# T H E S I S

# CARBON DIOXIDE AND HYDROGEN-ION CONCENTRATION AS FACTORS INFLUENCING THE GERMINATION OF SPORES OF COVERED SMUT OF OATS USTILAGO LEVIS (K & S) MAG.

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Submitted by

Mary F. Howe

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Colorado Agricultural College

Fort Collins, Colorado

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THIS THESIS HAS BEEN READ

APPROVED AND RECOMMENDED

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Head of the Department of Botany

Colorado Agricultural College

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THE DEGREE OF MASTER OF SCIENCE

[redacted]

Chairman and Professor of Zoology

[redacted]

Professor of Horticulture

[redacted]

Professor of Veterinary Pathology

Committee on Advanced Degrees
Colorado Agricultural College
Fort Collins, Colorado

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# Carbon Dioxide and Hydrogen-ion Concentration As Factors Influencing the Germination of Spores of Covered Smut of Oats Ustilago levis (K & S) Mag.

## I N T R O D U C T I O N

Recently several experiments have given consideration to the stimulation of the germination of fungus spores by plant tissue. Not only has this stimulation occurred where the plant tissue was in contact with the spores, or in contact with the drop in which the spores floated, but experiments showed that even volatile substance from plant tissues strongly effected the spore germination.

Blackman and Welsford (1) first found that spores of Botrytis cinereaproduced strong well-nourished hyphae when sown in turnip juice on the leaf to be infected.

Brown (2) further states that germination of Botrytis cinerea is increased by the action of volatile substances arising from certain plant tissues.

Durrell (3) also was able to stimulate the spores of Basisporium gallarum in a similar way with tissues of the corn plant.

Noble (4) working with Urocystis tritici, Kcke, confirms Brown's results on the favorable effect of plant tissue and distillates on spore germination.

Griffiths (5) obtained much the same results as Noble and Brown in that leaf tissue stimulated germination

of Urocystis tritici Kche. Moreover, she noticed that in some instances germinating seeds stimulated spore germination.

Leach (6) in working with spores of Colletotrichum lindemuthianum found that they germinated poorly in distilled water, but readily when small portions of the host tissue were present.

Though the above mentioned papers record instances of stimulation of spore germination by plant tissue, no analysis or explanation of the phenomenon is attempted.

Using the spores of a convenient fungus Ustilago levis, the following experiments were therefore undertaken to further demonstrate the stimulatory effect on germination of spores by a volatile substance given off from plant tissue. Tests were also made to determine the identity of this substance and to show the nature of its effect on the culture drops and the suspended spores.

## MATERIAL AND METHODS

Ustilago levis (K & S) Mag. (7), covered smut of oats, was used for the various experiments. This material was collected near Fort Collins, Colorado, during the summers of 1923 and 1924. The spores from the different samples were shaken from the smutted heads of oats into a piece of cheese cloth, then dusted thru the cloth

onto a clean paper. They were then put into a small bottle which was kept corked. This composite sample was used for all the tests.

Since the experiments dealt primarily with the germination of the smut spores, several methods were tried to determine the one best suited for the purpose. An optimum temperature 15°-25° C. was used (8) during germination and the cultures were held for thirteen to sixteen hours (9).

One of the usual methods of spore germination was first tried, ie., drops of solution containing the spores were placed upon slides in a petri dish as shown in fig. 1.

This method did not prove very satisfactory because despite the moisture in the dish, the drops often dried before the time for germination of the smut spores.

The method described above was replaced by the use of Van Tieghem cells. (10). However, instead of placing the same substance in the bottom of the Van Tieghem cell as in the hanging drop, germinated wheat and small pieces of tomato and apple tissue were placed in the bottom of the cell with spores in drops of either tap water, or an 0.8 per cent solution of gelatin, above the tissue. The Van Tieghem cells arranged in this way were then put in a moist chamber, Fig. 2. By placing the Van Tieghem cells in a moist chamber there was small possibility of the drops drying before the spores had germinated. This

method did not prove altogether satisfactory, however, because the seedlings often pushed off the cover glass or fungus, contamination over-ran the crushed tissue and effected the drop before the time for germination of the smut spores.

The method described by Noble (11) proved the most successful. About 3 cc. of the spore suspension were placed in Syracuse dishes. These dishes were placed in a moist chamber under the plant tissue which was on a wire rack as shown in Fig. 3. This method of using the Syracuse dishes also simplified reading the spores, because the dishes were easily placed on the mechanical stage of the microscope (fig. 4). The germinated spores were not disturbed and the dish was readily moved so that the entire field could be examined. This last method was used for the major portion of the experiments.

For measuring the definite per cents of carbon dioxide the apparatus shown in Fig. 5 was used. The carbon dioxide was measured in two gas burettes. (Fig. 5, A & A'). A Kipp generator was used for the production of the carbon dioxide which was washed and passed into the measuring burette. When the burette A' was filled with gas, the clamps D and F were closed. The Kipp generator was then detached and the tube at E fastened to the container G; by opening the clamp at F, carefully regulating the clamp

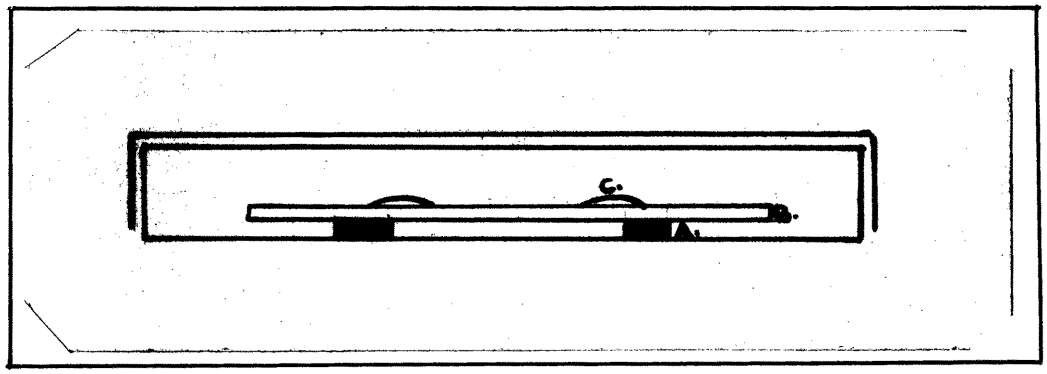


Fig. 1.- Diagram showing position of spore suspension (C), on slide (B), which is placed on small pieces of glass (A). A few cc. of water are put in the bottom of petri dish to maintain humidity and prevent evaporation of the drop.


Fig. 2.- Showing arrangement of Van Tieghem cells in a moist chamber. The plant tissue is placed in the bottom of the cell with hanging drops of spore suspension on a cover glass over the cell. The cells are then placed on glass racks in moist chamber.

