RELATION OF TEMPERATURE TO SPORE GERMINATION AND GROWTH OF UROCYSTIS CEPULAE

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INTRODUCTION

In a previous paper (21) the relation of soil temperature and soil moisture to infection by the onion-smut fungus (Urocystis cepulæe Frost) was considered. As the onion seed germinates the cotyledon, functioning as the first leaf, is readily invaded by the parasite until it becomes mature. This is usually a period of two to three weeks, following which the mature cotyledon and the successively maturing true leaves serve as protecting sheaths which exclude further infection. The soil environment during this susceptible period is therefore a critical one in determining the extent of infection.

Invasion by the smut organism occurred at temperatures as low as those at which germination and growth of the host ordinarily takes place (10° to 12° C.), but whether or not there was an extremely low point at which the host might escape the parasite was not determined. Infection occurred regularly at 25° C., but above that point the amount of disease rapidly declined, until at a constant temperature of 29° it was completely inhibited. During these studies the question arose as to the exact nature of this temperature inhibition, i.e., whether it was a case of inhibition of the parasite or of stimulation of greater resistance on the part of the host, or both. With the methods in use at that time it was impossible to separate these factors. It has become necessary, therefore, to consider the relation of environment to the development of the parasite as apart from the host. This paper embodies results of a study of the effect of temperature on the germination and growth of Urocystis cepulæ and its bearing upon infection.

PREVIOUS WORK ON RELATION OF TEMPERATURE TO SMUT SPORE GERMINATION AND INFECTION

Considerable attention has been given by previous workers to the temperature relations of the smut fungi, especially those attacking the cereal crops. Much of the earlier literature has been amply reviewed by Jones (10). Several more recent contributions are of special interest. In a critical study of corn smut (Ustilago zeæe (Beckm.) Unger) Jones (11) found that spore germination occurred throughout a temperature range of 8° to 38° C., the optimum lying between 26° and 35°. In another study the same writer (10) found the range of spore germination of Ustilagoavenæe (Pers.) Jens. to extend from 4° to 34°, with the optimum at 15° to 28°.

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2 Reference is made by number (italic) to "Literature cited," p. 145.

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(133)
Numerous recent infection studies on the relation of environment to the development of cereal smuts bring together evidence that infection takes place over a comparatively wide range, but in general the amount of infection is found to decrease at the extremes. Involved in the environmental complex, however, are the interdependent relations of soil temperature, soil moisture, and soil reaction, all of which should be considered in the final interpretation of the influence of any single factor. Working with the loose smut of oats \((\text{Ustilago avenae})\), Bartholomew and Jones (3) showed that low temperatures \((8°\) to \(12°\) C.) during the susceptible period of the host might reduce infection somewhat, but the reduction was more marked at the upper limit of the range of spore germination. When a soil temperature of \(31°\) to \(32°\) was combined with high soil moisture, the inhibition of the fungus was complete. In a parallel study with covered smut of oats \((\text{Ustilago levis} (\text{K. and S.}) \text{Magn.})\), Reed and Faris (17) found this organism to correspond very closely with \textit{Ustilago avenae} in behavior.

The covered smut \((\text{Sphacelotheca sorghi} (\text{Link}) \text{Clint.})\) and loose smut \((\text{Sphacelotheca cruenta} (\text{Kühn}) \text{Potter})\) of sorghums were studied by the same workers (16). The two organisms were found to behave very similarly, infection occurring from \(12°\) to \(37.5°\) C. There was a reduction of the amount of infection at the extremes, but here again the degree of reduction was influenced by soil moisture. Faris (6), working with covered smut of barley \((\text{Ustilago hordei} (\text{Pers.}) \text{K. and S.})\), found infection to occur over a range of \(5°\) to \(30°\), with a decided reduction in the amount of disease as the temperature approached the two extremes.

