

Viability of Sclerotia of *Sclerotinia minor* After Passage Through the Digestive Tract of a Crossbred Heifer

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ABSTRACT

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Peanut hay of the cultivar Florunner infested with *Sclerotinia minor* and containing sclerotia was fed for 10 days to a crossbred heifer. Fecal and ruminal samples were collected 6-9 days after the feeding began. Sclerotia of *S. minor* were recovered from fecal and ruminal samples by wet-sieving on a series of metal screens. Sclerotialike bodies retained on the 0.84-mm screen were collected, surface-sterilized in 0.5% NaClO for 3 min, and plated on potato-dextrose agar containing 100 µg/ml of streptomycin sulfate. Viable sclerotia were recovered from fecal and ruminal samples regardless of collection time. However, survival of sclerotia was greater in ruminal samples than in fecal samples. Viable half-life in the rumen was about 20 hr. Cultures of *S. minor* from fecal and ruminal sclerotia were pathogenic to peanut cultivar Tamnut 74 under greenhouse conditions.

Sclerotinia blight of peanut (*Arachis hypogaea* L.) caused by *Sclerotinia minor* Jagger has been endemic in Oklahoma since 1972 (7). White, cottony, fluffy mycelium appear on the base of diseased stems. Infected branches become chlorotic and eventually die. The pathogen produces numerous sclerotia on the surface and within infected stems, pegs, and roots. Sclerotia also can form between the shell and seed of infected peanut pods.

S. minor overwinters as sclerotia. Germinable sclerotia have been found in soil throughout the plow layer (the top 20 cm) of fields having a previous history of Sclerotinia blight even after a field has not been planted to peanuts for 4 yr. One sclerotium per 100 g of soil is sufficient to cause severe infection under favorable conditions for disease development (6).

Sclerotia of *Sclerotium rolfii* Sacc.

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ensure a high population of sclerotia in the hay, sclerotia of *S. minor* produced on oat seeds were added to the infested hay before feeding cattle.

Production of sclerotia on oat seed.

Fifty grams of oat seed and 50 ml of deionized water were mixed in 500-ml flasks, autoclaved for 1 hr, allowed to cool, and inoculated with four disks (6 mm in diameter) from the periphery of a 3-day-old culture of *S. minor* grown on potato-dextrose agar (PDA). The flasks were incubated for 5 wk at 25 ± 2 C in darkness. Then the contents of each flask were spread out to dry at 25 ± 2 C.

Feeding cattle and collection of samples.

Two crossbred heifers fitted with ruminal cannulas (8) were housed in individual pens with free access to water at all times. One of the heifers was fed 4.55 kg of infested hay plus 0.34 kg of infested oats per day for 10 days. The other heifer was fed noninfested hay and oat seed in a similar fashion. Four fecal and ruminal samples were collected daily for 4 days, commencing the sixth day after feeding. The ruminal samples were collected via cannula. Several 100-g portions of the fecal and ruminal samples were placed on 8-mesh (2.36-mm pores) and 20-mesh (0.84-mm pores) screens and wet-sieved under a stream of running tap water. Material retained on the 8-mesh screen was discarded. Sclerotia of *S. minor* retained on the 20-mesh screen were collected and placed on Whatman No. 1 filter paper in open 9-cm petri dishes at 25 ± 2 C for 24 hr to dry.

Sclerotial viability. Sclerotia collected from the fecal and ruminal samples were surface-sterilized in an aqueous solution of 0.5% sodium hypochlorite for 3 min and plated on PDA containing 100 mg/L of streptomycin sulfate (SPDA). A maximum of six sclerotia was plated in each 9-cm petri dish and incubated at 25 ± 2 C for 5 days in darkness. The number of germinating sclerotia was then recorded.

Survival of sclerotia in the rumen.

Sclerotia of *S. minor* were mass-produced on oat seeds as described previously. Oat seeds containing numerous sclerotia were separated by sieving

were reported to pass through the digestive tract of sheep and cattle without loss of viability (2). In sheep and cattle, 8-28% of sclerotia consumed survived passage as whole sclerotia through the rumen; 0.7-15% were viable and undoubtedly could disseminate the disease into uninfested areas of sugar beets (*Beta vulgaris* L.) (2). Similarly, livestock were found to spread *Sclerotinia sclerotiorum* (Lib.) de Bary, the causal agent of Sclerotinia on lettuce (1). When infected heads of lettuce containing large quantities of sclerotia were fed to sheep, 1-5% of sclerotia survived as whole sclerotia but viability was less than 1% (1).

In a peanut field in Hughes County, OK, we observed a pattern of distribution of Sclerotinia blight that was not uniform. We suspected disease spread within this field by cattle that were fed Sclerotinia-infested peanut hay. Peanut hay is sometimes fed to cattle as a source of roughage during winter months as they pasture on winter small grains being grown as a cover crop on peanut land. The objective of this study was to test the survival of sclerotia of *S. minor* after ingestion by cattle. A preliminary report of this study was presented previously (5).

MATERIALS AND METHODS

Peanut hay. Peanut hay of cultivar Florunner was collected after harvest from a field that had severe Sclerotinia blight infection in 1984. The hay contained numerous sclerotia of *S. minor*. The infested hay was stored in a building at 18-24 C from the middle of December 1984 to the end of February 1985. To

through the 8-mesh screen. The sclerotia were collected and allowed to dry at 25 ± 2 C for 5 days. About 80 sclerotia were placed in nylon mesh bags (100- μ m pores) to which weights were attached to keep the bags submerged in fluid in the rumen. Bags were placed into the rumen via cannula. Four bags were placed into the rumen of one of the heifers at each time. The bags containing the sclerotia were removed after ruminal fluid incubation times of 0, 12, 24, 36, and 48 hr. Sclerotia in similar nylon bags were submerged in McDougal's artificial saliva, pH 4, in 250-ml flasks and incubated in a water bath at 39 C for similar time periods (3). After the desired incubation period, 50 sclerotia were randomly removed from each bag, washed with deionized water, surface-sterilized in sodium hypochlorite solution, and plated on SPDA to determine viability.

