# SOME STUDIES IN THE BIOLOGY OF LOOSE SMUT OF BARLEY

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By

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## Some Studies in the Biology of Loose Smut of Barley

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## Introduction

Loose smut of barley, caused by Ustilago nuda (Jens.) Kell. and Sw., results in a considerable annual loss to the barley growers of this continent. In Canada it is the most important barley smut with which the growers have to contend. According to the Canadian Seed Growers Association(6) all fields throughout the Dominion show a trace of the disease, and they feel that the apparent difficulty of controlling the organism causing the disease is responsible for its general distribution.

Floral infection of barley(Hordeum sativum L.) by Ustilago nuda has been considered an established fact for many years. Since the discovery of this type of infection by Maddox (32) in 1895, the hot-water seed treatment has been accepted as the only means of controlling the disease. Recently, however, formaldehyde, and some of the organic mercury compounds have been found to control infection in certain varieties (24, 26, 59, 60, 61). This lead Tisdale(58) to believe that there yet remained important facts to be learned regarding the infection of barley by Ustilago nuda: consequently, in 1922, he made some studies on the possibility of seedling infection by this organism. His results were so striking that he followed up the work with a number of experiments the following year. These gave results which were even more convincing in suggesting that the fungus infects the seedlings of certain varieties and causes severe injury to seedlings when heavily infected.

It is only quite recently that the question of seedling infection has assumed any importance. Consequently, this

new light on the life cycle of the organism demands further investigation.

## Review of Literature

Long before the discovery of the difference in the fungi causing the smuts of barley, Hoffman (20), in 1866, mentioned the possibility of floral infection. Jensen (25), in 1888, after many years of investigation, found that there were two smuts of barley. Brefeld (2), about the same time, also encountered the same phenomenon.

Kellerman and Swingle (31), in 1889, described the two species and attributed the loose smut disease to the organism Ustilago nuda (Jans.) K & S.

Maddox (32), in 1895, from carefully conducted experiments, concluded that loose smut of barley could not be produced by inoculating the seed or by mixing spores with the soil.

In 1909, Freeman and Johnson (15) made certain studies on loose smut of barley and found that infection takes place from the time when the stamens are still green to the time when the ovary is one-third its mature size. They found the optimum time for infection to be when the flower is in full bloom or when the ovary is just commencing to develop following fertilization.

It was assumed, then, that infection occurred only at flowering time or shortly afterwards. It was generally believed that spores from infected heads lodge between the glumes of normal heads when the glumes are open at the flowering period. These spores, if viable, germinate, infect the

young ovaries and eventually penetrate the young embryos. Here the mycelium remains dormant in the matured seed until the grain germinates following planting. It is then revived, awakened from its dormancy and grows up through the young plants causing the abnormal or smutty heads.

On the other hand,the results of Tisdale's (58) investigations prove very conclusively that,at least in some varieties, a certain amount of infection may be seedling,caused by spores borne on the seed. By inoculating apparently smut-free dehulled seed previous to planting he succeeded in producing as high as one hundred percent infection in certain varieties. Moreover,he showed that copper carbonate,chlorophol,and formalin checked infection to a considerable extent. The effect of such seed treatments is in itself sufficient to indicate the possibility of seedling as distinct from floral infection. These results were amazing and decidedly opposite to the previous conception of the nature of infection. If his findings be true,the question of infection at once assumes a greater significance and suggests various new phases for investigation.

## Object of Work

The purpose of this investigation was to study the viability of the spores,the physiology of spore germination and the effect of various fungicides as means of control,all of which are cardinal features worthy of experimentation in view of Tisdale's findings. As the work progressed,other related problems arose; and these,although secondary in nature,

have been as adequately dealt with as time would permit.

## Experimental Procedure

### Materials

Collections of smutted heads were made from the fields at different dates after the first appearance of the smut during the summer months of 1929. Three infected heads, previously hooded in glassine paper bags, were detached from the stem daily and left in the field until August 17th. Each head was supported by a stiff wire (which took the place of the stem) pushed into the ground, all of them being, thereby, exposed to the same environmental conditions. As the plots were visited daily the collection is more or less complete, covering a comparatively wide range from July 5th to August 17th. Moreover, samples of smutted heads infected with Ustilago nuda were also obtained from different sources across Canada, including Kentville, N.S., Fredericton, N.B. Ottawa and Guelph, Ont., and Alameda, Kincaid and Indian Head, Sask. Supplementing this collection were a number of infected heads representing three different varieties of barley taken from the fields of the Macdonald College farm.

### Methods

Isolation: Isolations were made from the individual infected heads collected from the various sources. A few spores were gently dusted over the surface of potato-dextrose agar poured in large Petri dishes. These plates were then covered, and the

spores were given a few days to germinate. At the end of about the third day the covers were removed and the spores were examined under the microscope for germination. Single isolated germinated spores were carefully removed with a sterile platinum needle and transferred to one hundred and fifty cubic centimeter flasks containing thirty cubic centimeters of potato-dextrose agar. Six cultures were made in this way from each infected head.

Preparation of media: The media, Czapek's, Richards' and Richards-Duggar's, whose formulae are given below, were prepared as follows: The required components were carefully weighed and placed in clean Erlenmeyer flasks, and distilled water was added, as recommended in the formulae. The solution was gently warmed to dissolve the various solids. Definite volumes of the media as required were poured then into Erlenmeyer flasks of adequate size. The flasks were plugged with cotton-wool and autoclaved at fifteen pounds pressure for fifteen minutes.

Extracts of certain plant juices were also employed. A pales extract of barley was prepared by boiling in a steam autoclave twenty-five grams of barley seed in seven hundred and fifty cubic centimeters of distilled water for one and one-half hour for three successive days. The resulting solution was passed through a cotton filter and then distributed into the required flasks, plugged with cotton-wool and sterilized as above. A carrot decoction was prepared by boiling in a steam sterilizer four hundred grams of scraped and macerated

carrot tissue in one liter of distilled water for fifteen minutes, then similarly filtering and sterilizing.

Peptone broth and the different agars, such as potato-dextrose, cornmeal and Richards-Duggars concentrated solid media, were all prepared as recommended by their respective formulae.

Czapek's Solution

| | |
|---|---|
| $MgSO_4$ ..... .. ..... | 0.5 grms. |
| $KH_2PO_4$ ..... | 1.0 " |
| KCl ..... | 0.5 " |
| $NaNO_3$ ..... | 2.0 " |
| Cane sugar ..... | 3.4 " |
| Water ..... | 100.0 c.c. |

Note: The pH of this medium is 6.8

Richards'(White) Solution

| | |
|---|---|
| $KNO_3$ ..... | 10 grms. |
| $KH_2PO_4$ ..... | 5 " |
| $MgSO_4$ ..... | 2.5 " |
| $FeCl_3$ ..... | 20.0 mgms. |
| Dextrose ..... | 50.0 grms. |
| Distilled water ..... | 1000.0 c.c. |

Note: The pH of this medium is 4.5

Richards-Duggar's (Conc.)Solution

| | |
|---|---|
| $KNO_3$ ..... | 10.0 grms. |
| $KH_2PO_4$ ..... | 5.0 " |
| $MgSO_4.7H_2O$ ..... | 2.5 " |

Sucrose ..... 50.0 grms.

Water ..... 1000.0 c.c.

The "Difco" products were used in making up both potato-dextrose and corn meal agars. Richards-Duggars' concentrated solid medium was made by adding three percent agar-agar to the nutrient solution.

Inoculations: Seed inoculation was carried out by placing a definite number of seeds in a small culture dish. A portion of a smutted head of sufficient size to carry enough spores to ensure coverixing of each seed was selected. All were placed in the culture dish and thoroughly shaken until the seeds became almost black.

In some cases it was found necessary to use a spore suspension. This was prepared by placing a smutted head in a shaker to which was added a few sterile glass beads and some distilled water. Judicious shaking to avoid undue injury to the spores, produced a uniform suspension. Coarse fragments of plant tissue remaining in this suspension were removed by the use of fine-pointed forceps. The required concentration of spores was obtained by proper dilutions.

In making transfers from the original stock cultures use was made of a four millimeter platinum loop to remove a portion of the mycelial growth from the margin of the culture as produced on solid media.

Spore germination studies were generally carried out by dusting a few spores over the surface of certain test solutions. A four millimeter platinum loop was immersed in water and

and carefully applied to a mass of dry spores so that a number would loosely adhere to the film of water across the loop. (It was found that if the spores were previously moist,undue clumping and agglutination was the result. Care was taken to avaid this as a uniform contact of spores within the solution is essential.) In this way the spores were transferred to the required solution. A thorough shaking of the needle in the solution removed the spores,and surface tension prevented them from sinking or becoming submerged,

Germination Counts: For making counts of spore germination a four millimeter platinum loop was used to remove a drop of the solution containing some spores to a carefully cleaned slide. A clean cover glass was then placed over the drop and the spores were examined under a microscope.

When counts were made of spores in suspension,a one cubic centimeter pipette was employed. By keeping the thumb over the top of the pipette until the end about reached the bottom of the test-tube,as many as desired of the spores could be drawn up. Drops of the suspension were placed under cover glasses on perfectly clean slides.

Spores that had not produced a germ tube twice the length of the diameter of the spore were not considered to have germinated.

All the figures given as a result of the various readings represent averages of counts of a number of fields,five drops being taken from each tube in a test. No consideration was given to clumps of spores.

Hydrogen-ion determinations: In making up each series of differing pH values, a titration curve was first prepared. In this way the required volumes of 3/10 N HCl and 3/10 N KOH which were to be added to definite volumes of the solution for each adjustment were determined.

Welch's electro-potentiometer was used throughout the investigation.

Dehulling of seeds: The seeds were given a preliminary soaking in sterile distilled water for one hour. The water loosened the hull and facilitated its removal. At the end of this period they were spread out on a bench, the surface of which was previously sterilized with corrosive sublimate. The seeds were dehulled immediately by carefully removing the glumes with a sharp-pointed instrument. The basal end of the seed was first broken and the glumes stripped off.

## Experiments and results

(1) The effect of certain seed treatments.

The experiments directed towards a control of the organism as affecting the seed and subsequent growth of the plant constituted a major part of the problem.

Jensen (25), in 1888, discovered the hot-water seed treatment for smuts, and for many years it was recommended as being the only treatment to control the loose smuts of wheat and barley.

Freeman and Johnson (15), in 1909, also recommended its use.

Johnson (27), in 1912, maintaining that the hot-water treatment was best questioned the efficiency of other agencies.

Johnson (26), in 1914, got perfect control of barley

smuts by using the modified hot-water method. He found that ordinary formalin treatment reduced smut infection to a mere trace. This is quite important,since heretofore it had been supposed that this seed treatment was ineffective in the control of this disease. He also found that the formaldehyde solution was more efficient than the gas treatment and that it did not have such an inhibatory effect on germination.

Humphrey and Potter (24),in 1918, mentioned that loose smut of barley is more or less controlled by the use of the formaldehyde treatment.

In 1923,Tisdale and Taylor (59) found that the dusts including copper carbonate were less effective than liquids in the control of barley and oat smuts.

Tisdale et al (60),in 1923, succeeded in obtaining equally as good control with formalin as with hot water,but found that both caused seed injury and that there was little or nothing gained by using them. From the standpoint of both smut control and of yield chlorophol was superior to formaldehyde, hot water,or copper carbonate. Copper carbonate failed to control barley smuts. He also claimed that there was a varietal difference in the behaviour of barley towards seed treatment. What might be good for one variety could not be recommended for another. He suggested that the efficiency of the treatment may vary under different local conditions.

In 1925,Tisdale et al(57) showed that several organic mercury compounds were superior to formaldehyde from the standpoint of seed germination,smut control,and yields of plants from treated seeds,but he still obtained traces of

of infection.

Gussow (18), in 1927, maintained that the hot-water treatment is the only method that is wholly effective.

Howitt (23), in 1929, also recommended the use of the hot-water treatment as the only efficient means of controlling this disease.

It was observed that the seed harvested from the Macdonald College fields in 1928 yielded quite a high percentage of infection the following summer. To estimate the possible infection in the 1929 seed was a matter of considerable speculation. However, seed was selected from the 1928 and its 1929 crop. The selection was made without using any particular physical property as a standard. The idea was to use seeds that displayed nothing more than average merits.

The seed was sown in shallow flats at a depth of three quarters of an inch (and placed on benches in the greenhouse) after having been treated as follows:

(1) Check, no treatment.

(2) Seed immersed for ten minutes in a flask containing one part of a 37-40 percent formaldehyde solution to three hundred and twenty parts water; and covered for four hours and spread out to dry overnight.

(3) Dusted thoroughly with Ceresan at the rate of one ounce per bushel of seed.

(4) Immersed in water at 86°F. for four hours, after which it was placed in water at 112°F. for twenty minutes, and finally dipped in water at 127-128°F. for exactly ten minutes. It

was then cooled and spread out to dry.

The results shown in Table I suggest that the hot-water treatment is the only efficient method of control for this disease. The control, however, by both Ceresan and formalin is quite marked. This might be taken to indicate that some spores are borne on the seed and hence are the means of part of the infection. Ceresan gave better control and higher germination than formalin. This increased germination was possibly due to disinfection of the surrounding soil rather than to a stimulative effect on the seed.

The lethal effect on the seed as a result of the various treatments was more or less serious. The plants which grew from the treated seed appeared to be more healthy than the checks, and less actual seedling injury following germination and early growth occurred. Tisdale (58) attributes this injury to heavy smut infection. It may be a reasonable assumption, since the percentage of seedling injury decreases with a decrease of smut infection.

The germination following the hot-water treatment was very low due to the severity of the treatment, since the seeds were subjected to the maximum temperatures for the entire time recommended. An experiment carried out later showed that a more satisfactory germination can be obtained by using the minimum temperatures which will at the same time give adequate control.

It was felt that the results of the above experiment were not sufficient to warrant any generalization. Consequently

Table I. The comparative efficiency of Formalin, Ceresan, and hot water as seed treatments for the control of Loose smut of barley.

| Treatment | Number planted | | Number germinated | | Number matured | | Number infected | | Percent infected | |
|---|---|---|---|---|---|---|---|---|---|---|
| | '28 | '29 | '28 | '29 | '28 | '29 | '28 | '29 | '28 | '29 |
| Check | 100 | 100 | 87 | 93 | 83 | 92 | 8 | 0 | 9.63 | 0 |
| Formalin | 100 | 100 | 76 | 91 | 75 | 91 | 3 | 0 | 4.0 | 0 |
| Ceresan | 100 | 100 | 79 | 94 | 79 | 94 | 2 | 0 | 2.53 | 0 |
| Hot water | 100 | 100 | 41 | 63 | 41 | 63 | 0 | 0 | 0.0 | 0 |

Note: The barley variety O.A.C. 21 grown at Macdonald College was used. It was treated October 16, 1929, planted the next day, and harvested February 10, 1930.

