THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Robert Jackson Noble final oral examination for the degree of Master of Science . We recommend that the degree of Master of Science be conferred upon the candidate.

Minneapolis, Minnesota

May 5 1922. E. E. Stedman

Chairman

E. M. Freeman

H K Hayes.

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Robert Jackson Noble for the degree of Master of Science. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science.

F. C. Stadtman
Chairman

E. M. Freeman

H. K. Hayes

Date May 5, 1922

STUDIES ON FLAG SMUT OF WHEAT

A thesis presented to the Faculty of the
Graduate School of the University of
Minnesota in partial fulfillment
of the requirements for the
Degree of Master of
Science

By

Robert J. Noble

May 1922

ACKNOWLEDGEMENT

The writer wishes to acknowledge his indebtedness to Professor E. C. Stakman of the Department of Plant Pathology, University of Minnesota, for suggestions and helpful criticism during these investigations.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

STUDIES ON FLAG SMUT OF WHEAT

SYMPTOMS

This disease has been reported only on wheat, where it forms characteristic leaden colored streaks between the veins on the leaf blades and leaf sheaths; the stems and even the glumes are sometimes affected, although less frequently. These streaks eventually become ruptured and then expose a black powdery mass of spores.

In the field, the effects of the disease are usually very destructive, as it causes stunting of the plants, distortion of affected parts, and practically always prevents the formation of the head.

HISTORY, DISTRIBUTION AND PREVALENCE

The disease was first reported from South Australia in 1868 (McAlpine, loc. cit.), although it is evident that it was of common occurrence in the wheat crops before this period. Flag smut has now been reported in Australia from Queensland, New South Wales, Victoria, and South Australia. There is as yet no record of it in western Australia or Tasmania.

It is particularly serious in South Australia, Victoria, and New South Wales, where, according to some estimates, it annually destroys about three percent of the crop. Cobb (5) reports that a ten percent loss is

12-21-6M

common, although fifty percent losses have been recorded in New South Wales. McAlpine (16) and Brittlebank (3) record losses up to seventy percent in Victoria.

Climatic conditions and cultural practices are closely correlated with the amount of damage caused by the disease in any particular locality in a given year; hence, although it is not possible to give an accurate estimate of the total losses due to flag smut in Australia, there is sufficient evidence to show that the disease is widespread in the wheat-growing states concerned, and that, under certain conditions which are as yet incompletely understood, it may reach the proportions of a more or less severe epidemic.

The disease was recorded by Hori in Japan in 1895 (McAlpine loc. cit.). Dr. Miyabe in a personal statement in 1921 remarked that the disease is still responsible for some considerable loss in that country.

Sydow and Butler (25) record it at Lyallpur, India, in 1906.

Butler (4), in 1918, reports that the disease is confined to the province of the Punjab in India; and in 1921, in a personal statement, he mentioned that the disease has not yet been reported as causing serious damage in that country, and that it is still restricted to the northwestern portion of the wheat area.

Flag smut is said to occur also in southern Europe (Reed and Dungan loc. cit.) .

The disease was found in the United States for the first time in May 1919, Humphrey and Johnson (11). It was found in a number of fields in the vicinity of Granite City, Madison County, Illinois. In 1920 a survey was made of wheat fields in the vicinity of Granite City and the disease was found over an area of approximately forty-seven square miles.

The degree of infection ranged from a trace to twenty-five percent, although in most fields infection was not severe.

In 1921 infected plants were found both in Madison and St. Claire Counties, Illinois, over an area of approximately 53 square miles. The degree of total infection was lower, fifteen percent being reported as the highest infection in any field.

Putterill (21) records the disease from the Marico district of the Transvaal, South Africa, in 1920. He states that the disease has probably been present for a number of years, and that, although the total loss is not great, fifty percent of the plants are infected in some fields.

ECONOMIC IMPORTANCE

In 1891 Cobb (5) stated that the disease was a serious plague, and that probably in no other part of the world did it cause such serious losses as it did throughout the wheat-growing areas of Australia. Apparently this is still the condition today.

Brittlebank (3) in 1920 states that the disease has increased to an alarming extent in recent years throughout the wheat-growing areas of Victoria, Australia. He remarks that it is one of those insidious diseases which cause loss throughout the growing season; and that often where its presence has hitherto been unsuspected, weather and other conditions are held responsible for the damage. In a comparison with the damage caused by black stem rust of wheat, he states that since the latter disease in certain years, under favorable weather conditions, may ruin a crop in a few days, many have considered it as the most serious disease of cereals in Australia; and but that flag smut is annually taking a toll of from five to

seventy percent of the crop, so that the total annual loss from rust sinks into insignificance when compared to that resulting from flag smut.

ETIOLOGY

Causal Organism

Flag smut of wheat is caused by Urocystis tritici Koern. It was first designated by Wolff (29) in 1873 as Urocystis occulta (Wallr) Rab., and the early records of the disease refer to it under this name. Koernicke (15) in 1877 first distinguished it as a separate species. The morphological and biological differences between the organisms causing flag smut of rye and flag smut of wheat were again noted by McAlpine (16) in 1910.

Spores

(1) Morphology

The spores occur either singly or in spore balls. The spore balls are dark brown in color, rather variable in shape, most frequently globose, occasionally irregularly oblong; dimensions 18 μ. - 52 μ. long x 18 μ - 45 μ broad. In a statistical examination of one hundred spores, the length falls within the limits 31 μ ± 5.4 and breadth 23 μ ± 4.7 . The spores themselves are spherical or oval in outline and vary within the limits 12 - 16 μ x 9 - 12 μ .

The spores are completely invested with a layer of sterile peripheral cells, pale brown in color, globose or ellipsoid in shape, dimensions 7 - 10 μ x 5 - 9 μ . Cf. Figs. III, IV and V.

(2) Germination

Upon germination each spore of the spore ball may produce a promycelium. Frequent splitting of the spore wall has been observed (cf. Fig. I, a,c,d, and f), although this was not noted in all cases. Under normal conditions

the promycelium usually attains dimensions of about 20 - 30 μ x 5 μ, although in one instance the promycelium grew to a length of 70 μ before forming sporidia (cf. Fig. I k and Fig. II o); then at the tip there are formed a number of small protuberances (cf. Fig. I 6, Figs. III and IV), which elongate to form a whorl of from one to six sporidia, two to four being most frequently found. The sporidia are somewhat cylinirical in shape and, when fully formed, are usually about 30 x 5 μ in size. At first the young sporidia remain aggregated at the tip of the promycelium, but, as growth proceeds, they gradually diverge to form a series of finger-like protuberances. Further growth most frequently is manifested by a prolongation of the sporidium while still attached to the promycelium (cf. Figs. I j and 1). In some cases additional "buds" may be formed at the tip of the promycelium at the base of the whorl of sporidia (cf. Figs. I c, g, h); and these may be prolonged into "germ tubes" before any are produced by the first formed sporidia (cf. Fig. I 1).

These prolongations, which may be considered as germ tubes or infection threads, have been observed to grow to a length of about 900 μ. They are fairly constantly about 2 - 3 μ in width. The protoplasm becomes concentrated at the tips, while, at the same time, the remainder of the mycelium may become multicellular (cf. Fig. I j and II o). When the septa are formed, however, the cells are devoid of protoplasm.

Fusion of sporidia was noted several times (cf. Fig. I d, e, f, and n); and the germ tubes also occasionally fused (cf. Fig. I j). It has not yet been possible to ascertain the behavior of the nuclei when fusions occur.

Under some conditions of germination other irregularities were observed. Occasionally no sporidia were developed, but the promycelium

developed into a "germ tube" (cf. Figs. I m and II g and u). In one case a secondary "promycelium" was produced instead of a sporidium. This grew to a length of about 60 μ and then produced a number of protuberances which may be considered as sporidia. (cf. Fig. II p.)

Sometimes, particularly when spores germinated at high temperatures, promycelia were formed but no normal sporidia were produced; the mycelium merely branched in an irregular manner (cf. Fig. II s and t). Several times a portion of the protoplasm was cut off in an offshoot from the promycelium (cf. Fig. I g and h).

Thus it is seen that the character of the germination of the spores of Urocystis tritici may vary considerably. It has been noted also that under certain circumstances sporidia may be formed readily in the depths of a nutrient medium; the sporidia, therefore, do not seem to be such definite morphologic structual units as those which are formed by Tilletia sp.

Inoculation and Infection

McAlpine (16) records the results of inoculation experiments in Australia during 1906 and 1907, in which he has shown that infection may occur as a result of spores adhering to the seed or as a result of planting clean seed in infected soil. The actual mode of infection has not yet been observed histologically, but it seems most probable that the germ tube of the fungus penetrates the plumule of the seedling in the earliest stages of growth.