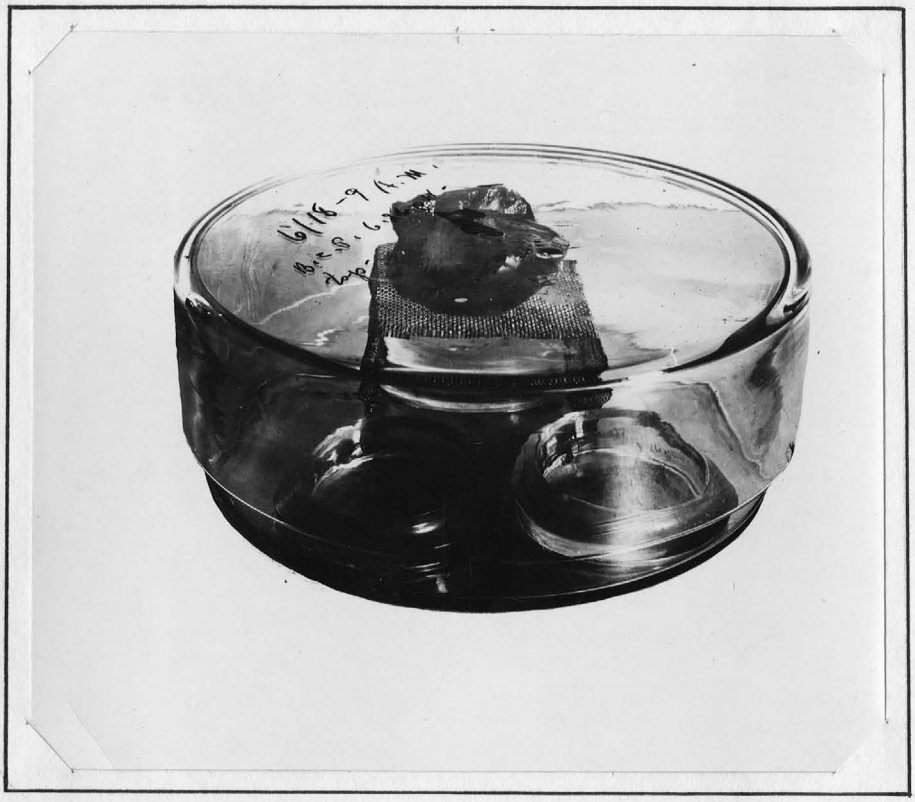

Fig. 3.- Showing moist chamber containing the Syracuse dishes of spore suspensions. The plant tissue is placed on a wire rack above these dishes.

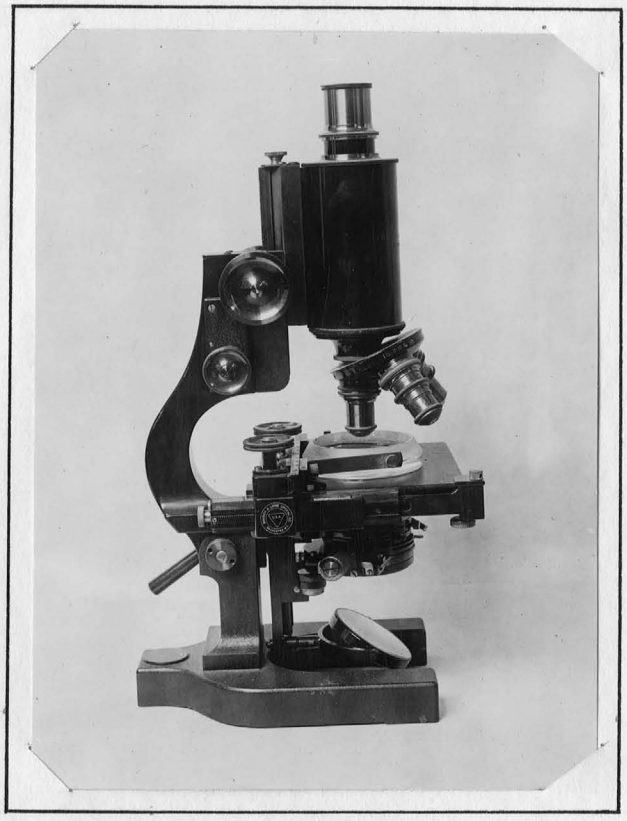

Fig. 4.- Microscope showing the Syracuse dish on the stage. The dish is readily moved by two screws at the left of the mechanical stage. A special clamp is screwed to the moving rack to hold the dish.

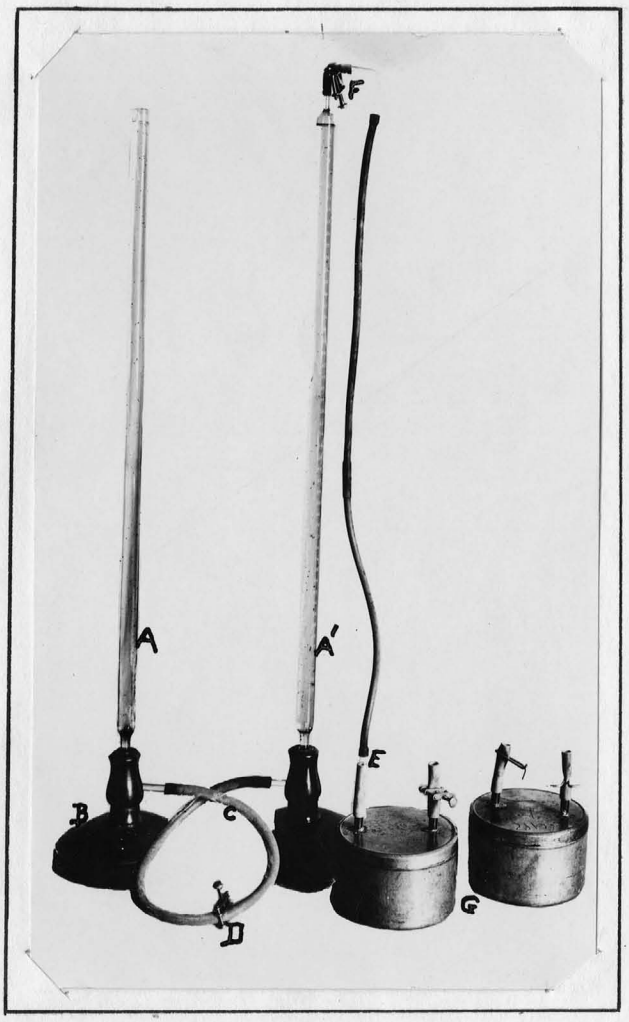


Fig. 5.- Gas burettes and two of the sealed culture chambers used in controlling the percentage of carbon dioxide in atmosphere of the spore suspensions.

D, and raising the burette A to the oil level of A', the necessary amount of carbon dioxide was forced into the sealed chamber (G) of 600 cc. capacity, containing the dishes of spore suspensions and indicator solutions.

The apparatus shown in Fig 6 was used for germinating spores in a continuous flow of carbon dioxide. The spore suspension was placed in a small dish (A) in a glass (b) and an indicator solution was placed in a small dish (A'( in a glass (B'). The glasses (B & B') were fitted with glass and rubber connections (c) to the wash bottle (D) which was filled with distilled water, to wash the carbon dioxide before passing into the spore suspensions. The glass tube from C to A and A' dipped just below the surface of the spore suspensions and indicator solutions in A and A'. This allowed the gas (carbon dioxide) to bubble directly into the liquid.

To determine the amount of carbon dioxide given off by a definite amount of plant tissue, an apparatus was arranged as shown in Fig. 7. The weighedplant tissue was placed on a wire rack in the glass (A). The end of the glass tube (B) was just below the rack holding the tissue. This tube (B) was fastened to the flask (C) which contained a perfectly clear solution of barium hydroxide. The flask was then fastened to a suction pump at D. The air was pulled through three wash bottles containing

Fig. 6.- Apparatus for continuous bubbling of carbon dioxide into the spore suspensions.

