

Spring black stem and leaf spot resistance screening in the USDA-ARS National Plant Germplasm

System's *Medicago* spp. genetic resources. Brian M. Irish¹, Lyndon Porter², Garrett Heineck³, and Deborah Samac⁴. 1) USDA-ARS PGITRU, Prosser, WA 99350; 2) USDA-ARS GLGPRU, Prosser, WA 99350; 3) USDA-ARS NSARU, Prosser, WA 4) USDA-ARS PSRU, St. Paul, MN 55108.



Introduction & Objectives

Alfalfa is the most important forage legume crop in the world and is used extensively in the U.S. as a hay crop destined for the dairy industry. Much of the alfalfa in the U.S. is grown in the Midwest where spring blackstem and leaf spot (SBS), caused by *Phoma medicaginis*, occurs on many of the modern-day susceptible cultivars (Samac et al., 2015). The USDA National Plant Germplasm System (NPGS) conserves close to 4,000 alfalfa germplasm accessions and many close relatives. In efforts to identify potential sources of disease resistance that could be useful in breeding, the following objectives were addressed:

- Optimization of inoculation protocols SBS of alfalfa;
- Evaluate Standard Check cultivars for reaction to SBS
- Define SBS host range in subsets of *Medicago* spp. taxa;
- Systematically screen alfalfa germplasm for SBS disease reaction;
- Make data, and associated information, publicly available



Figure 1. L-R: Two-week old healthy alfalfa seedlings prior to inoculation; dew chamber used for incubation post-inoculation, and; susceptible seedling (rating ~4.5) 10 days post inoculation with *P. medicaginis*.

Methods

Methods followed the NAAIC-published Standard Tests protocol for “Spring Blackstem and Leafspot Resistance” (Leath and Salter, 1991) with optimizations. Adjustments included 1) reducing inoculum spore concentrations considerably (to - 5×10^4 spores/ml) based on serial dilution assays; 2) adding water agar Petri dishes to check for spore viability and 3) developing a detailed modification of the original 1-5 rating scale to incorporate half ratings that better reflected symptoms (**Figs. 1 & 2**). A total of 8 isolates were tested for pathogenicity and one that was identified as moderately aggressive was chosen for subsequent evaluations.

Score	Class	Description ¹
1.0	Resistant	No lesions, chlorosis, or defoliation. Plant is symptom-free.
1.5	Resistant	5 or less pinpoint lesions (~0.25 mm), with <5% leaflet area affected. No chlorosis, stem/petiole lesions or defoliation. Plant is mostly symptom-free.
2.0	Resistant	>5 pinpoint to small lesions (~0.25 to 0.5 mm), with 5-10% leaflet area affected. None to very mild chlorosis. No lesions on stem/petioles or defoliation.
2.5	Moderately resistant	Small lesions (~0.5 mm), 11-20% of leaflet area affected. None to mild chlorosis. Few small stem/petiole lesions may be present. No defoliation. Plant rated overall between resistant and susceptible.
3.0	Susceptible	Small to medium sized lesions (0.5-2 mm), 21-30% leaf area affected. Mild to moderate chlorosis. Small stem/petiole lesions may be present. Single leaflet defoliation. Overall plant is mildly susceptible.
3.5	Susceptible	Medium sized lesions (1-2 mm), 31-40% leaflet area affected. Mild to moderate chlorosis. Mild stem/petiole lesions, no girdling of stem, but may have petiole girdling. One/two leaflet and/or petiole defoliations. Overall plant is moderately susceptible.
4.0	Susceptible	Medium to large sized lesions (>3 mm), with 41-50% leaflet area affected. Moderate chlorosis. Mild to moderate stem/petiole lesions, girdling of petioles may occur. Mild to moderate defoliation of leaflets and abscission of petioles. Overall plant is above average in susceptibility.
4.5	Susceptible	Medium to large-sized lesions with >50% leaflet area affected. Extensive chlorosis. Moderate to severe stem and/or petiole lesions. Moderate defoliation of leaves and/or girdling on multiple petioles. Overall plant is highly susceptible.
5.0	Susceptible	Many large lesions, extensive chlorosis, severe defoliation. Overall plant is in extremely susceptible or dead.