Table II. Further tests on the comparative efficiency of Formalin and Ceresan as seed treatments for the control of the Loose smut of barley.

| Treatment | Number planted | | Number germinated | | Number matured | | Number infected | | Number(%) infected | |
|---|---|---|---|---|---|---|---|---|---|---|
| | '28 | '29 | '28 | '29 | '28 | '29 | '28 | '29 | '28 | '29 |
| Check | 450 | 450 | 345 | 385 | 327 | 364 | 23 | 3 | 7.03 | 0.82 |
| Formalin | 360 | 360 | 263 | 326 | 256 | 302 | 24 | 2 | 9.37 | 0.66 |
| Ceresan | 360 | 360 | 266 | 327 | 265 | 321 | 15 | 0 | 5.66 | 0.00 |

Note: The barley variety O.A.C.21 grown at Macdonald College was used. It was treated February 20,1930, sown the next day and harvested May 1,1930.

a similar experiment was conducted but on this occasion the hot-water treatment was omitted. The results are shown in Table II.

With one exception the results obtained from the above experiment corresponded very favourably with that of the previous one shown in Table I. The outstanding feature to be noted is the uncertainty of the formalin treatment. In this instance no control whatsoever was offered by the formalin treatment. In fact more infection was found in the 1928 formalin-treated seed than in the check. Ceresan reduced infection somewhat but was by no means entirely effective except in the 1929 seed where infection was very low in the check.

The results, however, indicate that the partial control obtained in Table I was due to the destruction of spores of the fungus which may be on the surface of the seed or that a similar effect was due to the action of the fungicide on superficial mycelia located in the outer seed coat. But as the seedling infection of this and other varieties was tested during this investigation and negative results were obtained in each case the theory that infection may be caused by spores borne in the seed remains unproven for these varieties. Then the partial control of infection by formalin and Ceresan as seed treatments can only be attributed to the presence of superficial mycelia in the outer tissues of the seed which became killed by the penetration of these fungicides.

(2) Seedling inoculation

In view of the results obtained by recent investigations (58), it was felt that further work should be done on this particular phase of the problem.

Maddox (32), in 1895, failed to produce infection by seedling inoculation or by mixing spores with the soil. He therefore concluded that the disease could not be produced in this way.

Brefeld (2), in 1905, obtained a one percent infection by inoculating the seedlings with germinated spores in only a single experiment. From all his work this was the only indication of seedling infection. He concluded that infection in the blossom is the predominant form of infection of the host plants, if not the only one.

Tisdale (58) showed, in 1924, that seedling infection by Ustilago nuda takes place abundantly in certain varieties of barley when the dehulled seed is inoculated. His results were so striking that he claims to have shaken the long-accepted theory that floral infection is the only type of infection by this organism.

A reference has been made by Sampson (50), in 1928, to unpublished data which is said to indicate a decrease of germination in direct proportion to the time the spores are kept. It is also felt that the spores maturing late are more viable and more capable of causing infection than the spores maturing somewhat earlier.

The following experiment was carried out with a purpose of investigating these claims and to determine the susceptibility of O.A.C. 21 barley to seedling infection. Its susceptibility to the disease has been an established fact for some years.

The seed was all subjected to the hot-water treatment as referred to above, with the exception that in this case the lowest temperatures recommended were used. They were then dehulled and dusted with loose smut spores in small culture dishes until nearly black.

The results as given in Table III would lead one to believe that this variety of barley, O.A.C. 21, is not susceptible to seedling infection. No infection was obtained when the seeds were inoculated with normal spores collected early or late in the season. This is of considerable importance since O.A.C. 21 is probably the most widely grown six-rowed barley in the Dominion of Canada.

Smut free seeds of this variety were inoculated in a similar manner with fresh spores that matured during the winter. Of four hundred seeds inoculated and planted, three hundred and sixty-nine germinated, two hundred and fifty-three matured and no infection was obtained.

The above results were disappointing, but, nevertheless, significant. Although the results are negative, they do show quite definitely that this variety of barley (which is of such great economic importance in the provinces of Ontario and Quebec) is not subject to seedling infection.

Table III. Susceptibility of O.A.C.21 barley to seedling infection, inoculated with spores maturing early and spores maturing late.

| Treatment | Number planted | | Number germinated | | Number matured | | Number infected | | Percent infected. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | '28 | '29 | '28 | '29 | '28 | '29 | '28 | '29 | '28 | '29 |
| Check | 300 | 300 | 207 | 236 | 205 | 231 | 0 | 0 | 0 | 0 |
| Early spores | 300 | 300 | 205 | 244 | 198 | 240 | 0 | 0 | 0 | 0 |
| Late spores | 300 | 300 | 194 | 223 | 188 | 217 | 0 | 0 | 0 | 0 |

Note: The barley used in the above experiment was grown at Macdonald College, Que., and the source of the inoculum was the same variety of barley. It was treated with hot water October 28, 1929, dehulled, inoculated and planted two days after, and harvested February 19, 1930.

Inoculation by Hyperdermic injection of inoculum

The use of a hypodermic needle for the injection of liquid cultures or spore suspensions into plants has become very prevalent during the last few years.

By the use of a pointed glass tube, Hitchcock and Norton(19) in 1896, injected liquid cultures of Ustilago zeae into different parts of the maize plant.

Reed and Faris (12) used this method in 1925 to produce loose kernel smut, Sphacelotheca cruenta, of sorghum in different parts of the plant.

In 1928, Griffiths (17) injected a spore suspension of Ustilago zeae into or near the terminal growing region of the plant and produced infection in certain resistant varieties of corn. In the following year, the same writer (73) produced loose smut infection in certain varieties of barley in a similar manner.

The susceptibility of O.A.C.21 barley to infection was tested by this method of inoculation. This particular variety was selected on account of its great popularity throughout Canada. Three hundred smut-free seeds were planted in shallow flats. When about half mature, one hundred and fifty were inoculated with a spore suspension by a very fine hypodermic injection needle. The spores were injected at or near the apical region of growth of the young tillers. Negative results were obtained, as no infection developed in the inoculated plants or in the checks. It may readily be assumed, therefore, that this variety is not susceptible to the disease by this type of inoculation.

(3) Viability of the spores

The question of the viability of spores has received considerably more attention than that of longevity. Reference has been made by Novopokrovsky and Skaskin (37) to unpublished data on certain species of Ustilago which are said to indicate a decrease in germination in direct proportion to the length of time the spores are kept. If this be true, the spores may not be of any significance as a connecting link between the seasonal attacks of this disease.

In a physiological study of a few species of Ustilago, Duggar (10), in 1901, obtained varying results with different species. Some spores germinated immediately on maturing, but U. striaeformis required a rest period. All germinated better in nutrient media than in water.

Stakman (55), in 1913, found that germination of U.hordei occurred earlier than U.nuda and that both germinated much better on five percent cane-sugar solution than on tap-water distilled water, or on any other nutrient used. He managed to obtain good germination of both organisms during the winter months.

Novopokrovsky and Skaskin (37), in 1925, stated that neither U.hordei nor U.nuda required a rest period for germination.

The problem of the longevity of the loose smut spores has received but very little attention, for only a limited amount of literature is available on this particular phase of the subject. The writer feels that, since the spores may constitute a primary source of infection, their viability at various times on different media should be investigated.

The media used were tap-water, distilled water, two percent cane-sugar solution, and extract of pales. Five cubic centimeters of each of the respective media were placed into ten cubic centimeter vials. The flotation method of inoculation, as referred to, was employed.

Table IV gives the percentage germination for counts taken at two different dates of spores of different age. The results show that maturity is a critical factor in determining the germination capacity of spores. Spores removed from the host before they are properly mature do not germinate after a period of five months, unless in the presence of very good nutrient media. Distilled water was more favourable to germination than tap-water, and an extract of pales was slightly better than a two percent cane-sugar solution. Both the extract and the sugar solution gave a considerably higher germination than the water. All the tests represented in Table IV were made at room temperature.

A similar test, but using distilled water, was continued throughout the winter months until the end of April. Germination counts were made monthly.

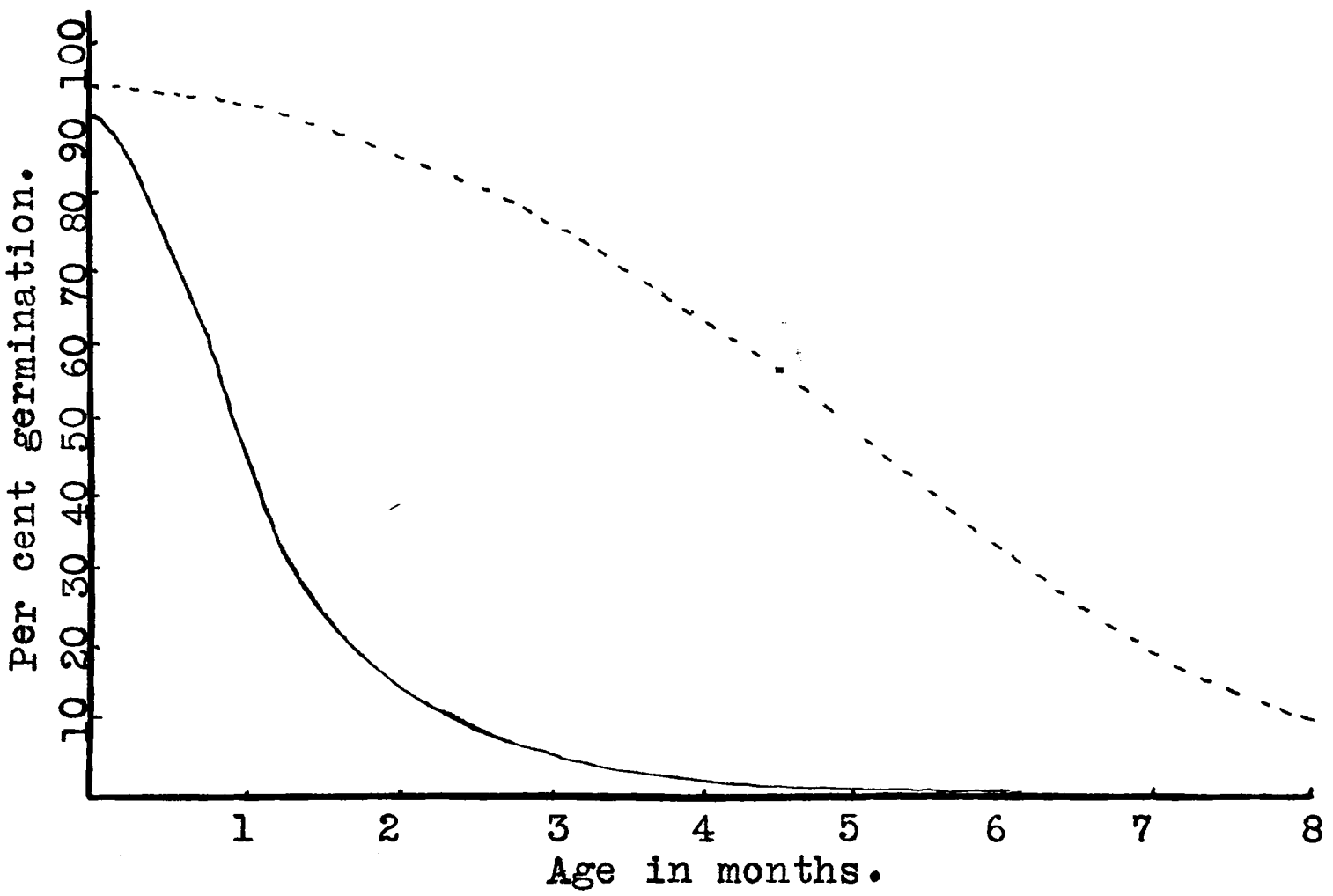
The results as graphically represented in Figure I. indicate that no rest period is necessary for germination. The spores which were detached from the host as soon as formed, as well as the spores allowed to mature on the host germinated at once. As the season progressed, both showed marked falling off in viability. This phenomenon was more rapid and marked in the case of the early spores. The results suggest that a low percentage of the normally matured spores alone will be capable of causing

Table IV. Percent germination in relation to time of maturity of spores

| Date of Maturity | Percent Germination | | | |
|---|---|---|---|---|
| | Tap water | Dist.water | 2% sucrose solution | Extract of Pales |
| | | October 9,1929. | | |
| July 5 | 3-5 | 4-8 | 25-30 | 30-35 |
| July 20 | 55-60 | 45-50 | 90-95 | 85-90 |
| August 4 | 80-85 | 85-90 | 90-95 | 90-95 |
| August 17 | 90-95 | 75-80 | 90-95 | 95-100 |
| | | December 9,1929. | | |
| July 5 | ----- | trace | 10-12 | 12 |
| July 20 | 10-15 | 35 | 60-75 | 75-80 |
| August 4 | 60-65 | 50-55 | 75-80 | 85 |
| August 17 | 60-65 | 60-65 | 75-80 | 75-80 |

Note: The incubation period lasted four and one half days.

Figure I. Viability of the Spores in Relation to Time.




..... fully matured spores.

_____ immature spores.

infection during the bloom period of 1930.

