Crown and Stem Rot of Alfalfa Caused by *Sclerotinia sclerotiorum*

R. G. GILBERT, Research Plant Pathologist, Vegetable and Forage Crops Production, Agricultural Research Service, U.S. Department of Agriculture, Prosser, WA 99350

ABSTRACT

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Sclerotinia crown and stem rot was observed at high incidence in several broadcast-planted alfalfa seed production fields in the Touchet-Gardenia area of southeastern Washington in 1982. The disease cycle and activity of *Sclerotinia* was characterized in test fields. Apothecia were produced from sclerotia in March and April. Symptoms of *Sclerotinia* infection and extensive mycelial growth on host tissue occurred from early May through June. Dormant and overwintering sclerotia were produced abundantly in June and July. Sclerotial inoculum density data were obtained from 1-m^2 canopy-soil profile samples of three fields. Each sample consisted of an individual 1-m^2 sample of alfalfa stem tissue, surface residue, and surface soil. From 30 to 55% of all sclerotia were detected inside alfalfa stems, and the remaining 45–70% were detected in or on plant residue and soil. Sclerotia ranged from 0.85 to 3.35 mm in diameter, with most of the smaller sclerotia produced within the alfalfa stems. Initially, the disease was assumed to be caused by *Sclerotinia trifoliorum*. However, ascospore dimorphism and number of ascospore nuclei indicated that the pathogen was *S. sclerotiorum*, which has not previously been reported on alfalfa.

Additional key words: forage, soilborne disease

Crown and stem rot of alfalfa caused by a species of *Sclerotinia* was observed in July 1982 in several broadcast-planted alfalfa stands used for seed production in the Touchet-Gardinia area near Walla Walla in southeastern Washington. Crown and stem rot of alfalfa, caused by *S. trifoliorum* Erikss., is known to occur in cool, humid areas of Europe and North America (1,4,5) but was not expected to occur in alfalfa fields in eastern Washington with its dry and hot desert climate. However, the dense plant canopy combined with the cool and wet conditions in April, May, and June of 1982 produced near-optimal conditions for a severe occurrence of this disease activity. During June and July, many fields throughout this production area showed considerable browning and dieback caused by *Sclerotinia*. There are no cultivars of alfalfa resistant to *Sclerotinia*, and the only control measures are cultural practices.

The major objectives of this study were to identify the *Sclerotinia* spp. causing this disease in southeastern Washington and to investigate the disease cycle and pathogenic behavior of the pathogen causing this damage in alfalfa seed fields. Portions of this report have been published (3).

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MATERIALS AND METHODS

Description of experimental alfalfa seed fields. Three alfalfa seed production fields were selected in 1982 for evaluation and characterization of the disease cycle and pathogenic activity of *Sclerotinia*. Fields 1 and 2 were 2- and 4-yr-old stands of Blazer and G777 (Funks) alfalfa, respectively. Field observations indicated both fields had a high incidence of disease. Field 3 was a 3-yr-old stand of G777 (Funks) that was adjacent to field 2; however, it was difficult to visually detect disease symptoms in this field. Similar cultural management practices applied to these fields included: winter burning (February through March), two overhead irrigations in the spring (May) and fall (October) with sprinkler sets of 48 hr each that applied 30 cm of water, and fertilizer, herbicide, and pesticide applications.

Collection of canopy-soil profile samples. Field observation and sampling schedules consisted of at least bimonthly examination (excluding winter months of December, January, and February) of fields infested with the pathogen. The temporal aspects of the disease cycle, including apothecial formation, disease symptoms, and sclerotia formation, were determined.

Sclerotia were observed both inside and on the surface of infected alfalfa stems. Those produced on the outside of alfalfa stems eventually dropped on the surface residue or soil surface. A 1-m^2 area of field surface consisting of alfalfa stems, surface residue, and surface soil was assayed to determine sclerotial numbers.

Duplicate canopy-soil profile samples were taken from three fields on three sampling dates: 21 September, 18 October, and 19 November 1982. Disease symptoms were visually detected in most fields examined, but their distribution was not necessarily uniform because of the topography of each field. Sample sites chosen were level with a similar topography and disease symptoms.