The influence of temperature upon flag smut of wheat \((\text{Urocystis tritici Koern.})\) has been studied recently by Noble (14). Spores pre-soaked in water for some days germinated at lower and at higher temperatures than those placed directly at fixed temperatures without pre-soaking. Such pre-soaked spores lost their viability more readily at the higher temperatures than did the spores which were not pre-soaked. Germination occurred at as low as \(5°\) and as high as \(32°\) C. Approximately the same percentage of spores germinated throughout the range from \(18°\) to \(27°\), but the maximum growth of germ tubes occurred at \(24°\). Incubation at constant temperatures above \(25°\) proved to be detrimental to growth following germination. In controlled experiments (15) optimum infection occurred at \(19°\) to \(21°\), the amount was decidedly reduced at \(24°\) to \(26°\), while at \(29°\) to \(31°\) it was practically inhibited. Tisdale, Dungan, and Leighty (20) in successive planting experiments in Illinois found that in seedings made after November 1 there was marked reduction in the amount of disease, due presumably to inhibitive effect of low soil temperature.

**METHODS OF EXPERIMENTATION**

The current conception of the mode of spore germination of \textit{Urocystis cepulae} has been modified by the recent work by Anderson (1). He secured germination readily as soon as spores became mature; a period of rest was unnecessary. Most satisfactory germination resulted from placing spores aseptically upon the surface of agar plates. Anderson shows that, contrary to previously held ideas,
there is no true promycelium produced upon germination of the chlamydospores, but instead a rounded vesicle with a diameter usually somewhat less than that of the central spore. From this vesicle hyphal branches are formed which extend and branch into a mycelial thallus without the expected production of secondary spores or sporidia. Somewhat later certain portions of the mycelium become many-septate and break up into detached cells. These "hyphal fragments" are germinable, and Anderson suggests that they may assume some of the functions of true sporidia. The present writers' findings agree with those of Anderson as to the morphology of the fungus as it grows on agar surfaces.

For germination studies, plates were prepared by first transferring spores aseptically from unbroken host lesions to tubes of onion decoction. Drops of this suspension were then transferred to the surface of plates of water agar or onion agar. Such plates were then transferred to constant temperatures of the desired range, and germination was estimated by counting several microscopic fields from each plate. Pure cultures of the fungus were obtained from such plates. From these cultures suspensions of hyphal fragments were readily secured. Drops of such suspensions were transferred to agar plates and germination at various temperatures determined, as in the case of chlamydospores. The rate of growth of hyphae was estimated by measuring the length of a certain number of germinating fragments in each plate at a given interval, and also by photographing the gross colonies from single drops of the hyphal-fragment suspension after a given period. (See pl. 1.)

EXPERIMENTAL RESULTS

GERMINATION OF CHLAMYDOSPORES

The process of chlamyospore germination is comparatively slow. Very little occurred before 5 days, and reliable comparative counts were not usually possible before 10 days. In some of the experiments increase in germination continued at certain temperatures even after two weeks. The tests were repeated a large number of times with concordant results in general, although there are occasional discrepancies. Some of the experimental results are given in Table I, and a representative graph is given in Figure 1. It will be seen that there is only slight germination at 9° C. This is slightly above the minimum for onion-seed germination and growth. It would appear that the fungus can develop at nearly as low a temperature as the host.

In the majority of cases the highest percentage of germination occurred at about 15° C. There were only slight decreases in germination, however, as high as 22°. The most rapid germination also occurred within this range. At 25° some detrimental effects of temperature are apparent in the reduced percentage of germination. Above this point there is a gradual but marked decline in germination and a decided retardation of growth of the hyphae. The extreme maximum is slightly above 32°. In many cases no germination was noted at 30° or above; in a few cases slight germination occurred, but the hyphae, in such cases, grew very little, and it is doubtful whether they would function in infection.
Plantings of distilled-water suspension of hyphal fragments were made on plates of onion agar. The plates were then placed in a series of constant-temperature incubator on February 22 (1924) and left until March 8. Resulting colonies from each temperature were then photographed.

