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In Vitro Apothecial Production of *Sclerotinia minor*

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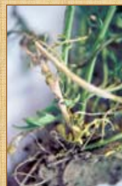
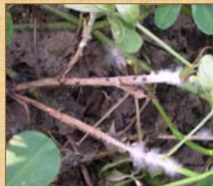
Introduction

Sclerotinia minor Jagger is an important pathogen of peanut (*Arachis hypogaea* L.) in many regions of the world. The fungus produces sclerotia that persist in the soil or on plant debris. These sclerotia germinate myceliogenically and infect peanut plants near the soil surface. Carpogenic germination and infection by ascospores are rare and unimportant during the peanut growing season. However, we have identified many winter weed species as hosts of *S. minor* and concurrently have observed apothecia of *S. minor* in harvested peanut fields during winter fallow. These observations suggest that weed infection by ascospores may support populations of *S. minor* between peanut crops.





Objective

The objective of this study was to identify differences in apothecial formation by isolates of *S. minor* found infecting weed hosts from fallow peanut fields.

Materials and Methods

Sclerotial production. Thirteen isolates of *S. minor* originally collected from weed hosts and two from peanut were observed for apothecial production. The isolates were cultured on sterile oat grains, dried, and stored in Nalgene bottles in the refrigerator.

An oat grain of each isolate was placed on potato dextrose agar (Pda) until mycelium emerged (2-3 days). Each isolate was then transferred to a fresh Pda dish and allowed to grow for 2 days.

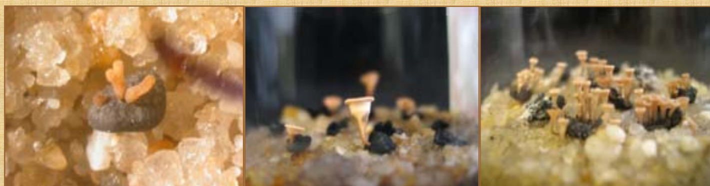
Isolates were grown on carrot discs to provide sclerotia > 2 mm diameter. A volume of 150 cm^3 of unpeeled carrot discs sliced 6 mm thick were placed in a 250 ml flask with 5 ml of deionized water and autoclaved. Mycelial plugs were added to the carrot discs and incubated in the dark at room temperature for 10 days without shaking.

Once black sclerotia had formed and white initials were no longer present, water was added to a 250 ml volume and flasks were shaken to dislodge the sclerotia. The suspension was poured over a 850 µm sieve nested on a 600 µm sieve to separate the sclerotia. Hyphal tissue was removed with the assistance of hand tweezers. Sclerotia were air-dried on paper towels for 48 h and separated by size on >2 mm and >1mm sieves. Sclerotia >2 mm were used for apothecia production. Weights of the sclerotia were recorded.

Stipe and apothecial production. Sterile coarse sand and deionized water (4:1 v/v), was prepared in 120 ml glass jars and autoclaved for 45 min. Ten sclerotia from each fungal isolate were placed on the sand surface in the jars. The jar lids were replaced with sterile small plastic petri dish lids (60 x 15 mm) and sealed with parafilm. The sclerotia were placed in a pattern so they could be repeatedly evaluated over time. Jars were arranged in a randomized complete block with 3 reps per isolate.

A three-stage conditioning process was used to induce production of apothecia. The sclerotia were incubated at 4°C in the dark for 30 days, checked for mycelial germination, and then moved to 12°C. After 14 days, sclerotia were checked for stipe formation. Jars remained at 12°C until 50% of sclerotia in a jar had stipes 5 mm long. After 62 days all jars were moved to a growth chamber at 16 ± 2°C with 12 h of diurnal light cycle under florescent lights until apothecia were fully developed. Formation of stipes and differentiation of apothecia were observed under the dissecting scope weekly for 12 weeks.

Chi-square and ANOVA analyses were applied to the data.



Ascospore evaluation. After 30 days of light treatment, selected apothecia were removed from the jars and placed on their side on blue medium to force ascospore release. The blue medium consisted of 30 g Pda, 20 mg PCNB (75% WP), and 50 mg pH indicator Bromophenol blue per liter deionized water. A yellow halo indicating the presence of ascospores aided in the microscopic observation. Single ascospores were isolated on Pda, and were later paired on Diana Sermons Medium (DSM) (Cubeta et al, 2001) for mycelial compatibility comparisons. Each pairing was performed twice. Pairings were scored as compatible or incompatible based on the mycelial reactions.

