ON THE GERMINATION PHYSIOLOGY OF CONIDIOSPORES OF THE WHEAT SCAB FUNGUS, *GIBBERELLA ZEAE* (SCH.) PETCH

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I. Introduction

Studies have been reported on the influence of temperature and humidity on the germination of conidiospores of *Gibberella Zeae* (Sch.) Petch (1, 15). Like other filamentous fungi, the fungus spores formed in slant cultures are rarely found germinated *in situ*. Likewise, germination in distilled water is also extremely poor. These phonomena are believed to be attributed to some internal or exterior causes. The authors experimented on some causes such as the density of spore, nutritive substance, and the age in culture. Results on them are reported below.

II. Influence of Spore Density on Germination

In a normal germination test, it is required that the density of the the spores be uniform. Spores of *Botrytis Allii*, *Cephalothecium roseum*, *Sclerotinia americana*, *Botrytis cinerea*, and others germinate less with an increase in spore density (4, 14). Others like *Pseudopeziza ribis*, on the other hand, show better germination at higher density. *G. Zeae* apparently reacts in the manner of the former, and some studies were made by the authors.

Van Tiehgem cell caused uneven settling of the conidiospores at the center of the droplet. The authors, taking the advantage of this fungus to germinate in submerged condition, used concave slides where test spore suspension held in the depression was covered with a cover glass and sealed with melted paraffin. The prepared slide was then kept in a constant temperature compartment held at 24°C in an inverted position so that the spores would settle to the surface of the cover glass. The germination was recorded after 4—8 hours.

G. Zeae strain No. 2089, a stock that formed abundant conidiospores on potato decoction agar medium was used in all experiments.

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A flattened platinum wire moistened with redistilled water was gently stroked over the surface of the culture to remove the spores. The spores were then suspended in sterile water or 2% glucose solution. All glass equipments used were previously cleaned with cleaning solution and finally rinsed with redistilled water.

On each sample of prepared slide, ten to twenty different fields were observed using a microscope magnified 160 times. The germination was expressed in percentage and finally transformed to $P=Sin^{2}\theta$ (2). Results are shown in table 1.

Table 1. *Influece of spore density on germination.**

Spore density**	258.2	165.2	113.9	80.9	44.2	20.5	Remarks
Number of spores tested	5163	3304	2277	1617	895	413	L. S. D. (0.05) = 2.62.
Number of spores germinated	14	13	178	699	599	348	2% solution of glucose.
Germination percentage (%)	0.7	0.4	7.8	43.3	66.6	84.3	Conidiospores formed after 17 days culture.
θ	2.19	2.50	15.41	40.89	55.50	66.90	

Spore density**	154.7	53.6	32.9	16.2	Remarks
Number of spores tested	1547	536	329	162	L. S. D. (0.05) = 4.49.
Number of spores germinated	13	27	86	80	2% solution of glucose.
Germination percentage (%)	0.8	5.0	26.4	49.4	Conidiospores used were taken from a 20 days' culture.
θ	4.08	12.13	30.51	44.81	

Spore density**	127.0	33.3	19.7	10.7	Remarks
Number of spores tested	508	500	508	521	L. S. D. (0.05) = 3.13.
Number of spores germinated	15	29	87	212	Redistilled water.
Germination percentage (%)	2.9	5.9	17.2	40.7	Conidiospores used were taken from a 14 days' culture.
θ	9.80	13.88	24.34	40.32	

* Results after 4 hours' incubation at 24°C.

** Spore density is shown by the average number of conidiospores found in a mieroscope's field under the magnification ×160.

The above results show that consistently better germination was obtained at lower density, where more than 100 per field markedly decreased the percentage. Conidia less than 40 per field resulted in wider variation among different fields, and it was not considered satisfactory for germination experiments. Conidia from 50 to 70 per field seemed ideal. The method

excluded air, but the germination was normal in every respect.

III. Influence of Nutrient Substances on Germination

It is known that the germination of spores is stimulated or promoted by the presence of various nutrient substances. The substance includes sugars, salts, and plant extracts. Furthermore, many fungi are known not to germinate or only poorly in distilled water (4, 5, 7, 10, 17).

Since the germination of spores of *G. Zeae* has been found to be very poor in distilled water, the influence of addition of various nutrient substances in the germinating medium was studied. In this test three to four drops of the spore suspensions on each slide glass were placed in a moist chamber made of inverted petri dishes 5 inches in diameter. The slides were laid on the glass rods placed at the bottom of the dish. To maintain a moist condition in the dishes, some water was introduced between the dish and the cover.

