Survival of Mycelia of *Sclerotinia sclerotiorum* in Infected Stems of Dry Bean, Sunflower, and Canola

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ABSTRACT

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Stems of sunflower, canola, and dry bean infected by *Sclerotinia sclerotiorum* were collected from fields and tested for survival of the pathogen in the mycelial state under field and laboratory conditions. In two field tests during 1986 and 1987, mycelia of *S. sclerotiorum* in sunflower, bean, or canola stems did not survive the winter months (November–March) in southern Alberta when infected stems were buried in soil at a depth of 7 cm. Mycelia survived the winter if the stems were placed on the soil surface; however, the survival rate was reduced to less than 69% after 4 mo (winter) and decreased rapidly during the spring. In laboratory experiments, mycelia of *S. sclerotiorum* in diseased stems survived longer at −10 C than at 20 C. Although the cold winter in southern Alberta favors survival of *S. sclerotiorum* mycelia, the study indicates survival of mycelia was too low for them to be a significant potential source of pathogenic inoculum.

Additional keywords: Sclerotinia stem blight of canola, Sclerotinia wilt of sunflower, white mold of bean.

Sclerotinia sclerotiorum (Lib.) de Bary is an important pathogen causing stem blight and pod rot of canola (*Brassica napus* L. and *B. campestris* L.) (6) and dry beans (*Phaseolus vulgaris* L.) (5) and wilt and head rot of sunflower (*Helianthus annuus* L.) (7) in western Canada. The disease is widespread; fields of these crops with more than 40% of the plants infected are common (5–7).

Sclerotia are known as primary survival structures of *S. sclerotiorum* (14). Mycelia in infected tissues can cause secondary spread of the disease by direct tissue-to-tissue contact during the crop season (1,4). Although mycelia of *S. sclerotiorum* can overwinter in bean seeds in Nebraska, mycelia are not an important source of initial inoculum (2). In western Canada, one of the symptoms of wilt of sunflower caused by *S. sclerotiorum* is a long basal stem canker (3). This may be a source of inoculum for the following crops because, after harvesting, stubble or basal stems of sunflower and canola are usually left in fields for the winter. Whether mycelia of *S. sclerotiorum* in infected stems can overwinter under prairie conditions remains unknown.

The objective of this study was to assess the overwintering ability of *S. sclerotiorum* in the mycelial state and to determine the importance of mycelia as a potential source of inoculum in southern Alberta.

MATERIALS AND METHODS

***S. sclerotiorum*-infected stems.** Field and incubator experiments were conducted with *S. sclerotiorum*–infected stems of dry bean, canola, and sunflower. For each crop, stems with long tan or white lesions were collected from fields near the end of the growing season (mid-August to late-September). The bleached stems were split longitudinally to remove any sclerotia of *S. sclerotiorum* in the pith cavity, cut into 5-cm segments, and stored dry at 4 C in paper bags. When each experiment (field or incubator) was initiated, 48 stem segments were taken randomly from each crop. The ends (~1 cm long) were cut from each segment, placed on potato-dextrose agar (PDA) containing streptomycin (200 ppm), and incubated at room temperature under light for 7 days to determine the viability of mycelia in the stem. The data were used to estimate mycelial survival in stem segments of each crop before the experiment started.

Field experiments. Stem segments from each crop were sealed in compartments of nylon mesh (2 mm) bags with one segment per compartment and eight segments per bag. Each compartment was 8 × 3 cm for dry bean and canola and 10 × 6 cm for sunflower.

Field experiments were conducted in a summer-fallow field previously cropped to cereals at the Lethbridge Research Station, Alberta, Canada, during October 1986 and 1987. The experimental plots were weeded several times during each summer. In each experiment, four replicate sample bags of stem segments from three crops (dry bean, sunflower, and canola) were buried ~7 cm deep in soil or placed on the soil surface for four storage periods (4, 8, 12, or 16 mo). To simulate the tall sunflower stubble (~0.6 m) farmers leave in their fields, a third treatment was included for sunflower. Sample bags were stapled to wooden stakes at ~20 cm above the soil surface. A split split-plot design (9) was used during each year, with the storage period as the whole-plot factor in four randomized complete blocks, the treatment (placement of stem segments) as the subplot factor, and the crop species as the sub-subplot factor. At the end of each storage period, bags were retrieved. Stem segments from each compartment were trimmed at both ends to leave middle sections ~1 cm in length. These were tested for mycelial survival on PDA.

