TEMPERATURE IN RELATION TO THE
FORMATION OF APOTHECIA OF SCLEROTINIA
SCLEROTIORUM (LIB.) de BARY

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The temperature factor in relation to the formation of apothecia of Sclerotinia sclerotiorum (Lib.) de Bary has been studied by several workers. According to Kheshwala (1934), whereas the sclerotia of this fungus formed in culture at 10°C developed apothecia, none were formed by those produced at 25°C.

Mundkur (1934) observed that temperatures of 22°C and below are necessary for the formation of stipes and apothecia. According to him, they were not formed at temperatures above 22°C. Also, according to Ramsey (1925), sclerotia germinate between 18°C and 22°C, but not above.

Opinions of different workers are also divided with respect to the effect of chilling of sclerotia on the subsequent development of apothecia therefrom. Thus, according to Lobik (1926), the previous chilling of sclerotia is not necessary for the production of apothecia. This observation is also supported by Ramsey (1925), and Coe (1949). Kheshwala (1934), on the other hand, observed that sclerotia, which were chilled in a frigidaire at 10°C, produced apothecia, when transferred to the laboratory temperature; while those kept at the laboratory temperature throughout failed to do so. He is, therefore, of the opinion that cooler temperatures than those ordinarily obtained in the room are necessary to give sclerotia the required stimulus for germination.

To verify and substantiate these results, the writer undertook detailed investigations to determine the factors governing the formation of apothecia by this pathogen. Another object of the investigation was to find out, if possible, a good explanation for the absence of the perfect stage of this fungus in the Punjab State, where apothecia have never been seen so far under natural conditions. Mehta, Singh and Bose (1946) recorded the
Formation of Apothecia of Sclerotinia sclerotiorum (Lib.) de Bary 245

occurrence of the apothecial stage of this pathogen in the United Provinces (now re-named Uttar Pradesh) in moist patches in some fields of *Eruca sativa* affected with it. This has been apparently the first record of the natural occurrence of apothecia of this fungus in India.

This paper deals with the temperature factor only.

1. Effect of different temperatures on the formation of apothecia from sclerotia.—Sclerotia produced on potato-dextrose agar at the room temperature (22° and 25° C.) were floated on water in Erlenmeyer flasks and incubated at 5°, 10°, 15°, 20°, 25° and 30° C. in constant-temperature cabinets receiving sufficient natural light during the daytime. The age of the sclerotia, when floated on water, was 3 weeks. Final observations on apothecia development were recorded at the end of about 8 weeks, and are given in Table I.

**Table I**

*Effect of different temperatures on apothecia formation from sclerotia*

<table>
<thead>
<tr>
<th>Temperature °C.</th>
<th><em>Percentage of sclerotia with stipes only (A)</em></th>
<th><em>Percentage of sclerotia with apothecia (B)</em></th>
<th><em>Percentage of total germination (A) + (B)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>65.8</td>
<td>0</td>
<td>65.8</td>
</tr>
<tr>
<td>10</td>
<td>80.0</td>
<td>3.3†</td>
<td>83.3</td>
</tr>
<tr>
<td>15</td>
<td>35.7</td>
<td>53.5</td>
<td>89.2</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>25</td>
<td>36.5</td>
<td>0</td>
<td>36.5</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Average of 3 replicates
† Abortive only

It may be seen from the data presented in Table I that sclerotia can germinate over a wide range of 5–25° C. What is to be particularly noticed is that at temperatures below 15° C. and above 20° C., the germination of sclerotia is almost totally restricted to the formation of stipes (Fig. 1). This, it is to be noted, is not effective germination, which implies the development of normal apothecia with ascospores therein.

B3
As judged from this experiment, 20°C. is the optimum temperature for apothecia formation. Although as many as 89.2% sclerotia germinate at 15°C., 35.7% of them form stipes only. Also, whatever apothecia are formed at this temperature are smaller and less vigorous than those developing at 20°C. (Fig. 1). The writer's results are, therefore, mainly in agreement with those obtained by Mundkur (1934).

Mundkur (1934) and Kheshwala (1934) also obtained only stipes and no apothecia at 10°C, and this result is in consonance with that obtained by the writer. It may be pointed out that the writer has not come across any reference in which stipe-formation has been recorded at a temperature as low as 5°C. As may be seen in Table I, as many as 65% sclerotia produce stipes at this temperature.

