SPORE GERMINATION TIME IN *FULIGO SEPTICA*[1, 2]

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ABSTRACT

Fuligo septica spores were selected randomly from six different aethalia collected within fifty feet of one another, on The Ohio State University campus. In each case the specimens were found growing on *Paygro*, a shredded hardwood-bark mulch used around most of the plantings of the O.S.U. campus. Germination time was determined for the spores of each individual aethalium and comparisons made. The number of spores per cubic millimeter, the pH, and the temperature were also recorded. Germination occurred in from 35 to 92 minutes in spores from all but two of the aethalia, where no germination had occurred as of two hours. The germination times for all spores taken from a single aethalium were found to vary by an average of about ±10 min. Spore viability seems to be related not so much to the age of the aethalium as to some condition or set of conditions during the formation of the fructification.

INTRODUCTION

Many investigators over the past one hundred years have reported on the time required for germination of plasmodial slime mold spores, particularly *Fuligo septica* (L.) Wiggers. This species has probably been popular because of the relatively large fructifications, the many spores available, and its cosmopolitan nature. It is for these reasons that it was chosen for this research.

Anton deBary (1887), who was probably one of the first to observe germination in slime molds, reported in general, "The spores are capable of germination as soon as they are ripe in most of the species in which this point has been examined . . .". He went on to describe the term "ripe" in a strictly morphological sense. Anton deBary seldom made mention of the medium used, or of other factors, such as pH, temperature, or age of collection.

A. J. McClatchie (1894), working with *Fuligo septica*, reported that spores from sporocarps one year old germinated in less than three hours in distilled water. E. Jahn (1905), on the other hand, had no success in germinating the spores in distilled water, a result he found to be true for most of the specimens with which he worked. The following year, J. C. Constantineanu (1906), reporting on *Aethalium septicum* (*Fuligo septica*), stated, "Die Sporen keimen ebenso leicht wie die Sporen von *Reticularia lycoperdon*, aber nicht so regelmässig bei allen Exemplaren." (The spores germinate just as easily as the spores of *R. lycoperdon*, however, not as regularly with all specimens.) He went on to say that he was able to get 50% germination in from 30 minutes to an hour and a half, and 100% germination in twenty hours. Cook and Holt (1928) agreed with Jahn, and reported finding *F. septica* ". . . very difficult to germinate at all."; however, in the same year, F. A. Gilbert (1928) reported successful germination of these spores in from one to four hours in distilled water, and the same author (1931) stated that the spores germinated in a little more than fifteen minutes in distilled water.

E. C. Smith (1929) concluded that "the age of spores is not a very important factor in the germination of spores." He also reported in the same article successful germination of *Fuligo septica* spores in tap water, at a pH of 6.8 and a temperature of 18 to 20°C., although the time required for germination was not given.

Robert F. Smart (1937) was one of the first investigators to concern himself

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with the possible contamination of the germinating medium and, when working with spores requiring more than two days for germination, to use a mercuric chloride solution to successfully control contamination. He was also one of the first to determine the optimum conditions for germination in spores of *Fuligo septica*. He found 95% germination in one hour with distilled water as a medium. The optimum pH was 5.5, but germination occurred over a range in pH from 2 to 10. The optimum temperature he found to be 29–30°C., but again there was a wide range over which germination would occur (2 to 36°C.). Smart also found that germination occurred more quickly if spores were placed in a medium in which germination had already occurred, suggesting the presence of some chemical compound that served to trigger or encourage germination.

It is evident, from the foregoing paragraphs, that there are several conflicting views as to the germination time for *Fuligo septica* spores. This variation in germination time was suggested earlier by Gilbert (1929), who had noticed the "surprising lack of uniformity" in the germination times reported by many researchers for specific slime molds. He attributed the variation to (1) the age of the spores, and (2) the degree of maturation of the spores. O. R. Collins (1961), while working with single-spore isolations of *Didymium iridis*, found that "The percentage of spore germination varied from 0.0 to 100%, depending on the sporangium from which the spores were isolated." Collins went on to say, "Inasmuch as the sporangia . . . were all approximately of the same age—never more than one month old—it is curious that such a wide variation in spore germination existed."

It was the work of Gilbert (1928) and of Collins (1961) which set the basis for this research. The problem was to determine the variation in germination time of spores taken from a single fructification of *Fuligo septica*, and subsequently the variation in germination time for spores taken from different aethalia of *F. septica*. Spore viability in relation to aethalium age was also investigated.