The white circles indicate no macroscopically visible growth. There is no evidence of growth at 9° C. There were small colonies at 12°; good growth at 15°, 18°, and 22°. There was evidence of retardation at 25°; very meager growth at 28°; and none at 32°. Note the prominent rugosity of the colonies at 22°. Rugosity evident to less degree at 15°, 18°, and 25°. Rugosity evident to less degree at 15°, 18°, and 28°, while at 12° and 28° the colonies have a velvety appearance. × 6/10
The temperature range of the germination of chalmydospores is very close to that for germination of onion seeds. The optimum germination of the smut is to be expected in a comparatively cool soil, i.e., 13° to 20° C. Soil temperatures as they increase above 25° become more unfavorable. Although some germination may occur up to 32°, the growth of the hyphae, even on favorable media, is so scanty above 28° that one would expect it to advance very slightly in the soil in competition with the other soil organisms.

![Graph](image)

**Fig. 1.—Relation of temperature to the germination of chalmydospores of Urocystis cepulae.** (See experiment 3 in Table I)

**Table I.—Relation of temperature to germination of chalmydospores of Urocystis cepulae**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Medium</th>
<th>Average temperature (° C.)</th>
<th>Estimated percentage of germination at various intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 to 14 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spores counted</td>
</tr>
<tr>
<td>1</td>
<td>Suspension in onion decoction on onion agar substrate.</td>
<td>4.0</td>
<td>1,012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.0</td>
<td>537</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.5</td>
<td>746</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.0</td>
<td>262</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.5</td>
<td>690</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32.0</td>
<td>490</td>
</tr>
<tr>
<td>2</td>
<td>Suspension in onion decoction on water agar substrate.</td>
<td>9.0</td>
<td>382</td>
</tr>
<tr>
<td>3</td>
<td>do</td>
<td>13.5</td>
<td>1,228</td>
</tr>
<tr>
<td>4</td>
<td>do</td>
<td>23.5</td>
<td>468</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30.5</td>
<td>305</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32.0</td>
<td>308</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.0</td>
<td>294</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22.0</td>
<td>279</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30.5</td>
<td>321</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22.0</td>
<td>373</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26.0</td>
<td>188</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30.5</td>
<td>429</td>
</tr>
</tbody>
</table>
GERMINATION OF HYPHAL FRAGMENTS

The hyphal fragments germinate very readily. Final results on germination were obtained within a week. The data from a representative experiment are given in Table II, and shown graphically in Figure 2. No germination occurred at 4° C.; a fair amount occurred at 9°. The optimum, at about 15°, is close to that of chlamydospore germination. There is distinctly less germination at 20°, while the temperature of 25° is still more detrimental. Above 25° there is a very abrupt check in germination, which agrees closely with the sudden decline in infection at these temperatures. The temperature relations of the hyphal fragments are very close to those of the chlamydospores. The former become slightly more active at 9°, but less so above 25°.

![Graph showing germination percentage vs. temperature]

**TABLE II.** Relation of temperature to germination of hyphal fragments of Urocystis cepulae

<table>
<thead>
<tr>
<th>Average temperature</th>
<th>Percentage of germination at various intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>0.0</td>
</tr>
<tr>
<td>9.0</td>
<td>4.3</td>
</tr>
<tr>
<td>15.5</td>
<td>90.7</td>
</tr>
<tr>
<td>20.0</td>
<td>92.9</td>
</tr>
<tr>
<td>25.5</td>
<td>96.4</td>
</tr>
<tr>
<td>28.0</td>
<td>3.0</td>
</tr>
<tr>
<td>32.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

VEGETATIVE GROWTH

The rate of growth of the mycelium was studied by measuring the hyphae from germinating hyphal fragments. The latter were floated onto onion agar in sterile distilled water, where germination took place within a few hours. Growth ensued quite rapidly and soon became difficult to follow because of the repeated branching of the hyphae.
The data from a single representative experiment run over a period of 52 hours are shown in Table III, and are represented graphically in Figure 3. No growth had occurred within this period at 9° C., but it was proceeding well at 12°. The growth maximum appeared to be around 18°, which is approximately 3° higher than in the case of germination of chlamydospores and hyphal fragments. Some growth occurred at 25°, while none could be observed after 52 hours at 28°. There is a notably larger amount of growth at 18° than at 15° or 24°. This curve ascends and descends more abruptly between those points than do the germination curves. Apparently growth is not arrested as quickly by the higher temperatures as is germination, but at 24° and 25° the hyphae became noticeably weaker and more attenuated in appearance.

