Longevity of Normal and Abnormal Sclerotia of *Sclerotinia sclerotiorum*

H. C. HUANG and G. C. KOZUB, Research Centre, Agriculture and Agri-Food Canada, P.O. Box 3000 Main, Lethbridge, Alberta, Canada T1J 4B1

ABSTRACT

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Morphologically abnormal and structurally deformed sclerotia of *Sclerotinia sclerotiorum* are common in samples of sclerotia collected from diseased (head rot) sunflowers grown in Manitoba and Alberta. Sclerotia collected from infected sunflowers were stored in paper bags at room temperature (20 ± 2 C) (four Manitoba samples) or at various temperatures (two Alberta samples). Subsamples of sclerotia were sorted and classified as normal, slightly abnormal, or grossly abnormal on the basis of surface characteristics and the degree of discoloration of medullary tissue and then were tested for viability. Viability of sclerotia decreased with degree of abnormality, duration of storage, and storage temperature. The difference in rate of viability decrease between normal and abnormal sclerotia was greater at above-freezing temperatures than at below-freezing temperatures. When sclerotia were air-dried and stored at 20 C for 62 mo, viability was 15% for grossly abnormal sclerotia and 65% for normal sclerotia.

Additional keyword: survival

Sclerotinia sclerotiorum (Lib.) de Bary is a soilborne plant pathogen that is highly destructive to oilseed crops, such as canola (*Brassica napus* L., *B. campestris* L.) (8,11), sunflower (*Helianthus annuus* L.) (3,10), and safflower (*Carthamus tinctorius* L.) (12), and to pulse crops, such as dry beans (*Phaseolus vulgaris* L.) (6) and dry peas (*Pisum sativum* L. var. *arvense* (L.) Poir.) (9). Sclerotia are the overwintering structures that serve as the primary source of inoculum for infection of crops in the Canadian prairies (7).

Huang (4) first discovered the occurrence of abnormal sclerotia of *S. sclerotiorum* in samples from diseased sunflower plants in Manitoba during 1977–1979. Abnormal sclerotia are readily recognizable by their wrinkled surface and discolored medullary tissues compared with normal sclerotia, which are smooth and have a white medulla (4). Further surveys in 1982 and 1985 revealed the presence of abnormal sclerotia in sclerotial samples from sunflowers grown in Alberta.

Huang (5) reported that structural malformation of abnormal sclerotia resulted in greater nutrient leakage than from normal sclerotia. The viability of abnormal sclerotia was directly associated with the degree of discoloration of medullary tissues (4,5). This study was undertaken to determine the relative longevity of normal and abnormal sclerotia of *S. sclerotiorum* at various temperatures.

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MATERIALS AND METHODS

Source of sclerotia. Seven bulk samples of sclerotia of *S. sclerotiorum* from confectionery-type sunflower were obtained from seed cleaning plants (Table 1). All samples had been previously air-dried. Four samples were from sunflowers grown in Manitoba in 1977, 1978, and 1979 and two were from sunflowers grown in Alberta in 1982 and 1985 (Table 1). Abnormal sclerotia with characteristic wrinkled surfaces (4) were found in all samples. The frequency of abnormal sclerotia among large sclerotia (longer than 10 mm) ranged from 13 to 30% (Table 1).

The large sclerotia in each sample were sorted into normal (smooth surfaced) and abnormal (wrinkled) sclerotia and stored in paper bags. The four Manitoba samples were stored at 20 C. The Alberta sample collected in 1982 was stored at room temperature (20 ± 2 C) for 18 mo, then a subsample was transferred to −20 C to test whether exposure to low temperatures affected viability. The 1985 Alberta sample was divided into subsamples that were stored at −40, −20, 0.5, 20, and 30 C to study the temperature, storage length, and sclerotial type interactions in more detail.

Viability of sclerotia. At various intervals, subsamples of sclerotia were removed from storage and tested for viability. Sclerotia were cut open, visually examined for discoloration of medullary tissue, and classified as normal, slightly abnormal, or grossly abnormal, according to the criteria of Huang (4). At each sampling date, 85–120 sclerotia from each of the three types were surface-sterilized in 70% ethanol for 90 sec, placed on potato-dextrose agar (PDA) containing streptomycin (200 ppm), and incubated at room temperature under light for 7 days. The viability of sclerotia was determined by examining development of the colony and formation of daughter sclerotia.