Some pot experiments have been carried out with a view of determining the limits of seedling growth within which infection is possible. Seed and seedlings of several Australian varieties of wheat were inoculated, and the results are recorded in Table I. When this test was carried out, it

was not possible to use other than ungerminated spores; neither was there sufficient material to carry out a test in infected soil.

The results would indicate, however, that infection takes place only when the wheat seedlings are very young. It is hoped that further information on this aspect of the question will be derived from experiments now in progress.

Under the conditions of the experiment, the first lesions appeared in from two and one-half to three months after inoculation. McAlpine (16) records infection in forty days in pot experiments, but, under field conditions, a much longer period may be required, although here much depends upon the normal growth period of the variety affected.

Table I

Effect of stage of growth of wheat seedlings on relation to infection by Urocystis tritici

Pot No.	No. of Individual	Variety	Age of Seedling	Stage of Growth	Date Planted 1921	Date of Appearance of lesions	Degree of Infection
Inoculum of dry spores applied at planting							
16	6	Bathurst 17		Dry seed	Oct. 6	Check	Nil
17	6	do		do	do	do	do
18	5	do		do	do	Jan. 4, 1922	1 plant
25	3	do		do	do	do	do
19	6	do	2 days	Plumules 3 mm. long	do	Jan. 4, Feb. 6	2 plants
20	6	do	3 days	Plumules 7 mm. long	Oct. 7		Nil
21	4	do	7 days	Plumules 4-5 cm. long	Oct. 11		do
22	5	do	10 days	1 cm. of green leaf showing	Oct. 14		do
26	6	Federation		Dry seed	Oct. 11		do
31	6	do		do	do		do
32	4	do	2 days	Plumule 4 mm. long	do		do
34	4	do	do	do	do	Jan. 4, and 28	3 plants
37	5	do	do	do	do	Jan. 4	1 plant
38	3	do	4 days	Plumule 1 cm. long	Oct. 3		Nil
39	4	do	do	do	do		do
15	4	do		Dry seed	Oct. 11	Check	do
51	7	Marshalls#3		do	Nov. 16	do	do
52	4	do		do	do		do
53	5	do		do	do	Feb. 6 and 12	2 plants
54	4	do		do	do	Check	Nil
55	6	do	4 days	Plumule 1 cm. long	Nov. 20		do
56	5	do	7 days	3/4 to 1 cm. of green leaf showing	Nov. 23		do

EPIDEMIOLOGY

It has been shown by MacAlpine (16) that infection of the crop may result from spores already in the soil. There is evidence to the same effect in the Illinois area, which would indicate that the spores may overwinter in the soil and infect the crop sown in the following year.

All the field evidence on the disease, under Australian conditions, confirms the view that spores in the soil constitute the main source of infection each year. In a recent report from Australia, Mr. R. G. May, Manager of the Bathurst Experiment Farm, states that clean seed of Clevelani wheat sown on an area of thirty acres at the Farm in 1921, produced a crop in which from twenty to twenty-five percent of the plants were affected with flag smut. This land was not cropped in 1920, but had borne a crop in 1919 in which the amount of flag smut present was estimated at from ten to fifteen percent. Seed from the same source, sown at the same time on another portion of the farm which had been under cultivation for only a few years, produced a crop which was free from infection.

It is generally conceded, in cases of soil infestation by smut organisms, that there is a fairly close correlation between the amount of smut in the crop and the amount of moisture present in the soil during the period prior to sowing and at germination.

McAlpine (16) reports that sowings made in dry, infested soil resulted in fourteen percent infection when germination of seed of wheat and the fungus spores occurred simultaneously, immediately following the first rains. When sowings were made in similar adjacent plots one month after rain, only one percent of the plants became infected.

Heald and Woolman (9) and Heald and George (10) have shown that soil moisture also influences the amount of infection by bunt or stinking smut of wheat -- Tilletia tritici (Bjerk) Wint. -- in the Palouse district in Washington. Under the prevailing conditions in that region, there is an almost constant shower of spores onto the soil during the latter part of August and September. Very little rain, as a rule, falls during this period, and early sown crops are invariably heavily affected with bunt. If sowing is delayed until four or five weeks after the first good, fall rains, the amount of infection is very much reduced.

It would appear that, under some circumstances, the spores of Urocystis tritici may retain their viability even in the presence of a considerable amount of soil moisture. A single pot containing spore material was placed in the greenhouse at this Station on October 11, 1921. It was watered frequently and sown with pickled seed of Hard Federation Wheat on November 15, 1921. Smut was observed on two of the six resulting plants on the 4th and 27th of January, 1922, respectively. The plants grown under similar conditions in dry soil did not become infected.

Laboratory tests made at this period indicated that the spores might require a considerable length of time for germination, ranging from four to sixteen days, and also that only very few spores appeared to be viable. These tests, however, cannot be considered conclusive since a high percentage of the spores from the same material subsequently germinated under favorable environmental conditions.

It has been observed that there are marked differences in the degree of varietal resistance of wheats to this disease, both in the United States and in Australia. Most of the main commercial varieties grown in Australia are, however, very susceptible.

Since it has been shown that outbreaks of the disease may be traced in practically every case to the presence of viable spores in the soil, and since control is not possible by present known methods of seed treatment, it is essential that we should have a more complete knowledge of the reaction of the pathogen to its environment, for the factors favoring germination and subsequent growth of the fungus must necessarily be related closely to those responsible for the development of the disease.

Hence, it becomes desirable as a first consideration that studies be directed toward a determination of the behavior of the spores under controlled conditions.

Spore Germination Studies

(a) Introduction

For several years McAlpine (16) made observations on the germination of spores of Urocystis tritici. He used tap water in his tests, and records germination which varied from a "small proportion" in material one month old, to forty percent in material which was apparently four or five months old.

Early studies made by the writer indicated that the spore germination was very capricious. Material from Australia and from the United States was used in a number of preliminary tests, but, under a series of varied conditions in trials extending over periods which ranged from several days to several weeks, only a few spores germinated. Usually only one spore in several thousand germinated; and frequently all of the spores failed to germinate.

It is well known that, as a general rule, many smut spores will

germinate only after a rest period. In a review of spore germination studies on Ustilago zeae, Stakman (24) reports that neither Kühn nor Brefeld (loc. cit) were able to secure germination of fresh spores, but that Brefeld secured germination in nutrient media, although the spores did not germinate in water until the following spring. Stakman found also that, with the exception of one spore lot, a rest period was required before the spores would germinate.

In a review of germination studies on Tilletia levis Kühn and Tilletia tritici (Bjerk.) Wint., the same investigator reports the uncertain and capricious germination observed by Prevost, DeCandolle, Tulasne, Kühn, Fisher von Waldheim, and Brefeld (loc. cit) .

Stakman (24) reports also that he was rarely able to secure germination of fresh spores, although he was successful in one instance (20% being recorded), and then only when distilled water was used; but that after the spores had passed through a rest period of about eight months duration, subsequent tests resulted in almost one hundred percent germination.

McAlpine's work already noted has shown that the age of the spore is a possible factor in relation to amount of germination which might be expected during the period following their formation.

Potter (19) speaks of the difficulty experienced in attempting to germinate spores of Sorosporium reilianum (Kuehn) McAlp. In repeated tests at different seasons and at various times of the year, he noted only slight and irregular germination, and states that rarely did more than fifteen percent of the spores germinate.

Prillieux (20) reports that the spores of Urocystis occulta germinate "easily" at the end of two orthree days. Few workers, however, have made accurate counts of the percentage of spores which germinate, and it is

generally agreed that, in the absence of complete knowledge of the factors favoring germination, great difficulty may be experienced in securing even slight germination.

(b) Description of Material and Methods

Flag smut spore material from the 1919 crop in Australia was first used, but, except where otherwise stated, the spores used in the following series of tests were obtained from the wheat varieties "Bobs" and "Federation", grown in the United States Department of Agriculture greenhouse, Arlington, Virginia, and collected in March 1921. When the results recorded below were obtained, this material was at least six months old; and the spores still germinated normally when they were fourteen months old.

It was found that most uniform results could be obtained by the use of Syracuse dishes. The spores were sown on the surface of 2 to 3 cc. of liquid contained in the dish. Diseased leaf tissue was broken into fragments about 5 mm. in length, and the spores were shaken out through the exposed ends of the unopened sori, and were therefore practically uncontaminated. This method resulted in saving of time and made it possible to use material from the same source in any series of tests.

(c) Nutrient solutions

The following substrata were used in this series of tests: distilled water (Barnstead still material), tap water, Cohn's solution (23), Cohn's modified solution (14), soil extract solution, and manure extract solution; plant decoctions; butyric, citric, and malic acids in separate series in concentrations ranging from 1 to .0001 percent, asparagin in .5 percent to .00125 percent solutions; potassium chloride, ammonium sulphate, ammonium tartrate and potassium di hydrogen phosphate each in 1 percent

solutions; caustic potash and sulphuric acid each in $\frac{N}{5}$ to $\frac{N}{1000}$ concentrations; saccharose in 1, 2 and 5 percent solutions; dextrose and levulose, each in a series of concentrations ranging from 1 to .0025 percent.