Following the 52-hour measurements the Petri dishes were returned to their respective incubators and growth was allowed to continue for some 15 days longer. By that time macroscopic colonies had developed at the favorable temperatures. Photographs after that period of five separate plantings of hyphal-fragment suspensions
on agar plates for each temperature are reproduced in Plate 1. Where no macroscopic growth resulted the point of inoculation is marked by a white circle. It is to be noted that no macroscopic growth had yet occurred at 9° C., while at 12° colonies were evident. It is apparent that growth is as abundant at 22° as at the earlier indicated optimum of 18°, and that the fungus grows very well over the range of 15° to 22°. At 25° there is evidence of retarded growth, while at 28° only one out of five plantings grew. A marked difference in the appearance of the colonies at optimum and at extreme temperatures was noticeable. At 22° the surface of colonies is prominently rugose, but somewhat less so at 15°, 18°, and 25°; at 12° and 28° the colonies have a raised velvety appearance, but no evidence of rugosity.

TABLE III.—Relation of temperature to growth of thalli of Urocystis cepulae

<table>
<thead>
<tr>
<th>Average temperature</th>
<th>Average length of 25 thalli after 52 hours incubation</th>
<th>Average temperature</th>
<th>Average length of 25 thalli after 52 hours incubation</th>
<th>Average temperature</th>
<th>Average length of 25 thalli after 52 hours incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>° C.</td>
<td>Microns</td>
<td>° C.</td>
<td>Microns</td>
<td>° C.</td>
<td>Microns</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>12</td>
<td>11</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>22</td>
<td>24</td>
<td>214</td>
<td>74</td>
<td>25</td>
<td>28</td>
</tr>
</tbody>
</table>

The question arose as to the viability of the hyphal fragments after exposure to near-maximum and near-minimum temperatures for this fortnight. It would be expected on a priori grounds that those at the low temperature would retain their germinative capacity, while at the higher temperatures the protoplast, as a result of increased metabolism, might expend its energy so rapidly as to have much-reduced vitality. Noble (14) showed for Urocystis tritici that spores germinating above 25° C. "spend themselves" much more rapidly than at lower temperatures. Plates which had been exposed to 3°, 6°, 9°, 28°, and 32° for the fortnight were therefore removed to room temperature (±20° C.). Examination 17 days later showed that normal colonies resulted on the plates held originally at 3°, 6°, and 9°, while on those held formerly at 28° and 32° no further growth occurred. It is evident, therefore, that temperatures of 28° or above not only prevent practically all growth on agar plates, but that hyphal fragments exposed to those conditions for two weeks in most cases die. On the other hand, prolonged exposure on agar plates to temperatures between 3° and 9°, though almost entirely preventing germination and growth, does not seem to be detrimental to the germinative capacity of hyphal fragments.

DISCUSSION OF EXPERIMENTAL RESULTS

The influence of external factors upon infection is expressed in the effect upon host and parasite separately or jointly. It also may or may not be cumulative. Previous studies (21) have shown that onion-smut infection occurs at as low a temperature as is ordinarily encountered by the host, and that abundant infection takes place
up to 25° C.; as one goes above 25° infection is rapidly lessened, until none occurs at 30°. The question at hand is: To what extent is this the result of direct inhibitive effects upon the fungus, and to what extent is it the result of host response?

Considering first the lower temperatures, we find that at about 10° C. germination and growth of the fungus are very slow. Due to the reduced temperatures, we expect that metabolic activity is also much retarded, but we find that, aside from the retardation in activity, the hyphae are quite as able to cause infection as at slightly higher temperatures. Along with this slowing down of the fungus there is a corresponding retardation of growth of the host cotyledon (21), and in consequence an extension of the susceptible period. Thus the coincident holding back of host and parasite due to low temperature only retards the progress of infection, but in the end does not appreciably reduce the amount of infection. As the temperature is increased up to 25° C. the rate of growth of both host cotyledon and parasite increases in somewhat the same proportion, and in consequence infection occurs as abundantly and the disease develops more rapidly.