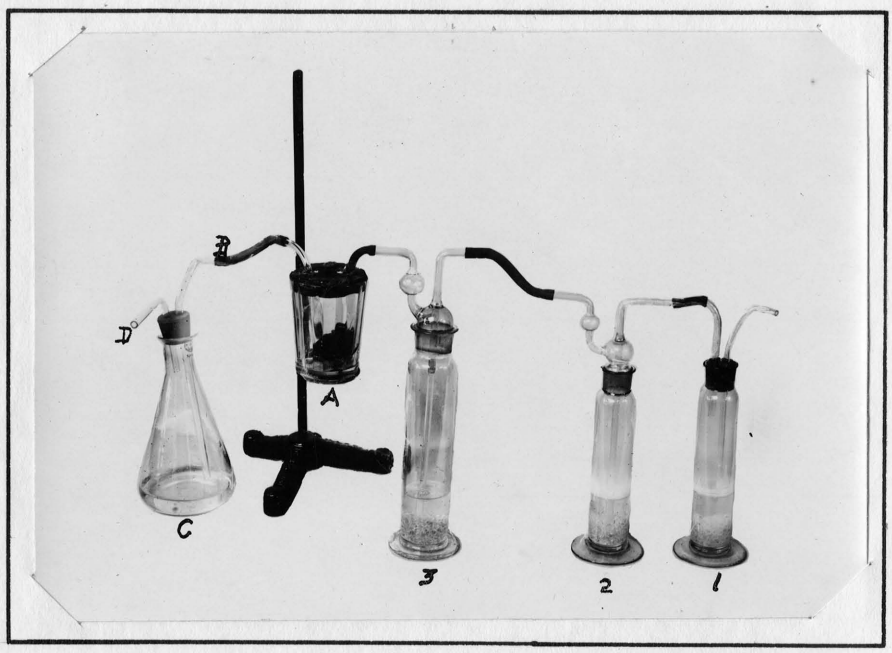


Fig. 7.- Apparatus used for determining the amount of carbon dioxide given off by a definite weight of plant tissue.

calcium hydroxide (1), barium hydroxide (2), and sodium hydroxide (3), in order to take out all the carbon dioxide from the air. Thus the carbon dioxide free air went into the glass (A) and the air containing the carbon dioxide from the plant tissue was pulled into the barium hydroxide in the flask (C) where it percipitated as barium carbonate. The amount of carbonate was then determined by the method used by Scott (12).

In determinations of acidity the colorometric method as described by Clark (13) was used. Standard solutions were made up and kept in a dark cabinet in sealed tubes. These standards were checked by electrometric titration.

All glassware used in the tests was carefully washed and due allowance was made for changes in ph during the tests. All cultures where acidity was determined were tested at time of making the spore suspensions and at the end of the period of germination.

## EXPERIMENTAL DATA

### The effect of plant tissue on the germination of spores of Ustilago levis.

It has been noted that plant tissue effects the germination of fungus spores and Brown (2), Leach (6), Noble (4), and Griffiths (5), have suggested that this is perhaps due to some volatile substance, perhaps an ester. However, diverse tissues produce similar

stimulation which indicates some more common substance as the stimulating agent.

The production of carbon dioxide by plant tissue is well known and Tashiro (14) has shown that this gas is evolved from minute quantities of tissue. The universality of carbon dioxide production from plant tissue and the stimulation of spore germination not only by odoriferous tissue such as apple peel but by other tissue as well, leads to the assumption that the carbon dioxide may play a part in the stimulatory action.

Using the Van Tieghem cells and methods previously described, forty-eight tests were made to demonstrate the stimulating action of plant tissue on the germination of spores of Ustilago levis. A count of 100 spores was made in each culture.

In the following table a summary of these results is given:

TABLE I. The effect of plant tissue upon the germination of spores of Ustilago levis in hanging drops of tap water. 16 hours 23° C.

| Plant Tissue | Percent Germination Tap Water |
|---|---|
| Tomato | 62.3 |
| Two wheat seedlings | 40.4 |
| Apple | 43.6 |
| Control (no tissue) | 14.6 |

In table I and the graph in Fig 8, it will be seen that the highest per cent germination was in the hanging

drop above a piece of tomato tissue. It may be noted that the apple (Fig. 8,2) and wheat seedlings (Fig. 8,3) also produced a higher germination than the control (Fig. 8,4). These figures indicate that plant tissue stimulates the germination of spores of Ustilago levis and further that this stimulation is not produced by any one kind of tissue.

Not only is the percentage of germination effected by the presence of plant tissue but the character of germination is influenced. Fig. 9 shows the type of promycellial germination produced by the plant tissue; illustrating again that the plant tissue stimulates germination of spores of Ustilago levis.

The diverse nature of the tissues used rather precludes the action of some specific aromatic substance, and presents the probability that the carbon dioxide given off by the plant tissue is absorbed by the hanging water drop producing a drop of carbonic acid. The results, therefore, may be due to the acidity of the culture drop.

Determining the acidity of drops suspended above pieces of plant tissue.

In order to determine whether carbon dioxide is absorbed by a culture drop suspended in a Van Tieghem cell over plant tissue with a resulting production of acidity, the following experiments were tried.

Using the colorimetric method described by Clark (13).

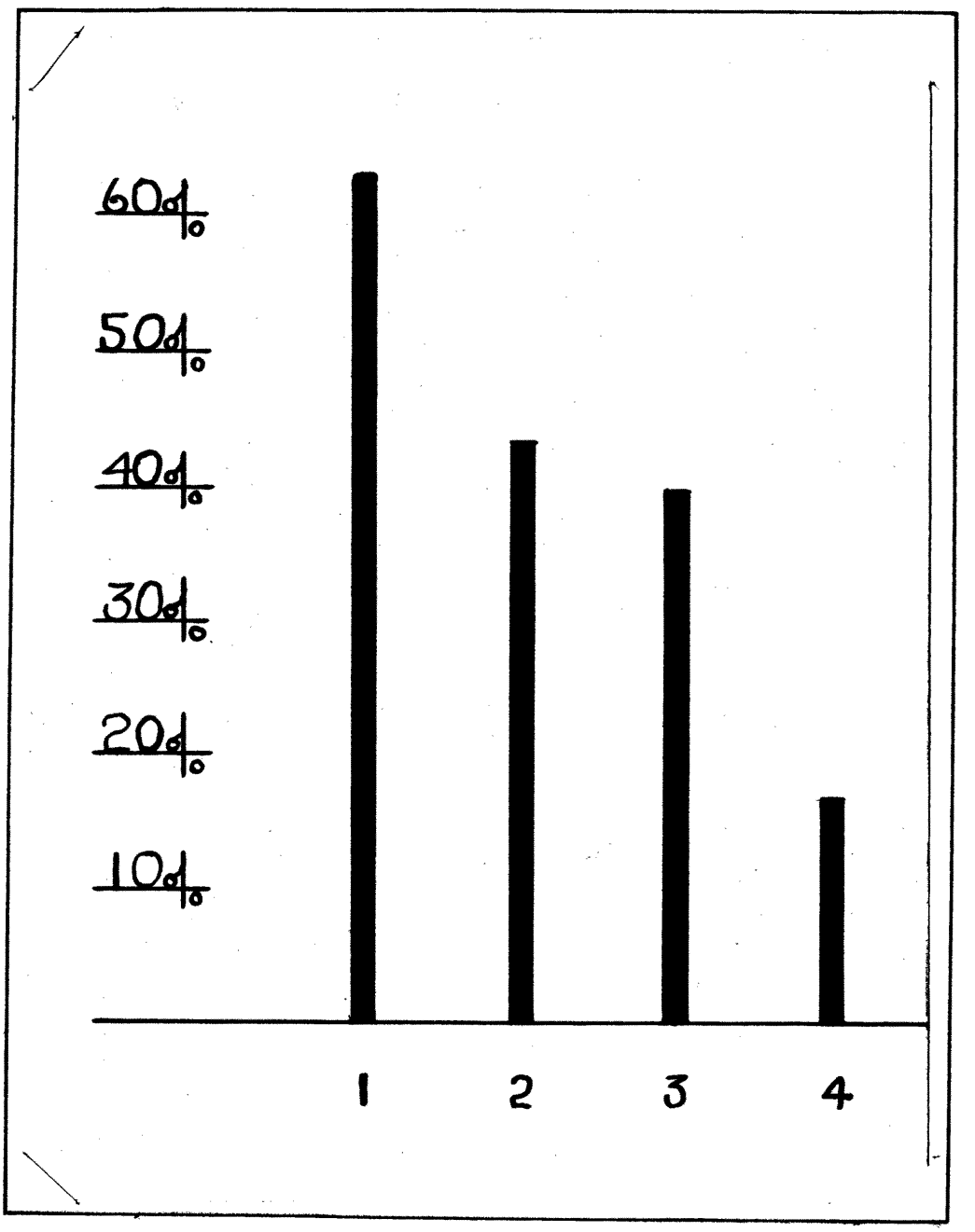


Fig. 8.- Histogram showing per cent germination of spores of Ustilago levis in spore suspensions of tap water.

