A Rapid Method for Evaluating Genotype Resistance, Fungicide Activity, and Isolate Pathogenicity of *Sclerotinia minor* in Peanut.[1]

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ABSTRACT

Peanut stem segments 8.5-cm long were inoculated at leaf nodes with mycelial plugs of *Sclerotinia minor* and incubated in moist chambers at 20 C. Water-soaked lesions were often visible after 24 hr. The rate of lesion elongation was used to quantitatively assess the physiological resistance of peanut genotypes to Sclerotinia blight, screen chemicals for fungicidal activity, and evaluate the pathogenicity of *S. minor* isolates. The age and/or developmental state of lateral limbs of plants had a marked effect on lesion development; 3 days after inoculation, mean lesion lengths were 58.8, 46.8, and 38.9 mm for terminal, median and basal parts of stems, respectively. The speed, simplicity and adaptability of this method make it a valuable tool for research on Sclerotinia blight of peanut.

Key Words: *Arachis hypogaea*, fungicide screening, germplasm evaluation, Sclerotinia blight.

Sclerotinia blight of peanut (*Arachis hypogaea* L.), caused by *Sclerotinia minor* (Jagger) Kohn (6), is a serious disease of peanut in Virginia, Oklahoma, northeastern North Carolina, and certain localities in Texas. Although not detected in the U.S.A. until 1971 (14), losses to this disease in Virginia alone have been estimated at 13% in years favorable for disease development (17).

Such losses have resulted in the immediate need for effective, economical strategies for disease management. Current disease management recommendations include 1) planting cultivars with partial disease resistance, 2) avoiding high seed rates which increase plant height and canopy density, 3) cultivating before June 15 or preferably not at all, 4) using the leafspot advisory to reduce negative non-target effects of chlorothalonil and vine injury by tractor tires, and 5) scouting fields weekly for early disease detection and fungicide treatment after vines touch in the middles between adjacent rows (9). Various laboratory, growth chamber and field tests have been used to evaluate and develop these measures (5,10,11,12,16).

Test procedures using detached plant parts have been developed to investigate other peanut diseases such as leafspot (8). Chun *et al.* (4) recently reported an excised stem technique to assess resistance in soybean to stem rot caused by *Sclerotinia sclerotiorum*. Such methods have the advantage of requiring little greenhouse space for evaluation of numerous genotypes, and results are obtained quickly. This paper reports on an excised stem technique that can be adapted for rapid evaluation of physiological resistance in peanut genotypes, fungitoxicity of chemicals, and pathogenicity of isolates of *S. minor*. An abstract of the method has been published (3) and a subsequent abstract by Melouk and Akem (7) reported using a detached shoot technique to differentiate reaction of peanut genotypes to *S. minor*.

Materials and Methods

Uniform lateral limbs were excised from field or greenhouse grown peanut plants and rinsed in tap water. A razor blade was then used to remove all leaves and pegs at their juncture with the stem. One 8.5-cm stem segment was cut from the same moist location on each limb, rinsed in distilled water, and then placed in most chambers consisting of 20 x 10 x 3.8 cm plastic boxes with hinged lids. The stems were supported at each end by a 18.5 x 1.3 x 1.3 cm pine slat, and near 100% relative humidity was maintained by adding 30 mL water to a paper towel in the bottom of each box.

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Isolates of *S. minor* were grown on plates of glucose-yeast extract agar (GYEA) consisting of dextrose, 20 g; yeast extract, 2.0 g; KH_2PO_4, 1.0 g; $MgSO_47H_20$, 0.5 g; agar, 20 g; and distilled water, 1000 mL. A cork borer was used to cut 5-mm-dia. plugs of agar and mycelium from the periphery of actively growing cultures of *S. minor*. The plugs were placed with the mycelium directly in contact with the stem either between nodes or on a node where a leaf or a peg had been removed.

Effect of wounds and stem development. Inoculations at internodes were made to assess the importance of wounding for infection by *S. minor*. Inoculum in these tests was applied to either a non-wounded internode or to a 3-mm-long by 1-mm-deep stem puncture made with a needle. The relative susceptibility of terminal (young), median, and basal (oldest) parts of lateral limbs was assessed by inoculation at stem nodes following the removal of a leaf or leaf and peg. Inoculated stems were incubated in moist chambers at 18-20 C. Stem lesion length was measured at 24 hr intervals.

Evaluation of isolate pathogenicity. Median segments of lateral limbs from 12-wk-old Florigiant plants were cut and inoculated as described previously. Four replications were used the test was repeated. Isolates from grower fields (S-1, S-2 and S-5) and dicarboximide-resistant subcultures obtained in earlier *in vitro* fungicide sensititivy tests were compared (2).