The various series were kept at a range of temperatures, but in practically every case only negative results were obtained. Germination in distilled water occurred, if at all, at about the tenth or twelfth day at room temperature (19° - 23° C). In many cases, no germination was noted, in others less than 1 percent, although in one case 20 percent of the spores germinated.

In soil extract, 10 to 20 percent of the spores germinated at 20° C five to eight days after sowing. Spores placed on moistened soil and floated off for examination did not germinate better than those which were in constant contact with the soil extract solution.

In the saccharose solutions less than 1 percent of the spores germinated after eight days. In 1 percent glucose, 10 percent of the spores germinated after thirteen days, trace germination appearing only in the .05 percent solution. Only a few spores germinated after seven days in 1 percent levulose, but in thirteen days fifteen percent had germinated. As a general rule, however, most of the spores germinated simultaneously, the process not being extended over a period of days.

In one hundredth normal potash, 3 percent of the spores germinated seven days after sowing; very few spores germinated in the lower concentrations and none in the higher.

It is of interest to note that the spores did not germinate readily when sown in wheat plant extracts, or when plant tissue was added to the dishes of distilled water simultaneously with the sowing of the spores, for as a general rule less than .1 percent of the spores germinated under these conditions.

(c) Action of Stimulatory Substances

Since it appeared that constant association of the spores with dissolved nutrients was on the whole apparently as ineffective as distilled water alone in inducing germination, a series of tests were devised to determine if any specific substance or method of treatment might act as a stimulus to germination.

Reference already has been made to the fact that the age of the spore is undoubtedly one of the factors in determining how well the spores will germinate at any given time. There are no data available as to the actual length of time which must elapse under any given set of circumstances before spores of the type of Urocystis tritici may be considered fully mature. As a general rule, however, a longer or shorter period of maturation is necessary, but it is considered from subsequent work that the spores used in these experiments were in all probability sufficiently mature to germinate strongly several months after formation, since, when they were twelve months old, they still germinated slowly and vicariously; while as a result of improved technique, from 80 to 95 percent of the spores germinated.

It is well known that the teliospores of Puccinia graminis will not germinate immediately after formation, but that under normal circumstances and after having passed through a rest period they will germinate fairly readily in the spring.

Exposure to low temperatures (0° - 5° C) for periods up to several weeks did not cause increased germination of spores of Urocystis tritici in distilled water.

In experiments designed to hasten the after ripening process in teliospores of Puccinia graminis, Thiel and Weiss (26) secured germination of the spores after preliminary treatment with dilute citric acid, several

months before germination could be obtained by ordinary methods. They report the work of Crocker, Eckerson, Denny, and others on after ripening in seeds and permeability of seed coats, in which it has been shown that germination of seeds may be delayed until certain definite physiological changes have taken place in the embryo, or, in other cases, mainly because of impermeability of the seed coats to water and to oxygen.

Thiel and Weiss state that the stimulus to germination of the teliospores apparently was not the result of increased permeability of the spore wall, but rather that the citric acid appeared to function as a specific activator of the protoplasm.

Spores of Urocystis tritici were subjected to somewhat similar treatment with dilute mineral and organic acids, but these substances did not stimulate germination.

Duggar (6) reports a number of substances which have a stimulatory effect on the germination of spores of Aspergillus flavus. These include ethyl alcohol, vaseline, phenol, organic acids, mineral acids, and salts of heavy metals.

Melhus and Durrell (18) report the stimulating effect of vaseline and of paraffin oil on the germination of urediniospores of Puccinia coronata Cda.

Spores of Urocystis tritici sown on distilled water germinated but slightly better than the checks when a slight trace of paraffin oil was added.

Anderson (1) has shown that the germination of pycnospores of Endothia parasitica is slight and uncertain when sown on tap water, rain water, or distilled water, but that they germinate readily in a decoction of chestnut bark. The action of chestnut bark was not specific, for decoctions of material from trees belonging to other genera, and various nutrient agars

produced the same effect.

Whitehead (27) reports that spores of Urocystis cepulae germinate slowly when sown in water, but they germinate much more rapidly and vigorously when they are sown in onion juice.

In studies on the longevity of spores, Duggar (6) has shown that the spores of Aspergillus flavus and Sterigmatocystis nigra would not germinate on the surface of distilled water, but when they were transferred to a decoction of beans they germinated readily. Spores of this kind, moreover, are known to germinate readily on a wide range of nutrient media.

Although the first tests with the spores of Urocystis tritici indicated that only slight germination could be secured by sowing in decoctions of green wheat plant material, and since it had been observed that a comparatively long period of time was necessary for the spores to germinate, it was thought that the protoplasm of the spore might become more responsive to some stimulus after a period of presoaking in water.

Spores were then sown on distilled water in four Syracuse dishes and kept in the laboratory for five days at a temperature range of from 18° to 23° C. None of the spores germinated during this period of time. On the fifth day, eight razor sections (about 20 μ thick) were cut from the young growing tissue of a wheat plant (var. Federation) and were then added to two of the dishes, the other two remaining untreated. Eighteen hours later the spores had germinated profusely in the dishes to which the tissue had been added, while no germination had occurred in the checks. (Cf. Table II)

Although in this instance approximately fifteen percent of the untreated spores germinated ten days after sowing, in many subsequent trials spores sown on distilled water showed only a trace germination -- less than one percent -- after periods ranging up to several weeks. Tests were devised

in order to determine at what period the addition of plant material might be most effective in stimulating germination. As no results obtained hitherto had indicated any optimum range of temperature for germination, these tests were continued at room temperature (18° - 23° C).

Table II

The effect of young wheat tissue on the germination of spores in distilled water

Dish No.	Date Sown	Date on which tissue was added	Percentage of Germination				
			Feb.20	Feb.21	Feb.23	Feb.24	Feb.25
55	Feb.15	Feb.20	0	over 50			
55a	do	do	0	do			
56	do	Check	0	0	0	0	17
56a	do	do	0	0	0	0	15

Percentages based on number of spores or spore balls which had germinated.

From the results shown in Table III it is seen that the stimulatory effect of plant material is much more potent when it is added to spores which have been presoaked for three or more days than when it is added simultaneously with them.

Some of the differences noted in the percentage of germination recorded after the third day are possibly due to differences in the amount of tissue added and to the degree of maturity of such tissue, although every effort was made to maintain uniform conditions in each case. This test was repeated and the same general results were obtained.

Spores sown on the surface of distilled water did not germinate

Table III

The effect on germination, of adding wheat tissue to distilled water in which spores had been presoaked for different periods of time.

Dish No.	Date on which spores were sown	Date on which tissue was added	Percentage of Germination						
			Feb.27	Feb.28	Mar.1	Mar.2	Mar.5	Mar.7	Mar.8
80	Feb. 24	Check	0	0	0	0	0	trace	
81	do	do	0	0	0	0	0	0	trace
82	do	Feb. 24	0	trace					
83	do	do	0	0	1				
84	do	Feb. 25	trace	2	2				
86	do	Feb. 27	0	30					
87	do	do	0	20					
88	do	Feb. 28	0	0	90				
89	do	do	0	0	85				
85	do	Mar. 1	0	0	0	65			
91	do	Mar. 4	0	0	0	0	70		

in the untreated dishes until ten days after sowing; in some instances germination was delayed until fifteen days had elapsed, and then less than one percent of the spores had germinated. Tissue added with the spores was either totally ineffective or much less effective than when applied two or more days later.

The spores remained in a receptive condition for at least twenty-eight days after sowing, although in this case the addition of tissue resulted in approximately 2 percent germination; while during the intervening period, at least from the third to the fifteenth day, from 70 to 80 percent of the spores germinated at from 23° to 25° C, twelve hours after the addition of plant tissue.

In subsequent tests, it was found that tissue derived from the plumules of young wheat seedlings was on the whole even more effective than the young growing tissue derived from older plants, although tissue derived from the actively growing portions of the plant invariably stimulated germination. It was found that all portions of the young growing seedling were equally effective in stimulating germination of presoaked spores. Spores sown on the surface of distilled water had not germinated after six days; sections of rootlets were then added to one series, and sections of plumules to another series, three dishes being used in each case. After incubation for twelve hours at 25°C, from 90 to 95 percent of the spores had germinated in each case. No spores germinated in the untreated dishes.

It was found also that the addition of germinated seed to dishes of distilled water containing presoaked spores stimulated germination almost as effectively as when sections of living tissue were added. Very young seedlings of Red Cross Wheat were added to Syracuse dishes containing distilled water and presoaked spores in the manner shown in Fig. VI. After

incubation for twelve hours at 20° C, very strong germination was always noted. As a general rule, germination did not take place as rapidly as when cut portions of seedling tissue were added, but in the cases observed (3 separate trials involving 2 treated and 2 untreated dishes each time) at least seventy percent of the spore balls had germinated with the production of sporidia in from 12 to 18 hours at 20°C. No germination occurred in the checks at any time during the tests.