Table 2A shows the results of germination in redistilled water, tap water and 2% glucose solution. Germination in the redistilled water was very poor in comparison to the tap water and 2% glucose solution. 2% glucose solution yielded best germination.

Table 2. *Influence of nutrient substance on conidiospore germination.*

A

		Number of spores tested	Number of spores germinated	Germination percentage (%)	θ
Redistilled water	a	110	7	6.4	8.93
	b	665	18	2.7	8.51
	c*	1085	60	5.5	13.22
Tap water	a	127	30	23.6	26.58
	b	338	112	33.2	34.99
	c**	313	177	56.4	48.33
2% solution of glucose	a	122	47	38.5	38.54
	b	320	121	37.8	39.25
	c**	375	245	65.4	54.60

Remarks: a: Results after 4 hours' incubation at 24°C. Spores used were formed after 8 days' culture. Number of spores found in 15 fields under the magnification ×160.
L. S. D. (0.05) = 7.21.
b: Results after 7 hours incubation at 24°C. Spores formed after 15 days' culture. Number of spores found in 10 fields under the magnification ×160.
L. S. D. (0.05) = 6.1.
c: Results after 8 hours' incubation at 24°C. Spores formed aftes 15 days' culture. Number of spores found in* 20** or 10 fields under magnification ×160.
L. S. D. (0.05) = 8.25.

Table 2. *continued.*

B

		Number of spores tested	Number of spores germinated	Germination percentage (%)	θ
Redistilled water	a	1012	92	9.1	17.5
	b	678	34	5.0	12.8
2% glucose	a	1061	322	33.0	33.3
	b	681	319	46.9	42.9
2% sucrose	a	1013	295	29.1	32.8
	b	678	292	43.1	41.0
2% levulose	a	1029	313	34.0	33.5
	b	680	322	47.4	42.7
0.5%KH_2PO_4	a	1004	36	3.6	10.9
	b	676	9	1.3	6.5
0.5%$MgSO_4$	a	1006	341	34.0	35.7
	b	671	96	14.3	22.2
Tap water	b	679	113	16.6	23.9
Potato decoction	b	687	554	80.7	64.0

Remarks: Results after 4 hours incubation at 24°C.
a: L. S. D. (0.05)=3.27. Spores formed after 5 weeks' culture.
b: L. S. D. (0.05)=2.74. Spores formed after 20 days' culture.

Table 2B includes also sucrose, levulose, KH_2PO_4, $MgSO_4$, and decoction of potato. Results are from 5 fields in (a), 3 fields in (b), replicated 4 times using about 50 spores to a field. There was no significant difference among the three sugars. KH_2PO_4 seemed to inhibit germination. $MgSO_4$ stimulated germination: in (a) it was about same as the sugars, but in (b) it approached the tap water. In other experiment not shown in the table, the effects of $MgSO_4$ and sugars were about same. A high percentage of germination was secured from a relatively weak decoction of potato (10 grams boiled for 20 minutes in 0.5 liter of tap water). The germination was 80.7% in this case compared to 14.6 of the more concentrated decoction (prepared from 20 grams in 0.1 liter of tap water). Gremination for this series in the redistilled water was 14.7%. The spores in concentrated decoction appeared to have lost their turgor, and those that had germinated were apparently abnormal.

Further experiment was made to determine whether the nutrients served as mere stimulants or were essential to their subsistence. The methods were as for table 2, but the glucose concentration was altered. Results are shown in table 3.

In table 3A glucose concentration of 0.25% showed considerable germination, but further increase in concentration did not show proportionate increase in germination. Results on concentrations below 0.25% are shown in table 3B. Here, the germination was influenced by the amount of glucose below 0.5%. These results did not decide definitely whether the glucose served as a stimulant or a nutrient. It is belived that further experiment is needed

Teble 3. *Influence of the concentration of glucose solution on conidiospore germination.* *

A

	Redistilled water	Glucose solution (%)					
		0.25	0.5	1.0	1.5	2.0	2.5
Number of spores tested	1346	974	1242	1090	942	976	1063
Number of spores germinated	52	168	268	235	233	269	311
Germination percentage (%)	3.9	17.3	21.7	21.6	24.7	27.6	29.3
θ	11.23	23.53	26.93	27.55	29.00	32.28	32.45