Evaluation of cultivar resistance. Stem segments from six peanut cultivars were evaluated for susceptibility to colonization by *S. minor*. Median stem segments were cut from 10-wk-old field-grown peanut plants, and inoculated with isolate S-2. The test was repeated for five of the cultivars with greenhouse-grown plants.

Evaluation of fungicides. The procedure was adapted to evaluate fungicides for the control of Sclerotinia blight. After preparing median segments from lateral limbs of Florigiant peanut, the stems were immersed in fungicide suspensions for 1 min. Stem segements were then removed and allowed to dry at room temperature prior to inoculations.

A range of concentrations of the fungicides, dicloran (2,6-Dichloro-4-nitroaniline as Botran 75W) and vinclozolin (3-(3,5-Dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxyazolidinedione as Ronilan 50W), were tested with two isolates of *S. minor*. A fungicide-sensitive field isolate (S-2) and a subculture (R-2C) of this isolate having *in vitro* resistance to dicarboximide fungicides (2) were compared with and without fungicide treatment. The percent inhibition of lesion expansion for each fungicide treatment was calculated on the basis of lesion lengths at 3 days after inoculation. Levels of inhibition were then plotted against fungicide concentration, and linear regression analyses were used to determine dosage levels for 50% inhibition of lesion expansion (ED_{50} values). Treatments were replicated five times.

Results and Discussion

Effect of wounds and stem development. Experiments demonstrated that wounding was necessary for infection. Nearly 100% infection was achieved with wounding and lesion expansion was at a uniform rate. Stems inoculated between nodes without injury at the site of inoculation usually did not develop lesions (Table 1). Stems inoculated at nodes where leaves had been removed developed longer lesions of more consistent length than did stems inoculated at wounds between nodes. The mean length of lesions at 2.5 days was 34.2 ± 4.9 mm for node-inoculated segments. Similar stem segments inoculated at a wound in the internode had lesions with a mean length of 22.4 ± 8.0 mm. Since node inoculation resulted in longer lesions of more consistent length, this method was used in all subsequent work.

These findings provide additional support for the contention that wounds are an important factor in pathogenesis by *S. minor* on peanut as well as soybean (13,15). It is not known if the few lesions that occurred on nonwounded stems were the result of direct tissue penetration or the presence of superficial abrasions made during collection and preparation of stems.

Table 1. The effect of wounding and tissue age on infection of excised peanut stems by *Sclerotinia minor*.

Stem segment	Lesion length (mm)[1] Test I[2]	Test II
Inoculated at node (wound)		
Terminal	63.2 a	58.8 a
Median	54.0 b	46.8 b
Basal	38.6 c	38.9 c
Inoculated at internode (no wound)		
Terminal	0.3 d	0.7 d
Median	0 d	1.1 d
Basal	0 d	0.3 d

[1] Time elapsed between inoculation and lesion measurement was three days. The peanut cultivar used was Florigiant.

[2] Test I represents the means of six S. minor isolates and Test II the means of nine isolates. Means in columns followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

Lesions lengths at 3 days after inoculation varied significantly according to the origin of a stem segment (Table 1). Segments from the youngest or terminal portions of lateral limbs were the most rapidly colonized by *S. minor*, followed by segments from median and basal parts, respectively. Since basal nodes had both leaves and pegs and terminal nodes had only leaves, differences could be a result of variations in susceptibility at the site of infection. However, the same trend in susceptibility was also demonstrated in a separate test wherein terminal, median and basal stem segments were inoculated at uniformly-wounded internodes (unpublished data). These results indicated that stem development and/or physiology was affecting susceptibility to infection. The basis for this difference in susceptibility is not known, but it may be related to increased lignification and/or decreased sugar content often associated with older tissues. From a disease control perspective, these findings reinforce the importance of broadcasting fungicide treatments over the entire width of the rows to protect the vine terminals which are most susceptible to colonization by the fungus and injury by tractor tires. Previous work has documented increased incidence and severity of Sclerotinia blight where vines have been injured by tractor tires (15).

Evaluation of isolate pathogenicity. Colonization rates for nine isolates of *S. minor* indicated significant differences in pathogenicity (Table 2). Although several of the *in vitro* fungicide-resistant strains had reduced pathogenicity, all proved to be capable of infecting stems and causing visible lesions. The two tests were highly correlated indicating the stem method gave consistent results ($r=0.91$, $P=0.01$). The pathogenicity of two of these isolates, R-2C and S-2, was quantified in a previous study using field microplots (1). Disease severity ratings in field microplots indicated no significant differences between the two isolates, which corresponds to results with excised stems.