It is not known whether the stimulus came from any particular part of the plant, or whether it was derived originally from the seed. Hurd (13) has shown that seed coat injury commonly occurs on grain which has been threshed by ordinary methods, so that in these tests there is a possibility that the stimulatory substance may have diffused through the seed coat. The only seedlings used in the experiment were those which had apparently germinated in a normal manner; they were then transferred so as not to injure the plumule or roots.

It is considered rather significant, however, that this stimulation was noted constantly under the conditions of the test. No tests have yet been made with varieties of wheat which are known to be resistant to flag smut, but these will be made as soon as such material becomes available. It would appear that stimulation might be expected in this case also, since it has been shown that the stimulus is not specific for wheat plant tissue.

In order to determine if this action was specific for wheat tissue, hand sections of tissue from other cereals and other plants were added to presoaked spores; results are given in Tables IV and V. The plant tissue was derived in each case from the growing portions of young plants. The spores were sown as before on the surface of distilled water in Syracuse dishes which were kept at room temperature (19°- 23° C).

Table IV

The effect of various cereal plant tissues on the germination of spores presoaked in distilled water.

Dish No.	Kind of tissue added	Percentage of Germination			
		Mar. 6	Mar. 7	Mar. 8	Mar. 9
44	None	0	0	0	60
45	do	0	0	0	15
46	Wheat	0	60		
47	do	0	80		
48	Barley	0	Light trace	trace	30
49	do	0	trace	do	25
50	Oats	0	0	0	40
51	do	0	0	0	30
52	Rye	0	36		
53	do	0	39		

Spores sown March 2

Tissue added March 6

Varieties used - Wheat Little Club

Barley Manchuria

Oats White Russian

Rye Winter

Table V

The effect of various plant tissues, other than wheat, on the germination of spores presoaked in distilled water.

Dish No.	Kind of tissue added	Percentage of Germination				
		Mar. 7	Mar. 8	Mar. 9	Mar. 10	Mar. 11
58	None	0	0	0	0	trace
59	Triticum vulgare	0	80			
60	Agropyron tenerum	0	30			
61	Agropyron repens	0	10			
62	Lolium subulatum	0	10			
63	Elymus canadensis	0	13			
64	Phleum pratense	0	20			
65	Linum usitatissimum	0	20			

Spores sown March 2.

Tissue added March 7.

The percentages of germination recorded in Tables IV and V cannot of course be considered entirely significant, although every effort was made to add the same quantity of tissue in each instance. It is of interest, however, to note in Table IV that, of the cereals, only wheat and rye showed an immediate effect which can be definitely attributed to some stimulatory action of the plant tissue. There is evidence that barley was slightly effective, although the trace of germination noted means that less than one spore per thousand germinated. In the other cases, germination occurred simultaneously with that in the check dishes.

In Table V, the greatest stimulatory effect noted is again that which is caused by addition of wheat tissue, but from the fact that germination apparently also was stimulated by other grasses and by flax tissue, it would appear that the stimulatory substance is not specific.

Tests were then carried out to determine whether the stimulatory substance was contained only in living tissues or whether it might also be obtained in wheat plant infusions which had been autoclaved. Tissue from the plumules of wheat seedlings was used to make up approximately one percent decoctions in distilled water. These were autoclaved for thirty minutes at fifteen pounds pressure, and the presoaked spores subsequently transferred to the extract thus obtained.

Vigorous germination was noted after incubation for twelve hours at 27° C. (Cf. Table VI)

This test was repeated several times and on each occasion the same results were obtained. It would thus appear that stimulation is not necessarily due to the action of enzymes present in the plant tissue.

Since practically negative results had been obtained when dry spores were sown on distilled water containing portions of plant tissue,

Table VI

The effect on germination of transferring prescaked spores to infusions of wheat plant tissue

Dish No.	Medium	Percentage of Germination		
		Apr. 7	Apr. 8	Apr. 10
83	Dist. water	0	0	0
84	do	0	0	0
87	Autoclaved infusion	0	80	
88	do	0	85	
89	Dist. water & fresh tissue	0	90	
90	do	0	90	

Spores sown on distilled water April 4

Spores transferred April 7

a test was then made in which dry spores were added to portions of the same infusion in which prescaked spores had germinated readily. With one exception, almost entirely negative results are noted again, as shown in Table VII.

It is known from temperature studies which were made simultaneously with these tests, that 25° C is very close to the optimum temperature for germination of spores which had been prescaked at lower temperatures, but it will be observed from Table VII that practically no germination occurred at this temperature when spores were sown in the plant infusion.

There is a possibility that incubation of the spores for long periods (i.e., over 12 hours) at 25° in the presence of moisture, changes

Table VII

The effect on germination of sowing dry spores on or in infusions of wheat plant tissue

Dish No.	Medium	Method of sowing	Temp.	Percentage of Germination					
				Apr.12	Apr.13	Apr.14	Apr.15	Apr.17	Apr.19
12	Infusion	Surface	25°C	0	0	0	0	0	0
13	do	do	do	0	0	0	0	0	0
18	do	Suspension	do	0	0	trace			
19	do	do	do	0	0	0	0	0	0
17	Dist.water	Surface	do	0	0	0	0	0	0
14	Infusion	do	19°-22°C	0	0	trace			
15	do	do	do	0	0	do			
20	do	Suspension	do	0	50				
16	Dist.water	Surface	do	0	0	0	0	0	0

Infusion made up on April 10

Spores sown April 10

(Compare with dish 87 and 88, Table VI.)

the character of the protoplasmic spore contents, for spores presoaked at this temperature were less responsive to the stimulus given by the addition of plant tissue than those soaked at room temperature range 19° - 22° C. (Cf. Table VIII)

Invariably from seventy to ninety percent of the spores which had been presoaked at lower temperatures for at least three to eight days germinated on the addition of the necessary stimulus. There is, however, the possibility also that this stimulatory substance is either volatile or somewhat unstable. This might perhaps more readily explain the fact that germination was so sparse when spores of Urocystis tritici and portions of wheat plant tissue were added simultaneously to distilled water, and it may explain also why germination was not more readily obtained when spores were sown in the infusions as shown in Table VII.

Working on this hypothesis, a test was made to determine if the stimulatory substance could be distilled from an infusion of young wheat plant tissue in water. Entirely satisfactory results were obtained, as shown in Table IX.

Plumules and roots of about fifty seedlings of Red Cross wheat were macerated in about 200 cc. of distilled water; after standing for five hours, this material was boiled and the distillate collected. Although this distillate is undoubtedly still a complex mixture, the results indicate that the stimulatory action of the plant tissue may yet prove to be due to the presence of some substance, or group of substances, of the nature of aldehydes, alcohols, or fatty acids.

The nature of the stimulus is still a matter for investigation, but since it is now possible to obtain strong spore germination at will,

Table VIII

Effect of the addition of tissue from growing wheat seedlings, on germination of spores sown on distilled water at 25° C.

Dish No.	Date on which tissue was added	Percentage of Germination					
		Apr.12	Apr.13	Apr.14	Apr.15	Apr.17	Apr.19
21	Apr. 11	0	0	trace			
22	do	0	0	do			
23	Apr. 12	0	0	do			
24	Apr. 13	0	0	0	trace		
25	Apr. 14	0	0	0	0	40	
26	do	0	0	0	0	30	
27	Apr. 18	0	0	0	0	0	10
28	do	0	0	0	0	0	8

Spores sown April 11

Table IX

The effect of distillate of wheat seedling infusion on germination of prescaked spores of Urocystis tritici

Dish No.	Medium	Percentage of Germination		
		Apr. 14	Apr.15	Apr.19
32	Distillate	0	90	
33	do	0	88	
34	do	0	92	
36	Residue	0	85	
37	do	0	85	
27	Dist.water and fresh tissue	0	95	
28	do	0	93	
A	Dist. water	0	0	0
B	do	0	0	0

Spores sown April 10

Spores transferred April 14

Counts made with material in sporidial stage, after 12 hours incubation at 25° C.

it has become possible to carry out further studies on the physiology of the organism.

(e) Temperature Studies

Fawcett (7) has pointed out that the cardinal temperatures of an organism may vary considerably, according to the conditions under which the determinations are made.