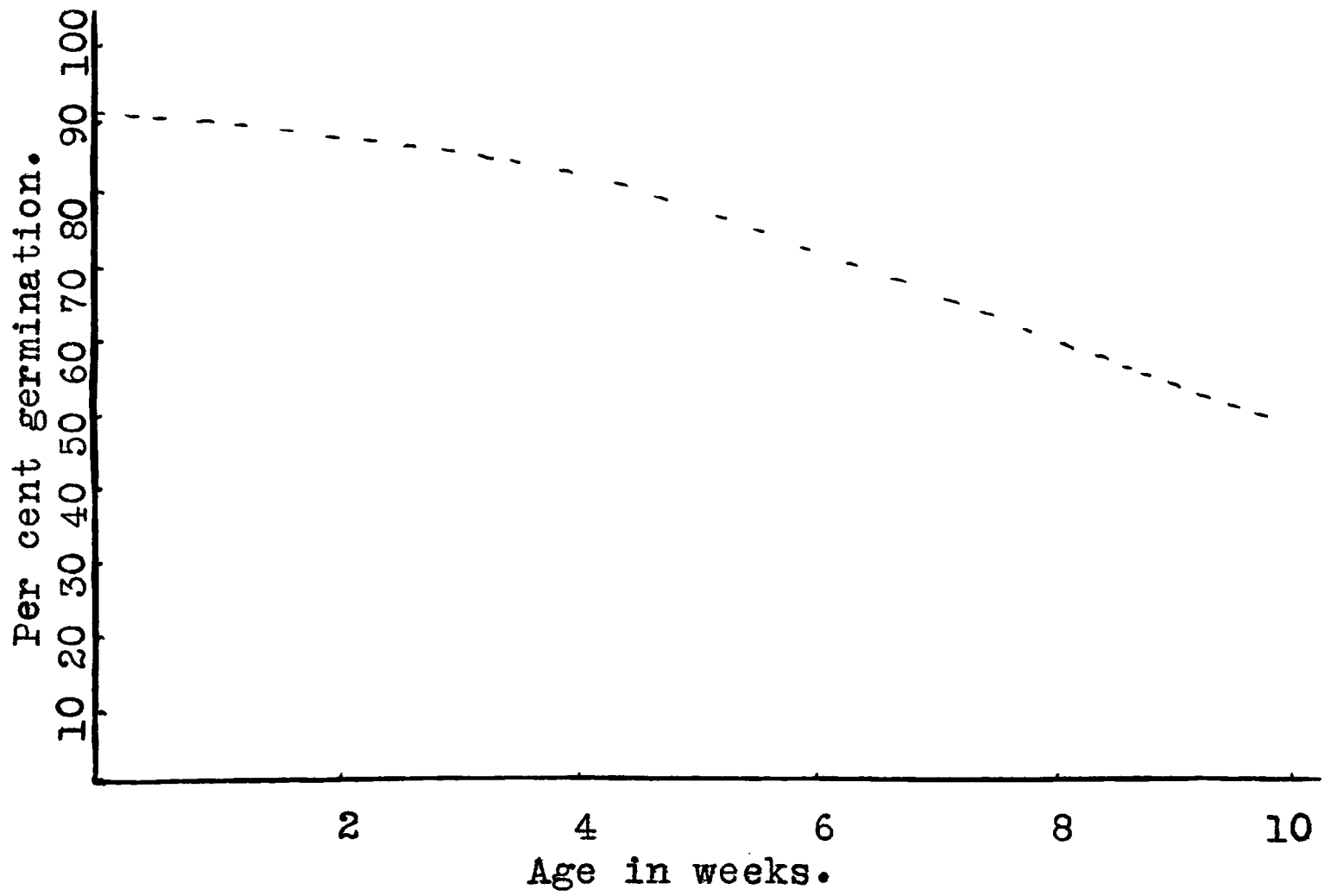
The spores used in the above test were stored in the laboratory at room temperature where the humidity was very low. It was felt that different storage conditions would affect the viability of the spores. Consequently one-half of the original collections of spores was placed in cold storage during the winter. The above test was duplicated,using the spores stored under these conditions. Similar results were obtained.

A difficulty in making germination counts wasencountered, as these spores up to the end of the fifth month actually germinated better,yet the germ tubes were decidedly shorter. Consequently,large numbers,falling below the standard length decided on for this study,had to be discarded. A standard for germination is essential if accurate comparisons are to be made. This is one difficulty with which investigators have to contend.

The germinating capacity of covered smut spores,U.hordei, was tested. It was found that the falling off in viability was by no means as marked,even when stored under the same conditions.

The viability of spores of U.nuda which matured during the month of February under greenhouse conditions was investigated. A very high percentage of germination was obtained immediately on water,and the falling off in viability was by no means as rapid as that shown for early spores in Figure 1. (See Fig.2).

Figure II. Viability of Spores Harvested Early in the Greenhouse, February 10th, 1930.



The temperature in the greenhouse was not allowed to exceed sixty-eight degrees Fahrenheit for any appreciable time. During this time the humidity was quite high. In view of the work reported it would seem that the fungus was developing under favourable conditions. This is well illustrated in experiment IV. This may in part account for the differences in the viability of spores of the same age.

(4) The effect of temperature on germination and growth

Considerable attention has been given by previous workers to the temperature relationships of the smut fungi, especially those attacking the cereal crops. Much of the earlier literature has been amply reviewed by Jones (29). A few of the more recent contributions are of special interest here.

In a critical study of corn smut, Ustilago zeae (Beckm.) Unger, Jones (28), in 1923, found that spore germination occurred throughout a temperature range of 8°C. to 38°C. with an optimum between 26 and 35 degrees. In another study, the same writer (29) found the range of spore germination of Ustilago avenae (Pers.) Jens. to extend from 4 to 34 degrees Centigrade, with an optimum at 15 to 28 degrees.

Bartholomew and Jones (1), in 1923, working with loose smut of oats, Ustilago avenae, showed that low temperatures (8 to 12 degrees Centigrade) during the susceptible period of the host might reduce the infection somewhat, but the reduction was more marked at the upper limit of the range of spore germination. When a soil temperature of 31 to 32 degrees

was combined with high soil moisture,the inhibition of the fungus was complete.

Reed and Faris (44),in 1924,in a similar study,found that Ustilago levis (K.& S.) Magnus corresponded very closely with Ustilago avenae in behaviour. The same workers (43) found that the covered smut,Sphacelotheca sorghi (Link) Clinton, and the loose smut,S.cruenta (Kuhn) Potter,of sorghums behaved very similarly,infection occurring at 12-37.5 degrees Centigrade. There was a reduction in the amount of infection at the extremes,but here again the degree of reduction was influenced by soil moisture.

Faris (13),in 1924,working with covered smut of barley, Ustilago hordei (Pers.) K.& S.,found infection to occur over a range of 5 to 30 degrees Centigrade,witha decided reduction in the amount of the disease as the temperature approached the extremes.

At about the same time,Noble (36) studied the influence of temperature on flag smut of wheat,Urocystis tritici Koern, and found that germination occurred as low as 5 degrees Centigrade,and as high as 32 degrees. Approximately the same percentage of spores germinated throughout the range from 18 to 27 degrees,but the maximum growth of germ tubes occurred at 24 degrees.

In 1926,Walker (66),working with onion smut,Urocystis Cepulae Frost, has shown that abundant infection occurs at temperatures as low as 10-12 degrees Centigrade. This temperature is almost the minimum for growth and germination of the onion itself,so that there appears to be no low point

at which the host escapes the disease. He found the optimum for germination and growth to lie between 13 and 22 degrees. Above 25 degrees germination became very light, and growth of the hyphae very slight with no infection occurring beyond a temperature of 29 degrees Centigrade.

It was felt that the temperature relations of the organism is an important factor influencing infection. It was with a purpose of determining these relations that the following few experiments were undertaken. This phase of the work may be considered under three headings, viz., the effect of temperature on germination, thermal death points, and the effect of temperature on growth.

(a) The effect of temperature on germination of spores. A four millimeter platinum loop, previously immersed in water, was carefully applied to a mass of dry but viable spores. A sufficient number of spores, loosely adhering to the thin film of water across the loop, were then transferred to 5 c.c. of sterile distilled water contained in 10 c.c. vials. A thorough shaking of the needle removed the spores from the loop and the surface tension of the water prevented tham from becoming submerged. If the spores happened to be moist, to begin with, unwarranted clumping and agglutination would result, but this must be avoided as a uniform contact of the spores with the solution is essential. This process was repeated until inoculations were made from the different sources in triplicate.

A differential thermostat, constructed of a well insulated copper trough divided by cross walls into twelve chambers heated at the end and cooled at the other, was used for

temperature studies. The temperature was controlled by a Dekotinsky Bimetallic regulator at one end and ice at the other. The thermostat was so adjusted that the heat generated from the bulbs employed produced a temperature of 60 degrees Centigrade in the upper chamber. The temperature in the ice chamber remained constant at 5 degrees Centigrade. The intervening compartments,producing a gradation of temperatures exhibiting differences of only 4 or 5 degrees,were selected for use throughout the experiment.

The vials containing the spores were then placed in the desired compartments so that an inoculum from all sources was represented in all chambers. A period of five days was allowed for germination.

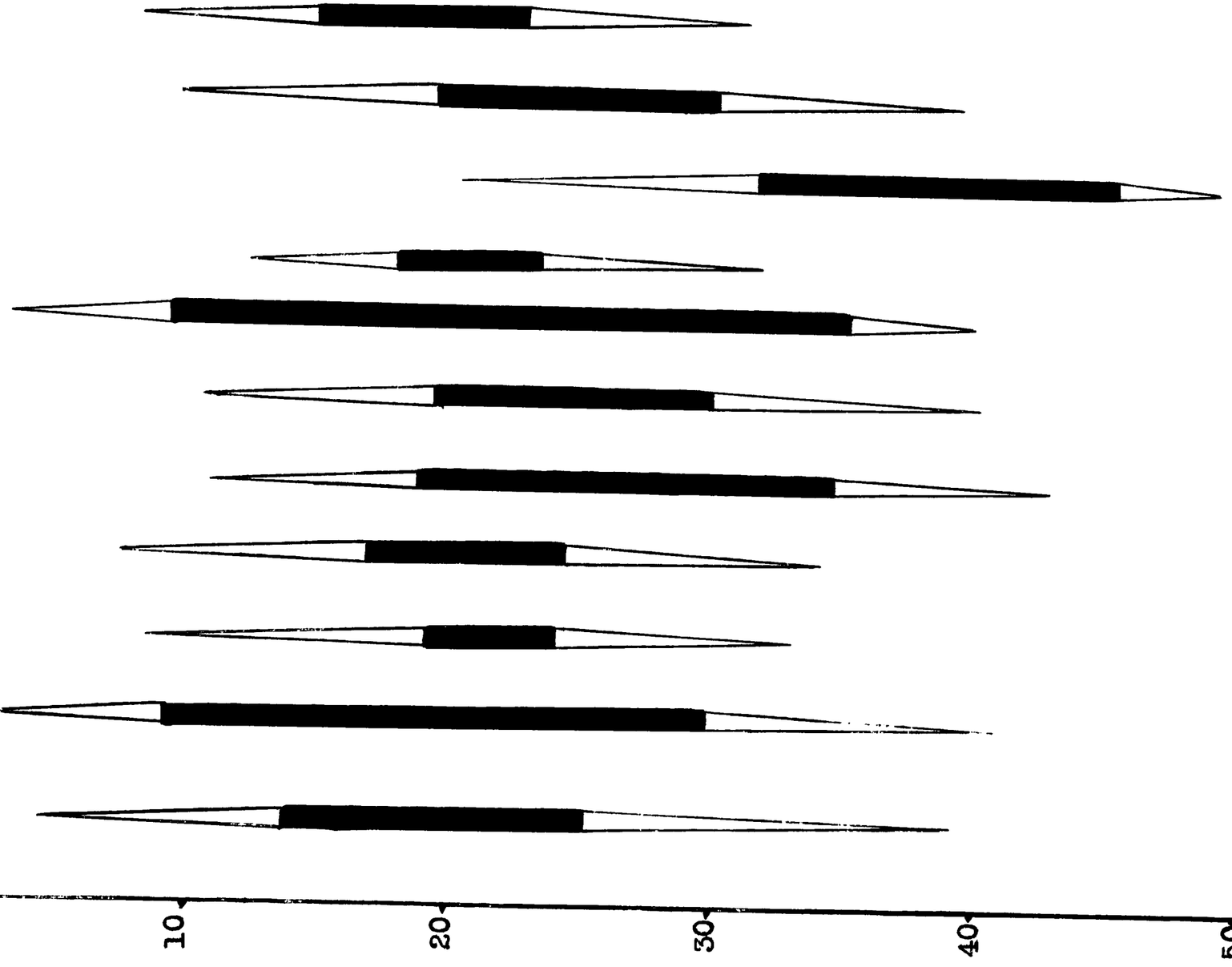
Figure 3 gives in graphic form the results of the experiments. The heavy shaded lines represent the range of tolerance,with only slight traces of germination on either side. Some of the spores in this test correspond very closely in behaviour to the results of Novopokrovsky and Skaskin (37). It will be noticed that there is considerable variance in their respective temperature requirements as regards germination,depending on the source of the material. It may also be observed that with the exception of No.3,there is a common optimum range existing between 19 and 21 degrees Centigrade. This experiment was further continued,giving the entire series a longer period of incubation. The results suggest that if the time factor were eliminated,the spores

exposed to the lower temperatures would ultimately germinate as well as those subjected to a temperature of approximately 20 degrees Centigrade.

(b) Thermal death points of smut spores As far as the writer was able to ascertain,no information has been published regarding the thermal death points of smut spores collected from various sources. The question then arises whether the temperatures as recommended in the hot-water treatment are inadequate or unnecessary as means of control.

Seventy-seven well-stoppered one cubic centimeter vials were three quarters filled with sterile distilled water and divided into seven groups of eleven vials each. Each vial contained different spores,one vial of each group being inoculated with spores from the same source. The same was repeated with spores from the other ten sources. The flotation system of inoculation was used. Hot water was placed in a Freas Electro-oven with constant temperature regulator, so that the temperature in the bath remained constant at 51 degrees Centigrade. One group of vials containing the inoculum from the various sources was then immersed in this bath. A signal clock was set to ring at the end of a period of ten minutes. When the bell rang,they were quickly removed and immediately brought to room temperature. Fifteen seconds was allowed for temperature changes.

By carefully adding small volumes of cold water,the temperature in the bath was lowered two degrees,and the same process was repeated for each one of the remaining groups at two degree intervals until a temperature of 39 degrees Centigrade




Source of Material

1. Kentville, N.S.
2. Ottawa.
3. Macdonald College B-2722
4. Macdonald College Success.
5. Macdonald College Var. O.A.C. 21.
6. Guelph O.A.C. 21
7. Indian Head, Sask. Var. Albert.
8. Indian Head, Sask. Var. Feeder.
9. Indian Head, Sask. Var. Junior.
10. Kincaid, Sask.
11. Alameda, Sask.

was reached. The spores were given five days to germinate,and germination counts were made as indicated in the above experiment.

The results,as given in Table V,show that there is a considerable difference in the vitality of the spores as collected from the various sources. Several were inactivated at 43 degrees Centigrade,while one collection required 49 degrees. Moreover they suggest that the temperatures as recommended in the hot-water treatment are not,to any great extent,above the actual requirements necessary for control.

(c) The effect of temperature on the growth of the fungus

The temperature requirements for mycelial growth were also determined. Using a 4 mm. platinum loop transfers were made from the outer edge of the stock cultures to potato-dextrose agar poured in small culture dishes. These transfers were made in quadruplicate from a few of the various sources. The new cultures were given five days to allow for a commencement of growth. At the end of this period they were placed in the chambered thermostat described above. A gradation of temperatures was so adjusted that the various compartments included a a range from 0 to 30 degrees Centigrade. Drying out of the media in the culture dishes subjected to the higher temperatures was avoided by placing the plates in stender dishes over water. The cultures were allowed to remain in the thermostat for a period of forty-five days.

