Second.—The age of the inoculating material in these experiments varied from two years and eleven months to four years and seven months, and from results obtained the germinative power lessens with age.

Third.—(a) Some media are decidedly favorable to the fungus in retaining its vitality. Example 1: Wort, all cultures from it having germinated in each of the six testing media. (b) Other media are favorable under certain conditions. Example: Wort-gelatine. Out of five cultures one grew in one of the testing media. (c) Still other media are decidedly unfavorable. Example: Dextrose, cultures from it having failed throughout the experiments.

Fourth.—Alcohol is not stimulating to the conidia of Aspergillus oryzae.

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Spore Resistance of Loose Smut of Wheat to Formalin and Hot Water.

William Stuart.

The comparative absence of any definite knowledge of the spore resistance of the loose smut of wheat to formalin and hot water, and the lack of any efficient method of preventing losses to the wheat crop from it, seem to invite some attention to this phase of the question. In a measure

the work which has been performed is simply a continuation of some investigations begun during the season of 1898, and reported in the Academy Proceedings for that year, pp. 64-70. At that time work was undertaken with both wheat and oats smut, but on account of the fact that the wheat smut spores did not remain viable under laboratory conditions this portion of the work had to be abandoned. Further opportunity for study of the wheat smut did not present itself till last summer. A considerable quantity of smutted heads was collected from last year's wheat crop just after the grain headed out, and before the spores were blown or washed off the rachis. This material was kept in the laboratory until needed for use. Spores mounted in hanging drop cultures over moist cells showed good germination in distilled or tap water at the time the material was collected, but in the course of two or three weeks failed to germinate. As these results corresponded with those of 1898, it was decided to try germinating them in some nutrient solution. Accordingly a Pasteur sugar solution was substituted for the water, with the result that a vigorous germination was obtained.

In order to insure a uniform lot of spores for the culture experiments, a sufficient quantity of them were jarred from the smutted heads, and after removal of the detritus by screening, they were thoroughly mixed and collected in a receptacle from which fresh supplies were drawn as desired.

Treatment of the spores.—The spores were treated in muslin sacks, one corner of which was weighted with a small quantity of shot in order to carry the sacks down into the solution and maintain them in proper position while being treated.

In treating the spores, especially in the formalin solutions it was found absolutely essential that only a minute quantity of spores be taken, otherwise they were apt to collect in masses, and in this way the solution did not readily permeate the whole mass. Whenever this occurred, in the shorter periods of treatment, spores taken from the interior of these masses would invariably show germination.

Formalin treatment.—The strength of formalin solutions used were .18, .25 and .5 per cent. The periods of treatment to which the spores were subjected in these solutions were one-quarter, one-half, one, and two hours, the four lots of spores being treated at the same time, each being successively removed without in any way disturbing the remaining ones. The treated spores were mounted as soon as possible after removal from

the formalin solution, and, after a microscopic examination, were placed in a moist chamber, the moist chamber being used in order to obviate the necessity of using vaseline to cement the cover slips to the glass cell. Better results seemed to be obtained when the culture had free access to moist air.

The data obtained from the formalin treatment which is given in Table I, shows that the quarter-hour treatment in the weaker solutions were apparently not effective. In the .18 per cent. solution with the quarter-hour treatment every culture made showed good germination, while in the .25 per cent. solution eight out of twelve showed germination in the quarter-hour treatment and one out of twelve in the half-hour.

TABLE I.

Germination Tests of Spores Treated With Formalin.

Strength of Solution.	Length of Treatment.	Number of Cultures.	Cultures Showing Germination.	Percentage of Same.
.18	¼ hour.	4	4	100
.18	½ hour.	4	0	.....
.18	1 hour.	4	0	.....
.18	2 hours.	2	0	.....
.25	¼ hour.	12	8	67
.25	½ hour.	12	1	8.3
.25	1 hour.	4	0	.....
.25	2 hours.	4	0	.....
.50	¼ hour.	3	0	.....
.50	½ hour.	3	0	.....
.50	1 hour.	3	0	.....
.50	2 hours.	3	0	.....
Not treated.	.....	10	10	100

The latter germination is probably accidental, owing to the fact that the half-hour treatment in the .18 per cent. solution showed no germination. Treatment in the .5 per cent. solution proved effective in all cases.