In studies of the temperature relations of Urocystis tritici, it was found that the nature of the treatment to which spores had been subjected had a considerable influence on their subsequent behavior at germination. In preliminary tests it was noted that after presoaking the spores for approximately three days at room temperatures, strong germination could always be secured on the addition of wheat plant tissue and subsequent incubation for twelve to eighteen hours at 25° or 27½° C. (over seventy percent germination, with sporidial formation well advanced . Cf. Figs. III and IV). It was found, however, that spores which were incubated at 27.5°C in the presence of moisture for at least thirty-six hours, failed to react to the stimulus normally noted on the addition of wheat plant tissue. (Cf.Table X)

This test at least confirms the fact already noted in Tables VII and VIII that the spores themselves become so altered by prolongeed exposure to moist heat at temperatures of 25° or above that they become less responsive to the stimulus of plant tissue.

The earlier studies on temperature relationships were not entirely satisfactory, since portions of plant tissue were used in order to test capacity for germination within each series, and there was thus the possibility that the results may have been vitiated because of lack of uniformity in the tissue used.

In one series the spores were sown on distilled water and incubated

Table X

The effect on germination of presoaked spores, of incubation for varying periods at 27½° C prior to the addition of wheat plant tissue

Dish No.	No. of hours at 27½° C	Percentage of Germination at 20° C
76 C	3	80
76 D	6	72
76 E	17	21
76 F	21	25
26	24	20
27	30	trace
28	26	0
76 G	41	0
29	45	0
66	Check*	90
30	do	80

Spores presoaked for 5 days at 20° C.

* Checks at 20° C throughout. Tissue added to all dishes simultaneously. Dishes removed to 20° C after incubation at 27½° C.

for three days at temperatures which ranged from 7° to 27° C. On the addition of portions of wheat plant tissue, it was found that germination commenced first at 18° C, then it occurred successively at 22°, 12° and 7° C, none being noted at 27° C.

As previously mentioned, wheat plant decoctions cannot be used over long periods of time in a test such as this, for radical changes apparently take place in the medium, particularly at the higher temperatures, so that there is again the possibility of lack of uniformity in the substrate by the time that the spores are ready to germinate.

More extensive experiments are now in progress in which the spores are being continuously incubated at fixed temperatures in the presence of moisture, and are being tested for germination at fixed intervals, by transference to "wheat plant decoction distillate" at the same temperatures. It is thought that by the use of more concentrated solutions, changes in the substrate may possibly be less complete within a given space of time and that more comparable results may yet be obtained.

Even with the use of fresh plant tissue, it was found that there was very little difference in the percentage of germination noted after continuous incubation within a temperature range of 19° - 24° C, but that the growth of germ tubes was a little more rapid at the higher temperatures.

In a number of tests involving the use of spores which had been presoaked at room temperature range (19° - 22° C), it was found that a few spores presoaked for six to eight days germinated even at 29° C; and in one case, a few abnormal promycelia were formed at 32° C, but none of the spores germinated at these temperatures when spores were presoaked for only three or four days.

With reference to low temperatures, it was found that germination

might occur at 5° C, although this test involved the use of ungerminated spores which had been presoaked for six days at from 18° to 22° C. (Cf. Table XI.) In one test it was found that the sporidia grew slowly even at 0° C. A dish of spores in the earliest stages of germination was placed in a water tight container and then totally immersed in a mixture of ice and water. The sporidia had germinated after immersion for twenty-four hours.

In a temperature range test with presoaked spores, it was found that the maximum growth of germ tubes took place at a temperature of 24° C, although there was very little difference in the percentage of germination throughout the range, 18° to 27° C. Under these conditions, the maximum temperature is apparently just below 32° C; and the minimum temperature is between 0° and 5° C. (Cf. Table XI)

It will be observed that spores germinated vigorously at 27° C; but, from previous tests, it is known that constant incubation at temperatures above 25° C is extremely unfavorable to spore germination. From the practical standpoint, however, it is interesting to note that germination may occur within a comparatively wide range of temperatures, but that the previous history of the spores immediately prior to germination has a considerable influence on their subsequent behavior.

It thus appears that spores which are incubated at fixed temperatures have lower optimum and maximum temperatures of germination than those which are noted for spores which have been presoaked at low temperatures, before exposure to a range of temperatures.

Under field conditions the spores are subject to a constantly changing environment, and it is apparent that their behavior under such conditions must vary considerably from that which might be assumed from laboratory tests

Table XI

Temperature relations in germination of spores of Urocystis tritici

Dish No.	Temp.	Percentage of Germination Apr.10 11am	Apr.11 9pm	Apr.11	Apr.13	
98	5° C	0	0	0	80	Promycelia in early stages of growth
99	5°	0	0	0	75	do
100	13°	0	0	98		do
1	13°	0	0	95		do
2	18°	0	90			Average length of promycelia and sporidia 30 μ
3	18°	0	92			do
4	20°	0	90			do 40 μ
5	20°	0	95			do
6	24°	0	85			do 105 μ
7	24°	0	91			do
8	27°	0	82			do 45 μ
9	27°	0	90			do
10	32°	0	trace			Few abnormal germ tubes
11	32°	0	0			

Spores sown on distilled water April 4 at 20° C

Transferred to wheat plant decoction and to incubators at 8 pm April 10

on temperature relationships, which are made only for extended periods at fixed temperatures.

(f) Relation to Humidity

Tests were made to determine the effect of different relative humidities upon the viability of spores. Relative humidities of 0, 10, 25, 35, 50, 65, 75, 90 and 100 percent were secured by the use of sulphuric acid in a series of dilutions, according to the method described by Wilson (28). The solutions were placed in a series of wide mouthed bottles and the spore material was suspended in small, paraffined containers fastened to the under surface of the corks, the latter being sealed down with paraffin. Since the relative humidities are practically constant within a range of about 10° C, the solutions were made up as if for determinations at 25° and the bottles were then kept at room temperature, from 19° to 24° C. Germination tests were made approximately at the end of each month, but the early figures cannot be considered as entirely significant, as at that time spores did not germinate well in any known nutrient solution.

Spores germinated after they had been kept for six months at relative humidities ranging from 0 to 75 percent. Drying over concentrated sulphuric acid appeared to accelerate germination in the early tests, but only a trace of the spores germinated until it was found that wheat plant tissues could be used in tests for viability.

(g) Reaction of Medium

Several extensive trials were made to determine the hydrogen-ion range within which the spores would germinate, but the results are inconclusive since the spores consistently failed to germinate normally. In one

instance in a potassium phosphate series (approximately 1.5 percent buffered solutions), when plant tissues were added, the spores germinated at hydrogen-ion concentrations varying from PH 5 to PH 7.9 .

(h) Relation of surface tension of medium

Tests were first made with the use of a soluble soap of an unsaturated fatty acid (sodium racineolate) in which surface tensions ranged from 73 dynes to 38 dynes, but no significant results were secured. Similar results were obtained when sodium oleate was used.

While engaged on these studies, a further series of trials was conducted with other soaps, which, with those already mentioned, were supplied by Professor W. P. Larson, Chief of the Department of Bacteriology and Immunology, University of Minnesota. It was found that vigorous germination could be secured by the use of very dilute solutions of potassium palmitate, sodium stearate, potassium stearate, and sodium oleate. From seventy to eighty percent of the spores germinated after nine days, in approximately .00005 percent solutions of these soaps. At the end of twelve days, only ten percent of the spores had germinated in distilled water.

It would appear that the slight reduction in surface tension in these cases was less important than some specific action of the soap, in which the fatty acid radicle may perhaps prove to be the activating component. These soaps, however, stimulate germination much more slowly than does the living plant tissue.

(i) Relation to oxygen supply

In preliminary studies it was found that the spores of Urocystis tritici very rarely germinated when in the depths of the fluid. This phenomenon has been noted by investigators for other types of spores; the

results of Melhus and Durrell (15) with urediniospores of Puccinia coronata are quite characteristic of the general experience. Duggar (6) also has referred to the phenomenon in connection with the germination of the spores of certain of the Basidiomycetes.

It was found here, however, that spores which were totally submerged in distilled water would germinate almost as readily and to practically the same extent as spores which were sown on the surface, on receiving a suitable stimulus after a presoak period. (Cf. Table XII) Spore suspensions were made by shaking up small quantities of spore material in distilled water. On subsequent transfer to Syracuse dishes, the spores soon sank to the bottom of the dish and remained there throughout the test. The test was conducted at 22° C.

There was a distinct tendency for the promycelia of the totally submerged spores to become abnormally elongated before producing sporidia, the average length of the promycelium being approximately 70 microns, while the average length of the promycelium developed by spores on the surface was approximately 30 microns. Sporidia were formed quite readily in the depths of the medium.

Subsequent growth, however, was more rapid on the surface. In this case the sporidial germ tubes grew to a length of 150 to 180 μ before any change was observed in the sporidia produced by the totally submerged spores.

But since sporidia were developed readily in the depths of the medium, perhaps they should be considered as branches of the promycelium, as Brefeld (2) has already suggested for Urocystis occulta, rather than as distinct morphologic entities.