1 tap, hanging drop spore suspensions above a piece of tomato in Van Tieghem cell; 2 tap, water spore suspensions above apple tissue; 3 tap, water spore suspensions above two wheat seedlings; 4 tap, water spore suspensions without plant tissue (control).

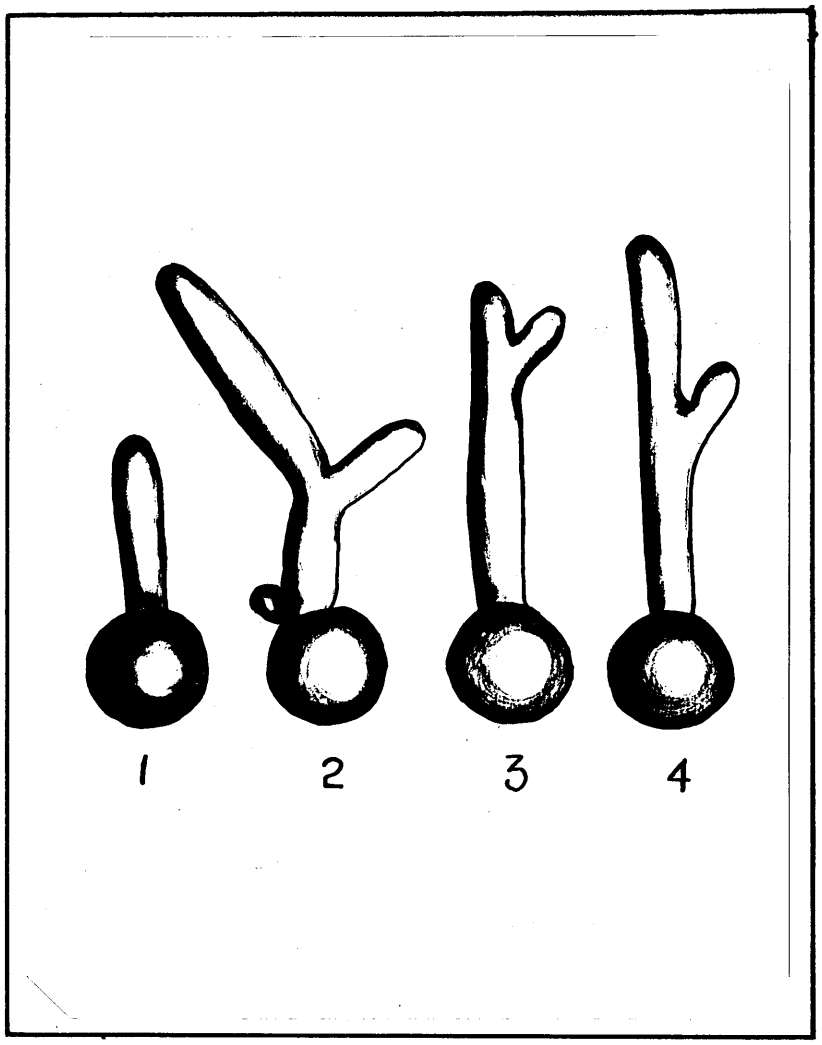


Fig. 9.- Showing types of germination produced in a hanging drop above the plant tissue in Van Tieghem cells.

1, type germination in control;
2, type of germination in presence of tomato tissue;
3, apple tissue;
4, wheat seeds (just germinated).

drops of tap water containing indicator were suspended in separate vials (1) over two wheat seedlings, (2) over a piece of tomato and (3) over apple tissue; in the latter two, fragments of tissue were used the same size as those used in the VanTieghem cells with the spore cultures. In the controls the drops of tap water with the indicator (methyl red) were suspended above barium hydroxide in one case and in air (without tissue) in the second instance.

In Fig. 10 is shown the device used in this experiment. Water containing the indicator (in amounts equivalent to that used in standards) was drawn into the glass pipette inserted through the cork of the vial. Pressure on the soft plasticene plugs caused drops of the water and indicator, comparable in size to those used in spore cultures, to be exuded into the vials containing the plant tissue. In this way conditions in the Van Tieghem cells were duplicated. After sixteen hours exposure the color of the drops was compared to drops of the standard solution.

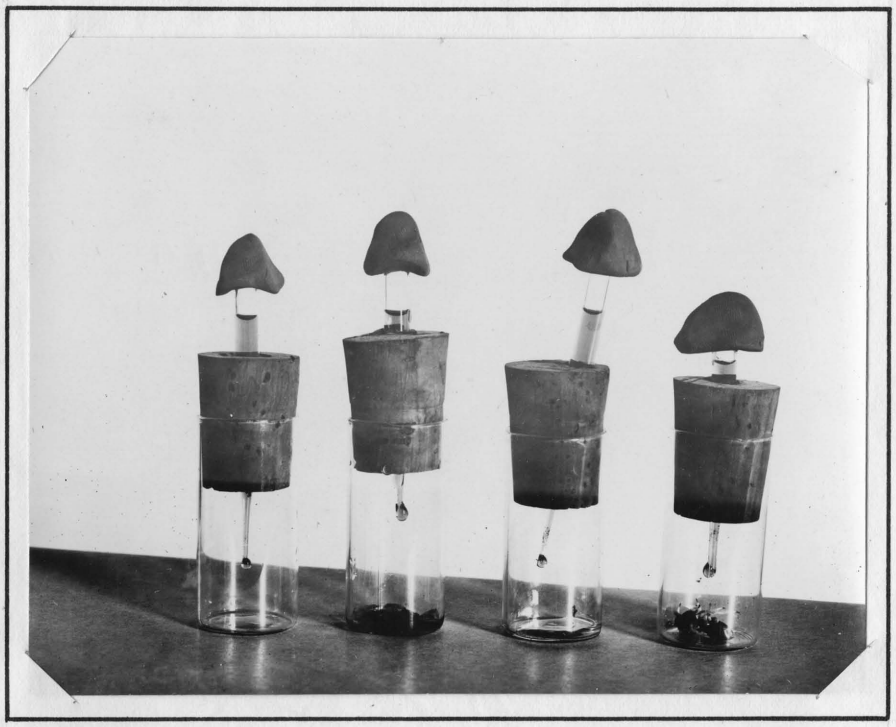

Fig. 10.- Drops (containing indicator) suspended above plant tissue. 1, Control; 2. Tomato tissue; 3. Control; 4. Wheat seedlings. These correspond to the hanging drops used in cultures of Ustilago levis spores.

The changes in pH obtained in these suspended drops are given in the following table which represents the mean of three trials:

TABLE II The effect of plant tissue in changing the pH of drops of tap water. 16 hours 23° C.

| Tissue | Tap Water | |
|---|---|---|
| | pH before | pH after |
| Tomato | 6.7 | 4.9 |
| Apple | 6.7 | 4.9 |
| 2 Wheat seedlings | 6.7 | 4.9 |
| Control, air | 6.7 | 5.5 |
| Control, Ba $(OH)_2$ | 6.7 | 6.7 |

Table II shows that the tissues in the vials with the suspended drops caused the pH of the tap water to change from 6.7 pH to 4.9 in the temperature and time used for all the spore cultures. The control over barium hydroxide remained unchanged while that in the air absorbed enough carbon dioxide to produce a change in pH. To 5.5

This leads to the conclusion that the carbon dioxide from the plant tissue is absorbed by the suspended drop and changes its acidity.

Comparison of acids in tissue and acidity produced by carbon dioxide.