Plate I shows that very little growth occurred at 8 or 27 degrees Centigrade. These temperatures approximate the

Table V. Thermal death points in distilled water at increments of ten minutes

| Source of Material | Temperature at which D.P. was reached. In Centigrade |
|---|---|
| 1. Kentville, N.S. | 43 |
| 2. Ottawa, Ontario. | 45 |
| 3. Macdonald College Var. B-2722 | 49 |
| 4. Macdonald College Var. Success | 43 |
| 5. Macdonald College Var. O.A.C. 21 | 45 |
| 6. Guelph, Ontario. Var. O.A.C. 21 | 43 |
| 7. Indian Head, Sask. Var. Albert | 43 |
| 8. Indian Head, Sask. Var. Feeder | 43 |
| 9. Indian Head, Sask. Var. Junior | 45 |
| 10. Kincaid, Sask. | 45 |
| 11. Alameda, Sask. | 47 |

lower and upper limits respectively. No growth whatever occurred at temperatures as low as five degrees or as high as thirty degrees. The optimum conditions of growth existed between the temperatures of 15.5 to 20 degrees Centigrade, with an abrupt dropping off above 23 degrees. Moreover, the results suggest that the higher temperatures favour dark pigment formation, which is characteristic of the older cultures. It seems, therefore, to have an ageing influence on the mycelial growth. The lower temperatures apparently favour the counter-clockwise development of the radial striations, which were not a feature of the normal growth of this particular form at room temperature.

(5) Effect of a diminished supply of oxygen on germination.

Oxygen is considered one of the chief requisites in the process of germination. It is generally believed that, in fungi where spores germinate by the production of a germ tube, the relation of oxygen to germination is essentially the same as in a germinating seed.

Melhus (34), in 1915, concluded that there was sufficient oxygen in the conidium of Phytophthora infestans to permit germination without the presence of free oxygen in water.

Murphy (35), in 1922, observed that presence of yeasts and bacteria in culture arrested germination of the conidia of P. infestans. He seemed to think that the competing organisms used up the oxygen in the water, thus retarding germination.

In 1922, Brown (5) maintained that, within very wide limits, variation of oxygen pressure had little effect on the

germination and growth of the ordinary fruit rot organisms, such as Botrytis,Fusarium,and Alternaria.

Uppal (65),in 1926,attacked this problem anew. From the results of his work on some species of the Peronosporales, he concluded that direct and indirect germination are two different phenomena,the former being a type of growth,and the latter a mere breaking up of the protoplasm into independent units. It seems that in the cases where the spore may germinate either directly or indirectly oxygen is necessary for direct germination. When the spore is characterized by only one of the types of germination,it may do so in the absence of Oxygen.

In 1928,Platz (40) conducted experiments to determine the relation of oxygen to spore germination of Ustilago zeae and found that atmospheres containing less than three percent of oxygen did not favour germination. He also found that spores completely immersed in water,tomato juice or gelatine,and kept in air tight vials did not germinate.

A few experiments were attempted to investigate the relation of oxygen to germination upon the spores of Ustilago nuda. In no case was any attempt made to determine the percentage of available oxygen.

Alkaline pyrogallol was used to remove the oxygen from the atmosphere above the solution where indicated. This was accomplished by connecting the pyrogallic solution with the nutrient,supporting the inoculum by means of air-tight tubing.

Table VI. The effect of a limited supply of oxygen on germination

| Method of Inoculation | Percent Germination |
|---|---|
| Flotation on water | 20-25 |
| Flotation on pales extract | 35-40 |
| Flotation on pales extract minus oxygen | 15-20 |
| Flotation on water minus oxygen | 1-2 |
| Suspension in water | 1-2 |
| Suspension in pales extract | 20-25 |

Table VI shows that the presence or absence of oxygen is a determining factor influencing germination. It is evident that maximum germination can only be obtained in the presence of an abundant supply of oxygen. Theresults also suggest that where sufficient pabulum is present the decrease in germination is not so great.

(6) The effect of the H-ion on germination and growth of smut

Recently the hydrogen ion ooncentration of culture media, whether liquid or solid,hass come to be regarded as a very important factor influencing physiological reactions. A great deal of literature is found on the toxic properties of the hydrogen and hydroxyl ions,but due to the lack of proper equipment the early investigators were unable to make accurate determinations of the hydrogen ion concentration.

Clark (8),in 1899,seems to have been the pioneer worker on the toxicity of acids and alkalis. Although the hydrogen ion concentration of his solutions was not known,he concluded that the OH ion was more toxic towards fungi than the H ion.

A few years later,Duggar(10) found that organic acids stimulated germination to some extent.

In 1912,Peltier (39) found that Botrytis cinerea grew best on acid media and gave poorest growth on strongly alkaline media.

Cooley (9),in 1914,working with Sclerotinia cinerea, obtained abundant spore production on acid media,with entire inhibition on the alkaline side.

Zeller (74),in 1916,found that Lenzites saepiaria was very sensitive to alkaline media but grew luxuriantly on an acid medium.

In 1918,Meacham (33),working with species of Lenzites, Fomes,Coniophora,and Merulius, asserted that growth was not inhibited until a very high H-ion concentration was reached. In general,growth proceeded in a straight line until about pH 2.6 and then decreased almost abruptly,the range 2.6 to 1.9 being termed the critical range. The limiting pH value seems to have been about 1.7,with the maximum growth at about 3.0.

Webb (69),during the following year,obtained a secondary maximum with a species of Fusarium at pH 7.4,and a minor secondary maximum with Penicillium cyclopium at pH 7.0 to 7.4.

Hopkins (22),in 1921,found that Gibberella saubinettii exhibited a double maximum of growth,with a minimum at pH 5.0 to 5.5. Fusarium isolated from "scabby" wheat gave a similar depression.

During the same year,Webb (67) stated that germination was a process strikingly supported by conditions of active acidity,and that usually relatively low germination percentages occurred in alkaline media. He also stated that growth and germination may not both be favoured by the same hydrogen ion concentration. The range of germination and the magnitude of the germination quantities,as influenced by the hydrogen ion concentration,depend upon both the organism and the medium.

In 1922,Hophins (21) found that Gibberella saubinettii

will tolerate a wide range of acidity and that it showed a minimum in the growth curve from pH 5.5. to 6.0 with a double maximum. It was found that this held in three series with striking similarity. He concluded that the effect on the growth was due to the hydrogen ion concentration and not to other molecules or ions.

Scott (53),in 1926,tested out germination of spores of Fusarium lycopersici in 0.001 M potassium phospahte adjusted to different values and found a primary maximum at pH 4.5, followed by a minimum at pH 5.0 to 5.5 anda secondary maximum at pH 7.0. The germination was greatly inhibited in solutions acid to pH 5.4 when toxic anions like cyanide and the acid dye Eosin were used with concentrations near the toxic limits. The reverse was true of the toxic kations such as mercury,copper,and the basic dye methylene blue. This fits into his idea of an iso-electric point,which phenomenon had already been brought to his attention as a result of experiments carried on afew years previously (52). In this earlier soil experiment conducted in the greenhouse,he found that a minimum of tomato wilt occurred at pH of 6.4 to pH 7.0, but that the maximum of infection occurred on either side.

Webb and Fellows (68),in 1927,working with Ophiobolus graminis,found that there was a tendency towards the formation of a bimodal curve in the growth characteristics of this fungus. A primary maximum occurred at pH 5.8 with a minor secondary maximum at pH 9.6.

At about the same time,White (70) tested out twenty-four

strains of Fusarium lycopersici. In general,the hydrogen ion growth curves were similar. The two maxima growth points in the curve were evident. The first maximum point was rather consistent with the strains,but the minimum growth point and the secondary maximum,as well as the limit of alkalinity tolerated,was found to be more or less variable.

Youden and Denny (71) believe the method used by Robbins to be unsatisfactory,as they can get the same shifts in pH values from water extracts of plant tissues which contain no colloids whatever. There is,nevertheless,no evidence denying the existance of an iso-electric point for plant tissues. Robbins and his co-workers (47) determined what they call the isoelectric point of proteins by placing pieces of plant tissues in a series of buffer solutions and then determining the changes in pH undergone by the buffer. The pH value of the buffer solution that remained unchanged after a period of contact was called the "isoelectric point".

From the above review of literature as published by the recent investigators,it seems that,for each particular organism,there are two specific ranges of H-ion concentration at which germination and growth are at the maximum. Moreover, it also suggests that,within the limits of tolerance,a slightly acid condition of the substrate is more favourable to germination and growth than an alkaline one. In fact it would appear that alkalinity is somewhat toxic to fungi. With these results in mind,the following experiment was carried out to determine the effect of the hydrogen ion on the germination and growth

of Ustilago nuda.

The first experiment performed was to determine the influence of the hydrogen ion concentration on spore germination. Two media,sterile distilled water,and the extract of pales, were used.

The spores were removed from a portion of an infected head by shaking in a small bottle containing a few glass sterile beads and filled with sterile distilled water. In this way a good spore suspension was obtained. If greater dilution was required to partly offset the effect of any contamination,more sterile water was added until the required concentration was obtained.

Two series of solutions of varying hydrogen ion concentrations were made up,one of water and the other of pales extract. This extract was prepared by steaming five grams of barley seed in one hundred and fifty cubic centimeters of water in the autoclave for one and one half hours. The adjustments were made by first preparing a titration curve to determine the exact amounts of .3 N HCl and .3 N KOH which must be added to five cubic centimeters of water or the extract to make up a series of varying hydrogen ion concentrations at increments of 0.4 from pH 9.4 to pH 3.0. The Quinhydrone Electro-potentiometer was used throughout to make the necessary determinations. A small quantity of the consentrated spore suspension was then added to each tube of a known pH value.

The results of the experiment as given in Figure 4 in graphic form show that the percentage germination is decidedly greater in the extract of pales. The pH range is also considerably increased. In water the range extends from pH 3.6 to pH 8.4, whereas in the extract it extends from pH 3.2 to pH 9.2. This stimulatory effect resulting in increased germination is possibly due to the presence of nutrient materials in the extract. The striking feature of the experiment is the production of a double optimum, with an apparent isoelectric point existing between pH 5.5 and pH 6.0. The hydrogen ion concentration of the primary and secondary maximum, as well as the so-called isoelectric point, varies slightly with the media. Webb (67), in an earlier and more detailed experiment, found the maxima phenomenon to be very promounced. Sideris (54) also found a similar phenomenon, but he termed it the "iso-metabolic point". His work is based on shifts in the reaction or changes in the hydrogen ion concentrations induced by plant tissues.

A second but similar experiment was conducted on potato-dextrose agar, adjusted in the same manner as above, in order to test the effect of the hydrogen ion concentration on the growth of this organism. The series was adjusted to include a range from pH 3.0 to pH 9.0, at increments of 0.5. The inoculations were made from pure cultures, using the same amount of inoculum in each case. The series was made up in duplicate.

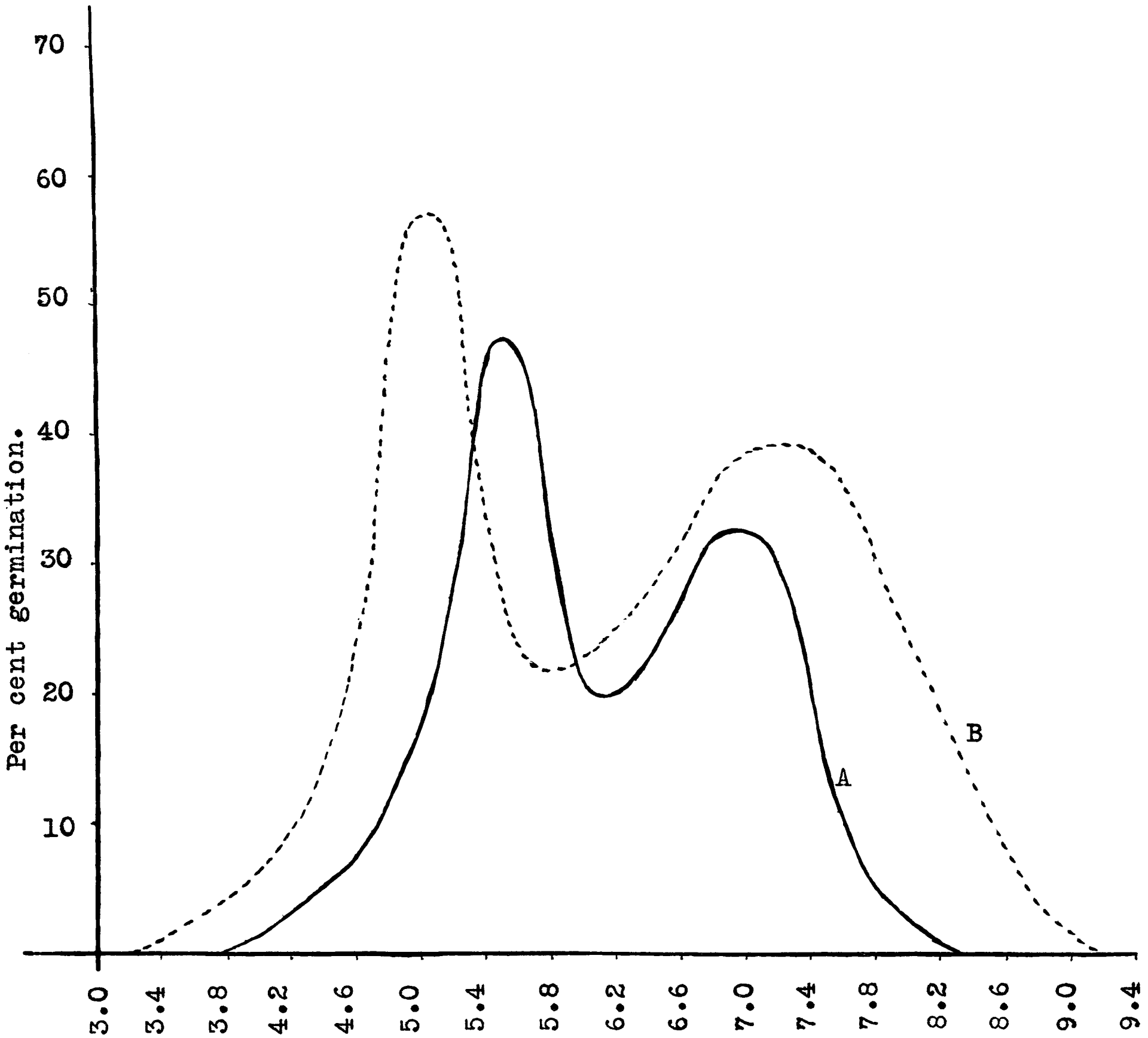
Figure 5 shows that an alkaline condition of the media

is more favourable to the growth of the fungus than an acid one. The hydrogen ion concentration conditions favouring growth are evidently the reverse to those favouring germination. This may be due to a difference in the nature of the media employed. There is,however,the double maximum,in this case,on the alkaline side.

Another series covering the same range was set up,using extract of pales as the nutrient medium. The adjustment of the various hydrogen ion concentrations and inoculations was made as indicated above.

