FUSARIUM WILT RESISTANCE

Test accepted: March 1991 **Test updated:** June 2024
Pathogen: *Fusarium oxysporum* Schlecht f. sp. *medicaginis* (Weimer) Snyd. & Hans. **Test authors:** S. Nygaard and D. K. Barnes

PLANT CULTURE

Greenhouse

Container..... Bench, pot, or flat deep enough to allow root development
Media..... Sand or soil mixture
Temp/Light..... 24 to 30°C; 16+ hour daylength
No. of Plants ..... 35 to 50 per replication
No. of Reps ..... 3 replications minimum
Other ..... Inoculate with *Sinorhizobium meliloti*, encourage vigorous growth

INOCULUM CULTURE

Source ..... Infected root tissue; axenic culture
Storage ..... Inoculated sterile soil cultures or mycelial plugs in cryoprotectant at -80°C
Temperature..... Soil cultures at 0 to 4°C, mycelial plugs in cryoprotectant at -80°C
Storage Life..... Several years in soil tubes, indefinitely at -80°C

INOCULATION PROCEDURE

Age of Plant ..... 8 to 10 weeks old
Type of Inoc. ..... Microconidia in suspension
Inoc. Culture ..... Potato dextrose broth or nutrient broth (2 g $NaNO_3$, 1 g KH_2PO_4, 0.5 g $MgSO_4$-$7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4$-$7H_2O$, 0.5 g yeast extract, and 15 g sucrose per 1 L distilled water); inoculate sterile broth with mycelial plugs from potato dextrose agar plates and incubate on a shaker for 4 days at about 21°C
Concentration.... 1.6 X 10^6 spores per mL or a 1:20 dilution of the shake culture
Method ..... Bare root soak
Time of Inoc. ..... 20 to 30 min

INCUBATION

Location ..... Transplant to field
Plant Counts ..... Approximately 2 weeks after transplanting, count all (alive and dead) plants as the base count for initial stand
Culture ..... Maintain vigorous growth, control insects, clip plants once or twice.
Spacing..... 0.15 m between plants, 1.0 m between rows.
Age at Rating ..... 3 months after transplanting

RATING

Plants are removed from the field and the tap root sectioned for rating.

0 Resistant..... No discoloration in the root
1 Resistant..... Small dark strands in the stele
2 Susceptible..... Small dark-brown arcs or rings in cross section of the stele
3 Susceptible..... Larger dark-brown areas, arcs or rings, or partial dark- brown ring in the outer stele
4 Susceptible..... Entire outer stele dark brown, plant alive
5 Susceptible..... Dead plant (stand loss)

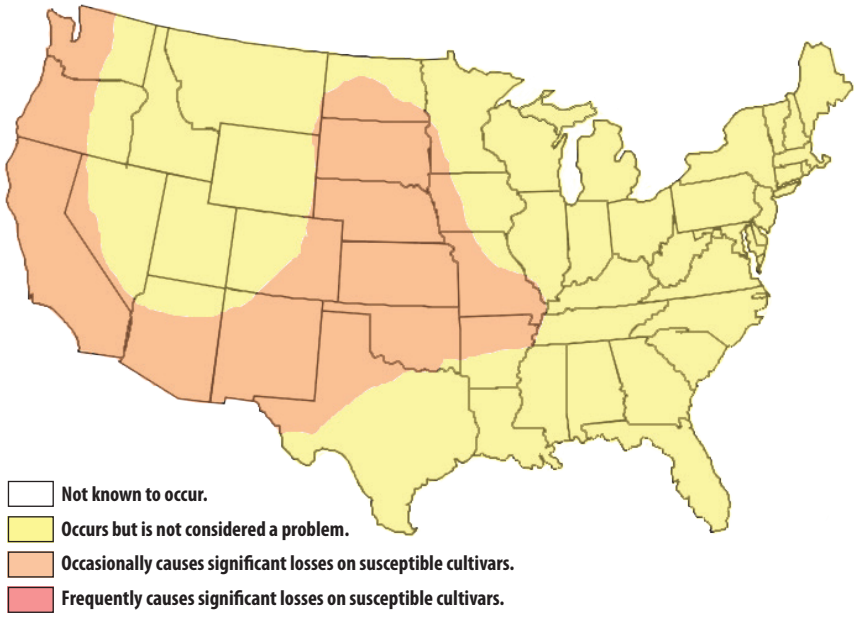
CHECK CULTIVARS

	Approximate Expected Resistance (%)	Acceptable Range of Reaction (%)
Resistant		
Agate**	54	45-65
Moapa 69	76	65-85
Moderately Resistant		
Narragansett	22	15-30
Susceptible		
MNGN-1**	4	0-8

Values for resistant standards are totals of 0's and 1's.
**Checks used by AOSCA Alfalfa and Miscellaneous Legumes Variety Review Board for variety certification.

(Click to see larger photo.)
Fusarium wilt symptoms in the root. Above ground Fusarium wilt symptoms.

DISTRIBUTION AND SEVERITY OF FUSARIUM WILT



Fusarium wilt, *Fusarium oxysporum* Schlecht f. sp. *medicaginis* (Snyd. & Hans.)
(Click on the map above for a larger version.)