(j) Light and darkness

The spores appeared to germinate equally well in the light and in

Table XII

Effect of total immersion, as compared with surface sowings on distilled water, on the germination of spores of Urocystis tritici

Dish No.	Method of Sowing	Treatment	Percentage of Germination		
			Apr. 7	Apr. 8	Apr. 9
50	Total immersion	Tissue[x] added on April 7	0	62	
51	do	do	0	65	
52	do	None	0	0	0
53	Surface Sowing	Tissue[x] added on April 7	0	70	
54	do	do	0	60	
55	do	None	0	0	0

Spores sown April 3

[x]Sections of plumules of wheat seedlings

the dark.

(k) Longevity of spores

Previous mention has been made of the fact that spores may live in the soil for at least two years. It is not definitely known how long the spores will retain their vitality under soil conditions although there is some evidence that some spores may live for a considerable number of years. McDiarmid (17) reports a case of infection in a crop after an interval of seven years. The seed in this instance was pickled with formalin and was sown on land which seven years previously had borne a disease crop but which had not been cropped since that time. Such evidence is, of course, far from conclusive, since there is the possibility that spores may have been blown onto the land in the meantime. McAlpine (16) reports some pot experiments in which he was unable to secure infection with spores that were more than twelve months old, although he does not consider these tests entirely conclusive.

In laboratory tests made by the writer it appeared that very few spores were viable after they were twenty-eight months old. No germination was noted in spore material derived from the 1914-1918 outbreaks in Australia, while from 80 to 90 percent of spores which were twelve months old germinated under the same conditions.

DISCUSSION AND CONCLUSIONS

The first prerequisite to the development of a disease is the inoculum. It is very evident, however, that susceptible plants may be inoculated with abundant inoculum but yet infection may not occur.

This fact often has been noted with respect to flag smut of wheat. Even though soil may be heavily infested with the spores of the causal organism, a clean crop sometimes may be grown. Under other conditions, however, a relatively small amount of inoculum may be sufficient to cause a serious outbreak of the disease.

The spores of Urocystis tritici have been known to germinate capriciously. In fact, it has been difficult to make pathogenicity studies on account of the erratic behavior of the spores.

It has sometimes seemed almost impossible to reconcile the abundant development of smut in the field with the apparent lack of viability of spores. Soil temperature, soil moisture and aeration are known to affect greatly the development of some smut fungi. But the evidence obtained from field observations and from spore germination studies seemed to indicate that these factors alone were relatively unimportant in influencing the infection of wheat by Urocystis.

It seemed, therefore, that a sequence of circumstances occurred under natural conditions which were not duplicated in experimental trials. Many attempts were made to hit upon the right combination, but they were unsuccessful until it was found that something in plant tissues, and, to some extent in soil infusions, stimulated spore germination in a most remarkable manner.

The stimulatory substances are contained in the living tissues of various plants and apparently diffuse out of uninjured living tissues. They will distill over when the plant tissue is boiled in water, and some of them apparently also remain in the residue. It is probable, although it has not been demonstrated, that they are ordinary chemical substances, possibly alcohols, fatty acids or aldehydes. Investigations are being made on this phase of the problem.

The stimulating substances must be present at just the right time in order to produce the maximum effect. This is shown clearly by the fact that they are not effective unless the spores have been presoaked. Neither are they effective when the spores have been in water too long or when they have been kept at an unfavorable temperature. The substances appear to be diffusible, and possibly unstable, because the germination of spores is not stimulated greatly if the plant tissues have been added to water some time before the spores are sown on it.

Temperature and other conditions influence spore germination, the rate of growth of the promycelium and the formation of sporidia in the presence of the stimulus, but without the stimulus they seem to be relatively unimportant. It is rather remarkable also that the stimulus is most effective if the spores have been presoaked not only for the proper length of time but also at the proper temperature.

The facts brought out in this investigation show clearly that a particular sequence of events is necessary in order that wheat may become infected with the flag smut pathogene. Soil moisture, soil temperature, soil aeration and the presence of a stimulating substance at any given time are not necessarily conducive to infection. These factors must operate

in the proper order and must be correlated chronologically. Sometimes the factors operate together in nature in such a manner as to cause the development of flag smut epidemics.

It is quite possible that the facts brought out may be of practical importance. For instance, since the spores of Urocystis tritici retain their vitality in the soil for several years, it is possible that they may be stimulated to germinate by growing some non-susceptible crop of the right kind on infested soil instead of fallowing, as is sometimes done now.

It is desirable that further studies be made, especially on the factors governing spore germination in the field. When more information is obtained, it is possible that it may serve as a basis for devising cultural practices, such as time of planting, depth of planting, etc., which may aid still more in controlling the flag smut disease.

In any case, facts brought out regarding the peculiar behavior of the spores of Urocystis tritici are extremely interesting and significant in assisting to an understanding of the complexities involved in the development of outbreaks of a serious and little understood plant disease.

SUMMARY

1. Flag smut of wheat, caused by Urocystis tritici Koern., was first found in Australia in 1868 and is now known to occur also in Japan, India, South Africa, southern Europe, and the United States.

2. It is considered one of the most destructive diseases of wheat in Australia. It is becoming more widely distributed each year and annually destroys approximately three percent of the total wheat crop. Losses up to seventy percent have been observed in individual fields.

3. Wheat seedlings in which the plumule is more than 4 mm. long did not become infected when they were inoculated with dry spore material.

4. Extensive spore germination studies were made, and it was found that there is considerable variation in the morphology of the promycelium and of the sporidia.

5. Practically no spores germinated in ordinary nutrient solutions. At the end of from four to sixteen days, from a trace to sixty percent of the spores had germinated in distilled water. Usually, however, less than one tenth of one percent of the spores had germinated at the end of twenty-one days.

6. When small portions of young wheat plants were added to distilled water in which the spores had been presoaked for from three to ten days, from seventy to ninety-five percent of the spores germinated.

7. Presoaked spores germinated when they were transferred to wheat plant infusions or to a distillate obtained from these infusions.

8. Young tissues of barley, flax, and several other plants also stimulated spore germination.

9. Uninjured wheat seedlings stimulated germination of presoaked spores

as well as did the cut tissues.

10. After spores had been soaked in water for twenty-eight days, they still responded to some extent to stimulating substances.

11. It is probable that the stimulating substances are fatty acids, alcohols, or aldehydes.

12. The minimum temperature for spore germination apparently is about 5° C. The germ tubes, however, will grow at 0° C. The maximum temperature appears to be a little less than 32° C. The optimum temperature for spore germination appears to be between 18° and 24° C.

13. The effect of temperature on spore germination varies with the previous treatment of the spores. When a stimulating substance is added to spores which have been presoaked for six days at 20° C the optimum temperature for growth of the germ tubes is about 24° C.

14. Spores which have not been presoaked germinate most quickly at about 18° C. When they are kept at temperatures of over 24° C for a considerable length of time they respond less readily to substances which stimulate germination.

15. When spores are dried during the first eight weeks after formation, they appear to germinate more quickly than those which have not been dried. However, spores will germinate after they have been exposed for six months to relative humidities ranging from 0 to 75 percent.

16. Spores did not germinate normally in sodium phosphate solutions of different hydrogen-ion concentrations. When plant tissues were added, however, the spores germinated at hydrogen-ion concentrations varying from PH 5 to PH 7.9.

17. Apparently the surface tension of the medium in which the spores

were placed did not affect germination. Dilute soap solutions, such as potassium palmitate, potassium stearate, sodium oleate and sodium stearate, however, stimulated germination.

18. Spores germinated readily while immersed in solutions to which a stimulating substance had been added. However, the germ tubes did not grow as well when immersed in the solution as they did when on the surface.

