Bacterial Stem Blight of Alfalfa: An Under-Recognized Disease Affecting Forage Yield & Quality

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Bacterial stem blight (BSB) of alfalfa, was first reported in 1904. However, most alfalfa producers and researchers did not recognize it as a production problem until 2016 when Steve Orloff started documenting widespread injury fitting the disease symptoms in Northeast California. Recent observations of damage from BSB in several states especially following a spring Roundup application to Roundup Ready alfalfa at the 6 to 12-inch growth stage suggests it is an emerging disease problem on alfalfa that can reduce forage yield. The pathogen causing BSB is caused by the ice-nucleation active bacterium *Pseudomonas syringae* pv. *syringae*, and disease was usually associated with frost damage. Our goal was to understand the disease cycle and develop tools for reducing losses due to the disease.

Methods were developed to identify alfalfa stems that had damaging levels of ice-nucleation active bacteria using a freezing assay. Bacteria were isolated from damage positive samples and a collection of 456 isolates were made from diseased plants in California, Utah, Oregon, Minnesota, and Ohio. A second bacterium, *P. viridiflava*, was shown to cause symptoms of BSB, but generally lacked ice nucleation activity. Phylogenetic analyses of 123 strains found that the *P. syringae* pv. *syringae* and *P. viridiflava* strains do not cluster by location, which serves as evidence that the populations are widespread and have a long history of association with alfalfa. Some indications for founder populations were identified, suggesting that the more recent epidemics could have been initiated from contaminated seeds. The high frequency of ice nucleation indicates that *P. syringae* pv. *syringae* moves in precipitation and would readily cause frost damage on alfalfa on which it is growing. Complete genome sequences of 12 strains of *P. syringae* pv. *syringae* and eight strains of *P. viridiflava* were assembled and will form the basis of understanding pathogenicity on alfalfa.

Field studies were conducted over two years in four locations to test the effectiveness of bactericides for reducing damage on Roundup Ready cultivars from BSB. Bactericide applications reduced disease symptoms and reduced bacterial populations measured by qPCR assays in some locations and years, but bactericides did not influence first cutting alfalfa yield. Roundup treatments did not increase internal or external populations. Seeding year stands had high levels of the pathogen indicating that the bacterium colonizes plants rapidly. Most locations had high levels of the bacterium that were pathogenic on alfalfa.

Reproducible greenhouse inoculations methods based on infiltration of leaves were developed for evaluating disease resistance. Resistant (ZG9830-21) and susceptible (ZG9830-25) plants from the cultivar ZG9820 were identified and used to create an F1 mapping population. Phenotypic and genotypic characterization of ~300 plants segregating for resistance to BSB identified QTLs and candidate genes for disease resistance. The standard tests for plant inoculation and DNA markers associated with resistance will be valuable tools for developing disease resistant cultivars.