The field sites were fenced throughout the experimental period to prevent disturbance by animals. Weather data, including temperature 10 cm below the soil surface, temperature 5 cm above ground within the canopy of grass regularly mowed to 10 cm (grass temperature), and air temperature 150 cm above the ground, were collected weekly from a weather station about 100 m from the experimental field. Weather data were smoothed using a moving average and plotted.

For some crop–treatment combinations, all replicates did not have stems with viable mycelia at the end of a storage period. Analyses of variance (ANOVAs) for a split-plot design were conducted on subsets of the data for each year to examine the effects of crop, treatment, storage period, and interactions among these factors on the proportion of stems with viable mycelia of *S. sclerotiorum*. The overall proportions of stems with viable mycelia for the crop–treatment–storage period combinations and their standard errors are presented graphically. All statistical analyses were conducted with SAS software (8).

Incubator experiments. Diseased stems collected from fields during 1986 (experiment 1) and 1987 (experiment 2) were air-dried, stored in paper bags, and used to study the effect of temperature on the survival of mycelia of *S. sclerotiorum* in infected tissues. In each experiment, stems from three crops (dry bean, sunflower, and canola), three storage temperatures (−10, 5, and 20 C), and four storage periods (4, 8, 12, and 16 mo) were used. For each crop–temperature–storage period combination, there were four replicate paper bags with eight diseased stem segments in each bag. At the end of each storage period, stems were retrieved and tested for viability of mycelia as previously described.

For each year, ANOVAs similar to those for the field experiments and based on a completely randomized design were performed on the proportions of stem segments in the bags that had viable mycelia of *S. sclerotiorum*. Linear regressions of the mean proportion of stems with viable mycelia on storage period were determined for each temperature within a crop, and the slopes were tested for homogeneity (11). ANOVAs also were performed over years by incorporating effects of each year and its interaction with crop, storage temperature, and storage period in the statistical model.

RESULTS

Survival of mycelia of *S. sclerotiorum* under field conditions. The two field experiments showed that survival of *S. sclerotiorum* in the mycelial state was affected by the placement of diseased stems, crop type, and storage period.

In the 1986 test, the proportion of stems with viable mycelia decreased with time for each of the treatments ($P < 0.01$). For sunflower, the rate of decrease was least for the above-ground treatment and most rapid for the buried stems (Fig. 1). Over the first 4-mo (winter) period, sunflower stems with viable mycelia decreased from 98 to 91% when stems were stored 20 cm above ground, compared to 59% of on-ground stems and 0% of buried stems. Sixteen months passed before the percentage of stems with viable mycelia among those hung above ground decreased to 0. When stems were placed on the soil surface, the decrease in the proportion with viable mycelia over 4 mo was less for sunflower (98 to 59%) than for bean (81 to 16%) and canola (79 to 9%), which were similar. Mycelia of *S. sclerotiorum* in all three crops did not survive the winter when buried.

In the 1987 test, the viability of mycelia decreased with length of storage but at rates different from those observed during 1986, except for buried stems. Over the winter, the proportion of stems with viable mycelia decreased from 90 to 47% in those stored 20 cm above ground and to 34% in stems stored on the ground (Fig. 2). After 12 mo in the field, almost none of the stems contained viable mycelia. Mycelia in stems of canola on the soil surface survived better than those in surface-stored bean and sunflower during 1987 (Fig. 2).

Mean daily temperature ranges for soil, air, and grass were less than or near freezing during the winter months (December–March) (Fig. 3). The grass temperature reached 20 C during the summer each year, and soil temperature was less than 10 C.

Survival of mycelia of *S. sclerotiorum* in controlled environments. The ANOVA of experiments 1 (1986 stems) and 2 (1987 stems) for the percentage of stems that had viable mycelia indicated there were significant interactions between experiment and crop type and temperature and storage period. Therefore, the effects of these factors were examined for each year separately.