No weighable quantities of mycelium were obtained in this experiment,but growth occurred approximately within the same limits with slightly decreased toleration,on the alkaline side, from pH 9.0 to pH 8.5. It was observed that only one flask in the entire seriescontained a growth that resembled the production of what might be called a normal mycelium. The hydrogen ion concentration of the solution in this flask was determined and found to have a pH value of 5.8. It was originally 6.0. Here the mycelium branched profusely and produced long hyphal threads. The entire appearance was that of a soft fleecy mat spreading over the bottom of the flask with no growth rising to the surface of the solution. In all the other flasks,on both the alkaline and acid side,the mycelium appeared whitish and more flaky,spreading only over the surface of the solution. A microscopic examination revealed wide abnormalities in mycelial growth,characterized by extensive budding (See Plate III,fig.8) of the yeast type,not the production of spores

Figure IV. Per cent Germination in Relation to H-ion Concentration.




A- Distilled water
B- Extract of pales
Incubation period 72 hours.

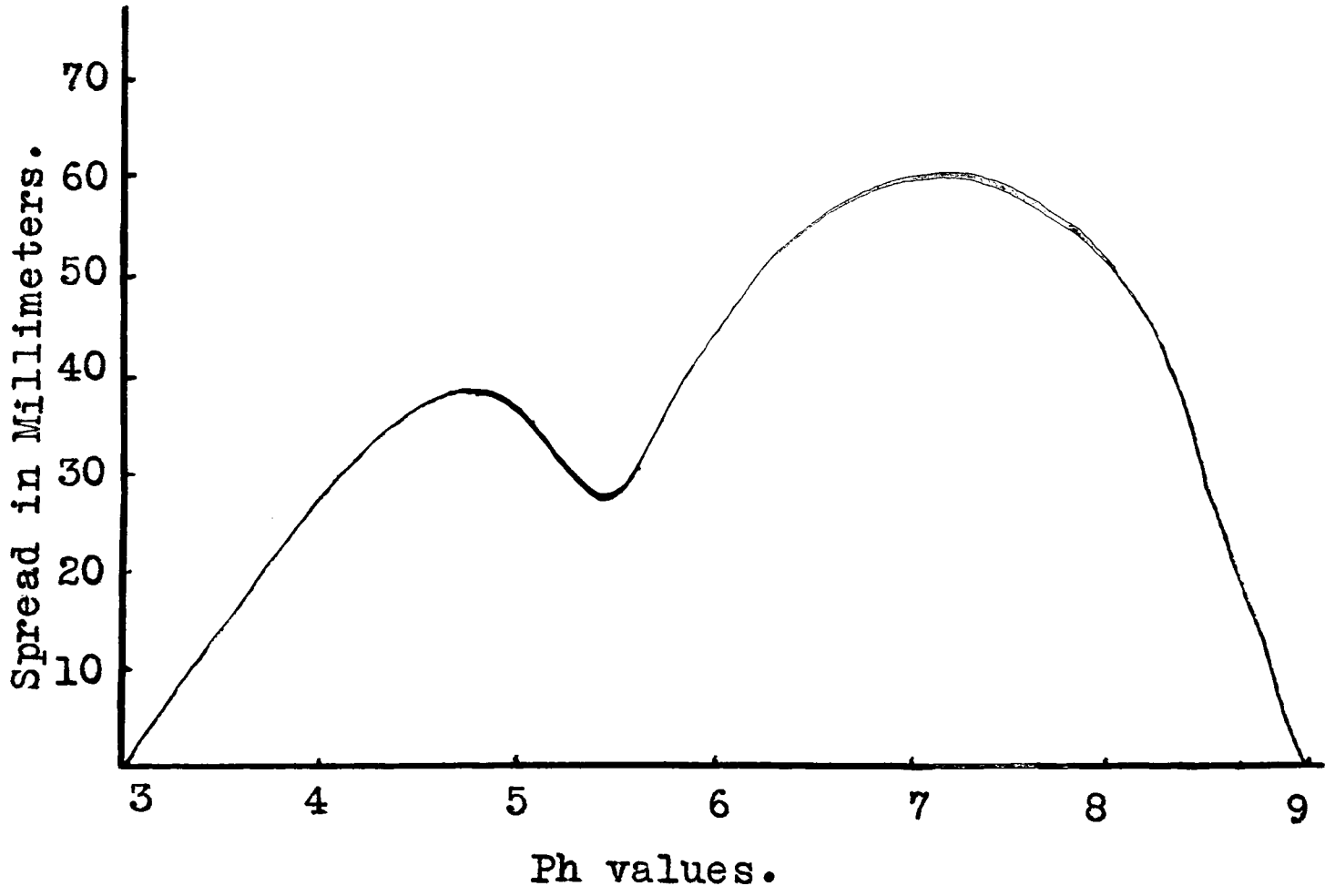
but merely a production of new cells. These cells varied in length and breadth and at the same time exhibited a pronounced tendency to continue budding It would seem that budding is a phenomenon closely related to or in some way dependent on the hydrogen ion concentration of thesubstrate or medium. It also appears that the pH value of 5.8 corresponds to the so-called isoelectric point,as referred to by Scott (52) and later by Robbins (48),because growth seems to be less here with a maximum on either side.

(7) The effect of toxic agents on germination in relation to the isoelectric point

Scott (51),working with Fusarium lycopersici, has shown that growth of the fungus was greatly inhibited in culture solutions initially acid to pH 5.5 when the toxic salts KCN or KI were present at or near their toxic limits. On the contrary he found that the addition of toxic salts ($HgCl_2$ or $CuSO_4$) to the nutrient solutions showed greatest inhibition of growth on the alkaline side of pH 5.5. He has also shown from a previous work (53),that pH 5.5 is a critical point for spore germination of this fungus as well as for the retention of acid and basic dyes by mycelial mats. In the interpretation of the results of his experiments,he maintained that the toxicity of the cations is more pronounced on the alkaline side of the equilibrium (pH 5.5),while the opposite is true of the toxic anions.

In view of this it was felt that the percentage germination of spores from loose smut infection produced in the extract of pales adjusted to different pH values would

Figure V. Growth of the Organism on Potato Dextrose Agar in Relation to H-ion Concentration.



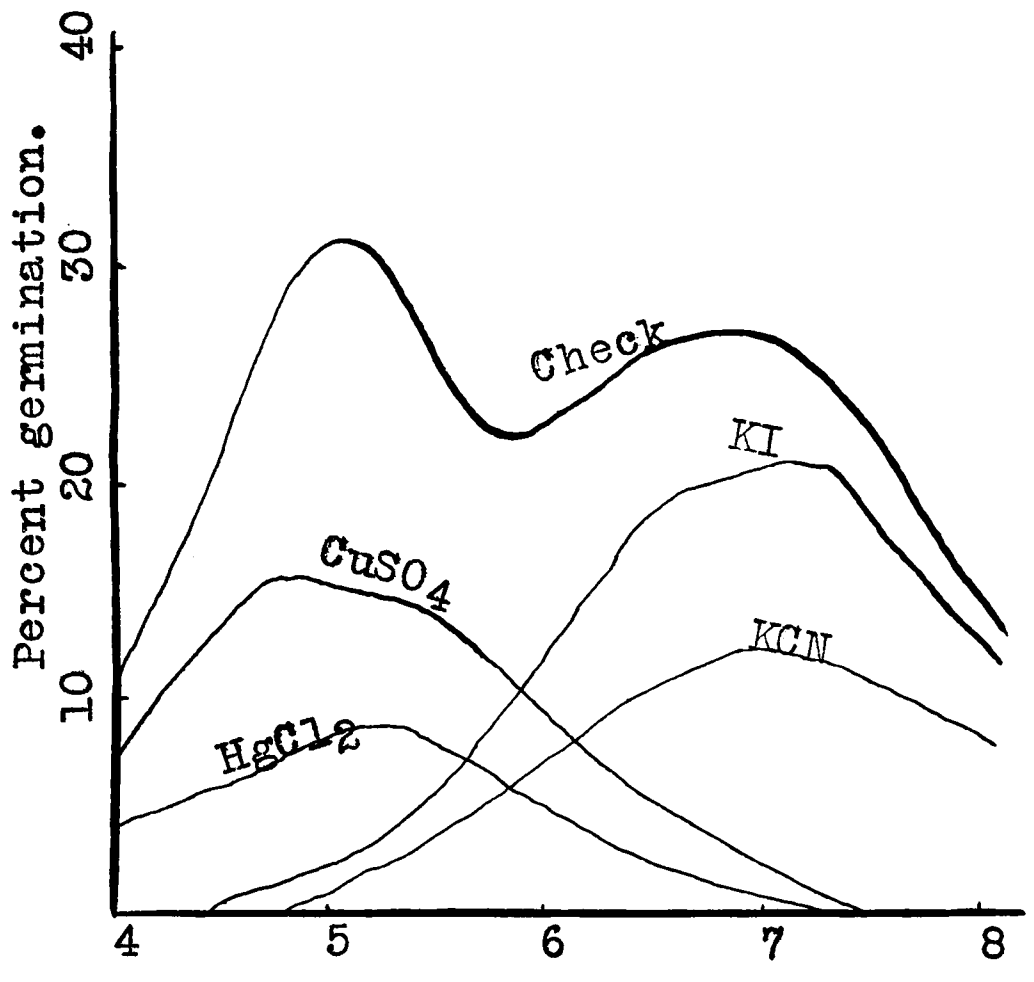
likewise show a differential response. Therefore a series of experiments was carried out in which the same toxic anions and cations as used by Scott (51) were added to culture solutions of different hydrogen ion concentrations. Potassium iodide and potassium cyanide furnished the toxic anions while mercuric chloride and copper sulphate furnished the toxic cations. Five cubic centimeters of the nutrient medium was used in small test-tubes. After the adjustment of the reactions and sterilization of the culture solutions, the toxic salts were aseptically added so as to give the following concentrations in the solution of each series: 0.006 % $HgCl_2$, 0.007 % $CuSO_4$(hydrated), 0.03 % KCN, and 1.5 % KI. Each culture was inoculated by adding one drop of a concentrated spore suspension, and incubated at room temperature for forty-eight hours.

Figure 6 gives the results of the above experiment graphically. These results are quite analogous to those obtained by Scott (51) in his work, and are explained, at least in part, by the assumption of a protein analogy for the fungous tissue and the existance of an isoelectric point at approximately pH 5.8.

(8) The effect of different media on growth

That the relative rate and amount of growth of any organism is largely dependent upon the substrate or the nutrient media to which it is exposed, has been an established fact for many years. As the comparative growth of this

Figure 6. Germination of spores of U. nuda in culture solution at different initial H-ion concentration with and without toxic salts as indicated.



organism has not been tested on various media,no extensive literature on this phase of the work is available.

Two hundred cubic centimeters of carrot decoction,extract of pales,peptone broth,Czapek's solution,Richards(White) solution,and Richards-Duggar's solution were each inoculated with equal amounts of inoculum. The inoculum in each case was placed on thin cork plates(floats) aseptically added to the solution. The cork,before being used,was boiled in water for several hours to remove all acids and then sterilized. These floats permitted aerobic growth when the solution itself was favourable to development. Several agars were also employed, potato-dextrose,Richards'(White),Richards-Duggar's,and corn-meal were selected. All cultures were made in triplicate, and a 0.4 mm. loop was used to transfer equal portions of mycelium from the outer edge of an original culture of the organism grown on potato-dextrose agar.

The carrot decodtion was considerably more favourable to growth than the extract of pales. In view of the results obtained from the use of the synthetic media,it would seem that dextrose,as a source of carbon, is more conducive to growth than sucrose. This was also borne out on the different agars.

Table VII shows the results obtained from this test.

(9) Physiologic specialization

Physiologic specialization has long been known in the smut fungi,although,in many cases,not definitely stated.

Table VII. Growth of the organism on different nutrient media

| Medium used | Amount of growth |
|---|---|
| Carrot decoction | 0.328 grms. |
| Extract of pales | 0.252 " |
| Peptone broth | 0.053 " |
| Czapek's solution | 0.000 " |
| Richards'(White) solution | 0.125 " |
| Richards-Duggar's solution | 0.000 " |
| Potato-dextose agar | ++++ |
| Richards'(White) agar | +++ |
| Richards-Duggar's agar | ++ |
| Cornmeal agar | + |

Note: The number of plus (+) signs indicates the relative growth obtained.

According to Rodenhiser (49), Ustilago nuda (Jens.) K. & S. and Ustilago tritici (Pers.) Rostrup really are physiologic forms and not true morphological species. Even Jensen (25) at first considered them as two varieties under the same species. It seems that the principal difference between them is physiologic and not morphologic. Moreover, it appears probable that there might be physiologic specialization within the so-called species of loose smut of barley.

Reed (45), in 1924, demonstrated the existence of strains with Ustilago avenae (Pers.) Jensen and U.levis (K. & S.) Magnus.

Faris (14), in the same year, showed that there was specialization within Ustilago hordei (Pers.) K. & S.

In 1926, Stakman and Christensen (7,56), also Tisdale and Johnson (64), showed that there were physiologic races within Ustilago zeae.

Rodenhiser (49), in 1926, demonstrated that there were very pronounced and permanent cultural differences in various forms of Ustilago nuda. He maintains that these forms are not mere varients but that they are permanent. Moreover, he states that the cultural resemblances between certain forms of U. nuda and U. tritici are so striking that some strains of U. nuda may possibly infect wheat.

Tisdale and Griffith (62,63), in 1927, also presented data which show that variants exist in this species and suggest that these variants apparently correspond to the so-called strains or physiologic forms. Their experiments were pathogenicity tests, and the results show that various smut

collections not only showed differences in their ability to produce smutted heads but they also affected germination and host development differently.

In the present work,five varieties of barley,namely O.A.C.21,Bearer,Star,Mensury 32 M.C.,and Guy Mayle 513 M.C. were selected. These are all six-rowed barleys commonly grown in Canada. All,with the exception of Guy Mayle,have proven to be sufficiently good to warrant a place in the provincial Comparative Tests. O.A.C.21 is probably the most widely grown six-rowed barley in Canada. Guy Mayle is a hulless variety and has been found to be the highest yielding variety of this type grown at Macdonald College.

O.A.C.21 originated at the Ontario Agricultural College at Guelph; Bearer,at the C.E.F. in Ottawa; Star,at the Svalof Plant Breeding Station,Sweden; Mensury 32 M.C. and Guy Mayle 513 M.C.(hulless) are both selections from Macdonald College.

Seven hundred and fifty seeds of each of the above varieties were dehulled and divided into five groups of one hundred and fifty each. One group in each variety was inoculated by the method outlined above for a preceding experiment,with viable spores from each of the following sources; Alameda and Kincaid,Sask.,Ottawa,Ont.,Macdonald College,P.Q., and Kentville,N.S.

The seeds were planted at a depth of three quarters of an inch in shallow flats,which were then placed on benches in the greenhouse.