In order to note the action of the formalin upon the smut after their removal from the solution, cultures were made of the spores at different periods after their removal, varying from a quarter to one and a half hours. The data obtained, which is presented in Table II, shows conclusively that the formalin proved effective in the quarter-hour treatments if given sufficient time to act upon the spores before mounting them in the liquid media. Spores treated a quarter hour in the weakest solution and mounted one hour after showed no germination.

TABLE II.

Germination of Spores Treated 1/4 Hour in Formalin Solution, Mounted Some Time After.

Time elapsed after removal from Formalin Solution.	Strength of Solution.	Number of Cultures.	Cultures Showing Germination.	Percentage of Same.
1 hour.	.18	2	0	....
1½ hours.	.18	2	0	....
¼ hour.	.25	2	2	100
½ hour.	.25	4	2	50
¾ hour.	.25	2	0	....
1 hour.	.25	3	0	....

In the quarter per cent. solution the treatment was effective if the spores were not mounted for three-quarters of an hour after their removal from the formalin. It would appear, therefore, that under ordinary conditions of farm practice in which the seed is allowed to dry before being planted, treatment with either strength of solution should prove effective. In actual practice, however, such a treatment does not prove effective. This has been amply demonstrated by some experiments which were reported by Dr. Arthur in the Thirteenth Annual Report of the Indiana Experiment Station, p. 21, January, 1901, in which seed treated a half hour in a .45 per cent. solution of formalin at an average temperature of

124.5 degrees F., showed over one per cent. of smutted heads in the resultant crop.

Hot water treatment.—Only two periods of treatment were tried with hot water; these were for five and ten minutes. The range of temperature tried was from 130 degrees F. to 100 degrees F. The highest temperature used was considered the lowest point at which the treatment of wheat seed could be expected to prove effective, and it was therefore taken as the starting point in the work. As this temperature proved effective in killing the spores, a lower one was tried and so on until the lower limit of effectiveness was reached. The results of the work, which are presented in Table III, show that the lower limit of effective treatment was 110 degrees F. for five minutes and 105 degrees F. for ten minutes.

TABLE III.

Germination of Spores Treated With Hot Water.

Temperature of Water.	Length of Treatment.	Number of Cultures.	Cultures Showing Germination.	Percentage of Same.
130° F.	5 minutes.	2	0	....
130° F.	10 minutes.	2	0	....
125° F.	5 minutes.	2	0	....
125° F.	10 minutes.	2	0	....
120° F.	5 minutes.	4	0	....
120° F.	10 minutes.	4	0	....
115° F.	5 minutes.	4	0	....
115° F.	10 minutes.	4	0	....
110° F.	5 minutes.	6	0	...
110° F.	10 minutes.	6	0	....
105° F.	5 minutes.	8	4	50
105° F.	10 minutes.	6	0	....
100° F.	5 minutes.	2	2	100
100° F.	10 minutes.	4	4	100
Not treated.	.....	19	19	100

The unusually low temperature at which the viability of the spores were impaired seems all the more remarkable when we take into account the fact that a treatment of the seed wheat for ten minutes at a temperature of 130 degrees F. is not effective in removing all the smut from the ensuing crop. The results obtained from both the formalin and hot water treatments would seem to indicate that the spores are easily killed, in weak solutions of formalin and in comparatively low temperatures of water, when brought in direct contact with these agencies.

The lack of success in treating the seed for smut seems to be due to the inability of the agency used to reach all the smut spores. This is probably due to the fact that the seed coat is somewhat impervious to liquid solutions; hence, all spores that are held in the interstices of the seed coat are reached with difficulty, if at all. Assuming this explanation to be correct, it would appear that a different treatment should be accorded wheat than that advocated for oats. Some preliminary treatment should be given with the object of softening the seed coat, to such an extent as to permit of the ready action of whatever disinfecting agency it is desired to employ. With this idea in view a series of experiments were undertaken in which the seed, intended for treatment either with formalin or hot water, was given a preliminary soaking in water at about 70 degrees F. The length of time in which the seed was allowed to soak in water varied somewhat inversely to the time in which it was to be treated in formalin and hot water. For example, in the formalin treatments in which four lots of seed were treated, the first lot was soaked a half hour in the water and two hours in the formalin solution, whereas the fourth lot was soaked three hours in the water and only a quarter-hour in the formalin solution. In the hot water treatment, somewhat the same method was followed, except that a shorter period of treatment was given.

