Spring Black Stem & Stemphylium Leaf Spot Resistance Screening in the USDA-ARS National Plant Germplasm System's *Medicago spp.* Genetic Resources

USDA-Agricultural Research Service- Irish

Project Award: $47,000

Justification:

- Alfalfa (Medicago sativa L.), is the fourth largest agricultural crop by planting area in the U.S. and is the most important forage legume crop in the world. Most alfalfa in the U.S. is grown in the Midwest with significant contributions from the north-eastern and irrigated western states. Despite its importance to the U.S., alfalfa and its close relatives, are not native and originally were domesticated and introduced from Central Asia, North Africa and parts of Europe. Modern day cultivated alfalfa in the U.S. was predominantly introduced from nine world regions and the trend to increase genetic diversity over the years has made the alfalfa crop less uniform and less susceptible to emerging pests (Barnes et al., 1977).

  The USDA-ARS National Plant Germplasm System (NPGS) Temperate-adapted Forage Legume (TFL) germplasm collection consists of over 13,000 accessions. Collection accessions represent mostly major crops species (e.g., alfalfa, red clover), but also includes many of their close crop relatives. Within the TFL collection, the Medicago genus, and alfalfa specifically, are represented by the largest number of accessions. Currently, there are 3,063 active alfalfa accessions in the collection and other closely related subspecies account for a significant number of accessions: M. s. subsp. falcata (454), subsp. varia (436) and caerulea (97). The TFL accessions are utilized by the stakeholder community to mine for traits useful in selection and breeding and in basic and applied research programs. An excellent example of the value of alfalfa germplasm comes from the identification and subsequent introgression of glandular hairleaf hopper resistance derived from several plant introduction (PIs) and related Medicago species (Bauchan and Green, 2001). The aim of the TFL project is to continue strategic acquisition, and to safeguard the collection making it available for distribution to the stakeholder community.

  Another role the TFL project plays is in the logistics of managing alfalfa Standard Check varieties/ inventories and their distributions. Standard Checks varieties are recommended by the industry and public research community to be utilized in Standard Test assays as references for comparison. As stocks run low, industry collaborators increase seed for future distributions. Regrettably, over time multiple inventories (e.g., different [year/source] increase seed lots) seem to have accumulated for several Standard Checks and reports of inconsistent results in Standard Tests have surfaced (M. Smith, pers. comm.). The identification of true-to-type varieties and corresponding inventories (e.g., lots) which perform correctly in Standard Tests was recently proposed during a joint Alfalfa Crop Germplasm Committee and Standard Test committees at the 2018 NAAIC meeting in Logan, UT. In addition, optimizing and possibly improving the Standard Tests would be useful. In some cases, species that cause alfalfa diseases are not clearly defined and isolates need to be tested for pathogenicity and virulence. Furthermore, a possibility exists that in some Standard Tests high spore concentrations in inoculations might be overwhelming plants and masking resistance (D. Samac, pers. comm.).

  Utility of plant germplasm in the collections increases as information associated to specific accessions is collected and made available (Byrne et al., 2018). An important TFL project goal is to characterize and evaluate germplasm collection accessions. If information, such as biotic or abiotic stressors and/or insect resistance, is associated with unique germplasm subsets these could be targeted by researchers

during requests. Some Medicago spp. accessions in the collections have characterization and/or evaluation data for important traits, however many accession lack this information. For example, between 1983 and 1991 close to 757 alfalfaaccessions were screened for leaf spot disease resistance by K. Leath. No resistant ratings (≤ 2; low number = more resistant on a 0-5 scale) were reported by Leath and only a few accessions showed moderate resistance 3.08 and 3.15 to spring black stem (SBS) and Stemphylium leaf spot (SLS), respectively. However, 3,063 alfalfa accessions are available many of which have never been screened. Stakeholders routinely performed germplasm evaluations, but many times this information is not shared. In-house characterization and evaluation efforts are routine, and data can be quickly made public. The NPGS makes the germplasm, and its associated information, easy to access through the Germplasm Resources Information Network (GRIN)-Global database and in other public and research mediums.

SBS is caused by an ascomycete fungus Phoma medicaginis and is an important pathogen of alfalfa (and its relatives) that affects plants mostly in temperate growing regions. Symptoms of the disease appear during cool, wet weather in the spring. The disease affects all plant parts, although it is most common on leaves and stems where it reduces dry weight and quality. New infections arise from pycnidia that overwinter in crop residue and pycnidial spores are dispersed by splashing water. Disease is polycyclic and infections can occur anytime environmental conditions are ideal (e.g., spring and fall). The organism has been reported to infect other legume crop species and may survive in soil on infested debris and even seed. Cultural management strategies have been to alternate to non-host crops and to harvest early when infection occurs to avoid losses. Limited information exists on fungicide application efficacy and varieties with good disease resistance are not available (Samac et al., 2015).

Another important leaf spot disease in alfalfa hay and seed production is SLS. The disease is caused by up to five reported species of Stemphylium, with S. botryosum most frequently associated with infections. In the U.S. disease occurs mostly in the Midwest (WI, MN) and the Pacific Northwest during wet periods of the year. Two biotypes of S. botryosum, warm (W-T) and cool (C-T), occur and can be distinguished by the temperate at which they infect the host and cause pathogenicity. Both biotypes reduce forage quality and in severe infestations the W-T can cause leaf defoliation affecting yield. The fungus survives through winter on seed, leaves and stems with primary inoculum being dispersed through ascospores in the spring. The disease is found more prevalently in the warm summer and fall months and disease management focuses on using low-to moderate disease resistance for the C-T biotype (Samac et al., 2015).

Objectives:

- The objectives of this project are to 1) Optimize inoculation protocols for fungal species causing SBS and SLS diseases of alfalfa; 2) Systematically screen through alfalfa germplasm for disease resistance to SBS and SLS; 3) Define host range for SBS and SLS diseases in subsets of representative Medicago spp. taxa; 4) Evaluate Standard Check variety and inventories for reaction to SBS and SLS diseases; and 5) Make data, and associated information, publicly available through presentations, publications and through the USDA-ARS National Plant Germplasm System's GRIN-Global database.