Above 25° C. there is to be noted a rather abrupt reduction in germination and growth of the parasite. In contrast to the retarded growth at low temperatures, there is probably at these higher temperatures less vigorous development and a more rapid exhaustion of the organism, due to increased metabolism. In further contrast, there is not a corresponding decrease in top growth of the onion nor increase in the length of the susceptible period. In other words, the parasite is being retarded due to the unfavorable temperature, and it continues more or less rapidly to exhaust its food reserve, while on the other hand the host through only slightly reduced growth is advancing promptly to the condition of immunity. It will be seen, therefore, that in comparison with the condition at low temperatures where reduced host and parasite activity combine to permit extensive infection, the combination of circumstances at the high temperatures leads to just the opposite result. Whereas we may justly say that the inhibition of infection at 27° C. and above is in a large measure due to the direct effect of temperature upon the parasite, the fact must not be overlooked that at this higher temperature the host is stimulated to relatively rapid top growth, and consequently the susceptible period is not as prolonged as at the extremely low temperatures.

The direct effect of the various soil temperatures upon the host tissue in relation to infection should be studied further. The question is still open as to whether or not the seedlings grown at constant temperatures above 25° are sufficiently different in constitution to render them more resistant to the parasite than those grown at temperatures lower than 25°. If such is the case the direct influence of temperature upon the host tissue may be a factor in the limitation of infection. Another problem of interest in this connection concerns a study of the changes which the cotyledon and leaves undergo between the young stage, when they are susceptible to smut invasion, and the time of maturity, when they are immune.
CORRELATION WITH DISTRIBUTION IN AMERICA AND EUROPE

It may be well to review, in the light of our present knowledge, the history of the appearance and gradual spread of onion smut both in Europe and America. The first record in America is that by Ware (22) in 1869, at which time the disease was of sufficient economic importance to attract the serious attention of onion growers in the Connecticut River Valley. With the wide dissemination of spores within or upon bottom sets, the fungus has undoubtedly been introduced at one time or another into all parts of this country. As pointed out previously (21), following this general distribution it is now evident that the disease has become established and has assumed destructive proportions in most of our intensive onion-growing sections of the northern and central States, where the crop is spring-sown. On the other hand, the southern onion-growing sections, where autumn sowing is the general practice, have apparently remained free. This is the more noteworthy because of the intensity of onion culture in certain southern areas, and also because of the fact that the smut is frequently and widely introduced into the South on northern-grown onion sets.

Although it has been generally assumed that onion smut was of American origin and was introduced thence into Europe, it is well to note that the first recorded collection of the fungus was made on R. ascalonicum in Denmark (12, p. 273) in 1864. During the two decades which followed this it was reported more widely in Europe than in America. Passerini described a form from Italy on Allium magicum in 1875 (19, p. 204–205). Hallier (9) reported Urocystis cepulae on onion from Germany in 1877. Schroeter (18, p. 378–379) collected it in southern France in the same year. Cornu (4) found it near Paris in 1879; Frank (8) near Leipzig, Germany, in 1880; and Malbranche (13) at Rouen, France, in 1881. So by 1880 it was widely distributed in western Europe, while in America it had not been reported outside of New England.

It is of interest, therefore, to compare 40 years later the regional distribution and destructiveness of the two diseases on the two continents. The senior writer had an opportunity to visit, with this point in mind, a number of the intensive onion-growing sections of western Europe in 1922. The onion crop north of central France is sown chiefly in the early spring, but to some extent in the autumn. In either case, however, there is probably at no time during the sowing or growing seasons a prevalent soil temperature which is unfavorable to onion-smut spore germination and growth. On the contrary, it would seem that conditions more nearly approach the ideal for its development than in any other location. As one goes southward,
however, there is a longer, drier, and warmer summer, which is more unfavorable for the establishment of the fungus accidentally introduced into the soil. There is along with this a general practice of sowing the onion seed toward the close of the warm summer, thus bringing on the young seedling during its period of susceptibility to smut in a warm soil and undoubtedly oftentimes at a temperature which would completely check the parasite. Although it must be true that onion-smut infection could occur in southern Europe if the plant were exposed under the proper conditions, it seems significant that in Europe, as in America, as one goes southward the combination of environmental conditions and cultural practices becomes the more unfavorable for the establishment and spread of the organism.

