Brown Root Rot Resistance

Phoma sclerotioides G. Preuss ex Sacc.

F.A. Gray, C.R. Hollingsworth, R.W. Groose, C.J. Reedy and R.C. Larsen

PLANT CULTURE

Greenhouse

Container ..... Stuewe and Sons, Inc., Tall One Treepots™ (top width = 10 cm, depth = 36 cm, volume = 2.83 L) (http://www.stuewe.com).
Media..... Pasteurized soil (1 part sand/1 part sandy loam soil).
Temp/Light..... 21C/15.6C (day/night); 12 to 16 hour day length.
No. of Plants ... One plant per pot and 24 plants per rep.
Planting..... Pregerminate seed and treat with *Rhizobium meliloti* prior to planting (http://www.nitragin.com).
No. of Reps..... 3 to 5 replications.
Other..... Since these pots are unstable, some type of support will be required. We have used metal milk racks which hold 12 pots each.

FUNGAL ISOLATION AND INOCULUM PRODUCTION

Source..... Diseased roots. Diseased roots in plastic bags can be stored under refrigeration (4C) for 1 month.
Isolation..... The fungus can be isolated from diseased root tissue placed in water agar using standard isolation techniques. Plates must be incubated at 10C.
Production ..... Culture fungus in Petri plates containing potato dextrose agar maintained at 10C. Place barley seed in a glass beaker, add water (250cc seeds/130 ml water in 600 ml beaker), cover with aluminum foil and autoclave for 60 min, allow to cool overnight and re-autoclave for an additional 60 min. When cool, transfer 2 mycelial plugs on to moistened seed and re-secure aluminum foil with parafilm. Maintain at 10C for 2 months. Following thorough colonization of barley seed, remove and spread out on clean surface and allow to air dry.
Storage..... Place barley seed inocula in plastic bags and store in freezer at -14C. Barley inocula should remain viable for up to 24 months.

INOCULATION PROCEDURE

Age of Plant.... 4 to 6 months old.
Type of Inoc ... Infected barley seed containing mycelium, pycnidia and conidia.
Amount..... 2 infected barley seeds/plant. Additional inocula should increase disease development.
Procedure..... Place barley seeds against the upper tap root, approximately 2.5 cm below the soil surface. Cover inocula with a plug of sterile cotton and replace soil. Plant injury is not required.

INCUBATION

Field Plot

Culture ..... After inoculation plants should be placed outside in the field in late summer to early fall. Plants are placed on top of the ground and surrounded by some type of insulation such as bales of hay to prevent roots from freezing. Root infection has been reported to occur during host dormancy from early fall to early spring. Plants should be watered as needed to maintain soil moisture.
Evaluation ..... Plants are left outside through the winter months. Plants can be evaluated for root rot in late spring to early summer. The disease is inactive during the warm summer months. An additional year will result in increased disease severity. Since the suggested standard test requires plants to be placed in field plots over one-half of the year, results may vary from year to year depending on local weather conditions.

RATING PLANTS

Field Plot

Evaluating plants for severity of root rot. Partially remove plants from pots, remove soil from upper 10 cm of root, and rinse under the spigot. Rate roots for disease response on a scale of 1-5 described below:

1 — No disease, roots healthy.
2 — Slight disease, root tissues exhibit localized discoloration.
3 — Moderate disease, well-defined lesions on root.
4 — Severe root rot, plant deterioration.
5 — Acute root rot, plant dead.

Alfalfa populations can be characterized for reaction to Brown root rot by the percent of resistance plants (1s and 2s).

CHECK CULTIVARS

Disease Reaction	Resistant Plants (%)*			
	Expected		Acceptable Range	
	Yr 1	Yr 2	Yr 1	Yr 2
Highly Resistant				
Peace (Canada Dept. of Ag.)	70	50	60-80	40-60
Moderately Resistant				
Multi-plier (Mycogen Seeds)	20	5	10-30	0-10

*Incidence and severity of BRR should increase with increasing number of test years. Peace should be Highly Resistant for years 1 and 2, while Multi-plier should be Moderately Resistant in year 1 and Susceptible in Year 2.

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CORRELATION TO FIELD TRIALS

A non-destructive forage yield trial, including the two BRR check cultivars, was conducted at a site in Wyoming naturally infested with *P. sclerotioides* (*P.s.*). Plant stands after three years were 86% for Peace and 16% for Multi-plier. An on-going repeat of the previously described trial conducted at a nearby *P.s.*-infested site, has produced similar but less dramatic results. Plant stands after three years were 97% for Peace and 67% for Multi-plier. A newly established third field trial, including the two standard check cultivars and our BRR-resistant breeding population, is currently underway at a nearby site. Plants will be removed from each replicate plot after one and two years and rated for BRR severity. Results will be compared to our previous experiments using sterile inocula.

PATHOTYPES

Pathotypes of *Phoma sclerotioides* have not been reported. Studies by the authors have shown one Canadian (ATCC #56515) and 13 Wyoming isolates of *P. sclerotioides* to all be pathogenic on alfalfa.

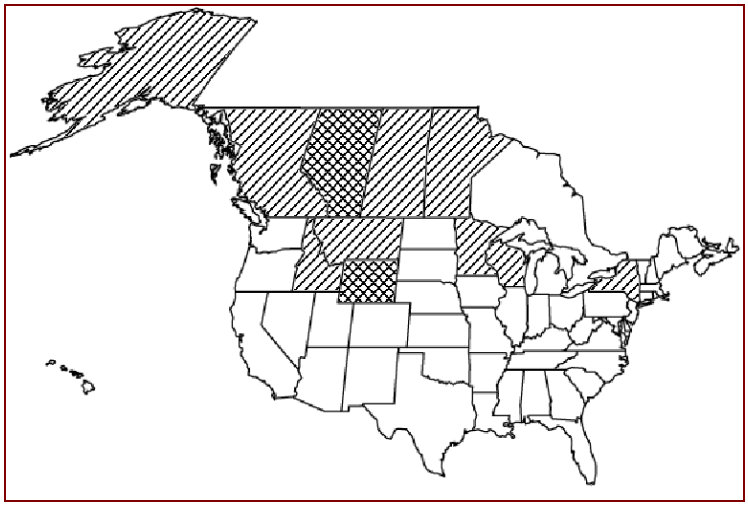
SOURCES OF INOCULUM

Barley grain inocula of Wyoming Isolate #2, for use in the establishment of *P. sclerotioides* cultures, is available on a limited basis from the senior author. A USDA/APHIS permit is required in states where BRR has not been reported. This isolate is also available from the American Type Culture Collection (ATCC), accession number ATCC #MYA-295 (http://www.atcc.org).

HELPFUL INFORMATION

BRR is widespread in the Canadian Province of Saskatchewan. It also has been reported from the provinces of Alberta, British Columbia and Manitoba, as well as from the state of Alaska. In 1997, it was reported in Wyoming where it has been found to be widely distributed. It was also found in east central Idaho and has been reported from Montana. Most recently it was found in Wisconsin, Minnesota and New York. The development of a PCR based SCAR probe for *P. sclerotioides* will undoubtedly prove helpful in determining the distribution of this root pathogen in the U.S.

DISTRIBUTION AND SEVERITY OF BROWN ROOT ROT



Crossed line fill = states or provinces where extensive surveys have been conducted or multiple reports have occurred. Single line fill = states or provinces where BRR has been found but an extensive survey has not been made.

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