In working with Ustilago tritici Noble (1) and Griffiths (5) placed fragments of plant tissue directly in the spore suspensions, which allows for the effect of such acids as may be in the tissue. In order to determine if the results from acids from tissue and carbonic acid are comparable, the following tests were made:

Using methods of Noble (1) cultures were made in Syracuse dishes, by placing pieces of plant tissue directly in the dish with the spore cultures and by placing plant tissue on racks in a chamber with the spore cultures. Tap water was used for the spore suspensions.

The following table shows a mean of the results of seventy-two cultures:

TABLE III. Comparison of per cent germination of spores of Ustilago levis in acids found in plant tissues and acidity produced by carbon dioxide from the plant tissue in tap water, 16 hours, 23° C.

| Tissue | Average Per cent Germination Tap Water |
|---|---|
| Tomato on rack above dishes of spores | 40.4 |
| Tomato in spore suspension | 64.7 |
| Fifty wheat seedlings on rack above dishes of spore suspension | 42.6 |
| Five wheat seedlings in dishes of spore suspension | 71.6 |
| Control | 26.1 |

In Table III above, it may be noted that in the control, germination is lower than where tissue is used, further, where tissue is actually in the cultures germination is greater than where the tissue is merely in the chamber with the cultures.

To determine the pH resulting under the conditions of the above experiments, Syracuse dishes of tap water, containing indicators, were placed in the same containers

with the cultures. Color changes indicated alteration of acidity as shown in the following table:

TABLE IV. The effect of plant tissue in changing pH of spore suspensions containing tissue, also with tissue above spore suspensions.

| Tissue | Average pH of Tap Water | |
|---|---|---|
| | Before | After |
| Tomato above spore suspensions | 6.7 | 5.9 |
| Tomato in spore suspensions | 6.7 | 5.2 |
| 50 Wheat seedlings on rack above dishes of spore suspensions | 6.7 | 5.9 |
| 5 Wheat seedlings in dishes of spore suspensions | 6.7 | 4.9 |
| Control | 6.7 | 6.7 |

The above figures in Table IV show that tissue in the culture medium produces conditions of acidity similar to those obtained where tissue is in the same chamber but not in contact with the medium. The tomato tissue and also wheat seedlings produced the same change in pH (6.7 pH to 5.9 pH) when placed on a rack above the spore suspensions in tap water. The changes in pH due to tissue directly in the spore suspensions are practically the same for both the tomato tissue and the wheat seedlings. These results are quite comparable to those shown in Table II.

The type of germination of the spores is affected by the acidity changes in a comparable manner to the present germination.

In comparing tables III and IV, it may be noted that

the tomato above the spore suspension produces an acidity of 5.9 pH with a resulting germination of 40.4 per cent. When the tomato was in the suspension, the pH reached 5.2 with a resulting germination of 64.7 per cent. Wheat seedlings above the suspension produced an acidity of 5.9 pH with 42.6 per cent germination while those in the suspension caused it to reach 4.9 pH and resulted in 71.6 per cent germination. Controls maintained the original pH of 6.7 and produced an average germination of 26.1 per cent. These figures indicate a correlation between pH concentration and per cent germination.

The germination of spores of Ustilago levis in different H-ion concentrations.

From foregoing data, the indications are that the acidity of the drop cultures materially affects the percent and character of germination of spores of Ustilago levis. It would seem logical, therefore, to determine the optimum acidity for germination of the spores of this organism and also the effects of various acids and buffers.

The following experiments were made, using each of five acids, succinic, citric, hydrochloric, lactic and malic. A series of these acids was set up in H-ion concentrations ranging from 4.0 pH to 7.5 pH in distilled water as determined colorimetrically (13). These were checked further by electrical titration.

The results of ninety-six tests are summarized in the following table.

TABLE V. The effect of H-ion concentration of various acids in distilled water on the germination of spores of Ustilago levis. 16 hours, 23° C.

| Acid | 4.0 pH | 4.4 pH | 4.9 pH | 5.5 pH | 5.9 pH | 6.4 pH | 6.9 pH | 7.5 pH |
|---|---|---|---|---|---|---|---|---|
| Succinic | 9.4 | 45.0 | 19.7 | 15.4 | 12.7 | 25.2 | 15.6 | 7.2 |
| Citric | 12.8 | 31.1 | 26.7 | 14.1 | 12.7 | 23.9 | 14.5 | 10.2 |
| HC l | 9.9 | 14.5 | 16.3 | 15.9 | 17.3 | 13.3 | 10.4 | 10.8 |
| Lactic | 12.7 | 12.8 | 34.4 | 18.0 | 16.5 | 24.2 | 17.3 | 13.6 |
| Malic | 19.3 | 46.0 | 43.4 | 24.4 | 15.2 | 29.7 | 22.9 | 15.4 |

In testing the germination of spores of Ustilago levis at different pH as indicated in Table V, the spores were placed in 3 cc. each of these solutions in Syracuse dishes which were stacked and left at room temperature (23° C.) for sixteen hours. No germination was obtained below 4.0 pH or above 7.5 pH. Two optimum points are indicated, one between 4.4 pH and 4.9 pH and the other at 6.4 pH. These results are graphically illustrated in Fig. 11.

McClendon (15) states that "the pH of water solutions --------- --- is changed by minute traces of impurities. --------- --- If exposed to the air, it becomes acid due to absorption of carbon dioxide and if kept in glass, it becomes alkaline, due to solution of the glass. ---- This difficulty in maintaining fixed hydrogen-ion concentrations is obviated by the use of buffers." An 0.8 per cent solution