The results of the experiment show that,under the existing

conditions,these varieties tested are not susceptible to seedling infection. Out of a total of 3750 plants,only two infected heads appeared,and the writer has every reason to believe that this infection was the result of an earlier natural floral infection and not the result of an artificial inoculation of the seedling. It is also felt that if an artificial inoculation of a seedling such as this is possible, a wider selection of more susceptible varieties would show varietal differences in their response to inoculation and infection with these spores from the various sources.

Isolations were made as indicated under the heading "Experimental Proceedure" from the collections of infected heads. The monosporous cultures were removed and transferred to fifty cubic centimeters of potato-dextrose agar in one hundred and fifty cubic centimeter Erlenmeyer flasks,and allowed to grow for several weeks.

Various cultures from the same sources exhibited wide morphological differences as to growth characters,e.g., aerial mycelium,fluffiness,spiral or whorling tendency,colour, etc. Plate II illustrates these cultural differences as described in Table 8,which gives,in brief, a description of seven variants or forms that show the greatest morphological differences. As far as the work went the differences in these forms remained constant and permanent throughout in triplicate cultures. They,therefore,should be considered as definite strains or physiological forms.

Table 8. Cultural Characteristics of Physiologic Forms of Ustilago Huda on Potato - Dextrose Agar.

| Source of Collection | Form | Age in Days | Size in mm. | Color | Topography | Surface | Aerial Mycelium | Margin |
|---|---|---|---|---|---|---|---|---|
| Kentville, N.S. | 1 | 25 | 12 | Whitish | Center umbonate, faintly radially striated, counter-clockwise to margin, margin appressed, covered with numerous small tufts. | Center coarsely velvety, rays waxy | X | Myceloid, almost entire |
| | | 75 | 51 | Dark cream, appearing buff-like | Raised to convex, center smooth, coarse radial striations, counter-clockwise, irregularly depressed with a few tufts | Waxy | X- | Entire |
| Indian Head, Sask. Var. Junior | 11 | 25 | 14 | Buff in center to a light tan | Slightly pulvinate - no striations, entire surface covered by numerous small tufts | Velvety | X | Myceloid, slightly undulated |
| | | 75 | 50 | Center dark cream becoming brown, margin light brown | Pulvinate - no striations - surface covered with large concise white tufts | Waxy, moist | X= | Not myceloid indefinite |
| Indian Head, Sask. Var. Albert | 111 | 25 | 16 | Cream | Center umbonate, covered with numerous fine tufts, radiations counter-clockwise coarse and uplifted | Moist and smooth | 0 | Myceloid, almost entire |
| | | 75 | 53 | Dark cream | Roughly umbillicate, surrounded by tufts of coarse rope-like strands, tufts large and aggregate | Waxy | 0 | Not myceloid, entire |
| Kincaid, Sask. | 1V | 25 | 15 | Light cream | Umbonate, center composed of numerous aggregate tufts, slight striations, direct to margin | Moist, waxy | 0 | Myceloid entire |
| | | 75 | 48 | Dark cream | Convex, roughly striated in sectors, subteranean mycelium showing through in dark patches, few isolated tufts. | Moist | 0 | Not myceloid, entire |
| Alameda, Sask. | V | 25 | 13 | Light becoming hyaline at edges | Pulvinate, center covered with fine dense tufts, not striated | Velvety | X | Myceloid, margin irregular |
| | | 75 | 47 | Black shading to brown | Hummocky, striations fine and sectored | Rough and waxy | 0 | Not myceloid, entire |
| Ottawa, Ont. | V1 | 25 | 16 | Dark cream becoming brown at margin | Almost flat with a central abrupt mound, striations very short and fine at margin | Waxy and smooth | 0 | Myceloid, entire |
| | | 75 | 55 | Dark cream peppered with brown and black spots grading to black and light brown at margin | Center thrown up into numerous papillae, grading off into long sharply defined coarse radiations | Waxy | 0 | Not myceloid, entire |
| Macdonald College, Var. O.A.C. 21 | V11 | 25 | 15 | Light cream | Umbonate, center composed of numerous aggregate tufts, slight striations, direct to margin | Moist, waxy | 0 | Myceloid, entire |
| | | 75 | 51 | Center black | Center thrown up into numerous ill-defined papillae grading off into medium well-defined unevenly striated | Waxy | 0 | Not myceloid, entire |

Note:- X= aerial mycelium abundant
X- " " medium
X " " slight
0 " " none

Transfers were made from the original stock cultures to potato-dextrose agar poured in small culture dishes. A 4 mm. platimum loop was used to transfer portions of inoculum of uniform size from the outer edge of the original cultures. Each plate was inoculated to study the association of the different forms on culture media. To accomplish this, two transfers were made of the same or different forms at a distance of one inch apart. All the combinations possible from the various sources were made, and the cultures were then placed under bell jars and allowed to develop for a period of forty-five days at room temperature.

Table IX gives the results of the experiment according to Porter's (41) classification. If the phenomenon of inhibition is correlated with growth of identical organisms, and the phenomenon of mutual intermingling is correlated with growth of different organisms, biologic forms must undoubtedly exist within this species.

(10) Toxic products of the fungus

A great amount of work has been done on the toxic properties of fungi, and the available literature dealing with the subject is proportionally as great. It is generally believed that some fungi do produce a certain amount of toxicity which may affect the host in various ways. The problem as related to the toxic effect of Ustilago nuda upon its host has not been sufficiently investigated to warrant a lengthy review of the general literature on the subject.

Table IX. Association of various cultures of different strains

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | C | A | A | C | C | B | A | A | A | A |
| 2 | A | E | B | C | B | B | A | A | A | A |
| 3 | A | B | C | A | A | C | A | A | A | C |
| 4 | C | C | A | E | A | A | A | A | A | A |
| 5 | C | B | A | A | C | C | C | B | A | B |
| 6 | B | B | C | A | C | C | A | A | A | A |
| 7 | A | A | A | A | C | A | C | A | A | A |
| 8 | A | A | A | A | B | A | A | C | A | C |
| 9 | A | A | A | A | A | A | A | A | C | B |
| 10 | A | A | C | A | B | A | A | C | B | E |

Legend:
1. Barley smut from Kentville,N.S.
2. " from Indian Head,Sask.,var. Junior
3. " " " " " " Albert
4. " " Alameda,Sask.
5. " " Indian Head,Sask.,var. Feeder
6. " smut from Kincaid,Sask.
7. " " " Ottawa,Ontario.
8. " " " Macdonald College,var. Success
9. " " " " " " B27-22
10. " " " " " " O.A.C.21

A - Mutually intermingling
B - overgrowing
C - Slight inhibition
D - Growth around
E - Inhibition at a distance

Tisdale (58), in 1924, maintained that a heavy smut infection caused by Ustilago nuda may kill the young seedlings. He has shown that with a definite percentage of inoculated seeds there was a pronounced retardation of germination, some of the germinated seeds even failed to mature. He has attributed this phenomenon to the toxic properties produced by the fungus in the host.

The present writer has observed in his investigations a similar condition. The dehulled seeds which were heavily inoculated with spores do in some cases show a pronounced retardation in germination and growth. At the same time, he believes that this condition cannot be definitely attributed to the effects of the toxic properties produced in the host by any particular fungus. The present method of inoculating seeds with smut spores is open to considerable criticism, because one cannot be sure that these spores are absolutely free from contamination. If, by any chance, spores of other organisms are present, they, too, may be partially or wholly responsible for the retarding effect upon germination and the ultimate failure of growth of the plants.

It was felt, however, that if toxic properties are produced by the organism in the host, the same should occur in pure culture as well. Two hundred cubic centimeters volumes of both carrot decoction and extract of pales were separately inoculated with equal portions of mycelium removed from the outer edge of pure cultures. Growth was allowed to continue

in these nutrients for a period of two and one half months. At the end of this time, the extracts were filtered through paper, and ten cubic centimeter volumes were aseptically distributed among various test-tubes. Small plugs of sterile cotton were forced into the tubes until they came into contact with the solution. Three barley seeds of the variety O.A.C.21 were then carefully dropped into each of the tubes. A control was also set up in a similar way, using water as the nutrient medium. The results are shown in Table X.

The extract of pales slightly increased germination. There appeared to be something in the carrot decoction which inhibited germination. This may be a toxic product produced by the fungus. If this be true, the supporting media must undoubtedly be a contributing factor as germination was not affected by the other solutions.

(11) Fungicidal coefficients

Rideal and Walker (46) have formulated a method of testing out the relative efficiency of bacteriological disinfectants. From the standpoint of a sanitarian and the commercial man it is of considerable value. Moreover, it facilitates the work of the bacteriological technician, for it has been found to be a convenient method of comparing the efficiency, under laboratory conditions, of a proposed disinfectant with that of some standard germicide such as pure phenol.

A detailed account of the procedure which should be

Table X. The effect of filtered cultured media in which Ustilago nuda had grown on germination of barley seed

| Medium | Barley seeds | | |
|---|---|---|---|
| | Number planted | Number germinated | Percent germinated |
| Carrot decoction | 54 | 22 | 42.9 |
| Distilled water | 30 | 24 | 80.0 |
| Extract of pales | 51 | 48 | 88.8 |
| Distilled water | 30 | 26 | 86.6 |

followed,with some suggestions to revise the original,is given by Eyre (11),who suggests that conditions as nearly uniform as possible should be aimed at in order that the work of different observers may be compared. His method has many points in common with the "drop" method,now known as the "Rideal-Walker Test".

Park and Williams (38),as well as others(16,30,42) emphasize the importance of the standardization of disinfectants and give in concise form the method of determining the relative efficiency of various bactericides and disinfectants.

Young and Cooper (72) have devised a method which is the nearest approach to real application in the field of plant pathology. Their method has been formulated to determine the fungicidal value of various fungicides in terms of a standard copper-sulphate solution in preference to phenol.

It has been felt for a considerable time that some such test would be of great value to the plant pathologist in aiding him to determine the comparative or relative values of various fungicides by testing them out on particular organisms against some standard fungicide. With this object in mind, the writer has attempted to make such revisions to the tests already designed in order that they may be more applicable in phytopathology. The modifications suggested are not great in any case,but it was felt that certain changes were essential to make it more adaptable to this purpose. A great deal more work may have to be done on this particular problem before a satisfactory test is finally completed. Fungicides

to control organisms other than Ustilago nuda will have to be subjected to the test before it can be entirely recommended. However,it is hoped that the method outlined below and the results obtained are of sufficient importanée to warrant further investigation.

The general considerations on this subject may be grouped under three headings: (1) Test Organism,(2) Fungicide, (3) Environment.

(1) Test Organism - Ustilago nuda

A portion of an infected head of grain is placed in a twenty cubic centimeter glass container with a few sterile glass beads. Sterile distilled water is then added. All are gently shaken together for a few minutes. Care must be taken not to destroy or unduly mechanically injure too great a number of spores. At the same time,a reasonably uniform spore suspension must be obtained. Large bits of the plant tissue left in the suspension can be removed by means of a sterile platinum loop.

(2) Germicide

Only the standard disinfectant used for comparison will be considered here. It should be one that is not subject to variation in its chemical composition. Copper sulphate has obtained almost universal use.

Prepare solutions of varying percentages by weighing out the quantity of copper sulphate required for each and dissolving in one hundred cubic centimeters of distilled water in an accurately standardised measuring flask. The solutions

must be prepared fresh as required each day. Determine what is the weakest concentration of the disinfectant that will produce a lethal effect on the organism within definite time limits of two and one half to thirty-five minutes.

(3) Environment

a. Temperature should be kept at eighteen to twenty degrees Centigrade,as the sterngth of the disinfectant varies with the temperature.

b. Relative proportional bulk of organism and disinfectant; five cubic centimeters is a convenient amount of the disinfecting solution to employ,and to this add one tenth cubic centimeter of the spore suspension.

c. Bulk of the sample removed from suspension plus disinfectant solution at the end of each of the time periods. One quarter of a cubic centimeter is sufficient to afford a fair sample of spores in suspension,and at the same time is not sufficient to exert any appreciable inhibitory influence on germination when transferred to the sub-culture medium.

d. Sub-culture medium.

Extract of pales provides a very satisfactory nutrient medium. It is best prepared by heating five grams of barley seed in one hundred and fifty cubic centimeters of distilled water for one and one half hours in a steam autoclave and then filtering. A fluid medium is essential in order to obtain immediate dilution of the disinfectant carried over. At the same time it is advantageous to employ a medium which favours the growth of the organism.

The amount of the medium present in each vial is a matter of some importance, since the medium not only dilutes but also provides a pabulum for the organism. Five cubic centimeters seem to be sufficient in most cases.

e. Temperature and incubation period

Incubation should be done at room temperature. In order to determine whether or not the organism has been destroyed, observations must always be continued - when growth appears to be absent- up to the end of three of four days before recording 'no growth'.

f. Apparatus required

Sterile 10 c.c. vials or test-tubes
Test-tuberack
Sterile graduated pipettes, 1c.c. (in tenths)
" " " 5 c.c. (in c.c.)
" corks to fit the glass vials containing the disinfectant. These corks should be pierced with 4 m.m. platinum loops and the loops should extend well into the vials.
Electric signal clock or stop watch
Sterile forceps
Sterilized glass beads
Shaking machine
Glass marking pencil

g. Material required

Percentage solutions of the fungicide to be tested
Spore suspension of Ustilago nuda
Vials of extract of pales

Preliminary tests

Determine the lethal effect of a series of five solutions of germicide - (say 1:100, 1:250, 1:500, 1:750, 1:1000) at contact times of $2\frac{1}{2}$, 5, 25, 35 minutes in the following manner:

1. Arrange five of the small test-tubes, marked A to E,

in the lower tier of the test-tube rack.

2. Into tube A pipette 5 c.c. germicide-x 1:100 solution.
" " B " 5 " " 1:250 "
" " C " 5 " " 1:500 "
" " D " 5 " " 1:750 "
" " E " 5 " " 1:1000 "

3. Arrange twenty tubes of the subculture medium in the upper tiers of the test-tube rack, preferably in four rows, those in front numbered consecutively 1 to 5, 6 to 10, 11 to 15, 16 to 20.

4. Place the spore suspension of about 10 c.c. close to the left of the test-tube rack.

5. Put down the forceps on the bench with the sterile points projecting over the edge. Without taking the tube from the rack, remove the cotton plug from the tuba A, and lower the pipette, with the rubber washer affixed, to the open mouth of the tube; with the help of the forceps to steady the washer, push the pipette on through the hole until the point of the pipette has reached to within a few millimeters of the bottom of the tube.

6. Adjust in the same way a pipette and washer in the mouth of each of the other tubes, B, C, D, and E.

7. Set the alarm signal clock to ring for the commencement of the experiment and at subsequent intervals of $2\frac{1}{2}$, 5, 25, and 35 minutes.