19. Light apparently did not affect spore germination.

20. Spores which were more than twenty-eight months old apparently had lost their viability.

BIBLIOGRAPHY

1. Anderson, P. J. The morphology and life history of the chestnut blight fungus. Commission for investigation and control of chestnut tree blight disease in Pennsylvania Bul. 7. Dec. 1913

2. Brefeld, O. Untersuchungen aus dem Gesammtgebiete der Mykologie XII Brandpilze III, p. 176. 1895

3. Brittlebank, C. C. Flag smut. Jour. Dept. Agr. Victoria, Australia, 18: 240-243. 1920

4. Butler, E. J. Fungi and disease in plants. Pp. 171-173. Calcutta. 1918

5. Cobb, N. A. Smut. Agr. Gaz. New South Wales, Australia, 2:676. 1891

6. Duggar, B. M. Physiological studies with reference to the germination of certain fungous spores. Bot. Gaz. 31: 38-66. 1901

7. Fawcett, H. S. The temperature relations of growth in certain parasitic fungi. Univ. of California Publications in Agr. Sci. 4: 183-232. 1921

8. Fromme, F. D. Diseases of cereal and forage crops in the United States in 1920. U. S. Dept. Agr. Plant Dis. Survey, Supplement 15: 126. 1921

9. Heald, F. D. and H. D. Woolman. Bunt or stinking smut of wheat. Wash. Agr. Exp. Sta. Bul. 126, pp. 12-21. 1915

10. Heald, F. D. and D. C. George. The wind dissemination of the spores of bunt or stinking smut of wheat. Wash. Agr. Exp. Sta. Bul. 151, p. 21. 1918

11. Humphrey, H. B. and A. G. Johnson. Take all and flag smut. U.S. Dept. Agr. Farmers' Bul. 1063. 1919

12. Hungerford, C.W. and A.E.Wade. Relation between soil moisture and bunt infection in wheat. Phytopath. 1: 53. 1920

13. Hurd, A. M. Seed coat injury and viability of seeds of wheat and barley as factors in susceptibility to molds. Jour. Agr. Res. 21: 99-122. 1921

14. Kellerman, W. A. and W. T. Swingle. Report on the loose smuts of cereals. 1889 Ann. Rept. Kans. Agr. Exp. Sta. 2: 230. 1890

15. Koernicke, F. Mykologische Beiträge. Hedwigia 16: 33. 1877

16. McAlpine, D. Smuts of Australia. Pp. 88-102. Melbourne, 1910

17. McDiarmid, R. W. Field experiments with wheat, 1911. Wagga Exp. Farm. Agr. Gaz. New South Wales, Australia, 23: 652. 1912

18. Melhus, I. E. and L. W. Durrell. Studied on the crown rust of oats. Iowa Agr. Exp. Sta. Res. Bul. 49. 1919

19. Potter, A. A. Headsmut of sorghum and maize. Jour. Agr. Res. 2: 341. 1914

20. Prillieux, E. Maladies des plantes agricoles 1: 189. Paris.

21. Putterill, V. A. Flag smut of wheat. Jour. Dept. Agr. Union South Africa 1: 252-257. 1920

22. Reed, G. M. and G. H. Dungan. Flag smut and take all. Ill. Agr. Exp. Sta. Circ. 242. 1920

23. Smith, E. F. Bacteria in relation to plant diseases 1: 197. Washington, D. C. 1905

24. Stakman, E. C. Spore germinations of cereal smuts. Minn. Agr. Exp. Sta. Tech. Bul. 133, pp. 34-41. 1913

25. Sydow, H. and P., and E. J. Butler. Fungi Indiae orientalis. Ann. Mycol. 4: 427. 1906

26. Thiel, A. F. and F. Weiss. The effect of citric acid on the germination of the teliospores of Puccinia graminis tritici. Phytopath. 10: 448-452. 1920

27. Whitehead, T. On the life history and morphology of Urocystis cepulae. 1920 Trans. Brit. Mycol. Soc. 7: 65-71. 1921

28. Wilson, R. E. Humidity control by means of sulphuric acid solutions with critical compilation of vapor pressure data. Jour. Ind. and Eng. Chem. 13: 326. 1921

29. Wolff, R. Beitrag zur Kenntniss der Ustilagineen. Bot. Zeitung. 31: 657. 1873.

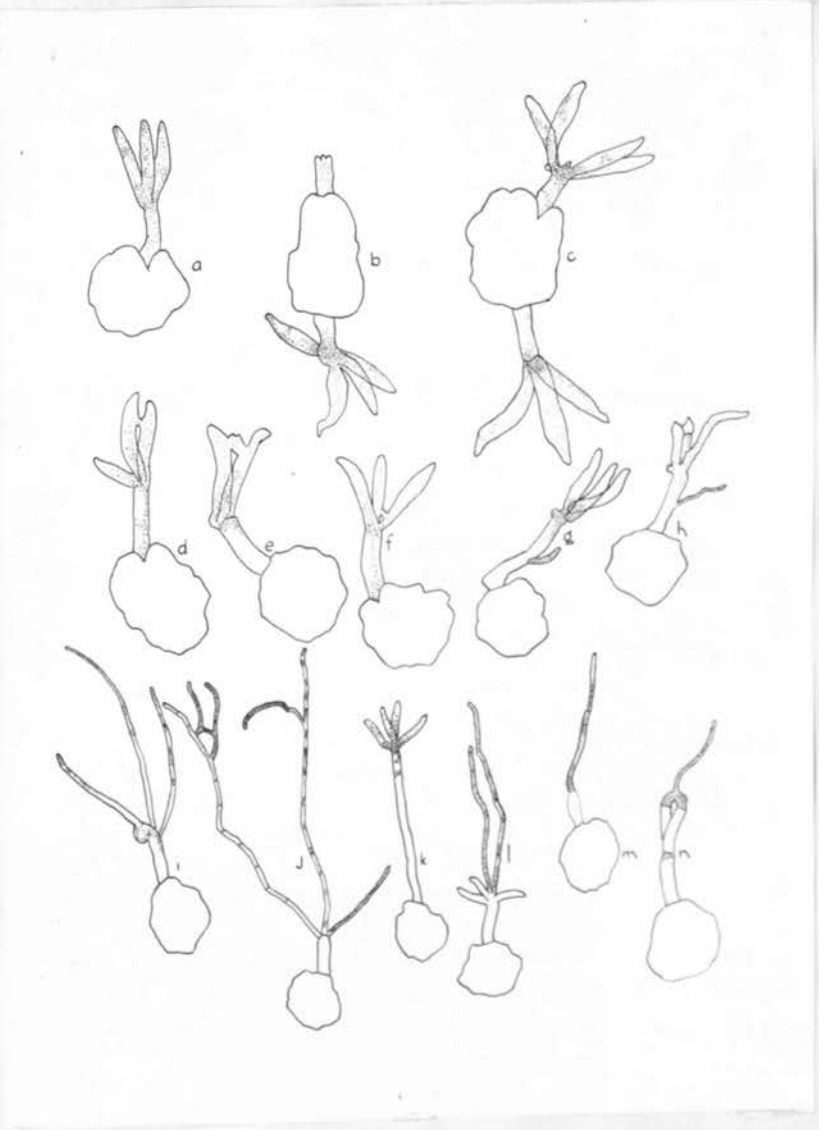

Figure I

Germination of spores of
Urocystis tritici

(Camera lucida drawings)

a - f x 450 g - h x 330

i - n x 250

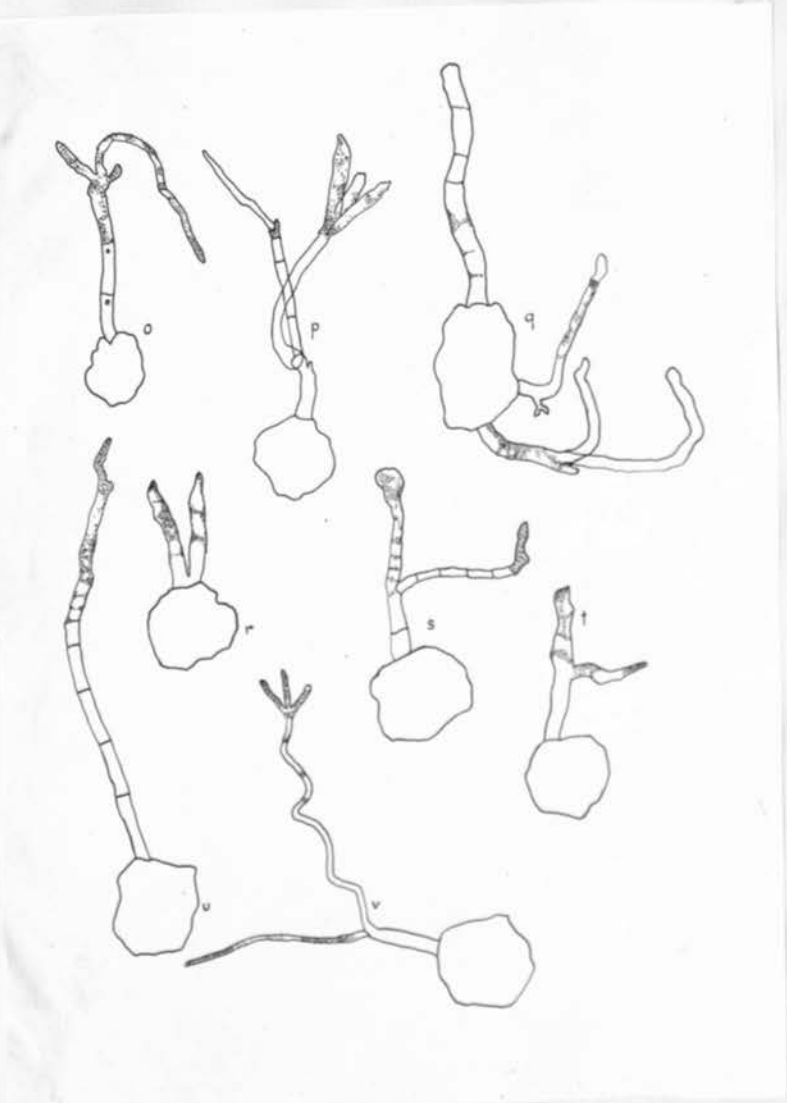

Figure II

Germination of spores of
Urocystis tritici
Less normal types

(Camera lucida drawings)

