ANTHRACNOSE RESISTANCE

Test accepted: March 1991 **Test updated:** June 2022
Pathogen: *Colletotricum trifolii* **Test author:** Nichole O'Neill

(Click each image to see larger photos.)

Anthracnose affects the stems and crowns of alfalfa growing in warm, humid environments. The crown rot phase is lethal to plants, making anthracnose one of the most serious diseases of alfalfa. It is a major disease in the United States, Argentina, Australia, and Europe.[12] Resistant cultivars for race 1 and race 5 are available.

Diamond-shaped lesions form on the stem and are straw colored with a brown margin. Stem wilting ("shepherds crook") leads to plant death. The symptoms are typically scattered throughout the field. The pathogen is spread by water splashed spores from infected to healthy plants. Disease occurs during periods of warm, wet weather. Symptoms for race 5 appear earlier in the season than those from race 1 or 2, which usually appear in mid- to late summer.

PLANT CULTURE

Growth Chamber/Greenhouse

Container ..... 10-cm plastic pots or 30 x 60 cm flats
Medium ..... Soil free potting mix
Temp/Light ..... 23°C; 16+ hour daylength
No. of Plants ..... 50 per replication
No. of Reps ..... 4 minimum
Other ..... Control insects and fertilize as necessary

INOCULUM CULTURE

Source ..... Cultures obtained from infected stem tissue (*See Notes*), reference strains, or other characterized *C. trifolii* strains.
Storage ..... Soil or silica gel at 4°C[8], -80°C in cryo-protectant solution (*See Notes*)
Storage Life ..... Up to several years

Reference Strains

Race 1 ..... Strains: 2sp2[10], WUPP, SM
Race 2 ..... Strain: SB2[10]
Race 5 ..... Strain: AN5

INOCULATION PROCEDURE

Plant Age ..... 7 to 14 days, take stand counts at 7 days. Plants should be approximately 2 inches (5-6 cm) tall and have one trifoliate leaf when inoculated.
Inoculum Type ... Spore suspension with 2 drops Tween 20 per L distilled water, taken from 7- to 14-day old cultures incubated at 23°C in ambient laboratory light on half strength Difco Oatmeal Agar or full-strength Difco Potato Dextrose Agar. PDA may be amended with 0.5 mg streptomycin sulfate/L[2, 12] (*See culture plate photos*).
Concentration .... 2 X 10^6 spores per mL. Use a hemocytometer to count spores repeating several times and taking the average.
Method ..... Spray onto plants to runoff, approximately 3 mL per pot or 20 to 40 mL per flat. Place in mist/dew chamber to maintain 100% relative humidity for 48 hours, 23°C. Keep lights off or provide shade. Alternatively, flats or pots may be covered with an inverted flat to block light or placed in a black plastic bag. Dark conditions are critical for obtaining standard responses.

INCUBATION

Location ..... Growth room, growth chamber, or greenhouse at 23°C
Rating Age ..... 10 to 14 days after inoculation

RATING

Resistance is assessed as a percent of the stand surviving 10 to 14 days after inoculation. To be considered susceptible, the plant must be dead. Additional details on host-parasite interactions have been published.[5, 9, 10]

	Arc	Saranac AR	Saranac	HvX-An5
Race 1	HR	R	S	HR
Race 2	S	R	S	HR
Race 5	S	S	S	HR

HR (High Resistance) = >50% survival
R (Resistance) = 31-50% survival
S (Susceptible) = <6% survival

Race 1

	Approximate Expected Resistance (%)	Acceptable Reaction Range
Arc**	58	40-70
Saranac AR	45	40-60
Saranac**	4	0-7
HvX-An5**	74	51-90

Race 2

	Approximate Expected Resistance (%)	Acceptable Reaction Range
Arc**	1	0-3
Saranac AR	45	40-60
Saranac**	1	0-7
HvX-An5**	62	51-80

Race 5

	Approximate Expected Resistance (%)	Acceptable Reaction Range
Arc**	2	0-5
Saranac AR	4	0-12
Saranac**	1	0-5
HvX-An5**	52	40-70