PROCEDURE

Spores were taken from four different regions within each of six *Fuligo septica* aethalia, found growing within fifty feet of each other in *Paygro* mulch (of shredded hardwood bark), on The Ohio State University campus. Fresh gatherings of new aethalia not present the day before were made daily, so that no spores were more than 24 hours old at time of collection.

The spores in each case were suspended in 4 ml of 0.5% TSP solution for several minutes to facilitate wetting, and centrifuged for 1 min at 4000 RPM. The supernatant was then poured off and the spores resuspended in 2 ml distilled water. These suspensions were then checked at 15-min intervals for germination. Germination in every case was preceded by a splitting of the spore wall, but it was not considered complete until the protoplast had completely emerged from the epispore.

A value for percentage germination was determined, based on the number of empty spore cases counted one hour after the first spore was seen to germinate. All observations were made using a Spencer Bright-Line Hemacytometer under magnifications of 100 and 430 power. The spores were counted at the beginning of each observation using the directions provided in the booklet accompanying the Hemacytometer (American Optical Company, 1470–101) (fig. 1).

The counting area was the center mm^2, consisting of 25 smaller squares, each of which is again divided into 16 squares. Spore counts were made in the five squares marked R in the diagram. These five counts were then averaged and multiplied by 25 to give the number of spores per mm^2. The depth of water in this region is 0.1 mm, so the count is equivalent to the number of spores per 0.1 mm^3. The spore count was made under 100X and is stated, in this report, in terms of spores/mm^3. The age of the aethalium, the pH of the spore suspension, and the temperature of the laboratory were also recorded. These data are shown in Table I.

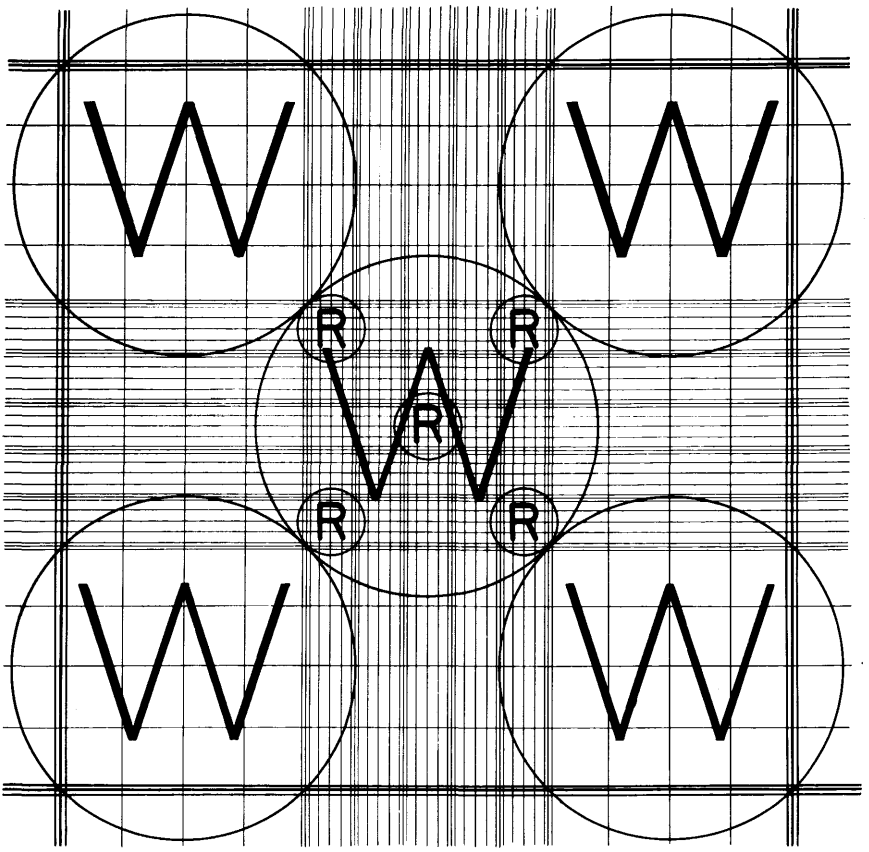

FIGURE 1. Counting areas used in spore count, from American Optical Booklet 1470–101. Permission for use granted by American Optical Corporation, December 15, 1970.

