

# BACTERIAL WILT RESISTANCE - FIELD TEST

Test accepted: March 1991

Test updated: June 2024

Pathogen: *Clavibacter insidiosus* (Syn. *Clavibacter michiganensis* subsp. *insidiosus*; *Corynebacterium insidiosum*)

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## PLANT CULTURE

### Greenhouse

Container ..... Bench 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 ..... 50 to 70 per replication

No. of Reps ..... 3 minimum

Other ..... Inoculate with *Sinorhizobium meliloti*; promote good growth by treating with insecticides and fertilizer as necessary

## INOCULUM CULTURE

Source ..... Infected root tissue

Storage ..... Ground up, washed roots

Temperature ..... -10°C

Storage Life ..... Up to several years if frozen

## INOCULATION PROCEDURE

Age of Plant ..... 8 weeks old

Concentration ..... 50 g ground root per 1L H<sub>2</sub>O

Inoc. Time ..... 20 to 30 min

Method ..... Bare root soak

Type of Inoc. ..... Bacterial water suspension

## INCUBATION

Location ..... Transplant to field (June)

Plant Counts ..... Count 2 to 4 weeks after establishment

Cultural ..... Maintain vigorous growth

Spacing ..... 0.15 x 1.0 m

Age at Rating ..... 5 months (3 months in field)

## RATING

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

0 Resistant ..... Root clean and white

1 Resistant ..... Very small yellow-brown spots visible in stele

2 Susceptible ..... Discoloration affecting up to one-third of stele

3 Susceptible ..... Nearly entire stele discolored, cortex white

4 Susceptible ..... Discoloration throughout stele and cortex, plant alive

5 Susceptible ..... Plant dead (based on plant count)

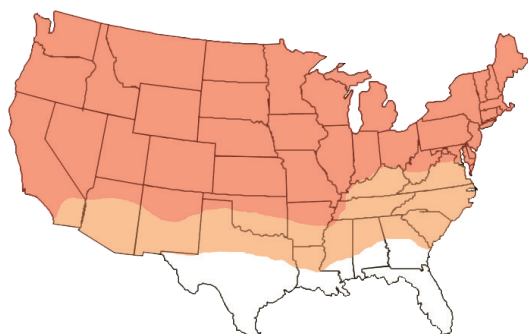
## CHECK CULTIVARS

	Approximate Expected Resistance (%)	Acceptable Range of Reaction (%)
<b>Resistant</b>		
Vernal**	42	30-50
<b>Susceptible</b>		
Narragansett**	1	0-5
Sonora**	2	0-5

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.

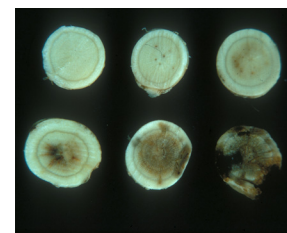
## DISTRIBUTION AND SEVERITY OF BACTERIAL WILT



- Not known to occur.
- Occurs but is not considered a problem.
- Occasionally causes significant losses on susceptible cultivars.
- Frequently causes significant losses on susceptible cultivars.

Bacterial wilt, *Clavibacter insidiosus*

(Click map to the left for a larger version.)



(Click to see larger photo.)  
Examples of ratings of root symptoms.



(Click to see larger photo.)  
Plant symptoms (foreground): stunting, leaf yellowing, leaflet upward cupping, leaf necrosis.

## SOURCE OF INOCULUM

**Deb Samac**

USDA-ARS PSRU

1991 Upper Buford Circle

495 Borlaug Hall

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## RACES

There are no known races of *Clavibacter insidiosus*.

## CULTURE OPTIONS AND RANGE OF CONDITIONS

Best results are obtained when the plants are grown under optimum conditions in the field and in the greenhouse. It is important to transplant healthy plants with well developed roots to assure good transplant survival and uniform tests.

## INOCULATION CONDITIONS AND RANGE OF CONDITIONS

Roots must not be allowed to dry out between pulling and inoculating. After inoculation, plant tops are trimmed to within 5 cm of the crown and roots to 10 to 12 cm. Several bundles can be wrapped together in paper or cloth towels to keep them moist until transplanting.

## HELPFUL INFORMATION

Plants may be stored in 1 to 2 cm water at 2 to 4°C for up to several days prior to transplanting. A tobacco transplanter or modified vegetable transplanter works well for trans planting. Plants are undercut at 15 cm and root sectioned for rating. A carrot or beet lifter also works well for removing the plants from the ground. Plants may be rated at any time between 12 to 16 weeks after transplanting. Ratings may be expressed as an Average Severity Index (ASI) or as a percentage adjusted to the long time average of Vernal (42%). The percentage of resistant plants adjusted to Vernal is very useful in comparing cultivars tested in different years.

## ALTERNATIVE METHODS

The root soak field evaluation method is most effective in determining resistance in alfalfa. However a combination of root soak and cotyledon wounding has proved effective for screening large numbers of seedlings in the greenhouse.<sup>(1,2,4)</sup>

## REFERENCES

1. Barnes, D.K., C.H. Hanson, F.I. Frosheiser, and L.J. Elling. 1971. Recurrent selection for bacterial wilt resistance in alfalfa. *Crop Sci.* 11:545-54.
2. Frosheiser, F.I. Alfalfa cotyledon inoculation with bacterial wilt inoculum prepared from infected alfalfa roots. *Phytopathology* 56:566- 567.
3. Kernkamp, M.F., and G. Hemerick. 1952. A deep freeze method of maintaining virulent inoculum of the alfalfa wilt bacterium, *Corynebacterium insidiosum*. *Phytopathology.* 42:13.
4. Krietlow, K.W. Infecting seven-day-old alfalfa seedlings with wilt bacteria through wounded cotyledons. *Phytopathology.* 53:800-803