8. Take up 0.5 c.c. of the spore suspension in a sterile pipette, graduated in tenths of a cubic centimeter, and stand by.

9. As soon as the bell rings, lift the pipette from tube A with the left hand, and from the charged pipette held

in the right hand deliver 0.5 c.c. of spore suspension into the 1:100 solution. Then replace the pipette and washer.

10. Raise the tube in the right hand and shake to ensure thorough mixing.

11. Repeat the process with tubes B,C,D,and E. The inoculation of the five tubes can be carried out very quickly, but a period of ten seconds should be allowed for each tube.

12. When the bell rings at 2½ minutes,blow through the pipette in tube A (this agitates the spores and disinfectant mixture and ensures the collection of a fair sample). Collect 0.3 c.c. of the mixture and transfer it to subculture No.1. Replace the washer pipette in tube A.

13. Repeat for the other four tubes. At the end of this operation,the subcultures numbered from 1 to 5 have been inoculated with spores which have been disinfected with various concentrations of the disinfectant for 2½ minutes.

14. Repeat these steps(12 and 13) when the bell rings at five,twenty-five and thirty-five minutes.

15. Incubate all the inoculated tubes at room temperatures.

16. Examine the tubes at the end of the incubation period and record the results in tabular form as shown in Table XI.

Final Test - Determination of the fungicidal coefficient

This comprises two distinct tests,one of the germicide-x the other of the standard copper sulphate.

1. Arrange six test-tubes clearly marked in the lower tier of the test-tube rack.

2. Pipette into each 5 c.c. respectively of six percentage solutions of x-disinfectant which the preliminary run has shown will include those affording lethal values at 2½ and 35 minutes. The writer has found that by adding one or more extra concentrations of the unknown disinfectant more accurate results can be obtained.

3. Arrange 36 tubes in six rows as indicated above.

4. Set the signal clock to ring for the commencement of the experiment and subsequently at 2½, 5, 10, 15, 25,and 35 minutes.

5. Complete precisely as indicated in the preliminary run.

Control Copper sulphate

Carry out a precisely similar experiment in which six percentage strengths of copper sulphate are arranged in the lower tier of the test-tube rack in place of the six strengths of the x-disinfectant.

Calculate the fungicidal coefficient by the following method:

(a) Divide the figure representing the percentage strength of the weakest lethal dilution of the copper sulphate control at the 2½ minute contact period by the figure representing the percentage strength of the weakest lethal dilution of the x-disinfectant at the same period. The quotient equals the copper sulphate coefficient at 2½ minutes.

(b) Similarly obtain the copper sulphate coefficient at the 35 minute contact period.

(c) Record the mean of the two coefficients obtained in

(a) and (b) as the mean copper sulphate coefficient,or simply as the fungicidal coefficient.

The details of the final determination are set out in Table XI.

The results of this experiment seem to indicate that a method similar to the Rideal-Walker test for disinfectants might be successfully worked out for fungicides. The attempt was made to perfect a satisfactory method by means of which the relative values of various fungicides could be determined in terms of a standard copper sulphate solution. The writer wishes to point out that the method is wholly arbitrary,and it is hoped that the significance of the various coefficients will not be misconstrued. It is intended that they should only represent the relative strengths of the various fungicides on this particular organism. It will be noticed that mercuric bichloride is by far the most effective,Ceresan,KCN,and Phenol being considerably weaker,yet all are stronger than copper sulphate.

The many obscure chemical changes which may take place in fungicides when used in this way have not been studied. No attempt has been made to determine the chemistry of the toxic effect on the fungus spores by the several solutions used.

The selection of the test organism constituted one of the unfortunate features of the work. For wholly adequate results,the test organism should be one of the first factors to be taken into consideration. The organism should be one which germinates and grows rapidly in culture.

Table XI. The Rideal - Walker Reaction

| Concentration of germicide in percent | Time in minutes | | | | | |
|---|---|---|---|---|---|---|
| | 2½ | 5 | 10 | 15 | 25 | 35 |
| Standard copper sulphate | | | | | | |
| 1.5 | x | - | - | - | - | - |
| 1.2 | x | x | x | - | - | - |
| 0.96 | x | x | x | x | x | - |
| 0.76 | x | x | x | x | x | - |
| 0.61 | x | x | x | x | x | x |
| 0.49 | x | x | x | x | x | x |
| Phenol | | | | | | |
| 0.8 | x | - | - | - | - | - |
| 0.64 | x | x | - | - | - | - |
| 0.51 | x | x | x | - | - | - |
| 0.4 | x | x | x | x | x | - |
| 0.32 | x | x | x | x | x | x |
| 0.25 | x | x | x | x | x | x |

Organism was Ustilago nuda grown on the extract of pales at 17°C. for 72 hours. (x) indicates germination, (-) indicates no germination.

$$\text{Fungicidal Coefficient} = \frac{\frac{1.5}{.8} + \frac{.76}{.4}}{2} = 1.93$$

In a manner illustrated in Table XI the following fungicidal coefficients were determined.

1. Mercuric bichloride ($HgCl_2$)

$$F.C. = \frac{\frac{1.5}{0.004} + \frac{.76}{0.00012}}{2} = 3354.16$$

2. Potassium cyanide (KCN)

$$F.C. = \frac{\frac{1.5}{.42} + \frac{.76}{.26}}{2} = 19.88$$

3. Ceresan

$$F.C. = \frac{\frac{1.5}{.012} + \frac{.76}{.008}}{2} = 110.00$$

## Discussion of Results

The effect of seed treatments, such as hot water, formalin, and ceresan, were investigated. It was found that formalin was wholly unsatisfactory to recommend, since very little, if any, control was secured by its use. The control offered by Ceresan was quite marked. It was concluded that this control was due to superficial mycelia present in the outer tissues of the seed which were rendered inactive by the penetration of the fungicide. Tisdale's (58) belief that it was due to the presence of spores on the surface of the seed was disproven as far as the varieties here tested were concerned because these varieties are apparently not subject to seedling infection. The hot-water treatment alone gave absolute control, and it is suggested that it is the only treatment which is wholly effective.

Tisdale (58) also maintained that a high percentage of infection could be obtained by inoculating the seed of certain varieties with spores previous to planting. It was felt that the susceptibility of varieties of barley commonly grown in Ontario and Quebec to seedling infection should be investigated. O.A.C.21 proved to be immune to infection from spores maturing either early or late. Bearer, Star, Mensury 32 M.C., and Guy Mayle 513 M.C. proved to be equally resistant to seedling infection. The results are undoubtedly such as show that the floral type of infection is the only one affecting these varieties.

Inoculation of young plants by hypodermic injection with a spore suspension failed to produce infection.

Contrary to the findings of Stakman (55) who maintained that good germination of the spores of Ustilago nuda was obtained after several months in storage, the present investigation revealed a pronounced dropping off in viability following the first few months. It was also found that no rest period was necessary as germination could be effected immediately. Environmental influences on the spores had little effect, if any, on this phenomenon: the loss in viability was as pronounced when the temperature was low and humidity high as when the temperature was high and humidity low.

Spores appearing early under greenhouse conditions did not show as marked a falling off in viability as those maturing in the field.. This may be due to the influence of a different environment during the maturing period.

The percentage germination varied with different media. Slightly higher germination was obtained on pales extract than on a two per cent cane sugar solution. Distilled water to a very small extent was more favourable to germination than tap water. It is suggested that the presence of chlorine in the tap water had some toxic effect on germination. Both of the nutrients used caused a decidedly higher germination than either water.

It was found that the temperature requirements for germination covered a wide range. Fair germination of one collection from Macdonald College was obtained at forty-five degrees Centigrade. The temperature was extremely high in this

case. In general the lower temperatures were more favourable to germination than the higher ones. At the high temperatures the spores seemed to have been rendered inactive with the elapse of time whereas at the lower temperatures (at approximately five degrees Centigrade) they ultimately germinate if the time factor were eliminated. A common optimum for germination apparently existed between the range 19-21 degrees Centigrade.

The Thermal Death Point of spores from several sources was determined. Spores from the majority of sources were inactivated at forty-three degrees Centigrade . The more viable spores required higher temperatures,none exceeding forty-nine degrees.

A study of the effect of temperature on the growth of the organism showed that there was an abrupt falling off in growth when exposed to temperatures above twenty-three degrees Centigrade. No growth occurred at twenty-seven or at five degrees. The optimum temperatures were apparently between fifteen and twenty degrees Centigrade.

In this connection it may be recalled that there was a marked difference in the percentage of smut produced from the 1928 and 1929 seed. Only a trace of infection was observed in the 1929 seed,while its parent crop produced approximately nine percent infection. The average maximum and average minimum temperatures during the bloom period of 1929 was 81.0°F. and 56.4°F. respectively. These high temperatures coupled with unusually low humidity is probably responsible for the decided decrease in infection.

The oxygen requirements of the spores for germination were quite marked. Spores floating on the surface of the solution, with access to oxygen, germinated much better than spores in suspension. Higher germination was obtained from spores immersed in a nutrient medium than from spores immersed in water. The exact percentage of available oxygen was not determined in any instance.

Experiments on germination and growth in relation to the hydrogen ion concentration constituted an important phase of this problem. In the presence of nutrient media traces of germination were found at an acid limit of pH 3.2 and an alkaline one of pH 9.2. Water adjusted in the same way reduced the limits of tolerance. Growth of the organism on solid media occurred approximately within the same limits(pH 3.0 to pH 9.0). On a nutrient solution of pales extract growth only occurred between pH 3.0 to pH 8.5. The results of all the experiments mentioned above definitely show a primary and secondary optimum condition for germination or growth. Between these two optima there exists a minimum point in the proximity of pH 5.8. The major optimum for germination occurred on the acid side; for growth on solid media on the alkaline side. No weighable amounts of mycelium were produced from the pales extract series, consequently, it cannot be definitely stated whether the acid or alkaline condition is more desirable. The pH value 5.8 undoubtedly corresponds to the so-called isoelectric point referred to by Scott and Robbins (47).

In agreement with the results obtained by Scott (51) it was found that toxic cations furnished by such salts as mercuric bichloride and copper sulphate were more toxic on the alkaline side of pH 5.8 and that toxic anions furnished by such salts as potassium iodide and potassium cyanide were more toxic on the acid side of pH 5.8. These results can be partly explained by the assumption of a protein analogy for the fungous tissue and the existence of an isoelectric point at approximately pH 5.8. It may be mentioned in this connection that the hydrogen ion concentration of any fungicide, capable of electrolytic dissociation, may be an important factor influencing the efficiency of the fungicide.

A more luxuriant growth of the organism was obtained with a carrot decoction than with pales extract. The results of the growth produced on the other media suggests that dextrose is a more desirable source of carbon than sucrose. The growth occurring from sucrose is possibly due to the inversion of the sugar as a result of sterilization.

Physiologic specialization appears to be a feature of this organism. Although seedling infection of several varieties failed to produce disease, cultural characteristics and association studies definitely indicated apparent differences. (See Table IX and Plate II.)

The organism when grown on carrot decoction for several weeks evidently produced some toxic product which decidedly inhibits seed germination. Tisdale's contention that the fungus has some toxic influence on its host is apparently a reasonable one. This phase of the problem, however, requires

further investigation.

Young and Cooper (72) have devised a method for determining the relative values of various fungicides in terms of copper sulphate solution of a standard strength. This method is a distinct contribution to science. Although it has many commendable features its application is limited. They suggest that the test organism involved should grow abundantly and rapidly in culture with a large spore production,and that growth in culture should present definite and quickly defined characters. If an organism which is to be subjected to the test must first satisfy these requirements one can readily see that its value will be insignificant.

The method outlined in this paper is wholly arbitrary. It is felt,however,that the fungicidal coefficients determined indicate in a comparative way the real value of these fungicides in relation to one another. Corrosive sublimate is apparently a very potent fungicide,while Ceresan,potassium cyanide and phenol come in the order named in reduced efficiency.

Summary

1. The hot-water treatment for loose smut is wholly effective. Ceresan offers some control. Formalin is entirely unsatisfactory.

2. Infection appears to be caused,in part,by superficial mycelia present in the outer tissues of the seed.

3. O.A.C.21, Mensury 32 M.C., Star, Bearer, Guy Mayle 513 M.C. are not susceptible to seedling infection.

4. Hypodermic injection of young plants with a spore suspension failed to produce infection in O.A.C.21.

5. No rest period for germination is required for spores of Ustilago nuda.

6. Viability of the spores decreases with the time, and produces a typical open S type of curve. The reduction in viability was more marked with spores maturing early.

7. The difference in germination of spores, exposed to different storage conditions, was negligible.

8. The media employed have a marked influence on germination. Nutrient solutions enhance germination.

9. No germination occurred above 45°C. or below 5°C., the optimum temperature for germination being 19-21 degrees.

10. With the elimination of the time factor the percentage of germination at the lower temperatures equals that of the optimum.

11. Thermal death points of the spores from various sources exist between 43 and 49 degrees Centigrade.

12. Optimum growth of the organism occurred between 15.5 and 20 degrees Centigrade, with an abrupt dropping off above 23 degrees, and with no growth at either 5 or 27 degrees Centigrade.

13. It is suggested that high temperatures, coupled with low humidity, during the bloom period of 1929, did not favour floral infection.

14. The oxygen supply is a determining factor influencing germination.

15. Germination is favoured by slightly acid media.

16. Studies on growth and germination in relation to the hydrogen ion concentration demonstrated the existence of a double optimum condition with an apparent isoelectric point at approximately pH 5.8.

17. The major maximum for growth on potato-dextrose agar occurred on the alkaline side.

18. Toxic cations are more effective on the alkaline side of the isoelectric point. Toxic anions are more effective on the acid side.

19. Carrot decoction was more favourable to growth than an extract of pales. Dextrose appears to be a better source of carbon than sucrose.

20. Germination of barley seeds on filtered carrot decoction in which was previously grown a culture of the organism was decidedly inhibited. This indicates the presence of a toxin produced by the fungus.

21. A method has been devised for determining the relative values of fungicides in terms of a standard copper sulphate solution. This method is based on the original Rideal-Walker reaction (46). The copper sulphate coefficients of a number of fungicides when applied against spores of Ustilago nuda are given.

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# PLATES

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## DESCRIPTION OF PLATES

Plate 1. Effect of temperature on growth of the organism

Upper row reading from left to right 8; 11; 15.5°C;

Lower row reading from left to right 20; 23; 27°C.

Plate 11. Cultural characteristics of the organism

Source of material -

a. Macdonald College - Var. Success.

b. Macdonald College - Var. B 2722.

c. Indian Head, Sask. - Var. Feeder.

d. Alameda, Sask.

e. Kincaid, Sask.

f. Kentville, N.S.

g. Ottawa, Ont.

h. Macdonald College - Var. O.A.C. 21.

i. Indian Head, Sask. - Var. Junior.

j. Indian Head, Sask. - Var. Albert.

