

VERTICILLIUM WILT RESISTANCE

Test accepted: March 1991

Test updated: June 2024

Pathogen: *Verticillium alfalfae* (Syn. *V. albo-atrum*)

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PLANT CULTURE

Greenhouse

No. of Plants 25 to 50 per replication

No. of Reps 4 replications

Soil Medium Autoclaved porous sand/soil mixture

Temp/Light..... Grow plants at 20 to 24°C; 12 to 14 hour photoperiod

Other Do not allow plants to be stressed by a water deficit

INOCULUM CULTURE

Source Infected stems

Storage Silica gel beads or mycelial plugs in cryoprotectant at -80°C⁽⁴⁾

Temperature..... 4°C

Storage life..... 2 to 3 years

INOCULATION PROCEDURE

Greenhouse

Age of Plant 6 to 8 weeks

Production Culture isolates on potato dextrose agar at 21°C, transfer mycelial plugs into Czapek's Dox broth in a 500 mL baffle sided Erlenmeyer flask; flasks are placed on a rotary shaker (120 rpm) at 20 to 23°C for 5 to 7 days

Type of Inoc. Conidia

Concentration.... 1x10⁶ conidia per mL of distilled water

Transplant Plants are lifted, washed free of excess soil, the roots trimmed to a length of ca. 8 cm, and the stems clipped to a height of 3 cm; plants are completely immersed in a suspension of conidia for 20 minutes, then transplanted into fresh steamed sand/soil mix^(1,2) or

Cut Stem..... Plants are inoculated by clipping the stems to 3 cm height with scissors dipped into a conidial suspension (8 X 10⁶ conidia per mL); the scissor is redipped before each clip; spray the freshly cut stems with the inoculum suspension amended with a wetting agent (polyoxyethylene 20 sorbitan monolaurate, 1 mL per L); stems are sprayed to run-off 4 times using a compressed air hand sprayer⁽³⁾

INCUBATION

Greenhouse

Culture Maintain inoculated plants at 20 to 24°C and 12 to 14 hour photoperiod; do not allow plants to be stressed by a water deficit

Age at Eval..... Incubate plants for 4 to 6 weeks

RATING

Plants are evaluated for severity of foliar symptoms.

1 Resistant..... No to minimal chlorosis of lower leaves

2 Resistant..... Chlorosis of lower and middle leaves, but no chlorosis or necrosis of terminal leaves

3 Susceptible..... Well developed symptoms of chlorotic, necrotic and twisted terminal leaflets on at least one, but not all mainstems

4 Susceptible..... Severe symptoms of chlorosis, necrosis, and twisting of all leaflets on all main stems

5 Susceptible..... Plant dead

Alfalfa populations can be characterized for reaction to *V. alfalfae* by calculating their mean disease severity index (DSI). Additionally, the percentage of resistant plants (i.e., combined percentage of plants rated as class 1 and 2) can be used to characterize alfalfa populations for reaction to *V. alfalfae*.

CHECK CULTIVARS

	Approximate Expected Resistance (%)	Acceptable Range of Reaction (%)
Resistant		
Vertus**	40	30-50
Oneida VR	60	55-65
Susceptible		
Saranac**	2	0-5

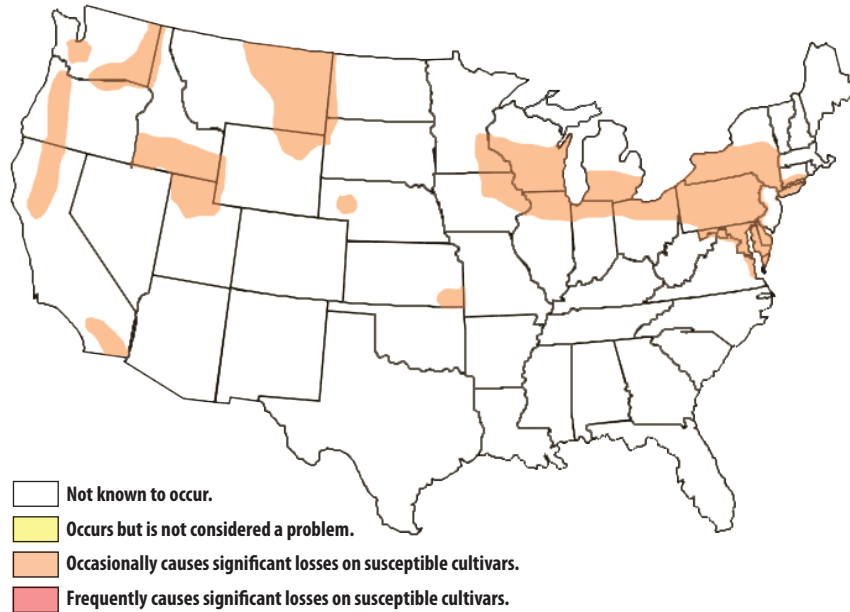
Values for resistant standards include total of 1's and 2's.

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



(Click to see larger photo.)
Foliar symptoms of Verticillium wilt.

DISTRIBUTION AND SEVERITY OF VERTICILLIUM WILT



Verticillium Wilt, *Verticillium alfalfae*.
(Click on the map above for a larger version.)

SOURCE OF INOCULUM AND SCIENTIST WITH EXPERTISE

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CORRELATION TO FIELD REACTION

There is a high correlation between results of this test and disease incidence in field environments.

RACES

Races of *V. alfalfae*, alfalfa strain, have not been reported.

PLANT GROWTH OPTIONS AND RANGE OF INCUBATION CONDITIONS

Air temperatures of 20 to 24°C are optimal for expression of foliar symptoms. Air temperatures above 24°C result in less symptom severity. The severity of foliar symptoms can be reduced if plants are subjected to nutrient deficiency and drought stress. It is important to control insects because their feeding damage can alter the expression of foliar symptoms.

FIELD EVALUATION

Alfalfa populations can be evaluated in field environments. Entries are seeded at a rate of 5 g of seed per 4.6 m row and rows are spaced 0.76 m apart. Replicate entries four times in a randomized complete block design. *Verticillium alfalfae* can be artificially introduced into plots at the second harvest by spraying the freshly cut alfalfa stubble with a suspension of conidia (8×10^6 conidia per mL) in the establishment year. Thereafter, plots are monitored for symptoms of Verticillium wilt. Plots are evaluated by counting healthy and diseased plants.

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 $1^{\circ}\text{C}/\text{minute}$.*
- 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. Christen, A. A., and Peaden, R. N. 1981. Verticillium wilt in alfalfa. *Plant Dis.* 65:319-321.
2. Graham, J. H., Peaden, R. N., and Evans, D. W. 1977. Verticillium wilt of alfalfa found in United States. *Plant Dis. Rep.* 61:337-340.
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4. Smith, D. 1984. Maintenance of fungi. Pages 87-88. In: *Maintenance of microorganisms, a manual of laboratory methods*. Ed. by Kirsop, B. E., and J. J. S. Snell, Academic Press.