Pathogenicity test. Fifteen isolates of *S. minor* were recovered from the ruminal and fecal samples and tested for pathogenicity on peanut cultivar Tamnut 74. Each isolate was tested by inoculation of six detached shoots of Tamnut 74. The basal end of 15-cm-long shoot-tips was immersed in Hoagland's solution in a 1 \times 14 cm test tube and was supported by a foam plug. All leaves, except about 1 cm of petiole, were removed from each shoot. A 4-mm-diameter plug of *S. minor* from the periphery of a 2-day-old culture on PDA was placed between the stem and a petiole in the middle of the shoot. The shoots were incubated in a fabricated polyethylene enclosure in a growth chamber at 28 ± 2 C during the day and 25 ± 2 C during the night. Relative humidity was maintained at 95–100% by lining the bottom of the enclosure with wet burlap (4). Lesions appeared on the shoots within 3 days after inoculation.

Statistical analysis. Data were analyzed using standard analysis of variance procedures. Linear regression was performed as deemed appropriate.

RESULTS AND DISCUSSION

Recovery of viable sclerotia of *S. minor*. Viable sclerotia of *S. minor* were recovered from ruminal and fecal samples collected at all sampling dates. On the sixth and ninth day after feeding, the viability (%) of sclerotia recovered from ruminal samples was significantly higher than that of sclerotia from fecal samples (Table 1). On the seventh and eighth days after feeding, there was no significant difference between the viability of sclerotia recovered from ruminal and fecal samples. In our test, the lowest viability of sclerotia recovered was 8% from the fecal sample of the crossbred heifer collected on the ninth day of feeding. This is considerably higher than the survival of sclerotia of *S. sclerotiorum* (<1%) recovered from fecal samples of sheep (1). The passing of germinable sclerotia in cattle feces after cattle are fed

Table 1. Viability of sclerotia of *Sclerotinia minor* from ruminal and fecal samples after feeding a crossbred heifer infested peanut hay

Days after feeding	Total sclerotia plated		Viability (%)		Probability
	Ruminal	Fecal	Ruminal	Fecal	
6	94	16	42	17	0.10
7	130	47	48	38	NS ^a
8	135	41	31	18	NS
9	87	19	35	8	0.01

^aNS = not significant.

Table 2. Survival of sclerotia of *Sclerotinia minor* in the ruminal fluid of a crossbred heifer and McDougal's artificial saliva^a

Incubation time (hr)	Viability (%)		Probability
	Rumen	McDougal's artificial saliva	
0	85	85	NS ^b
12	57	62	NS
24	47	10	0.01
36	4	7	NS
48	3	9	NS

^aLinear regression equations for rumen: $Y = -1.80 X + 82.10$, $R^2 = 0.87$; for McDougal's artificial saliva: $Y = -1.73 X + 76.10$, $R^2 = 0.78$.

^bNS = not significant.

infested hay seems to be a viable means for spreading the sclerotia of *S. minor* and Sclerotinia blight in peanut fields. Even though the passing of sclerotia of *S. minor* through the digestive tract of ruminant animals can inactivate or reduce viability by as much as 92%, enough sclerotia remain viable to initiate new infections. Porter (6) reported that only one sclerotium per 100 g of soil is sufficient to cause severe infection under favorable conditions for Sclerotinia blight development.

Survival of sclerotia in the ruminal fluid and McDougal's artificial saliva. Sclerotia of *S. minor* incubated in the rumen and in McDougal's artificial saliva remained viable after 48 hr of incubation (Table 2). After 24 hr of incubation in the rumen, 47% of the sclerotia were viable. Sclerotia incubated in McDougal's artificial saliva for 24 hr at 39 ± 0.1 C were only 10% viable (Table 2). After 36 and 48 hr of incubation in the rumen and in McDougal's artificial saliva, the viability of sclerotia was reduced drastically (Table 2). Linear regression of viability against time of incubation in the rumen and McDougal's artificial saliva buffer revealed a negative relationship, $R^2 = 0.87$ and 0.78, respectively. Ruminal retention time for hay particles typically ranges from 20 to 30 hr.

Pathogenicity of isolates of *S. minor* recovered from viable sclerotia. Fifteen isolates of *S. minor*, from sclerotia recovered from the fecal and ruminal samples, were pathogenic to the peanut cultivar Tamnut 74 by the detached shoot technique. Eight isolates were from sclerotia recovered from fecal samples across the four sampling dates. The pathogenicity of these isolates was comparable to a standard isolate of *S. minor* that is used routinely in our lab for

preliminary screening of peanut genotypes to identify resistance. Therefore, the ingestion of the sclerotia of *S. minor* by ruminant animals does not appear to affect their pathogenicity on peanut. Passage through the digestive tract has very important ramifications in the spread of sclerotia of *S. minor* in peanut fields.

This study demonstrates that caution should be exercised by peanut growers when feeding ruminant animals peanut hay that is infested with sclerotia of *S. minor*. Survival through the digestive tract would be expected to enhance the spread of Sclerotinia blight from infested areas to clean areas within a field or from infested fields to clean fields. Therefore, the possibility of spreading sclerotia of *S. minor* by ruminants cannot be ignored as a means of disease dissemination.

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