

INFLUENCE OF TEMPERATURE ON THE SPORE GERMINATION OF *USTILAGO ZEA*¹

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Temperature has been regarded by investigators as important among the environmental conditions influencing the infection of corn by *Ustilago zea* (Beckm.) Unger. Maire (5)² found that the spores of the corn smut fungus "germinate more quickly if the temperature is a little raised (20° to 25°)" and that the optimum temperature for sporidial and filamentous development is 20° to 25° C. Piemeisel (7), in considering the "phenomenon of infection and the optimum conditions for the propagation of the fungus," found the optimum temperature for the budding of sporidia to lie between 20° and 26° C., the maximum at about 40° and the thermal death point near 46°. He also found that incubation at 24° to 38° did not seem to influence the rate or amount of germination of the smut spores.

As the germination of the spores themselves would seem to be at least as significant as the budding of sporidia, and as the above investigators do not give detailed experiments on this stage of fungal activity, a study of the influence of temperature on the spore germination of *Ustilago zea* was undertaken in order to throw additional light upon the relation of temperature to this early, significant stage in the life history of the fungus. The studies of which the results are presented in this paper have been carried on under the direction of Dr. L. R. Jones.

In searching for suitable media for spore germination it was attempted to duplicate natural soil conditions. Brefeld (2, p. 67-75) performed experiments which indicated that the germination of the *Ustilago zea* spores takes place in the soil. The conception of soil, especially that containing manures, as a usual medium of germination of the spores also has been held even down to the present time. In attempting to find a soil medium like that obtaining in nature, the method of Thompson (8) was tested. When a tap-water suspension of spores was poured onto loam, no germination took place, whereas, when a suspension of spores in Pasteur's solution was introduced in the same way, germination followed. It seemed that the conditions for spore germination in such soil were not suitable, whereas they were satisfactory in the Pasteur liquid. In an attempt to find a soil on which the spores would germinate, seven modifications of loam and manure were employed, and the spores of *Tilletia tritici* (Bjerk.), Wint., were used as controls. On one pot which contained cow manure and loam, 1:2, there was considerable germination, and on another pot, one germinating spore was observed. Another test gave slight germination. In all these tests the bunt spores germinated abundantly, but, as a result of all the 27 attempts to germinate the spores of *Ustilago zea* on soil, no satisfactory method was found.

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² Reference is by number (italic) to "Literature cited," p. 596-597.

An artificial medium was used, therefore, in the study of the effect of temperature on spore germination. Arthur and Stuart (1), Brefeld (2), Clinton (3), Hitchcock and Norton (4), Maire (5), and Norton (6) germinated the spores in many artificial media. Of these, Pasteur's solution was found most favorable for the following experiments.

The spores from a pustule formed in an anther were suspended in a tube of medium, mounted in Van Tieghem cells, and subjected to 13 different temperatures between 8° and 37° C. in thermo-regulated incubators. Duplicate mounts were placed at each temperature and the experiment performed 11 times. To determine the cardinal temperatures for germination, the percentage of germination was counted; the sporidial production, the number of cells in the promycelia, and the cell contents were observed; and the length and width of the germ tubes measured.