B

	Redistilled water	Glucose solution (%)					
		0.005	0.05	0.5	1.0	2.0	5.0
Number of spores tested	677	629	653	657	665	658	660
Number of spores germinated	44	93	180	285	339	404	463
Germination percentage (%)	6.5	14.3	27.0	43.4	51.0	61.3	70.2
θ	14.7	22.4	31.4	41.1	45.7	51.6	58.1

Remarks: * Results after 4 hours' incubation at 24°C.
A: Spores formed after 5 weeks' culture. L. S. D. (0.05)=4.23.
B: Spores formed after 19 days' culture. L. S. D. (0.05)=7.23.

to clarify the above problem.

VI. Influence of Age of Condiospore to Germination

Changes in the germination capacity or viability of spores as influenced by the time after maturation are known to exist. Experiments were made to determine how the spores of *G. Zeae* are affected by time. Table 4 shows the formation and maturation of spores produced on cultures of potato decoction agar medium. Data are the averages of 5 petri dish plate cultures.

Table 4. *Influence of temperature on the growth of hyphae, the formation and the maturation of conidiospores.*

		Days after						
	°C	1	2	3	4	5	6	7
Diameter of colonies	20	0.74	1.50	2.48	3.45	4.82	6.20	7.56
	24	0.92	1.80	2.76	3.66	4.90	7.10	8.83
	27	1.06	1.98	2.55	3.25	4.03	6.43	7.30
	30	0.72	1.38	1.90	2.63	3.24	4.04	4.80
Formation and maturation of conidiospores	20	−	−	±(±)	+(±)	++(+)	++(+++)	++(+++)
	24	−	−	+(±)	+(+)	+++(+++)	+++(+++)	+++(+++)
	27	−	−	±(±)	+(±)	+++(++)	+++(+++)	+++(+++)
	30	−	−	−	−	−	±(+)	+(+)

Remarks: Number of plus signs shows the degree of conidiospore formation, the more the + sign the more the formation. Minus sign shows no formation. Plus sign in brackets shows the maturation.

Color on the fnugus mycelium began to appear after two to three days. The formation of spores on the colony was determined by taking samples of the mycelium at a distance of 1 cm from the center. There were no spores formed on mycelium that did not show pinkish coloration. On incubating at 20°, 24°, and 27°C, spores began to appear after the third day, but most of them were not mature morphologically as distinguished by distinct septations until the fourth day. At 24° and 27°C they matured after five days. At 20°C it took six days, and at 30°C there were very few even after the sixth day.

In test tube slants after seven days of incubation at 24°C the mycelium covered the medium and produced the pinkish coloration. This was selected as the stage the spores were mature. Table 5 shows the decline in the germination of mature spores by the elapse of time. From these results, although it is not possible to establish any definite law, there was a definite tendency for the decline in viability as affected by time. Effects of temperature and humidity on the matured spores, as determined in preliminary test using 10°, 20°, 24°, and 30°C for 7 days, indicated that: on the temperature, the higher the temperature the spores were exposed to after maturation, the loss of viability increased; and on the humidity, the lower the humidity, the less viable they became.

Table 5. *Influence of the aging of conidiospore on germination.* *

Number of days after maturation	5	35	63	Remerk
Number of spores tested	931	932	1001	L. S. D. (0.05) = 9.32.
Nnmber of spores germinated	721	305	26	
Germination percentage (%)	77.5	32.7	2.6	
θ	60.87	34.96	9.24	

Number of days after maturation	3	12	42	Remerk
Number of spores tested	618	654	612	L. S. D. (0.05) = 5.07.
Number of spores germinated	513	245	153	
Germination percentage (%)	83.1	37.4	3.0	
θ	64.63	38.01	29.82	

Number of days after maturation	4	20	30	36	69	Remerk
Number of spores tested	428	417	419	412	412	L. S. D. (0.05) = 4.16.
Number of spores germinated	348	329	318	256	75	
Germination percentage (%)	81.3	79.0	76.0	62.1	18.2	
θ	64.28	62.95	60.68	52.10	25.25	

* Results after 4 hours' incubation at 24°C in 2% solution of glucose.