After a site was selected, the plants surrounding the 1 m^2 were trimmed with hedge clippers to isolate the sample. The canopy sample was clipped near the soil surface, gently compressed, rolled, and placed in a paper bag for transport and storage. After removal of the 1-m^2 canopy (alfalfa stems), surface residue was gently raked into a pile and collected in a plastic bag for transport to the laboratory. Last, surface soil (about 0–2 cm deep) was scraped into a central pile with a hoe and shovel, collected in a plastic bag, and carried to the laboratory. Residue and soil samples were removed from the plastic bags, spread evenly on

Table 1. Comparative identification of unknown Washington alfalfa isolate of *Sclerotinia*, based on apothecial characteristics[a]

Apothecial ascospore characteristic	*S. sclerotiorum*	*S. trifoliorum*	Unknown species
Occurrence of apthecial production	Spring	Fall	Spring
Ascospore dimorphism	No	Yes	No
Ascospore nuclei	2	4	2
Ascospore size range (L × W [μm])	10–14 × 4–5	10–20 × 6–9 Small: 10–13 × 6–7 Large: 13–20 × 7–9	10–14 × 5–7 $\bar{x}$ = 3 × 6.4[b]
Characteristics of apothecial ectal excipulum region	Prosenchyma cells oriented perpendicularly to apothecial surface	Globose cells oriented perpendicularly to apothecial surface	Prosenchyma cells oriented perpendicularly to apothecial surface
Ascospore length:width ratio	>2.0	Usually ≤2.0	1.76 ± 0.05[c]

[a] From Kohn (7,8).
[b] Mean average ($\bar{x}$) of ascospore length and width based on measurements of 248 ascospores.
[c] Mean length:width ratio of ascospores calculated from measurements of 248 ascospores and subjected to a two-way analysis of variance on $\log_e$-transformed data.