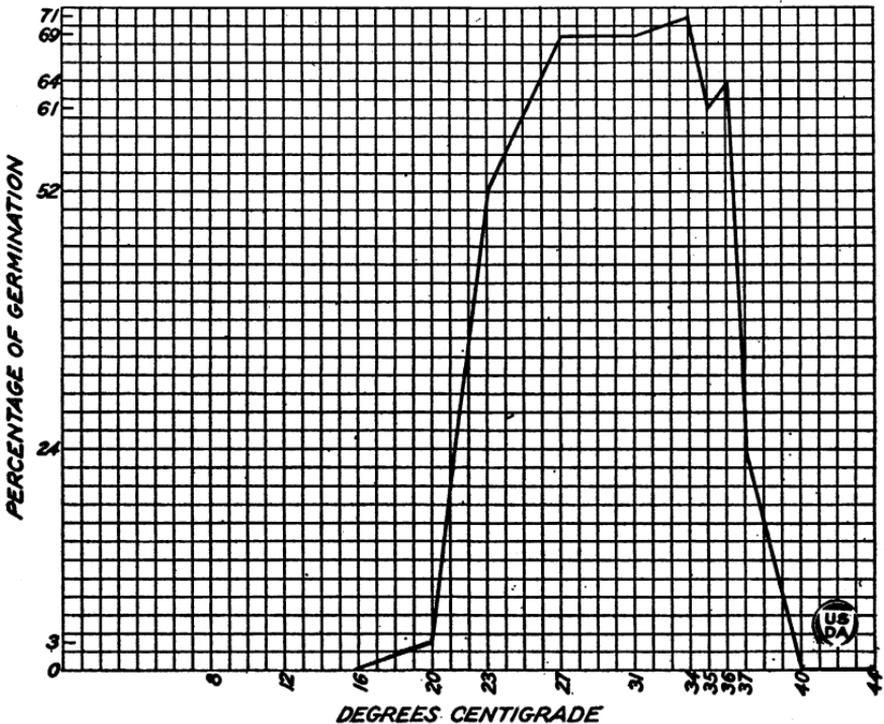


FIG. 1.—Graph showing percentages of germination of chlamydospores of *Ustilago zae* at different temperatures.

The exact minimum for spore germination of *Ustilago zae* was not determined. Four experiments showed germination at an average temperature ranging from 7.9° to 8.4° C., the germ tubes appearing on the 12th, 15th, 16th, and 21st days, respectively. The temperature range of the individual tests was greater than desired. That, together with the daily removal of the mounts from the incubators, may have given sufficient opportunity for eventual germination. The optimum temperature for germination is arbitrarily defined as that at which there is the highest percentage of germination within 24 hours together with a production of such germ tubes as behave normally, especially with respect to sporidial production. The highest percentage of germination within 24 hours occurred between 26.5° and 34.7° C. as is shown in Table I and figure 1, which give the average results of all experiments. As

determined by the number produced on first observation, the optimum temperature for sporidial production lies between 26.5° and 34.7° C. The maximum lies between 36° and 38°. Of 20 observations above 34° only 4 showed abundant production.

At the low temperatures, wherever germination took place sporidia also were produced. The number of cells in the basidia and the width of basidia were alike at the different temperatures and so could not be used as criteria in establishing the optimum temperatures. The lengths of basidia and condition of cellular contents, as shown in Table I, differed at 36.1° to 37.8° C. from those at other temperatures, indicating a deviation from normal at this high temperature. As below 26.5° the percentage of germination is decreased, and, as above 34.7° the sporidial production is decreased and germ tubes are abnormal, the optimum temperature for spore germination may be stated as between about 26° and 34° C. As Table I shows that the maximum temperature rests between 37.1° and 37.8° and as six experiments show no germination at temperatures above 38° after two weeks, the maximum temperature for germination apparently lies between 37° and 38° C.

Attention should be called to the fact that this optimum temperature for germination is much higher for *Ustilago zeae* than for other smuts which have been studied in this laboratory. For example, *Ustilago avenae* germinates best between 15° and 28° C., and, according to Volkart (9), *Tilletia tritici* (Bjerk.) Wint. and *T. laevis* Kühn germinate best between 16° and 18° C.

SUMMARY AND CONCLUSIONS

(1) In 27 trials to germinate *Ustilago zeae* spores in soils containing different amounts of manure, there was considerable germination in only one, slight germination in another, and germination barely occurring in a third. The spores germinated readily in many artificial media, from among which Pasteur's solution was chosen for the experiments to determine the relation of temperature to spore germination.

(2) The optimum temperature for the germination of *Ustilago zeae* spores was found to lie between about 26° and 34° C., the maximum between 36° and 38°, and germination was observed to occur at the minimum temperature of 8°. The optimum for sporidial production lies also between about 26° and 34° C., the maximum between 36° and 38°, and sporidia are produced at the lowest temperatures at which germination was observed.

(3) The experiments showing that high temperature is most favorable for spore germination indicate that infection likewise is favored by hot weather whether the germination takes place in the soil or in water held upon the host plant.

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