Statistical methods. Sclerotial viability data for samples collected during 1977–

1979, 1982, and 1985 were analyzed separately. For the 1977–1979 samples, the percentage of viable sclerotia was determined for each sample, sclerotial type, and storage period combination. Since all sclerotia were air-dried, their viability responses were considered independent, and categorical data analysis (2) was used to examine the magnitude of effects due to sample, sclerotial type, and storage period on the proportion of viable sclerotia. Interactions among these factors were included in the statistical model. The CATMOD procedure in SAS (13) was used to perform the calculations. Logistic regressions (1) were also fitted to data to summarize the relationship between viability response and storage period for each sclerotial type.

For sclerotia collected in 1982, categorical analysis was performed to examine effects of sclerotial type and storage period on viability of sclerotia for samples stored at 20 C. For the storage periods of 37, 50, and 62 mo, temperature and interactions involving temperature, sclerotial type, and storage period were added to the statistical model.

For sclerotia collected in 1985, similar categorical data analyses were performed to examine effects of sclerotial type, temperature, and storage period on viability of sclerotia. Depending on the temperature involved, linear, exponential, or logistic regressions of percent viability on storage period were fitted to summarize response patterns.

RESULTS

The categorical analysis of viability of sclerotia from the four samples collected during 1977–1979 indicated a significant ($P < 0.001$) sclerotial type × storage period interaction that was consistent for samples collected in different years. The logistic regressions for pooled data indicated that viability of abnormal sclerotia was lower initially (0 mo) and decreased more rapidly with storage period than that of normal sclerotia (Fig. 1). The percentage of viable sclerotia had decreased to 10% by 30, 40, and 60 mo for grossly abnormal, slightly abnormal, and normal sclerotia, respectively.

For sclerotia collected in 1982 and stored at 20 C, there was a significant ($P < 0.001$) sclerotial type × storage period interaction. As with the 1977–1979 samples, viability of abnormal sclerotia was lower initially (2 mo) and decreased more rapidly than that of normal sclerotia (Table 2). The percentage of viable sclerotia decreased over the 60-mo storage period by 35, 50, and 73% for normal, slightly abnormal, and grossly abnormal sclerotia, respectively. During the 37- to 62-mo period, a significant ($P < 0.001$) storage temperature and sclerotial type interaction was present. A larger difference in viability between −20 C and 20 C was present for abnormal than for normal sclerotia at all storage periods. An interaction was also evident between storage temperature and period ($P < 0.001$) as a result of a decrease in viability with increasing storage period for sclerotia stored at 20 C that was not evident for sclerotia stored at −20 C (Table 2).

For sclerotia collected in 1985, there was a highly significant ($P < 0.001$) sclerotial type × temperature × storage period interaction. For the storage period of 0–70 mo, the response was linear and similar at −20 and −40 C, with the rate of decrease in viability with increasing storage period being greater ($P < 0.01$) for abnormal than for normal sclerotia (Fig. 2). By 70 mo, viability of the abnormal sclerotia had decreased to 93% but the viability of normal sclerotia did not decrease. As storage temperature increased from 0.5 to 30 C, viability of all sclerotia decreased, and within each temperature, the rate of decrease in viability with increasing storage period increased with the degree of abnormality of sclerotia (Fig. 2). At −40 and −20 C, more than 90% of normal sclerotia were viable after 70 mo. At 0.5 C, the viability of normal sclerotia had decreased to 30% after 70 mo, and at 20 and 30 C, the viability was less than 10%.

DISCUSSION

The results of this study indicate that structural abnormality is an important factor affecting survival of *S. sclero-*

Table 1. Frequency of abnormal sclerotia of *Sclerotinia sclerotiorum* from diseased sunflowers in Manitoba and Alberta[a]

Date	Abnormal sclerotia (%)	Source
1977	30	CSP Foods Ltd., Altona, Manitoba
1978	13	Reimer's Seeds, Winkler, Manitoba
1979-1	15	CSP Foods Ltd., Altona, Manitoba
1979-2	18	CSP Foods Ltd., Altona, Manitoba
1982	27	Alberta Sunflower Company, Bow Island, Alberta
1985	24	Alberta Sunflower Company, Bow Island, Alberta

[a]More than 1 kg of sclerotia were collected from each sample of confectionery-type sunflowers with Sclerotinia head rot.