¹New rating scale and description takes into consideration lesions size, % leaflet area affected, chlorosis, stem/petiole lesions as well as petiole and leaflet abscission

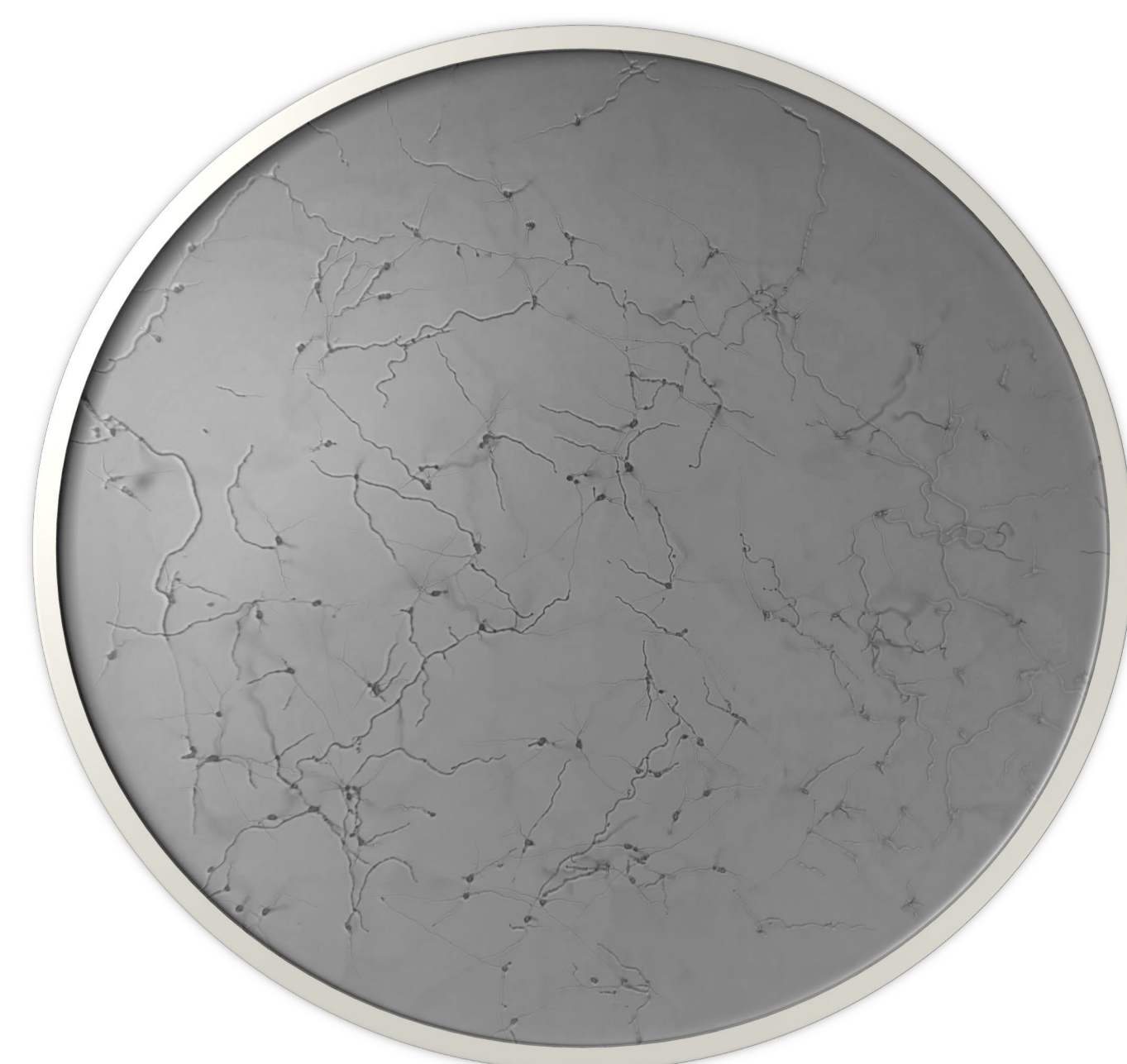
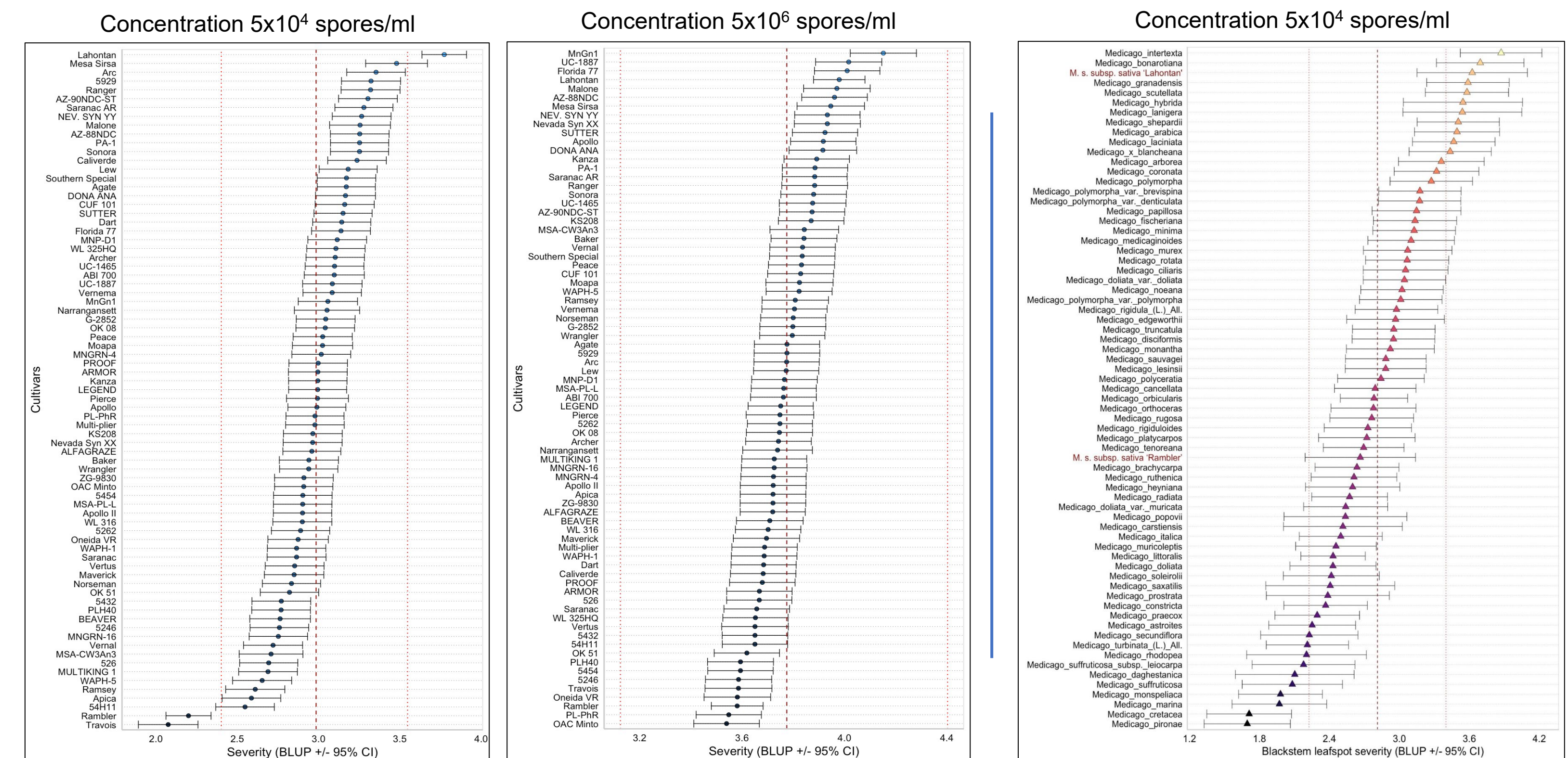