The temperature-range for the formation of stipes and apothecia is narrower than that for the formation of sclerotia, which are formed even at 30°C. If the range for effective germination of sclerotia, culminating in the development of apothecia, is considered, it is, however, extremely narrow, being 15-20°C.

(2) Effect of different temperatures employed to produce sclerotia on the development of apothecia therefrom.—For this experiment, sclerotia were produced at 5°, 10°, 15°, 20°, 25° and 30° C. on potato-dextrose agar. At the time of floating the sclerotia on water, they were about 3 months old. The Erlenmeyer flasks containing the floating sclerotia were placed at the temperature-range of 17-21°C., which is suitable for apothecia formation. The results, as recorded at the end of 8 weeks, are set out in Table II.

An examination of Fig. 2 and the data set out in Table II show that the temperatures of 15°C. and below, employed to produce sclerotia, inhibit their reproductive capacity. Whereas the sclerotia produced at the highest temperatures of the series, i.e., 30°C., form only a few abortive stipes, those produced at 25°C. not only develop apothecia in 100% cases, but are also much larger than those arising from the sclerotia produced at 20°C. It may also be noticed in Table II that only 50% of the sclerotia produced at 20°C. germinate and about 9% of them produce only stipes, which do not expand into apothecia; and thus remain abortive.

The importance of different temperatures employed to produce sclerotia on the subsequent development of apothecia therefrom was also observed by Kheshwala (1934), but his observations are quite the reverse of those made by the writer. As already mentioned in this paper, according to him, the sclerotia formed only at 10° and 15°C. developed apothecia, which
Formation of Apothecia of Sclerotinia sclerotiorum (Lib.) de Bary 247

were not formed by those produced at 25°C. Maybe, Kheshwala's race of *S. sclerotiorum* was different from that used by the writer in these studies.

### TABLE II

*Effect of different temperatures employed to produce sclerotia on the formation of apothecia therefrom*

<table>
<thead>
<tr>
<th>Temperature employed to produce sclerotia °C.</th>
<th>Percentage* of sclerotia producing stipes only (A)</th>
<th>Percentage* of sclerotia producing apothecia (B)</th>
<th>Percentage* of total germination (A) + (B)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>..</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>..</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>..</td>
</tr>
<tr>
<td>20</td>
<td>9.3</td>
<td>40.0</td>
<td>49.3</td>
<td>Apothecia began to appear on the 19th day after floating.</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>Apothecia began to appear on the 13th day after floating.</td>
</tr>
<tr>
<td>30</td>
<td>8.0</td>
<td>0</td>
<td>8.0</td>
<td>Some stipes which, however, did not develop into apothecia appeared on the 26th day after floating.</td>
</tr>
</tbody>
</table>

* Average of 3 replicates.

(3) *Effect of the refrigeration of sclerotia.*—For this purpose, sclerotia, which were 32 days' old, were soaked in water for a few hours and were subjected to 0°C and −15°C for one week. Sclerotia, soaked in water, but not chilled, served as the control. The results of the refrigeration treatment of the sclerotia on the development of apothecia therefrom are given in Table III.

The data in Table III show that all the sclerotia chilled at 0°C. for 7 days produce apothecia at the end of 3 weeks; while, most of the sclerotia not so treated have stipes only, apothecia having been produced only by about 24%. Sclerotia frozen at −15°C. for a week do not germinate at
Effect of the refrigeration of sclerotia at 0° and −15° C. on the development of apothecia therefrom, as recorded at the end of 3 weeks

<table>
<thead>
<tr>
<th>Treatment</th>
<th>*Percentage of sclerotia producing stipes only (A)</th>
<th>*Percentage of sclerotia producing apothecia (B)</th>
<th>*Percentage of total germination (A) + (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sclerotia exposed to 0° C. for a week</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2. Sclerotia exposed to −15° C. for a week</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3. Sclerotia not refrigerated (control)</td>
<td>72</td>
<td>24</td>
<td>96</td>
</tr>
</tbody>
</table>

* Average of 3 replicates.

The experiment was continued beyond 3 weeks. Whereas, eventually, all the sclerotia in the control series formed apothecia, those exposed to −15° C. remained ungerminated even at the end of 10 weeks, when the experiment was terminated. The viability of the ungerminated sclerotia was tested and was found to be 100%, showing, thereby, that refrigeration at −15° C. had not killed them, but had destroyed their reproductive capacity. Sclerotia frozen by the writer under ice for 4 months from the 15th November, 1950, to the 15th March, 1951, at the University Farm, St. Paul, Minnesota, also failed to produce apothecia, when tested. During this period, atmospheric temperature on some days fell to −10–20° C.