TABLE I

Germination Time for spores taken from Fuligo septica *aethalia of different ages. Percentage Germination determination made one hour after first spore was seen to germinate. The pH of the spore suspension was 7, and the laboratory temperature was 25–27 °C.*

Spore Sample	Avg. Spore Count/mm³	Germination Time in Minutes	Percentage Germination
Aethalium No. 1 collected 7–27–1970.		Spore Age: 20 Days Wetted 8–15–1970	
1.	2875	45	>50
2.	4000	65	>50
3.	2625	40	>50
4.	2375	50	>50

Avg. 50

TABLE I—*Continued*

Spore Sample	Avg. Spore Count/mm³	Germination Time in Minutes	Percentage Germination
Aethalium No. 2 collected 7–27–1970.		Spore Age: 21 Days Wetted 8–16–1970	
1.	5875	35	>50
2.	3376	35	>50
		Avg. 41	
3.	3125	45	>50
4.	1500	50	>50
Aethalium No. 3 collected 8–12–1970.		Spore Age: 1 Day Wetted 8–12–1970	
1.	3375	65	>50
2.	8875	78	>50
		Avg. 72	
3.	5125	55	>50
4.	5000	92	>50
Aethalium No. 4 collected 8–18–1970.		Spore Age: 2 Days Wetted 8–19–1970	
1.	3000	No germination at the end of 2 hrs.	
2.	3500	"	
3.	2750	"	
4.	3500	"	
Aethalium No. 5 collected 8–18–1970.		Spore Age: 2 Days Wetted 8–19–1970	
1.	5750	No germination at the end of 2 hrs.	
2.	3800	"	
3.	4200	"	
4.	3500	"	
Aethalium No. 6 collected 8–20–1970.		Spore Age: 1 Day Wetted 8–20–1970	
1.	3500	40	>50
2.	3750	42	>50
		Avg. 41	
3.	2750	40	>50
4.	3650	40	>50

TABLE II

Germination Time and Percentage Germination for spores taken from the same aethalia as shown in Table I after a period of two weeks.

Spore Sample[a] (Aethalium)	Spore Age in Days	Avg. Spore Count per mm³	Germination Time in Minutes[b]	Percentage Germination
1.	34	1000	45	>50
2.	35	2000	40	>50
3.	15	5250	60	>50
4.	16	2750	No Germination	
5.	16	1750	No Germination	
6.	15	1000	45	>50

[a]Only one spore sampling was taken from each aethalium.
[b]All spores were wetted 9–1–1970.

DISCUSSION

The 0.5% TSP solution used to "wet" the spores had no toxic effects on the spores, so that, after 72 hours, the swarm cells were quite active and apparently unharmed in any way. E. W. Elliott (1949) had used TSP in his research, but had discarded it because it failed to bring consistent germination results with any species except *Enteridium rozeanum* (*Reticularia splendens*). He suspected that the TSP might be having some toxic effects on the spores. This research does not support his suspicion, at least in regard to *Fuligo septica*.

The spore walls usually showed some signs of splitting from 30 to 45 minutes after they had been wetted, and the protoplast emerged completely some 10 to 20 minutes after that. Once emerged, the protoplast immediately broke free from the spore wall and moved slowly, in an amoeboid fashion, remaining rather close to the vacated epispore. After 5 to 10 minutes the myxamoeba elongated, began to quiver, and then moved off in a spiral fashion similar to some of the flagellated protozoa. This flagellated stage is commonly called a "swarm cell."

Germination of spores could be seen along the outer regions of the hemacytometer, in almost every case, before germination was seen within the counting zone. By the time the first cell germinated in the counting zone, many active swarm cells were visible along the edges of the hemacytometer cover slip. Spores taken from aethalia nos. 4 and 5 were the only exception to this. These spores not only failed to show signs of germination in the counting zone, but also showed no signs of germination anywhere on the hemacytometer. These spores measured 7.98 microns in diameter, were minutely spinulose, and appeared in every way to be morphologically mature. Because aethalium no. 6 was similar in age to nos. 4 and 5, and produced viable spores capable of germination in 40 minutes, it seems probable that age is not the determining factor. This view is supported by the data in Table II, which show the germination of spores from the same six aethalia after a period of two weeks.

Morphological maturity is apparently no guarantee of physiological maturity in the slime mold *Fuligo septica*, based on the data presented. If the spores of an aethalium are viable (or non-viable), they are consistently so. Spore viability seems to be related not so much to aethalium age as to conditions existing during the formation of the fructification.

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