V. Effect of Washing on the Germination

The surface of the conidiospore is coated with a gelatinous substance

which has an affinity for moisture. The effect of removal of this film on its germination was studied. The spores were subjected to various length of washing by suspending them in redistilled water, and finally precipitating with a centrifuge. The precipitated spores were then placed in 2% glucose solution for observing the germination. Table 6 shows a portion of the results repeated several times.

Results of table 6 indicated that washing and centrifuging had no significant effect on their germination.

Table 6. *Germination of the centrifuged conidiospores**

Period of centrifugation (in minutes)	0	1.5	1.5×2	1.5×3	0**	1.5×3**	Remerks
Number of spores tested	485	477	464	484	488	479	Spores used were formed after 44 days culture.
Number of spores germinated	256	267	255	271	4	5	2000 rpm
Germination percentage (%)	52.8	56.0	55.0	56.1	0.8	1.0	**Rediltilled water.
θ	46.91	48.37	48.44	48.50	4.20	4.69	L. S. D. (0.05) = 7.44.

Period of centrifugation (in minutes)	0	1.5	1.5×2	1.5×3	Remerks
Number of spores tested	616	619	614	616	Spores used were formed after 6 weeks culture.
Number of spores germinated	246	234	241	231	2000 rpm
Germination percentage (%)	39.9	37.8	39.2	37.5	L. S. D. (0.05) = 9.78.
θ	40.70	37.21	39.78	37.68	

* Results after 4 hours' incubation at 24°C in 2% soultion of glucose.

Table 7. *Germination of condiospores in supernatanted liquid**

	Redistilled water	Dilution			Remerks
		Original	×2	×10	
Number of spores tested	660	633	646	660	Conidiospores taken from an 8 days' culture were centrifuged at 1500 rpm for 1 minute, and the supernatant liquid was secured.
Number of spores germinated	5	302	108	27	
Germination percentage (%)	0.8	47.7	16.7	4.1	
θ	4.30	43.68	23.68	11.30	L. S. D. (0.05) = 5.47.

* Results after 4 hours' incubation at 24°C in 2% solution of glucose.

It was found in a preliminary test that the centrifuged supernatant liquid from spore suspension was pinkish in color, and this liquid aided the germination of conidiospores. From this, a stronger solution was prepared by dissolving a large quantity of spores in redistilled water, centrifuged at 1,500 rpm for one minute and the supernatant liquid diluted with redistilled water to 1/2 and 1/10 the original concentration. To this, spores were suspended and the effect on the germination was tested by comparing with those in which only the redistilled water was used. This result shown in

table 7 proved that even at a dilution of 1/10 yielded a better germination than the redistilled water. Smaller the dilution, the greater the germination, which apparently indicates that the substance coating the surface of the spores promotes spore germination.

VI. Discussion

Germination of spores of *Gibberella Zeae* and others as well is conditioned by internal and external factors. This report takes up a few of the important factors. It was found from results obtained on the relation of spore density to germination that the gemination decreased with the increase in density.

This result conformed with studies made on other fungi by other investigators, although no new explanation was able to be drawn from this experiment. The reason for poor germination in distilled water was also unexplainable. It is believed that if some specific substance was proved as a stimulant for activiating dormant spores, effects of spore density and distilled water may be explainable. The presence of glatinous substance covering the spores apparently promoted germination from our experimental results. On this subject further detailed studies are required.

VII. Summary

The present studies on *Gibberella Zeae* (Sch.) Petch cover results obtained on the effects upon germination by spore density, nutritive or stimulative substances, age of spores, and washing.

1. On the density of the suspending conidiospores, there was a tendency of higher the density of conidiospores, the lower the germination. A density of approximately 100 spores per field of a microscope magnified 160 times yielded a very poor germination. It was found that, with the above magnification, a density of between 50 to 70 per field was most appropriate for experimental purposes.

2. Conidiospores germinated very poorly in redistilled water. An addition of glucose, sucrose, levulose, magnesium sulfate, and decoction of potatoes supported germination. A glucose concentration of as low as 0.005% still showed higher germination than the redistilled water.

3. The fungus formed and matured conidiospores most readily when grown at temperatures between 24° and 27°C. At 30°C they were formed slowly as well as poorly. The viability of conidiospores declined with time. After 6 to 8 weeks, the percentage of germination decreased 30 or more percent.

4. Washing conidiospores in redistilled water and precipitating with a centrifuge did not cause significant loss in viability.

5. A gelatinous substance covering the conidiospores supported germination.

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