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ON THE REST PERIOD AND ITS SHORTENING IN SMUT SPORES (PRELIMINARY REPORT)

BY

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It is generally recognized that spores of certain smut fungi such as *Ustilago nuda* (PERS.) KELL. et Sw., *U. Tritici* (PERS.) JENS., *U. Hordei* (PERS.) KELL. et Sw., *U. grandis* Fr., *U. Avenae* (PERS.) JENS., and *Sorosporium Panici-miliacei* (PERS.) TAKAHASHI, germinate with ease, immediately after they have been produced, requiring no period of after-ripening.

So far as the writer's germination experiments are concerned, however, only small percentage of fresh spores of the above mentioned smut fungi germinated in most cases very sparsely, whereas the spores which had been kept at room temperature for several days germinated very readily showing a high percentage of germination. Consequently, it seems that the spores of these fungi may require a rest period before they are ready to germinate.

Aiming to ascertain the rest period and to find a method which will shorten the dormant period and accelerate germination of fresh spores, a series of germination experiments were carried out.

The present paper is a preliminary report of the results of these experiments with special reference to the effect of etherization upon the germination of smut spores.

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Rest Period of Smut Spores

Certain investigators of smut fungi such as BREFELD, (2), PARAVICINI (8), KNIEP (6), STAKMAN (10), DAVIS (3), and some others recognized a rest period of spores in certain smut fungi, e. g., *Urocystis Anemones* (PERS.) WINT. (2, 6, 8) *Tilletia laevis* KÜHN (10), *Cintractia Caricis* (PERS.) MAGN. (2), and *Ustilago striaeformis* (WESTEND.) NIESS. (3).

In general, however, spores of smuts, particularly in *Ustilago nuda*, *U.*

Tritici, *U. Avenae*, *U. Hordei*, and *U. grandis* are regarded as germinating readily either in water or nutrient solution.

As to *Ustilago Zeae*, KÜHN (7) and BREFELD (1) stated that fresh spores could not germinate in water, but did so readily when they had passed through a period of after-ripening.

On the other hand HITCHCOCK and NORTON (4), experienced no difficulty in making fresh spores germinate in water.

For the purpose of finding whether or not the spores of these fungi require a rest period, a series of germination experiments was performed with fresh spores of the smuts at intervals of 1 to 131 days. The germination tests were made by hanging drop cultures with 2 % sugar solution which were incubated at 25°C.

For the germination of the spores of *U. Hordei* distilled water was used incubating the cultures at 20°C. All the hanging drop cultures were examined on the next morning. The results of these experiments are shown in Table 1.

Table 1. Results of germination tests of smut spores
(Number of examined spores is 300 in each test)

Name of Smut	<i>Ustilago Tritici</i>	<i>U. Avenae</i>	<i>U. muda</i>	<i>U. grandis</i>		<i>U. Zeae</i>	<i>U. Hordei</i>	<i>Sorosporium Paniculacei</i>
Date of collection	July 6 1933	July 4 1933	June 25 1933	Oct. 12 1932	Oct. 19 1933	Aug. 16 1933	Aug. 31 1931	July 31 1933
Date of test	July 11 1933	July 11 1933	June 27 1933	Oct. 19 1932	Oct. 22 1933	Aug. 23 1933	Sept. 23 1931	Aug. 2 1933
Number of germinated spores	21	13	58	13	8	39	few	64
Percent. of germination	7.0	4.3	19.3	4.3	2.7	13.0		21.3
Date of test	July 14 1933	July 26 1933	July 4 1933	Oct. 20 1932	Oct. 25 1933	Aug. 30 1933	Jan. 23 1932	Aug. 4 1933
Number of germ. spores	43	29	94	47	14	44	168	52
Percent. of germ.	14.3	9.7	31.3	15.7	4.7	14.7	62.3	17.3
Date of test	July 18 1933	Aug. 4 1933	July 11 1933	Oct. 21 1932	Nov. 1 1933	Sept. 19 1933	June 3 1932	Aug. 15 1933
Number of germ. spores	53	47	100	66	28	42	218	25
Percent. of germ.	17.7	15.7	33.3	22.0	9.0	14.0	72.2	8.3

Date of test	July 26 1933	Aug. 15 1933	July 14 1933	Nov. 18 1932	Nov. 16 1933	Oct. 10 1933	July 5 1932	Aug. 30 1933
Number of germ. spores	56	69	114	111	48	32	169	19
Percent. of germ.	18.7	23.0	38.0	37.0	16.0	10.7	56.3	6.3
Date of test	Aug. 4 1933	Aug. 30 1933	July 20 1933	Dec. 17 1932	Dec. 7 1933	Nov. 16 1933	Sept. 7 1932	Sept. 15 1933
Number of germ. spores	67	41	122	67	91	23	143	22
Percent. of germ.	22.3	13.7	40.7	22.3	30.3	7.7	47.7	7.3
Date of test	Aug. 15 1933	Sept. 19 1933	July 26 1933	Jan. 25 1933	Jan. 14 1934	Dec. 7 1933	Oct. 19 1932	Oct. 10 1933
Number of germ. spores	44	20	197	61	78	31	146	13
Percent. of germ.	14.7	6.7	35.7	20.3	26.0	10.3	48.7	4.3
Date of test	Aug. 30 1933	Oct. 10 1933	Aug. 4 1933	Feb. 23 1933		Jan. 14 1934	Nov. 18 1932	Nov. 16 1933
Number of germ. spores	23	13	49	53		17	144	27
Percent. of germ.	7.7	4.3	16.3	14.3		5.7	48.0	9.0
Date of test	Sept. 19 1933	Nov. 16 1933	Aug. 15 1933	Mch. 15 1933			Dec. 7 1932	Dec. 6 1933
Number of germ. spores	0	23	13	58			102	19
Percent. of germ.	0	7.7	4.3	19.3			34.0	6.3
Date of test	Oct. 10 1933	Dec. 6 1933	Aug. 30 1933	April 20 1933			Jan. 21 1933	Jan. 17 1933
Number of germ. spores	3	18	16	50			101	14
Percent. of germ.	1.0	6.0	5.3	16.7			33.7	4.6
Date of test	Nov. 16 1933	Jan. 13 1934	Sept. 19 1933	May 27 1933			Feb. 23 1933	
Number of germ. spores	0	11	0	44			90	
Percent. of germ.	0	3.3	0	14.7			30.0	

