

Brown Root Rot Resistance

Phoma sclerotoides G. Preuss ex Sacc.

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PLANT CULTURE

- Container Stuewe and Sons, Inc., Tall One Treepots™ (top) width = 10 cm, depth = 36 cm, volume = 2.83 L) (<http://www.stuewe.com>)
- Plant Growth
- Medium Pasteurized soil (1 part sand/1 part sandy loam soil)
- Planting Use pregerminate seed and treat with *Sinorhizobium meliloti* prior to planting (<http://www.nitragin.com>)
- No. of Plants One plant per pot and 24 plants per replicate
- No. of Reps 3 to 5 replications
- Plant Care Maintain in the greenhouse at 21°C/15.6°C (day/night); 12 to 16 hr. day length for 4-6 months. Since these pots are unstable, some type of support is required. We have used metal milk racks, which hold 12 pots each.

FUNGAL ISOLATION AND INOCULUM PRODUCTION

- Source Brown root rot (BRR) diseased alfalfa roots. Diseased roots in plastic bags can be stored under refrigeration (4°C) for 1 month.
- Isolation *Phoma sclerotoides* can be isolated from diseased root tissue placed in water agar using standard isolation techniques. Plates must be incubated at 10°C for 2 months or until pycnidia are mature prior to sub-culturing.
- Production Culture fungus in Petri plates containing potato dextrose agar maintained at 10°C until pycnidia are mature. Place 250 cc barley seed in a 600 ml glass beaker and add 130 ml water. Cover beaker with aluminum foil and autoclave for 60 min. Allow seed to cool overnight and reautoclave for an additional 60 min. When cool, transfer two mycelia plugs on to moistened seed and secure aluminum foil with parafilm. Maintain at 10°C for 2 months. Following thorough colonization of barley seed, remove and spread out on clean surface and allow to air dry.
- Storage Place colonized barley seed in plastic bags and store at -14°C. Inoculum should remain viable for up to 24 months or longer.

PLANT INOCULATION

- Age of Plant 4 to 6 months old
- Type of Inoc..... Infected barley seed containing mycelia and mature pycnidia

- Amount Two infected barley seeds/plant. Additional inoculum should increase disease development.
- Procedure Inoculate plants in late summer to early fall. Remove soil from upper taproot. Place infected barley seeds against root, approximately 2.5 cm below the soil surface. Cover inoculum with a plug of sterile cotton and replace soil. Plant injury is not required.

INCUBATION OF INOCULATED PLANTS

- Culture After inoculation, potted plants are placed outside on top of the ground and protected from cold temperatures by some type of insulation such as bales of hay to prevent roots from freezing. The experiment should have a rectangular design with only one bale in width to provide maximum protection. Loose hay should be placed into all openings to insure good insulation. Root infection has been reported to occur during host dormancy from early fall to early spring. Cardinal temperatures for fungal growth are 0°C (minimum), 15-16°C (optimum), and 27°C (maximum). Plants should be watered as needed to maintain soil moisture.

If straw bales do not provide sufficient insulation to prevent winterkill, one of the following two alternatives are suggested.

- a) Place potted plants in the ground (dug-in) up to the top of the pots. A straw bale barrier of one bale high is still recommended for a windbreak, as well as the placement of loose straw over the plants, or
- b) Field plots could be direct seeded, 6-inch row spacing, thinned to one plant every 6 inches, inoculated as previously described, and evaluated the following spring. If this alternative is used, other plant pathogens and/or pests present in the test site may interfere with the results.

- Evaluation Plants are left outside through the winter months. Plants can be evaluated for root rot in late spring to early summer. The fungus is inactive during the warm summer months. Since this test requires plants to be placed outside over one-half of the year, results may vary from year to year depending on local weather conditions.

RATING PLANTS FOR BROWN ROOT ROT

Evaluating plants for BRR severity. Partially remove plants from pots, remove soil from upper 10 cm of root, remove cotton plug and rinse under running water. Where potted plants have been ‘dug-in’ or where plants have been direct seeded, additional effort will be required for rating plants for BRR.

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

Check Cultivars and Expected Results

Disease Reaction	Fall Dormancy Rating	Resistant Plants (%)*	
		Expected	Acceptable Range
Highly Resistant			
Peace (Canada Dept. of Ag.)	1	60	40-60
Moderately Resistant			
Multi-plier (Mycogen Seeds)	3	20	10-30

*Alfalfa populations can be characterized for reaction to brown root rot by the percent of resistant plants with scores of 1 and 2 compared to standard check cultivars.

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Note: Other scientists with expertise are given under References.

CORRELATION TO FIELD TRIALS

A non-destructive forage yield trial, including the highly resistant and moderately resistant brown root rot check cultivars, was conducted at a site in Wyoming naturally infested with *P. sclerotoides*. 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. sclerotoides*-infested site, has produced similar but less dramatic results. Plant stands after three years for the BRR standard resistant and susceptible check cultivars were 97% for Peace and 67% for Multi-plier.

PATHOTYPES

Pathotypes of *Phoma sclerotoides* is available have not been reported. Studies by the authors have shown one Canadian (ATC #56515) and 13 Wyoming isolates of *P. sclerotoides* to all be pathogenic on alfalfa. Also, a *P. sclerotoides* isolate from cicer milkvetch (*Astragalus cicer* L.) was equally pathogenic on alfalfa when compared to an alfalfa isolate from Wyoming.

SOURCES OF PHOMA SCLEROTIOIDES AND SEED OF CHECK CULTIVARS

A culture of *Phoma sclerotoides* is available from the American Type Culture Collection (ATCC) #MYA-295 (<http://www.atcc.org>). A USDA/APHIS permit is required in states where BRR has not been documented.

Small quantities of seed (10g) for the two standard check cultivars sufficient to conduct the BRR resistant test can be obtained from:

- Dave Stout
- USDA/ARS/PWA
- 49 Johnson Hall
- Washington State University
- Pullman, WA 99164-6402
- Phone (509) 335-3510
- e-mail: w6ds@ars.grin.gov

HELPFUL INFORMATION

Brown root rot of alfalfa is not a seedling disease. Good infection has occurred with four- to six-month-old plants. Also, disease development of inoculated plants has not been observed under normal greenhouse environmental conditions.

DISTRIBUTION

First reported on alfalfa in Canada in 1933, BRR of alfalfa has a circumpolar distribution in continental and alpine climates with severe winters. In North America, it occurs in Alaska, Yukon, the Northwest Territories, the four western provinces of Canada (British Columbia, Alberta, Saskatchewan, and Manitoba), as well as in Nova Scotia. First reported in the U.S. in Wyoming in 1969, it has since been reported from Idaho, Montana, Colorado and New Mexico in the West, Minnesota and Wisconsin in the Midwest, and New Hampshire, New York, and Vermont in the Northeast. To date, its distribution has occurred in Zones 1-4 of the USDA Plant Hardiness Zone Map. <http://www.usna.usda.gov/Hardzone/ushzmap.html>

DISTRIBUTION AND SEVERITY OF BROWN ROOT ROT

Crossed line fill = states or provinces where extensive surveys of alfalfa fields have been conducted or multiple reports have occurred. Single line fill = states or provinces where BRR has



been found on alfalfa but an extensive survey of alfalfa fields has not been made.

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