Plate 111. Germination of spores and mycelial development.

a. 36 hours in pales extract ( pH 5.5).

b. 36 hours in pales extract ( pH 5.8).

c. 48 hours in water.

d. 72 hours in water.

e. Fusion of mycelium.

f. Normal mycelial growth at pH 5.8.

g. Normal mycelial growth in solution more acid or alkaline than pH 5.8.

Plate lV. Association of cultures -

a. Marked inhibition of identical cultures.

b. Slight inhibition of different cultures.

c. Very slight inhibition of identical cultures.

d. Mutual intermingling of different cultures.

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# Plate 1

# Plate 2

A B

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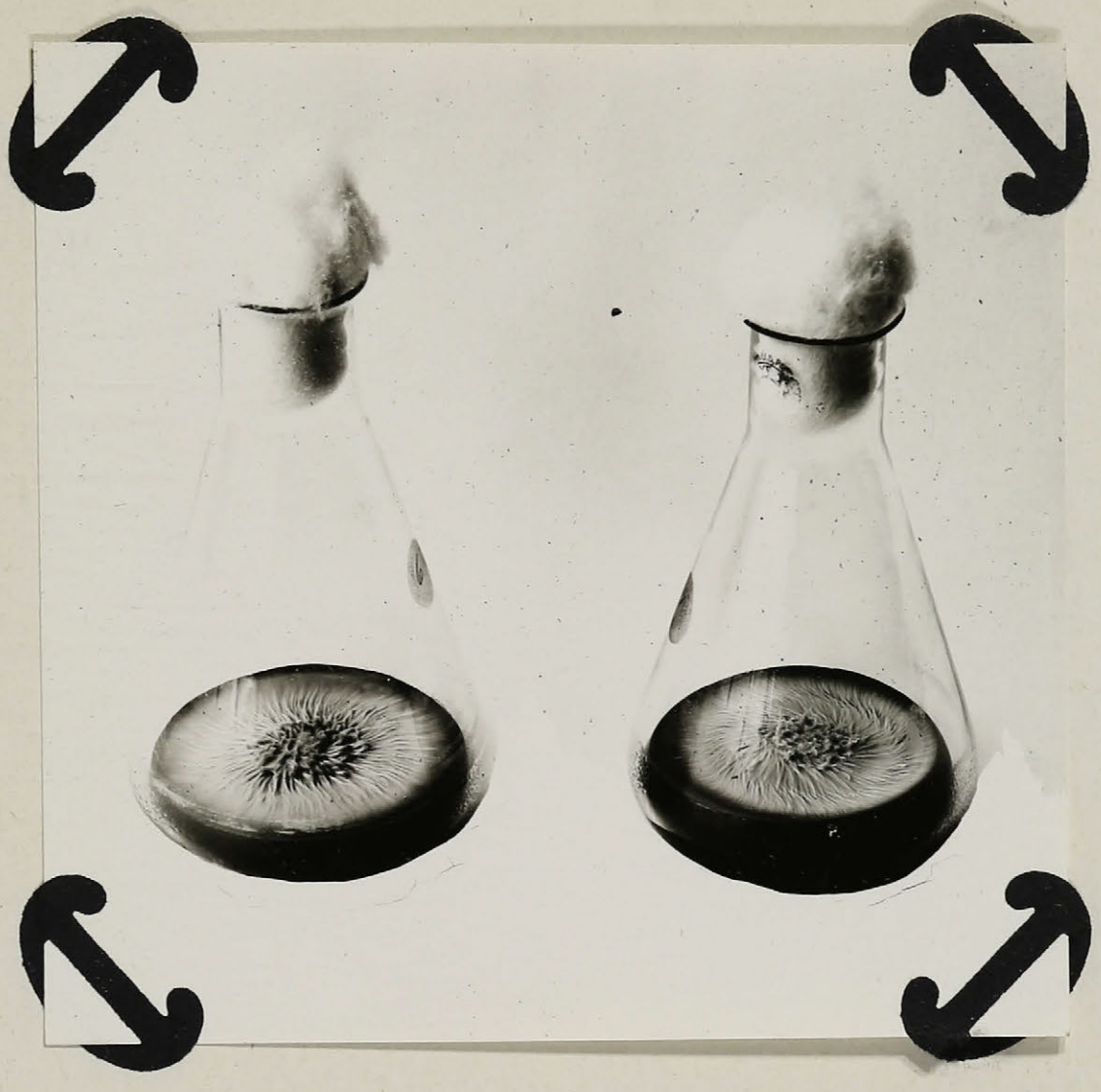
# Plate 2 cont'd



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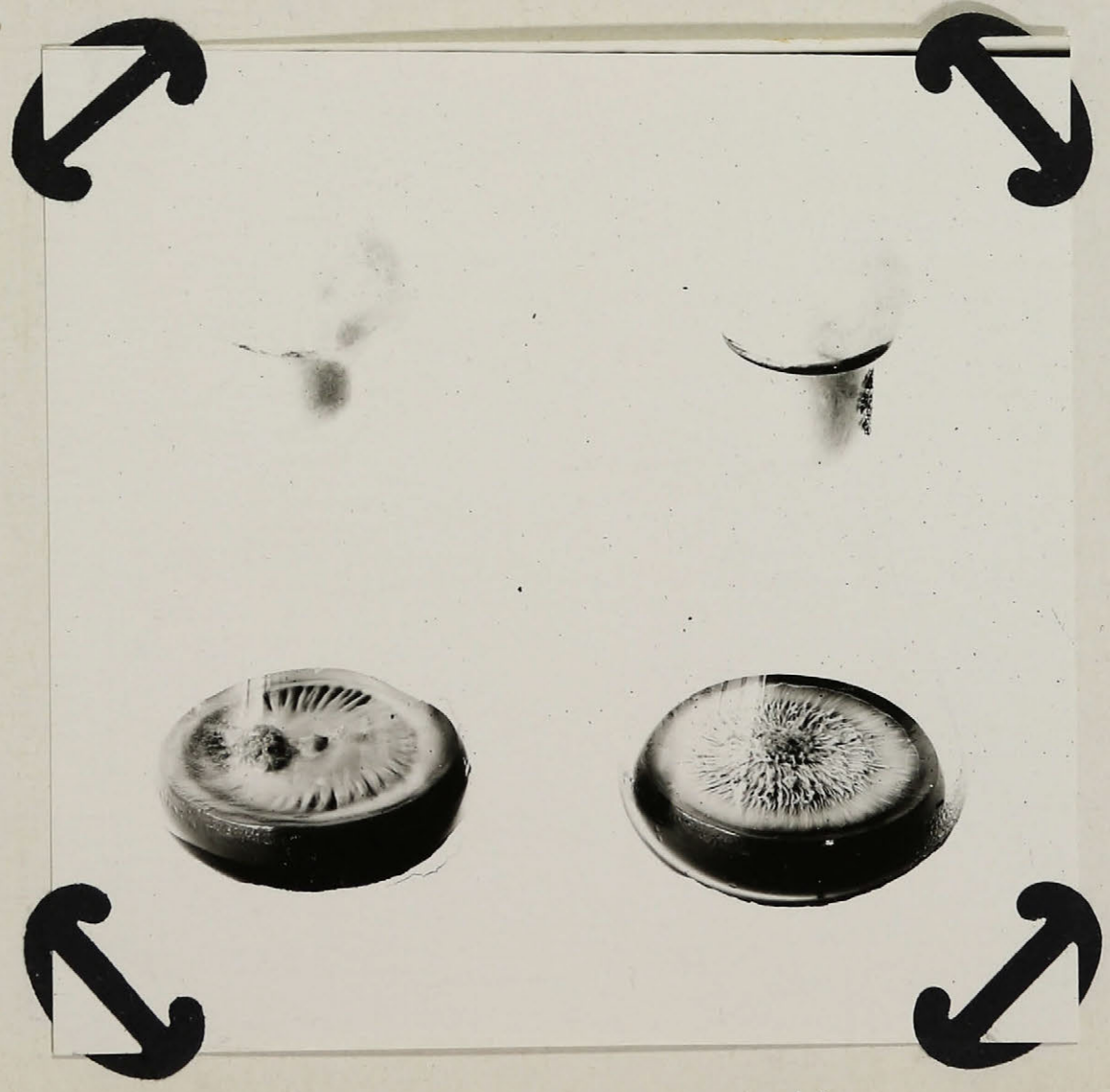

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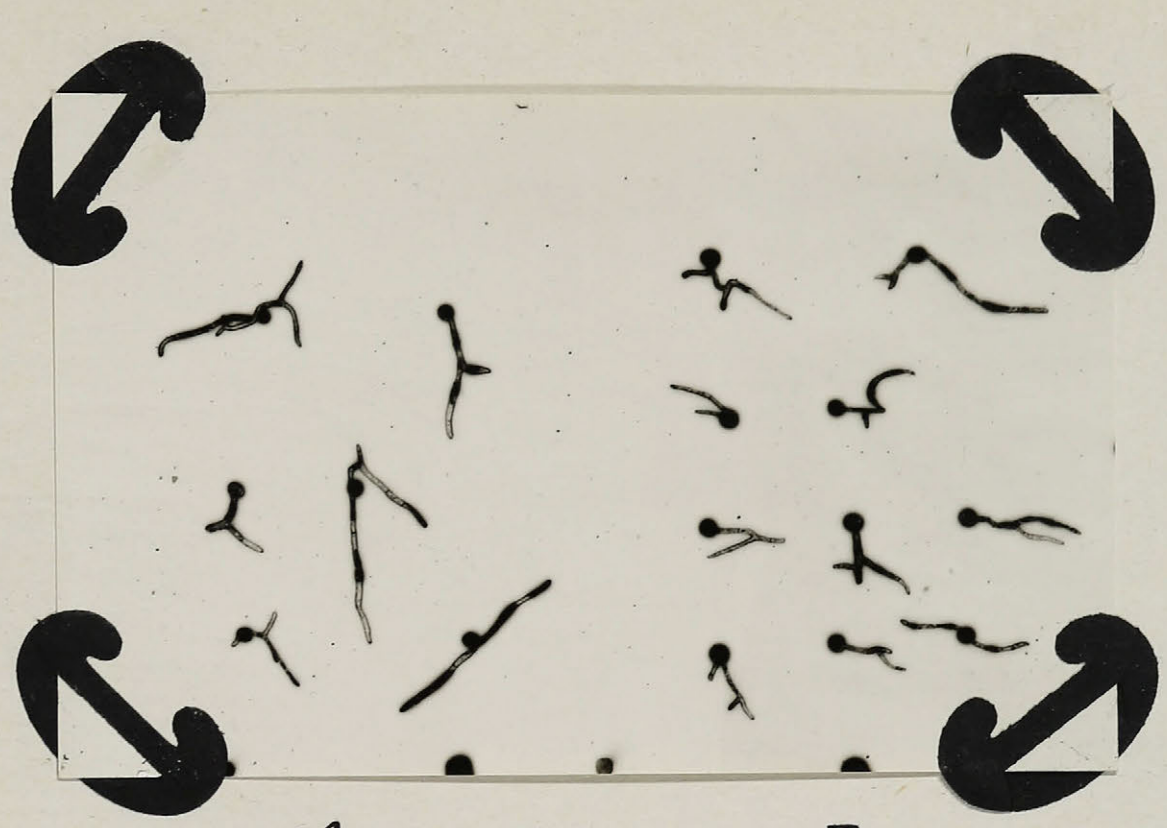
# Plate 3



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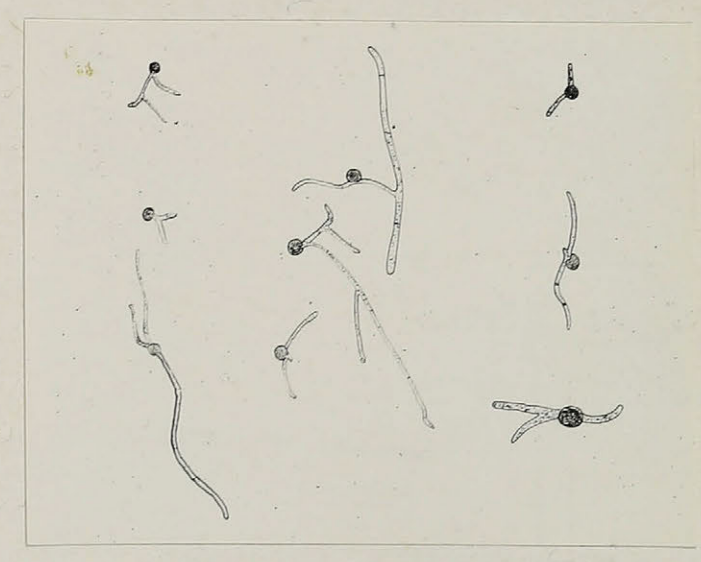

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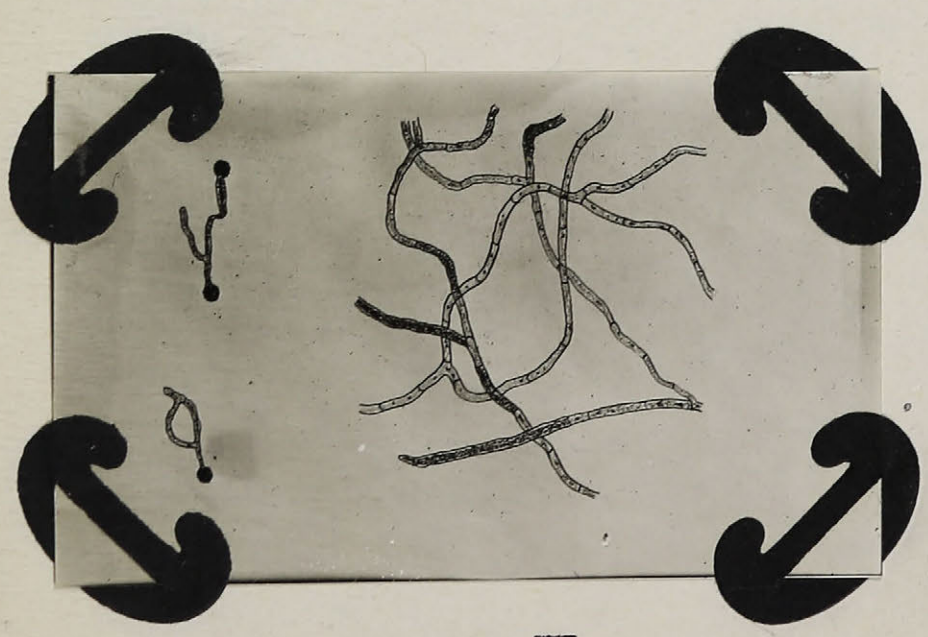

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# Plate 4

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