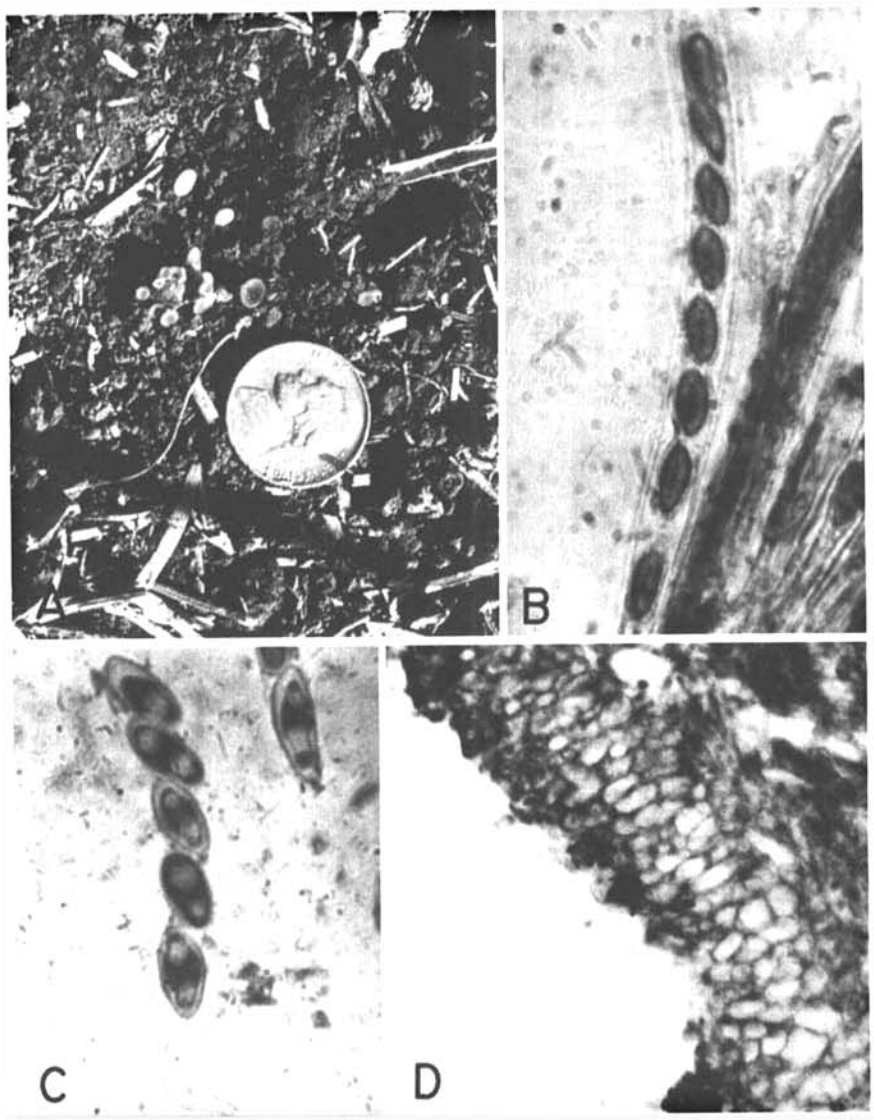

Fig. 1. Identifying characteristics that delineate *Sclerotinia sclerotiorum* as the species causing Sclerotinia crown and stem rot in broadcast-planted stands of seed alfalfa. **(A)** Apothecial production from sclerotia on the soil surface in the field during the spring months of March, April, and May. **(B)** Mature ascus showing eight ascospores of similar size with no obvious dimorphic development and segregation of ascospores in the ascus. **(C)** Dinucleated ascospores. **(D)** Ectal excipulum at margin of apothecium composed of prosenchyma cells oriented perpendicular to the apothecial surface.

paper, and air-dried in the laboratory for 5–7 days at about 25 C and about 30% relative humidity.

Sclerotial analysis. Alfalfa stems were air-dried for 10–14 days in the paper collection bags. Each sample was spread in a thin, uniform layer and gently crushed with a rolling pin to remove sclerotia inside the stems. The residue was then serially passed through three screens with openings of 12.7, 6.3, and 0.85 mm. The residue collected on the 0.85-mm screen was stored at room temperature (about 25 C) in a plastic bag until processed.

Residue and soil samples were wet-sieved with the same series of screens used for assay of stem samples. Material from each sample collected on the 0.85-mm screen was also air-dried at room temperature for 5–7 days and stored in separate plastic bags until processed.

A South Dakota Seed Blower was used to remove the air-dried lighter plant residue from each sample. The heavier residue, which contained the sclerotia, remained on the bottom screen and was stored in a plastic bag until sclerotia were visually separated and isolated. Processed samples were observed at ×10–100 to determine density of sclerotia per square meter from each collection site.

Identification of *Sclerotinia* species. Field-produced (naturally occurring) apothecia were collected and the following taxonomic criteria, as developed by Kohn (7,8), were used: 1) morphology and segregation of ascospores in the ascus, 2) nucleate condition of ascospores, 3) cellular structure of the apothecium, and 4) length:width ratio of ascospores.

The time of year when apothecia were produced was determined by field observations on a bimonthly schedule as previously described. Morphology and segregation of ascospores in the ascus were determined by micrometer measurements using bright-field and phase-contrast microscopy at ×400. Only ascospores within a mature, intact ascus were measured. Two hundred forty-eight ascospores were measured to calculate the average mean length:width ratio. The data was subjected to a two-way analysis of variance on $\log_e$-transformed data. Only apothecia freshly collected from the field were examined to determine the nucleate condition of the ascospores (2,10). The apothecial cellular structure was determined by microtechnique procedures described previously (6). Slide preparations were examined microscopically for cellular characteristics that differentiated species of *Sclerotinia* (8).

RESULTS AND DISCUSSION

Identification of *Sclerotinia* species. Apothecial anatomy and ascospore morphology were compared with those published for *S. trifoliorum* and *S. sclerotiorum* (Table 1). No dimorphism of ascospores was ever observed (Fig. 1B).