**Standard checks used for reporting resistance to the AOSCA Alfalfa & Misc. Legumes Variety Review Board.

INOCULUM SOURCE

Deb Samac
USDA-ARS PSRU
1991 Upper Buford Circle
495 Borlaug Hall
St. Paul, MN 55108
(612) 625-1243
debby.samac@usda.gov

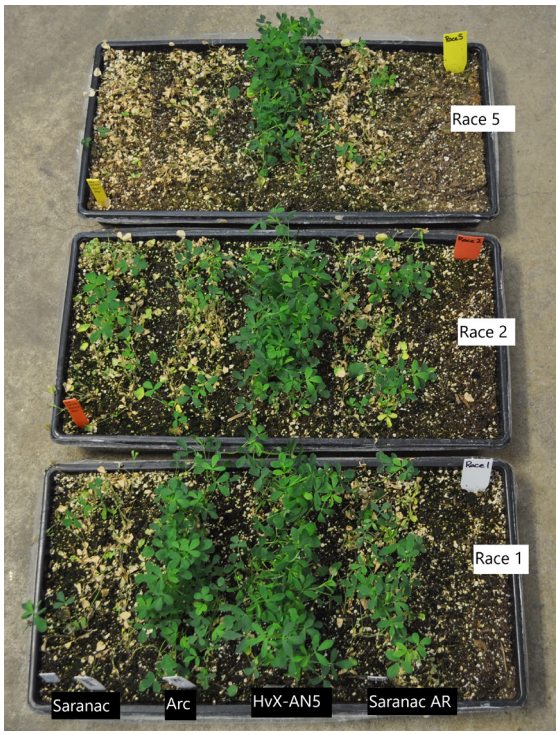
CHECK CULTIVAR SOURCE

Alfalfa Standard Check Seeds

Brian Irish
USDA-ARS PGITRU
24106 North Bunn Road
Prosser, WA 99350-9687
(509) 786-9316
brian.irish@usda.gov

Proprietary Race 5 Check (HvX-An5)

Dave Whalen
Forage Genetics International
N5292, Gills Coulee Road South
West Salem, WI 54669
(608) 786-2121
dwhalen@foragegenetics.com



(Click image to see larger photo.)

CONTROLLED TEST CORRELATION TO FIELD REACTION:

Cultivars occasionally appear more resistant in the field than indicated by seedling tests, but generally, good correlations are observed between greenhouse and field tests.[6]

RACES

Race 1 of *Colletotrichum trifolii* has been found wherever alfalfa is grown.

Race 2 was discovered in a limited area in 1975. It has since been found in Wisconsin in 2014.[13]

Race 3 was identified in Oklahoma in 1982[1] but subsequently reclassified as *C. destructivum*.

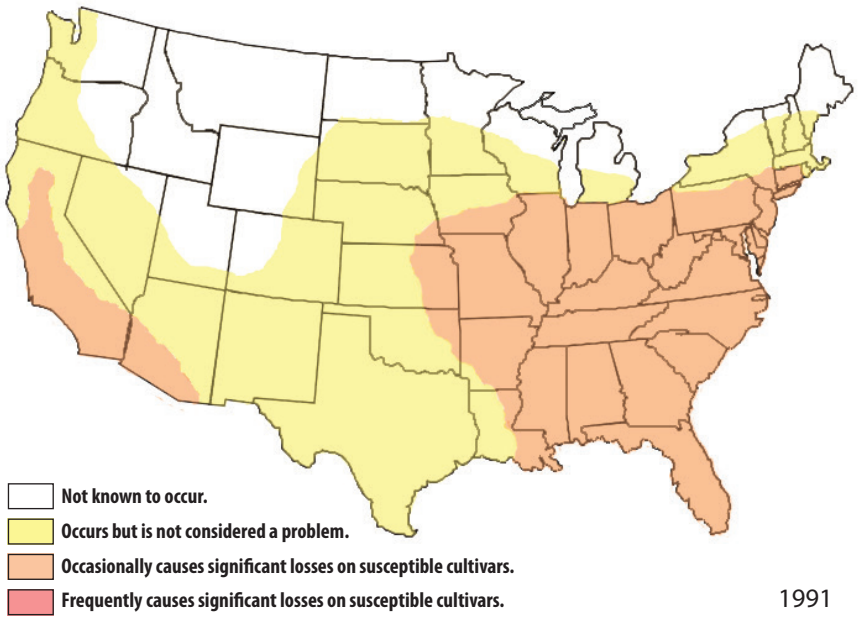
Race 4 was identified in Ohio in 2006.[3]

