BACTERIAL WILT RESISTANCE – GREENHOUSE/CONTROLLED ENVIRONMENT

Test accepted: March 1995 **Test updated:** June 2024
Pathogen: *Clavibacter insidiosus* (Syn. *Clavibacter michiganensis* subsp. *insidiosus; Corynebacterium insidiosum)*
Test authors: J.E. Brummer and S.L. Nygaard

PLANT CULTURE

Container ..... Bench or tub with adequate depth for root development (6 to 8")
Media ..... Soil Mix (1:2:1; Top soil: sand: sphagnum/perlite) adequate lime and fertilizer (0:24:24)
Temp/Light ..... 24° to 30°C.; 16+ hour daylength
No. of Plants ..... 50 to 60 per replication
No. of Reps ..... 4 replications
Other ..... Inoculate with *Sinorhizobium meliloti*

INOCULUM CULTURE

Source ..... Infected root tissue
Storage ..... Washed frozen root tissue
Temperature ..... -10°C
Storage Life ..... Several years if tissue remains frozen

INOCULATION PROCEDURE

Age of Plant ..... 8 weeks old
Concentration .... 100 g ground infected root per 1L H_2O
Method ..... Clip roots (6 to 8 cm from crown) and bare root soak
Type of Inoc. ..... Bacterial water suspension
Time of Inoc. ..... 20 min at 24°C or 12 hours at 16°C.

(Click to see larger photo.)

Examples of symptoms of bacterial wilt from a greenhouse test: plants are stunted with yellowed and necrotic leaves.

INCUBATION

Location ..... Greenhouse; transplanted into benches or tub
Temp/Light ..... 20° to 24°C; 16 hour daylength
Culture ..... Clip top growth twice at 5 to 6 week intervals (at bud or bloom) maintain good growth and control insect pests; fertilize (0-24-24) after each clipping.
Spacing ..... 2.5 cm between plants and 6 cm between rows
Age at Rating ..... 12 to 14 weeks after transplanting

RATING

Plants are removed from benches and tap root is sectioned for rating.

Resistant ..... Symptomless root or less than one-fourth of the stele expressing a yellow-brown discoloration (discrete small spots).
Susceptible ..... Discoloration in the root greater than one fourth of the entire root (stele and cortex); dead plant.

CHECK CULTIVARS

	Approximate Expected Resistance (%)	Acceptable Range of Reaction (%)
Resistant		
Vernal**	40	30-50
Susceptible		
Narragansett**	2	0-5
Sonora**	2	0-5

**Checks used by AOSCA Alfalfa and Miscellaneous Legumes Variety Review Board for variety certification.

CORRELATION TO FIELD REACTION

There is a good correlation between results of this test and visual root scores and plant vigor in naturally infested fields.

SCIENTIST WITH EXPERTISE AND SOURCE OF INOCULUM

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ALTERNATIVE METHODS

A mixture of 10 or more individual bacterial strains can be used instead of ground infected roots as the inoculum. Bacteria are cultured on nutrient broth agar plates for 7 to 10 days and then suspended in sterile water. Adjust the concentration to an OD600 = 0.1 and use for root soak inoculation for 20 minutes at 24°C. Bacterial strains should be stored in 20% glycerol at -80°C.

REFERENCES

See Bacterial Wilt field protocol; In: Standard Tests to Characterize Alfalfa Cultivars.