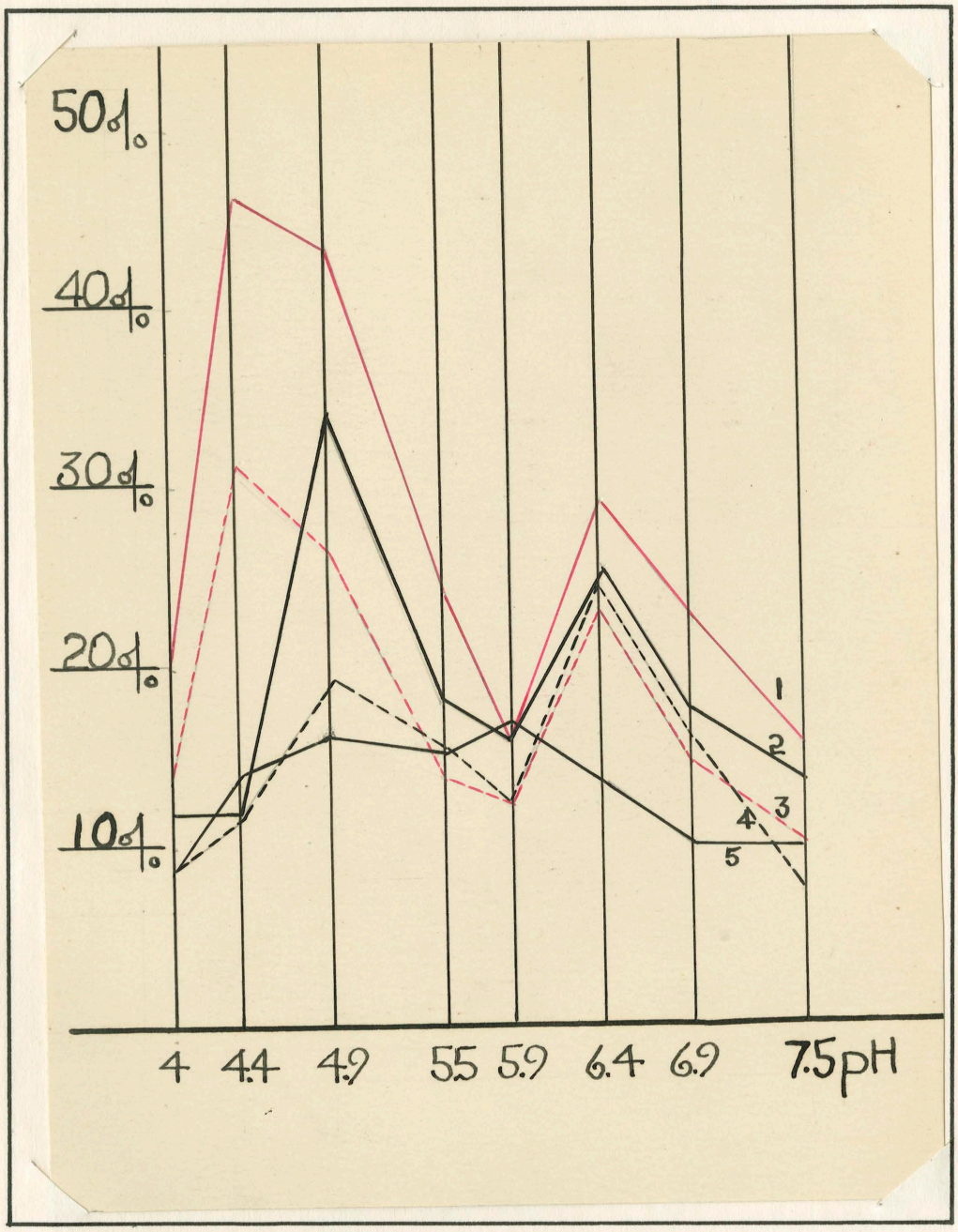


FIG. 11.- Curves showing per cent of germination of spores of Ustilago levis at various H-ion concentrations of - 1. malic; 2. lactic; 3. citric; 4. succinic; and 5. hydrochloric acids. The spores were placed in 3 cc. of each of these acid solutions for 16 hours at 23° C.

of gelatin in tap water was then used for the following tests.

Using a buffer solution of gelatin a similar series of H-ion concentrations was made. The gelatin gave a higher per cent germination. Lactic, succinic, citric and hydrocloric acids (with Na OH to neutralize) were used. One series using potassium acid phosphate ($K_2$ H $PO_4$) with citric acid was set up; the $K_2$ H $PO_4$ acting as the buffer instead of the gelatin in this case. Three cc. of each of these solutions with the spores were placed in Syracuse dishes and stacked at room temperature (23° C.) for sixteen hours. At the end of the germination period tests were made of the solutions for possible change in acidity. All cultures showing such change were discarded and not used in the following table.

In Table VI and Fig. 12 the results of 120 cultures are summarized.

TABLE VI. The effect of H-ion concentration of varying acids in 0.8% gelatin solution on germination of spores of Ustilago levis. 16 hours, 23° C.

| | Per cent Germination | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Acid | 4.0 pH | 4.4 pH | 4.9 pH | 5.5 pH | 5.9 pH | 6.4 pH | 6.9 pH | 7.5 pH |
| Succinic | 33.8 | 89.7 | 78.0 | 74.0 | 75.5 | 61.8 | 52.0 | 36.1 |
| Citric | 23.8 | 81.2 | 78.0 | 61.3 | 63.8 | 36.2 | 28.6 | 16.7 |
| HC l | 28.5 | 81.0 | 88.4 | 73.6 | 70.5 | 64.4 | 72.3 | 38.3 |
| Lactic | 21.3 | 78.7 | 66.7 | 56.8 | 60.3 | 43.4 | 30.0 | 37.5 |
| $KH_2$ $PO_4$ and Citric | 25.2 | 46.5 | 49.1 | 27.8 | 28.6 | 26.2 | 25.1 | 36.1 |

The above table and accompanying curve show results similar to those obtained in Table V except that there is a greater degree of germination. This might be due to the greater stability of the solution buffered by the gelatin; however, in the present experiments opportunity was not given for consideration of the effect of the buffer.

One of the most interesting results obtained in these tests was the double maximum point as illustrated in Fig. 11 and Fig. 12. It will be seen in these curves that one maximum point of germination lies between 4.4 pH and 4.9 pH and the other between 5.9 pH and 6.4 pH. While these points do not coincide and are not definite, the tendency toward two optimum points is shown in all the solutions. A greater number of tests would doubtless correct this discrepancy.

The character of the promycelium developed at these concentrations is illustrated in Fig. 13. There is a marked correlation between the per cent germination and the type of promycelium at the different acidities. Further, the character of the promycelium at the optimum point is identical with that obtained with plant tissue.

### The effect of certain per cents of carbon dioxide on the germination of spores of Ustilago levis.

The highest maximum point of germination for spores of Ustilago levis as above indicated lies between 4.4 pH

and 4.9 pH. The question arises, what amount of carbon dioxide will produce this maximum pH and resulting germination?

Five and ten per cents of carbon dioxide were put in chambers of measured volume (600 cc.) by the method described and illustrated in Fib. 5. Each chamber contained two small dishes, in one of which was 3 cc. of a spore suspension in distilled water and in the other 3 cc. of indicator solution (methyl red). The control was set up in the same way with the exception that no carbon dioxide was added.

The results of this test showed 18 per cent germination with 5 per cent carbon dioxide in the air of the chamber; and 16 per cent germinatio n where 10 per cent carbon dioxide was present. The control showed a germination of 13 per cent. The similarity of these figures raises a doubt as to the stimulating action of carbon dioxide. However, on examination of the dishes containing indicator, it was found that little of this carbon dioxide had gone into solution, the indicator having changed only from 6.7 pH to 6 pH. It can be seen from the curves shown in Fig. 11 that this acidity is not favorable for the highest maximum germination of the spore of Ustilago levis producting approximately 15 and 20 per germination.