Race 5 has been identified in Wisconsin in 2014 and Minnesota in 2017.

DISEASE PREVALENCE AND SYMPTOMS

Anthracnose races 1 and 5 largely affect the eastern half of the United States, from southeastern Minnesota, Iowa, Missouri, Arkansas, and eastern Louisiana to the East Coast except for the New England states. So far, race 5 has been limited to the Midwest and East, where high humidity and moisture is present during the growing season.

DISTRIBUTION & SEVERITY OF ANTHRACNOSE (RACE 1)



(Click on the map above for a larger version.)

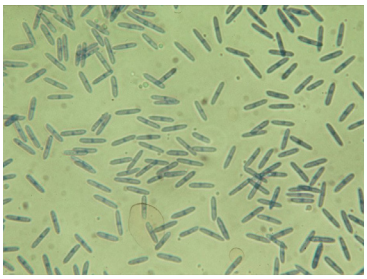
COLLETOTRICHUM SPECIES THAT CAUSE ANTHRACNOSE

At least four races of *C. trifolii* have been reported in the United States and Australia.

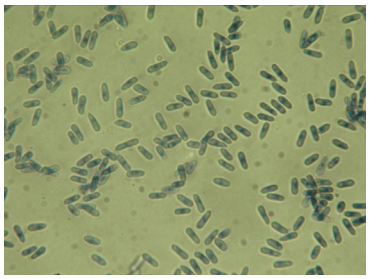
In Canada, anthracnose symptoms have been shown to be caused by *C. destructivum*.

In the mid-Atlantic states of the United States both *C. destructivum* and *C. dematium* f. sp. *truncatum (C. truncatum)* have been found on diseased alfalfa and were weakly pathogenic in greenhouse tests. Additionally, *C. gloeosporioides* has been found to be pathogenic on alfalfa cultivars with resistance to *C. trifolii*.[12]

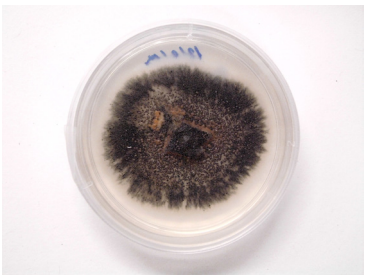
CONIDIA *(click each image to see larger photos)*

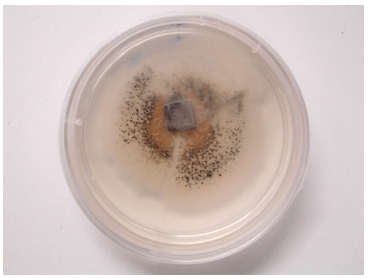


Colletotrichum destructivum



Colletotrichum trifolii





C. trifolii mycelia may appear from white to black on growth media with orange spore masses appearing slimy. Setae (hairs) may often be seen arising from mycelium.

Notes:

- Fungal strains should be stored on silica gel or as mycelial plugs at -80°C in cryoprotectant because strains can lose virulence after several transfers on agar media.
- Using mixtures of isolates will minimize error due to differences in virulence among isolates.
- Alternate methods to be used for research purposes only, not in standardized resistance test: Succulent stems of mature plants can be tested for susceptibility by needle inoculation.[11] Individual seedlings can be evaluated by cotyledon assay.[4] A very young seedling test also gives good results very quickly if only percent resistance is required.[7]