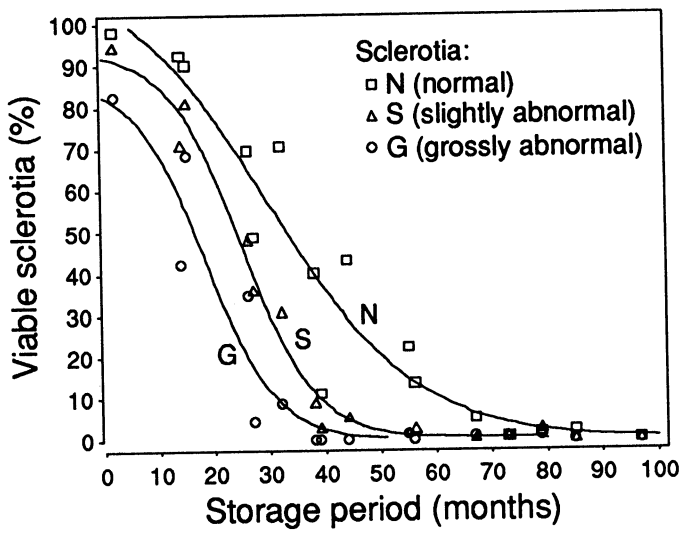

Fig. 1. Viability of normal, slightly abnormal, and grossly abnormal sclerotia of *Sclerotinia sclerotiorum* stored for different durations at 20 C (1977–1979 samples).

Table 2. Viability of normal, slightly abnormal, and grossly abnormal sclerotia of *Sclerotinia sclerotiorum* collected from sunflowers in Alberta during 1982 and stored at 20 and −20 C[a]

Storage period (mo)	Sclerotial viability (%, SE) and temperature (C)					
	Normal		Slightly abnormal		Grossly abnormal	
	20	−20[a]	20	−20[a]	20	−20[a]
2	100.0 ± 0.0	...	95.0 ± 2.2	...	88.0 ± 3.3	...
19	98.0 ± 1.4	...	88.0 ± 3.3	...	83.0 ± 3.8	...
37	91.0 ± 2.9	96.0 ± 2.0	57.0 ± 5.0	85.0 ± 3.6	49.0 ± 5.0	77.0 ± 4.2
50	58.8 ± 5.0	95.0 ± 2.2	41.0 ± 4.9	92.5 ± 2.9	9.8 ± 2.9	86.0 ± 3.5
62	65.0 ± 4.8	98.0 ± 1.4	45.0 ± 5.0	91.0 ± 2.9	15.2 ± 3.6	92.0 ± 5.4

[a]A subsample of sclerotia was transferred to −20 C after 18 mo of storage at 20 C.

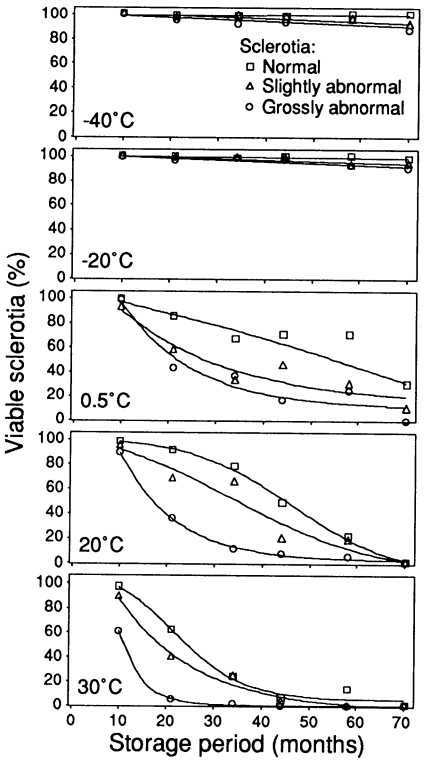

Fig. 2. Viability of normal, slightly abnormal, and grossly abnormal sclerotia of *Sclerotinia sclerotiorum* stored for different durations at −40, −20, 0.5, 20, and 30 C (1985 sample).

tiorum sclerotia and confirm previous findings (4) that abnormal sclerotia lose viability more rapidly than normal sclerotia and that the reduction in longevity is proportional to the degree of malformation. When air-dried sclerotia were stored at 20 C for 62 mo, the viability of grossly abnormal sclerotia was 15% compared with 65% for normal sclerotia.