Results and Discussion

The average time to first stipe appearance ranged from 62 to 84 days. The average number of days to the first apothecia appearance ranged from 95 to 107 days. There were significant differences among isolates in every variable observed.

All isolates of *S. minor* produced stipes and apothecia from sclerotia. However, the proportion of sclerotia producing stipes and apothecia varied among isolates (Table 1). On average, the 13 isolates from weeds produced more stipes and apothecia than the two isolates from peanut.

Isolate W8 from mouseear chickweed produced the most stipes and isolate W9 from swinecress produced the fewest stipes. Isolates W1 from yellow nutsedge and W10 from cutleaf evening primrose had the most sclerotia to produce stipes. Isolate W8 had the largest sclerotial size by weight and also produced the most stipes per sclerotia. Isolate W1 from yellow nutsedge and isolate W5 from smallflowered bittercress produced more stipes/apothecia than predicted and isolate P13 from peanut produced fewer than predicted by sclerotial size. Even though isolates W8 and W10 produced a larger number of stipes, many of them never made apothecia. Sclerotia of several isolates germinated myceliogenically in addition to producing stipes and apothecia.

In general, the number of stipes per sclerotium, total number of stipes, and number of apothecia was related to the size of sclerotia by weight. However, several isolates did not fit this pattern for apothecia/stipe and stipes/sclerotia. Isolate W1 from yellow nutsedge and isolate W5 from smallflowered bittercress produced more stipes than predicted by size and isolate P13 from peanut produced fewer stipes than predicted.

All single ascospores from within an apothecium were scored compatible in the mycelial compatibility pairings.

Table 1. Stipe and Apothecial Production

Isolate	Sclerotia Wt	Sclerotia with Stipes	Stipes	Apothecia	Stipe / Sclerotia	Apothecia / Stipe
W08	116.8167 a	7.0000 ab	44.5000 a	34.1667 a	6.3790 a	0.6966 bcd
W10	99.2667 ab	8.6667 a	35.1667 ab	23.6667 ab	3.9741 ab	0.6133 cd
W04	83.1500 bc	4.5000 bcd	9.1667 cd	8.5000 bc	1.7500 bc	0.9658 ab
P13	82.0333 bc	2.1667 d	3.6667 d	3.8333 c	1.0833 c	0.5602 cd
P58	80.9500 bc	3.3333 bcd	12.0000 cd	4.0000 c	2.9056 bc	0.5102 d
W12	78.6667 bc	4.1667 bcd	5.0000 d	5.1667 bc	1.1738 c	1.0069 ab
W07	77.7167 bc	3.1667 bcd	7.3333 cd	7.1667 bc	1.8393 bc	0.9650 ab
W01	76.2167 bc	8.6667 a	27.3333 abc	23.3333 ab	3.0415 bc	0.8133 a-d
W13	75.6333 bc	2.8333 cd	5.8333 d	5.5000 bc	1.0222 c	0.9590 abc
W02	75.5167 bc	5.8333 a-d	13.3333 cd	12.3333 bc	1.7669 bc	0.9232 abc
W11	72.8833 bc	2.3333 d	4.3333 d	3.8333 c	1.4861 bc	0.8632 abc
W05	71.0000 c	6.8333 abc	17.5000 bcd	15.5000 abc	2.4181 bc	0.9336 abc
W06	66.1500 c	2.5000 d	5.0000 d	4.6667 bc	1.3583 bc	0.8175 a-d
W09	61.7167 c	2.5000 d	3.1667 d	3.3333 c	1.0111 c	1.0454 a
W03	61.2167 c	3.6667 bcd	4.3333 d	3.8333 c	1.1548 c	0.8944 abc

Conclusions

All isolates of *Sclerotinia minor* observed were capable of producing stipes and apothecia even though there were differences in the numbers produced. It is possible that infection of weed species and apothecial production may support populations of *S. minor* between peanut crops.

References

Cubeta, M. A., Sermons, D. N., Cody, B. R. 2001. Mycelial interactions of *Sclerotinia minor*. Phytopathology 91:S19. Publication no. P-2001-0138-AMA.

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