Citing now the actual findings, it was noted that onion smut is now common and destructive at Edinburgh, Scotland, and in Northumberland (5), the most northerly county of England. Although the disease is now restricted by local quarantine in England, it has been found to assume destructive proportions where introduced in Northampton (5), a short distance north of London. In Denmark it has been reported occasionally, but the minor importance of the onion crop in that country would prevent its ever being more than of incidental occurrence. In the intensive onion-growing section of Germany, near Calbe, the disease is very destructive (23). In Holland it occurs in the onion-growing region of Zealand. Though it is apparently being checked by judicious crop rotation, the writers' observation of a single field where continuous cropping of onions had occurred for several years left no doubt that conditions were very favorable for the multiplication of the parasite. The disease is common in northern France. In southern France it is apparently little known among onion growers. It had not been found in the onion-seed-growing section at St. Remy, although the government inspector had searched repeatedly for it. In Spain the only authentic report of the disease, according to Dr. Gonzalez-Fragoso, of Madrid, is a single collection at Seville. It has not been found at Valencia, which is the largest and most intensive onion-producing section of Europe, or at La Coruna, a smaller but very intensive section in the northwest of Spain. Personal survey of these localities by the senior writer failed to reveal the disease. In Italy no authentic report of the disease was found other than the early collections of Passerini (19, p. 204–205) and Kühn on Allium magicum, already cited.

It would appear, therefore, that although onion smut was present in Europe from Italy to Denmark four decades ago, the disease has multiplied to the point of economic significance only in the more northern, cooler areas. Lack of dissemination alone could hardly account for this restriction. Moreover, the almost exact coincidence of this condition with that in America, where we know that onion smut is being widely disseminated annually on bottom sets, leads us to believe that climatic differences have an important bearing. The longer period of high temperatures in the upper layers of soil probably restricts the establishment of the fungus, while in many cases the temperature immediately after seed is sown is sufficient to check or completely inhibit infection.
SUMMARY

The soil environment, and especially the soil temperature during the short period following germination when the onion seedling is susceptible to infection by the smut fungus, *Urocystis cepulae* Frost, has an important bearing on the amount of disease which results. Previous studies have shown that abundant infection occurs at temperature as low as 10° to 12° C., which is nearly as low as germination and growth of onion ordinarily occurs. There appears thus to be no low point at which the host escapes the disease. Infection occurs equally well up to 25°, but above that a rapid reduction occurs, while at 29° or above the onion seedling will grow free from the disease.

The purpose of the present study has been to determine whether this temperature inhibition is the result of direct effect of the temperature upon the parasite, of host response to temperature, or the combined effect of both.

By studying the germination and growth of the fungus on artificial media the effect of temperature on the parasite under these conditions was determined. It was found that the minimum temperature for germination and growth of *Urocystis cepulae* was very close to that of the onion plant. The optima for chlamydospore germination, hyphal-fragment germination, and vegetative growth of the thallus lie between 13° and 22° C. Above 25° there is a decided reduction in the amount of germination which occurs, and the growth of the hyphae becomes more meager as the temperature rises. Such hyphae as do appear above 28° not only grow very slowly, but soon lose their viability upon continued exposure to these higher temperatures. On the contrary, protracted exposure to near-minimum temperatures does not so affect the slowly growing thallus.

There is no appreciable change in the rate of growth of the host or in the length of its susceptibility when grown slightly above or below the critical point (29° C.) above which no infection occurs. Further study is needed to determine whether there are changes in the host tissue at the high temperatures which render it immune to infection. The marked inhibitive effects of temperatures above 25° upon the parasite, with the maximum lying only slightly above 29°, show the direct influence of temperature upon the parasite to be a primary factor in the limitation of infection.

Although the smut organism is being continually introduced throughout the country on northern-grown onion sets, the disease has as yet never been recorded in the intensive southern onion-growing regions, while it has become established in most of the intensive northern sections. Likewise, in Europe the disease is largely confined to the northern, cooler areas. The results of this study strengthen the theory advanced earlier that temperature is one of the important factors which bring about this regional limitation of the disease.
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