

# A Greenhouse Method to Screen for Resistance to Charcoal Rot in Soybean

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## Introduction

The fungus *Macrophomina phaseolina* (Tassi) Gold is the causal agent of charcoal rot disease of soybean (*Glycine max*(L.) Merr.), one of several susceptible host species. The pathogen invades the roots, colonizes the vascular system, and interferes with water transport. Under conditions favorable for disease, such as low soil moisture and high ambient temperatures, significant economic losses up to 77% have been reported in soybean (1). Partial resistance to the disease has been found in soybean and other host species (2). Field screening methods have been primarily used to identify partial resistance in soybean genotypes.

The main objective of this study was to develop a greenhouse method for screening soybean germplasm for resistance to charcoal rot that can be combined with protocols for screening other pathogens in a multiple disease resistance screening program.

## Materials and Methods

### 1. Plant materials:

- LS92-1088 5.1, LS94-3207 4.7, LS97-1218 4, LS98-0373, LS98-0719 4E, LS98-1430 4E, LS981612, LS98-2248 4L, LS98-2574 4E, LS98-3257 4L. These lines were provided by Jason Bond, Southern Illinois University.
- USDA Stoneville MS, provided DT97-4290.
- USDA Germplasm Collection, Urbana, Illinois provided 'Spencer'.

### 2. Experimental design:

- Three identical experiments with different randomizations.
- Randomized complete block design, with four blocks and 16 plants per experimental unit.

### 3. Preparation of plants:

- Test plants were grown to V2 stage in multi-pot flats (8 x 12; Hummert, Inc. St. Louis, MO) in soil-less mix, in the greenhouse at 30 °C with a 12-hour photoperiod.
- Soybean stems were cut 5 cm above the second node (Fig. 1).

### 4. Inoculation Method:

- Mycelium, five days old, of *M. phaseolina* was used.
- Micropipette tips (200 µL) were used to remove and place 5 cm mycelia plugs (100 µL) on cut stems (Fig. 2).
- Plants were incubated in a growth chamber at 30 °C with a 12-hour photoperiod.

### 5. Evaluation:

Mean percentage death at 14 days after inoculation and rank correlations between the experiments.

### 6. Analysis:

Data was analyzed with JMP 5.1 (2).



Figure 1. Plants at growth stage V2 inoculated with *Macrophomina phaseolina* using pipette tips on cut soybean stems.



Figure 2. Plants at growth stage V2 14 days after inoculation with *Macrophomina phaseolina*.



Figure 3. Plants resistant (Rows 1 and 2) and susceptible (Rows 3 and 4) to infection by *Macrophomina phaseolina*.

## Results

- Percentage of dead plants ranged from 0 to 89% (Figure 3. & Table 1).
- Spearman's  $\rho$  rank-correlation analysis indicated that ranks of entries in all three experiments were significantly correlated (table 2).
- Two entries, Spencer and LS98-3257 4L, had the best mean rankings.

## Conclusions

- The pipette tip inoculation method was repeatable across three experiments.
- Greenhouse results of the soybean lines used in this study must be compared with field responses in order to validate the use of the procedure.

Table 1. Percentage of dead plants and ranking within experiment, after challenge with *Macrophomina phaseolina*.

Test entries	Exp. 1	Exp. 2	Exp. 3
DT97-4290	12 (6)	8 (4)	2 (3)
LS92-1088 5.1	24 (8)	10 (5)	2 (2)
LS94-3207 4.7	57 (10)	17 (8)	47 (11)
LS97-1218 4	21 (7)	32 (11)	51 (12)
LS97-0373	89 (12)	32 (12)	31 (9)
LS98-0719 4E	7 (4)	10 (6)	14 (6)
LS98-1430 4E	7 (5)	2 (1)	25 (7)
LS98-1612	79 (11)	21 (9)	4 (5)
LS98-2248 4L	27 (9)	24 (10)	33 (10)
LS98-2574 4E	6 (3)	15 (7)	30 (8)
LS98-3257 4L	0 (1)	2 (2)	2 (4)
Spencer	1 (2)	4 (3)	2 (1)
Mean	22	14	17
LSD (0.05)	17	21	18

Table 2. Correlations of ranks of test entries between experiments.

Comparison	Spearman $\rho$
Exp. 1 vs. Exp. 2	0.39***
Exp. 1 vs. Exp. 3	0.28*
Exp. 2 vs. Exp. 3	0.29*

## References

1. Hartman, G.L., Sinclair J.B., & Rupe J.C. 1999. Compendium of Soybean Diseases.
2. Smith, G.S., & Carvil, O.N. 1997. Field screening of commercial and experimental soybean cultivars for their reaction to *Macrophomina phaseolina*. Plant Disease 81:363-368.
3. SAS Institute. JMP Version 5.1. Release 5.1 SAS Institute INC., Carv. NC 27513. USA.