Table 2. Pathogenicity of *Sclerotinia minor* isolates on excised peanut stems (cv. Florigiant).

Isolate[1]	Lesion length (mm) at day 3
S-2	47.3 abc[2]
R-2A	50.3 ab
R-2C	50.3 ab
S-5	56.5 a
R-5A	43.0 bcd
R-5B	33.0 d
S-1	49.7 ab
R-1A	37.5 cd
R-1D	53.2 ab

[1] 'S' indicates a fungicide-sensitive field isolate and 'R' indicates an in vitro dicarboximide-resistant laboratory subculture of S. minor.

[2] Mean of four replications, each being a median stem segment. Means followed by same letters are not significantly different (P=0.05) according to Duncan's multiple range test.

Evaluation of peanut cultivar resistance. The excised stem method proved useful for screening peanut genotypes for physiological resistance to infection by *S. minor*. Results of a test of stem segments from field-grown plants are shown in Table 3. Evaluation of these cultivars in a similar test with plants grown in the greenhouse gave comparable results with the exception that NC 7 was not as susceptible as in the test with field-grown plants and NC 8C was not as resistant. VA 81 Bunch and AD 1, cultivars having partial field resistance to Sclerotinia blight, exhibited partial resistance in the excised stem test. Both VA 81 Bunch and AD 1 have an open canopy which is thought to lower susceptibility by allowing sunlight penetration and more efficient air circulation, thus suppressing fungal growth (5). Results of the excised stem tests suggested that these cultivars may also possess some physiological resistance. Earlier work (16) showed Florigiant to be moderately susceptible to Sclerotinia blight; however, its field resistance was not as great as suggested by the excised stem evaluation. This apparent discrepancy might be due to the dense canopy characteristic of Florigiant which promotes environmental conditions favorable for fungal growth.

Table 3. Evaluation of six peanut cultivars to infection by *Sclerotinia minor* using the excised stem method.

Cultivar	Lesion length (mm)[1]
NC 7	53.8 a[2]
NC 6	53.0 a
NC 8C	47.6 b
Florigiant	46.2 b
VA 81 Bunch	45.4 b
AD 1	43.2 b

[1] Fungicide sensitive isolate S-2 was used; data are means of five replications measured on day 3 after inoculation.

[2] Means followed by same letters are not significantly different (P=0.05) according to Duncan's multiple range test.

Evaluation of fungicides. The sensitivity of isolates S-2 and R-2C to fungicides was similar but R-2C was less inhibited by either dicloran or vinclozolin. The mean ED_{50} values for two tests with isolate S-2 were 36.6 and 4.6 mg/L for dicloran and vinclozolin, respectively. The ED_{50} values for isolate R-2C in these tests were 53.7 and 5.9 mg/L. All curves fit a linear model with R^2 values between 0.92 and 0.99 (Fig. 1).

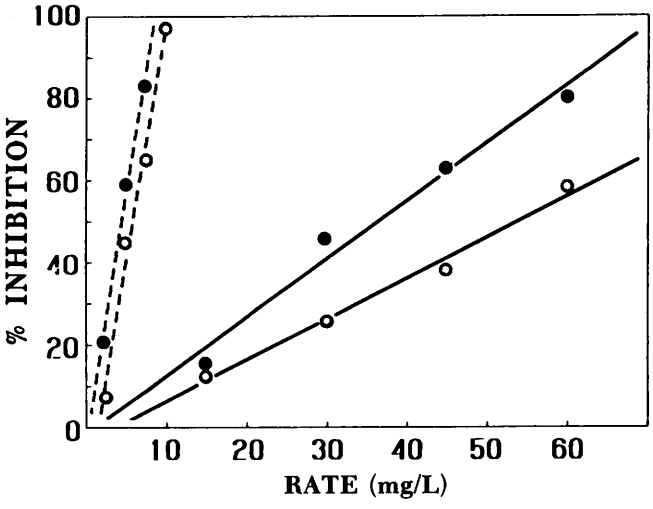

Fig. 1. Dosage-response effect of dicloran (—) and vinclozolin (---) on lesion expansion in excised stem tests with *Sclerotinia minor* isolates S-2 (●) and R-2C (○).

Although somewhat higher, ED_{50} values for isolate R-2C were reasonably close to those of its fungicide-sensitive parent isolate, S-2. Certainly the difference in S-2 and R-2C was much less than that observed in tests with fungicide-amended agar medium wherein R-2C was still able to grow at fungicide levels of 1000 mg/L(2). This correlates well with findings in fungicide-treated microplots where fungicide-resistant isolates were controlled to the same degree as were sensitive field isolates (1).