Figure 2. Proposed and utilized optimized SBS rating scale (1-5) with half-scale values and Petri dish germination of *P. medicaginis* conidia after incubation.



Estimated mean disease reaction ratings to *P. medicaginis* for alfalfa standard check cultivars at **a)** low and **b)** high spore concentrations and **c)** *Medicago* spp. taxa. Analyses used best linear unbiased predictions (BLUP) along with 95% confidence intervals and a modified 1-5 rating scale.

Results

- At reduced inoculum concentrations (5×10^4 spores/ml), disease reaction differences across accessions was more evident (**Fig. 3a/b**);
- Rating scale modification improved resolution and better captured observed symptoms at scoring;
- A few check cultivars were more resistant than the recommended moderately resistant check in the standard test protocol;
- Several alfalfa relative taxa were considerably more resistant (<2.0) than much of the alfalfa germplasm being screened (**Fig. 3c**);
- Much of the USDA NPGS alfalfa germplasm (>2,500 accessions) was screened with several accessions showing low disease ratings
 - e.g., PI 315478 (mean rating; 1.3) and PI 467885 (mean rating; 1.4)

Discussion

Identifying sources of disease resistance to SBS has been a high priority as modern cultivars lack this trait. Modified protocols optimized in our research and subsequent germplasm screening has identified plant selections that are being used in the development of SBS resistant pre-bred alfalfa germplasm (**Fig. 4**). The germplasm, and its associated information, will become freely available through NPGS.



Figure 4. Crossing block of SBS disease resistant alfalfa (*Medicago* spp.) plant selections used in pre-breeding efforts.

Acknowledgements



Special recognition goes to technical staff who play important roles in project implementation. This project was supported by funding from the U.S. Alfalfa Farmer Research Initiative program and by USDA-ARS National Program 301.

References: Slater and Leath, 1991. Spring Blackstem and Leafspot Resistance Standard Test Protocol - www.NAAIC.org; Samac et al., 2015. Compendium of Alfalfa Diseases. APS, St. Paul, MN.