March, 1991

Lepto Leaf Spot Resistance

Leptosphaerulina briosiana (Poll.) Graham and Luttrel Kenneth Leath

PLANT CULTURE
Greenhouse

Container ..... Flats or tool carts, 31x62x7.5cm
Medium ..... Not critical
Temp/Light ..... 18 to 24°C; daylength not critical light, intensity >1000 Mmol $m^{-2}sec^{-1}$\
No. of Plants ..... 20 to 25 per replication
No. of Reps ..... 4 minimum
Other ..... Inoculate with *Rhizobium meliloti* Dang or fertilize as needed; insect control, none within 1 week of inoculation.

INOCULUM CULTURE

Source ..... Infected leaves
Storage..... 6 months; longer on silica gel
Temperature..... 4°C

INOCULATION PROCEDURE

Age of Plant..... 6 to 8 weeks or 4 to 5 trifoliates
Type of Inoc ..... Sporulating V-8 juice agar plate cultures, 3 to 10 days old, 19 to 23°C, cw fluorescence >20µmol m^2sec^{-1}
Method ..... Cultures inverted 30 to 60 cm above plants, approximately one culture per 900 cm^2 of plant material, in place until 10 spores per cm^2 collected in trap slides; culture plates should be relocated every 15 to 30 min to ensure uniform coverage; plants are sprayed with water when plates are removed.
Length ..... 2 to 4 hours, variable
Conditions ..... Saturated R.H., 20+1°C, dark

INCUBATION

Duration..... 48 hours at 100% RH, 20°C
Location..... Move from moist conditions to green house after leaves have dried slowly out of direct sunlight.
Measurement ..... Type and size of leaf spot, usually 10 to 14 days after inoculation.

RATING

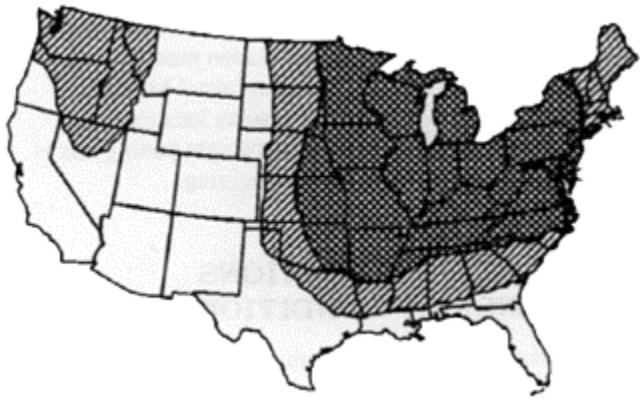
1 Resistant ..... No spots
2 Resistant ..... Barely visible black pepper spots, 1 mm diam. Or less
3 Susceptible ..... Spots >lmm, with or without tan center, no halo
4 Susceptible ..... Spots with tan center, halo
5 Susceptible ..... Spots >3mm, with tan center, halo, spots coalesced, leaf withered

CHECK CULTIVARS

	Approximate Expected Resistance (%)	Acceptable Range of Reaction (%)
Resistant		
MSA-PL-L	25	
Susceptible		
Ranger	5	0-10
Moapa 69	5	0-10

Values for resistant standards are totals of l's and 2's

DISTRIBUTION AND SEVERITY OF LEPTO LEAF SPOT



Lepto leaf spot, *Leptosphaerulina briosiana* (Poll.) Graham & Luttrell

Click on the map above for a larger version. See also the KEY.

SOURCE OF INOCULUM AND SCIENTIST WITH EXPERTISE

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CORRELATION TO FIELD REACTION

Good correlations to field; no exceptions reported.

RACES

No races are known.

CULTURE OPTIONS AND RANGE OF CONDITIONS

Light is critical for spore production. Fluorescent or near UV is as effective as natural daylight. Some glass petri dishes do not pass sufficient light below 340 nm wavelength for good sporulation. Cultures can be started either by placing pieces of agar containing fungal hyphae onto agar surface or by spreading a spore suspension, (prepared by scraping surface of mature culture in water), over agar plate surface. The latter is the quicker method, but if plates contain bacterial contaminants they will not be usable. When cultures are ready to use, ascospores discharged onto petri dish lid are visible. These can serve as contaminant free source for subsequent cultures.

PLANT GROWTH OPTIONS AND RANGE OF CONDITIONS

Vigorous plants are essential for expression of susceptible response. Light intensity after inoculation must exceed 1000 mole $m^{-2}sec^{-1}$. Supplemental light (metal halide or other) is necessary during winter at some locations to produce vigorous plants. Use of lightweight potting mix is best if plants are to be pulled during scoring.

INOCULATION CONDITIONS AND RANGE OF CONDITIONS

Temperatures from 15 to 25°C are probably usable. Light during infection not required. Lower temperatures slow infection but seldom cause failure; too high temperatures or drying leaf surfaces will result in failure.

HELPFUL INFORMATION

Plants may be cut back at scoring and regrowth used for different disease evaluation. Isolation of fungus is usually done by direct transfer of spores from sporulating pycnidia produced on leaf tissue. Infection does not kill stems or plants.

ALTERNATIVE METHODS

Field evaluations may be possible but field infections are rarely of sufficient purity, uniformity, and severity to facilitate satisfactory selection. Inoculations have been made by spraying spores onto leaves. Cultures are scraped in water, comminuted, and filtered through cheesecloth to remove large particles. This method often results in a light inoculation.

REFERENCES

1. Leath, K.T. 1971. Quality of light required for sporulation by *Leptosphaerulina.* Phytopathology 61:70-72.

2. Leath, K.T., and R.R. Hill, Jr. 1974. Large incubation chamber suited for use in selection for disease resistance. Crop Sci. 14:901-903.

3. Leath, K.T., and R.R. Hill, Jr. 1974. *Leptosphaerulina briosiana* on alfalfa: relation of lesion size to leaf age and light intensity. Phytopathology 64:243-245.