Bacterial Stem Blight of Alfalfa: Connection with Frost Damage, Development of Resistant Germplasm, and Mapping Resistance Genes

Deborah A. Samac[1]; Long-Xi Yu[1]; Rob Wilson[2]
[1]USDA-ARS, [2]University of California

ABSTRACT

Alfalfa is susceptible to frost damage once it breaks dormancy. The risk of frost damage increases when bacteria with ice nucleation activity occur on foliage. Often associated with frost damage is damage from bacterial stem blight (BSB), caused by the ice nucleation-active bacterium *Pseudomonas syringae* pv. *syringae*. The bacterium promotes frost formation, penetrates stems at frost injury sites, and subsequently decays leaves and wilts stems. Currently there are no cultivars with resistance to BSB and little is known about the disease in the field. Our goal is to understand the epidemiology of this disease and develop tools for reducing losses due to the disease. The project has the following objectives:

(1) Obtain information on the extent of bacterial stem blight damage occurring in commercial alfalfa production fields, the association of disease with frost damage, and the relationship of disease with bacterial populations.
(2) Identify DNA markers and candidate genes associated with disease resistance loci.
(3) Develop germplasm with enhanced resistance to bacterial stem blight.

The research will help ascertain how common the disease is in frost-prone areas and will help educate alfalfa researchers and producers on the importance of this disease, how to recognize it, and ultimately how to avoid it. This research should improve yields in frost-prone areas, which represent a significant percentage of the alfalfa acreage nationwide.

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Need for Research

Alfalfa is vulnerable to frost damage, but the cause of a recent surge in frost damage in the Intermountain West on alfalfa still needs to be elucidated. Some bacteria residing on plants makes them more susceptible to freezing damage by initiating the formation of ice. Associated with frost damage is damage from the disease bacterial stem blight (BSB), caused by an ice nucleation-active bacterium. The bacterium promotes frost formation, penetrates stems at frost injury sites, and subsequently decays leaves and wilts stems.

This research is necessary to understand the disease cycle and develop tools for reducing losses due to the disease. We will be conducting surveys to determine the extent of the disease and to educate growers about the disease and the relationship with frost damage. We will perform experiments to determine the source of the pathogen and follow population growth over several years. This information will be valuable for determining when protective action can be taken to reduce bacterial populations.

One of the most effective means to combat plant diseases is genetic resistance. We will identify the regions in the alfalfa chromosomes that have genes for resistance to the disease and develop markers that will allow plant breeders to quickly and efficiently identify plants with resistance. We will develop methods for selecting disease resistant plants and develop novel germplasm with resistance to the disease.

The research is necessary to understand how common the disease is in frost-prone areas and will help educate alfalfa researchers and producers on the importance of this disease, how to recognize it, and ultimately how to avoid it. By creating BSB resistant cultivars, we can provide improved frost tolerance to producers. This research should improve yields in frost-prone areas, which represent a significant percentage of the alfalfa acreage nationwide.

Products of Research

- Disease identification guide
- Knowledge of distribution of disease and damage caused
- Characterization of the pathogen
- New plant germplasm with disease resistance
- A standard test for selection and characterization of alfalfa germplasm with disease resistance
- Peer-reviewed journal articles
- Students trained in molecular plant breeding

Bacterial Stem Blight (BSB)

Figure 1. Foliar blight symptoms.

- Leaves become water soaked, followed by irregular patches of chlorosis and necrosis
- Stem lesion become amber colored with dried bacterial exudate that blackens with age
- Associated with frost damage due to ice nucleation activity of pathogen
- Can reduce forage yield by 50%

Figure 2. Stem lesions and wilt symptoms.

Figure 3. Chlorosis, wilting, and stunting symptoms in the field.

Pseudomonas syringae pv. *syringae*

- Broad host range pathogen with over 200 hosts
- Found world-wide
- Ice nucleation-active that causes frost to form at higher temperatures
- Capable of long-distance aerial movement
- The bacterium penetrates alfalfa stems primarily at frost injury sites

Epidemiology of BSB

- Newly established alfalfa in two locations in northern California with a history of bacterial stem blight will be monitored for *P. syringae* populations over time.
- Leaves, stem internodes, and stem nodes will be processed separately to determine the primary location of *P. syringae* on alfalfa foliage.
- Rainwater collectors will be placed in several locations within the test plots. Filter to collect bacteria and extract total DNA. Use DNA in quantitative PCR assays specific for *P. syringae*.
- Weather data will be recorded throughout the season to enable correlations between environmental conditions, pathogen populations, and disease development.

Resistant hypersensitive response.

Susceptible spreading lesion 5 days post-inoculation.

Figure 4. Infiltrating leaflets with *Pseudomonas syringae* pv. *syringae* quickly identifies resistant and susceptible plants.

Resistance Gene Mapping

Resistant and susceptible plants were identified in ‘Maverick’ and ‘ZG9830’

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Biparental F1 mapping populations were developed in each genetic background

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Phenotype F1 plants

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Use genotyping by sequencing (GBS) to identify disease resistance loci

Development of Resistant Germplasm

- Use phenotypic recurrent selection with a target of ≥ 50% resistant plants.
- Use three broad germplasm sources varying in fall dormancy as source germplasms.
- Plants will be inoculated twice and resistant plant retained and intermated.
- Seed will be collected by female parent and mixed in equal proportions.
- Plants from the mixed seed will be screened for resistance to a mixture of strains.