For experiment 1, ANOVA of the percentage of stems with

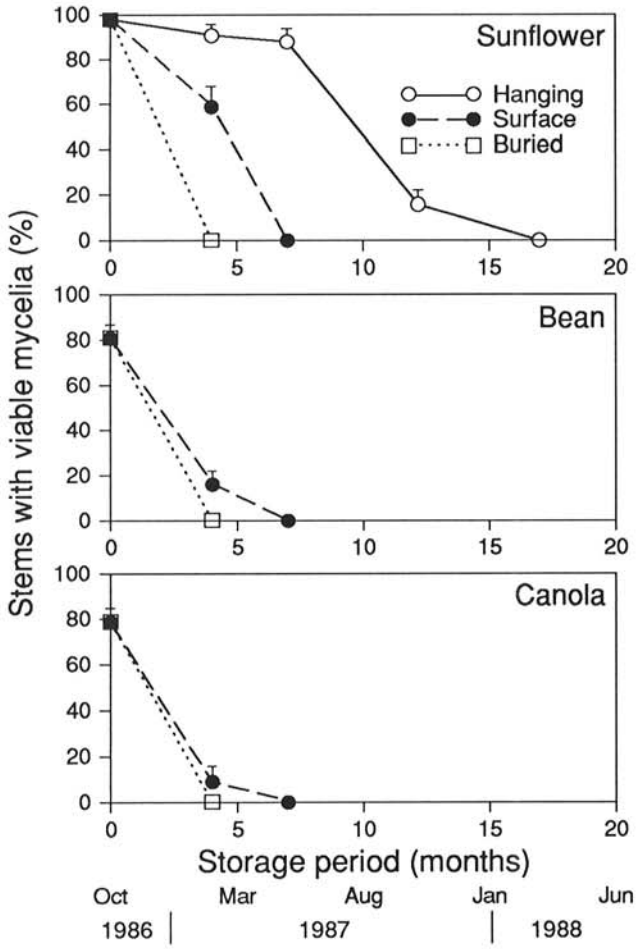

Fig. 1. Survival of mycelia of *Sclerotinia sclerotiorum* in diseased stems of dry bean, sunflower, and canola (1986 field experiment). Bars indicate standard errors. ○—○, stems 20 cm above the ground; ●—●, stems on soil surface; and □—□, stems buried in soil.

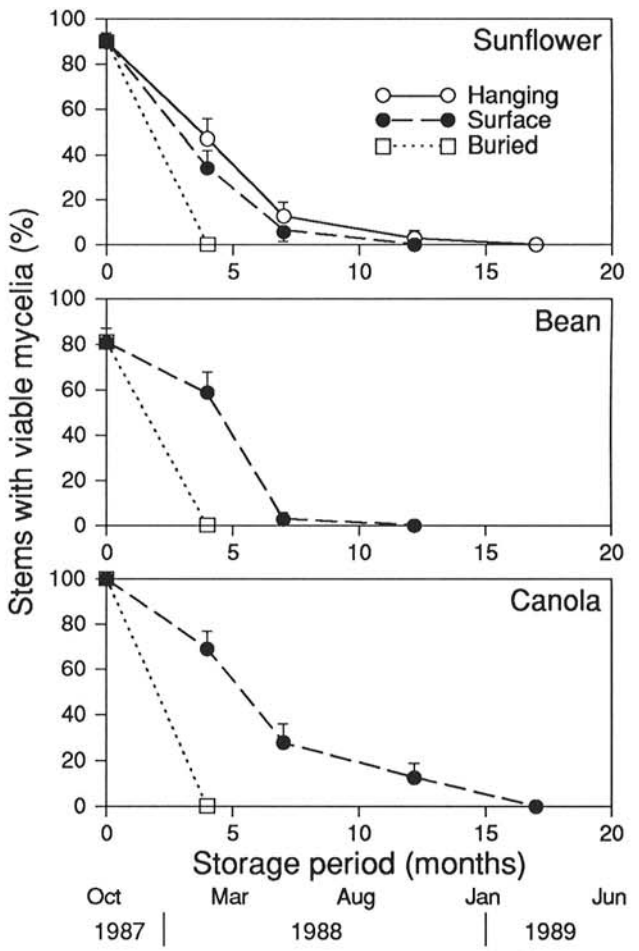

Fig. 2. Survival of mycelia of *Sclerotinia sclerotiorum* in diseased stems of dry bean, sunflower, and canola (1987 field experiment). Bars indicate standard errors. ○–○, stems above the ground; ●–●, stems on the soil surface; and □–□, stems buried in soil.

viable mycelia indicated that there were significant main effects of temperature and storage period ($P < 0.01$) for stems from each crop type and, for bean, a significant temperature–storage length interaction ($P < 0.01$) as well. The slopes of linear regressions of the percentage of stems with viable mycelia on storage period differed between temperatures for each crop ($P < 0.10$). The rate of decrease was slowest when storage was at −10 C for each crop and faster, but similar, at 5 and 20 C (Fig. 4).

For sunflower, the percentage of stems with viable mycelia was 98 at the beginning of the experiment and decreased by 1.3, 2.9, and 3.5% for each month of storage at −10, 5, and 20 C, respectively. After 16 mo of storage at −10, 5, and 20 C, 81, 58, and 44%, respectively, of stems contained viable mycelia. For bean, the percentage of stems with viable mycelia was 81 initially and decreased at 2.3, 4.3, and 5.1% per month of storage to 33, 10, and 1% after 16 mo at −10, 5, and 20 C, respectively. For canola the percentage of stems with viable mycelia was 79 initially and fell to 70, 13, and 15% after 16 mo at −10, 5, and 20 C, respectively.

