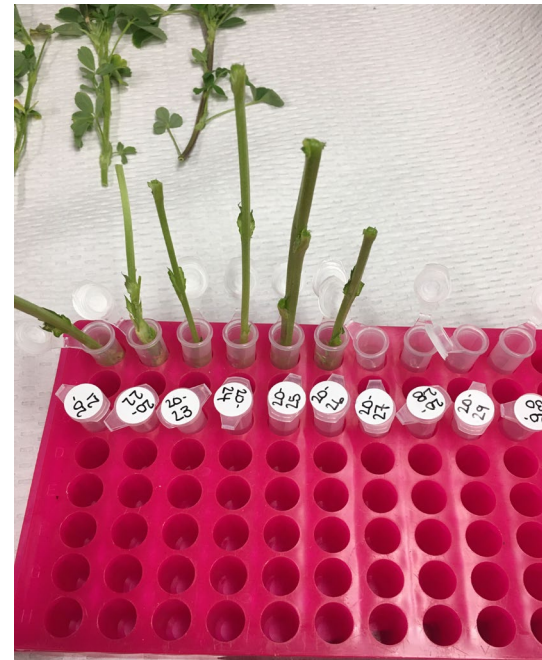
- ▶ Identified resistant and susceptible plants in Maverick and ZG9830
- ▶ Created F1 mapping populations
- ▶ Use genotyping by sequencing to identify DNA markers associated with resistance
- ▶ Resistance is not strain specific
- ▶ Resistant plants have lower endophytic bacterial populations



Check	Cultivar	Avg. Disease Severity	Percent Resistant Plants
FD1	Maverick	2.3	60
FD2	Vernal	2.7	36
FD3	5246	3.2	0
FD5	Archer	3.1	11
FD6	ABI700	3.7	0
FD7	Dona Ana	3.5	10
FD8	Pierce	3.5	18
FD9	CUF101	3.9	0
FD10	UC1887	4.1	0
FD11	UC1465	4.2	0
WS1	ZG9830	2.2	60
WS2	5262	2.8	33
WS3	WL325HQ	3.0	17
WS4	G-2852	3.6	0

BSB and glyphosate on Roundup Ready Alfalfa

- ▶ Tested effect of glyphosate and bactericide (Kocide DF + Manzate Max)
 - ▶ 2019, 2020
 - ▶ 4 locations
 - ▶ Yield, injury, stem height, disease, internal and external bacterial populations



Effect of glyphosate vs. control: 2020

Location-pressure	Yield	Stem height	Injury	Leaf disease	External population	Internal population
Tulelake-High	↔	↓	↑	↔	↑	↔
Scott Valley-High	↓	↓	↑	↔	↑	↔
Utah-High	↓	↔	↔	↔	↔	↔
Susanville-Low	↔	↓	↑	↔	↔	↔

Glyphosate did not affect BSB symptoms but did increase external populations and freezing of stems in some locations.

Effect of glyphosate + bactericide vs. glyphosate: 2020

Location-pressure	Yield	Stem height	Injury	Leaf disease	External population	Internal population
Tulelake-High	↔	↑	↔	↔	↓	↓
Scott Valley-High	↑	↑	↔	↔	↓	↓
Utah-High	↑	↔	↔	↔	↓	↔
Susanville-Low	↔	↔	↔	↔	↔	↔

Bactericide decreased effect of glyphosate on yield and height, decreased external and internal populations in some locations. No effects on disease scores.

Some of the injury from late spring glyphosate application can be attributed to BSB.

BSB and glyphosate on Roundup Ready Alfalfa

- ▶ Controlling winter weeds in established alfalfa
 - ▶ Spring application should be made when alfalfa plants are less than 4 inches high
 - ▶ Application to larger plants risks frost and disease damage, stunting
 - ▶ Yield losses up to 0.8 t/A
 - ▶ Second harvest recovers



Steve Orloff



Tulelake, May 2016



Ohio, May 2019

Summary

- ▶ *Pseudomonas syringae* on alfalfa promotes frost damage by causing ice formation.
- ▶ Bacteria then enter the plants and cause additional damage (bacterial stem blight).
- ▶ *Pseudomonas syringae* and *P. viridiflava* cause BSB symptoms but do not have a synergistic interaction.
- ▶ Standard test developed for identifying resistance to BSB in alfalfa.
- ▶ Resistance identified in 'Maverick' (FD1) and 'ZG9830' (WS1) checks while FD9, 10, 11 checks have no resistance.
- ▶ Glyphosate did not affect BSB symptoms but did increase bacterial populations and freezing of stems in some locations.
- ▶ Some of the injury from late spring glyphosate applications can be attributed to BSB.