Germination tests were made of the treated seed in a Geneva germinator. The treatment of the seeds and the data obtained from the germinator tests which are presented in Tables IV and V, show that the formalin treatments injured the viability of the seeds somewhat more than that of the hot water. In neither case, however, was the seed appreciably injured.

TABLE IV.

Germination of Seed Wheat Soaked in Water, Then Treated With Formalin.

Soaked in Cold Water.	Treated in .18% Formalin.	Per Cent. of Germination in					Total Per Cent. Germination.
		1 day.	2 days.	3 days.	4 days.	5 days.	
½ hour.	2 hours.	35.5	74	82.5	86.—	87.—	87
1 hour.	1 hour.	22.5	67	80.5	85.5	....	85.5
2 hours.	½ hour.	36	76.5	84.5	87	88	88.—
3 hours.	¼ hour.	34	81	81	85.5	87.5	87.5
Untreated.	.....	0	36.5	72.5	81.5	95.—	95.—

TABLE V.

Germination of Seed Wheat Soaked in Water, Then Treated in Hot Water.

Soaked in Cold Water.	Treated in Water at 120° F.	Per Cent. of Germination in					Total Per Cent. Germination.
		1 day.	2 days.	3 days.	4 days.	5 days.	
1 hour.	½ hour.	10.5	64.5	92	....	93	93
2 hours.	¼ hour.	31.5	76	93.5	....	94	94
3 hours.	10 minutes.	45.5	81.5	91.5	....	..	91.5
4 hours.	10 minutes.	46	92	94.5	....	97	97.—
Untreated.	.....	....	....	....	....	..	95

The delayed germination of the untreated seed was due to the fact that it had not been soaked in water previous to putting it in the germinator, hence it took some time to absorb sufficient moisture for germination.

In the formalin treatment seed soaked three hours in water and then treated a quarter-hour in an .18 per cent. solution of formalin, was not materially injured, there being but 7.5 per cent. less germination than from the untreated. That soaked one hour in water and one hour in the formalin solution showed slightly more injury than any of the others.

For the hot water treatment a temperature of 120 degrees F. was chosen, on the supposition that though considerably lower than that used

in ordinary practice it was nevertheless sufficiently high to insure killing all spores with which it came in contact. Four lots of seed were treated, for periods varying from one to four hours in the cold water and from ten to thirty minutes in the hot water. The highest germination obtained was from seed which had been soaked four hours in cold water and ten minutes in the hot water.

SUMMARY.

A careful consideration of the evidence at hand would seem to indicate that in themselves smut spores are easily destroyed by either formalin or hot water treatments.

Owing to the somewhat impervious nature of the seed coats of wheat, and the not improbable fact that spores find lodgment in the interstices of them, it is difficult to reach and kill all the spores with any ordinary method of treatment.

To render the seed coats of wheat susceptible to such agencies as are commonly employed for the prevention of smut, it appears to be necessary, even imperative, that they should be soaked for some time in cold or tepid water prior to treatment.

A three hours' soaking in cold water and a quarter-hour treatment in an .18 per cent. formalin solution did not materially injure the viability of the seeds.

Seeds soaked four hours in cold water and then treated ten minutes in water at 120 degrees F. gave slightly better germination than the untreated seeds.

Some Additions to the Flora of Indiana.

William Stuart.

The accompanying list of flowering and fungous plants are some which have been collected by the writer during the past two seasons. In the flowering plants, out of a list of five, three are far removed from the range to which they are accredited.

In presenting the list it has been thought desirable to append a few notes under each species, giving the locality and soil in which they were collected, together with such other observations as might be deemed of interest.