For experiment 2, ANOVA of the percentage of stems with viable mycelia indicated there were significant main effects of storage temperature and period ($P < 0.01$) for each crop type and a significant interaction between temperature and storage period for sunflower ($P < 0.05$) and canola ($P < 0.01$). Within a crop, storage-temperature effects on the rate of decrease in the percentage of stems with viable mycelia were more noticeable than in experiment 1 for sunflower ($P < 0.01$) and canola ($P < 0.05$) (Fig. 5). For sunflower, the rate of decrease was similar at −10 and 5 C but was considerably greater at 20 C. The percentage of stems with viable mycelia was initially 90 and was 68, 76, and 0 after 16 mo of storage at −10, 5, and 20 C, respectively. In bean, the percentage of stems with viable mycelia decreased at 1.0, 2.3, and 3.8% per month for −10, 5, and 20 C. Correspondingly, the percentage of stems with viable mycelia was reduced from the initial 81 to 62, 41, and 17 by 16 mo. For canola, there was little decrease in the percentage of stems with viable mycelia over the 16-mo period, but at 20 C, stems with viable mycelia decreased from the initial 100 at 3.7% per month to 40 at 16 mo.

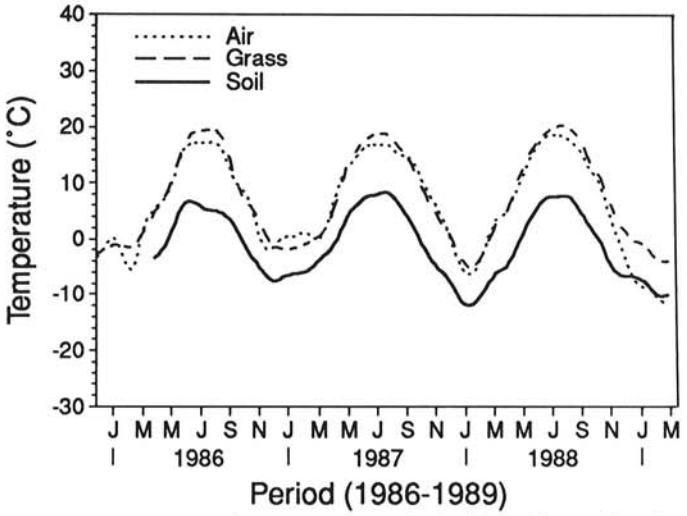

Fig. 3. Soil, grass, and air temperatures at Lethbridge, Alberta, Canada, from January 1986 to March 1989.

DISCUSSION

Our finding that the mycelia of *S. sclerotiorum* in diseased stems of dry bean, sunflower, and canola can overwinter in southern Alberta if the stems are left on the soil surface or above

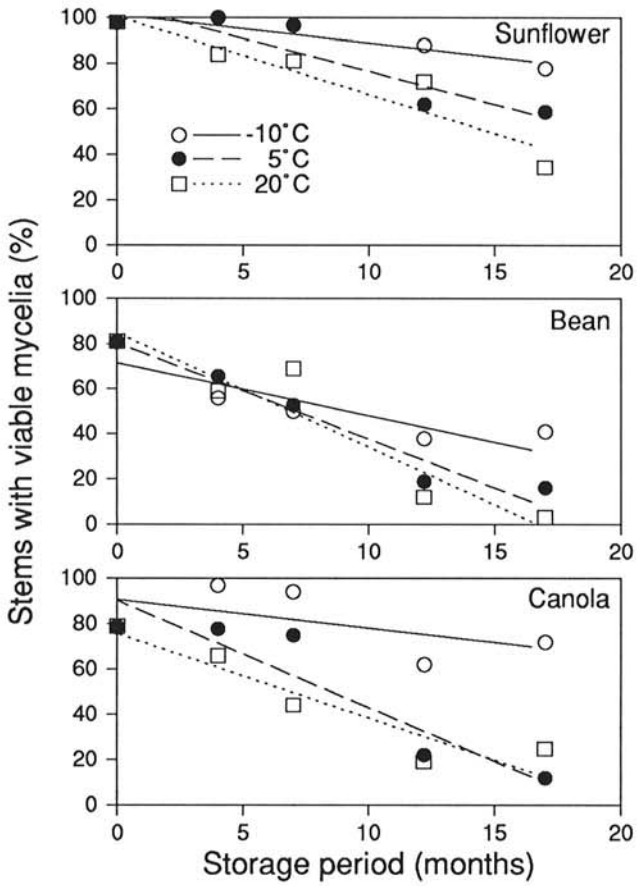

Fig. 4. Linear regressions of survival of mycelia of *Sclerotinia sclerotiorum* in diseased stems of dry bean, sunflower, and canola stored at different temperatures (experiment 1).