Refrigeration at the relatively higher temperature of 0° C., on the other hand, accelerated apothecial formation. This agrees with the results obtained by Ezekiel (1921), who states that cold tends to hasten the production of apothecia of Sclerotinia cinerea. It must, however, be noted that chilling is not at all necessary as a preliminary to the development of apothecia from sclerotia, as shown by the results of this experiment. Also, according to Lobik (1926), and Coe (1944), chilling of sclerotia is not a prerequisite to their germination to produce apothecia.

(4) Effect of freezing and thawing of sclerotia on the formation of apothecia therefrom.—Sclerotia frozen at −15° C., and thawed at the laboratory temperature (22–25° C.) for a week on alternate days, were also tested for the formation of apothecia. No stipes or apothecia developed from sclerotia so treated, though they were found to be viable.
DISCUSSION OF RESULTS

The temperature employed to produce sclerotia affects their reproductive capacity to a surprising degree. In the writer's experiments, sclerotia produced at temperatures of 15°C and lower failed to produce apothecia, and only 50% of those produced at 20°C germinated, and out of them also, some did not develop apothecia. On the other hand, those produced at 25°C produced large and vigorous apothecia in 100% cases. Sclerotia produced at 30°C produced only a few abortive stipes.

Sclerotia germinate over a wide temperature-range of 5–25°C, but their germination above 20°C and below 15°C is almost totally restricted to the formation of stipes. This is not effective germination, as it does not imply the development of normal apothecia containing asci and ascospores. The optimum temperature-range for apothecial development is rather narrow, being 15–20°C. This restricted temperature-range also explains the absence of the apothecial stage in the Punjab State. Here, the disease appears in the cold months of December and January, and by the time the sclerotia are old enough to form apothecia in the end of February or the beginning of March, the weather becomes too hot to be congenial for the development of apothecia therefrom.

Freezing of sclerotia at a very low temperature, like —15°C, or their burial under snow during winter months, does not kill them, but destroys their ability to produce apothecia. Walker (1950) states that sclerotiniaose of vegetables is very uncommon in the northern and the central states of the United States of America, even though the causal fungi have excellent means of surviving cold winters in the form of sclerotia. He does not give any explanation for this. The writer's observation, that the freezing of sclerotia under snow during winter months under conditions prevailing in Minnesota destroys their reproductive capacity, and thus puts an end to the dissemination of the pathogen through ascosporal inoculum, can be a good explanation for the very uncommon incidence of sclerotiniaose of vegetables in similar places in the United States.

Chilling of sclerotia at the relatively higher temperature of 0°C, on the other hand, has a stimulatory effect on the development of apothecia from them. Pre-chilling of sclerotia, however, is not necessary for their germination to produce apothecia.

SUMMARY

1. The temperature employed to produce sclerotia affects their reproductive capacity to a surprising degree. Sclerotia produced at 15°C and
lower do not produce apothecia. The best development of vigorous and well-developed apothecia takes place in the case of sclerotia produced at 25° C.

2. Sclerotia germinate over a wide temperature-range of 5–25° C., but at temperatures below 15° C. and above 20° C., their germination is almost totally restricted to the formation of stipes, which remain abortive. The optimum temperature-range for apothecia formation is 15–20° C.

3. Freezing of sclerotia at a very low temperature, like — 15° C., or burying them under snow during winter months does not kill them, but destroys their reproductive capacity. Chilling of sclerotia at 0° C. for a week, on the other hand, hastens the formation of apothecia from them.

ACKNOWLEDGEMENT

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REFERENCES


Fig. 1. Effect of different temperatures on apothecia formation. Notice that although sclerotia germinate over the wide range of 5°C-25°C, the range for effective germination resulting in the development of apothecia is extremely narrow, viz., 15°C-20°C, only.
Fig. 2. Effect of different temperatures employed to produce sclerotia on the subsequent development of apothecia therefrom. Notice the total absence of germination in the case of sclerotia produced at 5°-15° C, and only a slight germination in the case of those produced at 30° C. All sclerotia produced at 25° C germinate and form large and vigorous apothecia. Only about 50% sclerotia produced at 20° C germinate and even some of them form stipes only. The much smaller size of apothecia from sclerotia produced at this temperature than that of apothecia from sclerotia produced at 25° C may also be noticed.