o, p, r, s, t, v x 330 q, u x 450

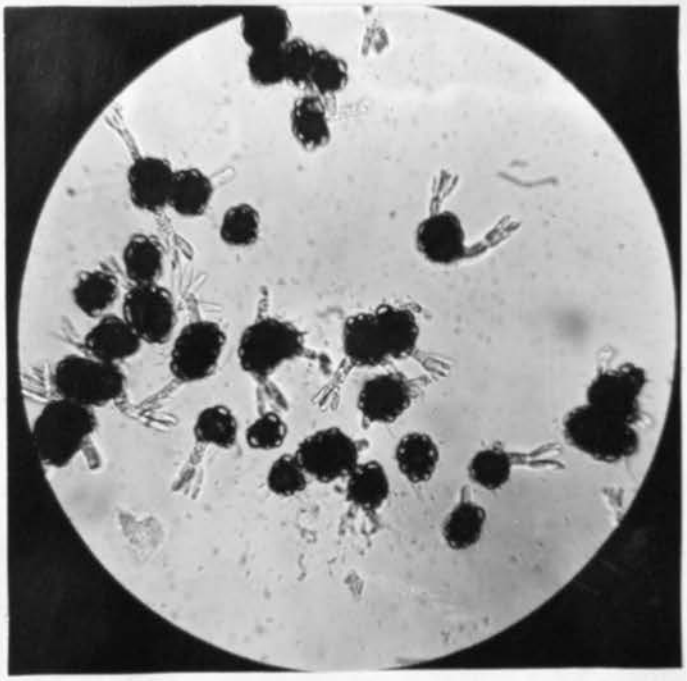

Figure III

Germination of spores of Urocystis tritici

Twelve hours after addition of plant tissue to prescaked spores.

Germination at 25° C

Photo x 200

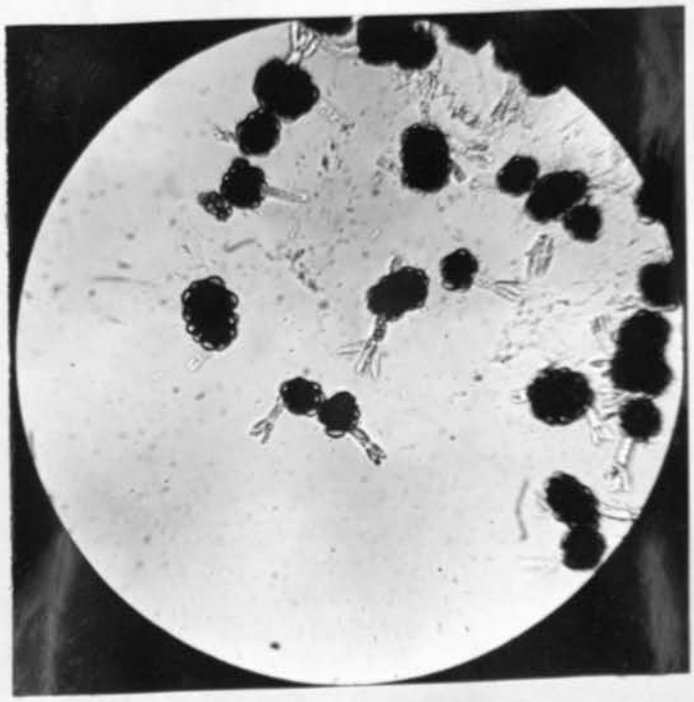

Figure IV

Germination of spores of Urocystis tritici

Twelve hours after addition of plant tissue to prescaked spores.

Germination at 25° C.

Photo x 200

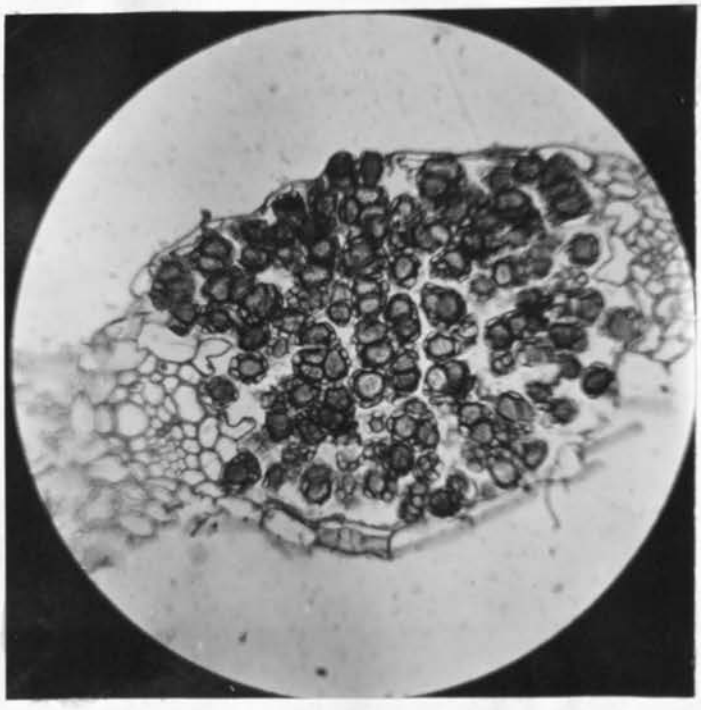

Figure V

Transverse section of leaf of
Federation wheat

Unopened sorus showing spores of
Urocystis tritici in section

Photo x 200

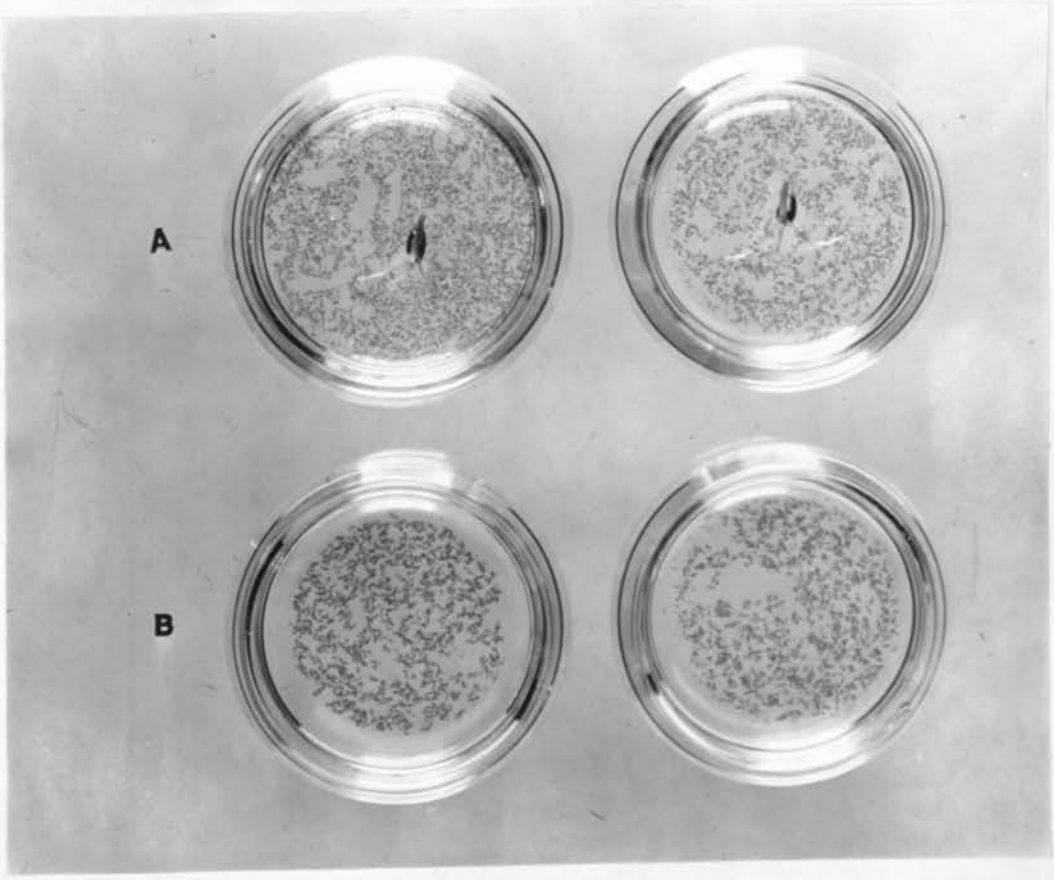

Figure VI

Syracuse dishes containing spores of Urocystis tritici on distilled water

A Wheat seedlings added to prescaked spores

B Untreated

Photo x 5/6