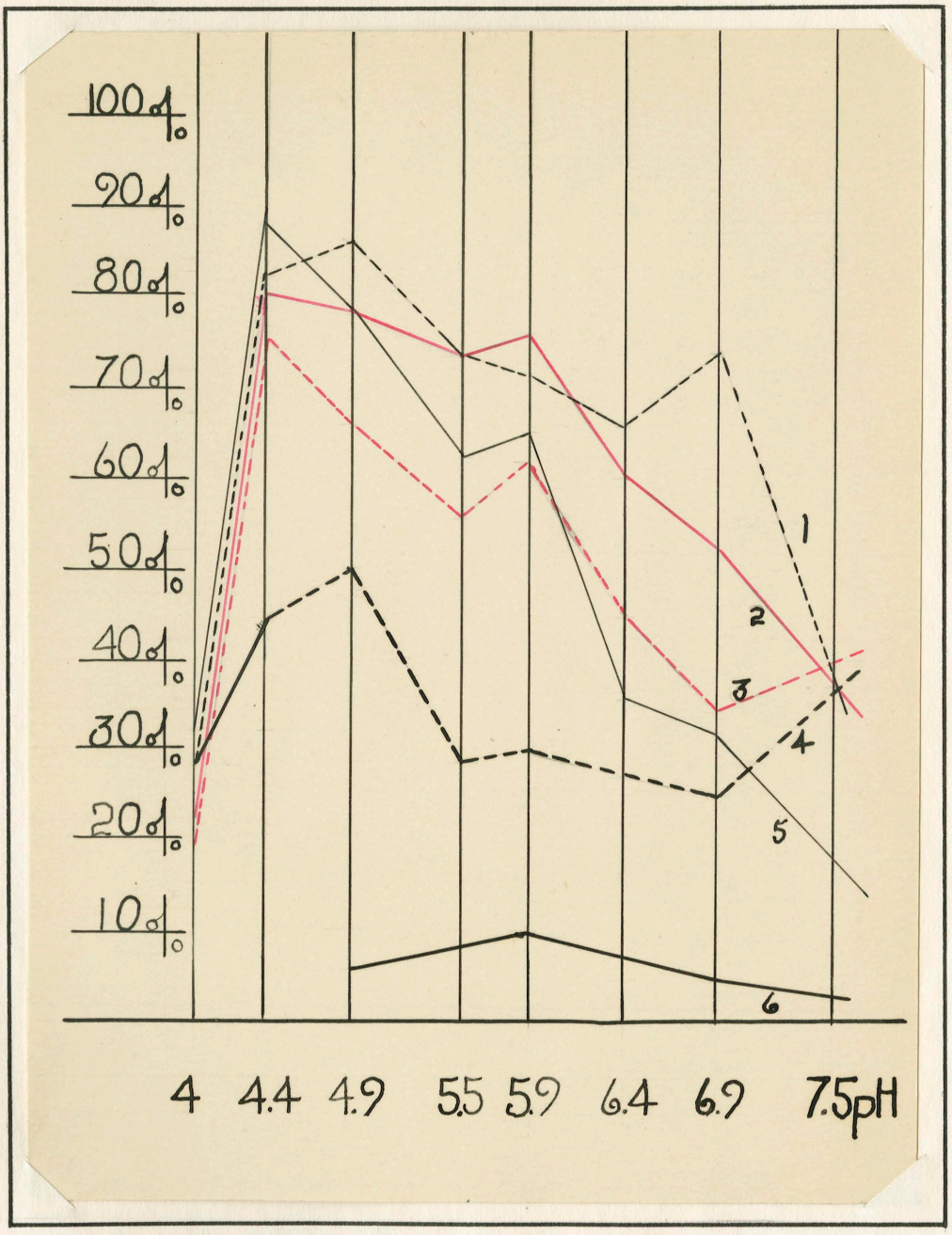


Fig. 12.- Curves showing per cent of germination of spores of Ustilago levis in various H-ion concentrations. The solutions were made using an 0.8 per cent gelatin solution with acids. 1. hydrochloric acid; 2. succinic; 3. lactic; 4. $K_2HPO_4$ and citric acid; 5. citric and 6. distilled water and hydrochloric acid.

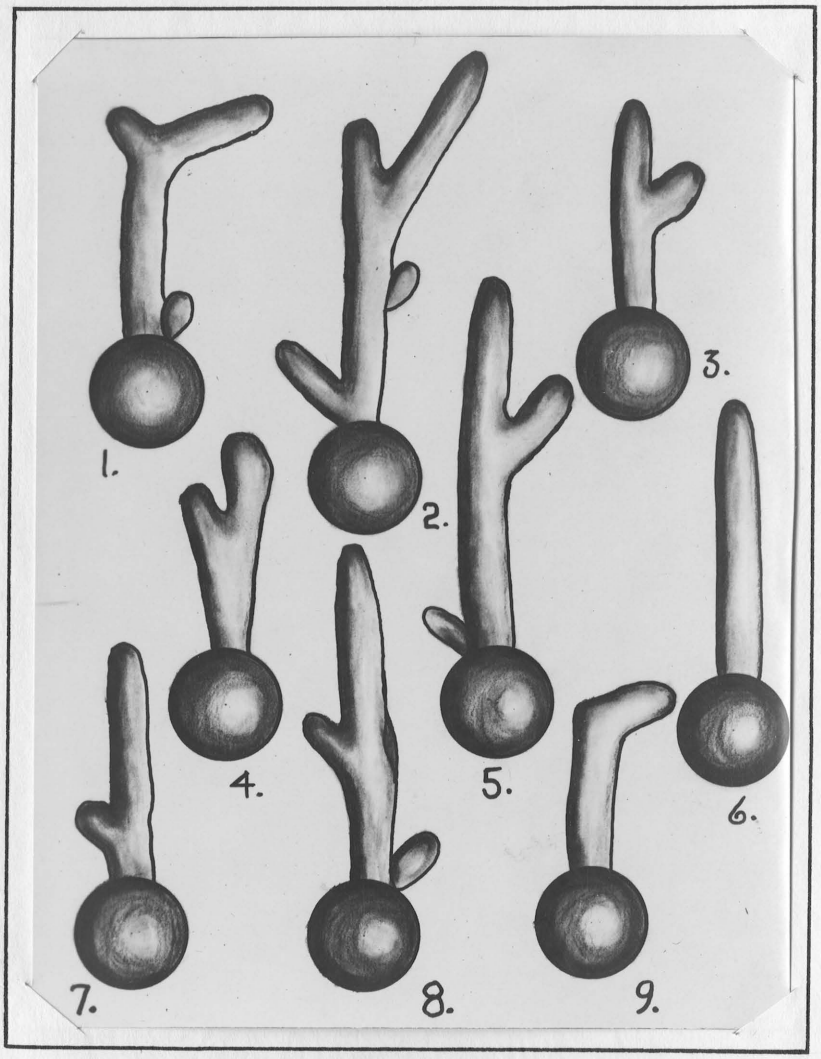


Fig. 13.- General types of spore germination of Ustilago levis in various H-ion concentrations.

1 - 4.0 pH; 2 - 4.4 pH; 3 - 4.9 pH;
4 - 5.5 pH; 5 - 5.9 pH; 6 - 6.4 pH;
7 - 6.9 pH; 8 - 7.5 pH.

According to Smith (16) a saturated solution of carbon dioxide in water attains a pH of 4.8 at 25°. It is evident that under the conditions of the experiment little carbon dioxide went into solution and but slight change in acidity resulted.

Since the definite per cents of carbon dioxide in the chamber did not produce the highest maximum acidity (4.4 pH to 4.9 pH) continuous bubbling of carbon dioxide through the spore suspensions was tried. In the first part of this experiment the solutions were very unstable owing to the dissociation of the acid. The continuous bubbling of carbon dioxide into the spore suspensions did away with this difficulty. The solution reached 4.4-4.9 pH as long as the gas was bubbling into it. In a very short time after the gas ceased to be bubbled into the indicator solution, it showed that the pH had changed toward alkalinity,due to loss of carbon dioxide.

The amount of germination in the spore suspension with continuous bubbling of carbon dioxide corresponds to the amount of germination in the acid spore suspension of the same acidity shown in Table V and Fig. 11.

In table VII and Fig. 14 are shown graphically a summary of the results above mentioned.