Table 2. Disease cycle of *Sclerotinia sclerotiorum* in broadcast-planted alfalfa seed production fields

Stage of disease cycle	Season	Observations
Apothecial production from sclerotia	Spring (late March to mid-May)	Abundant apothecia produced from sclerotia on surface soil and alfalfa stem residue/multiple apothecia (2–10) frequently produced from single sclerotium Apothecia produced from sclerotia buried in soil formed cuplulate receptacle at soil surface Seventy to 90% of sclerotia had produced apothecia
Pathogen activity: Infection and disease	Late spring to early summer (late May to mid-July)	White masses of vigorous mycelial growth and disease symptoms easily observed in infected seed fields Pathogen activity was restricted to soil surface-plant canopy interfacial region Only alfalfa stem tissue was diseased/no infected alfalfa crowns were observed Ascosporic or sclerotial origin of infecting mycelium not established
Sclerotial production	Late spring to early summer (early June to mid-July)	Abundant sclerotia produced during June and July on and in infected alfalfa stems Pathogen activity stopped with onset of hot summer temperatures that reduced canopy humidities and dried fields No sprinkle irrigations applied to seed production fields during summer
Overwintering and sclerotial dormancy	Late summer–fall–winter (late July to early March)	Dormant sclerotia overwintered during December, January, and February/alfalfa crowns dormant and surface soils partially frozen or periodically covered with snow No field samples obtained during December, January, and February

Production of apothecia in the spring was not typical of *S. trifoliorum* (Fig. 1A). Moreover, all ascospores were binucleate not tetranucleate (Fig. 1C), and the apothecial cellular structure was characteristic of *S. sclerotiorum* (Fig. 1D).

The identifying characteristics for *Sclerotinia* species formulated by Kohn (7,8) indicated the unknown *Sclerotinia* species was *S. sclerotiorum*. The exception was the ascospore length:width ratio of 1.8 ± 0.1. *S. sclerotiorum* usually has an ascospore length:width ratio of >2.0. Ascospore sizes ($10–13 \times 5–7$ μm) are more similar to the small-sized ascospores ($10–13 \times 6–7$ μm) from *S. trifoliorum*. However, the ascospores of the unknown species were not dimorphic (8,12) or size-segregated in the ascus (13), and the ascospores were dinucleate. These important characteristics strongly favor the identification of the unknown species as *S. sclerotiorum*. This *Sclerotinia* species has not previously been reported as pathogen of alfalfa (11).

Disease cycle in alfalfa seed fields of southeastern Washington. Bimonthly field examinations illustrated each phase of the disease cycle (Table 2). Apothecia were produced from early March through April. Apothecia produced from sclerotia buried in the soil formed a cupulate receptacle at the soil surface. Multiple apothecia were frequently produced from an individual sclerotium on the surface residue or soil (Fig. 1A). Extensive white masses of mycelial growth on host tissue and disease symptoms occurred from early May through June. Sclerotia were again produced abundantly in June and July on host tissue.

The type of *Sclerotinia* inoculum responsible for initiating infection in alfalfa seed fields was not determined. Two types of inoculum, germinated ascospores and mycelium from sclerotia or ascospores, can initiate infection (9). In some *Sclerotinia*-caused diseases, ascospores are considered the primary source of inoculum. However, mycelial infection in most *Sclerotinia*-caused disease, rather than infection directly from germinated ascospores, appears to be the primary means of host penetration. In addition, a source of organic matter for inoculum nutrition usually is a prerequisite for penetration, whether the original source of inoculum is germinated ascospores or sclerotia. After penetration and extensive colonization of tissue, ramifying hyphae emerge from the host tissue. White masses of mycelia form into clumps of cells that give rise to mature sclerotia. Sclerotia may form on the surface of the host, in the pith, or under decaying plant parts on the soil surface. With the formation of sclerotia, the disease cycle is completed.

The disease cycle of *Sclerotinia* in alfalfa seed fields was identical to the processes of infection, colonization, and *Sclerotinia* disease development described previously. The *Sclerotinia* disease, though present in all alfalfa seed fields examined, was more severe in fields with plant residue remaining on the soil surface from the last harvest. The residue would contain numerous sclerotia (Fig. 2B) and would serve as colonizing substrate for inoculum nutrition and enhancement of mycelial infection from germinating ascospores or sclerotia.