Conclusions

The excised stem method affords rapid, efficient evaluation of breeding lines, fungicides, or isolates with a small amount of plant material. This is a major benefit during early screening procedures when compared to field studies which require a large investment of land, labor, and time.

Germplasm screening results are probably the most difficult to relate to field performance, because plant growth and canopy development can have a marked influence on disease severity. The method appears to be most efficient for screening fungicides for potential value in Sclerotinia blight control, because the pathogen uses physiological processes required for parasitism rather than the saprophytic processes required for *in vitro* growth on synthetic media.

As suggested by Melouk *et al.* (8), tests with excised tissues are not a substitute for field evaluation. Plant architectural factors are believed to be important traits which may enable cultivars to escape infection by *S. minor*. Future work to develop peanut cultivars resistant to Sclerotinia blight should focus on combining genes which convey both physiological and architectural traits for disease control. Considering the speed, simplicity and adaptability of the excised stem method, it should be useful for research on Sclerotinia blight of peanut.

Acknowledgements

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Literature Cited

1. Brenneman, T. B., Phipps, P. M., and Stipes, R. J. 1987. Sclerotinia blight of peanut: Relationship between in vitro resistance and field efficacy of dicarboximide fungicides. Phytopathology 77:1028-1032.
2. Brenneman, T. B., Phipps, P. M., and Stipes, R. J. 1987. Control of Sclerotinia blight of peanut: Sensitivity and resistance of *Sclerotinia minor* to vinclozolin, iprodione, dicloran, and PCNB. Plant Dis. 71:87-90.
3. Brenneman, T. B., Phipps, P. M., and Stipes, R. J. 1984. A rapid technique to assess pathogenicity of *Sclerotinia minor* on peanut. Phytopathology 74:815 (Abstr.).
4. Chun, D., Kao, L. B., and Lockwood, J. L. 1987. Laboratory and field assessment of resistance in soybeans to stem rot caused by *Sclerotinia sclerotiorum*. Plant Dis. 71:811-815.
5. Coffelt, T. A., and Porter, D. M. 1982. Screening peanuts for resistance to Sclerotinia blight. Plant Dis. 66:385-387.
6. Kohn, L. M. 1979. A monographic revision of the genus *Sclerotinia*. Mycotaxon 9:365-444.
7. Melouk, H. A., and Akem, C. N. 1986. A detached shoot technique for evaluating reaction of peanut genotypes to *Sclerotinia minor*. Proc. Am. Peanut Res. Educ. Soc. 18:57 (Abstr.).
8. Melouk, H. A., and Banks, D. J. 1978. A method of screening peanut genotypes for resistance to Cercospora leafspot. Peanut Sci. 5:112-114.
9. Phipps, P. M. 1987. Disease Control. Pages 32-46 in 1987 Peanut Production Guide. Publ. MA197, Va. Coop. Ext. Ser., VPI&SU, Blacksburg.
10. Phipps, P. M. 1987. An evaluation of cultural and chemical practice for management of Sclerotinia blight of peanut in Virginia. Biol. & Cult. Tests 2:52, American Phytopathological Society, St. Paul, MN.
11. Phipps, P. M. 1980. Soil plate and field evaluation of fungicides for control of Sclerotinia blight of peanuts. Phytopathology 70:692 (Abstr.).
12. Phipps, P. M. and Coffelt, T. A. 1986. Susceptibility of peanut cultivars to Sclerotinia blight, 1985. Biol. & Cult. Tests 1:50, American Phytopathological Soceity, St. Paul, MN.
13. Phipps, P. M., and Porter, D. M. 1981. Sclerotinia blight of soybean caused by *Sclerotinia minor* and *Sclerotinia sclerotiorum*. Plant Dis. 66:163-165.
14. Porter, D. M., and Beute, M. K. 1974. Sclerotinia blight of peanuts. Phytopathology 64:263-264.
15. Porter, D. M., and Powell, N. L. 1978. Sclerotinia blight development in peanut vines injured by tractor tires. Peanut Sci. 5:87-90.
16. Porter, D. M., Beute, M. K., and Wynne, J. C. 1975. Resistance of peanut germplasm to *Sclerotinia sclerotiorum*. Peanut Sci. 2:78-80.
17. Porter, D. M., Powell, N. L., and Phipps, P. M. 1983. Disease detection and crop loss assessment in peanut fields with aerial infrared photography. (Abstr. 86). Page 22 in: Proc. Int. Congr. Plant Pathol., 4th.

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