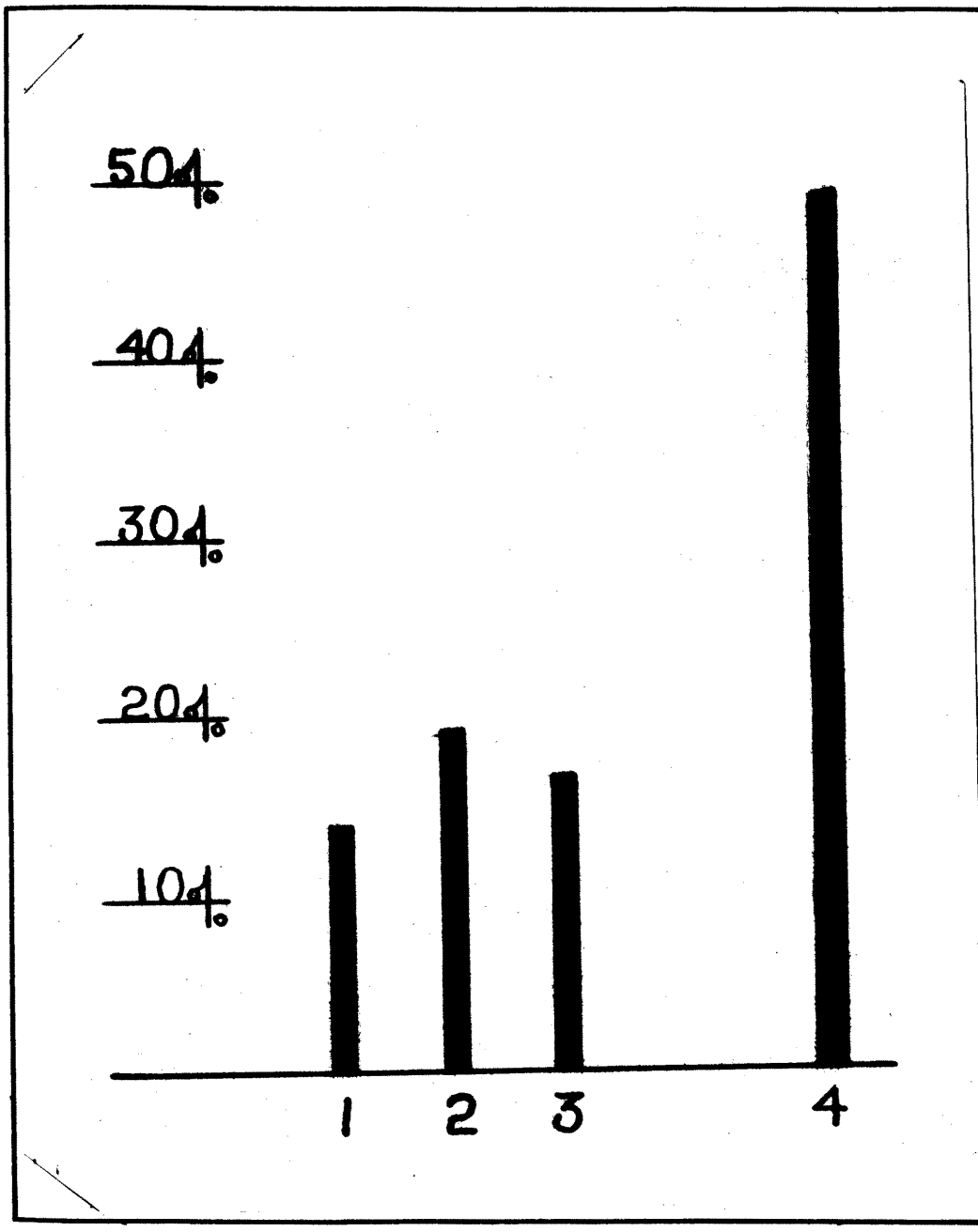


Fig. 14.- The effect of different amounts of carbon dioxide on the germination of spores of Ustilago levis in a spore suspension of distilled water.
1. effect of 5 per cent carbon dioxide; 2. effect of 10 per cent carbon dioxide; and 3. control. 4. shows the effect of continuously bubbling carbon dioxide into the spore suspensions.

TABLE VII. The effect of certain per cents of $CO_2$ and continuous bubbling of $CO_2$ in spore suspension of distilled water on the germination of spores of Ustilago levis. 17 hours, 23°C.

| Carbon Dioxide | Per cent Germination |
|---|---|
| 5% $CO_2$ | 18 |
| 10% $CO_2$ | 16 |
| Control, air | 13 |
| Continuous bubbling $CO_2$ | 48 |

The above figures suggest that if continuous bubbling of carbon dioxide into the spore suspensions produces the highest maximum point of acidity and germination, then plant tissue continuously giving off carbon dioxide should produce like results.

In the foregoing experiments variable results were obtained from plant tissue, also as just shown it is necessary that large and continuous amounts of carbon dioxide be present in order to keep up the acidity of the spore suspension to the point of highest maximum germination of the spores.

It seemed necessary, therefore, to determine the amount of carbon dioxide given off from definite amounts of plant tissue.

By the use of the apparatus shown in Fig. 7, determinations were made of the amount of carbon dioxide given off by a definite amount of plant tissue. It was found that 25 grams of tomato or apple tissue and 30 wheat

seedlings gave carbon dioxide sufficient to produce 4.9 pH in each of three dishes containing 3 cc. of tap water.

## C O N C L U S I O N S

The work of Brown (17) and that of Leach (6), Noble (4) and Griffiths (5) have suggested that fungus spores are stimulated not only by plant tissue in the infection drop but by some volatile substance given off by plant tissue. However, no effort has been made by these workers to offer an explanation of this stimulation or to correlate it with other related phenomena.

Tests with the germinating spores of Ustilago levis indicated that germinating seeds and other plant tissues do effect the spore germination of that fungus as to increased percentage and type of promycelium. This occurs not only when the tissue is in the culture drop but when the seed or tissue is in the same chamber as the culture drop.

It has been found that under both these conditions the acidity of the infection drop is changed varying in proportion to the amount of tissue used. Where tomato and wheat seedlings were placed in the spore suspensions the acidity was raised to 4.9 pH. Corresponding to this was the change where seedlings or tissues were put in the same chamber as the culture drop. In these the acidity was changed from 6.7 pH to 4.9 pH where tap water was used.

In the germination of spores of Basisporium gallarum Durrell (27) found that the carbon dioxide from plant tissue stimulated spore germination, and suggested that the carbon dioxide produced acidity in the drop which influenced the permeability of the spore membrane and thus resulted in germination.

In the present study of Ustilago levis it has been found that germination is practically the same in a drop containing plant tissue and in a drop exposed to carbon dioxide from plant tissue. Further titration of the spore suspensions in these experiments shows approximately equal changes in acidity. Definite determinations show that 30 wheat seedlings or twenty-five grams of tomato pulp produce carbon dixoide sufficient to bring 3 cc. of spore suspension to 4.9 pH and to produce optimum germination.

In the case of citric, lactic, succinic, malic and hydrochloric acids, the maximum germination of the spores occurred at about 4.9 pH which point is identical with that produced by plant tissue in the culture drop and with plant tissue in the same chamber with the drop.

The action of acids on the cell and their effect on its permeability is widely recognized, indicating that acidity may strongly effect the germination of spores.

Hind (18) shows that dilute solutions of acids or at any rate their hydrogen-ions, readily enter plant tissue.

Crozier (19) says that the relative effect of hydrogen-ion concentration on the speed of penetration is augmented with increasing concentration of acid; while according to Stiles and Jorgensen (20) the rate of exosmosis of each organic substance depends on the concentration of the substance employed; the higher the concentration the more rapid the exosmosis.

The effect of acidity on spore germination has been studied by Clark (21), Duggar (10), and Webb (22), indicating that certain acid concentrations are optimum for spore germination as well as for cell permeability.

Webb (23) and Hopkins (24) working with spores of several species of fungi also found that in many cases maximum germination occurred at two distinct acid concentrations.

In the present study of the germination of the spores of Ustilago levis, maximum germination was likewise found at two distinct concentrations, 4.9 pH and 6.4 pH. This phenomenon suggests the isoelectric points of protein as mentioned by Bayliss (25) and Freundlich (26) and are assumed to be the points of greatest cell permeability.

If the carbon dioxide from plant tissue produces the changes in the acidity of the infection drop as above noted and thereby increases germination, it should be possible to duplicate these results by the use of carbon dioxide from a generator.

Germination was increased in this manner. However, preliminary tests with definite per cents of carbon dioxide in the atmosphere gave negative results. This was due to the small concentration of carbon dioxide used and to the unstable character of the carbon dioxide solution which is readily effected by vapor tension and buffers that may be used in the solutions.

The pH in infection drops where five per cent and ten per cent of carbon dioxide atmosphere was used did not reach a concentration optimum for spore germination. However, where carbon dioxide was continuously bubbled through the spore suspensions, saturation was approached. According to Smith (16) a saturated solution of carbon dioxide and water attains a pH of 4.8. In the experiments carried out with Ustilago levis such continuous bubbling of carbon dioxide caused the spore suspension to reach a pH of 4.9 and stimulated spore germination to the maximum.

The general conclusions may be made that with spores of Ustilago levis, acid in weak concentrations stimulates germination. The optimum concentration being in the neighborhood of 4.9 pH. Where plant tissue in the spore suspension or in the chamber with the spore suspension has successfully stimulated spore germination it has been found that the acidity of the spore suspension has been changed to 4.9 pH.

Carbon dioxide approaching saturation in solution produces an acid concentration of about 4.9 pH and under such conditions stimulates spore germination.

It would appear then that the stimulation of spore germination by plant tissues resolves itself into a matter of acidity of the spore suspension as influenced by the tissues.

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Botanical Laboratory
Colorado Agricultural College
Fort Collins, Colorado.