As shown in the above table, fresh spores of the smuts germinated very sparsely except these of *Ustilago Zeae*, *Sorosporium Panici-miliacei*, but with the lapse of days, the percentage of germination gradually increased to attain the maximum and then decreased again eventually diminishing to zero, showing that these spores may require some rest period before their germination.

It seems that the length of rest period of each spore is different to some extent because the germination of the spores did not take place simultaneously.

As regards *Ustilago Zeae*, in the writer's previous experiments, it was found that the fresh spores of this fungus sometimes germinated scarcely at all as pointed out by KÜHN and BREFELD, while in other cases they germinated pretty well as HIRCHCOCK and NORTON reported working with the same smut. This may be partly due to the fact that some of the fresh spores require a longer rest period, while the others have passed through the dormant period and are ready to germinate. The spores of *Ustilago Zeae* as well as those of *Sorosporium Panici-miliacei* did not germinate abundantly in most cases, but they retained their germinating power during a period longer than a year.

Effects of Etherization upon Smut Spores

Certain investigators have succeeded in shortening the dormant period of fungous spores. According to THIEL and WEISS (11), citric acid solution was effective to accelerate the germination of teleutospores of *Puccinia graminis tritici*.

Carbon dioxide was used by PLATZ (9) to shorten the rest period of the spores of *U. Zeae* and likewise chloroform vapor and citric acid solution were used by DAVIS (3), for the spores of *Ustilago striaeformis*.

Among various methods tested by the writer the ether-forcing method originally devised by JOHANNSEN (5) for the purpose of forcing lilac flowers was found most effective in causing prompt germination of the fresh spores of the smut fungi under consideration. The procedure employed was as follows. In a tight glass jar the spores were etherized, using from 0.3 to 0.5 cc. of ether per 1 liter of space. After 20 hours' etherization, the spores were taken out and their germinating power was tested by the hanging drop cultures with 2 % sugar solution, incubating about 20 hours at 25°C. On the next morning these drop cultures were examined and the germinated spores counted. It was found, as will be shown in the following table, that etherized spores germinated more readily than the untreated ones. The results of the experiments are shown in Table II.

Table II. Effect of ether-vapors upon the germination of Smut spores.

Name of Smut	Date of collection	Date of test	Amount of ether per liter (in cc.)	Number of spores examined	Number of germinated spores	Percentage of germinated spores	Number of germinated spores untreated	Percentage of germinated spores untreated
<i>Ustilago Tritic</i>	July, 6 1933	July 14 1933	0.5	300	148	49.3	43	14.3
"	"	July 20 1933	0.3	300	74	24.0	42	14.0
"	"	Aug. 5 1933	0.4	300	121	40.3	110	36.7
<i>U. Avenae</i>	July 4 1933	July 14 1933	0.5	300	78	26.0	42	14.0
"	"	July 20 1933	0.3	300	64	21.3	19	6.3
"	"	Aug. 5 1933	0.5	300	101	30.4	51	17.0
<i>U. nuda</i>	June 25 1933	July 14 1933	0.5	300	143	47.7	114	38.0
"	"	Aug. 5 1933	0.3	300	138	46.0	122	40.7
<i>U. grandis</i>	Oct. 19 1933	Nov. 1 1933	0.3	300	53	17.7	28	9.3
"	"	"	0.5	300	97	32.3	28	9.3
<i>U. Zeae</i>	Aug. 16 1933	Aug. 23 1933	0.4	300	191	63.7	39	13.0
"	"	Nov. 2 1933	0.5	300	139	46.3	29	9.7
"	"	Nov. 2 1933	0.3	300	47	15.7	28	9.3
"	"	"	0.5	300	63	21.0	28	9.3
"	"	Nov. 8 1933	0.3	300	45	15.0	29	9.7
"	"	"	0.5	300	61	20.3	29	9.7
<i>Sorosporium Panic-milacei</i>	July 31 1933	Aug. 5 1933	0.4	300	57	19.0	7	2.3
"	"	Nov. 2 1933	0.3	300	38	12.7	18	6.0
"	"	"	0.5	300	34	11.3	18	6.0
"	"	Nov. 8 1933	0.3	300	40	13.3	17	5.7
"	"	"	0.5	300	62	20.7	17	5.7

Summary

1. Fresh spores of certain smut fungi such as *Ustilago nuda*, *U. Triticci*, *U. Hordei*, *U. grandis* and some others germinated less readily than those which had been kept at room temperature for several days, indicating that these smut spores may require a period of after-ripening.

2. Ether vapor was remarkably effective in causing prompt germination of these smut spores.

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