Temperature is also an important factor affecting survival of sclerotia of *S. sclerotiorum*. Viability of sclerotia remained high when stored at −20 and −40 C for up to 70 mo but declined rapidly when stored at above-freezing temperatures, especially at 30 C. At above-freezing temperatures, survival of normal sclerotia was greater than that of slightly abnormal or grossly abnormal ones. The reduced survival of abnormal sclerotia at higher temperatures suggests that survival of these sclerotia under field conditions would be impaired and their impact as a primary source of inoculum considerably reduced.

This and previous studies (4,5) indicate that a significant proportion of sclerotia of *S. sclerotiorum* produced on sunflowers in Manitoba and Alberta are abnormal. The abnormality is not a heritable character, as daughter sclerotia produced from abnormal sclerotia on PDA are morphologically normal (4). Whether this type of sclerotial abnormality also occurs on other hosts or in other geographic regions warrants further investigation.

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LITERATURE CITED

1. Draper, N. R., and Smith, H. 1981. Applied Regression Analysis. 2nd ed. John Wiley & Sons, Toronto.
2. Grizzle, J. E., Starmer, C. F., and Koch, G. G. 1969. Analysis of categorical data by linear models. Biometrics 25:489-504.
3. Hoes, J. A., and Huang, H. C. 1976. Importance of disease to sunflower in Manitoba in 1975. Can. Plant Dis. Surv. 56:75-76.
4. Huang, H. C. 1982. Morphologically abnormal sclerotia of *Sclerotinia sclerotiorum*. Can. J. Microbiol. 28:87-91.
5. Huang, H. C. 1983. Histology, amino acid leakage, and chemical composition of normal and abnormal sclerotia of *Sclerotinia sclerotiorum*. Can. J. Bot. 61:1443-1447.
6. Huang, H. C., Kokko, M. J., and Phillippe, L. M. 1988. White mold of dry bean (*Phaseolus vulgaris* L.) in southern Alberta, 1983-87. Can. Plant Dis. Surv. 68:11-13.
7. Huang, H. C., and Kozub, G. C. 1993. Survival of mycelia of *Sclerotinia sclerotiorum* in infected stems of dry bean, sunflower, and canola. Phytopathology 83:937-940.
8. Huang, H. C., Phillippe, L. M., Kokko, M. J., and Topinka, A. K. 1988. Distribution of sclerotinia stem rot of irrigated canola in southern Alberta, 1984-87. Can. Plant Dis. Surv. 68:108-109.
9. McKenzie, D. L., and Morrall, R. A. A. 1975. Diseases of specialty crops in Saskatchewan: II. Notes on field pea in 1973-74 and on lentil in 1973. Can. Plant Dis. Surv. 55:97-100.
10. McLaren, D. L., Rimmer, S. R., and Huang, H. C. 1988. Survey of sclerotinia wilt and head rot of sunflower in southern Alberta. Can. Plant Dis. Surv. 68:125.
11. Morrall, R. A. A., Dueck, J., McKenzie, D. L., and McGee, D. C. 1975. Some aspects of *Sclerotinia sclerotiorum* in Saskatchewan, 1970-75. Can. Plant Dis. Surv. 56:56-62.
12. Mündel, H.-H., Huang, H. C., and Kozub, G. C. 1985. Sclerotinia head rot in safflower: Assessment of resistance and effects on yield and oil content. Can. J. Plant Sci. 65:259-265.
13. SAS Institute. 1989. SAS/STAT User's Guide. Version 6. 4th ed. SAS Institute, Cary, NC.