REFERENCES

1. Allen, S.J., G.L. Barnes, and J.L. Caddel. 1982. A new race of *Colletotrichum trifolii* on alfalfa in Oklahoma. Plant Disease 66:922–924.
2. Ariss, J. 2005. Pathological factors affecting persistence in alfalfa with emphasis on diseases incited by *Fusarium* and *Colletotrichum* species. PhD Dissertation, Ohio State University.
3. Ariss, J.J., and L.H. Rhodes. 2006. A new race of *Colletotrichum trifolii* on alfalfa identified in Ohio. Plant Disease 91:1362.
4. Cucuzza, J.D., and J. Kao. 1986. In vitro assay of excised cotyledons of alfalfa (*Medicago sativa*) to screen for resistance to *Colletotrichum trifolii*. Plant Disease 70:111-115.
5. Elgin, J.H., Jr., and S.A. Ostazeski. 1982. Evaluation of selected alfalfa cultivars and related *Medicago* species for resistance to race 1 and race 2 anthracnose. Crop Science 22:39-42.
6. Grau, C.R., D.C. Arny, and S.L. Nygaard. 1989. Assessment of alfalfa cultivar reaction to anthracnose in controlled and field environments. Plant Disease 73:167-170.
7. Morrison, R.H. 1977. A seedling test for evaluating alfalfa for resistance to anthracnose. Plant Disease Reporter 61:35-37.
8. Nakasone, K.K., S.W. Peterson, and S.-C. Jong. 2004. Preservation and distribution of fungal cultures. Pages 37-47 in: Biodiversity of Fungi: Inventory and Monitoring Methods. G.M. Mueller, G.F. Bills, MS. Foster, eds. Elsevier Academic Press, Burlington.
9. O'Neill, N.R. 1996. Pathogenic variability and host resistance in the *Colletotrichum trifolii*/*Medicago sativa* pathosystem. Plant Disease 80:450–457.
10. O'Neill, N.R., J. H. Elgin Jr., and C.J. Baker. 1989. Characterization of induced resistance to anthracnose in alfalfa by races, isolates, and species of *Colletotrichum*. Phytopathology 79:750-756.
11. Ostazeski, S.A., and J.H. Elgin Jr. 1982. Use of hypodermic inoculations of alfalfa for identifying host reactions and races of *Colletotrichum trifolii*. Crop Science 22:545-546.
12. Rhodes, L.H. Anthracnose. 2015. Pages 27-30 in: Compendium of Alfalfa Diseases and Pests, Third Edition. D.A. Samac, L. H. Rhodes, and W. O. Lamp, eds. APS Press, St. Paul.
13. Samac, D.A., Allen, S., Witte, D., Miller, D., Peterson, J. 2014. First report of race 2 of *Colletotrichum trifolii* causing anthracnose on alfalfa (*Medicago sativa*) in Wisconsin. Plant Disease 98(6):843.

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.

ISOLATION OF *COLLETOTRICHUM TRIFOLII* FROM PLANT MATERIAL

1. Symptomatic stems from the field or controlled inoculation may be used.
2. Cut stems to lengths that contain 1 to 2 lesions.
3. Place a piece of sterile filter paper into the lid of a petri plate and moisten with sterile water. Pour off any standing water not absorbed by the filter paper.
4. Put stem sections onto filter paper, seal with parafilm, and place at 4°C for 24 to 48 hours. Spores may form on lesions in the cold. Observe lesions for orange spore masses using a dissecting microscope.
5. If no spores are seen, place petri dishes at room temperature under ambient light for an additional 1 to 5 days.
6. Once spores are observed, use a sterile needle to pick up a small amount of the spore mass in a lesion. Suspend the spores in 10 µl of sterile water in a petri dish.
7. Pipette the spore suspension onto 1% water agar letting the water run down the plate forming a track of spores. Spores for additional lesions can be placed on the same plate. Incubate at room temperature.
8. Observe spores after 24 to 48 hours under a dissecting microscope. Cut individual germinating spores or hyphal tips out of the agar using a scalpel blade and transfer to potato dextrose agar (PDA). Incubate at room temperature.
9. Observe PDA plates for bacterial contamination and discard contaminated plates.
10. Store new isolates on silica gel or at -80°C.