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Pythium Seed Rot and Damping-off Resistance

Pythium spp.

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PLANT CULTURE

Growth chamber

Container ..... 9 cm-diameter petri plates
Medium ..... 1.5% water agar (previously inoculated with Pythium)
Temp/Light..... 18 C; 14 hour daylength
No. of Seeds ..... 25 per plate (replication)
No. of Reps..... 3 minimum

INOCULUM CULTURE AND PREPARATION

Source..... Baiting with alfalfa seedlings in infested soil. (See sources of inoculum section).
Storage/Temp ..... Isolates stored on cornmeal agar (4 C) will remain viable for 4-6 months. Isolates stored in sterile water (room temperature) will remain viable for at least 12 months.
Production ..... Cornmeal agar inoculated with an isolate from stored cultures and incubated at 24 C for 2-3 days.
Preparation..... A 3 mm-diameter disc of inoculum is removed from the periphery of the 2-3 day-old colony growing on cornmeal agar and placed in the center of a separate 9 cm-diameter petri plate containing 1.5% water agar. Plates are incubated 3 days at 24 C prior to plating seeds.

INCUBATION

Plating Seed..... Surface-sterilized seeds are equidistantly spaced in a radiate pattern on Pythium-inoculated agar surface using a vacuum template. Test checks include surface-sterilized seeds placed on uninoculated plates of water agar for determining expected numbers of dead seed. Hard seed are not used in test calculations.
Location..... Environmentally controlled chamber maintained at 18C. Temperature during incubation is critical. Lower temperatures will increase disease severity and accuracy of disease ratin may be compromized, higher temperatures favor alfalfa seedling growth causing less disease severity (1,2). Average soil temperature during alfalfa seeding dates for a target area may be considered when choosing a temperature to use during a plant resistance selection program.
Age at Rating..... 5 days after plating seeds.

RATING

Score each individual seedling.

1 Resistant.....healthy seedling: primary root free of necrosis; a slight discoloration of the primary root may occur

2 Resistant.....infected seedling: prirnary root tip necrotic but firm

3 Moderately Susceptible...infected seedling: prirnary root tip soft and rotted

4 Susceptible .....dead seedling: gerrninated seed with emerged radicle rotted

5 Susceptible .....dead seed: unger ninated seed rotted

Ratings for each plate may be expressed as percentage Resistant Plants and as an Average Severity Index (ASI). The rating method is similar to one used to evaluate alfalfa germplasms and flax germplasms for seedling darnping-off caused by *Rhizoctonia solani* Kuehn (3,4).

$$\text{Resist. Plants} = 100 \times \frac{\text{total of seedlings in classes 1 and 2}}{\text{Number (N) of seeds expected to germinate in the uninoculated check (calculated by subtracting the number of dead seed from the total number of swollen seed)}}$$

$$\text{ASI} = \frac{(\text{N class 5 seeds-N dead seeds in ck.})5 + (\text{N class 4})4 + (\text{N class 3})3 + (\text{N class 2})2 + \text{N class 1}}{\text{N}}$$

CHECK CULTIVARS

	Approximate Average Severity Index (ASI)/Isolate		
	W3	GR1	L3
Resistant			
Florida 77	2.6	4.2	4.8
Alfagraze	2.7	4.2	4.7
Wrangler	2.8	4.2	4.8
Susceptible			
Saranac	4.5	5.0	5.0

	Approximate Expected Resistance (%)/Isolate		
	W3	GR1	L3
Resistant			
Florida 77	55	0	1
Alfagraze	42	0	0
Wrangler	43	0	0
Susceptible			
Saranac	0	0	0

	Acceptable Range of Resistance
	W3
Resistant	
Florida 77	45-65
Alfagraze	32-52
Wrangler	33-55
Susceptible	
Saranac	0-5

CORRELATION TO FIELD REACTION

Because the culture plate method maximizes inoculurn pressure, correlation to field reaction should be satisfactory if the laboratory and field isolates are similar.

SPECIES AND RACES

The following *Pythium* species have been reported (1) to be highly pathogenic to alfalfa seedlings in North America: P. *debaryanum* Hesse, P. *irregulare* Buisman, P. *paroecandrum* Drechsler, P. *splendens* Braun, P. *sylvaticum* Carnpbell & Hendrix, and *P. ultimum*Trow. No races are known.

SOURCE OF INOCULUM AND SCIENTIST WITH EXPERTISE

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