SOURCE OF INOCULUM AND EXPERTISE

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CULTURE OPTIONS AND RANGE OF CONDITIONS

Mixtures of *Fusarium oxysporum* and *Clavibacter michiganense* subsp. *insidiosum* inocula will not influence the severity of Fusarium wilt symptoms, but it will often produce reduced symptoms of bacterial wilt.[1]

PLANT GROWTH OPTIONS AND RANGE OF CONDITIONS

The same procedure can be used in the greenhouse with acceptable accuracy. Plants are inoculated and then transplanted into pots, benches, or celled flats. Greenhouse evaluations can be rated 6 to 8 weeks after transplanting.

INOCULATION OPTIONS AND RANGE OF CONDITIONS

Eight to 10 week-old plants are lifted from the soil, washed in tap water, and about 50 plants for each plot are tied in a bundle. The roots are kept in tap water until all plants for all plots in a replicate are prepared. The bundled plants are immersed in the inoculum, tops trimmed to ca. 4 cm from the crown and the roots are trimmed to ca. 12 cm length. Roots are kept in moist paper towel until transplanting.

HELPFUL INFORMATION

If not planted immediately, inoculated plants may be stored at 3 to 4°C for up to a week. A tobacco planter or modified vegetable planter works well for transplanting. It can be expected that many of the highly susceptible plants will die within 5 to 6 weeks after inoculation and transplanting. Plants are undercut at 15 cm and roots are sectioned for rating. A carrot or beet lifter or sod undercutter works well for this purpose. Ratings may be expressed as an ASI or as a percentage adjusted to the long-time average of the standard check cultivar. Seed of the susceptible check (MNGN-1) can be obtained .from USDA-ARS National Plant Germplasm System (NPGS) from the main seed storage facilities at Washington State University - Pullman. See details at: https://www.naaic.org/resource/checkseed.php.

The primary method used for evaluating resistance to Fusarium wilt has been a root soak method of inoculation followed by transplanting to the field and the scoring of root symptoms about 3 months later.[1,2]

CRYOSTORAGE PROTOCOL

Materials

1.8 mL Nunc cryovials with lids (Nunc 377267); Skim milk/glycerol cryoprotectant; Cryorack; Sterile pipette tips and pipetter; Sterile glass pipettes or #5 cork borer; Cryopen (Nunc 343850); Nalgene Cryo 1°C Freezing Container (Nalgene Cat. No. 5100-0001); Isopropyl alcohol; Cryoboxes

Methods

1. Preparing skim milk/glycerol cryoprotectant

- Prepare a 17% nonfat skim milk in distilled water by measuring 17 mL of dry skim milk into a 100 mL graduated cylinder. Pour this into a clean flask and add 50 mL of distilled water to the dry skim milk. Mix until everything has dissolved. Bring final volume to 100 mL in a 100 mL graduated cylinder.
- Prepare a 20% glycerol solution by measuring 20 mL of glycerol into a 100 mL graduated cylinder and filling to 100 mL with distilled water. Mix well.
- Autoclave both solutions (in separate flasks) for 20 minutes on slow exhaust. The milk solution should be a light brown after autoclaving.
- After they have cooled, mix the solutions together in a sterile 250 mL bottle while in a laminar flow hood or biological safety cabinet. Refrigerate resulting solution.

2. Introducing cultures into sterile cryovials for mycelium

- Label 6 cryovials (Nunc 377267) with cryopen (Nunc 343850). Label with Name, Strain Number, and Date.
- In hood, use the base of a sterile Pasteur pipette or sterilized #5 cork borer to make 24 plugs in growing margin of culture.
- Transfer 4 plugs into each cryovial.
- Fill each cryovial with milk/glycerol solution to the 1.8 mL line of vial using sterile pipettes.

3. Freezing and storing cultures

- Prepare Cryo freezing container "Mr. Frosty" (Nalgene Cat. No. 5100-0001) by filling with 100% isopropyl alcohol. Alcohol can be used up to 5 times.
- Transfer the cryovials to the cryo freezing container and place at -80°C overnight. *Note: when filled with isopropanol, the freezing container will provide the desired freezing rate of 1°C/minute.*
- Remove frozen tubes from unit and place in cryoboxes in a permanent, long-term storage freezer (e.g., -80°C or below).

4. Thawing and plating samples (testing viability)

- Thaw tube at room temperature.
- Plate onto two petri dishes of appropriate medium by pouring contents of vial onto the first petri dish, then transfer plugs onto the second petri dish.
- Incubate and check for growth every 2 days for 1 week.

REFERENCES

1. Frosheiser, F. I., and D. K. Barnes. 1978. Field reaction of artificially inoculated alfalfa populations to the Fusarium and bacterial wilt pathogens alone and in combination. Phytopathology 68:943-946.
2. Hijano, E. H., D. K. Barnes, and F. I. Frosheiser. 1983. Inheritance of resistance to Fusarium wilt in alfalfa. Crop Sci. 23:31-34.