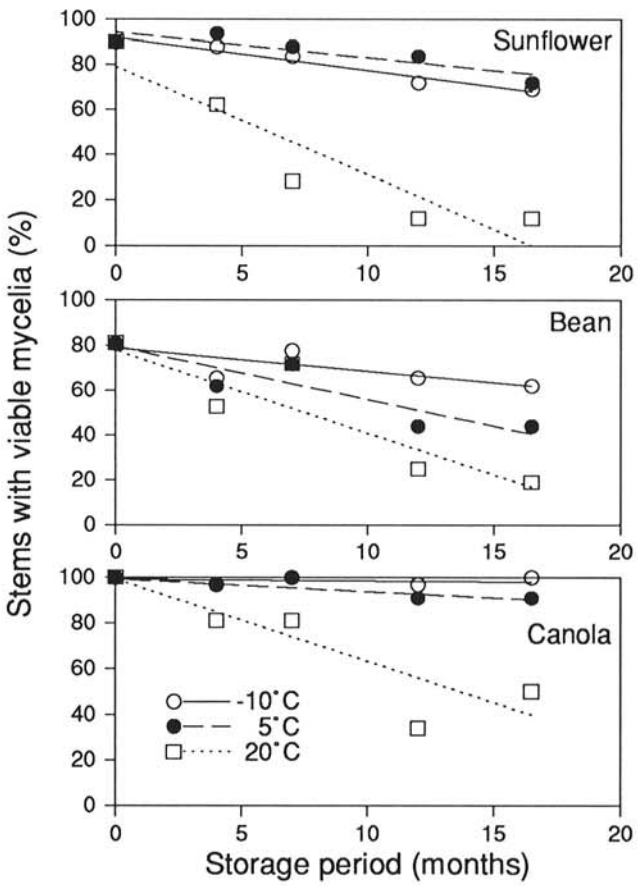

Fig. 5. Linear regressions of survival of mycelia of *Sclerotinia sclerotiorum* in diseased stems of dry bean, sunflower, and canola stored at different temperatures (experiment 2).

the ground is similar to the finding of Cook et al (2) that mycelia of this organism can overwinter in infected bean seeds in western Nebraska. Mycelia in diseased stems cannot survive the prairie winter if the stems are buried in the soil; however, poor survival of mycelia of *S. sclerotiorum* in buried stems may be due to the effects of humidity and microorganisms in the soil.

Our study suggests that mycelia in infected stems of dry bean, canola, and sunflower are not the primary source of inoculum for the disease in southern Alberta. The overwintered mycelia in diseased stems on the soil surface lost viability rapidly during spring–early summer (after 4–8 mo in the field). The few surviving overwintered mycelia in the field could serve as the primary inoculum for seedling infection only if the emerging seedlings were in direct contact with diseased stems on the soil surface. This agrees with the findings that in Nebraska mycelia in infected bean seeds are not an important source of initial inoculum for white mold of dry bean, despite the ability to overwinter in the field (2).

Apparently, sclerotia are the primary survival structure of *S. sclerotiorum* in soil, as previously reported (2,10,13). Although burial of diseased stems may accelerate the death of mycelia, this may not be an appropriate cultural practice, because numerous sclerotia are produced in diseased stems, particularly in sunflower (3). These sclerotia can survive in the soil, serving as a primary source of inoculum. Removal of diseased stems after harvest to reduce the level of sclerotial inoculum in the field remains a good practice.

Temperature and moisture are important factors that affect survival of mycelia of *S. sclerotiorum*. In the absence of nutrients and under 100% relative humidity, mycelia of *S. sclerotiorum* survived for less than 12 mo at 0 C and less than 7 mo at 20 C (12). Similar temperature effects were found in our controlled environment experiments, which showed that mycelia of *S. sclerotiorum* in air-dried stems survived better at −10 C than at 20 C. Thus, the subfreezing temperatures of prairie winters may prolong mycelial survival in diseased stems. However, mycelia of *S. sclerotiorum* loses viability rapidly at 20 C. This means that mycelia from the basal stem canker that remains after harvest of sunflower is an unlikely source of inoculum for the following crops in southern Alberta.

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