Determination of sclerotial density in canopy-soil profile samples. Conventional soil-sampling techniques were not con-

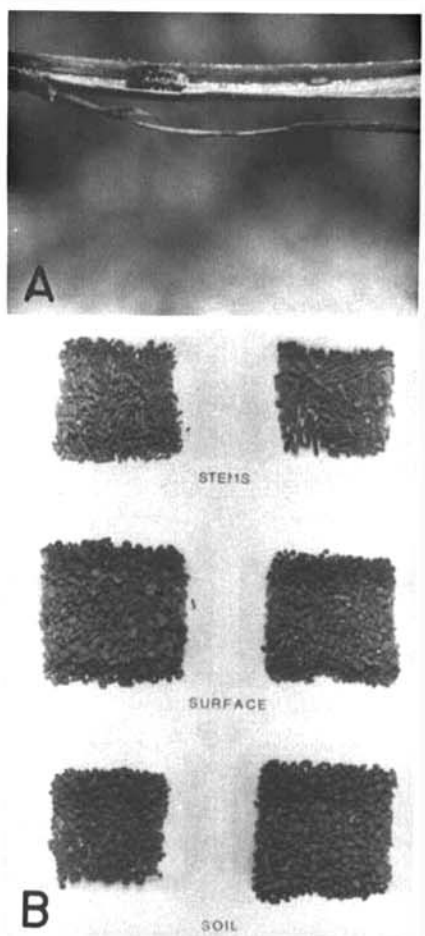

Fig. 2. Detection and isolation of sclerotia of *Sclerotinia* from alfalfa seed production fields. **(A)** Sclerotia in the center pith region of the alfalfa stem tissue. **(B)** Illustration of relative amount of sclerotia removed with canopy-soil profile samples from two highly infested broadcast-planted stands of seed alfalfa.

Table 3. Size distribution of sclerotia of *Sclerotinia sclerotiorum* in canopy-soil profile samples from a broadcast-planted stand of seed alfalfa[a]

Canopy-soil profile samples	Average numbers of sclerotia retained on sieves per square meter								
	6[b] (3.35)[c]	8 (2.36)	10 (2.00)	12 (1.70)	14 (1.40)	16 (1.18)	18 (1.00)	20 (0.85)	Total
Alfalfa stems	1	23	52	113	149	113	43	52	546 (37)[d]
Surface residue	11	149	115	139	133	69	26	10	652 (44)
Surface soil (0–2 cm deep)	2	61	45	59	60	33	14	3	277 (19)
Total sclerotia/m^2	14	233	212	311	342	215	83	65	1,475 (100)

[a] Averages of three samples of alfalfa stems and six samples each of surface residue and surface soil collected on 21 September, 18 October, and 19 November 1982.
[b] Sieve size (no.).
[c] Pore size (mm).
[d] Numbers of parentheses in this column represent the relative percent occurrence of sclerotia in each type of sample compared with the total sum of sclerotia per square meter isolated from the complete canopy-soil profile sample.

sidered satisfactory for a disease that concentrated its pathogenic activity above the soil line. Therefore, it was decided, for detection and evaluation of sclerotial density, to obtain canopy-soil profile samples of 1 m^2 of field area that consisted of individual samples of all alfalfa stem tissue, surface residue, and surface soil. The results from six canopy-soil profile samples collected from three fields indicated 30–55% of the total sclerotial population detected in these fields was found inside alfalfa stems (Table 3, Fig. 2A,B). The remaining 45–70% were detected on the outside of diseased tissue on or in plant residue or soil.

Sclerotial size in canopy-soil profile samples. Distribution of sclerotial sizes from all samples ranged from 0.85 to 3.35 mm in diameter (Table 3). Alfalfa stems contained most of the smaller sclerotia, and conversely, more of the larger sclerotia were present in the samples of surface residue and soil (Table 3, Fig. 2B).

Sclerotia are sometimes abundantly present in alfalfa seed freshly harvested from fields infested with *Sclerotinia* (R. G. Gilbert, *unpublished*). Commercial alfalfa seed lots are produced by standard cleaning procedures and are composed almost entirely of seed collected on screen opening sizes ranging from 1.7 to 1.4 mm (nos. 12 and 14). Sclerotia comparable in size to alfalfa seed account for about 40–50% of the total number of sclerotia collected per square meter (Table 3). From these results, it was not surprising that sclerotia were found in the two commercial seed lots examined from *Sclerotinia*-infested solid-planted stands of seed alfalfa in eastern Washington. Therefore, development of additional seed-cleaning processes other than clipper, gravity, rice mill, and magnetic separators may be needed to remove sclerotia from seed lots contaminated with *Sclerotinia*.

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